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Mercury and methylmercury intake estimation due to seafood products for the Catalanian population (Spain)

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3 **Mercury and methylmercury intake estimation due to seafood**
4 **products for the Catalanian population (Spain)**
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Abstract

This study estimates mercury and methylmercury levels in fish and fishery products commercialized in the city of Barcelona from 2001 to 2007. Combining data of mercury levels in food with the consumption data of 2158 people (as the median of two 24-hour-recall), the total Mercury intake of the Catalonian population was calculated. Mercury was detected in 32.8% of analyzed samples. The general population average weekly intake of total mercury in Catalonian population was 0.783 µg/kg of body weight. This value is clearly lower than the Joint FAO/WHO Expert Committee on Food Additives (JECFA) provisional tolerable weekly intakes (PTWI) of 5 µg/kg of body weight. The fish group was the main contributor to this value, mainly due to predatory species.

Key words: mercury, methylmercury, dietary intake, fish, fishery products, food safety, Spain

Introduction

Heavy metals like mercury (Hg) and specially its organic form (methylmercury, MeHg) are environmental contaminants of special concern due to a wide distribution in the environment and likely adverse effects for human health. (Silva-Pereira et al. 2005; Toimela and Tahti 2004).

Deleted: (hg)

Deleted: (methylmercury, meHg)

For the general population (non-occupationally exposed population) the main route of exposure to mercury is via the diet, mainly through fish and seafood consumption (World Health Organisation (WHO) 1990). Other food sources than fish and seafood products can contain traces of mercury but are considered of lower concern. The methyl mercury content in fish and shellfish varies, but it is generally assumed that it is over 90% (European Commission 2004). In marine sediments, elemental or inorganic Hg can be easily methylated by microbiological activity and it can be accumulated in animal tissues and increase in concentration through the food chain.

Deleted: mostly in the inorganic form

Following human ingestion, methylmercury, unlike elemental or inorganic forms, is almost completely absorbed from the gastrointestinal tract and distributed into all tissues, and readily crosses the hemato-encephalic barrier and the placenta. Central nervous system is considered to be the target organ for methylmercury. Prenatal exposition to methylmercury may cause disorders on the brain development. (EFSA, 2004). Therefore, the European Commission has made some consumption recommendations for protecting vulnerable groups of the population, such as infants, children, pregnant women and breast-feeding mothers. (European Commission 2004). European legislation set a maximum level for total mercury in fish and fishery products of 0.5 mg/kg wet weight, except for some predatory species where the limit was 1 mg/kg wet weight. (European Commission 2006).

On the other hand, the joint FAO/WHO Expert Committee in Food Additives (JEFCA) established a provisional tolerable weekly intake (PTWI) for total mercury of 5 µg/kg body weight (bw) and also for methylmercury at 1.6 µg/kg bw. Exposure to mercury depends on the diet habits and geographical location.

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1 Spain is the first fish market in the EU, with an average per capita consumption
2 of 40kg/person-year (Failler 2007).
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5 The main purpose of this study is to calculate mercury levels in fish and fishery
6 products commercialized in the city of Barcelona and combine this data with
7 seafood products consumption data, to estimate if there may be population
8 groups in Catalonia with a mercury or methylmercury intake that exceed the
9 PTWI recommended by the WHO.
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14 **Materials and Methods**

15 *Sampling:*

16 Food samples were collected as part of the IQSA program (Food Health Quality
17 Research Program) (Arqués 2003). This surveillance program developed by the
18 city public health services started in 1984 with the aim of monitoring additives
19 and pollutants, both chemical or microbiological, in the food sold in the city.
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22 Foodstuffs taken for the IQSA program were obtained in order to reflect food
23 consumed in the city, to monitor levels of food hygiene and safety and
24 compliance with existing regulations; thus sampling covers both diverse
25 production origins (local, national and EU, imported from other countries...), and
26 different marketing channels (municipal markets, supermarkets, and other retail
27 stores).
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30 From 2001 to 2007, 688 samples of 45 different fish and seafood products were
31 obtained from the retail and wholesale commerce of the city. They were
32 classified in six groups: fresh fish (93 samples of 21 species), canned fish (36 of
33 4 species), bivalves (185 of 7 species), cephalopods (185 of 3 species),
34 crustaceans (175 of 9 species) and gastropod (14 of 1 species).
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37 Mercury determination:

38 The method used for total mercury determination in food was developed in the
39 Barcelona Public Health Agency Laboratory and has been fully validated
40 evaluating parameters such as trueness precision and linearity, along the
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Deleted: Total mercury

1 working range. This method is accredited by the National Accreditation Body
2 (ENAC [Entidad Nacional de Acreditacion]) since 2000.
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5 For the determination of total mercury, an Advanced Mercury Analyser AMA-
6 254 (LECO) was used. This is an elemental analyzer based on an atomic
7 absorption spectrophotometer that analyses the mercury levels in triturated and
8 homogenized samples without any previous chemical pre-treatment.
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13 An aliquot of 0,1 g of the homogenized sample is weighted into a nickel boat
14 which is introduced into a sealed combustion/catalyzed tube where the
15 decomposition process at 750 C by catalytic oxidation takes place. Products
16 from the decomposition are carried out to the amalgamator using an oxygen
17 flow where only mercury vapors are trapped onto the gold-plated ceramic tube.
18 Once all byproducts have passed through the measuring cell, the amalgamator
19 is flash heated at about 300 C and the mercury is dosed to the measuring cell,
20 using an atomic absorption spectrophotometer at 253,7 nm for each
21 determination. The absorbance obtained for the sample is compared with the
22 standards calibration, which is formed by the following standards, injecting
23 200µgHg/L: 10µg/L, 25µg/L, 50µg/L, 75µg/L, 100 µg/L,150µg/L, 200µg/L, 250µg/L,
24 300µg/L, 400µg/L, 500µg/L, 750µg/L, 1000µg/L, 1250µg/L and 1500 µg/L. There
25 are two ranges of calibration; the low one corresponds to the standards 10 –20
26 and the high one 200-1500.
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37 Different seafood products were used to validated the method. The validated
38 concentration level for total mercury were 0.10 mg/Kg, 1 mg/Kg and 3 mg/Kg,
39 and 12 replicates were analysed for each level. Mean recovery of the process
40 was 99,2%, precision was 11% and uncertainty 22%. The limit of quantification
41 validated was 0,10 mg/Kg.
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45 Complementarily, the laboratory participates regularly (5-10 times a year) in
46 proficiency testing for Hg in fish and seafood products as a tool to ensure the
47 quality of generated data, obtaining always satisfactory results (z-score< 2.0).
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1 The analytical method for methylmercury determination was not validated in the
2 Barcelona Public Health Agency Laboratory when this study was developed. So, total
3 mercury was determined in fish samples, and the results were multiplied for a factor of
4 0.90. (European Commission, 2004). However, in 2007 some preliminary results were
5 obtained in the Barcelona Public Health Agency Laboratory showing that
6 methylmercury was 89% of the total mercury. This analytical method for MeHg is
7 based in a extraction of 1g of sample with clorhidric acid (HCl 5M) and CH₂Cl₂
8 (dichlormethane). Inorganic mercury elutes with the acid phase, which is analyzed with
9 CV-AAS. MeHg is calculated extracting the inorganic Hg from total Hg. Recovery for
10 this method are 90-110 %.

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17 *Mercury intake estimation:*

18 In order to estimate the health risk associated to fish and seafood consumption,
19 the weekly dietary mercury intake for the Catalan population was estimated as
20 the product between the mean mercury concentration for each species (IQSA
21 data) and its weekly consumption. As the number of samples with non-
22 quantifiable mercury residues was large (67,2%), it was decided not to assign a
23 value of one half the limit of quantification (0.050 mg/kg) to these samples in
24 order to avoid overestimation of the amount of mercury ingested. (Joint
25 FAO/WHO 2005).

Deleted: Mercury concentrations under limit of quantification were assumed to be 0 mg/kg.

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31 Individual food consumption and other individual data (weight, sex and age)
32 was taken from the latest Catalan Nutrition Survey (ENCAT, 2002-03) (Serra-
33 Majem et al. 2007) conducted by the Catalan Government Department of
34 Health. Dietary intake was assessed by means of two 24-hour recalls on non-
35 consecutive days. A probabilistic sample of 2158 individuals from 83 cities (995
36 males and 1163 females) aged 10-80 years was interviewed in 2002-03.

