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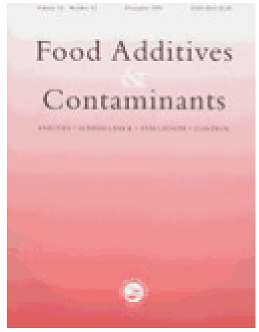

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Mercury and methylmercury bioaccessibility in swordfish

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Running head: Mercury bioaccessibility in swordfish

ABSTRACT

Concentrations of mercury (Hg) in swordfish (*Xiphias gladius*) present a food safety problem for many countries. This study analyzes total Hg concentrations in 27 samples of swordfish marketed in Spain in 2005 and in their bioaccessible fractions (soluble concentration in gastrointestinal medium), obtained after applying an *in vitro* digestion method. Methylmercury (MeHg) was also determined in the bioaccessible fractions. Total Hg concentrations in the samples were 0.41–2.11 mg kg^{-1} wet weight (ww), with a mean value of 0.96 ± 0.47 mg kg^{-1} ww. 37% of the samples exceeded the Hg limit set by Spanish legislation (1.0 mg kg^{-1} ww). Bioaccessible total Hg concentrations were 0.17–1.72 mg kg^{-1} ww (0.63 ± 0.4 mg kg^{-1} ww), corresponding to 38–83% (64 ± 14%) of total Hg. Bioaccessible MeHg concentrations, representing 94% of the bioaccessible total Hg concentrations, were 0.16–1.53 mg kg^{-1} ww, with a mean value of 0.49 ± 0.32

mg kg^{-1} ww. Children and adults who regularly consume this product in Spain have Hg and MeHg intakes that exceed the Tolerable Daily Intake limits recommended by the FAO/WHO and USEPA. These results show the need for recommendations about swordfish consumption by population groups at risk in Spain.

Keywords: *mercury, methylmercury, bioaccessibility, swordfish, intake, risk assessment*

INTRODUCTION

Consumption of fish offers an excellent source of protein and essential nutrients such as iodine and selenium. Also, some species of oily fish provide significant amounts of long-chain, polyunsaturated omega w-3 fatty acids, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), which help to reduce the risk of cardiovascular disease (Fussenegger et al., 2007). However, fish can also be a source of substances harmful to the body, as they contain polychlorinated biphenyls, dioxins, and methylmercury (MeHg) (Virtanen et al., 2007).

It is currently considered that consumption of fish is the main path for human exposure to mercury (Hg) (EFSA, 2004). In predatory marine fish, about 90% of mercury is in the methylated form (methylmercury, MeHg) (WHO, 2008), while the remainder consists of small or undetectable quantities of inorganic mercury [Hg(II)], ethylmercury, and phenylmercury (Branco et al., 2007; Chang et al., 2007). The MeHg/Hg ratio is lower in freshwater fish (WHO, 2008). Methylmercury compounds are considered by the International Agency for Research on Cancer as possibly carcinogenic to humans, Group 2B (IARC, 1993). Research has shown that MeHg produces adverse neurological effects such as mental retardation, seizures, vision and hearing loss, delayed development, language disorders, and memory loss (WHO, 2007). Prenatal mercury exposure may produce alterations affecting children's neurodevelopment (Gao et al., 2007; Jedrychowski et al., 2007).

Predatory fish such as swordfish, shark, and tuna have the highest concentrations of MeHg. Therefore, the European Food Safety Agency (EFSA), the Food and Drug Administration (FDA), and the Environmental Protection Agency (EPA) have advised vulnerable population groups (women who may become pregnant, pregnant women, nursing mothers, and young children) to avoid some types of fish that might accumulate high levels of MeHg, such as large predators, and eat fish and shellfish that are lower in mercury (EFSA, 2004; FDA, 2004). Hg and MeHg have been quantified in various species of fish (Mira and Lanfer-Marquez, 2005; Morgano et al., 2005; Burger and Gochfeld, 2006; Sivaperumal et al., 2006; Afonso et al., 2007; Cortes and Fortt., 2007); the influence of the physiological characteristics of the fish (sex, age) on the Hg concentrations has been evaluated (Monteiro and Lopes, 1990; Afonso et al., 2007; Storelli et al., 2007), and intakes of Hg have been calculated for child and adult populations (Llobet et al., 2003; Wilhelm, 2003; Muñoz et al., 2005; Falcó et al., 2006; Farias et al., 2006; Marti-Cid et al., 2007; Sahuquillo et al., 2007; Rubio et al., 2008).

To make a more realistic evaluation of the toxicological risk involved in intake of Hg from consumption of fish, it is necessary to estimate not only the concentration in the product as consumed but also its bioavailability (the fraction of the intake that is absorbed and reaches the systemic circulation and is thus available to exercise its effect on the receiving organism). For an element to be absorbed by the intestinal epithelium, the first requisite is that it should be soluble. It is therefore interesting to know the bioaccessible content, i.e., the maximum soluble content in simulated gastrointestinal media that is available for subsequent processes of absorption into the intestinal mucosa. The term bioaccessibility also indicates the relation between the bioaccessible content of a substance and the total content of the substance present in the sample. Bioaccessibility can be used as an indicator of maximum oral bioavailability (Versantvoort et al., 2005). Static and dynamic *in vitro* gastrointestinal models can be used to determine bioaccessibility of nutrients. The static models simulate transit through the human digestive tract by sequential exposure to simulated mouth, gastric, and small intestinal conditions (pH, temperature, time, enzymes, etc.) (Oomen et al., 2002), and they are the ones that have been most used in studying the bioaccessibility of trace elements from food samples (Intawongse and Dean, 2006).

