Food Control 22 (2011) 778–785

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Assessment of trace elements in fishes of Japanese foods marketed in São Paulo (Brazil)

Marcelo A. Morgano [a,*], Luana C. Rabonato [a], Raquel F. Milani [a], Luciana Miyagusku [a], Simone C. Balian [b]

[a] Centro de Ciência e Qualidade de Alimentos, Instituto de Tecnologia de Alimentos – ITAL, Av Brasil 2880, CP 139, 13070-178 Campinas, SP, Brazil
[b] Departamento de Medicina Veterinária Preventiva e Saúde Animal, Universidade de São Paulo – USP, Av. Prof. Dr. Orlando Marques de Paiva 87, 05508-27-SP, Brazil

ARTICLE INFO

Article history:
Received 2 August 2010
Received in revised form
21 October 2010
Accepted 6 November 2010

Keywords:
Arsenic
Mercury
Japanese food
ICP OES
Inorganic contaminants

ABSTRACT

In recent years, there has been increasing fish consumption in Brazil, largely due to the popularity of Japanese cuisine. No study, however, has previously assessed the presence of inorganic contaminants in species used in the preparation of Japanese food. In this paper, we determined total arsenic, cadmium, chromium, total mercury, and lead contents in 82 fish samples of Tuna (*Thunnus thynnus*), Porgy (*Pagrus pagrus*), Snook (*Centropomus* sp.), and Salmon (*Salmo salar*) species marketed in São Paulo (Brazil). Samples were mineralized in HNO_3/H_2O_2 for As, Cd, Cr and Pb, and in $HNO_3/H_2SO_4/V_2O_5$ for Hg. Inorganic contaminants were determined after the validation of the methodology using Inductively Coupled Plasma Optical Emission Spectrometry (ICP OES); and for Hg, an ICP-coupled hydride generator was used. Concentration ranges for elements analyzed in mg kg^{-1} (wet base) were as follows: Total As (0.11–10.82); Cd (0.005–0.047); Cr (0.008–0.259); Pb (0.026–0.481); and total Hg (0.0077–0.9681). As and Cr levels exceeded the maximum limits allowed by the Brazilian law (1 and 0.1 mg kg^{-1}) in 51.2 and 7.3% of the total samples studied, respectively. The most contaminated species were porgy (As = 95% and Cr = 10%) and tuna (As 91% and Cr = 10%). An estimation of As, Cd, Pb, and Hg weekly intake was calculated considering a 60 kg adult person and a 350 g consumption of fish per week, with As and Hg elements presenting the highest contribution on diets reaching 222% of provisional tolerable weekly intake (PTWI) for As in porgy and 41% of PTWI for Hg in tuna.

1. Introduction

Raw seafood such as fish, shellfish and shrimp is the main ingredient of a Japanese dish called *sashimi*. In recent years, fish consumption *in natura* has been increasing in Brazil, with raw fish (*sashimi*) being more and more consumed in specialized restaurants. Japanese cuisine may be considered one of the main contributing factors in the increase of fish consumption, introducing and innovating the preparation of raw seafood in regions where it could rarely be found before. Several Brazilian cities have now become gastronomic centers with a lot of restaurants providing Japanese food (de Moura Filho et al., 2007).

Nevertheless, it is known that fish-based food may be considered one of the main sources of human contamination since it may have traces of inorganic contaminants in its composition, usually known as heavy metals. According to Sellanes et al. (2002), fish consumption may be considered the main cause of human contamination by mercury.

Results obtained in studies of several sea fish and freshwater fish species suggest that chemical constituent contents, whether nutrients or toxic metals, diverge between different species and even between individuals of the same species due to time, catching place, habitat, gender, age, among other factors (Maia, Oliveira, & Santiago, 1999; WHO, 1989). Of particular interest is the finding that toxic metals may interact inside the body of these fishes, markedly affecting the metabolism of some essential constituents such as copper, zinc, and selenium (Curcho, 2009, pp. 1–204).

High toxicity rates of some metals for organisms even at low concentrations, associated with their respective ability to enter and remain in trophic chain for long periods, emphasize the relevance of studies determining their maximum concentrations allowable in aquatic environments (Azcue, 1987).

Inorganic contaminants such as Hg, Cd, Pb, As, and Cr are among the most toxicologically studied contaminants. These metals react with diffusing ligands, macromolecules, and ligands present in membranes, which mostly provide bioaccumulation and biomagnification properties in the food chain, persistence in the

* Corresponding author. Tel.: +55 19 37431178.
E-mail address: morgano@ital.sp.gov.br (M.A. Morgano).

0956-7135
doi:10.1016/j.foodcont.2010.11.016

environment, and disorders in the metabolic processes of living organisms. Bioaccumulation and biomagnification transform a concentration considered normal into a toxic concentration for a different biota species as well as human beings. Persistence ensures long-term effects even after emissions are interrupted (Tavares & Carvalho, 1992).

The trend of such toxic metals to accumulate in fish tissues and their high affinity and interaction with a large number of ligands and macromolecules are important factors that determine the toxicity of these elements regarding living organisms. According to Jobling (1995), the toxicity levels observed for 9 of the main inorganic contaminants in fish decrease in the following order: Hg, Cd, Cu, Zn, Ni, Pb, Cr, Al, and Co. However, this sequence is subject to changes depending on the physicochemical conditions of the environment directly affecting the chemical speciation of these elements, such as temperature, pH, and hardness of water. Other factors such as adsorption on cation exchange sites, flocculation, precipitation, co-precipitation, and complexation with organic molecules also constitute important mechanisms that determine the availability of these metal elements in aquatic environments (Costa, 2007).

Mercury is a metal that may undergo methylation in natural environments mainly in sediments (Anderson et al., 2004; Campbell et al., 1988, 298 p.; Niencheski, Window, Baraj, Wells, & Smith, 2001). Metal methylation in sediments through abiotic reactions occurs by transference of methyl groups to heavy metals. However, when biologically mediated, this reaction becomes more relevant since it is more efficient, significantly increasing the availability of metals in the food chain (Curcho, 2009). Lowenstein et al., (2010) has studied *sushi* portions prepared with tuna from five different species and sold in restaurants and supermarkets in the United States. The author observed mercury contents above the level authorized by Canada, the European Union, Japan and the World Health Organization legislations in several samples.

