Dried Blood Spots as a Tool to Assess Exposure to Mercury Species

Andrea Santa-Rios

Department of Natural Resource Sciences

McGill University, Montreal

August 2020

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Doctor of Philosophy

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

All humans are exposed to some levels of mercury (Hg), a pollutant of concern now being acted upon worldwide through the Minamata Convention on Mercury. In general, humans are exposed to methylmercury (MeHg) through the consumption of fish and shellfish, and to inorganic mercury (InHg) through occupational routes. The health impacts of Hg vary according to which Hg species one is exposed to hence a need for species-specific exposure methods. Further, the analysis of exposure to Hg species has been traditionally studied through the analysis of total Hg levels in biospecimens (mainly blood, urine, and hair). These traditional biomarkers of total Hg exposure can be limited (e.g., logistic requirements, external contamination, type of Hg species they reflect) thus posing constraints to the assessment of Hg exposure, particularly in vulnerable populations situated in remote or resource-limited settings. Dried blood spots (DBS) have been studied as a feasible alternative to whole blood sampling with most studies focusing on the analysis of total Hg (THg). Knowledge and methodological gaps associated with the limited studies examining THg in DBS prevent widespread adoption with key gaps including high method detection limits (MDL), lack of methods to speciate Hg in DBS, differences between the source of blood used for the collection of DBS (capillary blood) and venous blood, uncertainties over the stability of Hg species, feasibility of sample collection under real world conditions as well as in populations with a range of exposure to Hg species. Thus, the objective of this thesis was to develop, validate, and apply a novel, accurate and precise method to use DBS to assess Hg species in diverse human populations with a range of exposures to Hg. First, an accurate and precise method was developed using blood reference materials for the analysis of Hg species in DBS with a MDL of 0.3 μg/L for MeHg and 1.9 μg/L for InHg. This new method was then validated with samples from 49 volunteers from the university community with low level Hg exposures taken in a clinical setting. In doing so, no significant differences between blood sources (venous versus capillary blood) or matrix effects (whole blood versus DBS). The median MeHg concentration in DBS was 0.8 μg/L (range <MDL-2.75 μg/L), though InHg could not be analyzed since 94% of the samples were below the MDL. Next, a second validation study was done to evaluate the differences in Hg levels between DBS collected in

field conditions versus those artificially created under controlled laboratory conditions. Whole blood and DBS samples were collected from 20 individuals in a contaminated field site (electronic waste recycling). There was no evidence of external contamination of the DBS filter paper during the field sampling, and no differences in Hg levels were found between DBS collected in the field (median MeHg 0.75μg/L, range 0.31-2.60μg/L) and DBS created in the laboratory (median MeHg 0.73μg/L, range 0.34-2.28μg/L). With the method evaluated in a real-world setting, it was next applied to characterize Hg exposure in 35 individuals from an artisanal and small-scale gold mining (ASGM) community from Colombia. ASGM community members are amongst the most highly exposed worldwide to both MeHg and InHg since Hg is used for gold extraction. With the analysis of Hg species in DBS and a standardized questionnaire, this aim was able to tease apart Hg exposures across occupational, environmental, and dietary sources. The exposure levels in the community were higher than found in other populations, with median levels of MeHg (1.7μg/L, range <MDL-5.9 μg/L), InHg (3.1μg/L, range <MDL-19.8 μg/L) and THg (4.6μg/L, range 1.1-22.4μg/L). The final chapter aimed to develop and validate a non-destructive method for blood volume estimation within DBS. A regression model was constructed with the area and color code of the spot and validated with samples from two of the aforementioned groups (university members and the ASGM community). We found that the derived model was accurate for the estimation of volume (accuracy 114 ± 9.4%), and a high association between the MeHg concentrations with estimated and known volume (R^2 range between 0.8 and 0.9), indicating that this non-invasive method may be used as a method to estimate blood sample volume of a full spot and thus avoid the volume and hematocrit bias which tend to affect DBS-based approaches. Taken together, this doctoral thesis demonstrated that DBS could be used for the analysis of exposure to Hg species in diverse populations (from background populations sampled in a clinical setting to highly contaminated ones sampled in remote and resource-limited settings). The developed method and its various applications helps overcome key knowledge gaps in the field such as differences in Hg levels across blood sources, concerns over matrix effects, real-world application in vulnerable communities located in resource-limited settings, and validation of a non-destructive method to overcome volume and hematocrit bias. This DBS method can be used in biomonitoring efforts to

increase knowledge on Hg exposures, particularly in vulnerable populations located in remote areas, and this can help address Article 22 of the Minamata Convention on Mercury that calls out the need to monitor trends in human populations.

