Oral bioaccessibility of arsenic, mercury and methylmercury in marine species commercialized in Catalonia (Spain) and health risks for the consumers

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1. Introduction

Diet is the most important route of exposure to environmental contaminants, such as toxic elements and persistent organic pollutants (Arnich et al., 2012; Bocio et al., 2005; Cheng et al., 2013; Jogsten et al., 2009; Martorell et al., 2012; Oguri et al., 2012; Perelló et al., 2014). Furthermore, fish and seafood have been identified as the foodstuffs with the highest contribution to the total dietary uptake of chemical contaminants by the general population (Baeyens et al., 2009; Domingo et al., 2012a; Llobet et al., 2003; Muñoz et al., 1999; Perelló et al., 2015a,b).

In 2000, we initiated an ambitious Total Diet Study (TDS) aimed at assessing the intake of a wide range of chemicals through food consumption by the population of Catalonia (Spain), as well as to evaluate the associated health risks for the consumers. After 4 sequential surveys, performed in 2000, 2005, 2008 and 2012 (Domingo et al., 2012b; Falcó et al., 2006; Llobet et al., 2003; Martí-Cid et al., 2008; Martorell et al., 2011; Perelló et al., 2014), the results of the TDS support that fish and seafood are the major contributors to the dietary exposure to As and Hg, as well as to cadmium (Cd) and lead (Pb). In a recent study, the concentrations of the As and Hg species of most concern (inorganic As and MeHg, respectively) were also quantified in foodstuffs, and their dietary exposure was calculated (Perelló et al., 2014).

Human exposure to chemicals is a core step in the process of risk assessment, being optimally performed through biomonitoring studies. However, and partially due to cost limitations, exposure is usually estimated by considering the ingestion of contaminants through food rather than by direct biomonitoring investigations. Dietary estimations are often derived from food consumption surveys and the chemical characterization of contaminant levels in a subsample of foodstuffs, combined through point estimates, or optimally by stochastic models (Kroes et al., 2002). Besides the limitations related with the uncertainty and population variability, this approach does not consider the behaviour of the contaminants once they enter the body, and more specifically, their fate through the gastrointestinal system (EFSA, 2006). Although the effect of gastrointestinal juices on toxic elements has been scarcely investigated, preliminary studies indicate that only a fraction of the ingested chemicals, known as bioaccessible fraction, could be finally absorbed (Calatayud et al., 2012; Maulvault et al., 2011). The remaining fraction may be embedded in the un-absorbable fraction and excreted or changed its chemical form through a speciation process (Versantvoort et al., 2005). To fill this gap, in recent years, *in vitro* gastrointestinal models with a varied complexity level, have been successfully developed and validated, offering a reproducible and economic approach (Maulvault et al., 2011; Cardoso et al., 2015).

Due to the lack of information regarding the bioaccessibility of toxic elements in fish and seafood, the purpose of this study was to characterize the bioaccessibility of As, Hg and MeHg in

the fish and seafood species most consumed in Catalonia (Spain). The potential implications of this parameter on risk assessment were also investigated by comparing dietary intake estimations with and without bioaccessibility factors.

2. Materials and methods

2.1. Samples

Fish and seafood species highly susceptible to accumulate toxic elements and being greatly consumed in Catalonia (Llobet et al., 2003; Martí-Cid et al., 2008; Martorell et al., 2011; Perelló et al., 2014), were selected. Fresh commercial samples were purchased in five different supermarkets and retail shops from Tarragona and Reus (Catalonia). The species included in the study were the following: swordfish (*Xiphias gladius*), tuna (*Thunnus thynnus*), mackerel (*Scomber scombrus*), sardine (*Sardina pilchardus*), seabream (*Sparus aurata*), monkfish (*Lophius piscatorius*), common sole (*Solea solea*), mussel (*Mytilus galloprovincialis*), prawn (*Penaeus kerathurus*), and cuttlefish (*Sepia* spp.). The little specimens were eviscerated and grilled entirely with minor processing, while the larger specimens, such as tuna, were purchased as fillets and grilled without further processing. Grilling was selected because it is one of the most extended cooking procedures for fish and seafood, exhibiting minor interferences by contact with oil or water (Perelló et al., 2008b). Unlike other species, mussels were steamed in a Teflon® saucepot with ultrapure water for 10 min at 100ºC, without addition of salt. Fish and seafood samples were grilled (without addition of salt) using a Teflon® grilling pan for 10-15 min, until the internal temperature reached 100ºC. The temperature was controlled using a non-contact infrared temperature gun digital thermometer. Edible portions of grilled fish were pooled, mixed, and homogenised in composites of equivalent proportion, irrespectively of their origin.

