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Methylmercury Measurements in Dried Blood Spots from Electronic Waste Workers Sampled from Agbogbloshie, Ghana

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Abstract: Biomonitoring methylmercury (MeHg) exposure is problematic in resource-limited settings and with difficult-to-access populations where traditional biomarker approaches present logistical, economic, and ethical issues. The present study aimed to validate the use of dried blood spots (DBS) to assess MeHg exposure in a real-world contaminated field setting. Whole-blood and DBS samples were collected from electronic waste workers ($n = 20$) from Agbogbloshie (Ghana) in 2017, and DBS were also artificially created in the laboratory using the field-collected blood. Whole-blood MeHg concentrations averaged 0.84 µg/L, which was not different from levels measured in the corresponding DBS samples (field-collected or artificially created). Whole-blood MeHg comprised 61% of the blood total Hg concentrations. Linear regression analysis revealed no differences in MeHg concentrations between whole-blood samples and field-collected DBS (slope 0.89, $R^2 = 0.94$) and between field and laboratory DBS (slope 0.89, $R^2 = 0.96$). The MeHg content in DBS punch blanks averaged 0.86 pg and thus was not of concern. These findings indicate that DBS are a suitable tool for assessing MeHg exposure in real-world environmental settings that may be heavily contaminated. *Environ Toxicol Chem* 2021;40:2183–2188. © 2021 SETAC

Keywords: Biomonitoring; Exposure science; Method development; Surveillance; Public health; Metals

INTRODUCTION

Mercury (Hg) is a toxic chemical of global concern that is found in the environment in different chemical forms (United Nations Environment Programme 2019). All peoples worldwide are exposed to some form of Hg, with the majority of the population being exposed to the methylmercury (MeHg) form via the consumption of fish and seafood (Mergler et al. 2007; United Nations Environment Programme and World Health Organization 2008; Sheehan et al. 2014). Chronic, low-level exposure to MeHg primarily targets the central nervous system, with increasing evidence of impacts on the cardiovascular, immune, and other physiological systems (Clarkson and Magos 2006; Karagas et al. 2012). Given that exposures to MeHg are widespread and that the chemical poses a risk to population health, there is a need to monitor MeHg levels in human populations. With the recent entry into force of the Minamata Convention on Mercury, there is now a legal mandate through Article 22 to assess the effectiveness of the convention which includes monitoring exposure trends in human populations as well as identifying and protecting populations at risk of high exposures (United Nations Environment Programme 2013).

Human exposures to MeHg can be ascertained through biomonitoring efforts that make use of biomarkers, specifically measures of total Hg content in blood and hair (Horvat et al. 2012; United Nations Environment Programme and World Health Organization 2008). However, these measures remain challenging for application in many populations, particularly those inhabiting resource-limited settings or remote locations (Basu et al. 2018) as well as those communities with high exposure to elemental Hg (e.g., dental workers and artisanal gold miners). Venous collection of whole blood is invasive and requires trained medical personnel along with bulky supplies and a cold chain for sample storage and transportation (Li and Lee 2014). The collection of hair in some communities is objectionable for cultural reasons (Basu et al. 2018). Although measures of total Hg in blood and hair serve as a proxy of MeHg concentrations, there are many situations in which this does not hold true. For example, in communities with elevated environmental and occupational exposures to inorganic Hg, total Hg in blood and hair can bias results by overestimating MeHg exposure because speciation profiles in whole blood can differ from those of the general population, and hair can be

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contaminated by exogenously adsorbed inorganic Hg (Akagi et al. 1995, 2000; Sherman et al. 2015).

