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Dinoraz Velez, Silvia Torres-Escribano, Rosa Montoro

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1 Mercury and methylmercury bioaccessibility in swordfish

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10 **Silvia Torres-Escribano, Dinoraz Vélez*, Rosa Montoro**

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14 Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC). Apdo 73, 46100,
15
16 Burjassot, Valencia, Spain.

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21 * To whom correspondence should be addressed (telephone (+34) 963 900 022; fax
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23 (+34) 963 636 301; e-mail: deni@iata.csic.es).
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29 **Running head:** Mercury bioaccessibility in swordfish

30 31 32 33 **ABSTRACT**

34
35 Concentrations of mercury (Hg) in swordfish (*Xiphias gladius*) present a food safety
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37 problem for many countries. This study analyzes total Hg concentrations in 27 samples
38
39 of swordfish marketed in Spain in 2005 and in their bioaccessible fractions (soluble
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41 concentration in gastrointestinal medium), obtained after applying an *in vitro* digestion
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43 method. Methylmercury (MeHg) was also determined in the bioaccessible fractions.
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45 Total Hg concentrations in the samples were 0.41–2.11 mg kg⁻¹ wet weight (ww), with a
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47 mean value of 0.96 ± 0.47 mg kg⁻¹ ww. 37% of the samples exceeded the Hg limit set
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49 by Spanish legislation (1.0 mg kg⁻¹ ww). Bioaccessible total Hg concentrations were
50
51 0.17–1.72 mg kg⁻¹ ww (0.63 ± 0.4 mg kg⁻¹ ww), corresponding to 38–83% (64 ± 14%)
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53 of total Hg. Bioaccessible MeHg concentrations, representing 94% of the bioaccessible
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55 total Hg concentrations, were 0.16–1.53 mg kg⁻¹ ww, with a mean value of 0.49 ± 0.32
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3 26 mg kg⁻¹ ww. Children and adults who regularly consume this product in Spain have Hg
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5 27 and MeHg intakes that exceed the Tolerable Daily Intake limits recommended by the
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8 28 FAO/WHO and USEPA. These results show the need for recommendations about
9
10 29 swordfish consumption by population groups at risk in Spain.
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15 31 **Keywords:** *mercury, methylmercury, bioaccessibility, swordfish, intake, risk*
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17 32 *assessment*
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25 35 INTRODUCTION

26
27 36 Consumption of fish offers an excellent source of protein and essential nutrients such as
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29 37 iodine and selenium. Also, some species of oily fish provide significant amounts of
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31 38 long-chain, polyunsaturated omega w-3 fatty acids, eicosapentaenoic acid (EPA), and
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33 39 docosahexaenoic acid (DHA), which help to reduce the risk of cardiovascular disease
34
35 40 (Fussenegger et al., 2007). However, fish can also be a source of substances harmful to
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37 41 the body, as they contain polychlorinated biphenyls, dioxins, and methylmercury
38
39 42 (MeHg) (Virtanen et al., 2007).

43
44 43 It is currently considered that consumption of fish is the main path for human
45
46 44 exposure to mercury (Hg) (EFSA, 2004). In predatory marine fish, about 90% of
47
48 45 mercury is in the methylated form (methylmercury, MeHg) (WHO, 2008), while the
49
50 46 remainder consists of small or undetectable quantities of inorganic mercury [Hg(II)],
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52 47 ethylmercury, and phenylmercury (Branco et al., 2007; Chang et al., 2007). The
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54 48 MeHg/Hg ratio is lower in freshwater fish (WHO, 2008). Methylmercury compounds
55
56 49 are considered by the International Agency for Research on Cancer as possibly
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58 50 carcinogenic to humans, Group 2B (IARC, 1993). Research has shown that MeHg
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3 51 produces adverse neurological effects such as mental retardation, seizures, vision and
4
5 52 hearing loss, delayed development, language disorders, and memory loss (WHO, 2007).
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8 53 Prenatal mercury exposure may produce alterations affecting children's
9
10 54 neurodevelopment (Gao et al., 2007; Jedrychowski et al., 2007).

11
12 55 Predatory fish such as swordfish, shark, and tuna have the highest concentrations
13
14 56 of MeHg. Therefore, the European Food Safety Agency (EFSA), the Food and Drug
15
16 57 Administration (FDA), and the Environmental Protection Agency (EPA) have advised
17
18 58 vulnerable population groups (women who may become pregnant, pregnant women,
19
20 59 nursing mothers, and young children) to avoid some types of fish that might accumulate
21
22 60 high levels of MeHg, such as large predators, and eat fish and shellfish that are lower in
23
24 61 mercury (EFSA, 2004; FDA, 2004). Hg and MeHg have been quantified in various
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26 62 species of fish (Mira and Lanfer-Marquez, 2005; Morgano et al., 2005; Burger and
27
28 63 Gochfeld, 2006; Sivaperumal et al., 2006; Afonso et al., 2007; Cortes and Fortt., 2007);
29
30 64 the influence of the physiological characteristics of the fish (sex, age) on the Hg
31
32 65 concentrations has been evaluated (Monteiro and Lopes, 1990; Afonso et al., 2007;
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34 66 Storelli et al., 2007), and intakes of Hg have been calculated for child and adult
35
36 67 populations (Llobet et al., 2003; Wilhelm, 2003; Muñoz et al., 2005; Falcó et al., 2006;
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38 68 Farias et al., 2006; Marti-Cid et al., 2007; Sahuquillo et al., 2007; Rubio et al., 2008).