Very little research has been done on Hg bioaccessibility (Cabañero et al., 2004, 2007; Shim et al., 2009). It is therefore of great interest to investigate how consideration of the bioaccessible content of total Hg (t-Hg) and MeHg instead of the content in the raw product affects exposure assessments. The aims of the present work were a) to quantify concentrations of t-Hg in raw swordfish and concentrations of t-Hg and MeHg in the bioaccessible fraction obtained after an *in vitro* gastrointestinal digestion method and b) to make risk assessments by estimating daily intake of Hg and MeHg resulting from consumption of this top predator.

MATERIALS AND METHODS

Equipment. A microwave accelerated reaction system (MARS) from CEM (Vertex, Spain) operating at a power of 800 W was used for digestion of samples prior to quantification of t-Hg. Teflon perfluoroalkoxy (PFA) vessels of 55 ml inner volume were employed. For t-Hg quantification, a continuous flow cold vapor generationatomic fluorescence spectrometer (CV-AFS) model PSA 10.025, Millennium Merlin (PS Analytical, UK), was used.

For Hg speciation analysis, the HPLC system employed (Hewlett Packard Model 1100, Spain) was equipped with a quaternary pump, an on-line degassing system, an automatic injector and a thermostated column compartment. Separations were performed on a Hamilton PRP-X100 anion-exchange column (10 µm, 250 mm × 4.1 mm i.d., Teknokroma, Barcelona, Spain). A guard column packed with the same stationary phase (12–20 µm; 25 mm × 2.3 mm i.d.) preceded the analytical column. A heated bath (Julabo model HC, Merck, Spain) was used to thermooxidize the outlet from the HPLC column. After thermooxidation the Hg content was quantified by CV-AFS. An analog–digital converter (Model 35 900 C, Hewlett Packard) was used to acquire the AFS signal, which was processed by the chromatographic software.

Other equipment used included a lyophilizer (FTS Systems, USA), a mechanical shaker (KS 125 Basic, IKA Labortechnik, Merck, Spain), a pH meter (pH 526, Multical, Spain), an ultrasonic bath (J.P. Selecta, Spain), a heating bath (HC Julabo, Merck, Spain), an orbital shaking water bath (Unitronic Orbital C, J. P. Selecta, Spain), and various centrifuges (Superspeed refrigerated centrifuge RC-5B Instrument, Du Sorvall, Sorvall Pont; Eppendorf 5810, Merck; Heraeus Biofuge Pico, Merck).

Reagents. In the determination of t-Hg and MeHg, deionized water (18.2 MΩ cm), obtained with a Milli-Q water system (Millipore Inc., Millipore Ibérica, Madrid, Spain), was used for the preparation of reagents and standards. All glassware was treated with 10% v/v HNO_3 for 24 h, and then rinsed three times with deionized water before use.

All chemicals used were of analytical or reagent grade. A standard solution of 1000 mg L^{-1} Hg (Merck) was employed. A standard solution of 1000 mg L^{-1} MeHg was prepared by dissolving a commercially available salt of MeHgCl (Sigma-Aldrich) in 50% (v/v) MeOH/H_2O mixture. Working standard solutions of Hg and MeHg were prepared daily by serial dilutions of standard solution.

Other reagents used were: hydrochloric acid (Merck); nitric acid (Merck); acetic acid (Probus); sulfuric acid (Panreac); sodium hydroxide (Merck); hydrogen peroxide (Prolabo); L-Cysteine (Merck); tin(II) chloride dihydrate (Scharlau Chemie S.A, Spain); sodium hydrogen carbonate (Panreac), and potassium peroxodisulfate (Prolabo).

Enzymes and bile salts were purchased from Sigma Chemical Co. (St. Louis, MO, USA): porcine pepsin (enzymatic activity 944 U/mg protein), porcine pancreatin (activity equivalent to 4 x US Pharmacopoeia specifications/mg pancreatin), and bile extract (glycine and taurine conjugates of hyodeoxycholic and other bile salts). Water of cellular grade (B. Braun Medical, S.A., Barcelona, Spain) was used throughout the *in vitro* digestion assay.

Samples. In Spain, consumption of frozen swordfish is much higher than consumption of fresh swordfish (94% as opposed to 6% in 2003, according to data provided by the Ministry of the Environment and Rural and Marine Environment's General Secretariat of the Sea). Reflecting the habits of the Spanish population, we analyzed 27 samples of frozen swordfish muscle. The swordfish muscle samples were provided in 2005 by companies that market this product in Spain. The samples were thawed, the skin was removed, and the edible portions were frozen, lyophilized, ground, and stored at 4 ºC until analysis. A reference material with certified contents for t-Hg and MeHg, DORM-2 sample (muscle of dogfish, National Research Council of Canada), was also analyzed. Each sample was analyzed in triplicate, for both t-Hg and MeHg and their respective bioaccessibilities.

