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


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This study estimates mercury and methylmercury levels in fish and fishery products commercialized in the city of Barcelona, Spain, from 2001 to 2007. Combining data of mercury levels in food with the consumption data of 2158 people (as the median of two 24-h recall), the total mercury intake of the Catalonian population was calculated. Mercury was detected in 32.8% of analysed samples. The general population average weekly intake of total mercury in the Catalonian population was 0.783 mg kg–1 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 mg kg–1 body weight. The fish group was the main contributor to this value, mainly due to predatory species.

Keywords: exposure; metals analysis; heavy metals – mercury; fish and fish products

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 (Toimela and Tahti 2004; Silva-Pereira et al. 2005).

For the general (non-occupationally exposed) population, the main route of exposure to Hg is via the diet, mainly through fish and seafood consumption (World Health Organisation (WHO), International Programme on Chemical Safety 1990). Other food sources than fish and seafood products can contain traces of Hg but are considered of lower concern. The MeHg 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.

Following human ingestion, MeHg, unlike elemental or inorganic forms, is almost completely absorbed from the gastrointestinal tract and distributed into all tissues, and it readily crosses the haematoencephalic barrier and the placenta. The central nervous system is considered to be the target organ for MeHg.

Prenatal exposition to MeHg may cause disorders on the brain development. (European Food Safety Authority (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 Hg in fish and fishery products of 0.5 mg kg–1 wet weight, except for some predatory species where the limit was 1.0 mg kg–1 wet weight (European Commission 2006).

On the other hand, the Joint Food and Agricultural Organization/World Health Organization (FAO/WHO) Expert Committee in Food Additives (JEFCA) established a provisional tolerable weekly intake (PTWI) for total Hg in fish and fishery products of 0.5 mg kg–1 body weight and also for MeHg at 1.6 mg kg–1 body weight. Exposure to Hg depends on diet habits and geographical location. Spain is the first fish market in the European Union, with an average per capita consumption of 40 kg per person per year (Failler 2004).

The main purpose of this study is to calculate Hg levels in fish and fishery products commercialized in the city of Barcelona and to combine these data with seafood products consumption data to estimate if there might be population groups in Catalonia with an Hg or MeHg intake that exceeds the PTWI recommended by the WHO.
Materials and methods

Sampling

Food samples were collected as part of the IQSA programme (Food Health Quality Research Program) (Arqués 2003). This surveillance programme developed by the city public health services started in 1984 with the aim of monitoring additives and pollutants, both chemical or microbiological, in the food sold in the city.

Foodstuffs taken for the IQSA programme were obtained in order to reflect food consumed in the city, to monitor levels of food hygiene and safety and to comply with existing regulations. Thus, sampling covers both diverse production origins (local, national and European Union, imported from other countries, etc.), and different marketing channels (municipal markets, supermarkets, and other retail stores).

From 2001 to 2007, 688 samples of 45 different fish and seafood products were obtained from retail and wholesale commerce in the city. They were classified in six groups: fresh fish (93 samples of 21 species), canned fish (36 of four species), bivalves (185 of seven species), cephalopods (185 of three species), crustaceans (175 of nine species), and gastropod (14 of one species).

Hg determination

The method used for total Hg determination in food was developed in the Barcelona Public Health Agency Laboratory and has been fully validated evaluating parameters such as trueness precision and linearity, along the working range. This method is accredited by the National Accreditation Body (Entidad Nacional de Acreditacion, ENAC) since 2000.