39
40 69 To make a more realistic evaluation of the toxicological risk involved in intake of
41
42 70 Hg from consumption of fish, it is necessary to estimate not only the concentration in
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44 71 the product as consumed but also its bioavailability (the fraction of the intake that is
45
46 72 absorbed and reaches the systemic circulation and is thus available to exercise its effect
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48 73 on the receiving organism). For an element to be absorbed by the intestinal epithelium,
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50 74 the first requisite is that it should be soluble. It is therefore interesting to know the
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52 75 bioaccessible content, i.e., the maximum soluble content in simulated gastrointestinal

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3 76 media that is available for subsequent processes of absorption into the intestinal
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5 77 mucosa. The term bioaccessibility also indicates the relation between the bioaccessible
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8 78 content of a substance and the total content of the substance present in the sample.
9
10 79 Bioaccessibility can be used as an indicator of maximum oral bioavailability
11
12 80 (Versantvoort et al., 2005). Static and dynamic *in vitro* gastrointestinal models can be
13
14 81 used to determine bioaccessibility of nutrients. The static models simulate transit
15
16 82 through the human digestive tract by sequential exposure to simulated mouth, gastric,
17
18 83 and small intestinal conditions (pH, temperature, time, enzymes, etc.) (Oomen et al.,
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20 84 2002), and they are the ones that have been most used in studying the bioaccessibility of
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22 85 trace elements from food samples (Intawongse and Dean, 2006).
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27 86 Very little research has been done on Hg bioaccessibility (Cabañero et al., 2004,
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29 87 2007; Shim et al., 2009). It is therefore of great interest to investigate how consideration
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31 88 of the bioaccessible content of total Hg (t-Hg) and MeHg instead of the content in the
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33 89 raw product affects exposure assessments. The aims of the present work were a) to
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35 90 quantify concentrations of t-Hg in raw swordfish and concentrations of t-Hg and MeHg
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37 91 in the bioaccessible fraction obtained after an *in vitro* gastrointestinal digestion method
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39 92 and b) to make risk assessments by estimating daily intake of Hg and MeHg resulting
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41 93 from consumption of this top predator.
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48 95 MATERIALS AND METHODS

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50 96 **Equipment.** A microwave accelerated reaction system (MARS) from CEM (Vertex,
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52 97 Spain) operating at a power of 800 W was used for digestion of samples prior to
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54 98 quantification of t-Hg. Teflon perfluoroalkoxy (PFA) vessels of 55 ml inner volume
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56 99 were employed. For t-Hg quantification, a continuous flow cold vapor generation-
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3 100 atomic fluorescence spectrometer (CV-AFS) model PSA 10.025, Millennium Merlin
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6 101 (PS Analytical, UK), was used.

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8 102 For Hg speciation analysis, the HPLC system employed (Hewlett Packard Model
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10 103 1100, Spain) was equipped with a quaternary pump, an on-line degassing system, an
11
12 104 automatic injector and a thermostated column compartment. Separations were
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14 105 performed on a Hamilton PRP-X100 anion-exchange column (10 μm , 250 mm \times 4.1
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16 106 mm i.d., Teknokroma, Barcelona, Spain). A guard column packed with the same
17
18 107 stationary phase (12–20 μm ; 25 mm \times 2.3 mm i.d.) preceded the analytical column. A
19
20 108 heated bath (Julabo model HC, Merck, Spain) was used to thermooxidize the outlet
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22 109 from the HPLC column. After thermooxidation the Hg content was quantified by CV-
23
24 110 AFS. An analog–digital converter (Model 35 900 C, Hewlett Packard) was used to
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26 111 acquire the AFS signal, which was processed by the chromatographic software.

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31 112 Other equipment used included a lyophilizer (FTS Systems, USA), a mechanical
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33 113 shaker (KS 125 Basic, IKA Labortechnik, Merck, Spain), a pH meter (pH 526, Multical,
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35 114 Spain), an ultrasonic bath (J.P. Selecta, Spain), a heating bath (HC Julabo, Merck,
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37 115 Spain), an orbital shaking water bath (Unitronic Orbital C, J. P. Selecta, Spain), and
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39 116 various centrifuges (Superspeed refrigerated centrifuge RC-5B Instrument, Du Sorvall,
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41 117 Sorvall Pont; Eppendorf 5810, Merck; Heraeus Biofuge Pico, Merck).

