Mercury speciation in the hair of pre-school children living near a chlor-alkali plant

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Abstract

Exposure to mercury species was assessed in the hair of 130 Spanish children (age 4) from the general population in two areas. Both areas are exposed to different sources of mercury: a point source in Ribera d’Ebre (northeastern Spain) and a diffuse source on the island of Menorca (northwestern Mediterranean). The median MeHg values in the hair of children from Ribera d’Ebre (RE) were nearly twice (0.631 μg/g vs. 0.370 μg/g) those of children from Menorca (MC) (p<0.05). Total Hg showed a similar trend (REmedian: 0.720 μg/g vs. MCMedian: 0.476 μg/g). Nevertheless, inorganic mercury levels were similar in the two groups of children (REmedian: 0.186 μg/g vs. MCMedian: 0.210 μg/g). Two subgroups of the Ribera d’Ebre group were defined: children living in Flix (a village near a chlor-alkali plant) (RE1) and children living on the outskirts of Flix with no clear, direct influence of the plant (RE2). The mercury concentrations in RE1 were also significantly higher than those in Menorca, but no significant differences were found between Menorca and the RE2 subgroup. We evaluated the fish consumption of RE1, RE2 and MC and found that the Menorcan children consumed significantly less fish (p<0.05) than the other two subgroups. Children who consumed fish more than three times a week had higher MeHg concentrations (β(SE)=0.991 (0.279) than those who ate it less than once a week. Nevertheless, the differences in MeHg levels between children from Ribera d’Ebre and Menorca remained statistically significant after adjustment for fish intake and other variables (β(SE)=0.779 (0.203) for children from RE1). In conclusion, local sources other than seafood contribute significantly to MeHg content in hair in the two Ribera d’Ebre subgroups.

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Keywords: Mercury exposure; Mercury speciation; Methylmercury; Children’s hair; Routes of mercury intake; Sex effect

1. Introduction

Mercury is a naturally occurring element that is widespread in the environment (Schuster et al., 2002; Gustin, 2003; Mather and Pyle, 2004). Human activity has increased its release, mobilization and distribution into the environment (Yokoyama et al., 2000; Heebink and Hassett, 2002). Once released into the environment, inorganic mercury (I-Hg) undergoes biotic and abiotic transformations to become methylmercury (MeHg), a more toxic organic form (Wood et al., 1968; Greenwood, 1985; Palheta and Taylor, 1995; Kinjo et al., 1996). MeHg
can accumulate in animal tissues and cause biomagnification in the aquatic food chain. Its concentrations are the highest in top predatory species (de Souza Lima et al., 2000). Therefore, fish-consuming populations are a high-risk group for MeHg exposure (Choi, 1989; IPCS, 1990). MeHg is primarily neurotoxic (Castoldi et al., 2001; Costa et al., 2004) and affects neurodevelopment, especially during early development, the most susceptible period (IPCS, 2000).

Humans are exposed to MeHg primarily through seafood consumption (Clarkson and Strain, 2003; Daniels et al., 2004). Some studies have demonstrated the neurological injuries caused by low-level MeHg exposure (Mendola et al., 2002; Grandjean et al., 2004). Recent prospective epidemiological studies in the Faroe Islands, the Seychelles Islands and New Zealand have assessed the developmental effects of lower-level MeHg exposure in fish-consuming populations caused by maternal and fetal exposure to MeHg (Cernichiari et al., 1995; Grandjean et al., 1997; Crump et al., 1998).

Mercury biomarker studies have analyzed different human body fluids and tissues such as blood, urine, nails and hair (Adimado and Baah, 2002; Mortada et al., 2002; Mahaffey et al., 2004). Hair is a suitable medium for monitoring human exposure to mercury, because it reflects organ mercury levels and dietary intake (McDowell et al., 2004; Yasutake et al., 2004). Several studies have reported a strong correlation between MeHg content in hair and MeHg content in blood. The hair-to-blood ratio in humans has been estimated at approximately 250:1 (IPCS, 1990; Gill et al., 2002). Furthermore, MeHg levels in hair and total Hg are linearly related (Pellizzari et al., 1999), with MeHg accounting for 70–80% of total Hg (Cernichiari et al., 1995).

