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Methylmercury in fish species used in preparing *sashimi*: A case study in Brazil

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ABSTRACT

The objective of this work was to determine the organic and total mercury contents in twelve fish species used in preparing *sashimi* in Japanese restaurants and estimate the exposure to organic mercury due to the consumption of this food. The mercury species were quantified by atomic absorption with thermal decomposition and amalgamation. Total mercury was analyzed directly, whereas organic mercury was quantified after extraction with toluene in an acid medium using a microwave assisted system. Needlefish and tuna showed the highest levels of mercury species and salmon and mullet the lowest levels. The mean ratios between $MeHg^+$/total Hg were 93% and 87% for sandperch and octopus, respectively, indicating that the most toxic form (organic Hg) predominates in these species. Exposure to methylmercury was estimated based on the PTWI and the results showed that the ingestion of 2 portions of needlefish and tuna exceeded the values established by 100%.

1. Introduction

Fish consumption has grown exponentially throughout the world in a search for healthier foods and improved quality of life. In a human diet they are one of the best sources of high-quality protein and of other nutrients such as selenium, antioxidants and essential fatty acids of the ω3 series (Costa et al., 2015; Galimberti et al., 2016).

Recent surveys have shown that dishes based on raw fish are more and more accessible in common and specialized restaurants, buffets and sushi bars. However, the ingestion of fish and derived products is considered to be the main source of mercury for human exposure (Barone et al., 2015; Costa et al., 2015). The consumption of raw fish such as sashimi can significantly influence the final concentration of ingested mercury, thus, is important to obtaining data concerning the ingestion and exposure to the species of mercury present in culinary dishes based on raw fish (Miguéis, Santos, Saraiva, & Esteves, 2015).

The predominant organic mercury form found in fish is methylmercury ($MeHg^+$), which is highly toxic, and can show the potential to bioaccumulation throughout the food chain (Eisler, 2000). Exposure to $MeHg^+$ can cause adverse effects on the nervous system and also compromise brain development in fetuses. The compound is also able to cross the placental barrier and establish itself in the blood cells, nerve endings and muscle tissues of fetuses. In 2001 the Food and Drug Administration (FDA) issued an alert destined for pregnant and/or breastfeeding women to reduce their consumption of fish such as swordfish, needlefish and dogfish, which, due to their mercury contents, could compromise the development of normal cognition in fetuses and children (FDA, 2001a). In 2002 the FDA recommended that the alert was extended to the consumption of tuna (CAC, 2015).

Toxic metal contaminants such as mercury can be classified as carcinogenic or non-carcinogenic. Recently studies on contamination by toxic metals have intensified and different methods to evaluate the potential health risk have been proposed. Evaluation of the Target Hazard Quotient (THQ) has been applied to risk evaluation studies (Wang, Sato, Xing, & Tao, 2005), providing indications of the risk associated with exposure to a non-carcinogenic contaminant, with qualitative information concerning an adverse effect. This method was applied with success by Chien et al. (2002) in a study concerning the contaminants arsenic, cadmium, copper and zinc in oyster samples collected in the coastal region of Taiwan, and was also applied in the present study.

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A quantitative method frequently used to evaluate the risk arising from exposure to contamination from the consumption of mercury species is based on the calculation of the Provisional Tolerable Weekly Ingestion (PTWI). In 2000 the value established by JECFA for the general population was 3.3 μg kg^{-1} b.w. (body weight), making provisions for developing children and fetuses since they are more sensitive to $MeHg^+$ (WHO, 2000). Subsequently the value for PTWI was reduced to 1.6 μg kg^{-1} b.w., this value being considered safe for the health of the population (WHO, 2004).

For quantification, the analytical methods most used to determine $MeHg^+$ use liquid or gas chromatographic techniques coupled to highly sensitive and selective detectors such as atomic absorption spectrometry (AAS) (Martorell et al., 2011), atomic fluorescence spectrometry (AFS), atomic emission detection spectrometry (Kuballa, Leonhardt, Schoeberl, & Lachenmeier, 2009) and inductively coupled plasma mass spectrometry (ICP-MS) (Pastorelli et al., 2012). Thermal decomposition and amalgamation atomic absorption spectrometry (TDA AAS) has been used to quantify the total mercury content (Morgano, Milani, & Perrone, 2015).

Thus the objectives of the present work were: i) determine the organic and total mercury contents using the TDA AAS technique in twelve fish species used in the preparation of *sashimi*; ii) determine the organic mercury: total mercury ratio in the different fish species; iii) estimate the risk of exposure to $MeHg^+$ by consuming fish, using qualitative (THQ) and quantitative (PTWI) methods.

2. Materials and methods

2.1. Samples

A total of 50 small portions of raw filleted fish commercialized as *sashimi*, prepared using the 12 different species of fish most commonly used to elaborate this dish in Japanese restaurants located in the southeastern region of Brazil (City of Campinas, State of São Paulo) were acquired. The species studied were: tuna (*Thunnus thynnus, n = 5*), salmon (*Salmo salar, n = 5*), octopus (*Octopus vulgaris, n = 5*), mullet *(Mugil platanus, n = 5)*, black anchovy *(Ruvettus pretiosus*, n = 5), barracuda (*Boulengerella maculata n = 5*), amberjack *(Seriola lalandi, n = 2)*, golden fish *(Coryphaena hippurus, n = 3)*, and some species of white fish such as sea bass (*Centropomus* sp., *n = 5*), needlefish *(Strongylura marina, n = 3)*, swordfish *(Trichiurus lepturus, n = 4)* and sandperch *(Pseudopercis numida, n = 3)*. Fig. 1 shows the probable origin of the fish species evaluated in the present study.

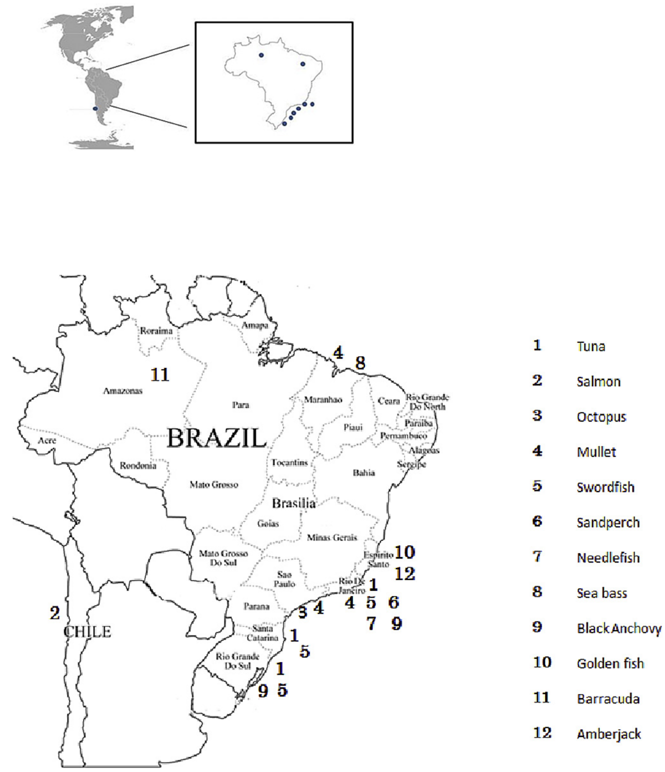

Fig. 1. A map displaying the states where fish samples were collected.