Fish is considered a good predictor of environmental pollution, and several studies have used some fish species to assess the contamination in some potentially affected regions, being considered bioindicators of environmental contamination (Kasper, Botaro, Palermo, & Malm, 2007).

Several agencies and organizations such as the US Food and Drug Administration (US FDA), the Food and Agriculture Organization (FAO), and the World Health Organization (WHO) establish limits on the human intake of trace elements. The Joint FAO/WHO Committee on Food Additives determined values for provisional tolerable weekly intake (PTWI), as well as daily intake values for food additives and some contaminants in food. Such determined values are important safety levels for human beings (COT-Committee, 2004, 222 pp.).

The previously mentioned increasing fish consumption in Brazil, largely due to the influence of Japanese cuisine, has heightened the need for an assessment of the quality of seafood. The objectives of this study were to validate methodologies using acid digestion and quantification by ICP OES, and to determine the arsenic, cadmium, chromium, lead and total mercury contents in fish samples of Porgy (*Pagrus pagrus*), Salmon (*Salmo salar*), Snook (*Centropomus* sp.), and Tuna (*Thunnus thynnus*) species, which are widely used in Japanese cuisine and were collected at the São Paulo General Warehousing and Centers Company (CEAGESP).

2. Material and methods

2.1. Samples

Samples of fish muscle from Tuna species (*Thunnus thynnus*) ($n = 21$), Porgy (*Pagrus pagrus*) ($n = 20$), Snook (*Centropomus* sp.) ($n = 20$), and Salmon (*Salmo salar*) ($n = 21$) were purchased at São Paulo General Warehousing and Centers Company (CEAGESP). The total of 82 samples were transported to the laboratory and were kept frozen (−22 °C) until analysis. All samples were analyzed in triplicate for the presence of As, Cd, Cr, Pb and total Hg.

2.2. Reagents and materials

All glassware used in the present study was previously washed with extran detergent (Merck, Darmstadt, Germany), decontaminated by immersion in a 20% (v/v) HNO_3 solution for 12 h and rinsed with purified water. Water was treated by reverse osmosis, resulting in water with resistivity 18.2 MΩ cm^{-1}. Nitric and sulfuric acids, hydrogen peroxide, stannous chloride, potassium permanganate, hydroxylamine hydrochloride, and vanadium pentoxide used were analytical grade obtained from Merck (Darmstadt, Germany). Multielement standard calibration of As, Cd, Cr, Pb, and Hg at concentration of 1000 mg L^{-1} was purchased from Merck (Darmstadt, Germany). The multielement standard solution was appropriately diluted and used to calibrate the ICP OES before metal determinations in the samples. Calibration curves for As, Cd, Cr and Pb were prepared in a 5% v/v HCl solution with concentration ranging from 0.0025 to 0.5 mg L^{-1}. As for Hg calibration curves were prepared in a 5% v/v sulphonitric solution with concentration ranging from 0.0005 to 0.05 mg L^{-1}.

2.3. Instrumentation

For the determination of the metals an Inductively Coupled Plasma Optical Emission Spectrometry (ICP OES) was used. The Vista – MPX CCD Simultaneous ICP OES was purchased from Varian Inc. (Mulgrave Victoria, Australia) and equipped with axial vision, a radio frequency (RF) source of 40 MHz, a CCD (Charge Coupled Device)-type simultaneous multi-elementary solid-state detector, a peristaltic pump, a nebulization chamber, and a sea spray nebulizer. The system was controlled by ICP Expert software using liquid argon as plasma gas with 99.996% purity (Air Liquid, SP, Brazil). For Hg determination, an ICP-coupled hydride generator from VARIAN – VGA77 (Mulgrave, Australia) was employed using argon as carrier gas and a 25% $SnCl_2$ (m/v) solution as reductant. Experimental conditions of ICP OES are outlined in Table 1.

Table 1
Experimental conditions used on ICP OES equipment to determine inorganic contaminants in fish samples.

ICP OES	Experimental conditions	
RF power (W)	1000	
Nebulizing flow rate (L min^{-1})	0.9	
Auxiliary argon flow rate (L min^{-1})	1.5	
Main argon flow rate (L min^{-1})	15	
Background correction	2 points	
Integration and reading time (sec)	10	
Replicate number	3	
Torch configuration	Axial	
Wavelength (nm)	As (II 193.696)	Pb (I 220.353)
	Cd (I 214.439)	Hg (II194.164)
	Cr (I 276.653)	
Hydride generator		
Sample rate	9 mL min^{-1}	
HCl 10 mol L^{-1} rate	1 mL min^{-1}	
25% $SnCl_2$ rate	1 mL min^{-1}	
Carrier gas pressure (Ar)	350 kPa	
Carrier gas flow (Ar)	100 mL min^{-1}	

(I) Analyte line ionic; (II) Analyte line atomic.

2.4. Digestion and analysis

2.4.1. As, Cd, Cr and Pb (Bordajandi et al., 2004)

In a glass tube for digestion block, 1.0 g of fish muscle (wet weight) was weighed and 15 mL concentrated HNO_3 were added. The glass tube with sample and HNO_3 was let to rest overnight. On the next day, 5 mL of 30% (w/v) hydrogen peroxide were added and tubes were placed in the digestion block, and heated at 100 °C for approximately 6 h. After digestion, tubes contents were quantitatively transferred to a 25 mL volumetric flask, with 5% (v/v) HCl solution.

2.4.2. Hg (Morgano, Gomes, Mantovani, Perrone, & Santos, 2005)

In a glass tube, 1.0 g of fish muscle (wet weight) was weighed, then 10 mL of sulphonitric mixture 1:1 with 0.1% (w/v) vanadium pentoxide were added and heated in water bath stirring at 90 °C for 3 h. After cooling, 5 mL of a 7% (w/v) $KMnO_4$ solution were added and heated for another 3 h. At the end of digestion, tubes were left to cool and afterwards 900 μL of 20% (w/v) hydroxylamine hydrochloride solution were added. Tubes contents were quantitatively transferred to a 25 mL volumetric flask with deionized water.