Therefore, there remains an outstanding need for suitable exposure science tools to biomonitor MeHg exposure (by the analysis of Hg speciation) in the general population, particularly in communities located in resource-limited settings or remote areas. The scientific community has started to study the use of dried blood spots (DBS) as an alternative to whole blood–based approaches (Resano et al. 2018). Sampling DBS is participant-friendly, is convenient, and overcomes restrictions related to field-based research, cutting the need for specialized equipment, cold-chain handling, and phlebotomists (Li and Lee 2014). Over the past decade, several research groups have demonstrated the potential of DBS-based methods to gauge Hg exposure by measuring total Hg levels in DBS (Chaudhuri et al. 2009; Funk et al. 2013; Funk 2015; Nelson et al. 2016; Basu et al. 2017; Nyanza et al. 2019a, 2019b). In recent years, these methods concerning total Hg have been extended to specifically measure MeHg levels in DBS (Basu et al. 2017; Santa-Rios et al. 2020), though these newer studies have focused on DBS obtained in clinical and well-equipped settings with population groups that have background (relatively low) exposures to Hg. These methods have yet to be validated in real-world environmental settings that may be confounded and challenged by logistical and technical issues (e.g., sampling, transport of materials, contamination). Therefore, the present study aimed to validate a previously developed method (Santa-Rios et al. 2020) for the analysis of MeHg in DBS under real-world, field-based conditions. Validation of this method was realized by studying DBS and whole blood collected from electronic waste (e-waste) workers from Agbogbloshie in Accra, Ghana. There are many hazardous substances found within the e-waste sector, with latest estimates calculating that 50 tons of Hg (largely in the inorganic form) flow through these sites (Forti et al. 2020).

METHODS

Sampling

Venous whole blood and DBS were collected from a subset of randomly selected e-waste workers ($n = 20$) from Agbogbloshie participating in the larger GEOHealth study described elsewhere (Nti et al. 2020; Takyi et al. 2020). These individuals came from the group that was sampled in 2017. Agbogbloshie is widely recognized as one of the world's most chemically contaminated locations (Basu et al. 2016; Landrigan et al. 2018) and thus was a good test site for us to address our study objective. Collected samples were transported to McGill University, where they were stored at –80 °C (whole blood) and room temperature (DBS). Human ethics approval for this work was obtained from the College of Health Sciences at the University of Ghana and McGill University.

Whole blood was collected by a phlebotomist in a 7-mL trace metal–free K2EDTA tube (BD Royal Blue cap) and stored frozen until analysis. Field DBS samples were collected following the biomarker field manual for DBS collection (US Agency for International Development 2012). Five drops of capillary blood were collected onto Whatman 903 protein saver cards (G.E. Healthcare) and left to dry at room temperature. In addition to these samples obtained from the field, we created DBS artificially in the laboratory by pipetting 40 µL of the whole blood collected in the field onto Whatman 903 protein saver cards. These laboratory-DBS samples were left to dry at room temperature for 4 h and then packed into a clean polypropylene bag with a desiccant pack.

MeHg analysis

We followed our previous method for the analysis of MeHg concentrations in DBS and whole blood (Santa-Rios et al. 2020). Briefly, for whole blood we used 20 µL of the sample, and for the field-DBS and laboratory-DBS we took 3 punches (each being 3 mm in diameter) from each DBS, assuming these to contain a total blood volume of 9.3 µL (Li and Lee 2014). In brief, samples were extracted with 200 µL of a solution containing 4 M HNO_3 (Omnitrace®; Millipore Sigma; NX0407-4) and 0.02% M L-cysteine (≥97%; Sigma-Aldrich; W326305) by placing them into pre-acid-washed (HCl 10%) 4.5-mL polypropylene tubes (Sarstedt; 60.557) and in an oven at 60 °C for 24 h. Samples were allowed to cool before analysis and diluted with ultrapure water so that the MeHg value would fall within the standard calibration range (0.02–6 ng/L). The analysis was carried out using a gas chromatography cold vapor atomic fluorescent spectrometer (Tekran® Series 2700; Tekran Instruments).

The quality analysis was carried out using a standard of MeHg (0.5 ng/L) at the beginning of the study (initial precision recovery [IPR]) and every 10 samples (ongoing precision recovery [OPR]), combined with method blanks according to Method 1630 (US Environmental Protection Agency 1998a). For quality control, we used laboratory-created DBS with 40 µL of human blood reference material PC-B-M1112 (MeHg = 8.68 µg/L) from the Institut National de Santé Publique du Québec. We also analyzed sample duplicates and blanks (method, field, and laboratory). Quality control samples were analyzed every 10 samples.

