MERCURY AND METHYLMERCUry IN HAIR OF SELECTED GROUPS OF CZECH POPULATION

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SUMMARY

As the concentration of methylmercury (MeHg) in the environment is insignificant, hair can be used as a suitable matrix to estimate endogenous MeHg exposure. A validated analytical method with AMA 254 spectrometer was used for the determination of inorganic mercury and methylmercury species in the hair of dentists, workers in fish industry and professionally non-exposed adults. ANOVA and QC Expert software was used for statistical evaluation. The number of amalgam fillings in oral cavity, consumption of fish, gender, smoking habits and age of the subjects were taken into account. A significantly higher level of inorganic bound mercury (Hg\text{in}) was found in the hair of dentists. The number of amalgam fillings had a slightly significant effect on Hg\text{in}; fish consumption had a significant influence on MeHg and slightly also on Hg\text{in}. Other parameters were not significant.

Key words: methylmercury, hair, sampling, contamination, storage, population groups

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INTRODUCTION

From the toxicological point of view, mercury belongs to the very toxic elements for humans. Most important species for living organisms are elemental form, divalent mercury and organic mercury compounds. Health risks of exposure to mercury and its compounds are well described in literature (1–9). From these publications it follows that urine, blood and hair are the most used biomarkers of mercury body burden (with some limitations). Urine is considered to be the best indicator of body burden from used biomarkers of mercury body burden (with some limitations). Long-term exposure to elemental and inorganic mercury (Hg\text{in}), urine is considered to be the best indicator of body burden from used biomarkers of mercury body burden (with some limitations). Blood is a good biomarker of short-term exposures. The use of hair analysis in environmental medicine is discussed in some reports (10, 11) and in materials of Agency for toxic substances and disease registry (12).

From these publications we can summarized that hair is well suited as a biomarker for the methylmercury (MeHg) endogenous exposure. Very detailed overviews concerning hair studies are done in monographs (13, 14). Many papers have been devoted to the hair mercury and fish consumption, to the problems connected with amalgam fillings, professional exposure in dentist’s surgeries and/or combination of various factors (15–26).

For the Czech population, more data are available on total mercury concentration in blood and urine (adults and children) and hair (only children) was low during the whole study and did not exceed values representing health problems. For example, in 2007 (adults) and 2006 (children) medians of total mercury concentration were as follows: blood adults male 0.85 μg.l\textsuperscript{-1}, female 0.89 μg.l\textsuperscript{-1}; urine adults 1.1 μg.g\textsuperscript{-1} of creatinine; children blood 0.45 μg.l\textsuperscript{-1}; children urine 0.3 μg.g\textsuperscript{-1} of creatinine; children hair 0.13 μg.g\textsuperscript{-1}. These results were lower than limits defined by the German Committee for Human Biological Monitoring (HBM I) – blood 5 μg.l\textsuperscript{-1}, urine 5 μg.g\textsuperscript{-1} of creatinine (34) and with the U.S. EPA limit for hair – 1 μg.g\textsuperscript{-1}, and did not represent health risks for general Czech population. As the average concentration of mercury found in air was about 0.001 μg.m\textsuperscript{-3} and median of mercury concentration in drinking water 0.1 μg.l\textsuperscript{-1} (35), the main source of the mercury intake of non-exposed population is food consumption. An average exposure to the mercury in diet was 0.08 μg Hg per kg b.w. and week, i.e. about 5% of the provisional tolerable weekly intake (PTWI), defined by Joint FAO/WHO Expert Committee on Food Additives (JECFA) for MeHg (36).

In connection with changes of the dietary habits of Czech population (higher frequency of fish consumption in the last years), the Scientific Committee for Foodstuffs in the Czech Republic decided in 2004 that a study of body burden of methylmercury is necessary even that the fish consumption is still low in comparison with seawood states: < 5 kg per reference man per year with seafood accounting for 63% of this amount (1, 36). Results from this study will serve as a starting point for future biomonitoring studies. For this purpose we used a rapid and very simple validated method described previously (31, 37).
MATERIAL AND METHODS

Instrumentation
All measurements were performed on a single-purpose spectrometer AMA 254 (Altec Prague Ltd. Czech Republic) by cold vapour atomic absorption spectroscopy (CV AAS) technique with a previous combustion of the sample in oxygen atmosphere and amalgamation preconcentration (38).

Reagents, Vessels
Demineralised water (Millipore), 18.2 MΩ cm⁻¹, nitric acid (Suprapur grade, Merck, Germany), concentrated and 2 mol.l⁻¹ hydrochloric acid (Suprapur grade, Merck), standard solution for AAS Hg 1.000±0.002 g.l⁻¹ (Merck), methylmercury chloride (analytical standard, Riedel de Haen, Germany), oxygen of medical purity (Linde, Prague, Czech Republic).

