Using human hair as an indicator for exposure to mercury

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Abstract

OBJECTIVES: Exposure to mercury, a risk factor for neuro-developmental toxicity, was evaluated in the Czech Republic by performing mercury determination using human hair as an indicator.
METHODS: Hair samples from Czechs (n=311; 2–66 years old) were analyzed for mercury content. Total mercury was analysed by mercury analyzer AMA 254.
RESULTS: The highest total mercury content found in sampled hair was 3.55 μg/g and the lowest content was 0.015 μg/g. No correlation was found between the mercury levels in the sampled hair and the subject's age, gender, and the amount of amalgam fillings. A total of 38 hair samples were analyzed for methylmercury content.
CONCLUSION: The results show a positive correlation between the total mercury content in human hair and the consumption of marine and freshwater fish. Hair are a very good indicator of fish consumption.

Abbreviations & units
AMA – advance mercury analyzer
ANOVA – analysis of variance
ANCOVA – analysis of covariance
MeHg – methylmercury
LOD – limit of detection
THg – total mercury

INTRODUCTION

Mercury is highly toxic to humans, posing a particular threat to the development of children in utero and infants. Most of the mercury in the environment results from human activity, particularly from coal-fired power stations, residential heating systems, waste incinerators (WHO, 2007) agricultural applications and from the atmosphere (Kowalski & Wiercinski, 2007). Mercury can undergo complex transformations within the human body. Elemental mercury is absorbed through the lungs while ionic mercury is absorbed through the intestines. Elemental mercury is commonly oxidized to divalent ionic mercury and targets the brains and kidneys (Selid *et al.* 2009). Chronic low levels of the organic form of mercury (methylmercury) have been associated with subtle learning difficulties in children. The developing brain and its neurons is a primary target of mercury in the body (Kowalski & Wiercinski, 2007). Methylmercury bio-accumulated in fish and consumed by pregnant women may lead to neuro-developmental problems in developing fetuses. Transplacental exposure is the most dangerous, as the fetal brain is very sensitive. Humans are exposed to mercury through the ingestion of contaminated water and food (WHO, 2007) especially freshwater and marine fish. It is common knowledge that cook-

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ing does not eliminate mercury from fish. Methylmercury is distributed through the tissues of the entire body. The de-methyllation of the organic form of Hg, to the less toxic inorganic form, takes place in the liver (Havelkova *et al.* 2008). Methylmercury is accumulated in scalp hair. To determinate the effect of MeHg on humans, it is preferable to use a biomarker that reflects the MeHg concentration in the brain (Cernichiari *et al.* 1995). Mercury concentration in hair reflects the MeHg concentration in the blood during hair formation and is frequently used as a biomarker for evaluating MeHg exposure. Hair reflects the average exposure over the growth period of the segment. Once incorporated into the hair, mercury is stable, and can provide a history of the exposure (Phelps *et al.* 1980). The advantages of mercury assessment in hair are that hair collection is non-invasive and good response rates can be achieved in population subgroups that are difficult to obtain blood specimens from, such as children. Human hair is a stable matrix with its ease of collection, low cost and is easily transported and stored. Hair can provide us information about short and long term exposure as well (Esteban & Castano, 2009).

The aim of the present study is to assess the total mercury content in human hair used as an indicator for exposure to mercury, and to establish the relationship to age, gender, region, amalgam fillings and the consumption of freshwater and marine fish. This study also focused on evaluating the role of fish consumption and its relationship to mercury exposure.

The hypotheses that have been proposed and tested in this study:

1. mercury content in hair depends on the consumption of fish, and preference for marine or freshwater fish is not negligible
2. mercury content in hair depends not only on consumption of fish but also on region
3. mercury content in hair does not depend on age, sex and the amount of amalgam fillings

MATERIAL AND METHODS

Characteristic of the groups and the answer sheet. A total of 311 hair samples were collected from volunteers from the Czech Republic. These samples were collected during a two-month period and were then prepared for analysis. The main characteristics of the volunteers and the means of THg in these groups are given in Table 1.

