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Development and application of a novel method to characterize methylmercury exposure in newborns using dried blood spots

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ABSTRACT

Background: Methylmercury (MeHg) is a pollutant of global concern. While there is a need to gauge early-life exposures, there remain outstanding ethical, financial, and practical challenges with using the preferred biomarker, whole blood, notably in pregnant women, infants, toddlers, and children. Dried bloodspots (DBS) may help overcome some of these challenges. Notably DBS are collected from newborns in many jurisdictions offering an institutionalized platform to efficiently characterize exposures.
Objective: To develop, validate, and apply a new method to measure MeHg levels in DBS with a specific aim to use this method to increase understanding of newborn exposures. Methods: Method development and validation was pursued by consulting U.S. EPA Method 1630 and other resources. The method was applied to measure MeHg levels in DBS from newborns (n = 675) from the Michigan BioTrust for Health program.
Results: The assay's detection limit (0.3 µg/L), accuracy (96–115% of expected), precision, linearity, and range met performance criteria guidelines. In the newborn DBS samples, the mean (SD) and geometric mean values of MeHg were 1.46 (0.90) and 1.25 µg/L respectively, and ranged from 0.09 to 9.97 µg/L. The values we report here are similar to cord blood mercury values reported elsewhere.
Conclusions: This is the first characterization of MeHg exposure in newborns, and thus fills an important data gap as prior studies have focused on pregnant women, cord blood, or toddlers. This method helps overcome technical challenges associated with other proposed approaches, and moving ahead there is great promise for applying this DBS-based method for population-level surveillance, particularly in resource-limited settings and for children's health.

1. Introduction

Mercury (Hg) is a pollutant of global concern now recognized under the multilateral UN Minamata Convention on Mercury (Evers et al., 2016; Gustin et al., 2016). Though Hg exists in multiple chemical forms the greatest concern is with the methylmercury (MeHg) form. Methylmercury is an established neurodevelopmental toxicant (Clarkson and Magos, 2006; Mergler et al., 2007), and a growing body of scientific evidence points to its disruptive effects on the cardiovascular, immune, and other physiological systems (Ha et al., 2016; Karagas et al., 2012).

Given the concerns over early-life exposures to MeHg there remains a need for suitable exposure science tools. While notable studies have characterized early-life exposures by sampling biomarkers from pregnant women or studying cord blood, thus allowing for assessment of exposure-outcome relationships and the establishment of reference doses and guideline values, the measurement and utility of MeHg biomarkers remain challenged owing to a range of technical, logistical, and biological factors (Basu et al., 2014a; Grandjean and Budtz-Jørgensen, 2011; Stern and Smith, 2003). In addition, beyond the prenatal period, there is little known about MeHg exposures during infancy (0–1 yr), which represents a tremendous knowledge gap. For example, while MeHg is measured in the U.S. National Health and Nutrition Examination Survey (NHANES, 2017) as well as the Canadian Health Measures Survey (CHMS) (Health Canada, 2013) the minimum age captured in these national surveys is 1 and 3 years, respectively.

Blood is the preferred biomarker to gauge MeHg exposures (Mergler et al., 2007) but it is not without its difficulties, particularly when trying to characterize early-life exposures. Blood sampling is invasive and ethically challenging for certain groups, including pregnant women, newborns, and infants. Sampling blood through venipuncture usually requires a clinical setting, as well as trained phlebotomists and specialized supplies (e.g., syringes, collection tubes). Storage and

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transport of blood necessitates cold-chain approaches and these add further logistical challenges and costs. Such challenges associated with blood-based measures, not only for MeHg but for other biomarkers, warrant the need to explore alternative methods in exposure science.

Dried blood spot (DBS) sampling emerged in the 1960s as a public health surveillance technique (Li and Lee, 2014). Over the past decade there has been an increasing interest in using DBS in exposure science (Olshan, 2007) particularly since DBS are a key component of newborn screening programs institutionalized in many jurisdictions and that residual DBS may be archived and available for research and surveillance purposes (Therrell et al., 2015). In addition, there are many ethical, practical, and economic advantages to using DBS to characterize exposures to toxic chemicals as well as biomarkers of health status (McDade et al., 2007). Sampling DBS can be more participant-friendly than venipuncture as less blood is collected in a less invasive manner. The approach is more conducive for field-based research, particularly in resource-limited settings (e.g., remote locations, developing countries) as there is no need for specialized equipment, cold-chain custody, and phlebotomists.