Working standards were prepared from the standard solution and stabilized by 1% v/v HNO₃ and 0.01% w/v potassium dichromate (reagent grade, Lachema, Czech Republic).

Reference materials of hair were CRM GBW 07601 (total mercury) and IAEA 085 (methylmercury).

Before use, glass vessels and tubes were washed as described in (31).

Analytical Method for Mercury Determination
To determine the Hgₜₒᵗ, Hgᵢₜ, and MeHg levels among various groups, scalp hair samples (about 0.2 g) from the occipital area were cut on about 4 mm pieces, homogenized, washed by the procedure, recommended by WHO/IAEA (acetone, 3 times demineralized water, acetone) and dried at about 50 °C in drying oven.

Total mercury concentration was determined directly without mineralization: about 10 mg of the sample was weighed into the boat of AMA 254 analyser, dried, combusted, and decomposed in a stream of oxygen on a catalytic column. After quantitative mercury trapping on the surface of gold amalgamator, the mercury was completely evaporated at 900 °C into the optical cell and measured at 253.7 nm.

Methylmercury was leached from the subsample of hair by hydrochloric acid (2 mol.l⁻¹, v/w=40ml/g) for 4 h. After centrifugation, 100 μl of leachate was pipetted into AMA 254 boat and measured by the same way as total mercury.

The content of inorganic bound mercury was calculated as a difference between Hgtotal and MeHg.

RESULTS

Characteristic of Population Groups
Groups of dentists, workers in fish industry and professionally non-exposed adults (altogether 60 persons) were included in our study. Filled-in questionnaires included the number of amalgam fillings, frequency of fish consumption, gender, age, smoking habits and informed consent. Descriptive data are shown in Table 1.

Table 1. Characteristic of the population groups (%)

<table>
<thead>
<tr>
<th>Age (mean)</th>
<th>44 years (range 17–77)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area</td>
<td>%</td>
</tr>
<tr>
<td>town</td>
<td>77</td>
</tr>
<tr>
<td>region</td>
<td>23</td>
</tr>
<tr>
<td>Gender</td>
<td>%</td>
</tr>
<tr>
<td>male</td>
<td>28</td>
</tr>
<tr>
<td>female</td>
<td>72</td>
</tr>
<tr>
<td>Profession</td>
<td>%</td>
</tr>
<tr>
<td>dentist</td>
<td>35</td>
</tr>
<tr>
<td>professionally non-exposed</td>
<td>48</td>
</tr>
<tr>
<td>fresh water industry</td>
<td>17</td>
</tr>
<tr>
<td>Smoking habits</td>
<td>%</td>
</tr>
<tr>
<td>non-smoker</td>
<td>92</td>
</tr>
<tr>
<td>smoker</td>
<td>8</td>
</tr>
<tr>
<td>Fish consumption</td>
<td>%</td>
</tr>
<tr>
<td>never or exceptionally</td>
<td>23</td>
</tr>
<tr>
<td>1–2 per month</td>
<td>45</td>
</tr>
<tr>
<td>1–2 per week</td>
<td>27</td>
</tr>
<tr>
<td>more than 3 times per week</td>
<td>5</td>
</tr>
<tr>
<td>No. of amalgam fillings</td>
<td>%</td>
</tr>
<tr>
<td>0–5</td>
<td>55</td>
</tr>
<tr>
<td>6–10</td>
<td>37</td>
</tr>
<tr>
<td>11–15</td>
<td>5</td>
</tr>
<tr>
<td>16–20</td>
<td>3</td>
</tr>
</tbody>
</table>

QA/QC
All validation criteria used in analytical method have been published previously (31). The limit of detection for mercury was 0.7 ng.g⁻¹, limit of quantification was 1.4 ng.g⁻¹. Uncertainty was about 7% for Hgᵢₜ and 10% for MeHg. The accuracy of the method was confirmed by the analysis of certified reference materials of hair: IAEA 85 (determined value for MeHg 22.4±2 μg.g⁻¹, certified value 22.9 μg.g⁻¹, 95% C.I. 21.9-23.9 μg.g⁻¹); GBW 07601 (determined value for total Hg 0.38±0.04, certified value 0.36±0.05 μg.g⁻¹). The control sample analysed with every set of samples throughout the study was used to ensure the accuracy and compatibility of the results (see Shewhart’s diagram on Fig. 1).

Fig. 1. Shewhart’s regulation diagram for methylmercury dashed line: ± 3SD, dot and dashed line: ± 2SD, full line: mean.
Statistical Evaluation

The results obtained for MeHg and Hg in of dentists, workers in “freshwater” fish industry and professionally non-exposed adults (about 20 persons in each group) were statistically evaluated by ANOVA and QC Expert, and Student’s t test at the level $\alpha \geq 0.95$ (39).