Total Hg analysis

We also measured total Hg concentrations in the whole blood samples to help us better interpret the MeHg data (i.e., what percentage of total Hg consists of MeHg). This measurement was performed using methods we have previously published (Santa-Rios et al. 2021) based on Method 7473 (US Environmental Protection Agency 1998b). A 200-µL subsample of whole blood was vortexed, placed into a quartz vessel, and then introduced into a direct total Hg analyzer (model MA-3000; Nippon Instruments). Sample replicates ($n = 3$), blanks ($n = 3$), and human urine reference material ($n = 3$) QM-B-Q1404 (assigned value of total Hg is 1.96 µg/L; Institut National de Santé Publique du Québec) were measured every 10 samples for quality control.

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Data analysis

Data were analyzed using JMP (Ver 14; SAS Institute). For quality analysis, we calculated the accuracy and precision of the IPR and OPR samples as percentage of recovery and the relative standard deviation (SD), respectively. For quality control, we calculated accuracy based on analysis of the reference material and precision based on sample duplicates. We used information from the blanks to help derive detection limits. These performance criteria were compared with the requirements established by Method 1630 (US Environmental Protection Agency 1998a).

To validate DBS measures taken in the field, we focused our study on 3 main comparisons. First, we compared MeHg concentration results from the field-DBS against those from the whole blood (i.e., the preferred biomarker). Second, we analyzed results from the field-DBS with DBS artificially created in the laboratory (from the corresponding whole blood) to identify any bias associated with sample collection under real-world settings. Third, we evaluated the potential for sample contamination by analyzing differences between field-DBS blanks and laboratory-DBS blanks. In each of these 3 steps, we performed the following statistical analyses: 1) calculated descriptive statistics and differences in mean values between samples using Wilcoxon signed-rank tests, 2) calculated constant bias and proportional bias through linear regressions, 3) derived Bland-Altman plots to identify differences in agreement, and 4) calculated coefficient of variances to study intraindividual differences.

RESULTS

The main results from quality analysis and quality control of the MeHg data are shown in Supplemental Data, Table S1. All of our MeHg analyses resulted in data that fell within parameters established by Method 1630 (US Environmental Protection Agency 1998a). Only one individual had DBS MeHg values that fell below the method detection limit (MDL) previously established as 0.3 μg/L (Santa-Rios et al. 2020). The MeHg concentrations across the sample types are summarized in Table 1. No significant differences were found between mean (±SD) levels of MeHg in whole blood (0.84 ± 0.54 μg/L), field-DBS (0.84 ± 0.54 μg/L), and laboratory-DBS (0.83 ± 0.45 μg/L). The median intraindividual coefficient of variation was 9%, and it ranged from 1 to 20%.

To help better interpret the blood MeHg concentrations, we also measured the samples for total Hg levels. The mean level of total Hg in whole blood was 1.58 μg/L (±1.33) and ranged from 0.26 to 5.88 μg/L. Therefore, in the present study population, MeHg was 61% (±17) of the total Hg content in whole blood, and it ranged from 40 to 102%. In addition, there was a significant correlation between the 2 measures of Hg content with an R^2 value of 0.97. The quality of the total Hg measures was deemed good, with a mean coefficient of variation of 2% for replicate analyses, analytical accuracy of 85% (through measures of the blood reference material), and a detection limit of 1.2 ng (blank value plus 3 × SD).

TABLE 1: Concentrations of blood methylmercury (MeHg; micrograms per liter) in e-waste workers ($n = 20$) from Agbogbloshie (Accra, Ghana) randomly sampled from the GEOHealth study in 2017[a]

Variables	Field DBS	Whole blood	Laboratory DBS
Median (IQR)	0.75 (0.48–0.96)	0.70 (0.45–1.16)	0.73 (0.50–1.00)
Mean ± SD	0.84 ± 0.54	0.84 ± 0.54	0.83 ± 0.45
Geometric mean	0.72	0.72	0.74
Minimum	0.31	0.24	0.34
Maximum	2.60	2.37	2.28
Blank range	(0.06–0.28)		(0.02–0.26)
Samples below the MDL	1	0	1

[a]Blood MeHg values are measured in dried blood spot (DBS) samples collected in the field, whole venous blood sampled from workers in the field, and artificially created DBS (using the whole venous blood) under laboratory conditions, with data on the field and laboratory DBS blanks ($n = 20$ for each).
IQR = interquartile range; MDL = method detection limit (0.3 for μg/L DBS samples and 0.2 μg/L for whole blood); SD = standard deviation.

