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Mercury content in hairs of mother-child pairs in Slovakia as a biomarker of environmental exposure

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Abstract: The mercury content in hairs was determined in the framework of the European funded projects COPHES and DEMOCOPHES to test the feasibility of an EU-HBM (*Human Biomonitoring*) approach generating comparable data. The aim of the Slovak participation in DEMOCOPHES was to obtain and contribute the Slovak data to the harmonization of Human Biomonitoring. Pre-analytical and analytical phase for mercury in hair measurements and activities developed for the harmonization analysis within COPHES/DEMOCOPHES projects were conducted under a strict quality assurance program (QA/QC). Total mercury in hair was determined by thermal decomposition-gold amalgamation atomic absorption spectroscopy (AMA-254 Advanced Mercury Analyzer.) Two interlaboratory comparison investigations (ICIs) and two external quality assessment schemes (EQUAS) were conducted before the beginning of the COPHES/DEMOCOPHES projects. The laboratory successfully completed both ICIs and EQUAS schemes and was allowed to analyze all DEMOCOPHES samples of the Slovak Republic. In summary, 129 mother-child pairs were recruited to pilot study of DEMOCOPHES in Slovakia from two different locations representing urban and rural environment. The analyzed data from Slovakia showed relationship between frequency of fish meals consumption (especially sea fish and sea food products) and mercury concentrations in hair of mothers and children. The exposure levels for a sampled population in Slovakia (children 0.092 (0.080–0.106) [$\mu g.g^{-1}$], mother 0.130 (0.113–0.148) [$\mu g.g^{-1}$]) are below the EU level (children 0.145 (0.139–0.151) [$\mu g.g^{-1}$], mother 0.225 (0.216–0.234) [$\mu g.g^{-1}$]) up to an order of magnitude lower than the limit medically important (FAO/WHO health-based guidance value of 2.3 $\mu g.g^{-1}$ JECFA – Joint FAO/WHO Expert Committee on Food Additives).

Keywords: mercury, hair, human biomonitoring, COPHES/DEMOCOPHES

Introduction

Biomonitoring is a scientific technique for assessing human exposures to natural and synthetic compounds in the environment. It is based on an analysis of human tissues and fluids. It provides the direct method of determining if people have been exposed to particular substances, what are the magnitudes of their exposures are, and how these may be changing over time. Human biomonitoring (HBM) involves collecting samples from human volunteers – urine, blood, hair, saliva, nails or other human tissue – and measuring in those indicators of chemical uptake and effect, known as biomarkers.

The mercury content in hair was determined in the framework of the European funded projects COPHES (*Consortium to Perform Human Biomonitoring on a European Scale*) and DEMOCOPHES (*Demonstration of a Study to Coordinate and Perform Human Biomonitoring on a European Scale*), (2012–2013) to test the feasibility of an EU-HBM (*Human Biomonitoring*) approach, generating comparable data. The aim of the Slovak participation in DEMOCOPHES was to obtain and contribute the Slovak data to the harmonization of Human Biomonitoring in response to one of the objectives of the Environment and Health Action Plan (SCALE, 2004).

From the toxicological point of view, mercury is a well-known, very toxic element and its presence in the environment and the human food chain is a rising matter of concern. Mercury is a naturally occurring element in the earth's crust. It is also released into the environment through man-made sources such as the burning of fossil fuels, waste incineration, forest fires and wastewater discharge from plastic production plants. The mercury released into the air and water is washed into seas and oceans, ending up in fish. Large fish at the top of the food chain are particularly affected by this pollution. Chronic exposure to mercury can cause damage to the central nervous system, kidneys and stomach. It also affects the immune system, blood pressure and may cause behavioral problems. Dur-

ing pregnancy, methylmercury compounds cross the placenta and can affect the development of the foetus, causing neurodevelopmental abnormalities and loss of Intelligence Quotient (IQ) (Final report DEMOCOPHES, Human biomonitoring on a European scale, 2012).