***In vitro* gastrointestinal digestion.** Samples of seafood products were digested using a simulated digestion process developed by our group in an earlier study (Laparra et al., 2003). A quantity of lyophilized swordfish sample, equivalent to 10 g of fresh sample, was weighed and cellular-grade water (90 mL) was added. The pH was adjusted to 2.0 with 6 mol L^{-1} HCl. After 5 min, the pH value was checked and if necessary readjusted to pH 2.0. Freshly prepared pepsin solution (1 g of pepsin in 10 mL of 0.1 mol L^{-1} HCl) was added to provide 0.01 g of pepsin/10 g fresh sample. The sample was made up to 100 g with water, and incubated in a shaking water bath (stroke rate 120 min^{-1}) at 37 ºC for 2 h to emulate the gastric stage of digestion.

Then, for the intestinal digestion, the pH value was raised to pH 5.0 by drop-wise addition of 1 mol L^{-1} $NaHCO_3$. The pancreatin–bile extract mixture (0.2 g of pancreatin and 1.25 g of bile extract in 50 mL of 0.1 mol L^{-1} $NaHCO_3$) was added to provide 0.0025 g of pancreatin/10 g fresh sample, and 0.015 g of bile extract/10 g fresh sample.

The incubation at 37 ºC continued for 2 hours. The pH was then adjusted to 7.2 by addition of 0.5 mol L^{-1} NaOH. Aliquots of 40 g of the digests were transferred to polypropylene centrifuge tubes and centrifuged (15000 rpm/30 min/4 ºC) to separate soluble and precipitate. The concentrations of t-Hg and MeHg were quantified in the soluble fraction.

Total mercury determination. An assisted digestion in microwave oven with subsequent quantification by CV-AFS was used for the determination of t-Hg in the samples of swordfish and in their bioaccessible fractions.

Lyophilized swordfish samples (0.2 g) or bioaccessible fractions (0.6 g) were placed in a Teflon PFA vessel and treated with 4 mL of HNO_3 concentrate (14 N) and 1 mL of H_2O_2. The Teflon PFA vessel was irradiated at 800 W (180 ºC, 15 min). At the end of the digestion program, the digest was placed in a 250 mL beaker and allowed to rest all night to eliminate nitrous vapor. It was then filtered through Whatman No. 1 paper and made up to volume with 5% HCl (v/v).

Mercury contents were determined by CV-AFS using the following analytical conditions: reducing agent, 2% (m/v) $SnCl_2$ in 15% (v/v) HCl, 4.5 mL min^{-1} flow rate; blank reagent, 5% HCl (v/v), 9 mL min^{-1} flow rate; carrier gas, argon 0.3 L min^{-1} flow rate; dryer gas, air 2.5 L min^{-1} flow rate.

Throughout the experiment, the quality assurance-quality control of Hg measurement was checked by analyzing the certified reference material DORM-2 with each batch of samples.

Methylmercury determination. For extraction of mercury species an ultrasonic acid extraction was employed**.** The lyophilized swordfish samples (0.2 g) or bioaccessible fractions (0.6 g) were weighed into a 50 mL centrifuge tube and 8 mL of 2.4 N HCl was added. The mixture was sonicated for 5 min, centrifuged (2000 rpm/15 min), and the supernatant was centrifuged again (12000 rpm/10 min). The resulting extract was filtered through 0.45 µm Whatman Nylon prior to species quantification by HPLC-thermooxidation-CV-AFS.

Samples were injected into the PRP-X100 column and, using PTFE tubing and T-joints, the eluate from the column was mixed with the persulfate solution. The mixture was thermooxidized by being passed through a loop of Teflon tubing placed in a heated bath. After cooling in an ice bath, the eluate was mixed with a continuous flow of $SnCl_2$. Using a gas-liquid separator and a continuous flow of argon, the arsines generated were introduced into the AFS. The hygroscopic-membrane drying tube used to transport the arsines allowed elimination of moisture by circulating a counterflow of air. Details of operating conditions are given in Table 1.

Signals were identified by coincidence of sample and standard retention times. The quantification was obtained from the peak area by interpolation of external calibration curves. Throughout the experiment, the quality assurance-quality control of MeHg measurement was checked by analyzing the certified reference material DORM-2 with each batch of samples.

RESULTS AND DISCUSSION

Total mercury in swordfish. The concentrations of t-Hg in the swordfish samples analyzed (Table 2) ranged between 0.41 and 2.1 mg kg^{-1} ww (mean ± standard deviation = 0.96 ± 0.47 mg kg^{-1} ww; n = 27). The analysis of Hg in seafood products marketed in Spain is a customary practice in Spanish food control laboratories, but few data are published in scientific journals. The range of concentrations obtained overlaps the few values reported in the journals for samples of swordfish marketed in Spain: 0.4–2.2 mg kg^{-1} ww (Cabañero et al., 2004; Falcó et al., 2006; Blanco et al., 2008). Furthermore, the values found in the present work are similar to those reported in the last five years for swordfish of various origins (Table 3). Only in samples acquired in Taiwan much higher mean concentrations have been found (3.6 ± 0.5 mg kg^{-1} ww) (Chen et al., 2007).