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48 119 **Reagents.** In the determination of t-Hg and MeHg, deionized water (18.2 M Ω cm),
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50 120 obtained with a Milli-Q water system (Millipore Inc., Millipore Ibérica, Madrid, Spain),
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52 121 was used for the preparation of reagents and standards. All glassware was treated with
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54 122 10% v/v HNO₃ for 24 h, and then rinsed three times with deionized water before use.

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58 123 All chemicals used were of analytical or reagent grade. A standard solution of
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60 124 1000 mg L⁻¹ Hg (Merck) was employed. A standard solution of 1000 mg L⁻¹ MeHg was

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3 125 prepared by dissolving a commercially available salt of MeHgCl (Sigma-Aldrich) in
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5 126 50% (v/v) MeOH/H₂O mixture. Working standard solutions of Hg and MeHg were
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8 127 prepared daily by serial dilutions of standard solution.
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10 128 Other reagents used were: hydrochloric acid (Merck); nitric acid (Merck); acetic
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12 129 acid (Probus); sulfuric acid (Panreac); sodium hydroxide (Merck); hydrogen peroxide
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14 130 (Prolabo); L-Cysteine (Merck); tin(II) chloride dihydrate (Scharlau Chemie S.A, Spain);
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16 131 sodium hydrogen carbonate (Panreac), and potassium peroxodisulfate (Prolabo).
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19 132 Enzymes and bile salts were purchased from Sigma Chemical Co. (St. Louis, MO,
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21 133 USA): porcine pepsin (enzymatic activity 944 U/mg protein), porcine pancreatin
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23 134 (activity equivalent to 4 x US Pharmacopoeia specifications/mg pancreatin), and bile
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25 135 extract (glycine and taurine conjugates of hyodeoxycholic and other bile salts). Water of
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27 136 cellular grade (B. Braun Medical, S.A., Barcelona, Spain) was used throughout the *in*
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29 137 *vitro* digestion assay.
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3 139 **Samples.** In Spain, consumption of frozen swordfish is much higher than consumption
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5 140 of fresh swordfish (94% as opposed to 6% in 2003, according to data provided by the
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8 141 Ministry of the Environment and Rural and Marine Environment's General Secretariat
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10 142 of the Sea). Reflecting the habits of the Spanish population, we analyzed 27 samples of
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12 143 frozen swordfish muscle. The swordfish muscle samples were provided in 2005 by
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14 144 companies that market this product in Spain. The samples were thawed, the skin was
15
16 145 removed, and the edible portions were frozen, lyophilized, ground, and stored at 4 °C
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18 146 until analysis. A reference material with certified contents for t-Hg and MeHg, DORM-
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20 147 2 sample (muscle of dogfish, National Research Council of Canada), was also analyzed.
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23 148 Each sample was analyzed in triplicate, for both t-Hg and MeHg and their respective
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25 149 bioaccessibilities.
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32 151 ***In vitro* gastrointestinal digestion.** Samples of seafood products were digested using a
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34 152 simulated digestion process developed by our group in an earlier study (Laparra et al.,
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36 153 2003). A quantity of lyophilized swordfish sample, equivalent to 10 g of fresh sample,
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38 154 was weighed and cellular-grade water (90 mL) was added. The pH was adjusted to 2.0
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40 155 with 6 mol L⁻¹ HCl. After 5 min, the pH value was checked and if necessary readjusted
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42 156 to pH 2.0. Freshly prepared pepsin solution (1 g of pepsin in 10 mL of 0.1 mol L⁻¹ HCl)
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44 157 was added to provide 0.01 g of pepsin/10 g fresh sample. The sample was made up to
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46 158 100 g with water, and incubated in a shaking water bath (stroke rate 120 min⁻¹) at 37 °C
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48 159 for 2 h to emulate the gastric stage of digestion.
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53 160 Then, for the intestinal digestion, the pH value was raised to pH 5.0 by drop-wise
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55 161 addition of 1 mol L⁻¹ NaHCO₃. The pancreatin–bile extract mixture (0.2 g of pancreatin
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57 162 and 1.25 g of bile extract in 50 mL of 0.1 mol L⁻¹ NaHCO₃) was added to provide
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59 163 0.0025 g of pancreatin/10 g fresh sample, and 0.015 g of bile extract/10 g fresh sample.
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3 164 The incubation at 37 °C continued for 2 hours. The pH was then adjusted to 7.2 by
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5 165 addition of 0.5 mol L⁻¹ NaOH. Aliquots of 40 g of the digests were transferred to
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8 166 polypropylene centrifuge tubes and centrifuged (15000 rpm/30 min/4 °C) to separate
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10 167 soluble and precipitate. The concentrations of t-Hg and MeHg were quantified in the
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12 168 soluble fraction.

