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Hair mercury levels in relation to fish consumption in a community of the Moroccan Mediterranean coast

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Abstract

Coastal populations with high seafood consumption in the Mediterranean have a significant exposure to dietary methylmercury and areas where environmental mercury pollution is an issue due to industrial activities are of special concern. The present study was undertaken with the aim of assessing methylmercury exposure through fish consumption in a community of north Morocco and characterizing the relevant health risk. Concentrations of total mercury were determined in human hair, a biomarker of methylmercury exposure, and in locally consumed fish by cold vapour atomic absorption spectrophotometry. Based on consumption

frequencies reported by the 108 subjects included in the study the weekly intake of methylmercury was estimated and biomarker data were evaluated in relation to the estimated intake and the sociodemographic characteristics of the population. Multiple regression analysis was employed for interpretation of hair mercury data in relation to fish consumption frequency, gender and age of individuals. Mercury concentrations in hair ranged from 0.22 to 9.56 $\mu\text{g/g}$ (geometric mean 1.79 $\mu\text{g/g}$) and were closely related to fish intake. Fisherman and their families consumed fish 3-5 times per week and were the most exposed population subgroup. A high proportion of women of childbearing age (50%) had relatively high levels of mercury in their hair (3.08-7.88 $\mu\text{g/g}$).

Keywords: Mercury, Hair, Fish, Multiple regression analysis, Weekly intake, Biomarkers of exposure, Exposure assessment, Risk characterization, Morocco, Mediterranean Sea.

Introduction

Environmental mercury levels have increased considerably since the on-set of the industrial age. Even regions with no significant mercury releases, such as the Arctic, appear to be affected due to the transcontinental and global transport of mercury (Muir et al. 1999; AMAP 1998). Elemental or inorganic mercury released into the air or water becomes methylated in the aquatic environment where it accumulates in animal tissues and increases in concentration through the food chain (WHO 1990). As a result of bioaccumulation in the aquatic food chain, fish contain the highest levels of mercury, largely as methylmercury. Food sources other than fish and seafood products generally contain low concentrations of mercury, mostly in the form of inorganic mercury. Based on the available data the contribution to methylmercury exposure from these foods is considered to be insignificant. Mercury toxicity depends on its speciation and inorganic mercury is considerably less toxic than methylmercury. Methylmercury is highly toxic particularly to the nervous system, and the

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3 1 developing brain is thought to be the most sensitive target organ for methylmercury toxicity
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5 2 (WHO 1990). Based on the outcome of large epidemiological studies of fetal neurotoxicity in
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8 3 fish-eating population of the Seychelles, the Faroes Islands and New Zealand, several national
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10 4 and international bodies have revised their health based guidance values for methylmercury
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12 5 (EFSA 2004). On the other hand, non-neurological risks for adults associated with
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14 6 methylmercury, including the potential for adverse cardiac outcomes, have not yet been
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16 7 incorporated into risk assessments.
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21 9 Total mercury concentration in hair is a reliable marker of methylmercury exposure through
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23 10 fish consumption (Berglund et al. 2005). Compared to blood, hair is easier to collect and
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25 11 analysis is easier to carry out due to the comparatively high mercury content (Airey 1983).
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27 12 Determination of total mercury is more straightforward than that of methylmercury and this
28
29 13 allows extensive human monitoring to be done at reasonable costs. Moreover, each cm of hair
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31 14 approximately represents one-month exposure so that a time record of previous mercury
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33 15 exposure can be established for a subject depending on the hair length.
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38 17 The Mediterranean region is located on a mercuriferous belt that extends itself until central
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40 18 Asia and has been interested by intensive mercury mining of cinnabar deposits since ancient
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42 19 times (Hylander and Meili 2003). Mobilization of mercury has lead to an increase of the
43
44 20 concentrations of this element in water bodies, the atmosphere and all of the environmental
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46 21 compartments. Mercury emissions to the atmosphere from natural sources are an important
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48 22 element of the biogeochemical cycle of mercury (Working group on mercury 2001).
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50 23 Volatilisation of elemental mercury from surface waters and emissions from volcanoes in the
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52 24 Mediterranean region represents a significant contribution to the total mercury budget
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54 25 released in Europe and to the global atmosphere (Pirrone et al. 2001). Recent measurements
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of dissolved gaseous mercury suggested the existence of a gradient with higher concentrations in the eastern sector of the Mediterranean with respect to the western sector (Gårdfeldt et al. 2003). Natural abundance of mercury in the Mediterranean, a sea with a very slow water turnover, has raised the question of the potential impact on marine food chains (UNEP/FAO/WHO 1987). According to some authors, there is evidence that marine organisms living in this basin have higher mercury body burdens than the same (or similar) species in the Atlantic and other Oceans (Renzoni et al. 1998).