2.2. Chemicals for the in vitro digestion

The chemical composition of artificial digestive juices was based on the protocol described by Maulvault et al. (2011). For artificial saliva, 900 mg of KCl (Sigma, Ref. 746436), 20 mg of KSCN (Sigma, Ref. P2713), 89 mg of NaH_2PO_4 (Sigma, Ref. S8282), 58 g Na_2SO_4 (Sigma, Ref. 239313), 30 mg of NaCl (Sigma, Ref. S7653), 170 mg of $NaHCO_3$ (Sigma, Ref. S5761), 20 mg of urea (Sigma, Ref. U5128), 20 mg of α-amylase (Sigma, Ref. 86250), 1.5 mg of uric acid (Sigma, Ref. U2625), and 2.5 mg of mucin (Sigma, Ref. M2378) were diluted to 100 mL with ultrapure water, being the pH adjusted to 6.8 using 0.2M NaOH.

Artificial gastric juice was prepared by diluting with 100 mL of ultrapure water, 250 mg of NaCl, 27 mg NaH_2PO_4, 82 mg KCl, 31 mg of NH_4Cl (Sigma, Ref. A9434), 250 mg of pepsin (Sigma, Ref. P7125), 65 mg of glucose (Sigma, Ref. D9434), 2 mg of glucuronic acid (Sigma, Ref. G5269), 8.4 mg of urea, 33 mg of glucosamine hydrochloride (Sigma, Ref. PHR1199) and 100 mg of bovine serum albumin (BSA) (Sigma, Ref. A7906), adjusting the pH to 1.3 by using 1M HCl.

Finally, artificial duodenal juice was prepared by diluting 700 mg of NaCl, 339 mg of $NaHCO_3$, 8 mg of KH_2PO_4 (Sigma, Ref. P5655), 56 mg KCl, 100 mg of urea, 100 mg of BSA, 900 mg of pancreatin (Sigma, Ref. P7545), and 150 mg of lipase (Sigma, Ref. L3126) in 100 mL of ultrapure water, and adjusting the pH to 8.1 by using 0.2M NaOH. Artificial bile was prepared by diluting 526 mg of NaCl, 579 mg $NaHCO_3$, 38 mg KCl, 25 mg of urea, 180 mg of BSA and 3 g of bile salt extract in 100 mL of ultrapure water, and adjusting the pH at 8.2 by adding 1M HCl.

2.3. In vitro digestion model

A static *in vitro* digestion model based on the 3 main gastrointestinal compartments (mouth, stomach and small intestine) was here applied (Maulvault et al., 2011). Briefly, 5 g of cooked sample (in triplicate) were mixed with 5 mL of artificial saliva for 5 min. Then, 12 mL of gastric juice were added and stirred for 2 h. Afterwards, 12 mL of duodenal juice were added and stirred for 5 min, prior to add 5 mL of bile juice, being the final extract digested for 2 h. The whole process was carried out in a rotating water bath (60 rpm; Memmert W1314, Germany) at 37 ºC. By the end of the whole process, the digestion was stopped by lowering the temperature down to 4ºC, and the bioaccessible fraction (i.e. supernatant) was separated from the non-digested fraction (i.e. pellet) by centrifuging at 10,000g for 10 min at 4ºC (Sigma 3k30, Postfach, Germany).

2.4. Quantification of total As

Each sample (~0.5 g) was treated with 5 mL of HNO_3 (65% Suprapur, Merck, Darmstadt, Germany) in hermetic Teflon vessels. Samples were kept for 8 h at room temperature, being subsequently heated at 80°C for 8 h. After cooling, solutions were filtered and made up to 25 mL with ultrapure water. The concentrations of As were determined by inductively coupled plasma-mass spectrometry (ICP-MS, Perkin-Elmer Elan 6000). The accuracy of the instrumental method and analytical procedure was checked by assessing the levels of As in duplicate per sample, as well as by using reference materials for trace elements (TORT-2 Lobster hepatopancreas, NRC, Canada). Rhodium was used as internal standard. Replicate measurements were performed. Uncertainty sources were identified and quantified according to the ISO 17025:2005 norm. The

limit of detection (LOD) for total As was 0.10 mg/kg, and the mean recovery percentage was 91% (Table 1).