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42 As it is shown below, the mercury weekly intake for each 2158 individuals was
43 calculated considering their body weight and their daily personal intake of 43
44 different species: 24 fresh fishes, 4 canned fishes; 6 crustaceans; 5 bivalve
45 mollusks; 3 cephalopods; 1 gastropods. For 13 species out of 43, a mercury
46 concentration was not available in the IQSA program. Therefore, a mean value
47 calculated for species of the same food group was used.

1 The weekly intake (WI) for each individual was calculated as the formula below:

2 **WI = Σ (Is*Cs) * 7days/ Kg body weight**

3
4 (Is= daily intake of fish and seafood products species; Cs = total mercury
5 content in these species ($\mu\text{g}/\text{Kg}$)
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9 Once the weekly intake of 2158 individuals was calculated, a general population mean
10 weekly intake was also estimated and also by sex and age (5 age groups were
11 considered: 10 -17, 18-24, 25-44, 45-64, >65). In order to estimate methylmercury
12 intakes, a factor of 0.90 was applied to mercury results (European Commission, 2004).
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15 *Statistical procedures*

16 All statistics were performed using SPSS 13.0 software. Mercury weekly
17 intakes were calculated for each individual, and its median intake in general
18 population as well as for sex and age group. Statistically significance were
19 analysed between this mercury intake and studied variables by U-Mann-
20 Whitney and Kruskal-Wallis test. Chi-square (χ^2) statistics tested differences
21 between subgroups to evaluate percentages of interviewed people above the
22 PTWI.
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29 **Results**

30 *Mercury levels*

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32 Mercury was detected in 32.8% of analyzed samples in the surveillance
33 program (226 of 688). By food groups (Table 1), mean concentration levels
34 were: fish (0.285 mg/kg) [non-predators=0.104 mg/kg; predators=0.750 mg/kg],
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canned fish (0.289 mg/kg), crustacean (0.082 mg/kg), gastropods (0.071
mg/kg), cephalopods (0.047 mg/kg) and bivalves (0.005 mg/kg).

Deleted: concentration levels

The highest mercury concentrations corresponded to two predatory species: In
both cases, the mercury levels exceeded the European limit of 1 mg/kg: a
porbeagle (*Lamna nasus*) and a little tunny (*Euthynnus quadripunctatus*) with
values of 7.6 mg/kg and 1.4 mg/kg respectively. Mercury concentration range in
the rest of fish samples was between 0 and 0.98 mg/kg.

1 Any bivalves or gastropods were above the maximum level of 0.5 mg/kg. Two
2 crustaceans (lobster: 0.6 mg/kg and prawn: 0.9 mg/kg) and one cephalopod
3 (octopus: 0.8 mg/kg) exceed the limit.
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7 *Weekly intake of mercury and methylmercury*

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9 The general population average weekly intake of total mercury in Catalanian
10 population (Table 1) was 0.783 µg/kg of body weight. Figure 1 and Table 1
11 show that the highest contribution to this value is provided by fish (0.440 µg/kg
12 body weight; 56.2% of the total intake) followed by canned fish (0.284µg/kg
13 body weight; 36.3%), crustaceans (0.032µg/kg body weight), cephalopods
14 (0.022µg/kg body weight), gastropods (0.004µg/kg body weight) and bivalves
15 (0.001µg/kg body weight). In terms of species (Table 3), the most important
16 contributors to the total mercury intake were canned tuna fish (0.27 µg/kg body
17 weight and week) and young hake (0.11mg/kg). It has been no possible to
18 calculate the contribution of porbeagle and little tuny because of consumption
19 data were not available in the ENCAT Survey.
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27 For methylmercury, the general population average weekly intake was 0.705
28 µgMeHg/kg body weight (Table 1). No differences between sex ($p=0.733$) were
29 found for mercury or methylmercury intake, but differences between age groups
30 existed in both cases ($p< 0.026$), being the youngest and the oldest those with
31 lowest levels of mercury and methylmercury intake.
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36 Overall, 31 individuals (1.4% of the population) could have intakes above the
37 PTWI for total mercury (Table 2a), and no differences were observed between
38 sex ($p=0.180$) or age group ($p=0.084$). On the other hand, 187 women (16.1%
39 of the population) would have methylmercury intakes above the PTWI for
40 methylmercury (Table 2b). No difference was observed between age groups
41 ($p=0.151$), although a slight significant difference was observed between sex
42 ($p=0.045$), being women the ones with a higher percentage.
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48 **Discussion**