The origin of most of the species was in the southeastern and southern regions of the Atlantic coast, especially in the states of Rio de Janeiro, Rio Grande do Sul, São Paulo, Santa Catarina and Espírito Santo. The states of Maranhão and Amazonas on the northern coast, contributed with the mullet, sea bass and barracuda fish, and the origin of the salmon was exclusively from the Pacific coastline, Chile being the main representative.

The samples were ground separately according to species, using a domestic processor to obtain a homogenous mass, and stored frozen (−18 °C) until analyzed. The mean weight obtained experimentally for the *sashimi* portions was 100 g, corresponding to 4 units (fillets) of fish. The mean standard size of the slices was 2.5 cm wide, 4 cm long and 0.5 cm thick. The total and organic mercury were determined in triplicate.

2.2. Reagents and standards

Only analytical grade reagents were used in this study. The water (18.2 MΩ cm) was purified by reverse osmosis (Gehaka, São Paulo, Brazil) and the nitric acid in a sub-boiling distiller (Distillacid, Berghof, Eningen, Germany). Toluene (Synth, Diadema, Brazil) and a 30% HCl solution (Merck, Darmstadt, Germany) were used for the extractions carried out by microwaves. A 2.5% L-cysteine solution (Sigma, Steinheim, Germany) was prepared to stabilize the organic mercury species. The analytical curves were constructed using certified 1.000 mg L^{-1} standard mercury solutions (Fluka, Sigma Aldrich, Steinheim, Germany) together with a 0.5% HNO_3 solution (v/v).

2.3. Instrumentation

The total and organic mercury contents in the *sashimi* samples were quantified by TDA AAS using a direct mercury analyzer (DMA-80, Dual Cell, Milestone, Sorisole, Italy). The samples were submitted to an initial heating step and subsequent determination of the mercury in an amalgamator containing gold.

2.4. Determination of total mercury and extraction of organic mercury (MeHg)

2.4.1. Determination of total mercury

The instrumental conditions used to determine total mercury (tHg) by TDA AAS were established based on the study developed by Morgano et al. (2015): drying at 200 °C for 60s; decomposition at 600 °C for 180s, desorption at 850 °C and detection at 253.7 nm. The homogenized samples were weighed into nickel recipients (60–100 mg for the fish species) for the subsequent determination of tHg.

2.4.2. Extraction of organic mercury (MeHg)

Organic mercury ($MeHg^+$) was determined using a closed microwave-assisted extraction system as described by Paiva et al. (2016): 1 g of sample was weighed into a Teflon PFA recipient followed by the addition of 8 mL toluene pa, 1 mL of demineralized water and 0.75 mL of a 30% HCl solution (v/v). The analytical conditions of the microwave extractor were: 1000 W power applied; (A) heating ramp to 110 °C in 10 min; (B) temperature constant at 110 °C for 5 min.

After extraction, a 4 mL aliquot of the organic phase was transferred to a centrifuge tube containing 2 mL of a 2.5% L-cysteine solution (m/v) and the mixture centrifuged at 3500 rpm for 6 min. A 100 mg portion of the L-cysteine phase was weighed into a quartz recipient and the mercury content determined in a DMA-80 analyzer. The optimized instrumental conditions for the determination of organic mercury were: drying of sample at 120 °C for 60s; decomposition at 300 °C for 180s; desorption at 850 °C for 12s; and the absorbance determined at 253.7 nm. The detection ranges for the two cells of the equipment were: 0.5–20 μg kg^{-1} and 20–1000 μg kg^{-1}.

2.5. Estimation of methylmercury exposure

Two parameters were considered to evaluate the potential risk of exposure to methylmercury: the Target Hazard Quotient (THQ) (Chien et al., 2002) and the Provisional Tolerable Weekly Ingestion (PTWI) ($MeHg^+$ = 1.6 μg kg^{-1} bw) (CAC, 2015).

- The methodology used to estimate THQ is described by US EPA (2007a), as from which calculations were carried out to evaluate the risk considering the following equation described by Chien et al. (2002). This index indicates the ratio between exposure and the reference dose and includes not only intake of metals but other significant data, as exposure frequency and duration, meal size, body weight and the oral reference dose. The equation is expressed as follows:

$$THQ = \frac{Ef \times Ed \times Ir \times C}{RfDo \times BW \times AT} 10^{-3} \quad (1)$$

where Ef is the exposure frequency (365 days/year); Ed is the duration of exposure (71 years for men and 78.6 years for women according to data of the Brazilian Institute of Geography and Statistics) (IBGE, 2013); Ir is the fish ingestion rate (g/person/day); C is the $MeHg^+$ concentration in the fish (μg kg^{-1}); Rf Do is the oral reference dose (mg/kg/day); BW is the body weight for adults (60 kg) and children (15 kg); AT is the mean exposure time to non-carcinogenic contaminants (365 days/year x years of exposure: 71 and 78.6 years for men and women, respectively); and 10^{-3} is the unit conversion factor (Wang et al., 2005).

A THQ <1 signifies that the level of exposure is less than that of the reference dose. It is thus presumed that the daily exposure to this level does not cause any negative effects to the health throughout the entire life of a human population (Barone et al., 2015). THQ values between 1 and 5 indicate that the consumers are already exposed to risks which could cause damage to the health, whilst THQ values above 5 denote a significant potential for risk to the health due to contaminant exposure (Wang et al., 2005).

- The estimated exposure to organic mercury as a function of PTWI was calculated as follows:

Estimated methylmercury exposure = [$MeHg^+$] x (portion/body weight); where [$MeHg^+$] (mean and interval of concentration, μg kg^{-1}); portions of 100 g and 20 g of *sashimi* were considered for adults of 60 kg and children of 15 kg, respectively. Hence the estimated exposure to $MeHg^+$ is given in μg kg^{-1} bw^{-1}.

The maximum tolerable number of portions per week for adults and children was indicated by values above 100% for PTWI.

$$\% \text{ PTWI} = 100 \times (\text{Estimated exposure to methylmercury})/(MeHg^+ \text{ PTWI}); \quad (2)$$

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

2.6. Statistical analysis

The mean results were evaluated for significance using one-way analysis of variance (one-way ANOVA) and Tukey's test, using XLSTAT software (Addinsoft, France).