2.5. Validation and uncertainty calculation

Both analytical methodologies applied for fish analyses were validated following INMETRO (2010) guidelines. The limit of detection (LOD) and limit of quantification (LOQ) for each metal were determined as follows: ten independent analysis of a blank solution spiked with metal at a level of lower concentration of the analytical curve were performed. The LOD and LOQ were calculated from the standard deviation (σ) of these determinations ($LOD = 3 \times \sigma$ and $LOQ = 10 \times \sigma$).

The accuracy of the analytical procedure was checked by the analyses of certified reference materials, Lobster Hepatopancreas (TORT-2) of the National Research Council (NRC), Canada, and Oyster Tissue (SEM-1566[a]) of the National Institute of Standards and Technology (NIST).

For uncertainty calculation, the following factors were evaluated as contribution source: weighing in analytical scale, volume measured in volumetric flasks and micropipettes, analytical curves linearity, standard solutions of inorganic contaminants studied, and methodology precision.

2.6. Statistical analyses

Descriptive statistic (mean, standard deviation, median, interval) and analyses of variance (ANOVA) were conducted using excel software. In order to verify if means obtained for inorganic contaminants from different fish species were statistically different at p level < 0.05, the Tukey's multiple comparison test was applied.

2.7. Inorganic contaminants intake estimation

Estimation of weekly inorganic contaminants intake (As, Cd, Cr, Pb, and Hg) was calculated and expressed as PTWI (provisional tolerable weekly intake) percentage. Calculations were made considering a mean fish mass of 50 g for a *sashimi* portion and a 60 kg body weight for adults.

3. Results and discussion

3.1. Method validation

From standard solutions used to elaborate analytical curves, work ranges linearity could be verified by analytical curves preparation for each element in its respective emission wavelength. Correlation coefficients and sensitivity values are shown in Table 2. We can observe that values obtained for correlation coefficients are above 0.999 for all elements, suggesting a good linearity of analytical curves in work ranges defined. This technique has demonstrated greater sensitivity for Hg, Cd, Cr, and Pb elements, being proportionately less sensitive for As element.

Table 2
Wavelength (λ), correlation coefficient (r), equation of straight line and sensitivity, limit of detection (LOD), and limit of quantification (LOQ) for inorganic contaminants studied.

Element	λ (nm)	r	Straight line equation	Sensitivity ($L\ mg^{-1}$)	LOD ($\mu g\ L^{-1}$)	LOQ ($\mu g\ L^{-1}$)
As	193.696	0.9998	$y = 79.5x + 5.899$	80	4	40
Cd	214.439	0.9999	$y = 6818.5x + 8.190$	6819	0.2	3
Cr	276.653	0.9992	$y = 4139.1x + 27.914$	4139	0.7	5
Hg	194.164	0.9998	$y = 15638.7x + 1.940$	15639	0.1	1
Pb	220.353	0.9998	$y = 598.6x + 10.311$	599	1	7

In order to verify method selectivity, samples spectra and analytical standard of contaminants were delineated in emission wavelength region selected for each element studied. Emission peaks profile of fish samples solution analytes is identical to respective profiles of standard solution peaks, suggesting that spectral or matrix interferences on determination of contaminants analyzed do not occur.

Method precision was evaluated by coefficient of variation of 7 analytical repetitions using the same measurement procedure, analysis, equipment, and repetitions in a short period of time. Intermediate precision was determined through assays performed in different times (2-week window) with the same examiner and same equipment, and expressed by coefficient of variation. Method precision has shown to be satisfactory with results achieved. For all elements coefficient of variation value was below 13% (Table 3). MAPA Normative Guideline no. 24/2009 recommends CVs of up to 20% for a sample concentration between 10 ppb and 100 ppb, up to 15% for a sample concentration between 100 ppb and 1000 ppb, and up to 10% for a sample concentration above 1000 ppb.

To assess the accuracy of the methodology applied, studies with certified reference material and recovery tests were conducted. A recovery test was performed in three concentration levels and in triplicate for each inorganic contaminant following MAPA NG no. 24/2009 instruction (Brasil, 2009, 7 pp.) using MRL (Maximum Residue Limit). From maximum limit allowed (MRL) of contaminants studied in the Brazilian legislation for fish (1 mg kg^{-1} for As, Cd and Cr, 2 mg kg^{-1} for Pb, and 0.5 mg kg^{-1} for Hg), samples were spiked in three concentration levels: low (0.5 MRL), mean (1 MRL), and high (1.5 MRL). Results obtained ranged from 98 to 106% for As; from 94 to 98% for Cd; from 97 to 108% for Cr; from 91 to 100% for Pb; and from 82 to 98% for Hg. As per the Ministry of Agriculture, Livestock and Food Supply (MAPA) (Brasil, 2009), recovery values between 70 and 110% are recommended for fortification levels used; therefore, results obtained are appropriate.

Results experimentally obtained for certified reference materials TORT-2 (Lobster Hepatopancreas) and Oyster Tissue (NIST 1566a) are shown in Table 4. For As, Pb, and Hg elements, experimental values obtained were more compliant with the certified values.

3.2. Uncertainty calculation

Uncertainty of analytical methodology to determine inorganic contaminants was estimated for the main factors that aggregate

Table 3
Descriptive statistic results for precision and intermediate precision evaluation of 7 analytical repetitions of inorganic contaminants found in fish sample.

Element	Mean (mg kg^{-1})	SD (mg kg^{-1})	Lowest value (mg kg^{-1})	Highest value (mg kg^{-1})	Precision CV (%)	Intermediate precision[a] CV (%)	CV max[b] (%)
Arsenic	8.04	0.02	7.97	8.08	0.2	4	10
Cadmium	0.015	0.002	0.010	0.021	13	11	20
Chromium	0.092	0.004	0.087	0.097	4	15	20
Lead	0.22	0.02	0.18	0.25	11	1	15
Mercury	0.16	0.01	0.15	0.17	4	6	15

[a] Intermediate precision in assays performed in different days of analysis ($n = 7$ repetitions) and assessed by coefficient of variation (CV).
[b] Acceptance criterion for coefficient of variation according to MAPA Normative Guideline no. 24 (Brasil, 2009).

variations in methodology such as sample digestion, dilution steps, and potential interferences during quantification (matrix effects, calibration curve linearity) as per guidelines EURACHEM (2002).