For the determination of total Hg, an Advanced Mercury Analyser AMA-254 (LECO Corp. St Joseph, MA, USA) was used. This is an elemental analyser based on an atomic absorption spectrophotometer that analyses the Hg levels in triturated and homogenized samples without any previous chemical pretreatment. An aliquot of 0.1 g of the homogenized sample was weighed into a nickel boat, which was introduced into a sealed combustion/catalysed tube where the decomposition process took place at 750°C by catalytic oxidation. Products from the decomposition were carried to the amalgamator using an oxygen flow where only Hg vapours are trapped onto the gold-plated ceramic tube. Once all by-products were passed through the measuring cell, the amalgamator was flash-heated at about 300°C and the Hg was dosed to the measuring cell using an atomic absorption spectrophotometer at 253.7 nm for each determination. The absorbance obtained for the sample was compared with the standards calibration, which was formed by the following standards, injecting 200 μg Hg l⁻¹: 10, 25, 50, 75, 100, 150, 200, 250, 300, 400, 500, 750, 1000, 1250 and 1500 μg l⁻¹. There were two ranges of calibration: the low one corresponded to standards 10–20 and the high one to standards 200–1500.

Different seafood products were used to validate the method. The validated concentration levels for total Hg were 0.10, 1 and 3 mg kg⁻¹, and twelve replicates were analysed for each level. Mean recovery of the process was 99.2%, precision was 11% and uncertainty 22%. The limit of quantification validated was 0.10 mg kg⁻¹.

The laboratory participates regularly (five to ten times a year) in proficiency testing for Hg in fish and seafood products as a tool to ensure the quality of generated data, always obtaining satisfactory results (z<2.0).

The analytical method for MeHg determination was not validated in the Barcelona Public Health Agency Laboratory when this study was developed. Therefore, total Hg was determined in fish samples. The results were multiplied by a factor of 0.90 (European Commission 2004). However, in 2007 some preliminary results were obtained from the Barcelona Public Health Agency Laboratory showing that MeHg was 89% of total Hg. This analytical method for MeHg is based on an extraction of 1 g of sample with chloridric acid (HCl 5 M) and CH₂Cl₂ (dichlormethane). Inorganic Hg elutes with the acid phase, which is analysed with CV-AAS. MeHg is calculated by extracting the inorganic Hg from the total Hg. The recovery for this method was 90–110%.

Hg intake estimation

In order to estimate the health risk associated with fish and seafood consumption, the weekly dietary Hg intake for the Catalan population was estimated as the product between the mean Hg concentration for each species (IQSA data) and its weekly consumption. As the number of samples with non-quantifiable Hg residues was large (67.2%), it was decided not to assign a value of one-half the limit of quantification (0.050 mg kg⁻¹) to these samples in order to avoid overestimation of the amount of Hg ingested (FAO/WHO 2005).

Individual food consumption and other individual data (weight, sex and age) were taken from the latest Catalan Nutrition Survey (ENCAT, 2002–2003) (Serra-Majem et al. 2007) conducted by the Catalan Government Department of Health. Dietary intake was assessed by means of two 24-h recalls on non-consecutive days. A probabilistic sample of 2158 individuals from 83 cities (995 males and 1163 females) aged 10–80 years was interviewed in 2002–2003.

As shown below, the Hg weekly intake for each of the 2158 individuals was calculated considering the individuals’ body weight and their daily personal
intake of 43 different species: 24 fresh fishes, four canned fishes; six crustaceans; five bivalve molluscs; three cephalopods; and one gastropod. For 13 species out of 43, an Hg concentration was not available in the IQSA programme. Therefore, a mean value calculated for species of the same food group was used.

The weekly intake (WI) for each individual was calculated using the following equation:

\[
WI = \Sigma (Is \times Cs) \times 7 \text{ days kg}^{-1} \text{ body weight}
\]

where Is is the daily intake of fish and seafood products species; and Cs is the total Hg content in these species (\(\mu g \text{ kg}^{-1}\)).

Once the weekly intake of 2158 individuals was calculated, a general population mean weekly intake was also estimated and also by sex and age (five age groups were considered: 10–17, 18–24, 25–44, 45–64, and >65 years). In order to estimate MeHg intakes, a factor of 0.90 was applied to Hg results (European Commission 2004).