Agreement between DBS and whole blood

All of the statistical results from our validation tests are presented in Supplemental Data, Table S2. We compared the results from field and laboratory DBS with those from whole blood. Wilcoxon signed-rank tests showed no significant differences in MeHg concentrations between field DBS and whole blood ($p = 0.81$) or between the laboratory DBS and whole blood ($p = 1.00$). Through analysis of linear regression relationships (Figure 1), DBS samples presented a close 1:1 relationship with whole-blood samples (slopes = 0.89 and 1.05 for field DBS and laboratory DBS, respectively) and significant correlations ($R^2 = 0.94$ and 0.95 for field DBS and laboratory DBS, respectively). The linear regression showed no constant bias (intercept 0.07 μg/L, 95% confidence interval [CI] –0.03 to 0.17 for field-DBS; intercept –0.05 μg/L, 95% CI –0.16 to 0.06 for laboratory DBS) or proportional bias (slope 0.89, 95% CI 0.78–1.00 for field DBS; slope 1.05, 95% CI 0.94–1.17 for laboratory DBS).

When analyzing the Bland-Altman plots (Supplemental Data, Figures S1 and S2), no differences were found for field DBS (bias –0.02 μg/L) and laboratory DBS (bias 0.01 μg/L) with whole blood. For this comparison, the limit of agreement ranged from –0.25 to 0.21 μg/L, and one sample fell outside the limit of agreement range. Further, all measured samples fell within the maximum allowed difference of 35% based on analytically relevant criteria established by Method 1630 (US Environmental Protection Agency 1998a).

Agreement between field DBS and laboratory DBS

We also compared MeHg values between field DBS and laboratory DBS. No significant differences were found between these 2 measures according to the Wilcoxon signed-rank tests. The linear regression analysis showed a strong correlation ($R^2 = 0.96$) but with a slight constant bias (intercept 0.12 μg/L, 95% CI 0.04–0.20) and proportional bias (slope 0.89, 95% CI 0.75–0.92). The Bland-Altman analysis (Supplemental Data,

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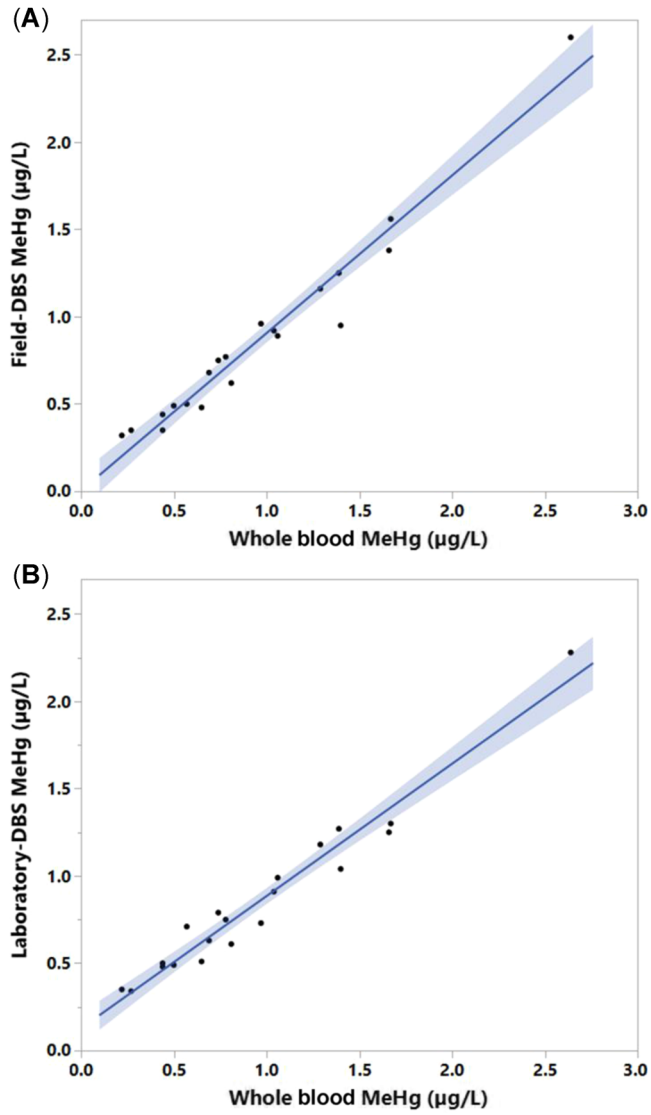