2.5. Quantification of total Hg and MeHg

Mercury (total and MeHg) concentrations in fish and seafood samples were quantified by atomic absorption spectrometry, using an automatic Hg analyser (AMA 254, Leco, St. Joseph, MI, USA). Briefly, for total Hg determination, 10-20 mg of solid sample or 100-200 µL of liquid sample were placed on a sample boat of the automatic analyser. After their drying and combustion, samples undergo to amalgamation at 700 °C. The elemental mercury (Hg^0) was pre-concentrated, released and detected at a wavelength of 254 nm. Mercury concentrations were calculated from linear calibration with a Hg (II) nitrate standard solution (1000 mg/L, Merck, Darmstadt, Germany) diluted in nitric acid (0.5 mol/L, Merck) at concentrations ranging between 0.10 and 40 ng of Hg. For the quantification of organic Hg (MeHg), seafood samples (~150 mg freeze-dried solid sample or 150 µL of liquid sample) were hydrolyzed in hydrobromic acid (10 mL, 47% w/w, Merck), followed by MeHg extraction with toluene (35 mL, 99.8% w/w, Merck). They were then removed from toluene using an aqueous solution of cysteine (1% L-cysteinium chloride in 12.5% anhydrous sodium sulfate and 0.775% sodium acetate) (Scerbo and Barghigiani, 1998), whose extracts were analysed in the automatic Hg analyser. Duplicate measurements of the analytes were performed, being blank samples also analysed in the same conditions as the samples. The LOD for both total Hg and MeHg was 5 µg/kg. Similarly to As, the method accuracy was checked through the analysis of a certified reference material (Table 1).

2.6. Exposure assessment and risk characterization

The dietary exposure of the Catalan population to the toxic elements here examined through the consumption of each species was estimated by using the following equation:

$$E_{ft} = C_f (X_{ft} B_{ft}) / BW$$

Where E_{ft} is the dietary exposure to the toxic element *t* in the general population through the fish or seafood species *f* (µg/kg body weight (bw)/day), C_f is the mean normalized consumption of the fish or seafood species *f* by the population (g/day), X_{ft} is the concentration of the toxic element *t* in the fish or seafood species *f* (µg/g), B_f is the bioaccessibility of the toxic element *t* in the fish or seafood species *f* (decimal basis), and BW is the body weight for the adult population (kg).

The concentrations of As, Hg and MeHg were determined in both cooked and digested samples, being oral bioaccessibility (in percentage) estimated by comparing the concentration in

the bioaccessible fraction with the amount found in the cooked sample prior to the *in vitro* digestion.

Consumption data were obtained from ENIDE, the most recent food consumption database in Spain (AECOSAN, 2011). ENIDE was conducted in men and women aged between 18 and 65 years. The methodology used 24 hours-recall, daily food records during 3 random days, as well as a food frequency questionnaire. Exposure levels were normalized by the body weight of 70 kg, widely implemented as a mean weight of European adults (EFSA, 2012a).

Regarding As, risk assessment was focused only on inorganic arsenic (InAs), which is the most toxic form. Concentrations of InAs were estimated by considering percentages of InAs vs. total As, recently reported for the same fish and seafood species (Perelló et al., 2015b). Since no data were available for seabream and monkfish, the InAs content was assumed to be 2% of total As according to estimations from the FAO/WHO (1993). For a suitable comparison with safety reference levels, exposure of Hg and MeHg was calculated on a weekly basis, while that of As was estimated on a daily basis.

Health risks of InAs intake were evaluated by comparison of the exposure estimates with the reference levels from the European Food Safety Authority's Panel on Contaminants in the Food Chain (EFSA-CONTAM Panel), which re-assessed the provisional tolerable weekly intake (PTWI) of 15 μg/kg bw, previously established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The expert panel established an overall range of 95% lower confidence limit of the benchmark dose of 1% extra risk ($BMDL_{01}$) values of 0.3 to 8 μg/kg bw per day. EFSA (2009) concluded that this range should be used instead of a single reference point in the risk characterisation for InAs.

