Marine Pollution Bulletin 186 (2023) 114464

Contents lists available at ScienceDirect

Marine Pollution Bulletin

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

Baseline

Are mercury levels in fishery products appropriate to ensure low risk to high fish-consumption populations?

Elsa T. Rodrigues[a,*], João P. Coelho[b], Eduarda Pereira[c], Miguel A. Pardal[a]

[a] Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal
[b] Department of Biology and CESAM and ECOMARE, University of Aveiro, Campus de Santiago, 3810-193 Aveiro, Portugal
[c] Department of Chemistry and CESAM/REQUIMTE, University of Aveiro, Campus de Santiago, 3810-193 Aveiro, Portugal

ARTICLE INFO

Keywords:
Mercury content in seafood
Methylmercury
Culinary treatments
Food quality
Health risk

ABSTRACT

The main goal of the present study is to determine the sources of methylmercury (MeHg) for high fish-consumption populations with the Portuguese population as showcase, as Portugal is the EU country with the highest fish consumption per capita (2019: 59.91 kg year^{-1}). Since limited information is available on the effective levels of mercury after culinary treatments, cooked and raw codfish (*Gadus morhua*), hake (*Merluccius merluccius*), octopus (*Octopus vulgaris*), horse mackerel (*Trachurus trachurus*) and sardine (*Sardina pilchardus*) were considered. The mercury concentration ranking Hake > Horse mackerel > Codfish > Octopus > Sardine was observed in all situations (cooked and raw samples) for both MeHg and total mercury (T-Hg). The gathered results reinforce the general assumption that the loss of moisture during cooking increases MeHg and T-Hg concentrations in fish, but the idea that MeHg in fish muscle tissue represents the bulk of T-Hg cannot be generalised, as our study determined a MeHg/T-Hg ratio of 0.43 for grilled sardines.

Mercury is considered a metal of concern in regard to its harmful effects on human health, since, when present, it disrupts normal cellular processes (Naganuma et al., 2002; Parran et al., 2001; Teixeira et al., 2018), alters histology and physiology (Asano et al., 2000; Sherwani et al., 2013), and has the potential to, among others, induce neurotoxicity (Ralstona and Raymond, 2018). Moreover, the organic methylmercury (MeHg) form, its main toxic form (IPCS, 1990), is considered one of the top ten environmental compounds suspected of causing learning disabilities and autism (Kern et al., 2016).

Humans are primarily exposed to MeHg through the consumption of fishery products, as it may bioaccumulate on the first levels of the aquatic trophic chain and undergo biomagnification (Bradley et al., 2017; Polak-Juszczak, 2018). Thus, it has been measured in substantial levels in predatory fish (Annibaldi et al., 2019; Barone et al., 2021; Shipley et al., 2019), but also in shellfish such as mussels and scallops (Fasano et al., 2018). Nevertheless, human health risk from mercury depends on the size of the fishery products, the amount eaten and the intake frequency, as well as individual susceptibility. Therefore, reflecting the human dietary exposure in Europe, a Tolerable Weekly Intake (TWI) value was set for MeHg: 1.3 μg kg^{-1} body weight (bw) (EFSA, 2012), and two maximum levels (MLs) were established: 0.5 mg kg^{-1} wet weight (ww) for fishery products and muscle meat of fish in general, and 1.0 mg kg^{-1} ww for specific species listed in *Annex*, section 3, of Commission Regulation 629 (2008). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has also established a provisional TWI for MeHg of 1.6 μg kg^{-1} bw for adults (WHO-TRS 959, 2010), which is higher than EFSA's TWI. All these regulatory limit values are expressed as total mercury (T-Hg) as this parameter is easier, quicker and less expensive to analytically measure than MeHg and, is therefore more often used. Nevertheless, Health Canada uses two Tolerable Daily Intake (TDI) values that could be expressed in MeHg or T-Hg, as this Agency follows the precautionary principle and for health risk assessment purposes considers that the 100 % of T-Hg level in fishery products are present in the form of MeHg. The TDI values are 0.2 μg kg^{-1} bw for sensitive populations (women of childbearing age and 12 years of age and younger), and 0.47 μg kg^{-1} bw for the general adult population (Health Canada, 2007). The US-EPA reference dose (RfD) of 0.1 μg kg^{-1} bw day^{-1} is also expressed in MeHg (US-EPA, 2001).

Recognising that fish constitutes an important part of a balanced and traditional Mediterranean diet and that Portugal is one of the countries with the highest fish consumption per capita in the world, and the highest at EU level (FAO, 2008), the risk determination of mercury for Portuguese citizens is key to guarantee human safety within Europe. Regarding consumption frequencies, >70 % of the Portuguese people

* Corresponding author.
E-mail addresses: etrodrig@uc.pt (E.T. Rodrigues), jpcoelho@ua.pt (J.P. Coelho), eduper@ua.pt (E. Pereira), mpardal@uc.pt (M.A. Pardal).

https://doi.org/10.1016/j.marpolbul.2022.114464
Received 23 June 2022; Received in revised form 30 November 2022; Accepted 1 December 2022
Available online 8 December 2022
0025-326X/

eat fishery products more than once a week (Pieniak et al., 2013), specifically two to three times (Fernandes, 2017; Nunes et al., 2014). A selection of 59 species is eaten, which are mainly consumed at home and usually boiled or grilled (Fernandes, 2017). Even though not native to the coastline of Portugal, codfish (typically dried and salted) is considered the most important fish in the Portuguese diet, representing ≈38 % of the fish demand (Almeida et al., 2015), followed by hake and canned tuna (Cardoso et al., 2013; Nunes et al., 2014). Additionally, in a recent survey, octopus, horse mackerel and sardines (also known as European pilchard) have also joined the previous group (Fernandes, 2017). This set of species, to which gilthead seabream has been added, also represents the items most consumed by Portuguese pregnant women (Nunes et al., 2014). Gender and geography are relevant, with men preferring cephalopods and sardines and women opting for hake, redfish and pink cusk-eel, and coastal populations and elderly people preferring wild fish (Cardoso et al., 2013, 2015). The Portuguese generations born from 1980s to mid-2000s (the ones shaping seafood consumption in the next decades) favour fresh fish (which is preferred over frozen, salted/dried, canned or smoked fish) as the most suitable food for a meal of fish for its taste, quality and freshness, even though they recognize that fish is not their favourite food. They still prefer wild to farmed fish, as well as Portuguese fish to imported fish (Madsen and Chkoniya, 2021).