Although the use of hair analysis in environmental medicine is discussed, hair is highly suitable for HBM studies due to non-invasive matrix (nature), ease to sample that can be transported and stored for long periods in a zipper bag or a paper envelope at room temperature. In addition, the analysis of hair can give information about exposure over the time and the seasonal variability by using sequential analysis. (Barr et al., 2005).

Hair is not a suitable matrix for many chemicals but it has a special application in the study of methyl-mercury exposure due to fish consumption (Harkins & Susten, 2003) and many studies in different populations had employed hair samples for this aim (Srogi, 2007). Scalp hair can be collected at different locations of the human head therefore Standard Operating Procedures must indicate the specific place for sampling, as variability has been observed in mercury levels in relation to where the sampling is done (Airey, 1983).

In some countries, HBM is already used extensively at national and regional level, but the results cannot be easily compared across projects and programmes. Improved comparability would allow a clearer understanding of exposure of the population to pollutants across Europe and would help identify potential high exposure populations and relations to possible sources, thus supporting the development of better regulations and preventive actions.

Material and Methods

Pre-analytical phase for mercury in hair and activities developed for the harmonization analysis within COPHES/DEMOCOPHES projects were conducted under a strict quality assurance program (QA/QC).

Hair samples were sampled according to Standard Operating Procedures which indicated the specific place for sampling on the head and specified different ways to proceed depending on the length of the hair. The hair was collected from the middle of the back of the head to the top. It was taken several strands of hair horizontally and roll it up to form a lock, fastened the lock with adhesive tape at 5–6 cm from the root of the hair. The sample was cut with the scissors as close to the scalp as possible, it was sealed to the end of the adhesive tape and labelled it with an arrow pointing to the end closest to the root. The hair sample was put into a paper envelope and labelled it with the sample identification code, packed into the ziplock plastic bag and delivered to laboratory. The 3 cm from the scalp segment was cut with the scissors in the polypropylene sample containers into the smallest pieces (COPHES – WP4 report, v5 19-10-2012).

Total mercury in hair was determined by thermal decomposition-gold amalgamation atomic absorption spectroscopy (AMA-254 Advanced Mercury Analyzer, Altec ltd., Prague). Measurement of Hg is based on the principle of mercury vapour generation and consecutive trapping and enrichment in gold amalgamator. After drying, the sample was ashed in an oxygen stream in a furnace at atmospheric pressure. The residues of ashing were taken through the amalgamator by the oxygen stream. The amalgamator was consecutively heated to high temperature and trapped mercury was released into measured cuvettes, where the absorption of Hg radiation at 254 nm was measured. No sample preparation is necessary. The SOP was validated and certified under ISO/IEC 17025: 2005 standard by the Slovak National Entity for Accreditation. The method had a limit of detection of 0.005 $\mu g.g^{-1}$ and limit of quantification 0.016 $\mu g.g^{-1}$.

Results and Discussion

The levels of the biomarkers measured in the general population are usually relatively low, in contrast to worker exposure to a specific known agent in a factory. Laboratory-introduced variations and detection limits must therefore be considered carefully. For the laboratories analyzing the DEMOCOPHES samples, more than a year of effective capacity building along with a strict quality assurance and control process made it possible to obtain comparable biomarker measurements. This was achieved through the Interlaboratory Comparison Investigations (ICI) and External Quality Assessment Scheme (EQUAS) organized by COPHES.

The ICI were seen as a first test for newcomers to HBM, to improve laboratory skills in measuring relatively low levels. Experienced laboratories could receive confirmation of their measuring skills. Laboratories received two control samples per round prepared from native biological samples with different concentrations of the target biomarkers and. During different rounds the range of expected concentrations was covered. The aim was to obtain comparability across the laboratories. In the EQUAS, the control samples were analyzed again in experienced reference laboratories. Their results were used to define an assigned value and tolerance ranges for each of the concentrations of biomarker in the samples. The aim was to attain

accuracy, i.e. the 'true' assigned values. Only those laboratories that successfully passed the quality assurance process, according to the criteria defined under COPHES/DEMOCOPHES, were allowed to analyze the DEMOCOPHES samples.