Linearity uncertainty calculation was based on least squares method considering straight line as $A_j = c_i B_1 + B_0$ and analyte concentration in stratum is provided by Equation (1).

$$c_0 = \frac{(I_j - B_0)}{B_1} \quad (1)$$

where I_j is $j$eth intensity measurement of $i$eth calibration standard solution, c_i is $i$eth calibration standard solution concentration, and B_i and B_o are angular and linear coefficients of calibration curve, respectively.

Analyte concentration in sample is given by (2):

$$C_{cont} = \frac{c_0 V_f}{M_{am}} \text{ or } C_{cont} = \frac{V_f (I_0 - B_0)}{M_{am} B_1} \quad (2)$$

where V_f is volume of flask where stratum was diluted, and M_{am} is mass of the sample used. In order to obtain uncertainty combined for inorganic contaminants evaluated Equation (3) was used, and in order to calculate expanded uncertainty a k coverage factor of 2 (confidence level of approximately 95%) was used.

$$\mu_c C_{cont} = \sqrt{\left(\frac{\mu V_f}{V_f}\right)^2 + \left(\frac{\mu c_0}{c_0}\right)^2 + \left(\frac{\mu M_{am}}{M_{am}}\right)^2} \quad (3)$$

where $\mu_c C_{cont}$ is uncertainty combined for contaminant.

Fig. 1 shows a graph with individual uncertainty contributions for each inorganic contaminant. We can clearly observe that the influence of uncertainty associated with micropipette used for standard dilutions and methodology precision was predominant in methodology total uncertainty value. The only element in which uncertainty contribution related to micropipette and methodology precision had approximately 50% contribution was mercury. For As, uncertainty contribution related to micropipette was 98%.

From this data, total uncertainty for each element was calculated as proposed by guideline EURACHEM (2002), and expanded total uncertainty was obtained using the factor $K = 2$. Table 5 shows such values.

Table 4
Certified values for reference materials TORT-2 (Lobster Hepatopancreas) for As, Cd, Cr, and Pb and Oyster Tissue (NIST 1566a) for Hg, and mean values experimentally obtained ($n = 7$).

Elements	Certified values (mg kg^{-1})	Results obtained (mg kg^{-1})	Recovery (%)
Arsenic	21.6 ± 1.8	21.103 ± 0.001	98
Cadmium	26.7 ± 0.6	23.7 ± 0.8	89
Chromium	0.77 ± 0.15	0.59 ± 0.03	78
Lead	0.35 ± 0.13	0.36 ± 0.02	103
Mercury	0.037 ± 0.001	0.035 ± 0.002	95

3.3. Inorganic contaminants in fish

Table 6 shows results obtained for inorganic contaminants As, Cd, Cr, Pb, and Hg in 82 samples analyzed. In Brazil, the maximum levels of inorganic contaminants in fish are established by the National Agency for Sanitary Surveillance (ANVISA) and the Ministry of Agriculture, Livestock and Food Supply (MAPA). For fish and fishing products, the maximum tolerance levels are: As = 1.0 mg kg^{-1}; Cd = 1.0 mg kg^{-1}; Cr = 0.1 mg kg^{-1}; Pb = 2.0 mg kg^{-1}; Hg = 0.5 mg kg^{-1} (except for predator fish) and 1.0 mg kg^{-1} (for predator fish). Of 4 fish species studied, only salmon is not a predator one. Considering the limits of the Brazilian legislation, no level above the maximum tolerance limit was found for Cd, Pb, and Hg.

The highest mercury concentration values were observed in tuna (0.9681 mg kg^{-1}) and porgy (0.7638 mg kg^{-1}) samples. Mercury may induce changes in normal brain development of babies, and at high levels may induce neurological problems in adults. It also causes toxicity to the liver and fetus development, being a potential human carcinogen (Commission of the European Communities, 2001).

Concentration levels found for Cd were low and the highest values were achieved for tuna (0.047 mg kg^{-1}), whereas for Pb the highest levels were found for salmon (0.481 mg kg^{-1}) and tuna (0.426 mg kg^{-1}). Cd element may accumulate in the human body and cause damages to the kidney, the bones and the reproductive system. The effects of lead in the body are related to a reduction in cognitive and intellectual development in children and blood pressure increase and cardiovascular diseases in adults (Commission of the European Communities, 2001).

For samples analyzed, As was the most predominant contaminant with contents above those established by the Brazilian law for tuna and porgy. For total individuals of species studied, the following have shown respective As contamination rates: porgy = 95%, tuna = 90%, salmon = 24%, and snook = 15%. The toxic effect of arsenic depends on its current chemical form. Arsenic inorganic compounds are toxic (As^{+3}) whereas organic compounds are considered of low toxicity. Arsenobetaine, organic form of As present in food of marine origin, constitutes the greatest arsenic

Table 5
Combined and expanded uncertainty applied to analysis of certified reference material, $k = 2$

Contaminant	Mean achieved[a] (mg L^{-1})	Combined standard uncertainty (mg L^{-1})	Combined standard uncertainty (%)	Expanded standard uncertainty (mg L^{-1})
Arsenic	21.10	2.07	9.81%	4.14
Cadmium	23.73	2.54	10.70%	5.08
Chromium	0.59	0.07	11.39%	0.14
Lead	0.35	0.04	10.44%	0.07
Mercury	0.035	0.021	5.97%	0.004

[a] Lobster Hepatopancreas Reference Material; NRC (National Research Council of Canada) (Ottawa, ON, Canada) for As, Cd, Cr and Pb, and Oyster Tissue Reference Material, NIST 1566[a].