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17 170 **Total mercury determination.** An assisted digestion in microwave oven with
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19 171 subsequent quantification by CV-AFS was used for the determination of t-Hg in the
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21 172 samples of swordfish and in their bioaccessible fractions.

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24 173 Lyophilized swordfish samples (0.2 g) or bioaccessible fractions (0.6 g) were
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26 174 placed in a Teflon PFA vessel and treated with 4 mL of HNO₃ concentrate (14 N) and 1
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28 175 mL of H₂O₂. The Teflon PFA vessel was irradiated at 800 W (180 °C, 15 min). At the
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30 176 end of the digestion program, the digest was placed in a 250 mL beaker and allowed to
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32 177 rest all night to eliminate nitrous vapor. It was then filtered through Whatman No. 1
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34 178 paper and made up to volume with 5% HCl (v/v).

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37 179 Mercury contents were determined by CV-AFS using the following analytical
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39 180 conditions: reducing agent, 2% (m/v) SnCl₂ in 15% (v/v) HCl, 4.5 mL min⁻¹ flow rate;
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41 181 blank reagent, 5% HCl (v/v), 9 mL min⁻¹ flow rate; carrier gas, argon 0.3 L min⁻¹ flow
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43 182 rate; dryer gas, air 2.5 L min⁻¹ flow rate.

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46 183 Throughout the experiment, the quality assurance-quality control of Hg
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48 184 measurement was checked by analyzing the certified reference material DORM-2 with
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50 185 each batch of samples.

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55 187 **Methylmercury determination.** For extraction of mercury species an ultrasonic acid
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57 188 extraction was employed. The lyophilized swordfish samples (0.2 g) or bioaccessible

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3 189 fractions (0.6 g) were weighed into a 50 mL centrifuge tube and 8 mL of 2.4 N HCl was
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5 190 added. The mixture was sonicated for 5 min, centrifuged (2000 rpm/15 min), and the
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8 191 supernatant was centrifuged again (12000 rpm/10 min). The resulting extract was
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10 192 filtered through 0.45 μm Whatman Nylon prior to species quantification by HPLC-
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12 193 thermooxidation-CV-AFS.

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15 194 Samples were injected into the PRP-X100 column and, using PTFE tubing and T-
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17 195 joints, the eluate from the column was mixed with the persulfate solution. The mixture
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19 196 was thermooxidized by being passed through a loop of Teflon tubing placed in a heated
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21 197 bath. After cooling in an ice bath, the eluate was mixed with a continuous flow of
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23 198 SnCl_2 . Using a gas-liquid separator and a continuous flow of argon, the arsines
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25 199 generated were introduced into the AFS. The hygroscopic-membrane drying tube used
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27 200 to transport the arsines allowed elimination of moisture by circulating a counterflow of
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29 201 air. Details of operating conditions are given in Table 1.

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31 202 Signals were identified by coincidence of sample and standard retention times.
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33 203 The quantification was obtained from the peak area by interpolation of external
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35 204 calibration curves. Throughout the experiment, the quality assurance-quality control of
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37 205 MeHg measurement was checked by analyzing the certified reference material DORM-
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39 206 2 with each batch of samples.

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42 208 **RESULTS AND DISCUSSION**

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44 209 **Total mercury in swordfish.** The concentrations of t-Hg in the swordfish samples
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46 210 analyzed (Table 2) ranged between 0.41 and 2.1 mg kg^{-1} ww (mean \pm standard deviation
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48 211 = 0.96 ± 0.47 mg kg^{-1} ww; n = 27). The analysis of Hg in seafood products marketed in
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50 212 Spain is a customary practice in Spanish food control laboratories, but few data are
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52 213 published in scientific journals. The range of concentrations obtained overlaps the few

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3 214 values reported in the journals for samples of swordfish marketed in Spain: 0.4–2.2 mg
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5 215 kg^{-1} ww (Cabañero et al., 2004; Falcó et al., 2006; Blanco et al., 2008). Furthermore, the
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7 216 values found in the present work are similar to those reported in the last five years for
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9 217 swordfish of various origins (Table 3). Only in samples acquired in Taiwan much
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11 218 higher mean concentrations have been found ($3.6 \pm 0.5 \text{ mg kg}^{-1}$ ww) (Chen et al., 2007).