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1 The general population average weekly intake of total mercury for Catalan
2 population was 0.783 µg/kg of body weight. This value is clearly below the
3 recommended PTWI (5 µg/kg bw).
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7 The main contribution to this intake was provided by fish, because of its higher
8 consumption and higher mercury concentration, mainly through predator fish:
9 fresh tuna is the most polluted, but its consumption is relatively low. However
10 the contribution of some species like young hake is due mainly to its high
11 consumption. Only considering tuna (canned and fresh), which represents 16%
12 of fish intake, it can be explained more than 42% of total mercury intake.
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17 Other studies corroborate this higher contribution provided by fish and similar
18 levels are found (Sahuquillo et al. 2007; SCOOP 2004).
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22 In recent years there has been published several studies of mercury intake
23 estimation. Nevertheless, methodological divergences, that a lot of times are
24 not specified, make comparisons tremendously difficult: foodstuffs included in
25 each food group, number of samples analyzed, different analytical methods and
26 quantification limits, the different statistical treatment given to negative results,
27 the different information sources used to estimate the consumption habits of the
28 geographical area being investigated, the average weights used to work out, the
29 amount of food ingested per Kg of body weight, etc.
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35 Always bearing in mind the caution required due to the different methodologies
36 used, we can observe that the present results are similar to the ones obtained
37 in other studies carried out in the same geographical area. In the study "Total
38 Diet" conducted by the Catalan Government in 2002-2003, an average weekly
39 ingestion of mercury of 0.892 µg/kg body weight was estimated. (Departament
40 de Salut. Agència Catalana de Seguretat Alimentaria. 2005). [Other studies
41 carried out in the same geographical area \(catalan population\) describe
42 mercury ingestions for the standard adult male \(70 Kg\) that range between 5.3
43 µg/day and 9.89 µg/day](#) (weekly ingestion per Kg of body weight = 0.53 –
44 0.989). (Bocio et al. 2005; Falco et al. 2006; Llobet et al. 2003).
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1 Following what appears to be a general trend, these studies give the negative
2 results half their limit of quantification, considering the possibility of mercury
3 traces in the samples with lower values than the laboratory limit. Nevertheless,
4 this system can lead to an overestimation of the amount of mercury ingested
5 and, if the foodstuffs considered in the study include some consumed by a high
6 percentage of the population, it could identify them as important contributors to
7 the metal ingestion even if all their analytical results were negative.
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13 On the other hand, unlike the current study, which uses the individual foodstuffs
14 consumption data and individual body weights, most of the studies considered
15 are carried out with aggregated consumption data and "standard body weights".
16 Recalculating the weekly mercury ingestion taking average consumption data
17 for a standard 70 kg individual, we would obtain a weekly ingestion of 0.239 μg
18 Hg per kg. Thus, if we calculate the ingestion using population standard values
19 of the variables "body weight" (70Kg) and "consumption", the result we obtain is
20 much lower, which would indicate an underestimation of the ingestion in this
21 type of studies.
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28 At European level (SCOOP 2004), a weekly mercury ingestion of 29.2 μg is
29 estimated through fish and fish products, for an average 70 Kg body weight
30 individual. This equals 0.41 $\mu\text{g}/\text{kg}$ body weight per week. We must bear in mind
31 however, that this result is the average of data belonging to 13 countries, where
32 in most of them (with the exception of Norway) the consumption of fish products
33 (between 10 and 53 g/day, depending on the country) is a lot lower than in
34 Catalonia (62.1 g/day).
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40 Focusing in the percentage of population whose PTWI is bigger than that fixed
41 by the WHO (5 μg total Hg/kg body weight), we observe that the 1.4%
42 calculated in the Catalan population is slightly bigger than, for instance, that of
43 the Basque Country (Sanzo et al. 2001), where a 0.1% of individuals have an
44 intake of over 75% of the PTWI.
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1 In a recent study in France (Agence Francaise de Sécurité Sanitaire dels
2 Aliments 2007), 35% of the individuals studied had an intake of metylmercury
3 that was above the PTWI (1.6 µg metylmercury/kg body weight).