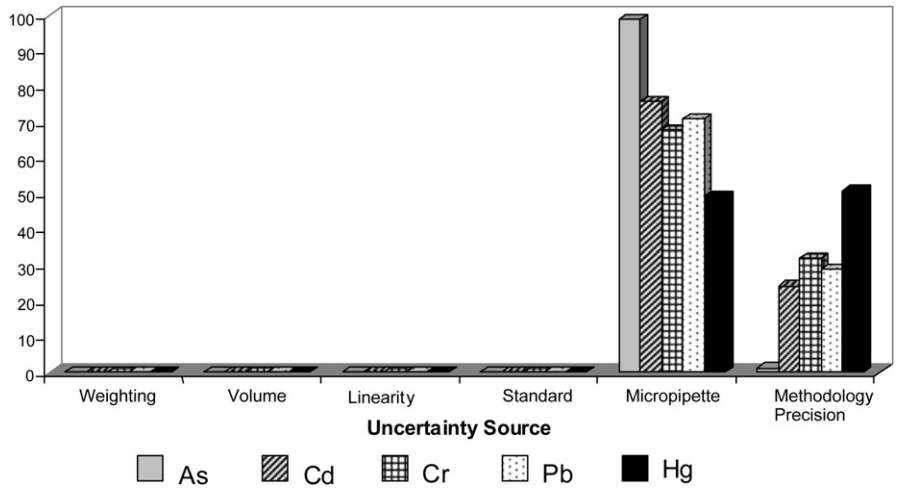

Fig. 1. Contribution of different uncertainty sources.

source in diet (Rossin, 2005). Chronic exposures to arsenic may cause dermatitis, keratosis of skin pigmentation with hyperkeratinization of exposed areas, warts, and may lead to lung cancer (Occupational Safety and Health Administration, 2004).

For chromium lower levels were found, with contents above those accepted by the Brazilian law: Tuna, Porgy, and Snook = 10%. Chromium is a mineral that can be found in nature in several manners, with trivalent and hexavalent being the most usual ones. Trivalent chromium is more stable and can be found in plants, and it is usually related to organic complexes that enhance insulin action in the organism influencing carbohydrates, lipids and proteins metabolism. Hexavalent chromium is considered toxic with carcinogenic potential (Institute of Medicine, 2002, 773 pp.).

3.4. Comparison with reported literature values

3.4.1. Arsenic

For all 4 fish species studied, As levels above the one established by the Brazilian Law (1 mg/kg) were found. A study conducted in Spain in canned and frozen tuna samples observed As levels ranging from 0.23 to 0.52 mg kg^{-1} respectively (Bordajandi et al., 2004). In the United States, Ikem and Egiebor (2005) have found As levels of up to 1.7 mg kg^{-1} for canned tuna samples, but for both pink and red canned salmon no As was detected. In the present study, As levels obtained for tuna ranged from 0.18 to 3.67 mg kg^{-1}, and for salmon values ranged from <0.04 to 1.53 mg kg^{-1}. For snook species, Curcho (2009) conducted a Trial in which As levels noted varied from 0.03 to 0.76 mg kg^{-1}, but a significant variation was observed in the present study, with values below the limit of quantification (<0.04 mg kg^{-1}) up to 5.87 mg kg^{-1}. Some countries such as Australia, New Zealand, and Hong Kong set forth a maximum content of inorganic As of 1, 2, and 6 mg kg^{-1}, respectively (Ikem & Egiebor, 2005). In Brazil, MAPA regulates maximum As level of 1.0 mg kg^{-1} in fish and fishing products.

3.4.2. Cadmium

Cadmium values found in the present study are close to the literature values. In Spain, Bordajandi et al. (2004) have observed Cd values of 0.01 mg kg^{-1} both in canned and frozen tuna. When Ikem and Egiebor (2005) analyzed USA samples, Cd levels between 0.0 and 0.05 mg kg^{-1} were obtained for canned tuna, whereas both pink and red canned salmon have not shown Cd presence. In another study performed in Spain, Yusà et al. (2008) noted Cd values of 0.003–0.02 mg kg^{-1} for tuna. In Brazil, Curcho (2009) verified mean Cd values of 0.003 mg kg^{-1} for snook. The results of the present study comply with the literature values for tuna (<0.003–0.05 mg kg^{-1}), salmon (<0.003–0.02 mg kg^{-1}), and snook (<0.003–0.02 mg kg^{-1}).

The European Community legislation determines a maximum Cd level of 0.05 mg kg^{-1}, and the Codex Committee on Food Additives and Contaminants recommends 0.5 mg kg^{-1} (Ikem & Egiebor, 2005), whereas in Brazil the Cd limit is 1.0 mg kg^{-1} for fish and fishing products. Therefore, all results obtained are below current legislations.

3.4.3. Chromium

Cr levels of 0.28 mg kg^{-1} were observed in canned pink salmon samples in the United States, and 0.09 mg kg^{-1} for canned red salmon (Ikem & Egiebor, 2005). When these values are compared, canned red salmon demonstrated more compliance with Cr level verified in the present study for salmon (0.03–0.09 mg kg^{-1}). On the other hand, in the same study of Ikem and Egiebor (2005), Cr values noted for canned tuna have ranged from 0.0 to 0.07 mg kg^{-1}, being lower than those obtained in the present study for tuna (<0.01–0.26 mg kg^{-1}). In another study conducted in Brazil, Cr levels observed for snook remained between 0.03 and 0.36 mg kg^{-1} (Curcho, 2009), which have demonstrated to be a little higher when compared to this study values of 0.01–0.19 mg kg^{-1}.

In Brazil, the maximum Cr level recommended by the legislation is 0.1 mg kg^{-1} while in the United States there is no tolerable level for Cr according to the Washington Medicine Institute in New York (Ikem & Egiebor, 2005), but only a tolerable intake level (AI) for men and women of 51–70 years old of 20 and 30 μg/day, respectively. Nevertheless, values verified both in the literature and in the present study when compared with the Brazilian legislation suggest some concern, since practically all species studied have demonstrated values above 0.1 mg kg^{-1}.

3.4.4. Lead

Generally, the results reported in the literature for Pb in all 4 species studied are lower than those obtained in the present study. In a study conducted in Spain, Bordajandi et al. (2004) have noted Pb values of 0.02 mg kg^{-1} for both canned and frozen tuna, a result close to the value observed for canned tuna by Ikem and Egiebor (2005) in the United States of 0.0–0.03 mg kg^{-1} which, in turn, was close to the tuna result verified by Yusà et al. (2008) in Spain of 0.02–0.09 mg kg^{-1} for Pb. In the present study, Pb levels ranging from 0.03 to 0.43 mg kg^{-1} were observed in tuna. In snook samples

Table 6
Results obtained for inorganic contaminants total As, Cd, Cr, and total Hg in 82 fish samples of Tuna ($n = 21$), Porgy ($n = 20$), Snook ($n = 20$), and Salmon ($n = 21$) species, and samples percentage above Brazilian law.