This study aims to assess mercury exposure through mercury speciation in the hair of 130 Spanish children (age 4). In order to evaluate the risks associated with a chlor-alkali plant and determine the role of fish consumption on mercury concentrations, we determined methylmercury (MeHg) and total mercury (T-Hg) levels in three subgroups living in different areas of northeastern Spain, one of which is near the plant.

2. Materials and methods

2.1. Areas of study

The survey was conducted in two different areas of Spain: the island of Menorca and Ribera d’Ebre. Menorca, located in the northwestern Mediterranean Sea, has a tourist economy with little industry. The Ribera d’Ebre area in northeastern Spain includes the village of Flix (ca. 5000 inhabitants), which is located near an electrochemical factory, and the rest of the towns of the administrative health district (ca. 12,000 inhabitants). The factory, built in 1898, has been producing chlorinated solvents for decades. Hg electrodes are used to produce chlorine gas (Cl2) and caustic soda. Unusually high atmospheric concentrations of hexachlorobenzene (HCB) have been found in Flix (Sala et al., 1999). The OSPAR Commission has reported mercury emissions in the area (OSPAR, 2004).

2.2. Population of study

The Ribera d’Ebre group (hereafter denoted RE) included children from the village of Flix (RE1) and its outskirts (RE2) born in the area’s main hospital between March 1997 and December 1999 for whom data on pregnancy, delivery, lactation and environment are available. A total of 102 of the 113 eligible children (90%) were enrolled and 71 provided complete exposure and outcome data up to the fourth-year visit (68.6%).

The Menorca group (hereafter denoted MC) included children born between January 1997 and March 1998. A total of 482 of the 513 eligible children (94%) were enrolled and 470 (97.5%) provided complete outcome data up to the fourth-year visit. Of these children, 59 (12.5%) were selected randomly to have the mercury in their hair measured. There were no differences between the children selected for mercury measurements and those who were not. RE was exposed to a large chlor-alkali plant located in Flix and MC was an external group with no point sources of mercury contamination, which was managed as a control group. We also considered that similar geographical populations may not necessarily have the same distribution of MeHg in hair because of different environmental conditions. Based on this assumption, RE was divided into two subgroups: the inhabitants of the village itself (n=38), denoted RE1, and the children from five other villages (n=33), denoted RE2.

The mothers of the selected children were contacted and the objectives and methods to be used were described to them. All of the mothers of the selected children agreed to participate and gave their consent. The study was approved by the ethics committee of the Institut Municipal d’Investigació Mèdica (IMIM, Spain). The selected children were all 4 years old and appeared healthy. None of them reported specific exposure to mercury or had major congenital anomalies or other diseases. The gender, weight and personal information of each subject were recorded. Each mother–infant pair was surveyed in person and the mothers were interviewed and asked to fill out a formal questionnaire about possible sources of mercury exposure such as
foods and beverages. The type and the quantity of foods and beverages ingested were recorded. The participants were asked about a range of aspects related to fish consumption, including the number of fish meals consumed per week, the type of fish and portion size. They were also asked how often they consumed different fish species in the last year, with the following choices: never, once per week, twice per week, 3 times per week, 4 times per week, 5–6 times per week, once per day or more. A lock of scalp hair approximately 5 cm long was obtained, usually from the nape. The samples were coded and stored in sealed plastic bags until analysis. Table 1 summarizes the results of the food-frequency questionnaires. The hair samples were analyzed for both MeHg and total mercury (T-Hg).

The RE subgroups were tested separately. Hence, the RE1 subgroup (children from the village near the electrochemical factory) and the RE2 subgroup (children from five villages surrounding Flix) were defined to test the differences in MeHg and T-Hg.