RÉSUMÉ

Tous les humains sont exposés à certains niveaux de mercure (Hg), un polluant préoccupant qui fait maintenant l'objet de mesures dans le monde entier par le biais de la Convention de Minamata sur le mercure. En général, les humains sont exposés au méthylmercure (MeHg) par la consommation de poissons et de crustacés, et au mercure inorganique (InHg) par des voies professionnelles. Les impacts du Hg sur la santé varient en fonction des espèces de Hg auxquelles on est exposé, d'où la nécessité de méthodes d'exposition spécifiques à l'espèce. En outre, l'analyse de l'exposition aux espèces de Hg a été traditionnellement étudiée par l'analyse des niveaux de Hg total dans les échantillons biologiques (principalement le sang, l'urine et les cheveux). Ces biomarqueurs traditionnels de l'exposition totale au Hg peuvent être limités (par exemple, les exigences logistiques, la contamination externe, le type d'espèce de Hg qu'ils reflètent), ce qui pose des contraintes à l'évaluation de l'exposition au Hg, en particulier dans les populations vulnérables situées dans des milieux éloignés ou à ressources limitées. Les taches de sang séché (DBS) ont été étudiées comme une alternative possible au prélèvement de sang total, la plupart des études se concentrant sur l'analyse du Hg total (THg). Les connaissances et les lacunes méthodologiques associées aux études limitées examinant la THg dans le DBS empêchent une adoption généralisée avec des lacunes clés telles que des limites de détection de méthode élevées (MDL), le manque de méthodes pour spécifier le Hg dans le DBS, les différences entre la source de sang utilisée pour la collecte de DBS (sang capillaire) et le sang veineux, incertitudes sur la stabilité des espèces Hg, faisabilité du prélèvement d'échantillons dans des conditions réelles ainsi que dans des populations avec une gamme d'exposition aux espèces Hg. Ainsi, l'objectif de cette thèse était de développer, valider et appliquer une nouvelle méthode précise et précise pour utiliser le DBS pour évaluer les espèces de Hg dans diverses populations humaines avec une gamme d'expositions au Hg. Tout d'abord, une méthode précise et précise a été développée en utilisant des matériaux de référence sanguins pour l'analyse des espèces Hg dans le DBS avec une MDL de 0,3 μg / L pour MeHg et 1,9 μg / L pour InHg. Cette nouvelle méthode a ensuite été validée avec des échantillons de 49 volontaires de la communauté universitaire avec de faibles expositions au Hg prélevés en milieu clinique. Ce faisant, aucune différence

significative entre les sources sanguines (sang veineux vs capillaire) ou les effets de matrice (sang total versus DBS). La concentration médiane de MeHg dans le DBS était de 0,8 μg / L (intervalle <MDL-2,75 μg / L), bien que l'InHg ne puisse pas être analysé puisque 94% des échantillons étaient inférieurs à la MDL. Ensuite, une deuxième étude de validation a été réalisée pour évaluer les différences de niveaux de Hg entre les DBS collectés dans des conditions de terrain et ceux créés artificiellement dans des conditions de laboratoire contrôlées. Des échantillons de sang total et de DBS ont été prélevés sur 20 personnes dans un site contaminé sur le terrain (recyclage des déchets électroniques). Il n'y avait aucune preuve de contamination externe du papier filtre DBS lors de l'échantillonnage sur le terrain, et aucune différence dans les niveaux de Hg n'a été trouvée entre le DBS collecté sur le terrain (médiane MeHg 0,75 μg / L, intervalle 0,31-2,60 μg / L) et le DBS créé en laboratoire (médiane MeHg 0,73 μg / L, intervalle 0,34-2,28 μg / L). La méthode ayant été évaluée dans un contexte réel, elle a ensuite été appliquée pour caractériser l'exposition au Hg chez 35 individus d'une communauté minière artisanale et à petite échelle d'or (ASGM) de Colombie. Les membres de la communauté ASGM sont parmi les plus exposés au monde à la fois au MeHg et à l'InHg puisque le Hg est utilisé pour l'extraction de l'or. Grâce à l'analyse des espèces de Hg dans le DBS et à un questionnaire standardisé, cet objectif a été en mesure de distinguer les expositions au Hg à travers les sources professionnelles, environnementales et alimentaires. Les niveaux d'exposition dans la communauté étaient plus élevés que ceux trouvés dans d'autres populations, avec des niveaux médians de MeHg (1,7 μg / L, intervalle <MDL-5,9 μg / L), InHg (3,1 μg / L, intervalle <MDL-19,8 μg / L) et THg (4,6 μg / L, plage 1,1-22,4 μg / L). Le dernier chapitre visait à développer et valider une méthode non destructive d'estimation du volume sanguin dans DBS. Un modèle de régression a été construit avec la zone et le code couleur du spot et validé avec des échantillons de deux des groupes susmentionnés (membres de l'université et communauté ASGM). Nous avons constaté que le modèle dérivé était précis pour l'estimation du volume (précision 114 ± 9,4%), et une forte association entre les concentrations de MeHg avec le volume estimé et connu (R2 compris entre 0,8 et 0,9), indiquant que cette méthode non invasive peut être utilisé comme méthode pour estimer le volume d'échantillon sanguin d'un point complet et éviter ainsi le biais de volume et d'hématocrite qui ont tendance à affecter les approches basées sur