Species	n		Inorganic contaminants (mg kg^{-1}, wet base)				
			Arsenic	Cadmium	Chromium	Lead	Mercury
Tuna	21	Mean ± SD	1.81 ± 0.79[a]	0.014 ± 0.013[a]	0.046 ± 0.054[a]	0.185 ± 0.094[a,b]	0.3506 ± 0.3504[a]
Thunnus thynnus		Median	1.83	0.012	0.034	0.195	0.1452
		Range	0.18–3.67	<0.003–0.047	<0.005–0.259	0.028–0.426	0.0251–0.9681
		% of samples above law levels	90.5	–	7.3	–	–
Porgy	20	Mean ± SD	5.70 ± 3.10[b]	0.008 ± 0.007[a,b]	0.059 ± 0.029[a]	0.101 ± 0.097[a]	0.2218 ± 0.2246[a]
Pagrus pagrus		Median	6.27	0.004	0.053	0.076	0.1284
		Range	0.28–10.82	<0.003–0.026	0.014–0.120	<0.007–0.337	<0.0010–0.7638
		% of samples above law levels	95.0	–	9.5	–	–
Snook	20	Mean ± SD	0.87 ± 1.22[a]	0.007 ± 0.005[b]	0.058 ± 0.039[a]	0.150 ± 0.091[a,b]	0.0202 ± 0.0341[b]
Centropomus sp.		Median	0.60	0.004	0.047	0.160	0.0128
		Range	<0.04–5.87	<0.003–0.017	0.011–0.185	<0.007–0.293	<0.0010–0.1536
		% of samples above law levels	15.0	–	10.0	–	–
Salmon	21	Mean ± SD	0.59 ± 0.45[a]	0.008 ± 0.005[a,b]	0.048 ± 0.018[a]	0.228 ± 0.127[b]	0.0127 ± 0.0055[b]
Salmo salar		Median	0.67	0.01	0.045	0.208	0.0098
		Range	<0.04–1.53	<0.003–0.016	0.027–0.092	<0.007–0.481	0.0077–0.0249
		% of samples above law levels	23.8	–	–	–	–

Equal letters in the same column are not significantly different at p level < 0.05.

from São Paulo State, Brazil, Curcho (2009) obtained Pb levels of 0.01 mg kg^{-1}, while in this study higher values of up to 0.29 mg kg^{-1} were found. For salmon, Ikem and Egiebor (2005) analyzed pink and red canned salmon, with no Pb being detected in both species; however, salmon samples have shown values of up to 0.48 mg kg^{-1} in this study.

The Brazilian legislaion (Brazil, ANVISA, 1998) recommends a maximum Pb level of 2.0 mg kg^{-1}, and the European Community (EC) regulates 0.2 mg kg^{-1} as maximum limit. Considering the limit value as per EC regulation, 38% out of 82 samples are above the maximum level accepted.

3.4.5. Mercury

Total Hg values found in tuna and snook species reported in the literature in comparison to this study have shown a great variation. Hajeb et al. (2009) studied Hg level in commonly consumed marine fishes in Malaysia and the tuna species had the highest level (4.15 μg g^{-1}).

In studies conducted in Spain, Bordajandi et al. (2004) found Hg levels of 0.30 and 0.28 mg kg^{-1} in canned and frozen tuna, respectively. Blanco, González, and Vieites (2008) also studied Hg in canned and frozen tuna, having obtained values ranging from <0.10 to 0.87 and from <0.10 to 0.49 mg kg^{-1}, respectively. Yusà et al. (2008) observed Hg values in tuna varying from 0.02 to 2.5 mg kg^{-1}. These results might indicate that total Hg contamination in species of tuna is increasing in Spain, in the last six years.

In the United States, Ikem and Egiebor (2005) also studied total Hg presence in canned tuna, and observed values ranging from 0.05 to 0.74 mg kg^{-1}. These same authors analyzed Hg in pink and red canned salmon, which showed levels of 0.022–0.079 and 0.015–0.067 mg kg^{-1}, respectively. Such values are lower than those obtained in a study conducted by Zhang et al. (2001), which have observed Hg values varying from 0.025 to 0.137 mg kg^{-1} for salmon. Both trials obtained higher levels than in the present study, which has levels from 0.01 to 0.02 mg kg^{-1}. For snook both in this paper and in Curcho (2009), which were conducted in Brazil, total Hg levels noted are similar: <0.001 to 0.15 and 0.02–0.18 mg kg^{-1}, respectively.

Lowenstein et al. (2010) analyzed samples from different tuna species destined to *sushi* preparation sold in New York, New Jersey and Colorado restaurants and supermarkets. The authors have observed higher levels of Hg in fish samples from restaurants, and *sushi* prepared with Bigeye Tuna (*Thunnus obesus)* species exhibited the highest levels of Hg (0.365–2.254 mg/kg). According to Hight and Cheng (2006), mercury from tuna *sushi* is predominantly present as methylmercury, being approximately 97% of total mercury.

Regarding the existing law for Hg, the European Community, Canada, and the FDA establish a total Hg level of 1 mg kg^{-1} (European Commission, 2008; FDA, 2000; Health Canada, 2007); Japan sets forth limit values of 0.4 mg kg^{-1} (Yamashita, Omura, & Okazaki, 2005), whereas in Brazil limit values are 0.5 mg kg^{-1} for non-predator fishes and 1.0 mg kg^{-1} for predator ones (ANVISA, 1998).

3.5. Estimated weekly intake

For As, Cd, Pb, and total Hg, fish consumption contribution was calculated by the estimated weekly intake of such contaminants based on PTWI (provisional tolerable weekly intake) values set forth by JECFA (WHO, 2004).

Table 7
Inorganic contaminants occurrence, estimated weekly intake, and estimated PTWI percentage for intake of 50 g daily fish portion (1/2 *sashimi* portion) based on mean values found in samples.