Each sample was accompanied with a questionnaire that required data on age, gender, the number of amalgam fillings and region (city/village). The important criterion was the consumption of fish (distinquishing between freshwater and marine fish) because fish are the primary source of mercury and methylmercury intake. Fish consumption was entered by using the following numerical codes.

Consumption of marine fish:
0 – none
1 – sometimes
2 – often
3 – several times a month
4 – several times a week

Consumption of freshwater fish:
0 – none
1 – rarely
2 – once a month

Two additional factors were given to test the proposed hypotheses: total consumption of fish (0 -never or rarely, 1 - occasionally, 2 - frequently) and the preference for either marine or freshwater fish (for ***Figs. 3, 4***).

Sampling and analysis of hair. Collected samples were taken from the area of the cranium 3 cm above the nape of the neck. Because head hair grows approximately 1 cm per month, this is where the 1–2 cm long samples were taken from. These samples were cut into small fragments (2–5 mm) and then washed according to the standard method WHO/IAEA (washed once in acetone, then three times in water, and once more in acetone). The samples were then dried overnight in room temperature.

About 5 mg of the prepared sample was eventually used for the mercury analysis. Each sample was measured twice, but where standard deviation was higher than 10% the sample was measure three times. The content of total mercury was determined (LOD 1 μg/kg) by the direct method of cold vapours atomic absorption spectroscopy technique using an AMA 254 analyzer (Altec Ltd., Czech Republic). For the determination of moisture in the hair samples, the following procedure was used: approximately 250 mg of hair was obtained by mixing, ten individual samples were dried at 80 °C for 6 h, and the ratios of wet weight/dried weight were calculated. The ratios varied from 1.08 to 1.18.

The accuracy of the results of Hg content was validated using the standard reference material CRM No.13 HUMAN HAIR (National Institute for Environmental Studies).

A method for determinate methylmercury content was developed and described by Cejchanova *et al.* (2008). Methylmercury in hair was analyzed using an AMA 254 (Altec Ltd., Czech Republic) after acid digestion with hydrochlorid acid. The LOD was 0.1 μg/kg.

Statistical analysis. Total mercury in groups of respondents defined by the total consumption of fish was tested for normal distribution using the Kolmogorov-Smirnov test (Zar, 1999). Similarly, methylmercury and the proportion of methylmercury in total mercury were tested for normal distribution. Since total mercury and methylmercury did not fit the normal distribution ($P < 0.05$), they were logarithmically transformed before entering the analyses (first the value of THg and MeHg was transferred into pg/g dry weight, then the logarithm was taken).

A two-way analysis of variance was used to test the relationship between THg and the consumption of fish with respect to preference for either marine or freshwater fish. Because of the small number of people in groups that were categorized due to their consumption

and preferences for marine or freshwater fish, a simpler interpretation for the next analysis was used without indicating preferences for a particular fish type. A two-way ANOVA was used to test the dependence of total mercury on the consumption of fish in relation to gender, and region, respectively. An analysis of covariance was used to examine the dependence of total mercury on the consumption of fish using a continuous predictor such as age, or the number of amalgam fillings. A one-way ANOVA was used to test the relationship between methylmercury and the consumption of fish, as well as that between the proportion of methylmercury in total mercury and the consumption of fish. Since methylmercury and the proportion of methylmercury in total mercury was measured in just 38 people, no general models with additional predictors were calculated. Data analyses were performed using Statistica software (StatSoft Inc, 2007).

RESULTS AND DISCUSSION

Relationship between total mercury and consumption of fish. The mean of THg in single groups (male, female, people who live in towns, village, etc.) is shown in the ***Table 1***. All results of the THg values are given in μg/g dry weight. The mean of THg in all samples was 0.243 μg/g, and the median was 0.146. This was very low in compared to the values found in coastal countries such as Brazil, Italy, India, Japan and Canada. Carvalho *et al.* (2009) compared mercury the levels in hair to those from various parts of the world. They found that people who live in the Singapure and Indonesia had the highest THg values (5.92 and 5.59 μg/g, respectively). Also mentioned were mercury levels for people living in Canada (0.93 μg/g) and in Brazil (1.44 μg/g). Sarmani & Alakili (2004) presented data from an adult population in Malaysia and the THg mean content was 4.01 μg/g. Similar content (4.27 μg/g) of THg in hair was found by Adimaho & Baah (2002). From this point of the view, it seems that the countries with the highest THg levels are in Asia where a different life style combines with a very high consumption of fish and fishery products.