**Statistical procedures**

All statistics were performed using SPSS 13.0 software. Hg weekly intakes were calculated for each individual, and its median intake in the general population as well as for sex and age group. Statistical significance were analysed between this Hg intake and studied variables by Mann–Whitney U- and Kruskal–Wallis tests. Chi-square (\(\chi^2\)) statistics tested differences between subgroups to evaluate the percentages of interviewed people above the PTWI.

**Results**

**Hg levels**

Hg was detected in 32.8% of analysed samples in the surveillance programme (226 of 688). By food groups (Table 1), mean concentration levels were: fish (0.285 mg kg\(^{-1}\)) [non-predators = 0.104 mg kg\(^{-1}\); predators = 0.750 mg kg\(^{-1}\)], canned fish (0.289 mg kg\(^{-1}\)), crustaceans (0.082 mg kg\(^{-1}\)), gastropods (0.071 mg kg\(^{-1}\)), cephalopods (0.047 mg kg\(^{-1}\)), and bivalves (0.005 mg kg\(^{-1}\)).

The highest Hg concentrations corresponded to two predatory species. In both cases the Hg levels exceeded the European limit of 1 mg kg\(^{-1}\): a porbeagle (Lamna nasus) and a little tuny (Euthynus quadripunctatus) with values of 7.6 and 1.4 mg kg\(^{-1}\), respectively. Hg concentration range in the rest of fish samples was between zero and 0.98 mg kg\(^{-1}\).

Any bivalves or gastropods were above the maximum level of 0.5 mg kg\(^{-1}\). Two crustaceans (lobster: 0.6 mg kg\(^{-1}\) and prawn: 0.9 mg kg\(^{-1}\)) and one cephalopod (octopus: 0.8 mg kg\(^{-1}\)) exceed the limit.

**Weekly intake of Hg and MeHg**

The general population average weekly intake of total Hg in the Catalanian population (Table 1) was 0.783 \(\mu g \text{ kg}^{-1}\) of body weight. Figure 1 and Table 1 show that the highest contribution to this value is provided by fish (0.440 \(\mu g \text{ kg}^{-1}\) body weight; 56.2% of the total intake) followed by canned fish (0.284 \(\mu g \text{ kg}^{-1}\) body weight; 36.3%), crustaceans (0.032 \(\mu g \text{ kg}^{-1}\) body weight), cephalopods (0.022 \(\mu g \text{ kg}^{-1}\) body weight), gastropods (0.004 \(\mu g \text{ kg}^{-1}\) body weight), and bivalves (0.001 \(\mu g \text{ kg}^{-1}\) body weight). In terms of species (Table 2), the most important contributors to the total Hg intake were canned tuna fish (0.27 \(\mu g \text{ kg}^{-1}\) body weight and week) and young hake (0.11 mg kg\(^{-1}\)). It was not possible to calculate the contribution of porbeagle and little tuny because consumption data were not available in the ENCAT Survey.

For MeHg, the general population average weekly intake was 0.705 \(\mu g \text{ MeHg kg}^{-1}\) body weight (Table 1). No differences between sex (\(p = 0.733\)) were found for Hg or MeHg intake, but differences between age groups existed in both cases (\(p < 0.026\)), being the youngest and the oldest of those with the lowest levels of Hg and MeHg intake.

Overall, 31 individuals (1.4% of the population) could have intakes above the PTWI for total Hg (Table 3a), and no differences were observed between sex (\(p = 0.180\)) or age group (\(p = 0.084\)). On the other hand, 187 women (16.1% of the population) would have MeHg intakes above the PTWI for MeHg (Table 3b). No difference was observed between age groups (\(p = 0.151\)), although a slight significant difference was observed between sex (\(p = 0.045\)), being women were the ones with a higher percentage.

**Discussion**

The general population average weekly intake of total Hg for Catalan population was 0.783 \(\mu g \text{ kg}^{-1}\) of body weight. This value is clearly below the recommended PTWI (5 \(\mu g \text{ kg}^{-1}\) body weight).