The EFSA-CONTAM panel also reassessed the JECFA provisional tolerable weekly intakes (PTWI) for MeHg and Hg. Regarding Hg, a tolerable weekly intake (TWI) of 4 μg/kg bw was recommended for the inorganic species, established on the basis of a $BMDL_{10}$ of 0.06 mg/kg bw per day, and considering an uncertainty factor of 100 to account for inter- and intra-species differences. In turn, a TWI for MeHg of 1.3 μg/kg bw was established based on the mean of the apparent non-observed effect level (NOEL) from the Seychelles nutrition cohort at 9 and 30 months (11 mg/kg maternal hair) and the $BMDL_{05}$ from the Faroese (Portugal) Cohort 1 at the age of seven (12 mg/kg in maternal hair) (EFSA, 2012b).

2.7. Statistics

One-way non-parametric analysis of variance (Kruskal-Wallis test) and multiple comparison tests were performed to compare the concentrations of the analysed elements between fish and

seafood species. Probability levels lower than 0.05 ($p<0.05$) were considered as statistically significant. Data treatment was performed by means of the software GraphPad Prism v.6.03.

3. Results and discussion

3.1. Concentrations of As, Hg and MeHg in commercial marine species

The concentrations of As, Hg and MeHg in cooked and digested samples of fish and seafood are summarized in Table 2. Sole showed the highest levels of As (6.40 mg/kg), followed by mussels and monkfish (2.85 and 2.60 mg/kg, respectively). The lowest concentrations were found in prawn (0.82 mg/kg), while the remaining species presented values ranging between 1.03 and 1.78 mg/kg. Martorell et al. (2011) reported similar As levels in swordfish (0.1 mg/kg), mackerel (1.1 mg/kg), sole (5.5 mg/kg), mussels (1 mg/kg), and prawn (3.5 mg/kg), also purchased in Catalonia. In contrast, the current element concentrations in swordfish, sardine, prawn, and cuttlefish were lower than those found by Perelló et al. (2014) also in the Catalan market (1.47 vs. 2.2, 1.78 vs. 3.8, 0.82 vs. 4.7, and 1.30 vs. 4.7 mg/kg, respectively). It is important to note that in both previous studies (Martorell et al., 2011; Perelló et al., 2014), fish and seafood was not cooked, while in the current study the levels of As were quantified in cooked samples. It is well known that cooking can affect the levels of toxic and essential elements in fish and seafood (Maulvault et al., 2012a,b). Thus, Perelló et al. (2008b) observed a clear tendency to increase metal concentrations in fish species (sardine, hake, and tuna) after cooking. Therefore, the effect of cooking may justify the differences found in As concentrations in the studied fish and seafood species (Domingo et al., 2011).

The highest levels of Hg were found in swordfish and tuna (0.866 and 0.185 mg/kg, respectively), being in agreement with the high concentrations of this element in these two species reported in the scientific literature (Storelli et al., 2010; Burger and Gochfeld, 2013). Monkfish, mackerel, seabream, sole and sardine showed moderate values (i.e. between 0.014 and 0.029 mg/kg). Mussels, cuttlefish and prawn had the lowest Hg levels (0.004-0.006 mg/kg). Comparing with previous surveys, current values are somehow lower. Martorell et al. (2011) reported higher Hg concentrations in raw samples of fish and seafood, namely swordfish, tuna, mackerel, mussels, and prawn (0.869, 0.554, 0.053, 0.016, 0.255, and 0.009 mg/kg, respectively). In turn, the results reported by the same authors in sardine and sole (0.019 and 0.029 mg/kg, respectively) were similar to those found in the present study (0.014 and 0.021 mg/kg, respectively). However, MeHg levels were not assessed by Martorell et al. (2011), being MeHg concentrations calculated according to estimated percentages with respect to total Hg. Similarly, Perelló et al. (2014) also found higher levels of Hg in raw seafood samples, which was likely due to the effect of cooking

(Domingo et al., 2011; Maulvault et al., 2012a,b). Methylmercury was detected in all samples, but with a different distribution pattern. The highest concentrations of MeHg were found in swordfish and tuna (0.623 and 0.142 mg/kg, respectively), while the highest percentage of MeHg over the total was found in prawn (84%). As abovementioned, swordfish and tuna generally present higher levels of MeHg. In a recent study, the concentrations of these toxic species in commercial samples of swordfish and tuna were experimentally found to be 1.1 and 0.52 mg/kg, respectively (Perelló et al., 2014). While the high levels of Hg and MeHg in the largest fish species are caused by a well-known process of bioaccumulation, there also exists an intra- and interspecies variability on the MeHg-to-Hg ratio, which is not completely understood yet. However, those species located in the upper stages of the trophic chain trend to have higher MeHg-to-total Hg ratios, with high variation among tissue levels (Forsyth et al., 2004; Lasorsa and Allen-Gil, 1995).