All results had log-normal distribution and therefore before statistical evaluation, the data were modified by logarithmic transformation. Calculated critical value of the determination coefficient ($R^2$) at the level $\alpha = 0.95$ equals to 0.102 (higher values signalised significance of parameter).

In the evaluation, smoking habits were excluded because of the very low number of smokers (<10% from all participants).

The results on the hair concentration of mercury species are summarised in Table 2.

**Table 2. Results of hair mercury species in groups of dentists and professional non-exposed population**

<table>
<thead>
<tr>
<th>Non-exposed population groups</th>
<th>Hg total $\mu$g.g$^{-1}$</th>
<th>MeHg $\mu$g.g$^{-1}$</th>
<th>Hg$_{in}$ $\mu$g.g$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>0.33</td>
<td>0.20</td>
<td>0.10</td>
</tr>
<tr>
<td>Max</td>
<td>2.38</td>
<td>1.56</td>
<td>0.90</td>
</tr>
<tr>
<td>Min</td>
<td>0.07</td>
<td>0.04</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Percentile 0.9</td>
<td>1.00</td>
<td>0.72</td>
<td>0.26</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dentists group</th>
<th>Hg total $\mu$g.g$^{-1}$</th>
<th>MeHg $\mu$g.g$^{-1}$</th>
<th>Hg$_{in}$ $\mu$g.g$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>0.51</td>
<td>0.29</td>
<td>0.23</td>
</tr>
<tr>
<td>Max</td>
<td>5.69</td>
<td>1.60</td>
<td>4.45</td>
</tr>
<tr>
<td>Min</td>
<td>0.28</td>
<td>0.07</td>
<td>0.11</td>
</tr>
<tr>
<td>Percentile 0.9</td>
<td>4.17</td>
<td>1.00</td>
<td>2.60</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Influences of various mutually independent parameters (age, number of amalgam fillings, dietary habits, professional exposure and gender) on methylmercury and inorganic bound mercury species were studied. Mercury species in human hair were determined in groups of dentists, workers in “freshwater” fish industry and professionally non-exposed adults with different dietary habits.

We found that differences among groups of workers in “freshwater” fish industry, other professionally non-exposed adults and celiatics (different dietary habits) in all parameters under study were statistically non-significant and these groups were henceforth taken as one “non-exposed” group. Dentists were treated as a professionally exposed group.

In all groups no significant influence of age and gender was found for both species.

A significant difference between non-exposed groups and dentists was found for inorganic mercury form. At dentists, the total mercury concentration $>1\ \mu g.g^{-1}$ (median $H_g = 0.51 \ \mu g.g^{-1}$) was found in 5 persons (i.e. 29%) and median of abundance of $H_g$ was about 60% while median of abundance of $H_g$ in “non-exposed” group was about 30%. Four dentists had the concentration of $H_g > 1\ \mu g.g^{-1}$. This fact can be explicated by the exogenous contamination of dentist’s hair by inorganic form of mercury presented in the atmosphere of the working place. All non-dependent parameters seemed to be statistically non-significant in the group of dentists but differences could be covered up by the higher mercury content.

In the professionally non-exposed group we found that the median $H_g$ was 0.33 $\mu g.g^{-1}$, median of abundance of MeHg was 70%. Two persons, having concentration of MeHg higher than 1 $\mu g.g^{-1}$ (1.5 and 1.2 $\mu g.g^{-1}$ resp.), consumed fish more than 3 times per week. Four persons have a concentration of $H_g > 1\ \mu g.g^{-1}$.

The influence of amalgam fillings was non-significant for MeHg (determination coefficient 0.019) but significant for inorganic Hg (determination coefficient 0.193) (Fig. 2). The influence of fish consumption was significant for both MeHg (determination coefficient 0.533) and $H_g$ (determination coefficient 0.189) (Figs. 3 and 4).
CONCLUSION

From the obtained results we can conclude that our results for non-exposed population are in agreement with non-exposed population in countries with similar dietary habits. The levels of mercury, obtained in the group of dentists, are also similar to the levels described in the literature, and represent both endogenous and exogenous exposure. The number of amalgam fillings was significant for Hg; the significant influence of fish consumption and exogenous exposure. The number of amalgam fillings was levels described in the literature, and represent both endogenous population in countries with similar dietary habits. The levels of non-exposed population are in agreement with non-exposed population.

The higher mercury level in dentist’s hair can be ascribed to the work with amalgams and contaminated area of the surgery; the higher values in the “fish-eaters” were caused by MeHg (abundance of this form more than 70%).