Apart from natural sources and mining, mercury has been extensively used in many products and industrial processes, with chlor-alkali production among the largest international uses of mercury in the world (Hylander and Meili 2003). In one of the common processes for chlor-alkali production – the mercury cell process – large quantities of mercury serve as a liquid cathode in electrolytic cells. The process releases mercury to the environment with both air emissions and water discharges, and mercury-containing solid and liquid wastes are generated. Today, emission controls and conversion to non-mercury technologies have led to a steady decrease in the consumption and releases of mercury in this industrial sector.

The small town of Martil in the Tetouan province is a harbour located on the Moroccan Mediterranean coast (35°37' N, 5°17' W). Several industrial plants, including one for chlorine and caustic soda production employing the mercury cell technology, are located in the area. As a result, mercury is released into the surrounding environment, mainly in the form of solid and liquid wastes (ca. 15 g Hg /kg dry matter) (Bryler-Iskane-Sheladia 1994). The extent of mercury contamination of the marine environment and its resources is actually an issue of importance due to fishing activities in this area and potential human exposure.

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3 1 The present investigation was undertaken with the aim of assessing the exposure of the local
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5 2 population to mercury through fish consumption and characterizing the relevant health risk.
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7 3 For this purpose, the occurrence of mercury in seafood was studied and the mercury human
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9 4 intake through fish consumption calculated. Hair was used as the biomarker of exposure and
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11 5 the focus of this paper is mainly on the relationship between hair mercury and fish
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13 6 consumption as well as the effect of other factors such as gender and age of individuals.
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15 7 Multiple regression analysis was employed for interpretation of hair mercury data in relation
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17 8 to the different independent variables.
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24 9 **Materials and Methods**

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26 10 *Subjects and biomarker assessment*

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28 11 Hair samples were collected from 108 individuals (68 males, 40 females) living in Martil
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30 12 from April to June 2001. The study area is shown in Figure 1. Each subject was asked to
31
32 13 complete a brief questionnaire detailing age, profession, fish species consumed, and fish
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34 14 consumption frequency (FCF) expressed as times per week. As far as possible, a random
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36 15 sample of the non-institutionalized civilian population of the study area was surveyed. People
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38 16 occupationally exposed to mercury (e.g. workers in chloral-alkali plants) were not included in
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40 17 the survey. Sociodemographic data are summarized in Table I.
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48 19 Hair samples (length = *ca* 2 cm) were cut from the occipital region of the scalp. Since hair
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50 20 treatments (e.g., bleaching, dyeing, artificial waving) may influence the mercury content of
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52 21 hair strands, only untreated hair was selected for the study. None of the surveyed subjects had
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54 22 dental amalgam fillings, a possible source of elemental mercury. Hair specimens were packed
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56 23 in polyethylene bags and stored at room temperature until analysis.
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1 *Fish sampling and treatment*

2 Based on information in the questionnaires, the most commonly consumed fish were pilchard
3 (*Sardina pilchardus* W., Clupeidae), common mullet (*Mugil cephalus* L., Mugilidae), and
4 hake (*Merluccius merluccius* L., Merlucciidae). Altogether about seventy samples of these
5 three species were collected between September 2000 and July 2001 in the fishing area shown
6 in Figure 1 and stored frozen in polyethylene bags pending analysis. Fillet was excised from
7 the left dorsal muscle and submitted to both dry weight determination and mercury
8 measurements. For the sake of comparison with literature values, mercury concentrations are
9 expressed hereafter on a fresh weight (*fw*) basis. Data can be converted to a dry weight (*dw*)
10 basis using *dw/fw* ratios of 0.38 for pilchard, 0.24 for common mullet, and 0.18 for hake.