Researchers have started to develop methods to measure Hg in human DBS samples, and we are aware of four relevant studies (Supplemental Table 1). Chaudhuri et al. (2008) analyzed 18 newborn DBS samples from the U.S. Rocky Mountain region, Funk et al. (2013) analyzed 49 newborn DBS samples from North Carolina State Laboratory of Public Health, Funk et al. (2015) analyzed DBS samples from 82 young individuals from Chicago, and Nelson et al. (2016) analyzed DBS samples from 48 newborn-mother pairs from Minnesota. Despite signifying that Hg can be successfully measured in DBS, collectively these studies have key limitations that may prevent the widespread adoption of the reported methods. These include the fact that key aspects required for validating an analytical method (e.g., precision, linearity, accuracy) were not well covered in each of the studies. The reported detection limits in the studies overlapped with the mean blood total Hg values of U.S. citizens (NHANES, 2017). Also, inductively coupled plasma mass spectrometry (ICPMS) was used to measure Hg despite the fact that this instrumental platform can be problematic for the analyses of this particular element (e.g., high ionization potential, multiple isotopes, volatility, adsorption, polyatomic interferences). Finally, total Hg levels were measured in the DBS. While a majority of the total Hg in blood is found in the MeHg form, the proportion can vary tremendously (e.g., range from 0% to 100% in NHANES; Mahaffey et al., 2004). Without being able to speciate Hg into organic and inorganic fractions and/or carefully characterize potential exposure sources, the measurement of total Hg levels in a DBS likely suffers from random measurement error as well as potential bias associated with certain aspects of the analytical method and study design.

The objective of this study was to develop, validate, and apply a new method to measure MeHg levels in DBS with a specific aim to use this method to increase understanding of newborn exposures to MeHg. Specifically we used a commercially available gas chromatography–cold vapour atomic fluorescence spectroscopy (GC-CVAFS) instrumentation platform to separate Hg species and quantify MeHg levels at the low part per trillion concentrations. Method development was pursued by consulting U.S. EPA Method 1630, which details a GC-CVAFS method to measure MeHg in water samples. Method validation for these DBS samples was carefully monitored by reviewing performance criteria established in US EPA Method 1630 as well as test parameters for method validation outlined by ICH (Reports Q2A and Q2B) and ISO 17025 as summarized in a resource from Huber (2007). Key test parameters we focused on included assay detection limit, linearity, range, precision, and accuracy. Assay specificity is not of concern as the instrumentation platform and method are designed solely for measurement of different Hg species, and here we focused on the MeHg spectral peaks. Following validation, we applied the method to the measurement of MeHg in DBS obtained from newborns (n = 675) from the Michigan BioTrust for Health program which oversees newborn DBS collected in the State and their use in health research. This represents, to our knowledge, the first study to characterize MeHg exposures directly in newborns and thus helps improve our understanding of early-life exposures to MeHg by focusing on a lifestage that was previously unstudied. The study also provides a novel method that can be widely applied given the ubiquity of DBS sampling in newborn screening programs.

2. Methods

2.1. General overview

The study was conducted in two main phases. First we developed an analytical method to measure MeHg levels in DBS, and used a range of artificially created DBS for this study aspect (i.e., DBS created in the lab with blood from reference materials and other study populations). The development of the method was based on U.S. EPA Method 1630 (designed for water samples). During the methods development phase a range of experimental parameters were examined including punches and whole spots sampled from DBS (3 mm diameter punches that included 1, 2, 4, and 8 punches, and full blood spots of 35 μL and 50 μL), as well as DBS digestion times (2, 3, 4 and 6 h), and digestion volumes and related dilutions (various conditions). The iterative and multi-factorial nature of methods development and resulting data are not conducive for simple representation here, and so we focus this paper on presenting the optimal method and reviewing its performance. Second, we applied this method to measure MeHg levels in DBS from newborns (n = 675) from the Michigan BioTrust for Health program. These analyses took several months and spanned 20 batch runs. Each batch run contained a range of quality control samples (i.e., a minimum of at least 6 blood reference materials; 4 method blanks; usually 36 individual newborn DBS samples; and 2 DBS samples from which replicate punches were taken and run separately).

2.2. DBS processing

All DBS cards in the methods development and application phases were Whatman 903 protein saver cards. For batches #1–6, two punches (3 mm diameter; 14.1 mm^2 area) were taken from near the edge of a single spot of a DBS card. For batches #7–20, the Michigan BioTrust for Health program provided us with rectangular punches that were approximately 2 mm × 6 mm in size (mean area 14.2 mm^2). We assumed that a single 3 mm punch contains 3.1 μL of blood based on Li and Lee (2014), and made this same assumption for the rectangular punches given the similar area. From 10% of all DBS cards a blank punch (i.e., did not contain blood) was taken from the edge of the card or supplied from the Michigan BioTrust for Health.

Punched samples from a single DBS card were placed into a single borosilicate glass vessel that was pre-cleaned with 10% HCl. The samples were digested using 8 ml of 25% potassium hydroxide (KOH) in methanol heated to a gentle boil (~ 140 °C) for 4 h. Cooled digests were filled to 30 ml with methanol and stored at − 20 °C until analysis. An aliquot of the sample digest (1.5 ml) was added to ultrapure water and adjusted to pH 4.0–4.5 using citrate buffer added in 200 μL increments. Thirty minutes prior to MeHg analyses, the sample was ethylated using 1% NaBEt4.

2.3. MeHg analysis

The measurement of MeHg in the digests was carried out using a GC-CVAFS unit (Tekran 2700, Tekran Instruments Corporation, Toronto) as outlined by Siedlikowski et al. (2016) as per U.S. EPA Method 1630. The volatile Hg species in the digest solution were purged, introduced to the machine via an autosampler (Model 2621-M, Tekran), and collected on a Tenax trap. The captured Hg compounds were thermally released from the trap (183 °C) and then separated in a

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