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Mercury analysis in hair: Comparability and quality assessment within the transnational COPHES/DEMOCOPHES project

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ABSTRACT

Human biomonitoring (HBM) is an effective tool for assessing actual exposure to chemicals that takes into account all routes of intake. Although hair analysis is considered to be an optimal biomarker for assessing mercury exposure, the lack of harmonization as regards sampling and analytical procedures has often limited the comparison of data at national and international level. The European-funded projects COPHES and DEMOCOPHES developed and tested a harmonized European approach to Human Biomonitoring in response to the European Environment and Health Action Plan.

Herein we describe the quality assurance program (QAP) for assessing mercury levels in hair samples from more than 1800 mother–child pairs recruited in 17 European countries. To ensure the comparability of the results, standard operating procedures (SOPs) for sampling and for mercury analysis were drafted and distributed to participating laboratories. Training sessions were organized for field workers and four external quality-assessment exercises (ICI/EQUAS), followed by the corresponding web conferences, were organized between March 2011 and February 2012. ICI/EQUAS used native hair samples at two mercury concentration ranges (0.20–0.71 and 0.80–1.63) per exercise. The results revealed relative standard deviations of 7.87–13.55% and 4.04–11.31% for the low and high mercury concentration ranges, respectively. A total of 16 out of 18 participating laboratories the QAP requirements and were allowed to analyze samples from the DEMOCOPHES pilot study. Web conferences after each ICI/EQUAS revealed this to be a new and effective tool for improving analytical performance and increasing capacity building. The

Abbreviations: AAS, Atomic Absorption Spectrometry; COPHES, Consortium to Perform Human Biomonitoring on a European Scale; CV-AAS, Cold Vapor Atomic Absorption Spectrometry; CV-AFS, Cold Vapor Atomic Fluorescence Spectrometry; DEMOCOPHES, Demonstration of a Study to Coordinate and Perform Human Biomonitoring on a European Scale; EQUAS, External Quality Assessment Schemes; ERM, European Certified Reference Material; ESBIO, Expert Team to Support Biomonitoring in Europe; HBM, Human Biomonitoring; IAEA, International Atomic Energy Agency Reference Material; ICI, Inter-laboratory Comparison Investigation; ICP-MS, Inductively Coupled Plasma Mass Spectrometry; LOQ, Limit of Quantification; NIES, National Institute for Environmental Studies Certified Reference Material; QAP, Quality Assurance Program; QAU, Quality Assurance Unit; RL, Reference Laboratory; RSD, Relative Standard Deviation; SOP, Standard Operating Procedures; TWI, Tolerable Weekly Intake

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procedure developed and tested in COPHES/DEMOCOPHES would be optimal for application on a global scale as regards implementation of the Minamata Convention on Mercury.

1. Introduction

Mercury is a well-known toxin and its presence in the environment and in the human food chain is a matter of increasing concern. In light of this, the Minamata Convention, a global action to protect human health and the environment by reducing exposure to mercury and mercury compounds, was signed in October 2013. Article 19 of this Treaty calls for harmonized methodologies for, amongst others, monitoring mercury levels in the population (http://www.mercuryconvention.org).

Mercury contamination is a worldwide problem because of its long-range transport and its ubiquity in global marine ecosystems, thus meaning that the entire global population is potentially exposed. Indeed, the general population, with non-occupational exposure, is mainly exposed to mercury via food, with fish being the major dietary contributor, and to a lesser extent by amalgam fillings (UNEP/WHO, 2002). According to data from the EFSA, high fish consumers may exceed the tolerable weekly intake (TWI) for methylmercury (MeHg) of 1.3 μg Hg/kg b.w. by up to sixfold (EFSA, 2012).