The main contribution to this intake was provided by fish, because of its higher consumption and higher Hg concentration, mainly through predator fish: fresh tuna is the most polluted, but its consumption is relatively low. However, the contribution of some species like young hake is due mainly to its high consumption. Only by considering tuna (canned and fresh), which represents 16% of fish intake, can it explain more than 42% of total Hg intake.

Other studies corroborate this higher contribution provided by fish and similar levels are found (SCOOP 2004; Sahuquillo et al. 2007).

In recent years there has been published several studies on Hg intake estimation. Nevertheless, methodological divergences, which a lot of times are not
Table 1. Mean concentration of total mercury (mg kg\(^{-1}\)) by food groups, daily intake of food groups (g day\(^{-1}\)) and mean weekly intake of total mercury and methylmercury (µg kg\(^{-1}\) week\(^{-1}\)), for the general population and by sex.

<table>
<thead>
<tr>
<th>Fish products</th>
<th>(n)</th>
<th>Total mercury mean and range concentration (mg kg(^{-1}))</th>
<th>Daily intake (g day(^{-1}))</th>
<th>Mean mercury weekly intake (µg kg(^{-1}) weight week(^{-1}))</th>
<th>Mean methylmercury weekly intake (µg kg(^{-1}) weight week(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total ((n = 2158)) Men ((n = 995)) Women ((n = 1163))</td>
<td></td>
<td>Total ((n = 2158)) Men ((n = 995)) Women ((n = 1163))</td>
<td>Total ((n = 2158)) Men ((n = 995)) Women ((n = 1163))</td>
</tr>
<tr>
<td>Fresh fish</td>
<td>93</td>
<td>0.285 ((0–7.60))</td>
<td>40.8</td>
<td>41.1</td>
<td>40.6</td>
</tr>
<tr>
<td>Canned fish</td>
<td>36</td>
<td>0.289 ((0–0.88))</td>
<td>7.1</td>
<td>7.8</td>
<td>6.5</td>
</tr>
<tr>
<td>Bivalves</td>
<td>185</td>
<td>0.005 ((0–0.20))</td>
<td>2.3</td>
<td>2.1</td>
<td>2.5</td>
</tr>
<tr>
<td>Cephalopods</td>
<td>185</td>
<td>0.047 ((0–0.81))</td>
<td>6.9</td>
<td>8.1</td>
<td>5.9</td>
</tr>
<tr>
<td>Crustaceans</td>
<td>175</td>
<td>0.082 ((0–0.90))</td>
<td>4.5</td>
<td>4.3</td>
<td>4.7</td>
</tr>
<tr>
<td>Gastropods</td>
<td>14</td>
<td>0.071 ((0–0.30))</td>
<td>0.5</td>
<td>0.6</td>
<td>0.5</td>
</tr>
<tr>
<td>Total</td>
<td>688</td>
<td>0.071 ((0–0.30))</td>
<td>62.1</td>
<td>63.9</td>
<td>60.6</td>
</tr>
</tbody>
</table>
specified, make comparisons tremendously difficult: foodstuffs included in each food group, the number of samples analysed, the different analytical methods and quantification limits, the different statistical treatment given to negative results, the different information sources used to estimate the consumption habits of the geographical area being investigated, the average weights used to work out, the amount of food ingested per kg of body weight, etc.

Always bearing in mind the caution required due to the different methodologies used, it can be observed that the present results are similar to those obtained in other studies carried out in the same geographical area.

In the ‘Total Diet’ study conducted by the Cataln government in 2002–2003, an average weekly ingestion of Hg of 0.892 \( \mu \text{g kg}^{-1} \) body weight was estimated (Departament de Salut, 
\textit{Agència Catalana de Seguretat Alimentaria} 2005). Other studies carried out in the same geographical area (the Cataln population) describe Hg ingestions for the standard adult male (70 kg) that range between 5.3 and 9.89 \( \mu \text{g day}^{-1} \) (weekly ingestion per kg of body weight = 0.53–0.989) (Llobet et al. 2003; Bocio et al. 2005; Falco et al. 2006).