### 2.3. Statistical methods

The differences between the groups were tested for significance using nonparametric tests (Mann–Whitney U). Multiple linear regressions were used to test how variables other than location determined MeHg concentrations. The statistical regression models were performed with Statgraphics (version 4.0, Statistical Graphic Corporation, Englewood Cliffs, NJ, USA). Simple regression was used to test different model types (linear, multiplicative, exponential, logarithmic, reciprocal, etc.) that contain just one independent variable. The software program fitted several curvilinear models to the data and listed the models in decreasing order of R-squared. Of the models fitted, the best was the one with the highest R-squared value. All other statistical analyses were conducted with SPSS, version 12.01 for Windows (SPSS Inc., Chicago, IL). All means are arithmetic unless otherwise noted. Statistical significance was defined as \( p \leq 0.05 \). Cases with values above or below 1.5 times the interquartile range were not considered for the statistical analysis, as defined by the Tukey method.

### 2.4. Analytical procedures

MeHg was measured using the method described above (Montuori et al., 2004). The hair samples were cut finely and placed in a 100-mL beaker that had been ultrasonically washed with a nonionic surfactant. Then, they were rinsed thoroughly and allowed to air dry. Subsamples (20 mg) were then digested in a hot (100 °C) nitric acid solution (350 μL). After the digest had cooled to room temperature, an aliquot was transferred to a glass vial with an acetate buffer solution. Dipentylmercury and phenylmercury were added as internal standard and ethylation quality control respectively. Finally, after derivatization with aqueous NaBEt₄, extraction was

<table>
<thead>
<tr>
<th>Table 1 Description of variables of interest by area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Menorca (MC) ( (n=59) )</td>
</tr>
<tr>
<td><strong>Mother</strong></td>
</tr>
<tr>
<td>Maternal age, years (mean)</td>
</tr>
<tr>
<td>BMI, kg/m² (mean)</td>
</tr>
<tr>
<td><strong>Child</strong></td>
</tr>
<tr>
<td>Gender (%)</td>
</tr>
<tr>
<td>Females</td>
</tr>
<tr>
<td>Males</td>
</tr>
<tr>
<td>Gestational age, weeks (median)</td>
</tr>
<tr>
<td>Birthweight, g (mean)</td>
</tr>
<tr>
<td>MeHg (mean±SD) (μg/g) (median)</td>
</tr>
<tr>
<td>IHg (mean±SD) (μg/g) (median)</td>
</tr>
<tr>
<td>Food consumption (%)*</td>
</tr>
<tr>
<td>Fish</td>
</tr>
<tr>
<td>Shellfish</td>
</tr>
<tr>
<td>Poultry</td>
</tr>
<tr>
<td>Pork</td>
</tr>
<tr>
<td>Eggs</td>
</tr>
</tbody>
</table>

*Normalized weekly intake frequency at the age of 4 years according to the different food categories.
accomplished using solid-phase microextraction (SPME). The final detection was carried out using a gas chromatograph equipped with a cold-vapor atomic fluorescence spectrometry system (GC-CVAFS). A certified human-hair reference material from the National Institute of Environmental Studies of Japan (NIES, CRM No. 13) was used to validate the method. The limit of detection (LOD) obtained (0.040 μg/g) was three times the SD of the blank. The limit of quantification was 0.080 μg/g, the lowest point on the calibration plot. Digestion recoveries were calculated using a reference material analysis, obtaining yields of 75±11%. Each sample was analyzed in triplicate.

T-Hg was measured using the method described by Chen et al. (2002), with minor modifications. Hair samples were quantified by external calibration with \( R^2 > 0.99 \). The LOD obtained was 0.090 μg/g and the limit of quantification of the method was estimated at 0.160 μg/g. The accuracy of this method was validated by reference material analysis (GBW 09101, Shanghai Institute of Nuclear Research), which obtained 88%±6% \((n=7)\) with respect to the certified value. Each sample was analyzed in triplicate. I-Hg, defined in several studies (Chen et al., 2002) as \([\text{I-Hg}]=[\text{T-Hg}]-[\text{MeHg}]\), was calculated in order to determine which type of mercury (organic or inorganic) was the most sensitive to the differences observed between the target groups. About 2–3% of the total number of samples analyzed were below the LOD.