12 *Mercury determination*

13 Hair sub-samples of approximately 300 mg were dried at 30°C for 24h and then digested with
14 4 ml of concentrated nitric acid and 2 ml of concentrated sulfuric acid (Suprapur, Merck,
15 Darmstadt, Germany). Both acids had negligible levels of Hg impurities. After digestion
16 (90°C, 4h), the analytical solutions were allowed to cool down and were diluted to 50 ml with
17 K₂Cr₂O₇ (1 ml) and deionized water obtained from a Milli-Q system (Millipore, Molsheim,
18 France). The same procedure was adopted for fish samples, but in this case subsamples of
19 about 1 g *fw* were digested.

21 Mercury was determined by cold vapour atomic absorption spectrophotometry using a Varian
22 Spectra AA20 equipped with a VGA-76 hydride generator. A stannous chloride solution was
23 used to reduce Hg to the elemental form.

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The quality control procedures included analysis of two reference materials (RMs), i.e. unspiked human hair (IAEA-086) and fish muscle (IAEA-407), both from the IAEA-MEL (Monaco). These RMs were regularly included in the analytical batches throughout the survey. The result of 14 independent replicate determinations of IAEA-086 was $0.59 \pm 0.03 \mu\text{g/g dw}$ (mean $\pm$ S.D.), compared to a target value of $0.573 \mu\text{g/g dw}$ with a 95% confidence interval (CI) of $0.534\text{--}0.612 \mu\text{g/g dw}$. The found value for IAEA-407 was $0.213 \pm 0.019 \mu\text{g/g dw}$, compared to a recommended value of 0.222 with a 95% CI of $0.216\text{--}0.228 \mu\text{g/g dw}$. Duplicate analyses were carried out throughout the study and the precision was always better than 5%. The method detection limit was $0.05 \mu\text{g/g dw}$ based on a sample weight of 0.5 g .

Analytical determinations were carried out at the Marine Environment Laboratory (IAEA, Monaco) and at the Department of Toxicology (National Institute of Health, Morocco).

Statistical Analysis

Mercury concentrations expressed on a *fw* basis were employed in all calculations. Distributions, extreme hair mercury values and correlations between variables were examined initially. After the initial analyses demonstrated that the hair mercury data were non-normally distributed, a logarithmic transformation was applied to normalize the distribution of data. Box plots, normal probability plots, and residual analysis were used to search for possible outliers; however it was not deemed necessary to eliminate any of the log-transformed values. Arithmetic means (AMs), geometric means (GMs), percentiles were calculated for the whole population sample and for several sub-groups identified on the basis of gender, age or FCF. In the comparisons between groups one-way ANOVA and ANCOVA on log-transformed data were used. Tests of statistical significance used a *p* value of 0.05.

The relationship between hair mercury and fish consumption as well as the effect of various independent variables that possibly influenced Hg concentrations were studied by multiple regression analysis. When age was included in regression models as an independent variable, five sub-groups were considered (i.e. 10-19, 20-29, 30-39, 40-49, >50 years). In all of the regression analyses $y' = \log_{10} (y+1)$, where y is the hair mercury concentration, was the dependent variable. Residual analysis showed that the logarithmic transformation substantially reduced deviations of the original dataset from normality, homoscedasticity and other assumptions of the linear regression model.

Statistical analyses were performed using Statistica (version 7.0, StatSoft, Inc.) and SPSS (version 13.0, SPSS Inc.).

Results and Discussion

Mercury in Hair

Table II summarizes descriptive statistics concerning mercury concentrations in hair. Fish consumption was firstly investigated as the most important predictor of hair mercury levels. In the population sample under study, the average weekly consumption of fish ranged from 0 to 5 times per week with an AM of 2.2 times per week (males 2.5, females 1.7 times per week). Nearly all subjects (96%) were fish consumers and 36% can be regarded as heavy consumers (consumption frequency of 3-5 times per week).

The original (untransformed) dataset of hair mercury concentrations is plotted in Figure 2 against FCF. It is clear that the relationship between the two variables is non-linear, as exemplified by a first order and a second order polynomial fit in Figure 2. As data deviated from the normal distribution required for the application of parametric statistics, a logarithmic

1 transformation of the independent variable was applied. Log-transformed data showed a linear
2 relationship to FCF according to the equation:
3
$$y' = 0.059 + 0.199x$$