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15 219 Some authors have found a linear correlation between the concentrations of Hg
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17 220 and the length and age of the fish, as most of the Hg is in the form of MeHg bound to
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19 221 the thiol groups of proteins, which increase with age (Monteiro and Lopes, 1990;
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21 222 Storelli and Marcotrigliano, 2001; Branco et al., 2004; Branco et al., 2007). A
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23 223 correlation has also been found between the concentration of Hg in the muscle and the
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25 224 area where the fish was caught (Branco et al., 2007). The size of the liver of predatory
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27 225 fish is another physiological variable that researchers have attempted to correlate with
28
29 226 Hg concentrations. However, further studies must be conducted in this regard as the
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31 227 results differ depending on the species of fish considered. Concentrations have been
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33 228 found in the liver of swordfish and bluefin that are between 2 and 3 times greater than
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35 229 the levels in muscle (Storelli et al., 2005; Branco et al., 2007), attaining $9.8 \mu\text{g g}^{-1}$ ww
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37 230 in the liver of swordfish from the area of Ecuador (Branco et al., 2007). In samples of
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39 231 shark, however, Hg concentrations in the liver have been shown to be less than those of
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41 232 muscle (Branco et al., 2007).

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48 233 With regard to the food safety of the samples analyzed, 37% exceed the value of 1
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50 234 $\mu\text{g g}^{-1}$ ww, the maximum limit of Hg permitted by Spanish legislation in swordfish
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52 235 (European Commission, 2006). It is important to emphasize that food alerts concerning
53
54 236 high Hg concentrations in fish have increased in Spain and other countries in recent
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56 237 years. This is shown in a report of the Rapid Alert System for Food and Feed of UE
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58 238 (RASFF, 2009), which gives details of the notification of 128 alerts concerning mercury
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3 239 in fish, 56% of which were for swordfish. Of the samples that exceeded the legislated
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5 240 values, 34% were of Spanish origin, which demonstrates the need for the application of
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8 241 rigorous health control measures for these products.
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10 242

11
12 243 **Total Hg and MeHg bioaccessible in swordfish.** The swordfish samples were
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15 244 subjected to a gastrointestinal digestion and t-Hg and MeHg were determined in the
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17 245 bioaccessible fraction. As the bioaccessible fraction is a very different matrix from the
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20 246 raw seafood, it was necessary to evaluate the suitability of the application of the
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22 247 methods for t-Hg and MeHg quantification developed previously in the laboratory.
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24 248 For this purpose, we evaluated the analytical characteristics (detection limit,
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26 249 precision, and recovery) of the t-Hg method, which includes *in vitro* digestion,
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29 250 microwave acid digestion of the soluble fraction, and quantification by CV-AFS. The
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31 251 results obtained endorse the suitability of the method for the aims proposed, and the
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33 252 absence of matrix interference: limit of detection = 0.02 ng g⁻¹, ww; precision = 10%;
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35 253 recovery = 98%. The quantification of MeHg in the bioaccessible fraction entails a
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37 254 more complex treatment, because after the *in vitro* gastrointestinal digestion it is
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39 255 necessary to lyophilize the soluble fractions and then perform an ultrasonic acid
40
41 256 extraction of MeHg. The analytical characteristics of the method endorse its use for the
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43 257 aims proposed: limit of detection = 0.11 ng g⁻¹, ww; precision = 5%; recovery = 87–
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45 258 104%.
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50 259 *Bioaccessible t-Hg.* The concentrations of bioaccessible t-Hg (Table 2) varied
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52 260 over a wide range: 0.17–1.72 mg kg⁻¹ ww (mean value = 0.63 ± 0.4 mg kg⁻¹ ww). In the
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54 261 study that was conducted, there was not a proportional relationship between the Hg
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56 262 concentration in the samples and their gastrointestinal solubility. This can be seen in
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58 263 various samples, including samples 17 and 22, where the t-Hg concentrations in the
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3 264 samples are almost identical (1.18 and 1.20 mg kg⁻¹ ww, respectively) but the
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5 265 bioaccessible concentrations are very different (0.48 and 0.84 mg kg⁻¹ ww,
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7 266 respectively). The bioaccessibility, the percentage relating the bioaccessible Hg
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9 267 concentration to the initial Hg concentration in the sample, varied between 38% and
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11 268 83%, with a mean value of 64 ± 14%. Given the suitable analytical characteristics of the
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13 269 method used for quantifying t-Hg in the bioaccessible fraction, the wide range of the
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15 270 bioaccessibility values found cannot be attributed to problems connected with the
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17 271 analytical method used. They might be the result of freezing rates, thawing conditions
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19 272 and storage temperature on protein denaturation. All this might affect the ability of the
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21 273 enzymes used in the gastrointestinal method to solubilize Hg from proteins in the
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23 274 swordfish. The samples were purchased in small retail outlets, where products might be
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25 275 stored in different time and temperature conditions.
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31 276 To our knowledge, the present study contributes the largest number of data in the
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33 277 scientific literature on the bioaccessibility of t-Hg in swordfish. Previously, Shim et al.
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35 278 (2009) obtained bioaccessibility values for king mackerel (68%), similar to the values
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37 279 presented here for swordfish. However, Cabañero et al. (2004) indicated much lower
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39 280 bioaccessibility values in three kinds of seafood products: 17% in swordfish, 13% in
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41 281 sardine, and 9% in tuna. The authors attributed the low bioaccessibility to the low
42
43 282 capacity of the enzymes in the *in vitro* gastrointestinal method to release mercury
44
45 283 complexed with Se (Cabañero et al., 2007). This hypothesis concerning the effect of the
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47 284 Se-Hg relationship on bioaccessibility needs to be corroborated in further studies.
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52 285 *Bioaccessible MeHg.* The bioaccessible MeHg concentration was determined in
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54 286 15 of the swordfish samples. The values ranged between 0.16 and 1.53 mg kg⁻¹ ww,
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56 287 with a mean value of 0.49 ± 0.32 mg kg⁻¹ ww (Figure 1). The MeHg in the bioaccessible
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3 288 fraction represents between 71% and 105% of the t-Hg in the solubilized fraction (mean
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5 289 value = $88 \pm 11\%$).