Some studies were focused on data for people living in Europe. Takagi *et al.* (1986) studied the mercury content in subject's hair from Poland (n=46). The mean value was 0.28 μg/g. It is practically the same amount found in our study (mean from all samples). On the other hand, Caroli *et al.* (1998) found mercury content in 95 Italians, and the geometric mean was 1.36 μg/g. This is more than four times the amount found in the Czech Republic or Poland. Kruzikova *et al.* (2008) examined hair from children in the Czech Republic from different sites (Praha, Jeseníky, Neratovice). They found analogous value (mean up to 0.3 μg/g) in comparison with our study. The lowest concentration of THg in hair in our study was 0.015 μg/g, the highest value was 3.55 μg/g. The median value is shown in the ***Fig. 1 and 2***.

Table 1. The main characterization of monitored groups.

		N	%	mean THg (μg/g)
Gender	♀	251	80.7	0.242
	♂	60	19.3	0.249
Region	city	236	75.9	0.269
	village	75	24.1	0.161
Consumption of marine fish	0	26	8.4	0.113
	1	95	30.5	0.159
	2	93	29.9	0.247
	3	86	27.7	0.254
	4	11	2.5	1.163
Consumption of freshwater fish	0	46	14.8	0.303
	1	232	74.6	0.214
	2	33	10.6	0.366
Total fish consumption (sum of consumption of freshwater and marine fish)	0	20	6.4	0.078
	1	18	5.8	0.156
	2	83	26.7	0.180
	3	87	28.0	0.229
	4	84	27.0	0.315
	5	11	3.5	0.538
	6	8	2.6	0.503

Figure 1 shows the content of THg in in relation to the consumption of marine fish. The median value of group 4 is impacted by the hair sample from the woman living in a town. This woman eats fish every day (this sample was included in the group where the consumption of marine fish was taken into account, code 4). We observed mercury exposure during a period when the women's consumption of fish was more varied. She spent one month in Madeira where the average consumption of marine fish is twice the amount than she ordinarily eats. A sample of her hair was taken a month after her return from Madeira. This sample was split into 3 parts (2 cm long) (sample code 204: normal intake of fish; sample code 205: intake of fish in the Madeira; sample code 206: back to normal intake) and these were analyzed similarly. The obtained results show a varying exposure to mercury from the time period in question. ***Table 2*** shows that the content of THg changed in the time period depending on fish intake. These results confirm that hair is a good indicator for monitoring mercury exposure, and can be used in forensic investigations for trace elements, to screen for the use of illicit drugs and for exposure assessments of occupational settings.

Figure 2 shows the content of THg in relation to the consumption of freshwater fish The content of THg rises with an increased intake of freshwater fish, and it is the same for the consumption of marine fish. There is a positive correlation between THg concentration in the

Table 2. The content of THg (mean value) in samples from the three time periods having different levels of marine fish consumption

consumption of marine fish	THg µg/g
normal intake of fish	1.828
intake of fish in the Madeira	3.455
return to normal intake	1.921

hair and the consumption of fish. The content of THg in hair for group 0 (consumption of fish none) is a little bit higher than in group 1 (consumption of fish rarely). The people in group 0 probably may eat marine fish althought did not eat freshwater fish and it may be reason why the THg in group wher are people who never eat freshwater fish have the higher amount of THg in hair.

The relationship between total mercury and preferences for eating marine or freshwater fish. The effects of marine/freshwater fish in the diet were examined. ***Fig. 3*** displays the relationship of the consumption marine/freshwater fish to total mercury in hair. When human diet consists mainly of marine fish, the THg content in their hair is statistically and significantly higher (p= 0.017) than those that prefer freshwater fish. This is in agreement with the study made by Pavlish (2004) in which he indicated that mercury exposure is higher when attributed to the consumption of marine fish.