Seeing that the recommended TWI can easily be exceeded by Portuguese citizens, and that EFSA recommends that each EU Member State considers its own consumption pattern of fishery products, especially the species of fish consumed, and assesses the risk of exceeding the TWI (EFSA, 2015), the main goal of the present study is to determine the effective sources of MeHg present in edible fishery products after culinary treatments, contributing to a more accurate human risk assessment of MeHg.

The estimation of MeHg levels have already been performed for canned tuna acquired in Portugal (Afonso et al., 2015). Thus, in the present study the MeHg levels were determined for the other above-mentioned fishery products most consumed by the Portuguese society: codfish (*Gadus morhua*), hake (*Merluccius merluccius*), octopus (*Octopus vulgaris*), horse mackerel (*Trachurus trachurus*) and sardine (*Sardina pilchardus*). Concerning codfish, evaluations were made nationwide, as this fish is not native to the coastline of Portugal, and is imported from Norway or Iceland and sold nationwide. Since ≈70 % of all codfish consumed in Portugal comes from Norway, the specimens used in the present study arrived from this country. Regarding hake, octopus, horse mackerel and sardines, the specimens used were all purchased fresh at the Coimbra municipal fish market, representing fish caught in the north-western coast region of the Portuguese mainland. It should be highlighted that, in Portugal, sardines are mainly captured by seine fishing, and the other species by trawler fishing (INE IP, 2018). Since fish size is relevant, as the amount of mercury is related to the age of the fish (EFSA, 2012), only the sizes most consumed by the Portuguese population were acquired: dried and salted codfish of ≈1.5–2.0 kg, fresh hake of 1.0–1.5 kg, fresh octopus of 1.0–1.5 kg, fresh horse mackerels of 25–30 cm, and fresh sardines of 15–20 cm; but larger specimens of the species that presented the highest risk regarding MeHg concentrations were also considered in an additional set of analyses: fresh hake of over 3 kg. The two most used culinary treatments, boiling (codfish, hake and octopus) and grilling (horse mackerel and sardine), were studied to determine the final fraction of MeHg ingested. Only the muscle tissue (the filet) was considered in the present study as it is the most consumed part of the fish, and because fish muscle has the highest levels of MeHg (Coelho et al., 2010; Polak-Juszczak, 2018). It is known that MeHg has high affinity with thiol groups and, thus, bonds with small, low-molecular weight and cysteine-rich proteins (Lemes and Wang, 2009). In order to reduce possible seasonal patterns regarding mercury accumulation, all the specimens were acquired between August, 31 and September, 11 of 2021; except fresh hake of over 3 kg that the specimens were acquired between October, 22 and November, 26, 2021, in both Coimbra and Figueira da Foz fish markets. All the culinary treatments were processed within one hour of acquisition, with no storage, as little is known about the effects of storage on the stability of MeHg in food samples (FAO/WHO, 2011). Hence, six individuals of each species (five individuals for hake of over 3 kg) were gutted and their heads and central spines removed, but their skin was kept. The fishmonger at the market executed all these procedures after biometric data were written down. Each specimen was washed with tap water and then individually placed in a plastic bag provided by the seller. Then, at the laboratory, each specimen was washed with distilled water and excess water was removed with paper. Two identical fillets were prepared by slicing each specimen in half using a ceramic knife. For the octopus, two tentacles (arms) were considered, as tentacle tissue is known to have the highest percentage distribution of mercury (Seixas et al., 2005). Regarding salted codfish, the fillets were soaked at 5 °C for 48 h (the water was renewed after 24 h) using a 1:9 fish:destiled water mass ratio. This procedure took place with the skin side up, without overlapping and without stirring. Then, for the same specimen, one sample was cooked and the other was left unprocessed as a raw sample, and to avoid uncertainty concerning different water content in cooked and unprocessed fillets, the moisture content of triplicate sub-samples was determined following standard official methods for food products, via a gravimetric approach, being the difference of the initial weight of the sample and the weight after oven drying at 60 °C for 24 h used to determine the percentage moisture of the sample (AOAC, 2007). In the individual boiling process of 10 min for codfish and hake, and 20 min for octopus, a 1:2 fillet:ultra-pure water mass ratio was used, and the water contained 2.0 g per 100 g of fillet of commercial marine salt without any additives (Vatel cooking sea salt, www.vatel.pt) (except for codfish, where no salt was added), whereas in the individual grilling process of 20 min (each side for about 10 min), fish was salted with 2.0 g of Vatel salt per 100 g 15 min prior to grilling, and a domestic griller (Phillips HD4419/20, 2400 W) operated in the position 4, the one recommended for fish, was used. Cooked and raw filets were immediately stored at −70 °C directly in glass mortars prior to lyophilisation (Martin Christ Alpha 1–2 Ldplus), and the dry product was ground into powder (except for lyophilised raw samples of octopus where stainless-steel tweezers and scissors were used), and then stored in new 40-mL amber glass vials with PTFE liners in the cap (Supelco 27,182). Since MeHg determinations were performed using lyophilised (freeze-dried) samples, the moisture of the fresh samples was estimated using the difference of the initial weight of the sample and the weight after lyophilisation. This evaluation was performed using six raw sub-samples.

Sample digestion and MeHg extraction were performed according to Válega et al. (2006) using a method established for the organic forms of mercury. Briefly, ≈200 mg of powder-homogenised samples (cooked and raw) were rigorously weighed and placed in 50-mL FEP (Nalgene 3114-0050) or PPCO (Thermo scientific 3119-0050) test tubes. Then, 5.0 mL of 18 % of potassium bromide (≥99 % trace metals basis, Sigma-Aldrich 221864) prepared in 5 % of sulfuric acid, and 1.0 mL of copper sulphate 1 M (Sigma-Aldrich C8027) were added. At that point, a quick vortex of 5 s was performed to promote full contact of the sample with the reagents, followed by a waiting time of 15 min. Then, 5.0 mL of toluene (Merck 1.08325) was added, and the test tubes were placed in an orbital shaker at 150 rpm for 15 min, centrifuged at 4,000 rpm for 12 min, and 3.0 mL of the organic fraction was carefully transferred to a 25-mL glass vial. This toluene step was repeated, but this time 5.0 mL of the organic fraction was collected and added to the respective 25-mL glass vial. Finally, 5.0 mL of sodium thiosulfate solution 0.002 M (≥99,99 % trace metals basis, Sigma-Aldrich 563188) was added to the organic fraction on the 25-mL glass vial and mixed for 5 min. Both fractions were stored at 5 °C for MeHg analyses, which were performed within 24 h. An extraction blank was considered in each extraction batch to assess possible contamination and to correct sample concentration values, as well as a positive control using the certified reference material (CRM) DORM-4 (National Research Council Canada) to estimate extraction efficiency.