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8 290 We are not aware of the existence of previous data of bioaccessible MeHg
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10 291 concentrations. There are only the reports by Cabañero et al. (2004, 2007) concerning
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12 292 the relation between Se and MeHg in the bioaccessible fractions of various species of
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15 293 fish. These data, which are expressed as [Se/Hg] bioaccessible molar ratios, show
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17 294 values ranging between 9.3 for swordfish and 126.3 for sardine, without providing data
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20 295 of bioaccessible concentrations for MeHg.

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22 296 If risk managers have an interest to consider bioaccessibility in establishing
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24 297 maximum limits of Hg and MeHg in foods, they should make an effort to increase the
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27 298 database and obtain a worse-case estimate of this parameter.

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31
32 300 **Evaluation of toxicological risk.** The toxicological risk associated with the intake of t-
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34 301 Hg and MeHg from consumption of the samples analyzed can be evaluated by using the
35
36 302 guideline values recommended by international organizations. The FAO/WHO
37
38 303 recommends a Provisional Tolerable Weekly Intake (PTWI) for Hg of less than 5 $\mu\text{g}/\text{kg}$
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40 304 body weight/week, of which MeHg should not be more than 1.6 $\mu\text{g}/\text{kg}$ body
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42
43 305 weight/week (WHO, 2003). The recommendation of the U.S. Environmental Protection
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45
46 306 Agency (USEPA) is much more restrictive, proposing a maximum MeHg intake of 0.1
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48 307 $\mu\text{g}/\text{kg}$ body weight/day (USEPA, 2001).

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51 308 Consumption of seafood products in Spain (44.5 kg/person/year) is among the
52
53 309 highest in Europe, exceeded only by Portugal (Laurenti, 2007). The study by the
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55 310 Ministry of Agriculture, Fisheries, and Food, reports that consumption of fish in the
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58 311 period between 1987 and 2003 was 102 g/person/day (MAPA, 2006).

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3 312 There are very few studies of the consumption of individual foods in Spain. The
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5 313 National Study of Nutrition and Diet (Varela et al., 1995) reports a mean consumption
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8 314 of swordfish by adults of 0.35 g/day, with substantial variations between different
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10 315 autonomous communities, ranging from areas where swordfish is not consumed to the
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12 316 areas of highest consumption, Murcia and the Valencian Community (1.06 g/day and
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14
15 317 1.17 g/day, respectively). More recently, a study by the Nuclear Safety Council (CSN,
16
17 318 2002) provided fresh data concerning the mean consumption of swordfish for the entire
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19 319 population of children (0.69 g/day) and adults (1.53 g/day), taking into account
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21 320 surveyees who consume swordfish and those who do not. This study also provides data
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23 321 concerning the real consumers of the product, who are only a small percentage of the
24
25 322 total surveyed: 1.3% of the children and 2.5% of the adults. For this population of
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27 323 consumers, the mean consumption is quite high, attaining 51.7 g/day in the children and
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29 324 60.3 g/day in the adults. The study also reports intake values for high consumers: 112.4
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31 325 g/day for children and 102.3 g/day for adults.