3.2. Bioaccessibility of As, Hg and MeHg in commercial marine species

The bioaccessible fraction of As was estimated in the range between 72% (mackerel) and 89% (sardine). These values fall well within the ranges of As bioaccessibility previously reported for a number of marine species (83-99%) (Table 3). The effect of cooking procedures seems to be a critical factor affecting the As bioaccessibility (Domingo et al., 2011). According to Maulvault et al. (2011), non-water methods (grilling and frying) seem not to affect the bioaccessibility of this element in brown crab and black scabbard, whereas boiling or steaming significantly decrease the amount of bioaccessible As.

With respect to Hg, the bioaccessibility in cooked fish and seafood was lower than that of As. However, there was a larger variation among species. Despite the high bioaccessibility of Hg that was found in cuttlefish and monkfish (77% and 61%, respectively), most species showed values below 50%. Cooked mussel and sardine showed the lowest bioaccessibility levels, being only 17% for both species. The amount of bioaccessible Hg for cooked tuna (35%) was higher than that reported by Torres-Escribano et al. (2011), being in the range of 6-10%. In contrast, they were in agreement with those reported by Afonso et al. (2015), who estimated a release of 49% in grilled tuna. The effect of temperature on protein conformation, reducing the enzymatic cleavage activity, seems to be the most plausible explanation for this process (Torres-Escribano et al., 2011).

The low levels of MeHg in most samples did not allow the quantification of this element in the digested fractions of most fish and seafood species. It would be due not only to analytical detection limits, but also to dilution factors involved in bioaccessibility assessment. Hence, MeHg was only detected and quantified in swordfish and tuna, showing their highest levels in cooked

samples. The fraction released to the gastrointestinal juice after the *in vitro* digestion accounted 42% and 57%, in tuna and swordfish, respectively. These levels are lower than those reported by Cabañero et al. (2007), who found a release ranging 92-93% in raw samples. The current results are also in agreement with those of Afonso et al. (2015), who estimated a recovery of 44% for MeHg in digested grilled tuna. Cooking methods seem to play a relevant role in Hg and MeHg bioaccessibility. The same authors reported a large difference of Hg released when comparing raw and cooked/canned tuna, decreasing its bioaccessibility from 78% (raw) to 44% (grilled) (Afonso et al., 2015). Denaturation of proteins was pointed out as a plausible reason of this marked reduction. Similar findings were also reported by Costa et al. (2015), who elucidated a reduction of bioaccessible fraction from raw (89.9%) to grilled (32.2%) salmon. Cooking might reduce the susceptibility of denatured proteins to be hydrolysed by proteases, strongly diminishing the release of Hg into the digested fraction.

The comparison of results obtained in different studies is rather difficult due to the variability of methodological approaches (Table 3). While most studies are based on a static approach, advisable for screening purposes, only Leufroy et al. (2012) implemented a dynamic model, which consisted of sequential leaching of samples by artificial saliva, gastric juice, and intestinal juice. Some authors use a bi-compartmental model (stomach and small intestine), while others add the mouth compartment, with different composition of artificial juices and duration of digestion. Anyhow, the most relevant difference is the use of raw or cooked samples on the digestions, as well as the different cooking procedures.

3.3. The role of bioaccessibility on the health risks of exposure to As, Hg and MeHg

In addition to the limitations of dietary estimates approaches related with the uncertainty and variability of exposure models, cooking procedures affect notably the final concentrations of not only nutrients, but also those of food contaminants. Relevant effects from grilling or roasting, among other cooking methods, have been noted (Domingo et al., 2011). On the other hand, little is known about the potential effects of the gastrointestinal events on the release and speciation of chemical contaminants from food matrices. The integration of bioaccessibility on risk assessment models has been previously emphasized to provide refinements on human dietary estimates (Marques et al., 2011). For comparison purposes, the exposure to InAs (Table 4), as well as to Hg and MeHg (Table 5) through fish and seafood consumption by the Catalan population was here calculated by comparing the model with and without bioaccessibility estimations.