FIGURE 1: Regression plot between methylmercury concentrations in venous whole blood and (**A**) dried blood spots (DBS) collected in the field and (**B**) DBS artificially created in the laboratory using venous whole blood. Points refer to values taken from individual study participants, and the blue band indicates the 95% confidence interval range. MeHg = methylmercury.

Figure S3) showed no differences in MeHg measures between field and laboratory DBS, with a mean bias of –0.01 µg/L and a narrow 95% CI (–0.07 to 0.05). A small shift in bias across the exposure range (CI of the slope –0.26 to –0.06) was calculated. One sample fell outside of the limit of agreement (which ranged between –0.26 and 0.24 µg/L), and all samples were between the maximum allowed difference.

Background contamination

A key objective of the present study was to validate the use of DBS in real-world contaminated sites, and thus we analyzed the differences in MeHg values between field-DBS blanks and laboratory-DBS blanks, recognizing that the former sample type was carried into the contaminated e-waste setting. Three punches of the blank filter paper per individual were used to calculate field-DBS and laboratory-DBS blanks. The MeHg content in field-DBS blanks averaged 0.86 ± 0.41 pg (range 0.14–1.59 pg), and in the laboratory-DBS blanks it averaged 0.86 ± 0.39 pg (range 0.36–1.73 pg). Considering that 3 punches are equivalent to approximately 9.3 µL whole blood (Li and Lee 2014), the expected MeHg concentrations in blanks (after taking into account dilution calculations) were all below the MDL, ranging between 0.02 and 0.28 µg/L.

DISCUSSION

The objective of the present study was to determine if a DBS-based method to evaluate MeHg exposure previously tested for use in laboratory- and clinic-based settings could be deployed in the real world for application in field sites that are contaminated and resource-limited. Our results demonstrate that such a method can be used in a field setting, with negligible differences found between MeHg measures taken from the preferred biomarker (whole blood) and field-collected DBS as well as between field-collected DBS and artificially created DBS in the laboratory. Further, the sampled population was one with exposures to both MeHg and inorganic forms of Hg. The method we describe has promise for application in Hg-contaminated sites worldwide, which may be of particular interest given the recent entry into force of the Minamata Convention on Mercury and an expressed interest of stakeholders to assess the effectiveness of the convention through human biomonitoring.

The quality analysis and quality control results for blood MeHg were similar to a previous study (Santa-Rios et al. 2020), and the calculated values were within Method 1630 (US Environmental Protection Agency 1998a) performance criteria. The blood Hg levels reported in the present study were similar to total Hg levels reported in a past study at the same e-waste site in Ghana (median whole-blood total Hg 0.9 µg/L, interquartile range [IQR] 0.7–1.3 [Srigboh et al. 2016]) and in the range of blood Hg values expected for populations sampled from across Africa from the 2018 United Nations Global Mercury Assessment (median whole-blood total Hg 1.3 µg/L, IQR 0.7–2.8 [Basu et al. 2018]). As expected, the MeHg levels reported in the present study were higher than median blood MeHg levels of the general adult population of the United States (0.53 µg/L for 2015–2016 [US Department of Health and Human Services 2019]) and Canada (0.57 µg/L for 2014–2015 [Canadian Health Measures Survey 2014]) based on national biomonitoring survey results. In most background populations, the MeHg content in whole blood is >70% of the total Hg concentration, though in populations with relatively high exposures to inorganic Hg this value may be lower. In the present study, the average MeHg content in whole blood was 61%, which is not surprising given the presence of inorganic Hg in products found within e-waste recycling sites (Forti et al. 2020).