Some authors have found a linear correlation between the concentrations of Hg and the length and age of the fish, as most of the Hg is in the form of MeHg bound to the thiol groups of proteins, which increase with age (Monteiro and Lopes, 1990; Storelli and Marcotrigliano, 2001; Branco et al., 2004; Branco et al., 2007). A correlation has also been found between the concentration of Hg in the muscle and the area where the fish was caught (Branco et al., 2007). The size of the liver of predatory fish is another physiological variable that researchers have attempted to correlate with Hg concentrations. However, further studies must be conducted in this regard as the results differ depending on the species of fish considered. Concentrations have been found in the liver of swordfish and bluefin that are between 2 and 3 times greater than the levels in muscle (Storelli et al., 2005; Branco et al., 2007), attaining 9.8 µg g^{-1} ww in the liver of swordfish from the area of Ecuador (Branco et al., 2007). In samples of shark, however, Hg concentrations in the liver have been shown to be less than those of muscle (Branco et al., 2007).

With regard to the food safety of the samples analyzed, 37% exceed the value of 1 µg g^{-1} ww, the maximum limit of Hg permitted by Spanish legislation in swordfish (European Commission, 2006). It is important to emphasize that food alerts concerning high Hg concentrations in fish have increased in Spain and other countries in recent years. This is shown in a report of the Rapid Alert System for Food and Feed of UE (RASFF, 2009), which gives details of the notification of 128 alerts concerning mercury in fish, 56% of which were for swordfish. Of the samples that exceeded the legislated values, 34% were of Spanish origin, which demonstrates the need for the application of rigorous health control measures for these products.

Total Hg and MeHg bioaccessible in swordfish. The swordfish samples were subjected to a gastrointestinal digestion and t-Hg and MeHg were determined in the bioaccessible fraction. As the bioaccessible fraction is a very different matrix from the raw seafood, it was necessary to evaluate the suitability of the application of the methods for t-Hg and MeHg quantification developed previously in the laboratory.

For this purpose, we evaluated the analytical characteristics (detection limit, precision, and recovery) of the t-Hg method, which includes *in vitro* digestion, microwave acid digestion of the soluble fraction, and quantification by CV-AFS. The results obtained endorse the suitability of the method for the aims proposed, and the absence of matrix interference: limit of detection = 0.02 ng g^{-1}, ww; precision = 10%; recovery = 98%. The quantification of MeHg in the bioaccessible fraction entails a more complex treatment, because after the *in vitro* gastrointestinal digestion it is necessary to lyophilize the soluble fractions and then perform an ultrasonic acid extraction of MeHg. The analytical characteristics of the method endorse its use for the aims proposed: limit of detection = 0.11 ng g^{-1}, ww; precision = 5%; recovery = 87–104%.

Bioaccessible t-Hg. The concentrations of bioaccessible t-Hg (Table 2) varied over a wide range: 0.17–1.72 mg kg^{-1} ww (mean value = 0.63 ± 0.4 mg kg^{-1} ww). In the study that was conducted, there was not a proportional relationship between the Hg concentration in the samples and their gastrointestinal solubility. This can be seen in various samples, including samples 17 and 22, where the t-Hg concentrations in the samples are almost identical (1.18 and 1.20 mg kg^{-1} ww, respectively) but the bioaccessible concentrations are very different (0.48 and 0.84 mg kg^{-1} ww, respectively). The bioaccessibility, the percentage relating the bioaccessible Hg concentration to the initial Hg concentration in the sample, varied between 38% and 83%, with a mean value of 64 ± 14%. Given the suitable analytical characteristics of the method used for quantifying t-Hg in the bioaccessible fraction, the wide range of the bioaccessibility values found cannot be attributed to problems connected with the analytical method used. They might be the result of freezing rates, thawing conditions and storage temperature on protein denaturation. All this might affect the ability of the enzymes used in the gastrointestinal method to solubilize Hg from proteins in the swordfish. The samples were purchased in small retail outlets, where products might be stored in different time and temperature conditions.

To our knowledge, the present study contributes the largest number of data in the scientific literature on the bioaccessibility of t-Hg in swordfish. Previously, Shim et al. (2009) obtained bioaccessibility values for king mackerel (68%), similar to the values presented here for swordfish. However, Cabañero et al. (2004) indicated much lower bioaccessibility values in three kinds of seafood products: 17% in swordfish, 13% in sardine, and 9% in tuna. The authors attributed the low bioaccessibility to the low capacity of the enzymes in the *in vitro* gastrointestinal method to release mercury complexed with Se (Cabañero et al., 2007). This hypothesis concerning the effect of the Se-Hg relationship on bioaccessibility needs to be corroborated in further studies.

Bioaccessible MeHg. The bioaccessible MeHg concentration was determined in 15 of the swordfish samples. The values ranged between 0.16 and 1.53 mg kg^{-1} ww, with a mean value of 0.49 ± 0.32 mg kg^{-1} ww (Figure 1). The MeHg in the bioaccessible fraction represents between 71% and 105% of the t-Hg in the solubilized fraction (mean value = 88 ± 11%).