Specie	Contaminant occurrence (mg/kg)	Estimated weekly intake (μg/kg body weight[a])	% PTWI[b]
Tuna	As = 1.81	10.6	71
	Cd = 0.014	0.082	1
	Pb = 0.185	1.079	4
	Hg = 0.3506	2.045	41
Salmon	As = 0.59	3.4	23
	Cd = 0.008	0.047	0.7
	Pb = 0.228	1.33	5
	Hg = 0.0127	0.074	1
Snook	As = 0.87	5.07	34
	Cd = 0.007	0.041	0.6
	Pb = 0.150	0.875	4
	Hg = 0.0202	0.118	2
Porgy	As = 5.70	33.25	222
	Cd = 0.008	0.047	0.7
	Pb = 0.101	0.589	2
	Hg = 0.2218	1.294	26

[a] Weight for adults (older than 17 years old): 60 kg.
[b] PTWI for As = 15 μg kg^{-1} week body weight^{-1}; for Cd = 7 μg kg^{-1} week body weight^{-1}; for Pb = 25 μg kg^{-1} week body weight^{-1}; for total Hg = 5 μg kg^{-1} week body weight^{-1}.

In estimation intake calculations, a 50 g fish/day (half of sashimi portion) and 60 kg as the body weight for adults were used. This portion is appropriate according to the United States Environmental Protection Agency (US EPA), which recommends a fish intake of 340 g/week, a quantity equivalent to 49 g/day (US EPA, 2004, 2 pp.). Values of weekly intake (Table 7) were calculated for each fish specie using mean values obtained for each inorganic contaminant, as shown in Table 6 i.e., estimated weekly intake in μg/kg body weight = mean element concentration (μg/kg) multiplied by consumed amount/week (kg) and divided by the mean weight of a subject (60 kg). Values obtained were compared to respective PTWI.

PTWI is expressed on a weekly basis and highlights a long-term exposure risk for contaminants that may accumulate in the human body. Inorganic contaminants that have mostly contributed to achieve PTWI values in 4 fish species were As and Hg; of note, As contaminant in porgy was 222% PTWI. For Hg the highest contribution has occurred in tuna, with 41% PTWI. Contributions to achieve PTWI for Cd and Pb elements were lower, not surpassing 1% for Cd in tuna and 5% for Pb in salmon.

4. Conclusion

The levels of trace metals in fish samples widely used in Japanese cuisine (porgy, salmon, snook and tuna) marketed in São Paulo were determined and assessed for their quality by comparing element levels in samples with maximum permitted levels stipulated by different agencies and organizations. The result from this study suggested that differences existed in the element concentrations even within the same fish species.

Four species assessed in this study have not shown Cd, Pb and Hg concentration levels above the limit established by the Brazilian law; however, for As and Cr all species have demonstrated concentrations above permitted levels (except for Cr in salmon), which is compliant with results obtained in other studies.

Inorganic contaminants that have mostly contributed to achieve provisional tolerable weekly intake (PTWI) values were As and Hg; of note, As contaminant in porgy was 222% PTWI. For Hg the highest contribution has occurred in tuna, with 41% PTWI.

It is extremely important to know the origin of the fish intended for the consumer market, since the quality of fish depends on the sea environment, fish feed, catching place, age, among other factors that may significantly influence on the levels of inorganic contaminants in fish. More research and assessments of seafood quality is needed to provide more data and help safeguard the health of humans and furthermore a study of speciation of arsenic is important since the different chemical forms of this element present different toxicity levels.

Acknowledgments

The authors acknowledge financial support of MAPA-SDA/CNPq (National Board of Scientific and Technological Development).

References

Anderson, H. A., Hanrahan, L. P., Smith, A., Draheim, L., Kanarek, M., & Olsen, J. (2004). The role of sport-fish consumption advisories in mercury risk communication: a 1998–1999 12-state survey of women age 18–45. *Environmental Research, 95*, 315–324.

Azcue, J. M. P. (1987). Possíveis vias de acesso de metais pesados provenientes do sistema hídrico do Rio Paraíba do Sul – Rio Grande, RJ, até o homem. 1987. Dissertação de Mestrado – Universidade Federal do Rio de Janeiro (IBCCFO), Rio de Janeiro.

Blanco, S. L., González, J. C., & Vieites, J. M. (2008). Mercury, cadmim and lead levels in samples of the main traded fish and shelfish species in Galicia, Spain. *Food Additives and Contaminants: Part B, 1*(1), 15–21.

Bordajandi, L. R., Gómez, G., Abad, E., Rivera, J., Fernández-Bastón, M. M., Blasco, J., et al. (2004). Survey of Persistent Organochlorine contaminants (PCBs, PCDD/Fs, and PAHs), heavy metals (Cu, Cd, Zn, Pb, and Hg), and arsenic in food samples from Huelva (Spain): levels and health Implications. *Journal of Agricultural and Food Chemistry, 52*, 992–1001.

Brasil. ANVISA – Agência Nacional de Vigilância Sanitária. (1998). *Portaria n° 685, de 27 de agosto de 1998. Limites máximos de tolerância para contaminantes inorgânicos*. Brasília, DF: Diário Oficial da União.

Brasil. MAPA – Ministério da Agricultura, Pecuária e Abastecimento. (2009) *Instrução Normativa n° 24, de 14 de julho de 2009. Define os requisitos e critérios específicos para funcionamento dos Laboratórios de Análises de Resíduos e Contaminantes em Alimentos integrantes da Rede Nacional de Laboratórios Agropecuários, Seção 1*. Brasília, DF: Diário Oficial da União.

Campbell, P. G., Lewis, A. G., Chapman, P. M., Crowder, A. A., Fletcher, W. K., Imber, B., et al. (1988). *Biologically available metals in sediments national research*. Ottawa.

Commission of the European Communities, 2001. (2002). Commisssion Regulation (EC) n. 221/2002 of the 6 February 2002 amending regulation (EC) n. 466/2002 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Communities*, . Brussels, 6 February.

Costa, J. R. da (2007). *Distribuição de metais em peixes marinhos ao longo do litoral sudeste do Brasil. Monografia*. Campos dos Goytacazes – RJ: Universidade Estadual do Norte Fluminense, Darcy Ribeiro.

Cot - Committee on Toxicity. (2004). *Advise on fish consumption: Benefits ans risks. Food Standards Agency and the Department of Health*. Norwich, UK: Her Royal Majesty's Stationary.

Curcho, M. R. da S. M. (2009). Avaliação de Micro e Macroelementos, Elementos Tóxicos (Cd, Hg e Pb) e Ácidos Graxos, em Peixes Disponíveis Comercialmente para consumo em Cananéia e Cubatão, Estado de São Paulo. São Paulo: IPEN. 2009. Tese para obtenção de título de Mestre em Ciências na Área de Tecnologia Nuclear – Aplicações, Instituto de Pesquisas Energéticas e Nucleares, São Paulo.