3. Results

Table 1 describes the characteristics of the population by study area. Children from MC were more likely to consume fish less than twice per week. Their mothers were more likely to have a lower body mass index (BMI) and be younger and more educated than the mothers from Ribera d’Ebre. Within the Ribera d’Ebre cohort, children from RE2 were more likely to be males and their mothers were more likely to be less educated.

The mean of MeHg hair concentrations of all of the children studied was 0.723±0.943 μg/g, ranging from 0.081 to 6.992 μg/g. The maximum concentrations were found in the children living in RE. The median value of MeHg concentrations in hair of RE was nearly twice that of MC (0.631 μg/g vs. 0.370 μg/g). The median value of MeHg concentrations of RE1 was more than twice that of MC and RE2. The same trends were observed for T-Hg, with a RE median of 0.720 μg/g (1.012 μg/g in RE1 and 0.610 μg/g in RE2) and a MC median of 0.476 μg/g. For I-Hg, similar concentrations were obtained for the different population groups, as shown in Table 1.

Multivariate analyses with the outlier and extreme data did not alter the results appreciably. The means and medians increased slightly when outlier and extreme data were included. Three outliers with mercury values above or below 1.5 times the interquartile range were removed from further analysis.

<table>
<thead>
<tr>
<th>Country</th>
<th>Year</th>
<th>n</th>
<th>Study group</th>
<th>Hair T-Hg (μg/g) ( \pm ) SD</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spain</td>
<td>2002</td>
<td>130</td>
<td>4 years</td>
<td>0.938±0.908 (0.189–5.627)</td>
<td>This study</td>
</tr>
<tr>
<td>Menorca (MC)</td>
<td>1998</td>
<td>59</td>
<td></td>
<td>0.720±0.664 (0.225–3.826)</td>
<td></td>
</tr>
<tr>
<td>Ribera d’Ebre</td>
<td>1998</td>
<td>71</td>
<td></td>
<td>1.093±1.016 (0.189–5.627)</td>
<td></td>
</tr>
<tr>
<td>Flix (RE1)</td>
<td>1996</td>
<td>38</td>
<td></td>
<td>1.259±1.079 (0.189–5.627)</td>
<td></td>
</tr>
<tr>
<td>outskirts Flix (RE2)</td>
<td>1996</td>
<td>33</td>
<td></td>
<td>0.922±0.948 (0.195–4.960)</td>
<td></td>
</tr>
<tr>
<td>Japan</td>
<td>2002–2003</td>
<td>327</td>
<td>7 years</td>
<td>1.64 (0.45–6.32)</td>
<td>Murata et al. (2004)</td>
</tr>
<tr>
<td>USA</td>
<td>1999–2000</td>
<td>838</td>
<td>1–5 years</td>
<td>0.22 (0.18–0.25) ( b )</td>
<td>McDowell et al. (2004)</td>
</tr>
<tr>
<td>Madeira Island</td>
<td>1998</td>
<td>113</td>
<td>7 years</td>
<td>4.09 (0.38–25.95)</td>
<td>Murata et al. (2002)</td>
</tr>
<tr>
<td>Germany</td>
<td>1996</td>
<td>245</td>
<td>8–10 years</td>
<td>0.23±0.20 (0.06–1.7)</td>
<td>Pesch et al. (2002)</td>
</tr>
<tr>
<td>Brazil, Amazon</td>
<td>1998</td>
<td>73</td>
<td>&lt;15 years</td>
<td>12.65±8.66 (0–44.53)</td>
<td>Barbosa et al. (2001)</td>
</tr>
<tr>
<td>Cururu</td>
<td>1998</td>
<td>86</td>
<td>&lt;10 years</td>
<td>4.76±2.09</td>
<td>Dorea et al. (2005)</td>
</tr>
<tr>
<td>Kaburu</td>
<td>1998</td>
<td>77</td>
<td>&lt;10 years</td>
<td>2.87±2.13</td>
<td>Dorea et al. (2005)</td>
</tr>
<tr>
<td>Seychelles Islands</td>
<td>1989–1990</td>
<td>708</td>
<td>5–6 years</td>
<td>6.5±3.3 (0.9–25.8)</td>
<td>Myers et al. (2000)</td>
</tr>
<tr>
<td>Faroe Islands</td>
<td>1986–1987</td>
<td>527</td>
<td>child, 12 months</td>
<td>1.12 (0.69–1.88)</td>
<td>Grandjean et al. (1997)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>903</td>
<td>child, 7 years</td>
<td>2.99 (1.7–6.1)</td>
<td></td>
</tr>
</tbody>
</table>