Since T-Hg is often used as a proxy for MeHg in seafood, T-Hg was also determined in the present study to add information regarding the MeHg/T-Hg ratio for the selected species. The analytical determination of MeHg (from the extraction aqueous fraction) and T-Hg (from the powder homogenised samples) was performed in a LECO AMA-254 apparatus by sample catalytic combustion, preconcentration by gold amalgamation, thermal desorption and atomic absorption spectrometry detection. The T-Hg method detection limit (MDL) was 0.01 ng absolute Hg, while for MeHg a MDL (mean + 3 × standard deviation (SD)) of 0.11 μg kg^{-1} and a method quantification limit (MQL, mean + 10 × SD) of 0.28 μg kg^{-1} were calculated from the blank signals. A duplicate analysis of the certified reference material DORM-4 for MeHg determinations, and a triplicate analysis of the certified reference material BB422 (European Reference Material, sample No. 0355) for T-Hg determinations were performed at the beginning and at the end of each working day, to confirm the calibration status of the LECO AMA-254 system and the quality of the analytical method. A recovery percentage above or equal to 90 % was considered as acceptance criterion. A minimum of two (MeHg) and three (T-Hg) sub-samples were analysed, and a coefficient of variation below or equal to 10 % was considered as acceptance criterion. All sample values were blank-corrected using the mean method blank concentrations within their corresponding batch.

In Portugal, the National Program for the Promotion of Healthy Eating of the Directorate-General of Health follows the food plate model (https://alimentacaosaudavel.dgs.pt/roda-dos-alimentos/), which shows the daily proportions of the seven different food groups that people need to eat in order to achieve a balanced diet. The recommended number of portions depends on individual energy requirements, e.g., children should be guided by the lower limits and active men by the upper limits. Hence, the Portuguese food wheel guide points to eating 1.5 to 4.5 portions of fish, meat or eggs per day, where one fish portion is 30 g of raw or 25 g of cooked fish. Since these days teenagers do not enjoy eating fish, in the present study MeHg dietary exposure was evaluated considering three age categories, namely children: 6–11 years (40 g day^{-1} of cooked filets), youths: 12–17 years (80 g day^{-1} of cooked filets), and adults: >18 years (112.5 g day^{-1} of cooked filets). The estimated daily intakes (EDI) of MeHg and T-Hg through the consumption of fishery products were determined using the equation:

$$\mathrm{EDI}\ \left(\mu\mathrm{g\ kg^{-1}\ bw\ day^{-1}}\right) = (\mathrm{C} \times \mathrm{IR})/\mathrm{BW}$$

where C is the MeHg or T-Hg concentration (μg kg^{-1}), IR is the daily ingestion rate (kg day^{-1}), and BW is body weight (mean values for children: 25 kg, youths: 50 kg and adults: 70 kg).

Species biometric data and moisture content obtained by the oven-drying gravimetric approach were summarized in Tables S1 and S2 (supplementary material), respectively. As expected, the moisture content in raw samples generally varied inversely with the total fat, ranking Hake (80.5 %) > Octopus (78.6 %) > Codfish (73.1 %) > Horse mackerel (72.5) > Sardine (61.4 %). The values of total fat obtained in literature for seafood consumed in Portugal rank Hake (0.2 g 100 g^{-1}) < Codfish (0.4 g 100 g^{-1}) < Octopus (1.2 g 100 g^{-1}) < Horse mackerel (1.4 g 100 g^{-1}) < Sardine (10.9 g 100 g^{-1}) (https://www.ipma.pt/resources.www/docs/publicacoes.site/pescado/inicio.htm). Using the same gravimetric method, similar mean values of moisture were obtained for raw horse mackerel (76.0 %) and sardines (50.9 %) collected on the Portuguese coast by Vieira et al. (2011). The applied culinary treatments (boiling and grilling) always decreased moisture content in a percentage between 8.0 % (hake) and 40.4 % (sardine). Compared to raw controls, cooking led to a significant loss of moisture content (Student's *t*-test, dependent samples: $P < 0.001$), with losses being more evident in grilling (mean percentage decrease: 30.8 %) than in boiling (mean percentage decrease: 9.4 %) treatments. Moisture decrease was also observed in grilled salmon (*Salmo salar*), in a percentage of 14.0 % (Costa et al., 2015), as well as in boiled (mean value of 30.3 %) and grilled (mean value of 45.1 %) Atlantic mackerel (*Scomber scombrus*), European seabass (*Dicentrarchus labrax*) and Black scabbardfish (*Aphanopus carbo*) (Mieiro et al., 2016). The effect of different drying techniques (oven- and freeze-drying) was only significant in sardines (Student's *t*-test, dependent samples: $P < 0.001$), where percentage moisture loss was 22 % higher for freeze- than for oven-dried sardines.