3. Results and discussion

3.1. Analytical characteristics

The figures showing the accuracy, precision, linearity of the analytical curves, detection limits and quantification limits were evaluated according to INMETRO (2011). The accuracy of the methods used to determine total and organic mercury was verified using certified reference materials for total (fish protein – NCR DORM-4) and organic (fish protein – NCR DORM-4 and oyster tissue – NIST SRM 1566b) mercury, the recovery values obtained being: 97± 14% for total mercury (DORM-4) and 94± 6% and 111± 2% for methylmercury (DORM-4 and NIST SRM 1566b). The precision of the method was evaluated by the coefficient of variation (16 analytical repetitions) obtaining values of 5.5% and 9.0%, respectively for total and organic mercury. The analytical curves for the determination of mercury presented linearity in the ranges from 0.5 to 20 μg kg^{-1} and from 20 to 1000 μg kg^{-1} ($R > 0.999$). The limits of detection (LOD) and of quantification (LOQ) for organic and total mercury were: LOD (3s) = 2.0 and 0.40 μg kg^{-1}; LOQ (10s) = 6.6 and 1.4 μg kg^{-1}, respectively; "s" being the value of the standard deviation for the concentrations of ten repetitions.

3.2. Results obtained for total mercury and methylmercury in sashimi

Table 1 shows the results obtained for tHg and $MeHg^+$ in the different fish species used in the preparation of *sashimi*. Different concentrations of total and organic mercury were found amongst the 12 fish species studied. The species that presented the highest mean contents of tHg and $MeHg^+$ were: needlefish (1010 μg kg^{-1} and 855 μg kg^{-1}); tuna (902 μg kg^{-1} and 683 μg kg^{-1}); barracuda (743 μg kg^{-1} and 476 μg kg^{-1}) and anchovy (517 μg kg^{-1} and 322 μg kg^{-1}), respectively.

The contents obtained for total and organic mercury in the different fish species were in the following order: needlefish > tuna > barracuda > anchovy > sandperch > amberjack > swordfish > octopus > golden fish > sea bass > salmon > mullet. Statistical analysis was performed between mercury and methylmercury levels in the fish species in order to verify significant difference among them. The fish species needlefish and tuna were those presenting the greatest contents of total mercury. Regarding methylmercury, high amounts were observed for needlefish, sandperch and tuna. Those mentioned mercury levels presented significant difference from others fish species (Table 1).

Of the 12 species studied used in the elaboration of *sashimis*, needlefish, swordfish and tuna are classified as predators and the others as non-predators. The mean mercury contents found in the swordfish were approximately five times less than those found in the needlefish and tuna. This fact could be related to the fish habitat, and in Fig. 1 it can be seen that the swordfish and anchovy (lower mean Hg levels) came from the same fishing region. According to Grandjean, Satoh, Murata, and Etoh (2010), the aquatic environment of the southern Brazilian coast, where these fish

Table 1
Total and $MeHg^+$ levels (μg kg^{-1}) observed in sashimi fish species.

(Fish species)	n		Total Hg (μg kg^{-1})	$MeHg^+$ (μg kg^{-1})
			Mean ± SD (Range)	
Needlefish	3		1010 ± 480 (644–1576)[a]	855 ± 416 (589–1379)[a]
		% samples > ML*	*33*	*33*
Sandperch	3		476 ± 44 (422–512)[b,c,d]	442 ± 20 (408–486)[b]
		% samples > ML*	*33*	*0*
Sea bass	5		36 ± 20 (4–61)[e]	24 ± 14 (17–46) [c,d]
		% samples > ML*	*0*	*0*
Tuna	5		902 ± 703 (303–1790)[a]	683 ± 523 (261–1395)[c]
		% samples > ML*	*40*	*40*
Salmon	5		12 ± 2 (8–14)[e]	7.5 ± 1.6 (2–12)[d]
		% samples > ML*	*0*	*0*
Amberjack	2		322 ± 260 (477–157)[c,d,e]	229 ± 175 (90–355)[c,d]
		% samples > ML*	*50*	*0*
Swordfish	4		189 ± 145 (102–410)[d,e]	145 ± 116 (76–348)[c,d]
		% samples > ML*	*0*	*0*
Barracuda	5		743 ± 285 (305–1036)[a,b]	476 ± 181 (192–745) [c,d]
		% samples > ML*	*80*	*40*
Octopus	5		130 ± 96 (67–304)[d,e]	113 ± 83 (54–261) [c,d]
		% samples > ML*	*0*	*0*
Black anchovy	5		517 ± 156 (319–732)[b,c]	322 ± 104 (192–504) [c,d]
		% samples > ML*	*60*	*0*
Golden fish	3		124 ± 39 (83–177)[d,e]	67 ± 29 (48–106) [c,d]
		% samples > ML*	*0*	*0*
Mullet	5		4.0 ± 2.5 (0.45–8.5)[e]	<6.6[d]
		% samples > ML*	*0*	*0*

*Samples above establish maximum limit (ML) by Brazilian legislation (RDC 42/ANVISA, 2013): 0.5 μg kg^{-1} of total Hg for fish and 1.0 μg kg^{-1} for predatory fish. The same levels were established for $MeHg^+$, according to CODEX (CAC, 2015).
Values followed by different letters on the same column differ significantly by the Tukey test ($p \leq 0.05$).

species predominate, is less polluted and has a lower concentration of methylating bacteria.

The World Health Organization (CAC, 2015) established maximum limits for $MeHg^+$ of 1 mg kg^{-1} for predatory fish and 0.5 mg kg^{-1} for non-predatory fish. In the present study, the species needlefish and tuna presented values varying from 589 to 1379 μg kg^{-1} and 261–1395 μg kg^{-1}, respectively, contents above the established levels. The $MeHg^+$ levels observed could be associated with the characteristics of these fish (predatory fish), showing a greater tendency to accumulate mercury.

For the non-predatory species such as barracuda and black anchovy, $MeHg^+$ contents above the maximum limit established by the CODEX committee for Food Contaminants (CAC, 2015) of 0.5 mg kg^{-1} were found, with intervals between 192 and 745 μg kg^{-1} and 192 and 504 μg kg^{-1}, respectively. Fish species such as sandperch, amberjack and swordfish showed $MeHg^+$ values below the maximum permitted limit, and even lower values were observed for $MeHg^+$ amongst the samples of sea bass, salmon and mullet, with respective mean values of 29 μg kg^{-1}, 7 μg kg^{-1} and not detected. For the species mullet, total mercury contents varying between 0.45 and 8.5 μg kg^{-1} were found and the $MeHg^+$ levels, were below the quantification limit of the method (6.6 μg kg^{-1}).