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5 In the present study 14.6% of the Catalan population is over the PTWI for
6 metylmercury, with an average weekly ingestion of 0.705 µgMeHg/kg body
7 weight.
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11 From a health protection point of view, fertile women and children (consumption
12 data no available for this group) are considered especially vulnerable to the
13 toxic effect of contaminants such as mercury. For that reason, most
14 consumption recommendations are addressed to them and they are the special
15 targets in toxic intake studies. According to Guallar et al. 2002 this advice
16 should be extended to the general adult population. However, great care must
17 be taken when providing health guidance related to fish consumption. While
18 some studies (Mozaffarian D et al. 2006) conclude that "among adults... the
19 benefits of fish intake exceed the potential risks" , others (Stern 2007) conclude
20 that there are easily available fish that offer both high polyunsaturated fatty
21 acids and low MeHg by choosing among the available fish, in order to maximize
22 the nutritional benefits, as for instance polyunsaturated fatty acids, and to
23 decrease the risks.

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33 In this way, it is important to stress that the population group with the highest
34 percentage of individuals that exceed the PTWI is the group of women aged
35 between 18 and 44: in the case of total mercury, 14 out of the 20 women (70%)
36 whose PTWI is higher than the recommended level are within this age group,
37 although this difference with the rest of them is not significant (p=0.11); in the
38 case of metylmercury, 107 out of the 187 women whose weekly intake is higher
39 than the PTWI belong to this age group (57%), a difference that is statistically
40 significant (p<0.02). Another vulnerable sector of the population is that with an
41 extremely high intake of fish.

42 **Conclusions**

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49 Total mercury was detected in 32.8% of 688 analyzed samples. The
50 concentration range oscillates between 0 and 0.98 mg/kg, with the exception of
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1 two predator fish samples with extreme values (1.4 and 7.6 mg/kg) The weekly
2 median intake of total mercury and methylmercury by general population in
3 Catalonia were estimated as 0.783 µg/kg and 0.705 µg/kg of body weight,
4 respectively. These values were similar to another studies set in the same
5 population, and fish group was the main contributor to these values. Women 18-
6 44 years old (fertile women group), had a higher percentage of individuals with
7 an intake above the PTWI for total mercury and methylmercury, which could
8 cause adverse effects to the developing foetuses in case of pregnancy.
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Table 1. Mean concentration of total mercury (mg/Kg) by food groups, daily intake of food groups (g/day) and mean weekly intake of total mercury and methylmercury ($\mu\text{g}/\text{kg}/\text{week}$), for the general population and by sex.

Fish products	n	Total mercury mean and range concentration (mg/Kg)	Daily intake (g/day)			Mean Mercury weekly intake ($\mu\text{g}/\text{kg}$ weight/week)			Mean Methylmercury weekly intake ($\mu\text{g}/\text{kg}$ weight/week)		
			Total (n=2158)	Men (n = 995)	Women (n=1163)	Total (n=2158)	Men (n = 995)	Women (n=1163)	Total (n=2158)	Men (n = 995)	Women (n=1163)
Fresh fish	93	0,285 (0- 7,60)	40,8	41,1	40,6	0,440	0,408	0,467	0,396	0,367	0,420
Canned fish	36	0,289 (0-0,88)	7,1	7,8	6,5	0,284	0,280	0,287	0,256	0,252	0,259
Bivalves	185	0,005 (0-0,20)	2,3	2,1	2,5	0,001	0,001	0,001	0,001	0,001	0,001
Cephalopods	185	0,047 (0-0,81)	6,9	8,1	5,9	0,022	0,025	0,019	0,019	0,022	0,017
Crustaceans	175	0,082 (0-0,90)	4,5	4,3	4,7	0,032	0,027	0,038	0,029	0,024	0,034
Gastropods	14	0,071 (0-0,30)	0,5	0,6	0,5	0,004	0,004	0,004	0,004	0,004	0,003
Total	688		62,1	63,9	60,6	0,783	0,745	0,815	0,705	0,671	0,734