4 with $R = 0.929$ and $p < 0.001$ ('model 1' in Table III).
5
6 The influence of gender and age was then considered and both factors were found to be
7 significantly related to the independent variable ('model 2', Table III). In the population
8 sample under study male subjects had higher concentrations in their hair due to higher FCFs.
9 When data were stratified for fish consumption, females averaged higher mercury
10 concentrations in their hair. This outcome might either be related to some specific
11 characteristics of the studied population or reflect a general pattern, e.g. differences in
12 physiology or metabolism. Higher levels of hair mercury in females than in males have been
13 found in several other studies (Benson and Gabica 1972, Sumino et al. 1975, Bulinski et al.
14 1979, Batista et al. 1996, Pellizzari et al. 1999, Babi et al. 2000, Agusa et al. 2005),
15 notwithstanding the general belief that male subjects exhibit a greater tendency to mercury
16 accumulation in their hair. However, failure to take into account the effect of fish
17 consumption (with stratification for FCF) and of possible other confounders (see Airey, 1983)
18 might have biased the results of most of the studies that investigated gender-specific
19 differences in hair mercury, so that no definitive conclusion can be drawn. Some authors also
20 found that gender is an insignificant factor for any form of mercury accumulation in hair
21 (Suzuki et al. 1976).
22
23 The effect of age on hair mercury is shown in Figure 3 for a selected population sub-group,
24 i.e. those with a FCF of 1 time per week. Figure 3 shows that mercury levels had a small
25 variability in females whereas in males a distinct trend towards a greater accumulation with

1 increasing ages was evident up to the age of 50 years. Thus separate regression models were
2 developed for males and females. As expected, age was a significant predictor of hair mercury
3 only in the case of male subjects ('model 3', Table III) whereas for females FCF was the only
4 independent variable acting on hair mercury ('model 4', Table III). A similar result was
5 obtained in studies of population groups in Cambodia and South Korea (Agusa et al. 2005;
6 Lee and Lee 1999). In particular, Lee and Lee (1999) reported that hair mercury levels of
7 Korean male subjects were significantly different according to their occupation and age
8 groups, but in females such variation was not detectable.

10 The dependence of hair mercury on age is possibly due to the greater fish consumption in
11 males, which results in an input rate higher than the mercury excretion rate with consequent
12 age-related accumulation. Twenty out of thirty subjects with hair mercury contents above 4
13 $\mu\text{g/g}$ were males, typically fishermen, with a FCF of 3-5 times per week. The hair content of
14 these thirty subjects exerted a great influence on the shape of the experimental plot as shown
15 in Figure 2. On the other hand age-related accumulation in males could be at least partially
16 due to other sources of exposure, outside the household, not accounted for by the present
17 study and possibly related to occupation and/or some specific habits (e.g. smoking).

19 A positive relationship between hair mercury levels and age has been reported by several
20 authors (Paccagnella et al. 1973, Sivalingam and Binti Sani 1980, Nakagawa 1995, Dickman
21 et al. 1998, Dumont et al. 1998, Pellizzari et al. 1999). Generally hair mercury has been found
22 to increase up to a given age, which changes depending on the population studied, and then
23 gradually decrease. This trend is consistent with that of subjects aged >50 years in this study,
24 i.e. almost constant levels of hair mercury in males and a decrease in females (not statistically
25 significant).

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1 *Mercury in Fish*

2 Table IV shows the results of mercury determination in muscle tissue of the most widely
3 consumed fish in the Martil area. These concentration values can be compared with
4 background concentrations for the same species in the Mediterranean. In doing this, attention
5 has to be paid to the size of organisms, as mercury accumulation in fish is generally an age-
6 related process.

7
8 Pilchards of a standard length of 15 cm from non-contaminated Mediterranean environments
9 generally have mercury concentrations in the range 0.08-0.12 µg/g (Del Prete e Bolletta 1984;
10 Buzina et al. 1989; Thibaud and Noel 1991; Pastor et al. 1994; Serrano et al. 1994; Bolletta
11 1996; Joiris et al. 1999; Storelli et al. 2003). Specimens from the study area had a length
12 range of 10-14 cm and a mean mercury concentration of 0.137 µg/g. In the case of common
13 mullet background concentrations for commonly exploited sizes (about 25 cm) are in the
14 range 0.05-0.09 µg/g. (Del Prete and Bolletta 1984; Crisetig et al. 1985; Focardi et al. 1991;
15 Cubadda et al. unpublished results), whereas in the study area individuals of this species had a
16 length range of 20-25 cm and a mean concentrations of 0.096 µg/g.