32 326 *Estimation of Hg intake from contents in raw swordfish.* To estimate the intake
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34 327 of t-Hg by the Spanish population (Table 4), we used the consumption data provided by
35
36 328 the CSN, and the minimum, mean, and maximum t-Hg concentrations found in the
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38 329 samples analyzed. The results obtained were compared with the reference value
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40 330 recommended by the WHO, 5 µg/kg body weight/week, equivalent to a Tolerable Daily
41
42 331 Intake of 0.71 µg Hg/kg body weight. On the basis of the mean consumption of the total
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44 332 child population (0.69 g/day), the daily intake would attain a maximum of 6% of the
45
46 333 TDI, which does not indicate the existence of a risk. If the estimate is based only on the
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48 334 intake of consumers of the product, however, the situation is very different. For the
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50 335 mean consumption by children (51.67 g/day), the intake of t-Hg is less than the TDI
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52 336 (85% of the TDI) if the lowest concentration of t-Hg found in swordfish is assumed, but
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3 337 greater than the TDI for the mean and maximum concentrations of Hg in swordfish. The
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5 338 situation is aggravated for children that are high consumers (112.37 g/day), whose
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8 339 consumption is much greater than the TDI: between 1.9 and 9.5 times. Adults face a
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10 340 similar situation: assuming the mean consumption of the entire population (1.53 g/day),
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12 341 intake is 7% of the TDI at most; for consumers of the product, the TDI is exceeded in
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14
15 342 the cases of mean consumption (60.26 g/day) and high consumption (102.34 g/day) of
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17 343 swordfish with mean or maximum concentrations of t-Hg.

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20 344 *Estimation of MeHg intake from contents in raw swordfish.* To determine the
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22 345 intake of MeHg (Table 5), we assumed that all of the Hg in raw swordfish is present as
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24 346 MeHg, providing a protectionist estimate for the consumer. This is also the general
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26 347 approach recently recommended by the WHO for exposure assessments of MeHg in
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28 348 fish (WHO, 2008). The MeHg intake values calculated on this basis were compared
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30 349 with the reference value recommended by the WHO for MeHg, (Tolerable Daily Intake
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32 350 = 0.23 µg MeHg/kg body weight). When the total population surveyed is considered,
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34 351 neither the mean consumption by children (0.69 g/day) nor the mean consumption by
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36 352 adults (1.53 g/day) lead to intake values that exceed the TDI. However, if only the real
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38 353 consumers of the product are considered, the mean consumption both by children (51.67
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40 354 g/day) and by adults (60.26 g/day) leads to MeHg intakes exceeding the TDI; between
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42 355 2.7 and 13.6 times the TDI in the case of children, and between 1.6 and 7.9 times the
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44 356 TDI in the case of adults. The most worrying situation is that of high consumers, both
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46 357 children and adults, whose intake of MeHg is 29.6 and 13.5 times greater than the
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48 358 recommended value, respectively. If the estimated intakes are compared with the MeHg
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50 359 reference value established by the USEPA (0.1 µg/kg body weight/day), 2.3 times less
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52 360 than the WHO value, the exposure situation is even worse.
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3 361 **Recommended safe intake.** The results presented indicate that there is a small
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5 362 percentage of the child and adult population in Spain, corresponding to consumers of
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7 363 swordfish, that is at risk as a result of exposure to Hg from this product. This is the case
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10 364 even when the swordfish has a concentration of Hg below the maximum limit
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12 365 established by the legislation (1 mg/kg ww). This should lead the authorities to make
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14 366 recommendations of maximum consumption of this product by children and adults. In
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17 367 view of the neurotoxic nature of MeHg and its transmission through the placenta and
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19 368 breast milk, some countries have adopted recommendations for groups at risk: women
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21 369 who may become pregnant, pregnant women, nursing mothers, and young children.
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24 370 This is the case in the UK, where the FSA recommends that babies and pregnant women
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26 371 should not consume swordfish and that breastfeeding women should not consume more
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28 372 than one portion per week (FSA, 2008). Another example is Canada, where Health
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30 373 Canada recommends a maximum consumption of 150 g of swordfish per month for
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32 374 certain women (those that are or may become pregnant or are breastfeeding), 125 g per
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34 375 month for children 5–11 years old, and 75 g per month for children 1–4 years old
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36 376 (Health Canada, 2008). In Spain, there are no recommendations in this regard. As Table
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38 377 6 shows, even if all the Hg quantified in the fish analyzed were in the form of MeHg,
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40 378 the weekly consumption of 25 g swordfish/week for Spanish children and 50 g/week for
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42 379 adults would give MeHg intakes lower than the PTWI recommended by the WHO.
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44 380 Even if the concentration of Hg in the fish (0.958 mg kg⁻¹ ww) were very close to the
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46 381 maximum value permitted by the legislation (1 mg kg⁻¹ ww), consumption of these
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48 382 quantities would not represent a health risk. Recommendations for the Spanish
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50 383 population concerning monthly consumption could be set at values very close to those
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52 384 stipulated in the UK and Canada.
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3 385 *Estimation of MeHg intake from bioaccessible contents.* It would be interesting to
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5 386 study whether taking bioavailability into account would alter the risk associated with
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7 387 consumption of this product. Assuming the quantities of bioaccessible MeHg found in
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9 388 the present study (0.164–1.53 mg kg⁻¹ ww; mean = 0.495) (Fig. 1), the mean estimated
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11 389 intake for children (51.7 g of swordfish/day) ranges between 8 and 79 µg MeHg/day
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13 390 (mean value = 32 µg MeHg/day), exceeding the TDI values recommended by the WHO
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15 391 by 1–10 times (mean = 3.2 times the TDI). For adult consumers of swordfish (60.3
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17 392 g/day according to the study by the CSN mentioned earlier) the estimated intakes of
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19 393 MeHg range between 10 and 92 µg MeHg/day (mean value = 38 µg MeHg/day), values
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21 394 that represent from 63% of the TDI to 6 times the TDI (mean = 1.9 times the TDI).
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23 395 When the value established by USEPA is used as the toxicological reference value, the
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25 396 limit is exceeded for both children and adults and over the entire range of bioaccessible
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27 397 MeHg concentrations found in the fish analyzed.