la DBS. Pris dans son ensemble, cette thèse de doctorat a démontré que le DBS pouvait être utilisé pour l'analyse de l'exposition aux espèces Hg dans diverses populations (des populations de fond échantillonnées en milieu clinique à celles hautement contaminées échantillonnées dans des milieux éloignés et à ressources limitées). La méthode développée et ses diverses applications aident à surmonter les principales lacunes dans les connaissances dans le domaine, telles que les différences de taux de Hg entre les sources de sang, les préoccupations concernant les effets de matrice, l'application dans le monde réel dans les communautés vulnérables situées dans des contextes à ressources limitées et la validation d'un non- méthode destructive pour surmonter les biais de volume et d'hématocrite. Cette méthode DBS peut être utilisée dans les efforts de biosurveillance pour accroître les connaissances sur les expositions au Hg, en particulier dans les populations vulnérables situées dans des zones reculées, et cela peut aider à répondre à l'article 22 de la Convention de Minamata sur le mercure qui appelle à la nécessité de surveiller les tendances des populations humaines.

ACKNOWLEDGMENTS

First of all, I would like to extend my gratitude to my supervisor, Dr. Niladri Basu, for his support and guidance throughout my Ph.D. In addition, I thank my committee members Drs. Jill Baumgartner and Geoffrey Sunahara.

I am thankful to all my colleagues that were part of the Basu Lab; in particular, I would like to thank, Dr. Benjamin Barst for his guidance, Jenny Eng and Gordana Martincevic for all her help in the laboratory. I also thank Dr. Marie Perkins and Jessica Pawly for their support in the study design and the sample collection, Veronica Saldana for her technical support, and Jessica Ewald for the support for the last chapter. I would like to thank the University of Cartagena for their support, in particular Lesly Tejeda.

Most importantly, I would like to thank my partner for his support and encouragement during this process and my family (my mom, dad, sister, and nephew (Gracias Familia!)) for being my forever cheerleaders. I want to thank my friends for always being by my side and providing support. I dedicate my thesis to my unborn child, I want you to learn that it is possible to accomplish every goal you set in life, and I can not wait to guide you.

This work was funded by a Graduate Excellence Fellowship from McGill University, as well as from the Canada Research Chairs (CRC) program, McGill University, the Canada Foundation for Innovation, and the program of Pasaporte a la Ciencia of the Colombian Government.

CONTRIBUTION TO ORIGINAL KNOWLEDGE

This thesis fills important knowledge gaps and contributes to the advancement of knowledge as follows:

As a brief background, all populations worldwide (especially notable vulnerable ones, including artisanal and small-scale gold miners largely situated remote settings in low- and middle-income countries) are exposed to mercury (Hg) which is now being acted upon legally through the UN Minamata Convention. There is thus a need (that spans human health to environmental quality to regulatory) to properly characterize exposure to this toxic metal. Human biomonitoring studies of Hg exposure tend to focus on measuring Hg levels in biological samples, with many researchers, scholarly works, and institutions favoring measures in whole blood. Despite the acceptance of whole blood as a biomarker of Hg exposure, there remain challenges to venipuncture thus posing a barrier from wider adoption. Notable challenges to venipuncture include ethical concerns in many populations and logistical difficulties (e.g., need for a clinical setting and trained phlebotomist).