The relationship between total mercury and region. Total mercury depends not only on the consumption of fish, but also on the particular region in question, it being higher in urban areas rather than in rural regions (***Fig. 4***). The effect of location. together with consumption of fish on total mercury in hair. was significant (P = 0.05). Kruzikova *et al.* (2008) discovered that children living in Prague statistically have a higher mercury content than children living in smaller towns or villages. This was determined by observing the higher level of consumption of fish. This effect could be caused by the fact that people living in bigger towns have healthier life styles. People in villages usually prefer traditional Czech

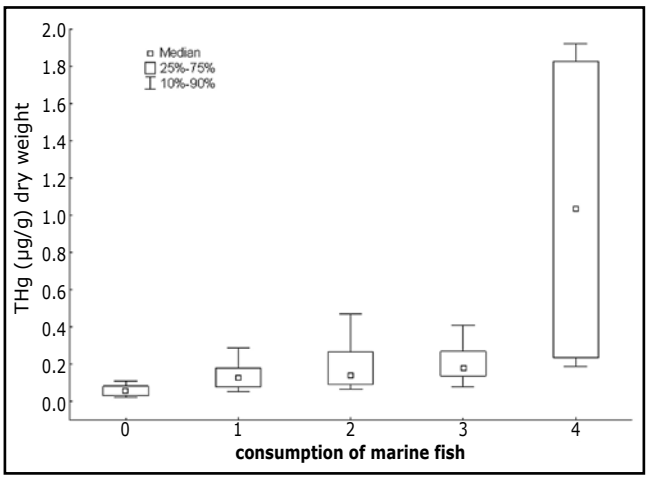

Figure 1. Content of THg (median value) in hair in relation to marine fish consumption

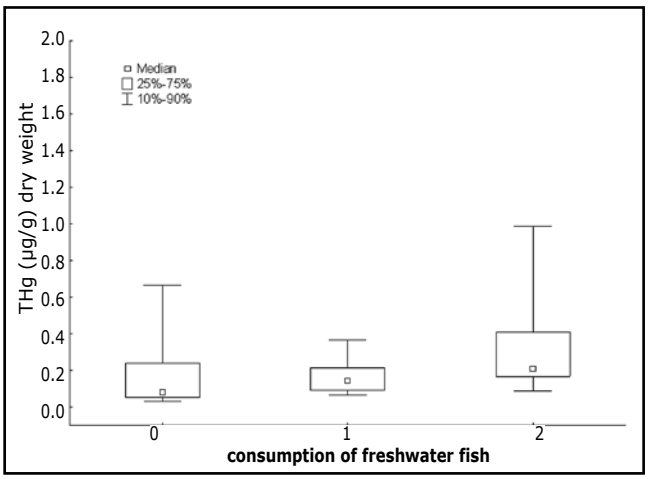

Figure 2. Content of THg (median value) in hair in relation to freshwater fish consumption

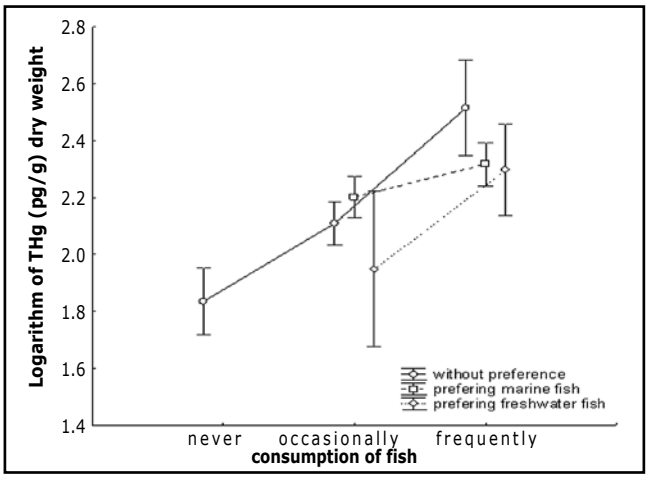

Figure 3. Result of two-way ANOVA. Means of total mercury (pg/g) dry weight (logarithmically transformed) in hair from groups of people according to the consumption of fish and their preference for marine or freshwater fish. Vertical bars denote 0.95 confidence intervals.