\( a \) Mean±SD.
\( b \) 95% CI (confidence interval).
Table 3 shows the results of the final linear regression model for mercury concentrations. The beta coefficients (\( \beta \)) are the coefficients from the multivariate regression analysis and the regression coefficient is the change in response per unit of change in the predictor. The standard errors (SE) are the standard errors of the regression coefficients.

Finally, considering the ratio of MeHg to total Hg in hair, similar results were found in MC (mean 75 ± 15, ranging from 37% to 98%) and RE (mean 76 ± 17, ranging from 35% to 98%). In all samples, the same trends were found when a reciprocal-x model of regression analysis was performed to determine the percentage of organic fraction of Hg (MeHg) in hair as a function of T-Hg content. The equation of the fitted model is

\[
\frac{\text{Hair-MeHg}}{\text{T-Hg}} = 97.2947 - 17.299.5/\text{T-Hg}.
\]

Since the \( P \)-value in the analysis of variance is less than 0.05, there is a statistically significant relationship between MeHg and T-Hg at the 95% confidence level. The R-squared statistics indicate that the model as fitted explains 70.83% of the variability in MeHg. The correlation coefficient of −0.8416 indicates a moderately strong relationship between the variables.

4. Discussion

We examined recent epidemiological studies of mercury exposure in children (Counter and Buchanan, 2004). Table 2 shows several T-Hg concentrations reported for other groups and/or cohorts of children worldwide. It is difficult to compare the data in this study with studies from other countries, because most of them are carried out with children of different ages (usually >10 years old).

Table 2 shows that the mean T-Hg levels in RE and MC are lower than those reported for populations exposed to MeHg through high-frequency fish consumption, such as the populations in the two most outstanding epidemiological studies of the past decade, carried out in the Seychelles Islands (Myers et al., 2000) and the Faroe Islands (Grandjean et al., 1997). The levels are also lower than those reported in the Amazon basin (Barbosa et al., 2001; Dorea et al., 2005), Japan and Madeira Island (Murata et al., 2002, 2004). In fact, the children in our study could be better compared to children in Germany (Pesch et al., 2002) and the US (McDowell et al., 2004). Spanish mercury levels are much higher that those in either study. In our research, the median Hg level among participants was over 5 times the median Hg level found in American children 1–5 years of age by the National Health and Nutrition Examination Survey (NHANES) (n=838). In addition, the mean T-Hg level was approximately 1/3 of the average T-Hg levels found in the Faroe Islands study. The USEPA's MeHg reference dose (RfD) is based on these results. The USEPA defines an RfD of 0.1 \( \mu g \) mercury/kg of body weight per day, which corresponds to a level of 1.0 \( \mu g/g \) hair (USEPA, 2005). Approximately 31% of the RE children (44.7% RE1 and 15.2% RE2) and about 6.8% of the MC children had mercury levels in their hair that exceeded this RfD, which shows the relevance of this contaminant.

We examined further interesting differences between the groups of children studied. Significant differences in T-Hg were found between RE and MC and between RE1 and MC (\( p < 0.05 \)). Surprisingly, the two RE subgroups were significantly different from each other and RE2 was not different from MC (\( p = 0.267 \)). Similar speciation results were obtained for MeHg. However, no significant differences in I-Hg (\( p > 0.05 \)) were found in the different groups of children.

These results indicate that children living in RE had higher concentrations of MeHg in their scalp hair than those in MC. Fish consumption (\( \beta \) (SE)=0.991 (0.279)) and sex (\( \beta \) (SE)= −0.424 (0.173)) were also associated with MeHg concentrations. This statement is in agreement with authors who have reported a significant association between the presence of MeHg in hair and being female. Some authors have reported that the children’s sex was the variable that most influenced Hg content in hair (Batista et al., 1996), with significantly higher Hg levels in girls than in boys. Similar findings were previously reported by several authors (Lie et al.,
1982; Watanabe et al., 1994), but Nakagawa (1995) found higher Hg concentrations in males than in females for a Japanese group.