The results obtained for the tested CRMs agree with their respective certified range (detailed information in Tables S3 and S4, supplementary material), presenting analytical recovery percentages between 89.9 and 97.0 % for MeHg (mean ± SD: 92.0 ± 2.0 %), and between 89.7 and 95.7 % for T-Hg (mean ± SD: 91.2 ± 2.3 %). The results of MeHg and T-Hg contents were determined taking into account the recovery of the analytical procedure, and are presented in Tables S5 and S6 of supplementary material, respectively. As expected, higher MeHg and T-Hg concentrations were detected in carnivorous species, while at the bottom of the food chain, sardines presented low MeHg and T-Hg concentrations (Fig. 1). Our T-Hg results of raw samples could be compared to several studies from the Central and North Atlantic coast for the same species. For instance, codfish collected in Norwegian fishery areas presented a mean concentration of 0.08 mg kg^{-1} ww in their fillets (Azad et al., 2019), and a similar value was determined in the present study (0.076 mg kg^{-1} ww). Moreover, octopus collected in Portugal in previous studies presented mean concentrations of 0.075 and 0.094 mg kg^{-1} ww in their arms (Cardoso et al., 2012; Seixas et al., 2005), which are consistent with our result of 0.055 mg kg^{-1} ww. Horse mackerel and sardines purchased randomly from several markets in the Porto Metropolitan area (Portugal) presented mean mercury levels slightly different, but still comparable with our results, of 0.172 and 0.018 mg kg^{-1} ww, respectively (Vieira et al., 2011). Also, sardines of around the same size (18–20 cm) collected in Essaouira (Marrocos) presented ≈0.035 mg kg^{-1} ww in their muscle (Chahid et al., 2014), and a comparable value was determined in our study: 0.026 mg kg^{-1} ww. In the present study, the gathered mean values of raw data never surpassed the current ML for mercury, laid down in the *Annex*, Section 3, of Commission Regulation 1881 (2006), which is 0.5 mg kg^{-1} ww (expressed as T-Hg) for the selected species. However, confirming that dietary exposure determinations should take into account the culinary treatments as part of the risk characterization of chemicals in food, if the data of cooked fish are considered, boiled hake of 1.0–1.5 kg does not conform with the regulatory value since a mean value of 0.53 mg kg^{-1} ww was determined. The present study revealed that culinary treatments (boiling and grilling) always increased MeHg and T-Hg levels (Student's *t*-test, dependent samples: $P < 0.044$) in percentages ranging from 31.7 (horse mackerel) to 108.3 % (sardines) for MeHg (Table S5, supplementary material), and from 33.7 (horse mackerel) to 119.2 % (sardines) for T-Hg (Table S6, supplementary material). It should be highlighted that this effect was likely due to the loss of moisture during the culinary treatments as, except for octopus for MeHg and horse mackerel for both MeHg and T-Hg, no significant differences between cooked and raw mercury dry weight data were observed (data not shown). Moreover, it should be stressed that, in Portugal, fishery products are usually bought fresh, and then cooked or eaten raw (e.g., oysters, or sashimi found in some sushi), and are rarely consumed dried. Despite some differences in the cooking methods (sample weight, amount of water used, cooking times, etc.), an increase of MeHg and T-Hg (wet weight basis) was also observed in boiled and grilled tuna (*Thunnus* spp.) (Afonso et al., 2015) and in boiled and grilled gilthead seabream (*Sparus aurata*) (Afonso et al., 2018), as well as in grilled salmon (*Salmo salar*) (Costa et al., 2015) and in grilled blue shark (*Prionace glauca*) (Matos et al., 2015). The same pattern for T-Hg was observed in grilled anchovy (*Engraulis encrasicolus*), hake (*Merluccius merluccius*) and squid (*Loligo vulgaris*) (Kalogeropoulos et al., 2012). Nevertheless, the mercury level increased after Perelló and collaborators boiled hake, but no change was observed after they grilled sardines (Perelló et al., 2008). Thus, despite some different results, the tendency for mercury increase (using wet weight data) after commonly used cooking treatments is currently generally accepted in review studies (e.g., Bradley et al., 2017). The gathered results also revealed

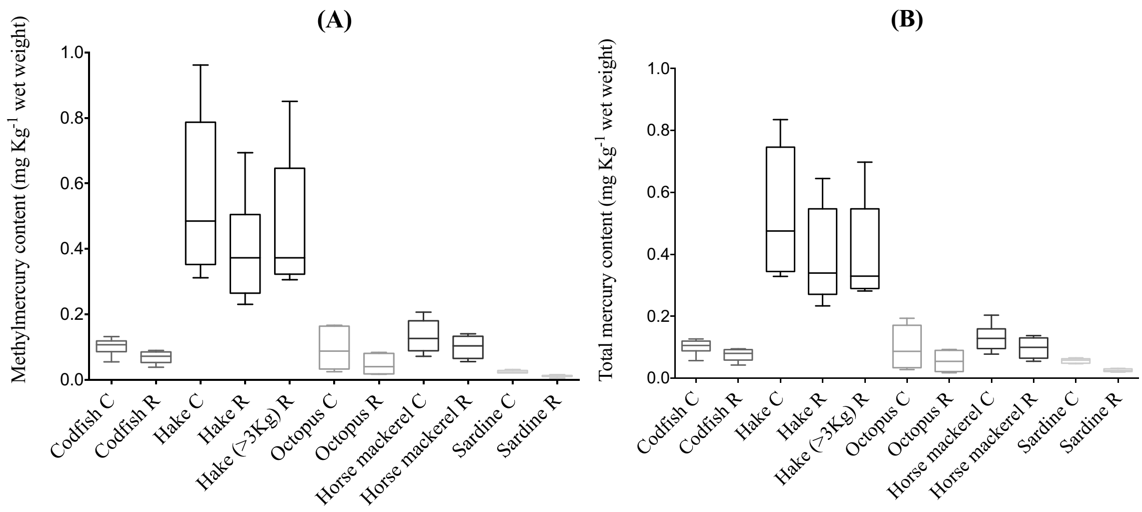

Fig. 1. Methylmercury (A) and total mercury (B) content (mg kg^{-1} wet weight) by experimental treatment: C, cooked and R, raw. Boxes show the median and 25th and 75th percentiles, and whiskers show minimum and maximum values.

that the amount of mercury appeared to increase with the duration of cooking time since the octopus, which was boiled for twice as long as the fish that underwent the same type of cooking - codfish and hake, had the highest percentage increase of both MeHg and T-Hg after cooking (see Tables S5 and S6, supplementary material). These results suggest that increasing the cooking time enhances the extractability of MeHg, which is possible due to further loss of moisture. In fact, from the three boiled species, the octopus presented the higher decrease of moisture content after cooking (see Table S2, supplementary material). The mercury concentration ranking Hake > Horse mackerel > Codfish > Octopus > Sardine was observed in all situations (cooked and raw samples) for both MeHg and T-Hg. In the present study, no significant differences between raw hake of 1.0–1.5 kg and raw hake of >3 kg were determined for MeHg (Student's *t*-test, independent samples: $P > 0.597$) or T-Hg (Student's t-test, independent samples: $P > 0.936$) (both on a wet weight or dry weight basis).