To evaluate the proportion of organic mercury present in the fish species used in the elaboration of *sashimi*, the ratios (%) between the organic and total mercury contents were calculated, and the results are presented in Fig. 2. The *sashimi* elaborated with sandperch showed the highest $MeHg^+$/tHg ratio with a value of 93%, and the *sashimi* samples made with octopus and needlefish also showed elevated $MeHg^+$/tHg ratios of 87% and 85%, respectively. With respect to the octopus *sashimi*, the result obtained in

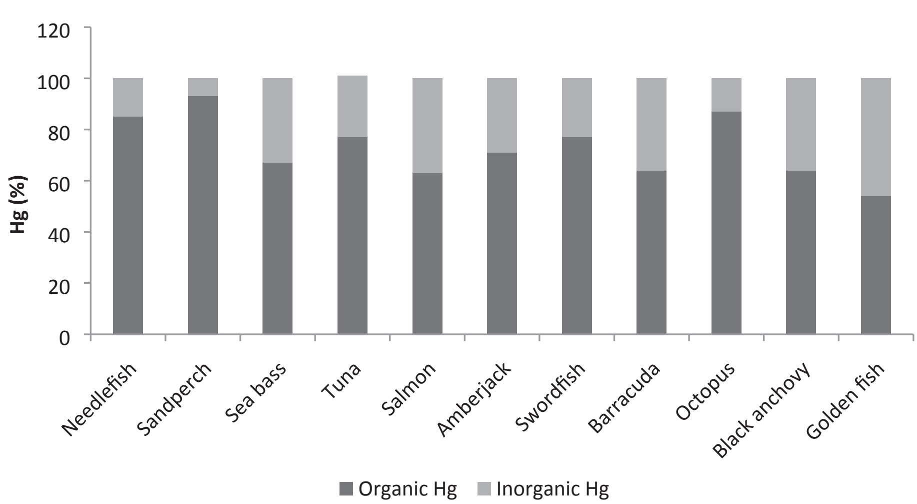

Fig. 2. Ratio between $MeHg^+$/iHg (%) in analyzed *sashimi* samples.

the present study was comparable with that observed by Raimundo, Carlos Vale, Canário, Vasco Branco, and Moura (2010), who found values varying between 70 and 99%. With respect to the tuna *sashimi*, Storelli, Stuffler, and Marcotrigiano (2002) found values for the $MeHg^{+}$/tHg ratio of between 77 and 100%, whilst Burger et al. (2013) found mean values of 90%. A value of 77% was found in the present work, in agreement with the values found by Storelli et al. (2002).

According to the literature data the comparison of total and organic Hg is presented in Table 2. Torres-Escribano, Calatayud, et al. (2010) and Torres-Escribano, Vélez, and Montoro (2010) found higher values for the $MeHg^{+}$/tHg ratio (89%) in swordfish *sashimi* than those found in the present study (77%), and for anchovy, Martorell et al. (2011) found an $MeHg^{+}$/tHg ratio of 93%, as against 64% found in the present study. For golden fish *sashimi* a value of 54% was observed in the present study, but to the best of the authors' knowledge, studies found in the literature with this fish species only reported the data for tHg.

For salmon *sashimi* a value for the $MeHg^{+}$/tHg ratio of 63% was found in the present study, inferior to those reported in the literature: Chung, Kwong, Tang, Xiao, and Ho (2008) found a mean value of 74%; Costa et al. (2015) observed a valor of 82%; and the group of Martorell et al. (2011) found a mean proportion of 100%. For amberjack *sashimi* a higher $MeHg^{+}$ content was found in the present study as compared to data available in the literature (71% for the $MeHg^{+}$/tHg ratio), whereas Gibičar et al. (2009) reported a value of 61%. The mullet *sashimi* presented $MeHg^{+}$ contents below the quantification limit of the method (6.6 μg kg^{-1}), and hence the $MeHg^{+}$/tHg ratio for this species could not be represented in Fig. 2. However, Chung et al. (2008) obtained a mean ratio of 64%.

According to Forsyth, Casey, Dabeka, and McKenzie (2004), factors related to the origin of the fish, location where fished, environmental conditions and anthropogenic activities could be associated with the availability of mercury in the environment, and consequently different proportions between the mercury species can be observed in the fish.

The $MeHg^{+}$ and total mercury levels observed in the present study were in agreement with those of other authors: Forsyth et al. (2004) found $MeHg^{+}$ levels varying from 61 to 1303 μg kg^{-1} in tuna samples coming from Canada; Storelli's group (2002) evaluated different tuna species (*Thunnus alalunga)* and *(Thunnus thynnus*) obtained from the Adriatic and Ionian seas, and obtained mean values of 430 μg kg^{-1} for $MeHg^{+}$; whereas samples of tuna *sashimi* collected from the New Jersey region (USA) presented mean values of 442 μg kg^{-1} for the organic mercury species (Burger, Gochfeld, Jeitner, Donio, & Pittfield, 2013).

Hosseini, Mohammad, Nabavi, and Parsa (2013) studied predatory species of needlefish from the Persian Gulf and reported total mercury contents of 50–710 μg $kg^{-1,}$ whereas in the present study the results varied from 644 to 1576 μg kg^{-1} for total mercury and from 589 to 1379 μg kg^{-1} for $MeHg^{+}$.

In general the values obtained in the present study for the total Hg and $MeHg^{+}$ contents of swordfish were similar to those found in studies carried out in other fishing areas: values between 638 and

Table 2
Comparison of total and organic Hg results for different fish species used for *sashimi* dishes (mean values or range and literature reference).