However, the differences in MeHg content between the children from RE and MC remain statistically significant after adjustment for fish intake and sex ($\beta$ (SE) = 0.779 (0.203) for children from RE). However, there is no association between the I-Hg levels in hair and the frequency of fish consumption. No other variables, such as maternal body mass index, age, education, social class, birth weight or gestational age, were associated with individual MeHg levels. In addition, previous studies have shown that the meat of animals fed with fish meal or other fish products is likely to contribute to MeHg exposure (Ask Björnberg et al., 2003; Lindberg et al., 2004). Nevertheless, since no significant correlation was found between MeHg exposure and pork, poultry and egg intake, these possible sources of toxic MeHg exposure were discarded in our survey (Table 3). The reference value (0.494 for mercury) refers to females from MC who consumed fish less than once a week. A boy from RE1 who consumed fish more than 3 times per week would have a mercury concentration of 1.84: 0.494 (reference)+ 0.991 (estimate for fish consumption >3 per week)+ 0.779 (estimate for residence in Flix)− 0.424 (estimate for male). A girl with the same residence and fish consumption would have a mercury concentration of 2.264. In summary, although fish consumption is the most important variable influencing Hg content in hair, the place of residence has an effect.

The two RE subgroups had significant differences in MeHg content. Since these two subgroups had similar food-consumption habits, the higher MeHg content in the hair of children from Flix may be attributed to the industrial activity that has caused Hg emissions near Flix for over a century. Furthermore, no significant MeHg differences were found between MC and RE2. Therefore, the RE2 group is more similar to MC than to RE1, despite the geographic and food-consumption differences.

Nevertheless, Horvat et al. (2003) performed a study in a major Hg-production area and concluded that the local population may be exposed via consumption of Hg-contaminated food and inhalation. Other studies have shown higher exposure to mercury for people working in or living near mercury-related industries (Aks et al., 1995).

Finally, the MeHg percentages with respect to T-Hg concentrations in hair found in our study are in accordance with other studies (Lee et al., 2000; Barbosa et al., 2001). In fact, we found a significant correlation between MeHg and T-Hg levels in the scalp hair of children. The organic mercury fraction in hair increased significantly as T-Hg increased (Fig. 1). Therefore, increased T-Hg in hair reflects exposure to organic Hg, which accumulates more easily in the organism. These results are in agreement with other studies of the relationship between T-Hg and MeHg in blood and emphasize the strong correlation between Hg content in hair and blood (Mahaffey et al., 2004). Presumably, this could be because inorganic Hg has a faster excretion time than MeHg because it is more liposoluble. In fact, Clarkson (1993) and WHO (IPCS, 1990) reported that MeHg has a half-life in the body of about 50 days, with a range of 20–70 days, and a half-life in the hair of about 65 days, with a range of about 35–100 days, indicating that MeHg leaves the body slowly. I-Hg remains

![Comparison of Alternative Models](image)

Fig. 1. Reciprocal-x model of MeHg proportion in T-Hg hair concentrations for total children. % MeHg = $97.2947 - \frac{17,299.5}{[\text{T-Hg}]}$; $R$-squared = 70.83%.
roughly constant in the organism and MeHg is the main form and can accumulate.

In summary, we determined the MeHg levels in children’s scalp hair in two geographical areas of Spain. We believe that the different MeHg concentrations in the three groups of children are related to fish consumption, area of residence and gender. Our general conclusion is that people living close to the chlor-alkali plant (RE1: Flix subgroup) have higher levels of MeHg than people living within 15 km (RE2: Flix outskirts subgroup). Even though we found that the intake of Hg through food consumption (fish in particular) was possibly the most important parameter for all of the groups studied, the living environment could not be disregarded. Populations with similar fish consumption may receive different levels of exposure to mercury due to their proximity to mercury-related industrial activities.

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