The MeHg/T-Hg ratio varied between 0.45 (sardines) and 1.15 (hake >3 kg) for raw samples, and between 0.43 (sardines) and 1.05 (hake 1.0–1.5 kg) for cooked samples (Table S6, supplementary material). Despite reportable, the gathered ratio values slightly above one are likely due to the fact that MeHg is determined from the powder samples prepared by acidic digestion and extraction to toluene, while T-Hg was determined directly from the powder samples. This differentiated pre-treatment may result in a MeHg/T-Hg ratio higher than one. Also, two different reference materials were used, one for MeHg determinations (DORM-4) and another for T-Hg determinations (BB422). These two different materials lead to a mean recovery slightly higher in the case of MeHg. Since the results of MeHg and T-Hg content in samples were determined taking into account the recovery of the analytical procedure of each working day, a slight overestimation of MeHg may have occurred in relative terms. Nevertheless, using muscle tissues of fish, MeHg/T-Hg ratios above one were also reported by Ikingura and Akagi (2003), Maršálek et al. (2006) and Reyes et al. (2009). The boiled samples always presented slightly higher ratios when compared with the corresponding ratios of raw samples, which indicates possible loss of inorganic mercury by this cooking process. Corroborating this finding, and by using a mass balance model to ascertain the main form of mercury loss in fishery products after cooking, Liao et al. (2019) found that, during boiling, inorganic mercury dissociated to a much larger extent than MeHg, and that loss of mercury was primarily due to volatilisation. Regarding the grilled samples, corresponding to the smaller species (Table S1, supplementary material), with higher fat content - horse mackerel and sardine - a slight decrease of the MeHg/T-Hg ratios were determined when compared with the corresponding ratios of raw samples. This is possibly due to the thermal denaturation of proteins, which released some MeHg by volatilisation, as grilling is associated with intense dry heating, which, in turn, was intensified by the small size of the grilled fillets; or because fat is known for inhibiting MeHg extraction (Miyamoto et al., 2010). For cooked filets, no ratio values were found in our literature review, but for raw samples similar ratios have been reported. For instance, a ratio of 0.84 was reported for 438–2,027 g codfish (38.3–56.4 cm) (Polak-Juszczak, 2018), a ratio of 0.75 was determined for the European hake (21.5–30.8 cm) (Barone et al., 2021), and a ratio of 83.9 was determined for octopus caught in Portuguese continental waters Cardoso et al. (2012). Moreover, it has been documented that MeHg/T-Hg ratio in fish muscle tissue varies with age and body mass (Coelho et al., 2010; Lescord et al., 2018; Polak-Juszczak, 2017), as well as between species (Barone et al., 2021; Lescord et al., 2018; Yoshimoto et al., 2016), which is confirmed by gathered results. However, our study reveals a ratio of 0.45 for sardines (raw samples) acquired in September (when growth and fat deposition is at its maximum for the beginning of the most active spawning period, in October (Nunes et al., 2011)), while Barone et al. (2021) reported data for sardines purchased in May–July (with a smaller size than the ones used in the present study) that allow the calculation of a higher MeHg/T-Hg ratio, of 0.67. Thus, our MeHg results for this species are possibly sub-evaluated due to the high fat content of samples since some authors point out that in fat-rich samples, as sardines, fat inhibits MeHg extraction (Miyamoto et al., 2010). Finally, the general assumption that >90 % of the mercury detected in fish muscle is in the form of MeHg should not be used for planktivorous fishes. Corroborating our findings, MeHg/T-Hg ratios of 0.56 and 0.62 were determined for Lebranche mullet (*Mugil liza*) by Kehrig et al. (2009) and Kehrig et al. (2002), respectively; and a ratio of 0.60 was determined for Brazilian sardinella (*Sardinella brasiliensis*) (Kehrig et al., 2010), all planktivorous fishes. Although more protective, the use of the same conversion factor for different species, especially if they have different trophic positions, to estimate MeHg levels from T-Hg concentrations may not provide accurate and realistic health risk assessments.

Since none of the species studied is consumed raw in Portugal (or in Europe), only MeHg and T-Hg Portuguese dietary intake results via the consumption of cooked fishery products are presented (Table 1). In line with our MeHg and T-Hg concentration data, boiled hake is associated with the highest MeHg daily intake, with 0.89 μg kg^{-1} bw day^{-1}, while the consumption of grilled sardines, planktivorous fish of smaller size, led to a much lower MeHg daily intake, of 0.04 μg kg^{-1} bw day^{-1}. In order to characterize potential risks from exposure to oral MeHg, the assumption that fish is the only food group making contributions to the

Table 1
Portuguese dietary intake (μg kg^{-1} bw day^{-1}) of methylmercury and total mercury via consumption of cooked fishery products by age category. Bold indicates non-compliance with the established US-EPA reference dose (RfD)[a] of 0.1 μg MeHg kg^{-1} bw day^{-1} for chronic oral exposure (US-EPA, 2001).

	Boiled codfish (1.5–2.0 kg)		Boiled hake (1.0–1.5 kg)		Boiled octopus (1.0–1.5 kg)		Grilled horse mackerel (25–30 cm)		Grilled sardine (15–20 cm)	
	MeHg	T-Hg	MeHg	T-Hg	MeHg	T-Hg	MeHg	T-Hg	MeHg	T-Hg
Children	**0.163**	0.163	**0.890**	0.848	**0.152**	0.158	**0.213**	0.210	0.040	0.091
Youth	**0.163**	0.163	**0.890**	0.848	**0.152**	0.158	**0.213**	0.210	0.040	0.091
Adults	**0.164**	0.164	**0.894**	0.852	**0.153**	0.159	**0.214**	0.211	0.040	0.092

MeHg, methylmercury; T-Hg, total mercury.
[a] Concentration of methylmercury which can be consumed on a daily basis over a lifetime without expectations of adverse effects.

MeHg exposure was made, i.e., that the contributions from drinking water and other dietary sources are negligible. Except for sardines, the EDI of MeHg was higher than the established US-EPA RfD of 0.1 μg MeHg kg^{-1} bw day^{-1}, originating a Hazard Quotient above one (i.e., exposure level exceeds the threshold), which indicates possible adverse effects due to the presence of MeHg in the codfish, hake, octopus and horse mackerel consumed by the Portuguese population throughout life.

The established European TWI for MeHg, which is 1.3 μg kg^{-1} bw $week^{-1}$ (expressed as T-Hg), was used to determine frequencies of consumption between age categories. Thus, this recommended limit could be surpassed if the Portuguese population (with all age categories covered) eats boiled hake of 1.0–1.5 kg (or higher) more than once per week, as well as if grilled horse mackerel is eaten more than five times per week. Nevertheless, considering the Health Canada guideline, which provide the values 0.2 μg MeHg kg^{-1} bw day^{-1} for sensitive populations and 0.47 μg MeHg kg^{-1} bw day^{-1} for youths and adults, our results indicate that Portuguese children (and women of childbearing age) should never eat boiled hake of 1.0–1.5 kg (or higher), boiled codfish of 1.5–2.0 kg and grilled horse mackerel of 35–40 cm, while youths and adults should never eat boiled hake of 1.0–1.5 kg (or higher).

Determined by risks to wildlife rather than risks to human health, as marine predators like otters and large fish, as well as marine birds, consume a much larger proportion of fish as part of their diet than humans, the European Water Framework Directive (WFD) established the environmental quality standard (EQS) for mercury at 20 μg kg^{-1} ww (expressed as T-Hg) in fish (Directive, 2013/39/EU). Since our raw samples also reflect coastal and marine wild feed items, the gathered T-Hg concentrations could be compared with the above-mentioned EQS threshold. In fact, the WFD EQS was exceeded in 100 % of the samples analysed, demonstrating an effective risk to marine top predators crossing Portuguese waters. Since mercury is ubiquitous in the environment and cannot be degraded over time, innovative technologies to remove it from the water compartment should be developed and implemented, an issue recently reviewed by Wang et al. (2020).