Two ICIs and two EQUAS schemes were conducted before the beginning of the COPHES/DEMOCOPHES projects. The telephone conferences and targeted communication were arranged after each round of ICI and EQUAS scheme. Our laboratory successfully completed all ICI and EQAS schemes and was allowed to analyze the all DEMOCOPHES samples of the Slovakia. In the laboratory obtained results and comparison with the result observed in the EU scale are shown in Table 1. The consensus values, both for low (C_{low}) and high (C_{high}) level of mercury in hair, calculated as the mean of the results of the participants after exclusion of the outliers, was used as reference. Number *n* of participating laboratories, relative standard deviation *RSD* and range of accepted value, equaled as twice of standard deviaton (SD), are given in the Table 1. To improve the accuracy of analytical results, in EQUAS scheme, the accuracy was evaluated by comparing results with the assigned values (calculated from the results of the reference laboratories — RLs are laboratories with a worldwide reputation and proven excellence through peer-reviewed publications and/or experience in organization of well-known international interlaboratory exercises) (Esteban, 2014).

Pre-analytical and analytical phases for mercury in hair were conducted under a strict quality assurance program (QA/QC). The hair samples were analyzed in series of max. 20 samples per day. QA/QC scheme included two checks of calibration point, two analysis of internal quality control samples (CRM), duplicated analysis of each sample and analysis of mother-child pairs samples in the same day.

In summary 129 mother-child pairs were recruited to pilot study of DEMOCOPHES in Slovakia from two different locations. Four schools in capital city Bratislava represented urban area and district Banská Bystrica in two villages Slovenská Ľupča a Brusno have been selected as rural environment. Mothers provided details on their living environment, nutrition, smoking behavior, way of life affecting exposure, employment social demography and other information that could help to explain the levels of the biomarkers measured in hair. Hair questionnaire survey included investigation of hair colors, structures, chemical treatments, washings, shampoos using.

Data in the COPHES/DEMOCOPHES project were based on samples from 17 European countries that participated in the study. In order to adjust for unequal sample numbers per country, a weighed geometric mean and a weighed 90th percentile was calculated as European exposure values. All countries were weighed in such a way that they contribute for 120 participants, except for Cyprus and Luxemburg who contribute for 60 participants. The values cannot be considered as 'European reference values' since the study population does not reflect a representative samples of the population within a country and since not all European countries are included.

The weighed geometric mean (95 % confidence interval, 95 % CI) for mercury in hair in the European study population equaled 0.145 (0.139—0.151) $\mu g.g^{-1}$ hair in children and 0.225 (0.216—0.234)

Tab. 1. Results of interlaboratory comparison investigations and external quality assessment schemes for mercury in hair.

	C_{low} [$\mu g.g^{-1}$]			C_{high} [$\mu g.g^{-1}$]		
	SK	EU	Range 2SD	SK	EU	Range 2SD
ICI 1	0.74	0.74	0.61—0.87	1.07	1.05	0.82—1.29
(n; RSD %)			*(12; 8.9)*			*(12; 11.3)*
ICI 2	0.20	0.18	0.18—0.23	1.45	1.47	1.28—1.66
(n; RSD %)			*(15; 13.6)*			*(13; 6.6)*
EQUAS 1	0.20	0.20	0.16—0.23	0.75	0.70	0.65—0.76
(n; RSD %)			*(16; 7.9)*			*(12; 4.0)*
Assigned value		0.20			0.76	
(n; RSD %)		*(16; 7.5)*			*(16; 4.0)*	
EQUAS 2	0.20	0.19	0.15—0.23	1.11	1.08	0.82—1.30
(n; RSD %)			*(17; 11.6)*			*(16; 9.0)*
Assigned value		0.18			1.08	
(n; RSD %)		*(16; 11.5)*			*(16; 9.3)*	

Tab. 2. Comparison values of Hg in hair of national values with EU exposure values.