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29 398 These results show that there would still be a situation of risk if bioaccessible
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31 399 MeHg concentrations were considered instead of using the concentrations in the
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33 400 product, although the TDIs are 2.5 times lower than those obtained from the contents in
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35 401 raw swordfish. If MeHg bioaccessibility were considered, therefore, perhaps the
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37 402 recommendations concerning consumption of swordfish could be modified by
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39 403 increasing the quantity consumed or the frequency of consumption.

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41 404 The results presented show the need for recommendations about swordfish
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43 405 consumption by population groups at risk in Spain, and the desirability of broadening
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45 406 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 \pm standard deviation ($n = 3$).

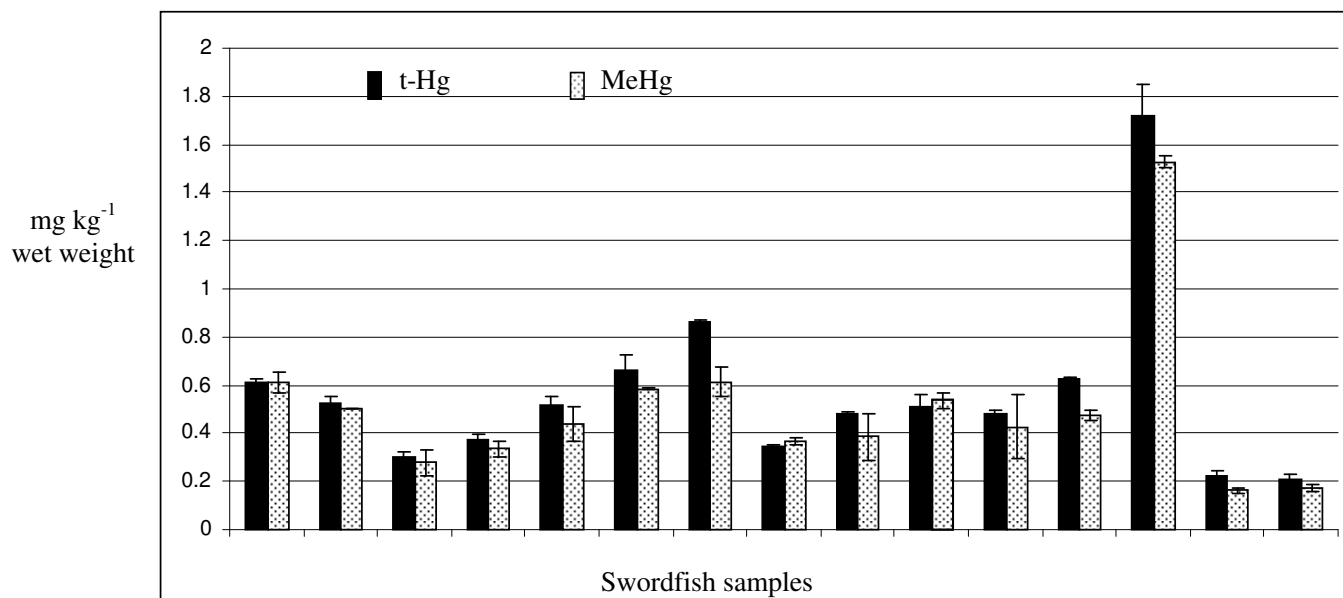


Table 1. Instrumental parameters used for the determination of methylmercury by HPLC-thermooxidation-CV-AFS

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 ₂ S ₂ O ₈ in 0.5% (mol L ⁻¹) H ₂ SO ₄ . Flow 2 mL min ⁻¹
Reaction loop	3 m x 0.5 mm i.d.
Bath temperature	150 °C
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 \pm standard deviation (n = 3).**

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

^a Bioaccessibility = $\frac{\text{(t-Hg in bioaccessible fraction of swordfish)}}{\text{(t-Hg in swordfish)}} \times 100$

(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)			n	Reference
	min	max	mean		
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).

^c Intakes calculated on the basis that all the Hg is present as MeHg.