With respect to InAs, the dietary intake for average consumers ranged between 0.058 and 2.424 ng/kg bw/day through consumption of cuttlefish and mussels, respectively. The highest consumption estimates (P99) provided intakes of InAs ranging 0.015-5.130 ng/kg bw/day. When

introducing the bioaccessibility outcomes in the exposure assessment model, a relevant reduction of these estimated values was noted, being the highest values 2.085 and 4.412 ng/kg bw/day, for average and high consumers of mussels, respectively. Although the sum of the highest exposure values, calculated by summing the P99 estimates from all species (14.872 ng/kg bw/day), is an improbable scenario, it is far below the $BMDL_{01}$ range of 300-8000 ng/kg bw/day established by the EFSA (2009). These estimates are in agreement with previous findings (Martorell et al., 2011; Perelló et al., 2014) and with those estimated by the CONTAM panel of EFSA (370-1220 ng/kg bw/day for European high consumers) (EFSA 2009).

In contrast to As, a clear overestimation of Hg and MeHg exposure was observed when considering bioaccessibility percentages. According to the current data, the highest mean exposure of Hg and MeHg corresponded to swordfish (0.171 and 0.123 μg/kg bw/week, respectively), being followed by tuna (0.0.30 and 0.023 μg/kg bw/week, respectively). As abovementioned, these were the only two fish and seafood species in which MeHg was quantified. Exposure to both Hg and MeHg were notably reduced when considering their bioaccessibility. In all cases, the estimated intakes for the mean consumers were below the respective TWI proposed by the EFSA for inorganic Hg and MeHg. However, P99 estimations might exceed these safety levels in swordfish, depending on the use of the bioaccessibility estimates. These findings elucidate the complexity related with the apparently simple equation implemented on dietary exposure assessment, supporting the urgent need for refinements addressing those chemicals of most concern.

It is important to remark that this approach has several limitations, mainly related with the gastrointestinal model, which could be improved using a dynamic approach and validated with *in vivo* data. Moreover, the study could be extended to more complex and realistic food matrices (i.e. combining fish and seafood with other food items such as cereals, vegetables, fruits, etc.) that are ingested together with fish and seafood and go through the gastrointestinal tract during the digestion process. Another issue that was not addressed in the present study is the bioaccessibility during different human life stages, as well as the physiological conditions that may modify the composition, concentration and duration of the different gastrointestinal digestion processes.

4. Conclusions

To the best of our knowledge, this is the very first study aimed at providing evidences on the bioaccessibility of As, Hg and MeHg in marine species of high consumption in Catalonia. This investigation elucidates the potential overestimation of health risks for the consumers, when the effects of bioaccessibility and cooking procedures are considered in risk assessment. Unlike As, for which an overestimation of risk was not found, Hg and MeHg showed a lower and variable

bioaccessibility in fish and seafood species. It means an obvious overestimation of health risks for the adult population. Further studies should be focused on the role of the bioaccessibility of mercurial species for children, the most sensitive population subgroup.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Table 1

Detection limits (DLs) and concentrations of As, Hg and MeHg in certified reference materials.

Element	DL	Certified reference material	Certified	Obtained	n	RSD (%)	Rec. (%)
As	0.1 mg/kg wet weight	Lobster hepatopancreas (TORT-2)	21.6 ± 1.8	19.44 ± 0.40	6	5	91
Hg	5 µg /kg dry weight	Dogfish muscle (DORM-2)	4.64 ± 0.26	4.71 ± 0.10	4	2	102
MeHg	5 µg /kg dry weight	Dogfish muscle (DORM-2)	4.47 ± 0.32	4.19 ± 0.21	4	5	94

Data are given as mean ± standard deviation. RSD: relative standard deviation. Rec.: recovery

Table 2

Concentrations of As, Hg and MeHg (mg/kg of wet weight) and bioaccessibility measured in 10 fish and seafood species.