Acknowledgement

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REFERENCES


Health and Conflict Prevention

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danger of infectious diseases. It is necessary to continue at the European level in the policy to develop and examine new vaccines, diagnostics and antibiotics and also the diagnostic and therapeutic strategies in order to protect people from possible future epidemics. Scott Ratzan vice president of Johnson and Johnson Company is discussing the role and possibilities of the private sector in global health problems in the 21st century. The tsunami 2004 disaster led to broad health diplomacy, which also led to enrolment of the Johnson and Johnson Company according to its commitments and responsibilities to customers, employees and communities. The author also stressed the role of modern media tools such as Google, America on Line and Yahoo in communication about acute serious global disasters and the possibilities to create direct links to organizations like the Red Cross, UNICEF or Oxfam. One of the concrete projects where the private sector was involved, was its support to a John Hopkins University project to support midwifery in Indonesian Aceh.

Jerker Liljestrand from Lund University and Jeffrey Lazarus from WHO Europe in Copenhagen describe the situation with HIV/AIDS infection. They demonstrate that in Botswana, Lesotho, Namibia, South Africa, Swaziland and Zimbabwe well over 20% of pregnant women are infected. “This creates millions of orphans and quadricentenaries, the teachers and health workers” (Koff Anan, former UN Secretary General, 2000). Social instability, worsening of security and slower development are consequences at the local level. The role of health care could not be isolated and without broader governmental and international involvement, success is not imaginable.

Zihe Rao from Tsinghua University in Beijing reports about combating SARS in China. He analyses the reasons, why the Chinese response was slow. The atypical pneumonia in Guangdong province was originally regarded as possibly caused by Chlamydia pneumoniae, which was the conclusion of a renowned senior microbiologist. On the contrary, the scientists at the Academy of Military Medical Sciences (AMMS) in Beijing isolated the virus in February 2003, but they were cautious to report it to the media or WHO network. Rao reports there was also some hesitation by the AMMS scientists to criticize the official Chlamydia story. The consequent Chinese reaction, according to Rao, was swift and efficient. 7,000 construction workers were quickly able to build a new hospital for 1,000 patients in Beijing. Parallel, in Beijing, which was particularly badly hit, schools and universities, theaters, and discos were closed. Still before the outbreak of SARS in China, the Chinese CDC was established in January 2002 to replace the Chinese Academy of Preventive Medicine. Epidemics of SARS led in China to important reforms in biomedicine, the life sciences and public health.

Anthony Zwi and Natalie Grove from New South Wales see the modern threats as a challenge to human security, and expressed reflections on health, fragile states and peace building. For the maintaining of human security, military forces are often needed to protect civilians and to quell the violence. There is a need of good projects for health care in affected areas. The responsible health sector has to build trust. Hans Blix and Manne Wangborg (Sweden) report about legal and diplomatic issues countering biological weapons. They describe some deficits in the Biological and Toxin Weapons Convention (BTWC) and were concerned about the lack of a verification mechanism in the early nineties. Although something has improved in last years, there is still a lot of reasons for public awareness in this field. Pekka Haavisto, EU Special Representative in Sudan and former Finnish minister for environment describes the EU response to the Darfour crisis. The EU supported the people in Darfour in the years 2004–2006 with nearly 500 million Euros. Anders Mellbourn (Halmstad University, former editor in chief of Stockholms daily newspaper Dagens Nyheter) in the last section of the book stresses the responsibility of media and journalists to report about the disasters in the world to allow proper and balanced action.

As reviewer, with the interest in diabetes and nutrition, I missed more remarks about nutrition, politics and economics. This became to be of great importance especially nowadays, when high prices of rice, wheat and oil are ruining large populations in Africa and South East Asia. The global epidemic of diabetes became a real danger not only for patients, but also for governments and for national economies. Perhaps questions on nutrition and on diabetes could be part of another book of this series.

To summarize: an important book showing the complexity of the multilayer modern world.

The book should be important reading for public health specialists, physicians in the fields of infection and tropical diseases, economists as well as for politicians.

The health issues do not know frontiers: an infection in one place of the world can lead, thanks to air traffic, to its outbreak in other place many thousands of kilometers apart. Changes in the climate, the fragility of food and energy resources are other fields which can influence human health. The book is fascinating reading for anybody, who observes the world from a wider perspective. There are not too many reasons for optimism – the early nineties. Although something has improved in last years, there is still a lot of reasons for public awareness in this field. Pekka Haavisto, EU Special Representative in Sudan and former Finnish minister for environment describes the EU response to the Darfour crisis. The EU supported the people in Darfour in the years 2004–2006 with nearly 500 million Euros. Anders Mellbourn (Halmstad University, former editor in chief of Stockholms daily newspaper Dagens Nyheter) in the last section of the book stresses the responsibility of media and journalists to report about the disasters in the world to allow proper and balanced action.

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Michal Anděl
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