Ellison, S. L. R., Rosslein, M., & Qilliams, A. (2002). *GUIA EURACHEM/CITAC: Determinando a Incerteza na Medição Analítica – 2a Edição*. Sociedade Brasileira de Metrologia.

European Commission (2008). Commission regulation (EC) No. 629/2008 of 2 July 2008 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs. *Off. F. EU*, 51, 4–10.

FDA. (2000). *Guidance for industry: Action levels for poisonous or deleterious substances in human food and animal feed*. Available from. http://www.fda.gov/Food/Guidance-ComplianceRegulatoryInformation/GuidanceDocuments/ChemicalContaminantsandPesticides/ucm077969.htm Accessed 19.05.10.

Hajeb, P., Jinap, S., Ismail, A., Fatimah, A. B., Jamilah, B., & Abdul Rahim, M. (2009). Assesment of mercury level in commonly consumed marine fishes in Malaysia. *Food Control, 20*, 79–84.

Health Canada. (2007). *Human health risk assessment of mercury in fish and health benefits of fish consumption*. Available from. http://hc-sc.gc.ca/fn-an/pubs/mercur/merc_fish_poisson-eng.php Accessed 19.05.10.

Hight, S. C., & Cheng, J. (2006). Determination of methylmercury and estimation of total mercury in seafood using high performance liquid chromatrography (HPLC) and inductively coupled plasma-mass spectrometry (ICP-MS): method development and validation. *Analytica Chimica Acta, 567*, 160–172.

Ikem, A., & Egiebor, N. O. (2005). Assesment 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*, 771–787.

INMETRO - Instituto Nacional de Metrologia, Normalização e Qualidade Industrial. (2010). Orientação Sobre Validação de Métodos Analíticos. DOQ-CGCRE-008. *Rev, 03*, 1–20, fev.

Institute of Medicine. (2002). *Dietary reference intakes for vitamin a, vitamin k, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium and zinc*. Constitution Avenue, NW, Washington, DC: Institute of Medicine of the National Academies, The National Academy Press, 2001.

Jobling, M. (1995). *Environmental biology of fish*. London: Chapman e Hall.

Kasper, D., Botaro, D., Palermo, E. F. A., & Malm, O. (2007). Mercúrio em peixes – fontes e contaminação. *Oecologia Brasiliensis, 11*(2), 228–239.

Joint FAO/WHO Expert Committee onf Food Additives. (2004). *Summary of evaluations performed by the Joint FAO/WHO Expert Committee on food additives (JECFA 1956-2003)*. Washington, DC: Food and Agriculture Organization of the United Nations and the World Health Organization, ILSI Press International Life Sciences Institute.

Lowenstein, J. H., Burger, J., Jeitner, C. W., Amato, G., Kolokotronis, S.-O., & Gochfelf, M. (2010). DNA barcodes reveal species-specific mercury levels in tuna sushi that pose a health risk to consumers. *Biology Letters, 6*(5), 692–695.

Maia, E. L., Oliveira, C. C. S., & Santiago, A. P. (1999). Composição química e classes de lipídios em peixe de doce curimatã comum, Prochilodus cearensis. *Ciência e Tecnologia de Alimentos, 19*(3), 433–437.

Morgano, M. A., Gomes, P. C., Mantovani, D. M. B., Perrone, A. A. M., & Santos, T. F. (2005). Níveis de mercúrio total em peixes de água doce de pisciculturas paulistas. *Ciência e Tecnologia de Alimentos, 25*(2), 250–253.

de Moura Filho, L. G. M., Mendes, E. S., e Silva, R. P. P., de Barros Góes, L. M. N., de Araújo Vieira, K. P. B., & de Paula Mendes, P. (2007). Enumeração e pesquisa de Vibrio spp. e coliformes totais e termotolerantes em sashimis de atum e vegetais comercializados na região metropolitana do Recife, Estado de Pernambuco. *Acta Scientiarum Technology, 29*(1), 85–90.

Niencheski, L. F., Window, H. L., Baraj, B., Wells, D., & Smith, R. (2001). Mercury in fish from Patos and Mirim lagoons, southern Brazil. *Marine Pollution Bulletin, 42*, 1403–1406.

Occupational Safety and Health Administration. (2004). *Toxic metals, occupational safety and health administration*. Available from. 200 Constitution Avenue, NW, Washington, DC: US Department of Labor. http://www.osha.gov Accessed 18.05.10.

Rossin, R. (2005). Especiação de arsênio inorgânico em águas por espectrometria de absorção atômica acoplada com gerador de hidretos. 44f. Dissertação (Mestrado em Geociências) – Universidade Estadual de Campinas, São Paulo.

Sellanes, A. G., Mársico, E. T., Santos, N. N., Clemente, S. C. S., Oliveira, G. A., & Monteiro, A. B. S. (2002). Mercúrio em peixes. *Acta Scientiae Veterinariae, 30*(2), 107–112.

Tavares, T. M., & Carvalho, F. M. (1992). Avaliação de exposição de populações humanas a metais pesados no ambiente: exemplos do recôncavo baiano. *Química Nova, 15*(2), 147–154.

US EPA – United States Environmental Protection Agency. (2004). *What you need to know about mercury in fish and shellfish*. EPA-823-F-04-009, Available from. http://www.epa.gov/waterscience/fish/MethylmercuryBrochure.pdf Accessed 18.05.10.

Who – World Health Organization. (1989). *Mercury*. In *Environmetal health criteria, n.86*. Geneve.

Yamashita, Y., Omura, Y., & Okazaki, E. (2005). Total mercury and methylmercury levels in commercially important fishes in Japan. *Fisheries Science, 71*, 1029–1035. doi:10.1111/j.1444-2906.2005.01060.x.

Yusà, V., Suelves, T., Ruiz-Atienza, L., Cervera, M. L., Benedito, V., & Pastor, A. (2008). Monitoring programme on cadmium, lead and mercury in fish and seafood from Valencia, Spain: levels and estimated weekly intake. *Food Additives and Contaminants: Part B, 1*(1), 22–31, June.

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*, 993–997.