The results of the present study reinforce the general assumption that loss of moisture during cooking increases MeHg and T-Hg concentrations in seafood. On the contrary, the idea that MeHg in muscle tissue represents the bulk of T-Hg cannot be generalised, and should be considered only for carnivorous fishes. In order to reduce risk of oral exposure, it is recommended that high fish-consumption populations such as Portuguese people should avoid eating boiled hake of over 1 kg (fresh weight) more than once a week since it is considered a possible hazard. Accordingly, we recommend that a complementary assessment of MeHg exposure should be carried out for the Portuguese population by biologically monitoring mercury levels in blood (reflects short-term exposure) and/or hair (reflects long-term exposure). Finally, our study also alerts for the effective risk to marine top predators (e.g., piscivorous fish and birds) that feed in Portuguese waters.

CRediT authorship contribution statement

Project administration: ET Rodrigues
Conceptualisation: ET Rodrigues
Supervision: JP Coelho
Investigation: ET Rodrigues
Writing- original draft: ET Rodrigues
Writing- review: MA Pardal, JP Coelho, E Pereira
Methodology: E Pereira
Funding acquisition: MA Pardal, JP Coelho, E Pereira
Formal analysis: ET Rodrigues, JP Coelho
Resources: MA Pardal, JP Coelho, E Pereira

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Raw data are presented as supplementary material.

Acknowledgements

The Portuguese Foundation for Science and Technology supported the present study through national funds under the scope of the Programme to stimulate scientific employment - individual support, which were attributed to Elsa T Rodrigues (CEECIND/03543/2017) and João P Coelho (CEECIND/01778/2020). This study was also supported through the PT2020 Partnership Agreement and COMPETE 2020 by the Centre for Functional Ecology (UIDB/04004/2020, LA/P/0092/2020) and CESAM (UIDP/50017/2020, UIDB/50017/2020, LA/P/0094/2020) Strategic Projects.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2022.114464.

References

Afonso, C., Costa, S., Cardoso, C., Oliveira, R., Lourenço, H.M., Viula, A., Batista, I., Coelho, I., Nunes, M.L., 2015. Benefits and risks associated with consumption of raw, cooked, and canned tuna (Thunnus spp.) based on the bioaccessibility of selenium and methylmercury. Environ. Res. 143, 130–137. https://doi.org/10.1016/j.envres.2015.04.019.
Afonso, C., Costa, S., Cardoso, C., Coelho, I., Castanheira, I., Lourenço, H., Gonçalves, S., Oliveira, R., Carvalho, M.L., Martins, M.F., Bandarra, N.M., Nunes, M.L., 2018. Bioaccessibility in risk-benefit analysis of raw and cooked seabream consumption. J. Food Compos. Anal. 68, 118–127. https://doi.org/10.1016/j.jfca.2016.10.003.
Almeida, C., Karadzic, V., Vaz, S., 2015. The seafood market in Portugal: driving forces and consequences. Mar. Policy 61, 87–94. https://doi.org/10.1016/j.marpol.2015.07.012.
Annibaldi, A., Truzzi, C., Carnevali, O., Pignalosa, P., Api, M., Scarponi, G., Illuminati, S., 2019. Determination of Hg in farmed and wild Atlantic Bluefin tuna (Thunnus thynnus L.) muscle. Molecules 24, E1273. https://doi.org/10.3390/molecules24071273.
AOAC, 2007. Official Methods of Analysis of AOAC International, 18th ed. AOAC International, Arlington.
Asano, S., Eto, K., Kurisaki, E., Gunji, H., Hiraiwa, K., Sato, M., Sato, H., Hasuike, M., Hagiwara, N., Wakasa, H., 2000. Review article: acute inorganic mercury vapor