We are not aware of the existence of previous data of bioaccessible MeHg concentrations. There are only the reports by Cabañero et al. (2004, 2007) concerning the relation between Se and MeHg in the bioaccessible fractions of various species of fish. These data, which are expressed as [Se/Hg] bioaccessible molar ratios, show values ranging between 9.3 for swordfish and 126.3 for sardine, without providing data of bioaccessible concentrations for MeHg.

If risk managers have an interest to consider bioaccessibility in establishing maximum limits of Hg and MeHg in foods, they should make an effort to increase the database and obtain a worse-case estimate of this parameter.

Evaluation of toxicological risk. The toxicological risk associated with the intake of t-Hg and MeHg from consumption of the samples analyzed can be evaluated by using the guideline values recommended by international organizations. The FAO/WHO recommends a Provisional Tolerable Weekly Intake (PTWI) for Hg of less than 5 µg/kg body weight/week, of which MeHg should not be more than 1.6 µg/kg body weight/week (WHO, 2003). The recommendation of the U.S. Environmental Protection Agency (USEPA) is much more restrictive, proposing a maximum MeHg intake of 0.1 µg/kg body weight/day (USEPA, 2001).

Consumption of seafood products in Spain (44.5 kg/person/year) is among the highest in Europe, exceeded only by Portugal (Laurenti, 2007). The study by the Ministry of Agriculture, Fisheries, and Food, reports that consumption of fish in the period between 1987 and 2003 was 102 g/person/day (MAPA, 2006).

There are very few studies of the consumption of individual foods in Spain. The National Study of Nutrition and Diet (Varela et al., 1995) reports a mean consumption of swordfish by adults of 0.35 g/day, with substantial variations between different autonomous communities, ranging from areas where swordfish is not consumed to the areas of highest consumption, Murcia and the Valencian Community (1.06 g/day and 1.17 g/day, respectively). More recently, a study by the Nuclear Safety Council (CSN, 2002) provided fresh data concerning the mean consumption of swordfish for the entire population of children (0.69 g/day) and adults (1.53 g/day), taking into account surveyees who consume swordfish and those who do not. This study also provides data concerning the real consumers of the product, who are only a small percentage of the total surveyed: 1.3% of the children and 2.5% of the adults. For this population of consumers, the mean consumption is quite high, attaining 51.7 g/day in the children and 60.3 g/day in the adults. The study also reports intake values for high consumers: 112.4 g/day for children and 102.3 g/day for adults.

Estimation of Hg intake from contents in raw swordfish. To estimate the intake of t-Hg by the Spanish population (Table 4), we used the consumption data provided by the CSN, and the minimum, mean, and maximum t-Hg concentrations found in the samples analyzed. The results obtained were compared with the reference value recommended by the WHO, 5 µg/kg body weight/week, equivalent to a Tolerable Daily Intake of 0.71 µg Hg/kg body weight. On the basis of the mean consumption of the total child population (0.69 g/day), the daily intake would attain a maximum of 6% of the TDI, which does not indicate the existence of a risk. If the estimate is based only on the intake of consumers of the product, however, the situation is very different. For the mean consumption by children (51.67 g/day), the intake of t-Hg is less than the TDI (85% of the TDI) if the lowest concentration of t-Hg found in swordfish is assumed, but greater than the TDI for the mean and maximum concentrations of Hg in swordfish. The situation is aggravated for children that are high consumers (112.37 g/day), whose consumption is much greater than the TDI: between 1.9 and 9.5 times. Adults face a similar situation: assuming the mean consumption of the entire population (1.53 g/day), intake is 7% of the TDI at most; for consumers of the product, the TDI is exceeded in the cases of mean consumption (60.26 g/day) and high consumption (102.34 g/day) of swordfish with mean or maximum concentrations of t-Hg.

Estimation of MeHg intake from contents in raw swordfish. To determine the intake of MeHg (Table 5), we assumed that all of the Hg in raw swordfish is present as MeHg, providing a protectionist estimate for the consumer. This is also the general approach recently recommended by the WHO for exposure assessments of MeHg in fish (WHO, 2008). The MeHg intake values calculated on this basis were compared with the reference value recommended by the WHO for MeHg, (Tolerable Daily Intake = 0.23 µg MeHg/kg body weight). When the total population surveyed is considered, neither the mean consumption by children (0.69 g/day) nor the mean consumption by adults (1.53 g/day) lead to intake values that exceed the TDI. However, if only the real consumers of the product are considered, the mean consumption both by children (51.67 g/day) and by adults (60.26 g/day) leads to MeHg intakes exceeding the TDI; between 2.7 and 13.6 times the TDI in the case of children, and between 1.6 and 7.9 times the TDI in the case of adults. The most worrying situation is that of high consumers, both children and adults, whose intake of MeHg is 29.6 and 13.5 times greater than the recommended value, respectively. If the estimated intakes are compared with the MeHg reference value established by the USEPA (0.1 µg/kg body weight/day), 2.3 times less than the WHO value, the exposure situation is even worse.