Concerns have been raised about the potential contamination of filter paper during its manufacturing (Resano et al. 2018), as well as external contamination of these

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cards, particularly if they are to be used in field locations that may be polluted. To our knowledge, only one study has previously examined the difference in total Hg in filter cards comparing between field blanks and laboratory blanks, and they found that total Hg concentrations in field-DBS blanks were 2-fold higher (mean = 0.06 ± 0.02 μg/L) than those in laboratory-DBS blanks (mean = 0.03 ± 0.02 μg/L; Nyanza et al. 2019b). For Hg, concerns over external contamination may be minimal because levels in the air can range from 0.87 to 1.91 ng/m^3 in rural areas (United Nations Environment Programme 2018) and as high as 1 million ng Hg/m^3 in heavily contaminated sites (Cordy et al. 2011), but the chemical form of Hg in the air is in an inorganic or elemental state and not in the organic MeHg form that we focused on. Nonetheless, it is still important to demonstrate background levels of MeHg in the cards (from manufacturing processes) and in blank cards taken to field locations (to account for exogenous contamination during use). In the present study, the background level of MeHg in the blank samples from both the field- and laboratory-DBS cards (mean 0.86 ± 0.40 pg or equivalent to 0.14 ± 0.06 μg/L) were similar to blank MeHg values previously reported in a study from a "clean" environment (mean 0.16 ± 0.02 μg/L; Basu et al. 2017). Because the present study site (Agbogbloshie, Ghana) is considered to be one of the most contaminated worldwide, such a finding increases confidence that DBS cards can be sampled in diverse field settings. Our study, coupled with previous studies (Basu et al. 2017; Santa-Rios et al. 2020), indicates that MeHg levels in blank filter cards are low and likely not of concern, though moving forward studies should continue to use field blanks and report on background levels.

The agreement on MeHg measures in whole blood and corresponding field-collected DBS (i.e., near 1:1 linear relationship, no constant or proportional bias) indicates that a DBS-based approach serves as an alternative to whole blood. The findings from the present study extend those of a previous study involving 49 volunteers in which a similar observation was made (Santa-Rios et al. 2020). Although both populations presented similar blood MeHg levels, the present study was performed in a resource-limited field setting that is notorious for being contaminated, whereas our past study was performed in a well-resourced clinical setting. Taken together these studies demonstrate the range of settings in which human biomonitoring of Hg exposures can be performed using DBS.

A major challenge when working with DBS is that in many studies the volume of blood within the filter paper is not known. In the present study, drops of capillary blood were collected onto the filter cards in the field without any data on sample volumes obtained. Such is the standard practice in most settings (especially in population studies situated in resource-limited settings), and thus an assumption is made that a single 3-mm punch contains 3.1 μL of blood (Li and Lee 2014). To help test the legitimacy of this assumption, we compared blood MeHg values between the field-collected DBS (in which the sample volume is not known) and DBS samples artificially created in the laboratory in which the whole-blood samples from the same individuals were spotted onto filter cards. In doing so, we found generally good agreement between the 2 measures but did calculate slight constant and proportional bias in the regression, suggesting that more effort is needed to try and improve the method.

The results of the present study coupled with those of our earlier validation study provide good support for the use of DBS for MeHg exposure assessment. Our research could be combined with other studies using DBS to understand
micronutrient status (Cu, Se, and Zn; Pawly et al. 2019) and exposure to other toxic elements (Cd, Pb; Nyanza et al. 2019b; Rodriguez-Saldana et al. 2020) to support environmental public health research studies and intervention activities in resource-limited and difficult-to-access areas. The present study was limited to a population with relatively low exposures to MeHg, so future studies are needed to apply the method outlined to study populations with relatively high exposures.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at https://doi.org/10.1002/etc.5121.

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Data Availability Statement—Data, associated metadata, and calculation tools are available from the corresponding author (Niladri.basu@mcgill.ca).

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