Fish specie	n	Total Hg (μg kg^{-1}) Mean or (Range)	$MeHg^{+}$ (μg kg^{-1}) Mean or (Range)	Literature reference
Swordfish	9	(638–3845)	(486–1492)	Forsyth et al., 2004
	27	(170–1720)	(160–1530)	Torres-Escribano, Calatayud, et al., 2010; Torres-Escribano, Vélez, et al., 2010
	6	(1025–1700)	–	Cortes & Fortt, 2007
	10	529	–	Pastorelli et al., 2012
	11	540	–	Olmedo et al., 2013
	4	(102–410)	(76–348)	Present study
Tuna	8	(77–2121)	(61–1303)	Forsyth et al., 2004
	33	491	442	Burger et al., 2013
	5	840	800	Storelli et al., 2002
	5	(303–1790)	(261–1395)	Present study
Salmon	3	34	25	Chung et al., 2008
	25	28	23	Costa et al., 2015
	24	10	10	Martorell et al., 2011
	5	12	7,5	Present study
Amberjack	200	301	184	Gibičar et al., 2009
	2	322	230	Present study
Needlefish	3	(50–710)	–	Hosseini et al., 2013
	3	(644–1576)	(601–1336)	Present study
Sandperch	1	454	–	Morgano et al., 2014
	3	476	442	Present study
Sea bass	4	1700	–	Farias et al., 2005
	5	36	29	Present study
Barracuda	7	(303–2129)	–	Bastos et al., 2008
	5	(305–1036)	(192–745)	Present study
Octopus	12	(130–760)	(110–750)	Raimundo et al., 2010
	1	443	–	Bonsignore et al., 2013
	5	20	–	Galimberti et al., 2016
	5	(67–304)	(54–261)	Present study
Black anchovy	24	140	130	Martorell et al., 2011
	5	517	332	Present study
Golden fish	20	(26–90)	–	Sellanes et al., 2002
	385	(12–550)	–	Adams, 2009
	57	(10–490)	–	Cai et al., 2007
	3	(83–177)	(48–106)	Present study
Mullet	3	14	9	Chung et al., 2008
	5	4.0	<6.6	Present study

3845 μg kg^{-1} were found by Forsyth et al. (2004) for total mercury and from 486 to 1492 μg kg^{-1} for $MeHg^{+}$ in samples acquired in Canadian markets; the group of Torres-Escribano, Calatayud, et al. (2010) and Torres-Escribano, Vélez, et al. (2010), who studied swordfish acquired from the Spanish coast obtained total mercury contents varying from 170 to 1720 μg kg^{-1} and $MeHg^{+}$ contents from 160 to 1530 μg kg^{-1}; Cortes and Fortt (2007) observed mean levels of 1362 μg kg^{-1} for total mercury in samples obtained on the Chilean coast, whereas Pastorelli et al. (2012) and Olmedo et al. (2013) found mean contents of 529 μg kg^{-1} and 540 μg kg^{-1} for fish from Italy and Spain, respectively.

With respect to the species barracuda, similar concentrations of total mercury were reported by Bastos, Rebelo, Fonseca, Almeida, and Malm (2008) in a study carried out in the Amazon basin, with values varying from 303 to 2129 μg kg^{-1}. For the species anchovy, Martorell et al. (2011) found contents of total and organic mercury of 140 μg kg^{-1} and 130 μg kg^{-1}, values below those found in the present work of 517 μg kg^{-1} and 322 μg kg^{-1}, respectively. Kuballa, Moellers, Schoeberl, and Lachenmeier (2011) in their study to assess methylmercury in fish and seafood from the southwestern German market verified close values to those of this work for the anchovy, with the oHg/tHg ratio obtained of 53%. Distinct values for concentration can be attributed to different fishing areas, determined geographically by the FAO fishing areas. According to this division, the fish samples used in the present work come from the Southeast Atlantic, whereas those in the study of Martorell et al. (2011) were from the Northeast Atlantic.

Although there is not a large volume of data available in the literature concerning the sandperch, Morgano, Rabonato, Milani, Miyagusku, and Quintaes (2014) analyzed the total mercury content in samples from the southeast coast of Brazil, obtaining mean values of 454 μg kg^{-1}. In the present study the total mercury and $MeHg^{+}$ contents were determined in 3 samples of *sashimi* of this fish and the mean contents obtained were 476 μg kg^{-1} and 442 μg kg^{-1}, respectively.

To the best of the authors' knowledge, there is little data available in the literature with respect to the presence of $MeHg^{+}$ in octopus. In the study carried out by Raimundo et al. (2010) to determine the mercury species in octopus from the Portuguese coast, significant levels of total mercury (130–760 μg kg^{-1}) and $MeHg^{+}$ (110–750 μg kg^{-1}) were encountered. Bonsignore et al. (2013) found medium values of 443 μg kg^{-1} of total mercury, whereas Galimberti et al. (2016) found 20 μg kg^{-1} for the same species of mercury in the south and north regions of Italy, respectively.

The sea bass samples analyzed in the present study presented low values of total mercury and $MeHg^{+}$, namely 36 μg kg^{-1} and 29 μg kg^{-1}, respectively. On the other hand, Farias, Azevedo, Fávaro, and Braga (2005) observed high levels of total mercury (1700 μg kg^{-1}, on average) in samples fished in the Bay of Santos, Brazil, but since this fish is not predatory, the elevated mercury content could be related principally to the localization of the estuary, just below the industrial complex of Cubatão, which is a direct, primary receptor of contaminated effluents.

With respect to samples with low Hg contents such as the golden fish, values of 26 and 90 μg kg^{-1} of total mercury were found by Sellanes et al. (2002) in samples collected on the Rio de Janeiro State coast, Brazil. Adams (2009) reported total mercury levels of between 12 and 550 μg kg^{-1} in samples from the southeast coast of the USA, whereas Cai, Rooker, Gill, and Turner (2007) obtained minimum values of 10 μg kg^{-1} and maximum values of 490 μg kg^{-1} in fish from the northern region of the Gulf of Mexico. In the present study, the golden fish samples collected in the southeast of Brazil presented total mercury and $MeHg^{+}$ contents varying from 83 to 177 μg kg^{-1} and from 48 to 106 μg kg^{-1}, respectively.

The species mullet presented the lowest concentration of total mercury. Chung et al. (2008) found mean values of 14 μg kg^{-1} for total mercury and 9 μg kg^{-1} for $MeHg^{+}$ in mullet samples commercialized in China, and in the present study, the mean value obtained for total Hg was 3.9 μg kg^{-1} and the levels for $MeHg^{+}$ were below the quantification limit of the method (LOD = 6.6 μg kg^{-1}). Since this species is small, the low Hg contents found could be associated with the correlation existing between the mercury concentration and size of the fish.

Therefore, in the present study, for the first time the concentration of total mercury and $MeHg^{+}$ were evaluated in 12 different sashimi samples from different Brazilian regions. Batista, Souza, Souza, and Barbosa (2011) also developed a regional study about arsenic speciation in Brazilian rice.

3.3. *Evaluation of the potential risk related to the ingestion of $MeHg^{+}$*

On considering the metal contents in marine organisms versus human consumption one of the most important aspects to be evaluated is their toxicity. With this objective in mind, different approaches to the estimation of health risks from the consumption of mercury have been proposed. In the food area, the approach most used has been the quantitative comparison with the PTWI values, which represent the amount of the substance that can be ingested during a lifetime without significant risks to the health, and which was defined by a mixed FAO/WHO committee of experts in food additives (JECFA) (WHO, 2008). One approach that has been used in environmental studies to evaluate the potential risk has been the determination of the target hazard quotient (THQ) (USEPA, 2014). This factor consists of a qualitative risk index that compares the levels of ingestion of a contaminant with a standard dose of the reference.