17
18 Comparison is less easily done in the case of hake because of the large spread of values found
19 in the literature, which is possibly due to the age-related changes in habitat and feeding habits
20 exhibited by this species. Juveniles are found in deep waters and feed on crustaceans
21 (especially euphausiids and amphipods), whereas adults move to coastal waters and mainly
22 feed on fish (including pilchard) and squids (Froese and Pauly 2006). This means that adult
23 individuals are most prone to mercury (and especially methylmercury) accumulation due to
24 their higher trophic level and preference for coastal environments, i.e., areas where mercury
25 contamination is more likely to occur. A realistic range of typical concentrations equal to

0.10-0.25 $\mu\text{g/g}$ can be estimated for specimens of 25-35 cm body length (Del Prete and Bolletta 1984; Horvat et al. 1989; Mikac e Picer 1985; Cannizzaro et al. 1986; Capelli et al. 1991; Vukadin et al. 1995; Bolletta 1996; Capelli et al. 2004; Storelli et al. 2005). In the study area hake specimens were for the most part of smaller size, nevertheless the mean mercury concentration was 0.101 $\mu\text{g/g}$ with a maximum of 0.124 $\mu\text{g/g}$.

Overall, these data are indicative of mercury environmental concentrations slightly above Mediterranean background levels in the Martil fishing area. However, none of the fish specimens analysed exceeded the maximum limit of 0.50 $\mu\text{g/g}$ established by the relevant EU regulation.

Exposure assessment

The GM of the hair mercury content of subjects who did not consume fish was 0.29 $\mu\text{g/g}$, whereas the least-square value obtained from the y-intercept of the regression line was 0.15 $\mu\text{g/g}$. These values reflect average background mercury exposures resulting from sources other than fish consumption, i.e. other foods and non-dietary sources. They are in good agreement with those reported for populations with no or marginal fish consumption in Sweden (0.04-0.32 $\mu\text{g/g}$, Lindberg et al. 2004), USA (0.29 $\mu\text{g/g}$, Pellizzari et al. 1999), Hong Kong (0.38 $\mu\text{g/g}$, Dickman et al. 1998), Albania (0.40 $\mu\text{g/g}$, Babi et al. 2000), and Chile (0.43 $\mu\text{g/g}$, Bruhn 1994).

The weekly intake of mercury as a function of FCF was estimated for the target population on the basis of concentration data in fish. As discussed above, the mercury content of commonly consumed fishes was similar on a *fw* basis and thus data were averaged for the purpose of intake estimation. Based on local uses, a fish serving amounts to about 200 g and this average figure was used in calculations. Methylmercury intake was estimated assuming that 90% of

mercury in fish is in the monomethylated form. The results are shown in Table V along with the corresponding mercury concentrations in hair. The latter were compared with those calculated by means of two simple models developed by WHO. Both assume a linear relationship between methylmercury intake and blood concentration of mercury at steady state, with subsequent conversion of blood concentrations to hair concentrations using a blood-to-hair ratio of 250. According to the first model, an intake of 1 µg of mercury per kg body weight per week in the form of methylmercury corresponds to a concentration of 10 µg Hg/L of blood and 2.5 mg Hg/kg of hair (WHO 2000). Alternatively, the quantitative relationship between exposure (intake) and concentration in hair can be expressed in a more meaningful way by means of a toxicokinetic model in the form of the following equation (adapted from WHO 1990):

$$C = \frac{d \times A \times f \times R}{b \times V \times 10^3}$$

where:

- C is the concentration of mercury in hair in µg/g,
- d is the daily dietary intake of methylmercury in µg,
- A is the fraction of methylmercury in the diet that is absorbed,
- f is the fraction of absorbed methylmercury that is found in the blood,
- R is the ratio of the concentration in hair (in µg/g) to that in blood (µg/L),
- b is the elimination constant,
- V is the blood volume in the body in L.

The hair mercury concentrations calculated by means of the first model (assuming a body weight of 60 kg) and the second model (assuming $A = 0.95$, $f = 0.05$, $R = 250$, $b = 0.01$, $V = 4.2$) were 0.83 and 0.81 µg/g, respectively, for a consumption frequency of 1 fish meal per week (weekly methylmercury intake = 20 µg), and 1.67 and 1.62 µg/g, respectively, for a

consumption frequency of 2 fish meals per week (weekly methylmercury intake = 40 µg). These figures agree closely with those reported in Table V. For consumption frequencies ≥ 3 , the calculated concentrations deviate substantially from the observed ones. WHO algorithms are based on single-compartment models and assume constant steady state blood-to-intake and blood-to-hair ratios. In the studied population this simple approach appears to be inadequate for the description of mercury biokinetics in heavy fish consumers, which resulted in a non-linear relationship between intakes and hair mercury levels (Figure 2).