	Moisture	As			Hg			MeHg			
	%	Cooked	Bioaccessible	Bioaccess (%)	Cooked	Bioaccessible	Bioaccess (%)	Cooked	% Total Hg	Bioaccessible	Bioaccess (%)
Swordfish	64	1.47±0.26[a]	1.21±0.04	83±3	0.866±0.011[a]	0.393±0.002	45±1	0.623±0.107[a]	72	0.354±0.031	57±2
Tuna	58	1.03±0.00[c]	0.79±0.03	76±2	0.185±0.008 [c]	0.064±0.008	35±3	0.142±0.019[c]	77	0.060±0.008	42±5
Mackerel	65	1.59±0.19[a]	1.14±0.05	72±3	0.020±0.001[b]	0.005±0.005	26±7	0.014±0.001[d]	68	<DL	-
Sardine	69	1.78±0.05[a]	1.59±0.13	89±7	0.014±0.000[a]	0.002±0.000	17±10	0.010±0.001[b]	70	<DL	-
Seabream	65	1.48±0.04[a]	1.23±0.11	83±7	0.029±0.001 [b]	0.011±0.002	38±3	0.022±0.001[e]	74	<DL	-
Monkfish	79	2.60±0.08[d]	2.18±0.08	84±3	0.016±0.000[b]	0.010±0.007	61±10	0.012±0.000[bd]	76	<DL	-
Sole	69	6.40±0.18[e]	4.90±0.27	76±4	0.021±0.001[b]	0.010±0.002	50±6	0.015±0.001[d]	72	<DL	-
Mussels	77	2.85±0.04[f]	2.45±0.15	86±5	0.006±0.001[bd]	0.001±0.002	17±6	0.003±0.002[f]	51	<DL	-
Prawn	69	0.82±0.07[b]	0.69±0.12	84±1	0.005±0.000[d]	0.001±0.000	21±2	0.004±0.000 [f]	84	<DL	-
Cuttlefish	80	1.30±0.08[a]	1.03±0.09	79±7	0.004±0.001[d]	0.003±0.003	77±6	0.003±0.002[f]	68	<DL	-

DL: Detection limit.
Different superscripts indicate significant differences between species (Kruskall-Wallis test, $p<0.05$)

Table 3

Bioaccessibility of As, Hg and MeHg in marine species reported in the scientific literature.

Fish species	Country	As (%)	Hg (%)	MeHg (%)	*In vitro* digestion	Reference
Butter clams (Raw)	Canada	108	50	Not detected	Static (Stomach, small intestine)	Laird and Chan, 2013
Chinook salmon (Raw)		57	49			
Sockeye salmon (Raw)		68	46			
Mussel [a]	France	84-65	NA	NA	Dynamic (Mouth, stomach, small intestine)	Leufroy et al., 2012
Shrimp [a]		72-67				
Salmon [a]		53-75				
Tuna [a]		81				
Canned tuna [a]		65				
Tuna (Raw)	Portugal	NA	78	78	Static (Mouth, stomach, small intestine)	Afonso et al., 2015
Tuna (Grilled)			39	44		
Tuna (Canned)			18-20	18-29		
Salmon (Raw)	Portugal	NA	89.9	Not detected	Static (Mouth, stomach, small intestine)	Costa et al., 2015
Salmon (Grilled)			32.2			
Black scabbard (Raw/cooked)	Portugal	NA	45	NA	Static (Mouth, stomach, small intestine)	Maulvault et al., 2011
Crab brown meat (Raw/cooked)		88-94	NA			
Anchovy (Raw)	Spain	77-86	NA	NA	Static (Stomach, small intestine), coupled with cell uptake assay	Calatayud et al., 2012
Hake (Raw)		62-68				
Sardine (Raw)		35-50				
Sole (Raw)		67				
Swordfish (Raw)		42-66				
Cuttlefish (Raw)		63-65				
Mussel (Raw)		38-69				
Swordfish (Raw)	Spain	NA	59-87	NA	Static (Stomach, small intestine)	Torres-Escribano et al., 2011
Tope shark (Raw)			43-69			
Bonito (Raw)			17-23			
Tuna (Raw)			13-19			
Swordfish (Cooked)			35-49			
Tope shark (Cooked)			34-47			
Bonito (Cooked)			12-17			
Tuna (Cooked)			6-10			
Tuna (Raw)	Spain	NA	NA	92	Static Stomach, small intestine)	Cabañero et al., 2007
Swordfish (Raw)				93		
Sardine (Raw)				92		

[a] Freeze-dried samples. NA: Not analysed.