3.3.1. *Target Hazard Quotient*

The THQ value proposed by USEPA (2014) is an integrated risk index by comparing the ingestion amount of a pollutant with a standard reference dose and has been widely used in the risk assessment of metals in contaminated fish (Wang et al., 2005). THQ <1 signifies that the level of exposure is lower than the reference dose, which assumes that a daily exposure at this level is not likely to cause any negative health effects during a lifetime in a human population.

Thus, the evaluation exposure risk to $MeHg^{+}$ was also carried out as a function of the THQ value, which expresses the ratio between exposure to the contaminant and its respective daily reference dose (DRD). The value used for the DRD of $MeHg^{+}$ was 0.1 μg kg^{-1} day^{-1} (USEPA, 2007a). Fig. 3 shows the results obtained for THQ using Equation (1) for each fish species used in the *sashimis* studied.

The results showed that 42% of the fish samples analyzed had THQ ≥ 5, that is, they were above the maximum limit for danger, indicating a high risk of the exposure to contamination by the consumption of some of the fish species. Of these, needlefish and tuna stood out with elevated THQ values, followed by sandperch, barracuda and anchovy. Fish of the species amberjack, swordfish, octopus and golden fish (33% of the samples) presented $1 < THQ < 5$, denoting a significant risk associated with the consumption of these fish species. THQ values < 1 were only obtained for salmon and sea bass, indicating no potential risk of contamination associated with the $MeHg^{+}$ contents present in these *sashimis*.

The values reported as a function of THQ provided evidence that the fish species used in the elaboration of the *sashimis* had a high capacity to retain mercury compounds, principally the toxic organic

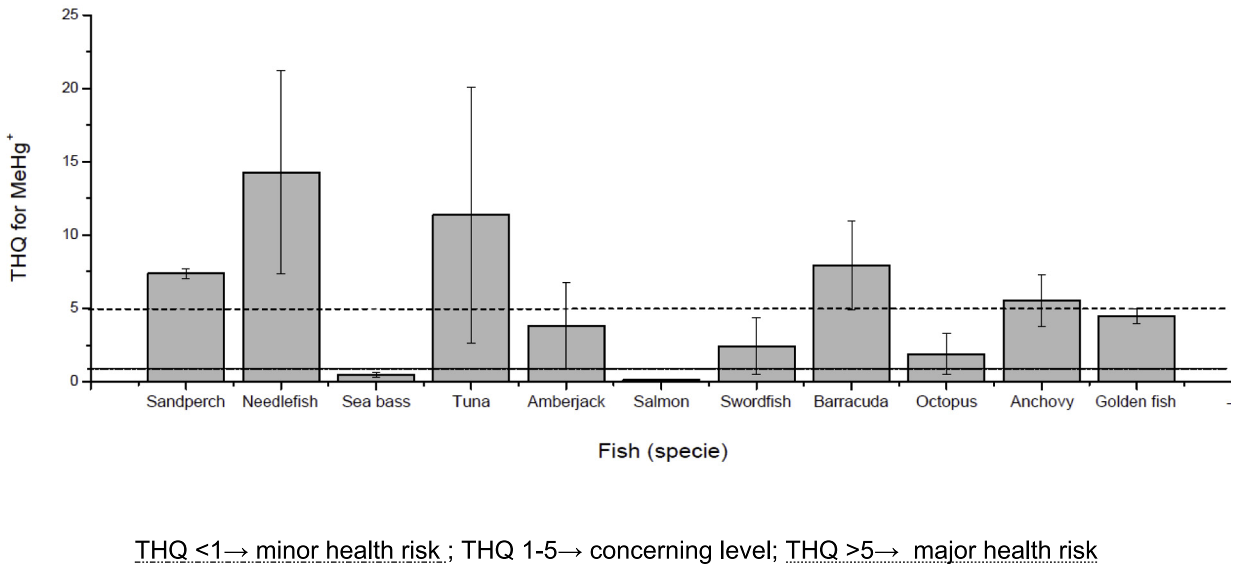

Fig. 3. The estimated target hazard quotient of $MeHg^+$ in fish species used in sashimi preparation.

species ($MeHg^+$). The analysis of the target hazard quotient also showed that 10 of the 12 fish species used in the *sashimis* evaluated in this study had THQ values > 1. Barone et al. (2015), working with fish samples from different regions of the Mediterranean Sea, observed THQ values of from 0.74 to 0.8 for mercury in tuna and swordfish, respectively. Although these THQ values were lower than those obtained in the present study, they were still close to 1 (minimum exposure limit).

The results obtained for THQ for the *sashimi* samples provided evidence of the need to promote consumer consciousness with respect to the ingestion of some fish species and fish-based dishes.

3.3.2. Estimate of the exposition to methylmercury: evaluation of the consumption of sashimi and calculation of the PTWI value

The estimate of the exposure to $MeHg^+$ from the consumption of *sashimi* from different fish species was calculated considering the mean weight of a *sashimi* portion determined experimentally as 100 g for adults and estimated as 20 g for children. The calculations were done using PTWI values for $MeHg^+$ of 1.6 μg kg^{-1}, considering the weight of an adult as 60 kg and that of a child (2–6 years old) as 15 kg, as recommended by FAO/WHO (2011). With the objective of comparing the values obtained with the PTWI established for $MeHg^+$, it was considered that this was the main organic mercury species present in the different fish species evaluated.

Table 3 shows the mean $MeHg^+$ content present in each of the fish species used to elaborate the *sashimis*, the value for the % of the PTWI reached by adults and children considering the consumption of one *sashimi* portion, and the number of *sashimi* portions necessary to reach 100% of the PTWI for $MeHg^+$.

The analysis of the values estimated for ingestion showed that some fish species had expressive $MeHg^+$ contents in the portion considered, and consequently contributed significantly to the calculation of the PTWI. Of these, *sashimis* elaborated with needlefish and tuna presented 86 and 68 μg kg^{-1} of $MeHg^+$, respectively. The consumption of just 1.1 and 1.4 weekly portions of *sashimi* made from these fish for adults, or 1.4 and 1.7 weekly portions for children, exceeded the PTWI value established for $MeHg^+$ (1.6 μg kg^{-1} bw) by 100%.

Fish such as sandperch, barracuda and anchovy also presented significant concentrations of the organic mercury species, where the mean consumption of three weekly portions exceeded the value of the PTWI established for $MeHg^+$ for both adults and

Table 3
Estimated exposure to $MeHg^+$ from sashimi consumption portions considering 100% of PTWI, adults of 60 kg and children of 15 kg.