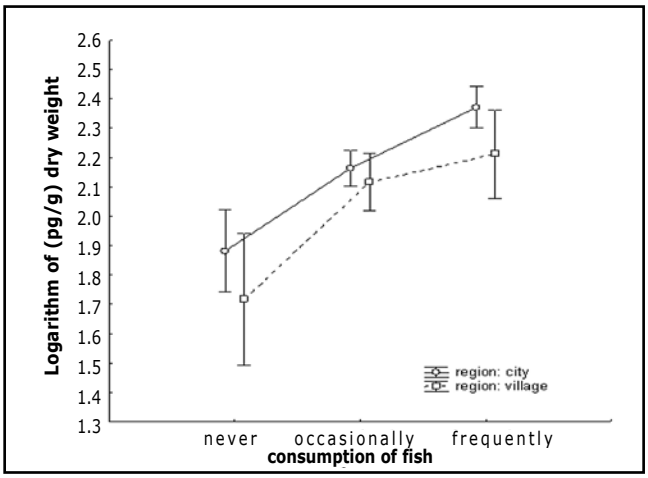

Figure 4. Result of two-way ANOVA. Means of total mercury (pg/g) dry weight (logarithmically transformed) in hair from groups of people in relation to the consumption of fish and region. Vertical bars denote 0.95 confidence intervals.

food which is not rich o the fish. So a regional influence might be a secondary effect on the amount of consumption. Another reason for this effect could be an exogenous contamination. Is very know that exogenous contamination of hair with mercury is not possible to remove from hair by washing (Catt & Katz, 1988). An exogenous contamination in the big town is very big and can have affect content of THg in hair.

The relationship between total mercury and other indices. In examining the possibility that mercury accumulation in hair might be affected by gender, our results show that total mercury levels were not different between male and female. Although dental amalgam is a potentially significant source of mercury exposure, since it can contain up to 50% elemental mercury, no correlation was found between mercury levels and the number of amalgam fillings in our study. Nevertheless, amalgam may represent an occupational risk for dentists and can cause the release of mercury into the atmosphere during cremation. Neither sex nor age has any additional effect on the THg content in hair. This agrees with a study by Diez *et al.* (2008) who studied the mercury content in people living in Naples (Italy). There was no correlation found between the mercury content in hair and hair dye (hair dye can include mercury) in a study by Kowalski & Wiercinski (2007). However, they did find that people who smoked cigarettes have significantly higher amounts of mercury in their hair ($0.14 \pm 0.09\,\mu g/g$) than do non smokers ($0.05 \pm 0.03\,\mu g/g$).

The relationship between methylmercury and the consumption of fish. The content of MeHg was analyzed in 38 hair samples. The mean of MeHg ranged from 0.062 to 0.129 μg/g. Similarl results were indicated by Cejchanova *et al.* (2008) (quantile 0.1 to 0.9 has value 0.07 to 0.19 μg/g) in children's hair from Kašperské Hory (Czech Republic). Comparable results for children from Starý Plzenec and Benešov (Czech Republic too) were found in this work (Cejchanova *et al.* 2008). In our study, the percentage of MeHg in THg ranged from 53% to 86%. Differences among similar groups were analyzed, but were not significant (one was ANOVA). It is wit agree of the study performed by Wranova *et al.* (2008) where the MeHg content in nonexposed population ranged up to 60% of THg. In the future, developing methods and data from this study could be used for the detailed monitoring of mercury specimens. This is important mainly for people working in dentistry and chemical laboratories.

Conclusions

The acquired data relating to mercury concentration in hair samples taken from Czech citizens indicates that the characteristics of the environment in which the testing took place are relatively free from harmful levels of this element. Our results confirm the influence of a diet rich in fish on people exhibiting high levels of mercury content. People who prefer marine fish instead of freshwater fish statistically have more mercury in their hair. However, the consumption of fish in the Czech Republic is very low compared to other countries, and the data indicates a low exposure to mercury in the Czech population. Our results also confirm that hair is an excellent indicator for mercury exposure and is sufficient for the monitoring of mercury over an extended period of time.

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