Hair has been used as the biomarker of mercury exposure in several studies carried out in the Mediterranean region; those published since 1995 are summarized in Table VI. Comparisons with concentration levels found in other studies should be carried out with caution due to differences in study design, sampling strategies, and the existence of several confounding factors. For instance, repetitive seasonal changes in diet and metabolism cause a yearly pattern in the amount of mercury excreted into the hair and this lead to a bias that is rarely accounted for when such comparisons are made. Bearing in mind that several potential confounders may exist, nevertheless it can be observed that hair concentration values obtained in this study were higher than those obtained in the largest study carried out so far in the area (Belloni et al. 1998), when adjusted for the estimated weekly fish consumption of the various population groups. Mercury levels in women's hair were lower than those found by Gibicar et al. (2006) in a mercury-rich area of the Aegean sea when subjects with a FCF of 0-1 times per week are considered, and higher for FCFs ≥ 2 . Levels in children aged 10-16 were similar to that found by Batista et al. (1996) when adjusted for FCFs. In general, mercury levels in childrens hair in this study were lower than those of adults for both sexes when adjusted for FCFs ($p < 0.001$).

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The highest levels of mercury recorded in this study (about 9.5 µg/g) were found in the hair from people who had a FCF of 5 times per week and lived near the Martil estuary, which is located 4 km downstream the plant for chlorine and caustic soda production. It is possible that these individuals had higher-than-estimated intakes due to fishing in an area influenced by a hot spot of mercury contamination. The fish samples collected for this study were meant to determine an exposure estimate for the entire community and not to address extreme exposures due to preferential fishing in highly contaminated areas. In the literature, maximum levels far above 10 µg/g have been reported for several populations (Table VI), but they typically refer to people with FCFs ≥ 7 times per week.

Risk Characterization

In 2003 the Joint FAO/WHO Expert Committee on Food Additives (JECFA) revised the Provisional Tolerable Weekly Intake (PTWI) for methylmercury to 1.6 µg/kg body weight, whereas it was previously 3.3 µg/kg body weight, based on the epidemiological studies in the Faroe Islands and the Seychelles that investigated the relationship between maternal exposure to mercury and impaired neurodevelopment in their children (WHO 2004). For a person with a body weight of 60 kg the PTWI is equal to 96 µg, a level that in the target population is slightly exceeded by the consumers with a FCF of 5 times per week (estimated weekly intake = 100 µg). However it should be noticed that in our population sample no women reported a FCF above 4 times per week.

JECFA used the average from the two above-mentioned studies, 14 µg/g maternal hair-mercury, as an estimate of the concentration of methylmercury in maternal hair that reflects exposures that would have no appreciable adverse effect on the offspring. Concentration in hair was converted into maternal methylmercury intake using the one-compartment model and

1 steady state blood-to-hair and blood-to-intake ratios. A total uncertainty factor (UF) of 6.4
2 was applied to derive a PTWI that was considered sufficient to protect developing fetuses, the
3 most sensitive subgroup of the population. Application of the same UF to hair mercury would
4 give a concentration of 2.2 $\mu\text{g/g}$.

5
6 In our population sample the maximum mercury concentration in hair was 9.56 (male
7 subject), but the GM of hair mercury concentrations of women aged 18-45 years was 2.05
8 $\mu\text{g/g}$ and the 95% percentile was 6.83 $\mu\text{g/g}$. If the subgroup of women aged 20-40 years is
9 considered, the GM of hair concentrations was 2.44 $\mu\text{g/g}$ and the 95% percentile was 7.16
10 $\mu\text{g/g}$. Fishermen's wives aged 20-40 years are clearly the population subgroup that deserve
11 the highest attention due to average FCFs of 4 times per week and hair mercury
12 concentrations (GM) of 6.51 $\mu\text{g/g}$ (range = 5.42-7.88 $\mu\text{g/g}$).