Fish species used in *Sashimi*	Adults			Children		
	$MeHg^+$ (μg kg^{-1})[a]	% PTWI	*Sashimi* portions to achieve 100% of PTWI	$MeHg^+$ (μg kg^{-1})[b]	% PTWI	*Sashimi* portions to achieve 100% of PTWI
Needlefish	86	89	1.0	17	71	1.4
Sandperch	44	46	2.0	9.0	37	3.0
Sea bass	3.0	0	33	0.6	2.4	41
Tuna	68	71	1.4	13.6	57	1.7
Salmon	0.7	0.7	140	0.14	0.5	175
Amberjack	23	24	4.0	4.6	19	5.2
Swordfish	14	15	6.6	3.0	12	8
Barracuda	48	49.5	2.0	9.5	39	2.5
Octopus	11	12	8.5	2.2	9.4	10.6
Black anchovy	33	34.6	3.0	6.6	27	3.7
Golden fish	6.7	7	14.2	1.3	5.6	18
Mullet	ND < 6.6	ND < 6.6	–	ND < 6.6	ND < 6.6	–

[a] Considering a sashimi portion of 100 g for adults of 60 kg.
[b] Considering a sashimi portion of 20 g for children of 15 kg.

children by 100%. In fish with smaller $MeHg^{+}$ levels such as golden fish, sea bass and salmon, the number of portions necessary to exceed the ingestion limits established were 14.2, 33 and 140 for adults, and 17.8, 41 and 175 for children, in this order, indicating that the consumption of these *sashimis* presented no potential health risk even when ingested with greater frequency and amounts.

The choice of the fish species used in the elaboration of *sashimi* should be made with care for those groups of the population more vulnerable to the toxic effects of the contaminant mercury, such as children and pregnant and/or gestating women. Due to their greater susceptibility to contamination by $MeHg^{+}$, the EFSA (European Food Safety Authority) recommends that this category includes a wide variety of fish in their diet, and controls the consumption of predatory fish such as tuna and needlefish (EFSA, 2004a). The results of the present study showed that the *sashimis* containing the species mullet, salmon, sea bass and golden fish were those presenting the lowest amounts of this contaminant.

In the study carried out by Afonso et al. (2015) involving fish from the Portuguese coast, it was observed that the monthly consumption of 160 g of raw fish (tuna) was associated with a 3%–5% probability of exceeding the maximum limits established for $MeHg^{+}$. According to Grandjean et al. (2010) the organic mercury contents present in fish species are greatly influenced by the distinct environmental conditions such as a contaminated marine habitat and the presence of methylating bacteria.

4. Conclusions

The study showed that the *sashimis* samples made from needlefish and tuna possessed greater amounts of total and organic mercury, whilst those made from mullet, salmon, sea bass and golden fish presented the lowest levels. The ratio $MeHg^{+}$/tHg was high for all the fish species used in the elaboration of *sashimi*, with values above 54%, the highest ratios being obtained for the *sashimis* from sandperch (93%) and octopus (87%), indicating a predominance of the more toxic mercury form ($MeHg^{+}$). With respect to the estimate of exposure to $MeHg^{+}$ from the consumption of *sashimi* made using the target hazard quotient (THQ), it was shown that 42% of the fish species presented THQ values ≥ 5 (among tuna and needlefish) indicating they were above the maximum limit for the danger of exposure. Regarding comparison with values of the provisional tolerable weekly ingestion (PTWI), it was confirmed that the ingestion of 2 *sashimi* portions (100 g) of needlefish or tuna exceeded the maximum values established for adults and children by 100%, whereas for mullet and salmon the contribution to the PTWI was insignificant. Therefore, the qualitative risk assessment index (THQ) and quantitative parameter of comparison (PTWI) confirms a high exposure contamination to some analyzed fish species.

The results presented highlighted the importance of evaluating the exposure to $MeHg^{+}$ from the ingestion of *sashimi*. Frequent consumers (>once a week) should check on the variety of fish in the diet in order to make a conscious choice, especially in the case of consumers belonging to groups at risk such as pregnant women, lactating mothers and children.