Recommended safe intake. The results presented indicate that there is a small percentage of the child and adult population in Spain, corresponding to consumers of swordfish, that is at risk as a result of exposure to Hg from this product. This is the case even when the swordfish has a concentration of Hg below the maximum limit established by the legislation (1 mg/kg ww). This should lead the authorities to make recommendations of maximum consumption of this product by children and adults. In view of the neurotoxic nature of MeHg and its transmission through the placenta and breast milk, some countries have adopted recommendations for groups at risk: women who may become pregnant, pregnant women, nursing mothers, and young children. This is the case in the UK, where the FSA recommends that babies and pregnant women should not consume swordfish and that breastfeeding women should not consume more than one portion per week (FSA, 2008). Another example is Canada, where Health Canada recommends a maximum consumption of 150 g of swordfish per month for certain women (those that are or may become pregnant or are breastfeeding), 125 g per month for children 5–11 years old, and 75 g per month for children 1–4 years old (Health Canada, 2008). In Spain, there are no recommendations in this regard. As Table 6 shows, even if all the Hg quantified in the fish analyzed were in the form of MeHg, the weekly consumption of 25 g swordfish/week for Spanish children and 50 g/week for adults would give MeHg intakes lower than the PTWI recommended by the WHO. Even if the concentration of Hg in the fish (0.958 mg kg^{-1} ww) were very close to the maximum value permitted by the legislation (1 mg kg^{-1} ww), consumption of these quantities would not represent a health risk. Recommendations for the Spanish population concerning monthly consumption could be set at values very close to those stipulated in the UK and Canada.

Estimation of MeHg intake from bioaccessible contents. It would be interesting to study whether taking bioavailability into account would alter the risk associated with consumption of this product. Assuming the quantities of bioaccessible MeHg found in the present study (0.164–1.53 mg kg^{-1} ww; mean = 0.495) (Fig. 1), the mean estimated intake for children (51.7 g of swordfish/day) ranges between 8 and 79 µg MeHg/day (mean value = 32 µg MeHg/day), exceeding the TDI values recommended by the WHO by 1–10 times (mean = 3.2 times the TDI). For adult consumers of swordfish (60.3 g/day according to the study by the CSN mentioned earlier) the estimated intakes of MeHg range between 10 and 92 µg MeHg/day (mean value = 38 µg MeHg/day), values that represent from 63% of the TDI to 6 times the TDI (mean = 1.9 times the TDI). When the value established by USEPA is used as the toxicological reference value, the limit is exceeded for both children and adults and over the entire range of bioaccessible MeHg concentrations found in the fish analyzed.

These results show that there would still be a situation of risk if bioaccessible MeHg concentrations were considered instead of using the concentrations in the product, although the TDIs are 2.5 times lower than those obtained from the contents in raw swordfish. If MeHg bioaccessibility were considered, therefore, perhaps the recommendations concerning consumption of swordfish could be modified by increasing the quantity consumed or the frequency of consumption.

The results presented show the need for recommendations about swordfish consumption by population groups at risk in Spain, and the desirability of broadening the study of the bioaccessibility of MeHg from these products.

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Figure 1. Bioaccessible total mercury (t-Hg) and bioaccessible methylmercury (MeHg) (expressed as Hg) in the soluble fractions of 15 swordfish samples (mg kg^{-1}, wet weight). For each sample values are expressed as mean ± standard deviation (n = 3).

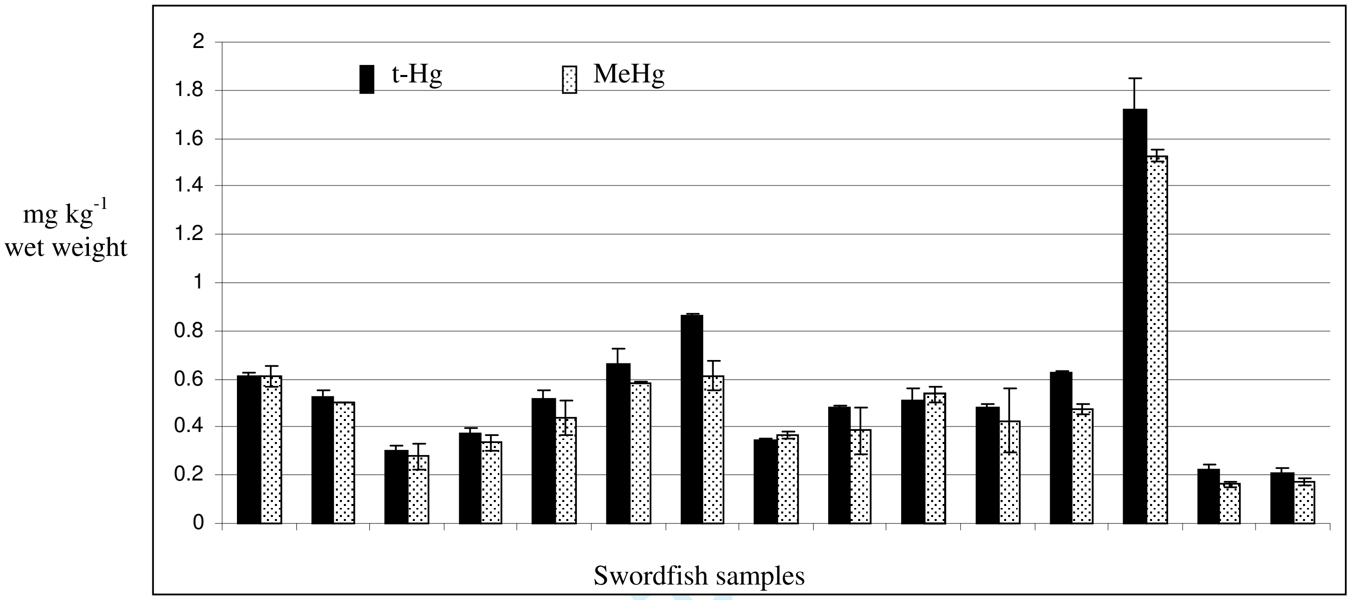

Table 1. Instrumental parameters used for the determination of methylmercury by HPLC-thermooxidation-CV-AFS