inhalation poisoning. Pathol. Int. 50, 169–174. https://doi.org/10.1046/j.1440-1827.2000.01032.x.
Azad, A.M., Frantzen, S., Bank, M.S., Nilsen, B.M., Duinker, A., Madsen, L., Maage, A., 2019. Effects of geography and species variation on selenium and mercury molar ratios in Northeast Atlantic marine fish communities. Sci. Total Environ. 652, 1482–1496. https://doi.org/10.1016/j.scitotenv.2018.10.405.
Barone, G., Storelli, A., Meleleo, D., Dambrosio, A., Garofalo, R., Busco, A., Storelli, M. M., 2021. Levels of mercury, methylmercury and selenium in fish: insights into children food safety. Toxins 9, 39. https://doi.org/10.3390/toxics9020039.
Bradley, M.A., Barst, B.D., Basu, N., 2017. A review of mercury bioavailability in humans and fish. Int. J. Environ. Res. Public Health 14, 169. https://doi.org/10.3390/ijerph14020169.
Cardoso, C., Lourenço, H., Afonso, C., Nunes, M.L., 2012. Risk assessment of methylmercury intake through cephalopods consumption in Portugal. Food Addit. Contam. 29, 94–103. https://doi.org/10.1080/19440049.2011.623284.
Cardoso, C., Lourenço, H., Costa, S., Gonçalves, S., Nunes, M.L., 2013. Survey into the seafood consumption preferences and patterns in the Portuguese population. Gender and regional variability. Appetite 64, 20–31. https://doi.org/10.1016/j.appet.2012.12.02.
Cardoso, C., Lourenço, H., Costa, S., Gonçalves, S., Nunes, M.L., 2015. Survey into the seafood consumption preferences and patterns in the Portuguese population: education, age, and health variability. J. Food Prod. Mark. 00, 1–15. https://doi.org/10.1080/10454446.2014.949982.
Chahid, A., Hilali, M., Benlhachemi, A., Kadmiri, I.M., Bouzid, T., 2014. Concentrations of heavy metals in muscle, liver and gill of Sardina pilchardus (Walbaum, 1792): risk assessment for the consumers. Journal of environmental and occupationalScience 3, 47–52. ISSN 2146–8311.
Coelho, J.P., Santos, H., Reis, A.T., Falcão, J., Rodrigues, E.T., Pereira, M.E., Duarte, A.C., Pardal, M.A., 2010. Mercury bioaccumulation in the spotted dogfish (Scyliorhinus canicula) from the Atlantic Ocean. Mar. Pollut. Bull. 60 (2010), 1372–1375. https://doi.org/10.1016/j.marpolbul.2010.05.008.
Commission Regulation 1881, 2006. Setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union L364.
Commission Regulation 629, 2008. Amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union L176.
Costa, S., Afonso, C., Cardoso, C., Batista, I., Chaveiro, N., Nunes, M.L., Bandarra, N.M., 2015. Fatty acids, mercury, and methylmercury bioaccessibility in salmon (Salmo salar) using an in vitro model: effect of culinary treatment. Food Chem. 185, 268–276. https://doi.org/10.1016/j.foodchem.2015.03.141.
Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy, n.d.Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy (n.d.). Official Journal of the European Union L226, 1-17. Available at http://eur-lex.europa.eu/legalcontent/EN/TXT/PDF/?uri=CELEX:32013L0039&from=EN.
EFSA, 2012. Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. EFSA J 10, 2985. https://doi.org/10.2903/j.efsa.2012.2985. Available at.
EFSA, 2015. Statement on the benefits of fish/seafood consumption compared to the risks of methylmercury in fish/seafood. EFSA J 13, 3982. https://doi.org/10.2903/j.efsa.2015.3982. Available at.
FAO, 2008. Fishery Statistical Collections. Consumption of Fish and Fishery Products. Available at. http://faostat.fao.org.
FAO/WHO, 2011. Safety evaluation of certain food additives and contaminants. In: Methylmercury, 63. WHO Food Additives Series, p. 605684.
Fasano, E., Arnese, A., Esposito, F., Albano, L., Masucci, A., Capelli, C., Cirillo, T., Nardone, A., 2018. Evaluation of the impact of anthropogenic activities on arsenic, cadmium, chromium, mercury, lead, and polycyclic aromatic hydrocarbon levels in seafood from the Gulf of Naples, Italy. J. Environ. Sci. Health A 53, 786–792. https://doi.org/10.1080/10934529.2018.1445075.
Fernandes, C.P., 2017. The Consumption of Seafood in Portugal-Preferences, Knowledge and Public Perception. Available at. Mestrado em Ecologia e Gestão Ambiental, Universidade de Lisboa, Faculdade de Ciências, Departamento de Biologia Animal. http://hdl.handle.net/10451/30629.
Health Canada, 2007. Human Health Risk Assessment of Mercury in Fish and Health Benefits of Fish Consumption. Available at. http://hc-sc.gc.ca/fn-an/pubs/mercur/merc_fish_poisson_e.html.
Ikingura, J.R., Akagi, H., 2003. Total mercury and methylmercury levels in fish from hydroelectric reservoirs in Tanzania. Sci. Total Environ. 304, 355–368. https://doi.org/10.1016/S0048-9697(02)00581-8.
INE IP, 2018. 2017 Fishery Statistics Compendium. Statistics Portugal and Directorate General for Natural Resources, Safety and Maritime Services. Instituto Nacional de Estatística, I. P., Lisboa, Portugal.
IPCS, 1990. World Health Organization & International Programme on Chemical Safety. Methylmercury/published under the joint sponsorship of the United Nations Environment Programme, the International Labour Organisation, and the World Health Organization. Available at. https://apps.who.int/iris/handle/10665/38082.
Kalogeropoulos, N., Karavoltsos, S., Sakellari, A., Avramidou, S., Dassenakis, M., Scoullos, M., 2012. Heavy metals in raw, fried and grilled Mediterranean finfish and shellfish. Food Chem. Toxicol. 50, 3702–3708. https://doi.org/10.1016/j.fct.2012.07.012.
Kehrig, H.A., Costa, M., Moreira, I., Malm, O., 2002. Total and methylmercury in a Brazilian estuary, Rio de Janeiro. Mar. Pollut. Bull. 44, 1018–1023. https://doi.org/10.1016/S0025-326X(02)00140-6.
Kehrig, H.A., Seixas, T.G., Palermo, E.A., Baêta, A.P., Castelo-Branco, C.W., Malm, O., Moreira, I., 2009. The relationships between mercury and selenium in plankton and fish from a tropical food web. Environ. Sci. Pollut. Res. 16, 10–24. https://doi.org/10.1007/s11356-008-0038-8.
Kehrig, H.A., Seixas, T.G., Baêta, A.P., Malm, O., Moreira, I., 2010. Inorganic and methylmercury: do they transfer along a tropical coastal food web? Mar. Pollut. Bull. 60, 2350–2356. https://doi.org/10.1016/j.marpolbul.2010.08.010.
Kern, J.K., Geier, D.A., Sykes, L.K., Haley, B.E., Geier, M.R., 2016. The relationship between mercury and autism: a comprehensive review and discussion. J. Trace Elem. Med. Biol. 37, 8–24. https://doi.org/10.1016/j.jtemb.2016.06.002.
Lemes, M., Wang, F., 2009. Methylmercury speciation in fish muscle by HPLC-ICP-MS following enzymatic hydrolysis. J. Anal. At. Spectrom. 24, 663–668. https://doi.org/10.1039/b819957b.
Lescord, G.L., Johnston, T.A., Branfireun, B.A., Gunn, J.M., 2018. Percentage of methylmercury in the muscle tissue of freshwater fish varies with body size and age and among species. Environ. Toxicol. Chem. 37, 2682–2691. https://doi.org/10.1002/etc.4233.
Liao, W., Wang, G., Zhao, W., Zhang, M., Wu, Y., Liu, X., Li, K., 2019. Change in mercury speciation in seafood after cooking and gastrointestinal digestion. J. Hazard. Mater. 375, 130–137. https://doi.org/10.1016/j.jhazmat.2019.03.093.
Madsen, A.O., Chkoniya, V., 2021. Tendencies regarding fish consumption - the case of Portugal (Europe's leader & 3rd in the world). Eur. J. Interdiscip.Stud. 7, 12–35. https://doi.org/10.26417/ejis.v6i1.p33-50.
Maršálek, P., Svobodová, Z., Randák, T., 2006. Total mercury and methylmercury contamination in fish from various sites along the Elbe river. Acta Vet. Brno Czech Republic 75, 579–585. https://doi.org/10.2754/avb200675040579.
Matos, J., Lourenço, H.M., Brito, P., Maulvault, A.L., Martins, L.L., Afonso, C., 2015. Influence of bioaccessibility of total mercury, methylmercury and selenium on the risk/benefit associated to the consumption of raw and cooked blue shark (Prionace glauca). Environ. Res. 143, 123–129. https://doi.org/10.1016/j.envres.2015.09.015.
Mieiro, C.L., Coelho, J.P., Dolbeth, M., Pacheco, M., Duarte, A.C., Pardal, M.A., Pereira, M.E., 2016. Fish and mercury: influence of fish fillet culinary practices on human risk. Food Control 60, 575–581. https://doi.org/10.1016/j.foodcont.2015.09.006.
Miyamoto, K., Kuwana, T., Ando, T., Yamamoto, M., Nakano, A., 2010. Methylmercury analyses in biological materials by heating vaporization atomic absorption spectrometry. J. Toxicol. Sci. 35, 217–224. https://doi.org/10.2131/jts.35.217.
Naganuma, A., Furuchi, T., Miura, N., Hwang, G.W., Kuge, S., 2002. Investigation of intracellular factors involved in methylmercury toxicity. Tohoku J. Exp. Med. 196, 65–70. https://doi.org/10.1620/tjem.196.65.
Nunes, C., Silva, A., Soares, E., Ganias, K., 2011. The use of hepatic and somatic indices and histological information to characterize the reproductive dynamics of Atlantic sardine Sardina pilchardus from the Portuguese coast. Mar Coast Fish 3, 127–144. https://doi.org/10.1080/19425120.2011.556911.
Nunes, E., Cavaco, A., Carvalho, C., 2014. Exposure assessment of pregnant portuguese women to methylmercury through the ingestion of fish: cross-sectional survey and biomarker validation. J. Toxic. Environ. Health A 77 (1–3), 133–142. https://doi.org/10.1080/15287394.2014.867200.
Parran, D.K., Mundy, W.R., Barone Jr., S., 2001. Effects of methylmercury and mercuric chloride on differentiation and cell viability in PC12 cells. Toxicol. Sci. 59, 278–290. https://doi.org/10.1093/toxsci/59.2.278.
Perelló, G., Martí-Cid, R., Llobet, J.M., Domingo, J.L., 2008. Effects of various cooking processes on the concentrations of arsenic, cadmium, mercury, and lead in foods. J. Agric. Food Chem. 56, 11262–11269. https://doi.org/10.1021/jf802411q.
Pieniak, Z., Vanhonacker, F., Verbeke, W., 2013. Consumer knowledge and use of information about fish and aquaculture. Food Policy 40, 25–30. https://doi.org/10.1016/j.foodpol.2013.01.005.
Polak-Juszczak, L., 2017. Methylmercury in fish from the southern Baltic Sea and coastal lagoons as a function of species, size and region. Toxicol. Ind. Health 33, 503–511.
Polak-Juszczak, L., 2018. Distribution of organic and inorganic mercury in the tissues and organs of fish from the southern Baltic Sea. Environ. Sci. Pollut. Res. 25, 34181–34189. https://doi.org/10.1007/s11356-018-3336-9.
Ralstona, N.V.C., Raymond, L.J., 2018. Mercury's neurotoxicity is characterized by its disruption of selenium biochemistry. Biochim. Biophys. Acta Gen. Subj. 1862, 2405–2416. https://doi.org/10.1016/j.bbagen.2018.05.009.
Reyes, L.H., Rahman, G.M.M., Kingston, H.M.S., 2009. Robust microwave-assisted extraction protocol for determination of total mercury and methylmercury in fish tissues. Anal. Chim. Acta 631, 121–128. https://doi.org/10.1016/j.aca.2008.10.044.
Seixas, S., Bustamante, P., Pierce, G., 2005. Accumulation of mercury in the tissues of the common octopus Octopus vulgaris (L.) in two localities on the Portuguese coast. Sci. Total Environ. 340, 113–122. https://doi.org/10.1016/j.scitotenv.2004.08.012.
Sherwani, S.I., Pabon, S., Patel, R.B., Sayyid, M.M., Hagele, T., Kotha, S.R., Magalang, U. J., Maddipati, K.R., Parinandi, N.L., 2013. Eicosanoid signaling and vascular dysfunction: methylmercury-induced phospholipase D activation in vascular endothelial cells. Cell Biochem. Biophys. 67, 317–329. https://doi.org/10.1007/s12013-011-9304-3.
Shipley, O.N., Lee, C.S., Fisher, N.S., Burruss, G., Frisk, M.G., Brooks, E.J., Zuckerman, Z. C., Herrmann, A.D., Madigan, D.J., 2019. Trophodynamics and mercury bioaccumulation in reef and open-ocean fishes from the Bahamas with a focus on two teleost predators. Mar. Ecol. Prog. Ser. 608, 221–232. https://doi.org/10.3354/meps12798.
Teixeira, F.B., de Oliveira, A.C.A., Leão, L.K.R., Fagundes, N.C.F., Fernandes, R.M., Fernandes, L.M.P., da Silva, M.C.F., Amado, L.L., Sagica, F.E.S., de Oliveira, E.H.C., Crespo-Lopez, M.E., Maia, C.S.F., Lima, R.R., 2018. Exposure to inorganic mercury