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References

Adams, D. H. (2009). Consistently low mercury concentrations in dolphinfish, Coryphaena hippurus, an oceanic pelagic predator. *Environmental Research, 109*, 697–701.
Afonso, C., Costa, S., Cardoso, C., Bandarra, N. M., Batista, I., Coelho, I., et al. (2015). Evaluation of the risk/benefit associated to the consumption of raw and cooked farmed meagre based on the bioaccessibility of selenium, eicosapentaenoic acid and docosahexaenoic acid, total mercury, and methylmercury determined by an in vitro digestion model. *Food Chemistry, 170*, 249–256.
Barone, G., Storelli, A., Garofalo, R., Busco, V. P., Nicoletta Cristiana Quaglia, N. P., Centrone, G., et al. (2015). Assessment of mercury and cadmium via seafood consumption in Italy: Estimated dietary intake (EWI) and target hazard quotient (THQ). *Food Additives and Contaminants, 32*, 1277–1286.
Bastos, W. R., Rebelo, M. F., Fonseca, M. F., Almeida, R., & Malm, O. (2008). *A description of mercury in fishes from the Madeira River Basin* (Vol. 38, pp. 431–438). Brazil: Amazon.
Batista, B. L., Souza, J. M. O., De Souza, S. S., & Barbosa, F. (2011). Speciation of arsenic in rice and estimation of daily intake of different arsenic species by Brazilians through rice consumption. *Journal of Hazardous Materials, 191*, 342–348.
Bonsignore, M. D., Manta, S., Oliveri, E., Sprovieri, M., Basilone, G., Bonanno, A., et al. (2013). Mercury in fishes from Augusta Bay (southern Italy): Risk assessment and health implication. *Food and Chemical Toxicology, 56*, 184–194.
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*, 1057–1075.
CAC - Codex Alimentarius Commission. (2015). *Discussion paper on maximum levels for methylmercury in fish. 9 th Session New Delhi, India*, 16 e 20 March http//ftp.fao.org/codex/Meetings/cccf/cccf9/cf09_13e.pdf (Accessed 08 June 2016).
Cai, Y., Rooker, J. R., Gill, G. A., & Turner, J. P. (2007). Bioaccumulation of mercury in pelagic fishes from the northern Gulf of Mexico. *Canadian Journal of Fisheries and Aquatic Sciences, 64*, 458–469.
Chien, L. C., Hung, T. C., Choang, K. Y., Yeh, C. Y., Meng, P. J., Shieh, M. J., et al. (2002). Daily intake of TBT, Cu, Zn, Cd and as for fishermen in Taiwan. *Science of The Total Environment, 285*, 177–185.
Chung, S. W. C., Kwong, K. P., Tang, A. S. P., Xiao, Y., & Ho, P. Y. Y. (2008). Mercury and methylmercury levels in the main traded fish species in Hong Kong. *Food Additives and Contaminants, 1*, 106–113.
Cortes, S., & Fortt, A. (2007). Mercury contents in Chilean fish and estimated intake levels. *Food Additives and Contaminants, 24*, 955–959.
Costa, S., Afonso, C., Cardoso, C., Batista, I., Chaveiro, N., Nunes, M. L., et al. (2015). Fatty acids, mercury, and methylmercury bioaccessibility in salmon (Salmo salar) using an in vitro model: Effect of culinary treatment. *Food Chemistry, 185*, 268–276.
EFSA (European Food Safety Authority). (2004a). Opinion of the Scientific Panel of Contaminants in the food chain on a request from the commission related to mercury and methylmercury in food. *EFSA Journal, 34*, 1–14.
Eisler, R. (2000). *Mercury, in handbook of chemical risk Assessment: Health hazard to human, plants and animals* (Vol. 1, pp. 313–409). Boca Raton, FL: Lewis Publishers. Metals.
FAO/WHO. (2011). *Report of the joint expert consultation on the risks and benefits of fish consumption*. Rome: FAO Fishery and Aquaculture Report.
Farias, L. A., Azevedo, J. S., Fávaro, D. I. T., & Braga, E. S. (2005). *Evaluation of mercury, selenium and methylmercury in fish consumed by Santos Bay communities, São Paulo, Brazil*. Santos, SP, Brazil: INAC.
FDA (Food and Drug Administration). (2001a). *FDA consumer advisory*. http://www.cfsan.fda.gov/Bdms/hgpdftoc.html (Accessed 13 June 2016).
Forsyth, D. S., Casey, V., Dabeka, R. W., & McKenzie, A. (2004). Methylmercury levels in predatory fish species marketed in Canada. *Food Additives and Contaminants, 21*, 849–856.
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.
Gibičar, D., Horvat, M., Logar, M., Fajon, V., Falnoga, I., Ferrara, R., et al. (2009). Human exposure to mercury in the vicinity of chlor-alkali plant. *Environmental Research, 109*, 355–367.
Grandjean, P., Satoh, H., Murata, K., & Etoh, K. (2010). Adverse effects of methylmercury: Environmental health research implications. *Environmental Health Perspectives, 118*, 1137–1145.
Hosseini, M., Mohammad, S., Nabavi, B., & Parsa, Y. (2013). Bioaccumulation of trace mercury in trophic levels of benthic, benthopelagic, pelagic fish species, and sea birds from Arvand river, Iran. *Biological Trace Element Research, 156*, 175–180.
IBGE (Instituto Brasileiro de Geografia e Estatística). (2013). *Expectativa de vida*. http://teen.ibge.gov.br/noticias-teen/7827-expectativa-de-vida (Acessed 11 June 2016).
INMETRO. (2011). *Orientação Sobre Validação de Métodos Analíticos*. Instituto Nacional de Metrologia, Normalização e Qualidade Industrial. DOQ-CGCRE-008. Rev.: 04, 1–20.
Kuballa, T., Leonhardt, E., Schoeberl, K., & Lachenmeier, D. W. (2009). Determination of methylmercury in fish and seafood using optimized digestion and derivatization followed by gas chromatography with atomic emission detection. *European Food Research and Technology, 228*(3), 425–431.
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.

Martorell, I., Perelló, G., Martí-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, 142*, 309–322.

Miguéis, S., Santos, C., Saraiva, C., & Esteves, A. (2015). Evaluation of ready to eat sashimi in northern Portugal restaurants. *Food Control, 47*, 32–36.

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*, 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*, 24–29.

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. *Environment International, 59*, 63–72.

Paiva, E. L., Alves, J. C., Milani, R. F., Boer, S. B., Quintaes, K. D., & Morgano, M. A. (2016). Sushi commercialized in Brazil: Organic Hg levels and exposure intake evaluation. *Food Control, 69*, 115–123.

Pastorelli, A. A., Baldini, M., Stacchini, P., Baldini, G., Morelli, S., Sagratella, E., et al. (2012). Human exposure to lead, cadmium and mercury through fish and seafood product consumption in Italy: A pilot evaluation. *Food Additives and Contaminants, 299*, 1–9.

Raimundo, J., Carlos Vale, C., Canário, J., Vasco Branco, V., & Moura, I. (2010). Relations between mercury, methylmercury and selenium in tissues of Octopus vulgaris from the Portuguese Coast. *Environmental Pollution, 158*, 2091–2100.

Sellanes, A. G., Mársico, E. T., Santos, N. N., São Clemente, S. C., Oliveira, G. A., et al. (2002). Mercury in marine fish. *Acta Scientiae Veterinariae, 30*, 107–112.

Storelli, M. M., Stuffler, R. G., & Marcotrigiano, G. O. (2002). Total and methylmercury residues in tuna-fish from the Mediterranean Sea. *Food Additives and Contaminants, 19*, 715–720.

Torres-Escribano, S., Calatayud, M., Ruiz, A., De La Flor, M. T., Devesa, V., Vélez, D., et al. (2010). The effect of cooking on mercury bioaccessible contents of predatory fish. *Toxicology Letters, 196*, 310.

Torres-Escribano, S., Vélez, D., & Montoro, R. (2010). Mercury and methylmercury bioaccessibility in swordfish. *Food Additives and Contaminants, 27*, 327–337.

USEPA - United States Environmental Protection Agency. (2014). *Risk-based concentrations table* [Internet]. [cited 2014 Oct 3] http://www.epa.gov/reg3hwmd/risk/human/index.htm (Accessed 16 May 2016).

USEPA (United States Environmental Protection Agency). (2007a). *Risk-based concentration table. Philadelphia PA*. Washington, DC: United States Environmental Protection Agency. https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0073.htm (Accessed 10 May 2016).

Wang, X. L., Sato, T., Xing, B. S., & Tao, S. (2005). Health risks of heavy metals to the general public in Tianjin, China via consumption of vegetables and fish. *Science of the Total Environment, 1*, 28–37.

WHO (World Health Organization). (2000). *Safety evaluation of certain food additives and contaminants. WHO Food additives series 44*. Lead. http://www.who.int/ipcs/publications/ehc/ehc_numerical/en (Accessed 09 June 2016).

WHO (World Health Organization). (2004). *Safety evaluation of certain food additives and contaminants, WHO. Food Additive Series: 52*. Geneva: International Programme on Chemical Safety, WHO.

WHO (World Health Organization). (2008). *Guidance for identifying populations at risk from mercury exposure*. Geneva (Switzerland): UNEP Chemicals Branch and WHO Department of Food Safety, Zoonoses and Foodborne Diseases. http://www.who.int/entity/foodsafety/publications/chem./mercury/en (Accessed 20 June 2016).