Following on from what appears to be a general trend, these studies give the negative results half their limit of quantification, considering the possibility of Hg traces in the samples with lower values than the laboratory limit. Nevertheless, this system can lead to an overestimation of the amount of Hg ingested and, if the foodstuffs considered in the study include some consumed by a high percentage of the population, it could identify them as important contributors to the metal ingestion even if all their analytical results were negative.

On the other hand, unlike the current study, which uses the individual foodstuffs consumption data and individual body weights, most of the studies considered are carried out with aggregated consumption data and ‘standard body weights’.

Recalculating the weekly Hg ingestion by taking average consumption data for a standard 70 kg individual, one would obtain a weekly ingestion of

### Table 2. Consumption, mercury concentration and mercury mean intake (\( \mu \text{g kg}^{-1} \) body weight week\(^{-1} \)) due to fresh fish and canned fish species and its contribution to the total mercury intake.

<table>
<thead>
<tr>
<th>Fish</th>
<th>Mean consumption (g day(^{-1} ))</th>
<th>Mean mercury concentration (mg kg(^{-1} ))</th>
<th>Mean mercury weekly intake (( \mu \text{g kg}^{-1} ) body weight week(^{-1} )) and contribution to the total mercury intake due to fresh fish (%) or canned fish (%)</th>
<th>Contribution to the total mercury intake (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young hake</td>
<td>10.82</td>
<td>0.11</td>
<td>0.113 (26)</td>
<td>14.5</td>
</tr>
<tr>
<td>Common sole</td>
<td>4.91</td>
<td>0.05</td>
<td>0.025 (6)</td>
<td>3.2</td>
</tr>
<tr>
<td>Cod</td>
<td>4.58</td>
<td>0.00</td>
<td>0.000 (0)</td>
<td>0.0</td>
</tr>
<tr>
<td>Hake</td>
<td>3.79</td>
<td>0.18</td>
<td>0.069 (16)</td>
<td>8.8</td>
</tr>
<tr>
<td>Angler fish</td>
<td>3.34</td>
<td>0.24</td>
<td>0.080 (18)</td>
<td>10.2</td>
</tr>
<tr>
<td>Sardine</td>
<td>2.76</td>
<td>0.11</td>
<td>0.033 (8)</td>
<td>4.6</td>
</tr>
<tr>
<td>Salmon</td>
<td>2.52</td>
<td>0.03</td>
<td>0.008 (2)</td>
<td>1.0</td>
</tr>
<tr>
<td>European anchovy</td>
<td>1.97</td>
<td>0.00</td>
<td>0.000 (0)</td>
<td>0.0</td>
</tr>
<tr>
<td>Tuna</td>
<td>1.27</td>
<td>0.46</td>
<td>0.061 (14)</td>
<td>7.8</td>
</tr>
<tr>
<td>Mackerel</td>
<td>1.15</td>
<td>0.11</td>
<td>0.013 (3)</td>
<td>1.6</td>
</tr>
<tr>
<td>Gilthead seabream</td>
<td>0.81</td>
<td>0.11</td>
<td>0.010 (2)</td>
<td>1.3</td>
</tr>
<tr>
<td>European seabass</td>
<td>0.79</td>
<td>0.00</td>
<td>0.000 (0)</td>
<td>0.0</td>
</tr>
<tr>
<td>Megrim</td>
<td>0.42</td>
<td>0.04</td>
<td>0.002 (0)</td>
<td>0.2</td>
</tr>
<tr>
<td>Trout</td>
<td>0.31</td>
<td>0.00</td>
<td>0.000 (0)</td>
<td>0.0</td>
</tr>
<tr>
<td>Other species</td>
<td>1.54</td>
<td></td>
<td>0.021 (5)</td>
<td>2.7</td>
</tr>
<tr>
<td>Total fish</td>
<td>40.8</td>
<td></td>
<td>0.440</td>
<td></td>
</tr>
<tr>
<td>Canned tuna</td>
<td>6.51</td>
<td>0.395</td>
<td>0.2713 (95.5)</td>
<td>34.7</td>
</tr>
<tr>
<td>Canned sardine</td>
<td>0.52</td>
<td>0.199</td>
<td>0.0100 (4.6)</td>
<td>1.7</td>
</tr>
<tr>
<td>Canned bonito</td>
<td>0.06</td>
<td>0.338</td>
<td>0.0025 (0.9)</td>
<td>0.3</td>
</tr>
<tr>
<td>Canned mackerel</td>
<td>0.02</td>
<td>0.168</td>
<td>0.0003 (0.1)</td>
<td>0.0</td>
</tr>
<tr>
<td>Total canned fish</td>
<td>7.11</td>
<td></td>
<td>0.2841</td>
<td></td>
</tr>
</tbody>
</table>
0.239 \mu g kg^{-1} Hg. Thus, if we calculate the ingestion using population standard values of the variables ‘body weight’ (70 kg) and ‘consumption’, the result obtained is much lower, which would indicate an underestimation of the ingestion in this type of studies.