causes oxidative stress, cell death, and functional deficits in the motor cortex. Front. Mol. Neurosci. 11, 125. https://doi.org/10.3389/fnmol.2018.00125.

US-EPA, 2001. Integrated Risk Information System (IRIS) Chemical Assessment Summary: Methylmercury (MeHg); CASRN 22967-92-6. U.S. U.S. Environmental Protection Agency, Washington, DC, NCEA-S-0930. Available at: http://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0073_summary.pdf.

Válega, M., Abreu, S., Pato, P., Rocha, L., Gomes, A.R., Pereira, M.E., Duarte, A.C., 2006. Determination of organic mercury in biota, plants and contaminated sediments using a thermal atomic absorption spectrometry technique. Water Air and Soil Pollution 174, 223–234. https://doi.org/10.1007/s11270-006-9100-7.

Vieira, C., Morais, S., Ramos, S., Delerue-Matos, C., Oliveira, M.B.P.P., 2011. Mercury, cadmium, lead and arsenic levels in three pelagic fish species from the Atlantic Ocean: intra- and inter-specific variability and human health risks for consumption. Food Chem. Toxicol. 49, 923–932. https://doi.org/10.1016/j.fct.2010.12.016.

Wang, L., Hou, D., Cao, Y., Ok, Y.S., Tack, F.M.G., Rinklebe, J., O'Connor, D., 2020. Remediation of mercury contaminated soil, water, and air: a review of emerging materials and innovative technologies. Environ. Int. 134, 105281 https://doi.org/10.1016/j.envint.2019.105281.

WHO-TRS 959, 2010. Seventy-second Report of the Joint FAO/WHO Expert Committee on Food Additives.

Yoshimoto, K., Van Anh, H.T., Yamamoto, A., Koriyama, C., Ishibashi, Y., Tabata, M., Nakano, A., Yamamoto, M., 2016. Simple analysis of total mercury and methylmercury in seafood using heating vaporization atomic absorption spectrometry. J. Toxicol. Sci. 41, 489–500. https://doi.org/10.2131/jts.41.489.