Mercury in hair	unit	GM (95 %CI)	
		exposure values in Slovakia	EU exposure values
Children	μg g^{-1}	0.092 (0.080–0.106)	0.145 (0.139–0.151)
Mother		0.130 (0.113–0.148)	0.225 (0.216–0.234)

μg.g^{-1} hair in mothers. The 90th percentile was 0.800 μg.g^{-1} hair and 1.200 μg.g^{-1} hair, respectively. Mercury in hair was measured in 1836 children and 1839 mothers. Since the hair samples were cut at approximately 2–3 cm from the scalp, the biomarker provides a measure for the exposure to mercury in the last two to three months (assuming a hair growth rate of 1 cm per month). In this study, 1.4 % of the children and 3.4 % of the mothers had mercury levels above the FAO/WHO health-based guidance value 2.3 μg.g^{-1} according to JECFA (Joint FAO/WHO Expert Committee on Food Additives). The guidance value was defined by JECFA at their 67th meeting in 2006 (Final report DEMOCOPHES, Human biomonitoring on a European scale, 2012).

Values of mercury in hair of Slovakia obtained in the laboratory as the weighed geometric mean and comparison with the results observed in the EU scale are given in Table 1. Results achieved by Slovakia are very similar to those achieved at European level. Higher values of mercury presence in adults compared to children show a gradual accumulation of mercury in body over the life. The analyzed data in Slovakia showed relationship between frequency of fish meals consumption (especially sea fish and sea food products) and mercury concentrations in hair of mothers and child. Moreover, data showed a significant relationship in the area of family residence and education level of parents. People who live in urban environment and have higher education level are more exposed to environmental mercury. This finding could be explained by naturally higher consumption of sea products in urban areas and also by higher educated people. To the contrary, results had disapproved the association between other mercury sources as amalgam fillings or broken mercury thermomethers and energy saving lamps, for mothers as well as children sampled in the pilot study (Final report DEMOCOPHES, Human biomonitoring on a European scale, 2012).

If we compare average values of exposure levels (Tab. 2), the levels for a sampled population in Slovakia are below the EU level and up to an order of magnitude lower than the medically important limit (2.3 μg.g^{-1} JECFA). This conclusion can be explained by relatively low consumption of fish in Slovakia (Jajcaj M, Halzlová K, National report on the implementation of the project including data analysis and integrated interpretation, *16. 11. 2012*).

Conclusions

The project COPHES/DEMOCOPHES demonstrated that a harmonized approach on HBM can be achieved in Europe. Selected biomarker – mercury in hair with samples from mother-child pairs recruited in 17 European countries is a strongly advisable biomarker for assessing the exposure to mercury to their largely recognized toxicity and the widespread exposure to methyl mercury through diet. Only strict quality assurance and control can guarantee comparable and reliable results. Targeted communication must include social science strategies. Training, helpdesk and telephone conferences were essential additional tools to guide and maintain the harmonized approach.

As a representative for Slovakia in the harmonized approach, we might say that we had not faced any significant difficulties and were delighted to take part in the project and acquire new experience.

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References

Airey D (1983): Mercury in human hair due to environment and diet: a review. *Environmental Health Perspectives*, 52: 303–316.

Barr DB, Wilder LC, Caudill SP, Gonzalez AJ, Needham LL, Pirkle JL (2005): Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. *Environmental Health Perspectives*, 113: 192–200.

Esteban M, Castaño A et al. (2014): Mercury analysis in hair: Comparability and quality assessment within the transnational COPHES/ DEMOCOPHES project, *Environmental Research*, in press.

Harkins DK, Susten AS (2003): Hair analysis: exploring the state of the science. Environmental Health Perspectives, 111: 576–578.

Srogi K (2007): Mercury content of hair in different populations relative to fish consumption. Reviews of Environmental Contamination and Toxicology, 189: 107–130.