HPLC	
Column	Anionic exchange Hamilton PRP X-100; polymer base; 10 µm; 250 mm x 4.1 mm i.d.
Precolumn	Hamilton PRP X-100; 12-20 µm; 25 mm x 2.3 mm i.d.
Mobile phase	0.04 mol L^{-1} cysteine in 0.1 mol L^{-1} acetic acid
Injection volume	100 µL
Flow	1 mL min^{-1}
Thermooxidation	
Oxidant	1% (m/v) $K_2S_2O_8$ in 0.5% (mol L^{-1}) H_2SO_4. Flow 2 mL min^{-1}
Reaction loop	3 m x 0.5 mm i.d.
Bath temperature	150 ºC
CV-AFS	
Reducing agent	2% (m/v) $SnCl_2$ in 15% (v/v) HCl. Flow 2 mL min^{-1}
Carrier gas	Argon. Flow 300 mL min^{-1}
Dryer gas	Air. Flow 2.5 mL min^{-1}
Amplification range	100
Filter	32
Wavelength	**254 nm**

Table 2. Total mercury (t-Hg) concentrations in swordfish samples (mg kg^{-1}, wet weight), bioaccessible t-Hg (mg kg^{-1}, wet weight), and t-Hg bioaccessibility. **Values are expressed as mean ± standard deviation (n = 3).**

Sample	t-Hg	Bioaccessible t-Hg	Bioaccessibility [a]
1	0.440 ± 0.006	0.166 ± 0.015	38
2	0.826 ± 0.027	0.608 ± 0.017	74
3	0.787 ± 0.051	0.299 ± 0.022	38
4	0.779 ± 0.043	0.522 ± 0.030	67
5	0.605 ± 0.007	0.301 ± 0.025	50
6	0.705 ± 0.073	0.542 ± 0.025	77
7	0.748 ± 0.071	0.371 ± 0.022	50
8	0.650 ± 0.023	0.517 ± 0.039	80
9	0.647 ± 0.016	0.466 ± 0.039	72
10	1.05 ± 0.060	0.665 ± 0.065	63
11	1.41 ± 0.01	0.862 ± 0.007	61
12	1.13 ± 0.04	0.622 ± 0.016	55
13	0.439 ± 0.011	0.348 ± 0.006	79
14	0.629 ± 0.002	0.479 ± 0.010	76
15	0.880 ± 0.021	0.514 ± 0.045	58
16	0.939 ± 0.049	0.716 ± 0.010	76
17	1.18 ± 0.04	0.482 ± 0.018	41
18	0.687 ± 0.012	0.486 ± 0.045	71
19	0.782 ± 0.016	0.502 ± 0.006	64
20	1.02 ± 0.09	0.624 ± 0.007	61
21	1.25 ± 0.12	0.928 ± 0.014	74
22	1.20 ± 0.04	0.844 ± 0.004	70
23	2.11 ± 0.18	1.41 ± 0.08	67
24	2.00 ± 0.01	1.66 ± 0.13	83
25	2.08 ± 0.01	1.72 ± 0.13	83
26	0.488 ± 0.031	0.226 ± 0.021	46
27	0.413 ± 0.024	0.206 ± 0.027	50

[a] Bioaccessibility = (t-Hg in bioaccessible fraction of swordfish) x 100

(t-Hg in swordfish)

Table 3. Summary of total Hg levels (mg kg^{-1}, wet weight) in swordfish reported in the literature since 2004.

Origin (specified in article)	t-Hg (mg kg^{-1}, wet weight) min	max	mean	n	Reference
Spain	–	–	0.42 ± 0.01	1	Cabañero et al., 2004
Canada (seafood outlets)	0.40	3.85	1.82	10	Forsyth et al., 2004
Mediterranean Sea	0.02	0.15	0.07 ± 0.04	58	Storelli et al., 2005
Supermarkets in USA	0.15	3.07	1.40 ± 0.18	18	Burger and Gochfeld, 2006
Spain	–	–	1.59-2.22	Composite of 20 samples	Falcó et al., 2006
Réunion Island	–	–	1.24 ± 0.83	7	Kojadinovic et al., 2006
Mozambique Channel	–	–	0.38 ± 0.26	37	Kojadinovic et al., 2006
Azores (Atlantic Ocean)	0.031	2.4	–	29	Branco et al., 2007
Ecuador (Atlantic	0.9	2.3	–	23	Branco et al., 2007
Indian Ocean	0.26	2.54	1.47 ± 0.63	171	Chen et al., 2007
Atlantic Ocean	0.06	3.97	1.20 ± 1.12	55	Chen et al., 2007
Acquired in Taiwan	–	–	3.64 ± 0.15	1	Chang et al., 2007
Chilean markets	1.25	1.7	1.53	6	Cortes and Fortt, 2007
Spain	-	1.74	0.68	24	Blanco et al., 2008
Spain	0.413	2.11	0.958 ± 0.475	27	This work

Table 4. Estimated daily intake of total mercury from Spanish consumption of the swordfish samples analyzed and comparison with the Provisional Tolerable Weekly Intake recommended by the WHO [a].