13
14 In carrying out any risk characterization it should be kept in mind that health based guidance
15 values are based on several assumptions, each associated with some degree of uncertainty. In
16 fact, using a different approach and different UFs, the US Environmental Protection Agency
17 (EPA) and US National Research Council (NRC) established a reference dose (RfD) of 0.1
18 $\mu\text{g/g}$ body weight per day or 0.7 $\mu\text{g/g}$ body weight per week (42 $\mu\text{g/week}$ for a body weight of
19 60 kg), which corresponds to a hair mercury level of about 1 $\mu\text{g/g}$ (NRC 2000).

20
21 As a further complication, it should be also noticed that ethnic factors might possibly exert an
22 influence on dose-response relationships and steady state biomarker ratios used in the
23 evaluations. This is particularly true in regard to the use of hair as a biomarker of exposure.
24 Based on structural differences, hair is usually categorized into three ethnic groups (African,
25 Asian, and Caucasian) (Wolfram 2003). As noted by Budtz-Jorgensen et al. (2004), available

information suggests that Asian and Caucasian hair, although structurally different, is quite similar in regard to the mercury hair-to blood ratio, but African hair, although extensively studied, has not yet been calibrated in regard to methylmercury intake and blood concentration.

Conclusions

This is the first published study on the exposure of Moroccan population to mercury through fish consumption. The establishment of baseline levels for the study area enables future monitoring to be carried out with the aim to detect changes in (methyl)mercury exposure, especially in relation to the impact of industrial and other human activities on the marine ecosystem.

Based on the combined evidence gained through dietary intake estimation and use of hair as the biomarker of exposure it appeared that only a few among fishermen (and possibly their relatives) were likely to exceed the JECFA PTWI regularly, but on the other hand about 50% of women aged 18-45 years had hair mercury concentrations above 2.2 µg/g. On the whole, the present results indicate a narrow margin of safety for a sizeable part of the adult population including many women of childbearing age and support efforts to reduce methylmercury exposure.

Fish and seafood products are important sources of energy, protein, and a variety of essential nutrients, such as vitamins, trace elements, and omega-3 fatty acids. Because of the beneficial effects of fish consumption, the long-term aim would not be to reduce fish intake in the Martil community, but to develop advisories for heavy consumers on recommended fish species with low methylmercury content and to reduce mercury emissions in the environment resulting from human activities.

The mercury content of fish from the study area was indicative of mercury environmental concentrations slightly above Mediterranean background levels. However it cannot be excluded that fishing resources directly impacted from point sources of mercury emissions had higher contamination levels and were nevertheless exploited by a part of the fishing community in the study area. Mercury releases from chlor-alkali operations can be either substantially reduced by installation of proper devices/techniques for removal of mercury at points of emission generation or entirely eliminated by converting to a mercury-free process such as the membrane process – a more energy efficient technology indeed – as already happened in most of the industrialized countries. In the interest of health protection of these fishing communities, a substantial reduction of mercury releases from chlor-alkali and other industrial facilities would represent an important step forward.

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Table I. Sociodemographic characteristics of the sample population

Sample description		
Age		
Mean (years)		34
Range (years)		10-61
10-19 (%)		19
20-29 (%)		27
30-39 (%)		19
40-49 (%)		19
>50 (%)		16
Occupation		
Fishermen and their families (%)		25
Farmers and their families (%)		21
Other occupations/No occupation (%)		28
Students (%)		26

Table II. Descriptive statistics concerning hair mercury concentrations ($\mu\text{g/g}$)

Sample description	N	GM	AM	Min	Max	10th	25th	Percentile			
								50th	75th	90th	95th
All	108	1.79	2.90	0.22	9.56	0.49	0.93	1.46	5.03	7.79	8.85
Male	68	2.07	3.25	0.29	9.56	0.53	1.14	1.71	5.75	8.63	9.28
Female	40	1.40	2.29	0.22	7.88	0.35	0.71	1.18	3.82	5.59	6.30
Fish consumers ^a	65	1.04	1.21	0.29	3.56	0.49	0.75	1.16	1.46	1.99	2.33
Heavy fish consumers ^b	39	5.30	5.97	1.38	9.56	1.97	4.72	6.22	8.07	9.25	9.42

^a Subjects with a FCF of 1-2 times per week

^b Subjects with a FCF of 3-5 times per week

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Table III. Hair mercury regression models