The analysis of mercury levels in hair could become a highly recommended tool for monitoring mercury levels in the general population. Methylmercury is the main chemical form of mercury in fish and its analysis in hair is accepted as a reliable estimate of the internal dose (Harkins and Susten, 2003). Methylmercury is incorporated into the follicle during hair formation and shows a direct correlation with blood levels (Clarkson and Magos, 2006; UNEP/WHO, 2008). Moreover, as it does not return to the blood once incorporated, this matrix can give information about the history of exposure by analyzing different longitudinal sections of hair strands (UNEP/WHO, 2008). Hair is a non-invasive matrix that is easy to sample and does not require highly trained technicians. In addition, hair samples can be transported and conserved at room temperature, thereby facilitating logistics during study fieldwork. With regard to storage, the methylmercury content in hair in long-term storage remains unchanged over many years provided the sample is stored under dry and dark conditions at room temperature (Horvat et al., 2012). However, the fact that methylmercury analysis is time-consuming and expensive means that its use in large human biomonitoring (HBM) surveys is limited. The measurement of total mercury in hair is generally accepted as a surrogate for methylmercury exposure as it is present in hair in a high percentage (approx. 80% or more) and analysis at different concentrations is simpler and cheaper (Clarkson and Magos, 2006; McDowell et al., 2004; Berglund et al., 2005; UNEP/WHO, 2008; Poulin and Gibbs, 2008; EFSA, 2012). In addition, reference material for mercury in hair analysis is available (e.g. NIES, ERM or IAEA), an aspect that is essential when it comes to validating analytical methods.

Despite the multiples advantages of using hair in HBM surveys, there are some relevant influencing factors, such as hair treatments (coloring, curling, etc.), race (ethnicity) and, most importantly, external contamination if participants are living in contaminated areas (hot-spots) (ATSDR, 2001; McDowell et al., 2004; Dakeishi et al., 2005; UBA, 2005). However, overall, and irrespective of the human matrix used (hair, urine, blood, etc.), the effect of the different sampling and sample-preparation procedures and analytical methods used can influence the results significantly (ATSDR, 2001).

Mercury concentration in hair can be determined by different methods, with the preferred technique being Atomic Absorption Spectrometry (AAS) (UNEP/WHO, 2008). Recently, direct mercury analyzers are becoming more and more popular given the advantage of not requiring sample pretreatment or extraction and the short analysis times, thus allowing high sample throughput. The hair analysis panel discussion arranged by the Agency for Toxic Substances and Disease Registry in 2001 (ATSDR, 2001) encouraged the development of Standard Operating Procedures (SOPs) to standardize sampling and chemical analysis as well as the need to collect exposure histories, establish quality-assurance protocols and develop external validation by means of proficiency testing as this would lead to more reliable and reproducible results in hair analysis. Some years later, the German Federal Environmental Agency again pointed out the need to establish external quality control for hair analysis as well as SOPs (UBA, 2005).

In a wider framework, the European Environment and Health Action Plan 2004–2010 identified the high variability in HBM activities in Europe as a problem and called for "*the development of a coherent approach to HBM in Europe*" (EHAP, 2004). Activities aimed at harmonizing HBM in Europe commenced in 2007 with the establishment of *Expert Team to Support Biomonitoring in Europe* (ESBIO) (ESBIO, 2009), a precursor to the EU twin-projects *Consortium to Perform Human Biomonitoring on a European Scale* (COPHES) and *Demonstration of a Study to Coordinate and Perform Human Biomonitoring on a European Scale* (DEMOCOPHES). Thus, the theoretical basis required for such harmonization was developed in COPHES (Joas et al., 2012; COPHES website) and tested through the DEMOCOPHES pilot study (Becker et al., 2014; DEMOCOPHES website). A total of 17 countries participated in this test phase, in which mercury in hair and cadmium, cotinine, phthalate metabolites and, for some countries, bisphenol A in urine were analyzed in samples from mother–child pairs under a strict quality-assurance program.

The activities for harmonizing urinary parameters were presented and discussed in a previous paper by Schindler et al. (2014). The objective of this manuscript is to show the measures taken to ensure the reliability and comparability of hair mercury data among countries participating in DEMOCOPHES.

2. Material and methods

A Quality Assurance Unit (QAU) was established within the framework of COPHES/DEMOCOPHES by the leaders of the work package in charge of sample handling, analysis and biobanking (WP 3). The QAU comprised experts from the Instituto de Salud Carlos III (ISCIII, Madrid, Spain) and the Institute for Prevention and Occupational Medicine of the German Social Accident Insurance – Institute of the Ruhr – University Bochum (IPA, Bochum, Germany).

The QAU was responsible for all tasks related to quality control and quality assurance of the pre-analytical and analytical phases and served as a reference point for all technical questions that arose during the DEMOCOPHES pilot study (Schindler et al., 2014).

The 18 laboratories participating in the quality assurance program were from Austria, Belgium, the Czech Republic, Denmark, Germany, Hungary, Ireland, Luxembourg, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland and the United Kingdom.

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