Table 4

Daily exposure of the adult Catalan population to InAs through fish and seafood, with and without bioaccessibility estimations.

Species	Daily intake[a] (g/day)		Levels of As[b] (µg/g)	Percentage of InAs vs As total	Levels of InAs (µg/g)	BioAc percentage	EDI of InAs (ng/kg bw/day)		EDI of InAs accounting BioAc (ng/kg bw/day)	
	Mean	P99					Mean	P99	Mean	P99
Swordfish	1.97	65	1.47	0.42	0.006	83	0.174	0.573	0.144	0.476
Tuna	1.62	50	1.03	0.46	0.005	76	0.110	0.338	0.083	0.257
Mackerel	0.50	8.33	1.59	1.99	0.032	72	0.226	0.377	0.163	0.271
Sardine	3.58	100	1.78	1.02	0.018	89	0.929	2.594	0.826	2.308
Seabream	2.89	100	1.48	2.00	0.030	83	1.222	4.229	1.014	3.510
Monkfish	1.45	50	2.60	2.00	0.052	84	1.077	3.714	0.905	3.120
Sole	4.32	100	6.40	0.03	0.002	76	0.118	0.274	0.090	0.208
Mussels	3.15	66.7	2.85	1.89	0.054	86	2.424	5.130	2.085	4.412
Prawn	5.07	72.1	0.82	0.42	0.003	84	0.249	0.355	0.210	0.298
Cuttlefish	7.85	19.7	1.30	0.04	0.001	79	0.058	0.015	0.046	0.012

[a]ENIDE study (AECOSAN, 2011).
[b]Levels in cooked fish and seafood.
[c]Assumptions according to FAO/WHO (1993).
As: arsenic; InAs: inorganic arsenic; BioAc; bioaccessibility; EDI: estimated daily intake; P99: 99th percentile.
PTWI: 15 µg/kg bw (or 2.14 µg/kg bw/day); $BMDL_{01}$: 0.3-8 µg/kg bw/day

Table 5

Weekly exposure of the adult Catalan population to Hg and MeHg through fish and seafood, with and without bioaccessibility estimations.

	Daily Intake[a] (g/day)		Levels of Hg[b] (μg/g)	BioAc (%)	EDI of Hg (μg/kg bw/week)		EDI of Hg accounting BioAc (μg/kg bw/week)	
Species	**Mean**	**P99**			**Mean**	**P99**	**Mean**	**P99**
Swordfish	1.97	65	0.866	45	0.171	5.63	0.077	2.53
Tuna	1.62	50	0.185	35	0.030	0.925	0.010	0.324
Mackerel	0.50	8.33	0.020	26	0.001	0.017	0.000	0.004
Sardine	3.58	100	0.014	17	0.005	0.140	0.001	0.024
Seabream	2.89	100	0.029	38	0.008	0.290	0.003	0.110
Monkfish	1.45	50	0.016	61	0.002	0.080	0.001	0.049
Sole	4.32	100	0.021	50	0.009	0.210	0.005	0.105
Mussels	3.15	66.7	0.006	17	0.002	0.040	0.000	0.007
Prawn	5.07	72.1	0.005	21	0.003	0.036	0.001	0.008
Cuttlefish	7.85	19.7	0.004	77	0.003	0.008	0.002	0.006
	Daily Intake[a] (g/day)		**Levels of MeHg[b] (μg/g)**	**BioAc (%)**	**EDI of MeHg (μg/kg bw/week)**		**EDi of MeHg accounting BioAc (μg/kg bw/week)**	
Species	**Mean**	**P99**			**Mean**	**P99**	**Mean**	**P99**
Swordfish	1.97	65	0.623	57	0.123	4.05	0.070	2.31
Tuna	1.62	50	0.142	42	0.023	0.710	0.010	0.298

[a]ENIDE study (AECOSAN, 2011).

[b]Levels in cooked fish and seafood.

Hg: total mercury; MeHg: methylmercury; BioAc; bioaccessibility; EDI: estimated daily intake; P99: 99th percentile.

TWI: for inorganic Hg: 4 μg/kg bw/week; for MeHg: 1.3 μg/kg bw/week.