			Hg content in swordfish [b]		
			Minimum $0.413\ mg\ kg^{-1}$ ww	Mean $0.958\ mg\ kg^{-1}$ ww	Maximum $2.11\ mg\ kg^{-1}$ ww
			Intake		
Children	**Total population**				
	Mean consumption [c]	0.69 g/day	1.14% of TDI	3% of TDI	6% of TDI
	Consumers only				
	Mean consumption [c]	51.67 g/day	85% of TDI	2 times the TDI	4.4 times the TDI
	High consumption [c]	112.37 g/day	1.9 times the TDI	4.3 times the TDI	9.5 times the TDI
Adults	**Total population**				
	Mean consumption [c]	1.53 g/day	0.9% of TDI	3% of TDI	7% of TDI
	Consumers only				
	Mean consumption [c]	60.26 g/day	51% of TDI	1.2 times the TDI	2.6 times the TDI
	High consumption [c]	102.34 g/day	86% of TDI	2 times the TDI	4.4 times the TDI

[a] WHO recommendation for Hg = 5 µg Hg/kg body weight/week (WHO, 2003). For Spanish children 7–12 years old, mean body weight is 34.48 kg; for this weight the TDI recommended by the WHO is 25 µg/day. For Spanish adults, over 17 years old, mean body weight is 68.48 kg; for this weight the TDI recommended by the WHO is 49 µg/day.

[b] Minimum, mean, and maximum concentrations of mercury for all the swordfish samples analyzed in this study (Table 2).

[c] Consumption of swordfish by the Spanish population (CSN, 2002).

Table 5. Estimated daily intake of methylmercury from Spanish consumption of the swordfish samples analyzed and comparison with the Provisional Tolerable Weekly Intake recommended by the WHO[a].

			Hg content in swordfish [b]		
			Minimum 0.413 mg kg^{-1} ww	Mean 0.958 mg kg^{-1} ww	Maximum 2.11 mg kg^{-1} ww
			Intake		
Children	**Total population**				
	Mean consumption [c]	0.69 g/day	4% of TDI	8% of TDI	18% of TDI
	Consumers only				
	Mean consumption [c]	51.67 g/day	2.7 times the TDI	6.2 times the TDI	13.6 times the TDI
	High consumption [c]	112.37 g/day	5.8 times the TDI	13.5 times the TDI	29.6 times the TDI
Adults	**Total population**				
	Mean consumption [c]	1.53 g/day	4% of TDI	9% of TDI	20% of TDI
	Consumers only				
	Mean consumption [c]	60.26 g/day	1.6 times the TDI	3.6 times the TDI	7.9 times the TDI
	High consumption [c]	102.34 g/day	2.6 times the TDI	6.1 times the TDI	13.5 times the TDI

[a] WHO recommendation for MeHg = 1.6 µg MeHg/kg body weight/week (WHO, 2003). For Spanish children 7–12 years old, mean body weight is 34.48 kg and the WHO TDI recommendation is 8 µg/day. For Spanish adults, over 17 years old, mean body weight is 68.48 kg and the WHO TDI recommendation is 16 µg/day.

[b] Minimum, mean, and maximum concentrations of mercury for all the swordfish samples analyzed in this study (Table 2). It is assumed that all of the Hg in raw swordfish is present as MeHg.

[c] Consumption of swordfish by the Spanish population (CSN, 2002).

Table 6. Weekly consumption of swordfish by Spanish children and adults that would give intakes of methylmercury below the Provisional Tolerable Weekly Intake value recommended by the WHO [a].

		Hg content in swordfish [b]			
		Minimum 0.413 mg kg^{-1} ww	Mean 0.958 mg kg^{-1} ww	Maximum 2.11 mg kg^{-1} ww	
	Consumption	**MeHg Intake[c]**			**MeHg PTWI[a]**
Children	25 g/week	10 µg/week	24 µg/week	53 µg/week	55 µg/week
Adults	50 g/week	21 µg/week	48 µg/week	106 µg/week	110 µg/week

[a] WHO recommendation for MeHg = 1.6 µg MeHg/kg body weight/week (WHO, 2003). For Spanish children 7–12 years old, mean body weight is 34.48 kg and the WHO PTWI recommendation is 55 µg/week. For Spanish adults, over 17 years old, mean body weight is 68.48 kg and the WHO PTWI recommendation is 110 µg/day.

[b] Minimum, mean, and maximum concentrations of mercury for all the swordfish samples analyzed in this study (Table 2).

[b] Intakes calculated on the basis that all the Hg is present as MeHg.