	Standardized coefficients		Non standardized coefficients		
	<i>Beta</i>	<i>SE^a</i>	<i>B</i>	<i>SE^a</i>	<i>p</i> -level
<i>Model 1 (All subjects)</i>					
<i>R² = 0.862</i>					
Intercept			0.059	0.020	0.003
Fish consumption	0.929	0.036	0.199	0.008	<0.001
<i>Model 2 (All subjects)</i>					
<i>R² = 0.878</i>					
Intercept			-0.065	0.041	0.111
Gender	0.080	0.036	0.047	0.021	0.028
Age	0.088	0.035	0.002	0.001	0.013
Fish consumption	0.946	0.036	0.203	0.008	<0.001
<i>Model 3 (Males)</i>					
<i>R² = 0.877</i>					
Intercept			-0.033	0.035	0.002
Age	0.142	0.044	0.003	0.001	0.002
Fish consumption	0.902	0.044	0.194	0.009	<0.001
<i>Model 4 (Females)</i>					
<i>R² = 0.889</i>					
Intercept			0.058	0.026	0.029
Fish consumption	0.943	0.054	0.219	0.013	<0.001

^a Standard Error

Table IV. Mercury concentration in muscle tissue of the fish most commonly consumed by the population of Martil

Fish species	<i>n</i>	Length (cm)	Hg concentration (µg/g)	
			Mean±S.D.	Range
Pilchard (<i>Sardina pilchardus</i>)	26	10-14	0.137±0.042	0.087-0.175
Common mullet (<i>Mugil cephalus</i>)	24	20-25	0.096±0.017	0.091-0.115
Hake (<i>Merluccius merluccius</i>)	18	20-38	0.101±0.031	0.081-0.124

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Table V. Estimated weekly intake of mercury by the target population through fish consumption and related biomarker data

Consumption frequency Times/week	Estimated Intake ^a µg	Hg hair _{Obs} ^b µg/g	Hg hair _{Reg} ^c µg/g
0	0	0.29	0.15
1	20	1.04	0.81
2	40	1.70	1.86
3	60	3.69	3.53
4	80	6.68	6.16
5	100	9.23	10.32

^a Assumptions: Hg concentration in fish equal to the average of concentrations found in the most consumed species (0.111 µg/g), 90% of mercury in fish is methylmercury

^b Observed mercury concentration in hair samples (GM)

^c Hair mercury concentration obtained through regression model 1

Table VI. Hair mercury concentrations found in surveys concerning Mediterranean populations ($\mu\text{g/g}$)

Country	Study area	N	AM	Min	Max	Sample	Ref.
Croatia	Island of Vis	91	5.69	0.33	19.30	Population with a mean FCF of 4.5 times/week	Buzina <i>et al.</i> (1995)
Italy	North Adriatic sea	77	4.58	0.69	19.87	Fishermen and their relatives	Moretti <i>et al.</i> 1995
		92	1.40	0.03	12.50	Control population	
Italy	Sicily	123	4.11	0.23	30.20	Fishermen	Valentino <i>et al.</i> (1995)
		22	2.09	0.40	21.70	General population with fish consumption	
Spain	Tarragona province	233	0.77 ^a	0.18	2.44	Children (FCF 1-2 times/week in most cases)	Batista <i>et al.</i> (1996)
Italy	Various areas	ca. 1000	2.64	0.23	14.28	Coastal population	Belloni <i>et al.</i> (1998)
			5.76	0.62	28.52	Fishermen and fish dealers	
			1.86	0.23	9.41	Women	
			1.73	0.23	9.41	Pregnant women	
			2.25	0.25	9.90	Newborns	
			3.46	0.84	8.80	Pregnant women with high FCF	
			2.25	0.74	6.89	Newborns from women with high FCF	
Albania	<i>n.a.</i>	25	0.40	0.19	1.70	Population with marginal FCF	Babi <i>et al.</i> (2000)
Greece	Eastern Aegean islands	246	1.36	0.05	17.50	Women (FCF 1-3 times/week in most cases)	Gibicar <i>et al.</i> (2006)

^aGM

Figure 1. Map of the study area

Figure 2. Plot of hair mercury concentrations versus FCF (untransformed data).

Review Only

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Figure 3. Hair mercury concentrations in different age groups of male and female subjects with a FCF of 1 time per week (GMs in $\mu\text{g/g}$ and their 95% C.I.).