Table 2a. Individuals with a total mercury weekly intake higher than the Tolerable weekly Intake (5 µg Hg/kg/week), by age and sex

Age groups (years)	General population		Men		Women	
	n	>PTWI n (%)	n	>PTWI n (%)	n	>PTWI n (%)
10-17	203	4 (2,0%)	114	2 (1,8%)	89	2 (2,2%)
18-24	309	8 (2,6%)	127	2 (1,6%)	182	6 (3,3%)
25-44	702	10 (1,4%)	326	2 (0,6%)	376	8 (2,1%)
45-64	602	9 (1,5%)	265	5 (1,9%)	337	4 (1,2%)
≥65	342	0 (0%)	163	0 (0%)	179	0 (0%)
Total	2158	31 (1,4%)	995	11 (1,1%)	1163	20 (1,7%)

Table 2b. Individuals with a methylmercury weekly intake above the PTWI (1,6 $\mu\text{g Hg/kg/week}$), by age groups and sex.

Age groups (years)	General population		Men		Women	
	n	>PTWI n (%)	n	>PTWI n (%)	n	>PTWI n (%)
10-17	203	26 (12,8%)	114	15 (13,2%)	89	11 (12,4%)
18-24	309	49 (15,9%)	127	16 (12,6%)	182	33 (18,1%)
25-44	702	115 (16,4%)	326	41 (12,6%)	376	74 (19,7%)
45-64	602	87 (14,5%)	265	37 (14,0%)	337	50 (14,8%)
≥ 65	342	38 (11,1%)	163	19 (11,7%)	179	19 (10,6%)
Total	2158	315 (14,6%)	995	128 (12,9%)	1163	187 (16,1%)

Table 3. Consumption, mercury concentration and mercury mean intake ($\mu\text{g}/\text{kg}$ body weight/week) due to fresh fish and canned fish species and its contribution to the total mercury intake.

	n	Mean consumption (g/day)	Mean mercury concentration (mg/Kg)	Mean mercury weekly intake ($\mu\text{g}/\text{kg}$ body weight/week) and contribution to the total mercury intake due to fresh fish (%)	Contribution to the total mercury intake (%)
Young hake	4	10.82	0.11	0.113 (26%)	14.5 %
Common sole	4	4.91	0.05	0.025 (6%)	3.2 %
Cod	1	4.58	0.00	0.000 (0%)	0.0%
Hake	6	3.79	0.18	0.069 (16%)	8.8%
Angler fish	3	3.34	0.24	0.080 (18%)	10.2%
Sardine	14	2.76	0.11	0.033 (8%)	4.6%
Salmon	9	2.32	0.03	0.008 (2%)	1.0%
European anchovy	2	1.97	0.00	0.00 (0%)	0.0%
Tuna	20	1.27	0.46	0.061 (14%)	7.8%
Mackerel	6	1.15	0.11	0.013 (3%)	1.6%
Gilthead seabream	3	0.81	0.11	0.010 (2%)	1.3%
European seabass	1	0.79	0.00	0.00 (0%)	0.0%
Megrim	2	0.42	0.04	0.002 (0%)	0.2%
Trout	1	0.31	0.00	0.00 (0%)	0.0%
Other species		1.54		0.021 (5%)	2.7%
Total fish		40.8		0.440	

	n	Mean consumption (g/day)	Mean mercury concentration (mg/Kg)	Mean mercury weekly intake ($\mu\text{g}/\text{kg}$ body weight/week) and percentage of contribution to the total mercury intake due to canned fish	Contribution to the total mercury intake (%)
Canned Tuna	13	6.51	0.395	0.2713 (95.5%)	34.7%
Canned Sardine	13	0.52	0.199	0.0100 (4.6%)	1.7%
Canned Bonito	6	0.06	0.338	0.0025 (0.9%)	0.3%
Canned Mackerel	4	0.02	0.168	0.0003 (0.1%)	0.0%
Total Canned Fish		7.11		0.2841	

Figure 1: Seafood group percentage of contribution to the mercury weekly intake of the Catalan general population.