At a European level (SCOOP 2004), a weekly Hg ingestion of 29.2 \mu g is estimated through fish and fish products for an average 70 kg body weight individual. This equals 0.41 \mu g kg^{-1} body weight week^{-1}. It must be borne in mind, however, that this result is the average of data belonging to 13 countries, where in most of them (with the exception of Norway) the consumption of fish products (between 10 and 53 g day^{-1}, depending on the country) is a lot lower than in Catalonia (62.1 g day^{-1}).

Focusing in the percentage of population whose PTWI is bigger than that fixed by the WHO (5 \mu g total Hg kg^{-1} body weight), we observe that the 1.4% calculated in the Catalan population is slightly greater than, for instance, that of the Basque Country (Sanzo et al. 2001), where 0.1% of individuals have an intake of over 75% of the PTWI.

In a recent study in France (Agence Francaise de Sécurité Sanitaire des Aliments 2007), 35% of the individuals studied had an intake of MeHg that was above the PTWI (1.6 \mu g MeHg kg^{-1} body weight).

In the present study, 14.6% of the Catalan population is over the PTWI for MeHg, with an average weekly ingestion of 0.705 \mu g MeHg kg^{-1} body weight.

From a health protection point of view, fertile women and children (consumption data are not available for this group) are considered especially vulnerable to the toxic effect of contaminants such as Hg. For that reason, most consumption recommendations are addressed to them and they are the special targets in toxic intake studies. According to Guallar et al. (2002), this advice should be extended to the general adult population. However, great care must be taken when providing health guidance related to fish consumption. While some studies (Mozaffarian and Rimm 2006) conclude that ‘among adults… the benefits of fish intake exceed the potential risks’, others (Stern 2007) conclude that there are easily available fish that offer both high polyunsaturated fatty acids and low MeHg by choosing among the available fish, in order to maximize the nutritional benefits, as for instance polyunsaturated fatty acids, and to decrease the risks.

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

Conclusions

Total Hg was detected in 32.8% of 688 analysed samples. The concentration range oscillates between zero and 0.98 mg kg$^{-1}$, with the exception of two predator fish samples with extreme values (1.4 and 7.6 mg kg$^{-1}$). The weekly median intakes of total Hg and MeHg by the general population in Catalonia were estimated as 0.783 and 0.705 μg kg$^{-1}$ of body weight, respectively. These values were similar to another study set in the same population, and fish group was the main contributor to these values. Women of 18–44 years of age (fertile women group) had a higher percentage of individuals with an intake above the PTWI for total Hg and MeHg, which could cause adverse effects to developing foetuses in the case of pregnancy.

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