The necessity of feasible and low invasive tools to study vulnerable populations in resource-limited areas has motivated interest in the use of dried blood spots (DBS) to assess Hg exposure. The research community has started to develop and apply methods to measure Hg in human DBS samples, and prior to my thesis research I was able to identify seven such studies. In a critical examination of these studies I determined that most of the studies (6 of 7) focused only on quantifying levels of THg, and most were limited with respect to high method detection limits (MDL), lack of documentation of key quality controls (as accuracy and precision), unknown differences between capillary and venous blood, lack of analysis of Hg species, and analysis of real-world samples. To my knowledge, a method to properly characterize Hg species (MeHg and InHg) in DBS has not been established which thus forms the basis for my doctoral thesis.

The first manuscript (Chapter 2) describes the development and validation of a novel method to assess MeHg and InHg levels in DBS. The paper includes detailed information on method

development following robust quality control (i.e., I carefully followed guidelines from US EPA 1630, Health Canada, and European Medicine Agency). I established a working method to characterize MeHg and InHg in DBS, and carefully validated the work using paired whole blood-DBS samples from venous and capillary sources from 49 volunteers with Hg exposures similar to background populations (i.e., MeHg in venous whole blood ranged from 0.2 to 3 µg/L with a median value of 0.8). To my knowledge, this is the first study to analyze Hg species in DBS, and also the first to analyze differences in Hg levels between blood sources (venous and capillary) and sample matrices (whole blood and DBS). Overall, this study helps validate DBS as a novel tool to assess MeHg exposure in human population studies. Though this study tackled and resolved key knowledge gaps concerning the use of DBS for Hg exposure assessment, an outstanding question remained pertaining to the high MDL for InHg; even under controlled conditions I was unable to improve this aspect. The manuscript was published in the Journal *Analytical Chemistry* [Santa-Rios A, Barst BD, Basu N. 2020. Mercury Speciation in Whole Blood and Dried Blood Spots from Capillary and Venous Sources. Analytical Chemistry. 92(5): 3605-3612. doi: 10.1021/acs.analchem.9b04407].

Chapter 2 validated the use of DBS for Hg exposure in the general population under controlled laboratory conditions. To help transition the applicability of the developed method into the real-world, Chapter 3 presents the results of a validation study in the field (i.e., contaminated site in Ghana) in a population with Hg levels and sampling conditions that differ from the first population (university members sampled in a clinical environment), to demonstrate the wider applicability of the method. Whole blood and DBS collected in the field, were compared with DBS created under controlled laboratory conditions for 20 electronic waste (e-waste) recycling workers. The results indicated negligible differences between MeHg concentrations in DBS collected in the field, laboratory-created DBS, and whole blood. The results of this study showed the feasibility of using DBS under real-world conditions in a contaminated site. The research was submitted for publication to the Journal *Environmental Toxicology and Chemistry* where it is currently under review. The study was also presented as a poster at the 2018 Joint International Society of Exposure Science -

International Society for Environmental Epidemiology Annual Meeting [Santa-Rios A., Barst D.B., Basu N. 2018. Method Development and Validation of Dried Blood Spots as a Tool for Mercury Exposure Assessment. ISES-ISEE Joint Annual Meeting. Ottawa, Canada. August 26-30].

A primary aim of this doctoral thesis was to develop and validate a novel DBS-method to characterize different chemical species of Hg so that it could be applied in a real-world setting where there exist serious concerns over Hg pollution; ASGM communities represent such a setting. Thus, Chapter 4 describes a study in which DBS are used to assess exposure to MeHg and InHg among 35 community members in an ASGM site in Colombia. To our knowledge, this is the first study to report Hg speciation in DBS in an ASGM community that is exposed to both forms of Hg from dietary habits (mostly MeHg), and occupational and environmental exposure to InHg. The use of DBS allowed us to assess a remote population and evaluate MeHg and InHg concentrations in miners and community members. The results showed that the developed Hg speciation methods involving DBS is a field-friendly, accurate, and precise tool to identify occupational, environmental, and dietary exposure in vulnerable populations in resource-limited areas. The manuscript was published in the Journal *Chemosphere* [Santa-Rios A, Barst BD, Tejeda-Benitez L, Palacios-Torres Y, Baumgartner J, and Basu N. 2020. Dried blood spots to characterize mercury speciation and exposure in a Colombian artisanal and small-scale gold mining community. Chemosphere. doi: 10.1016/j.chemosphere.2020.129001].

The last chapter (Chapter 5) of the thesis was to develop and validate a simple and non-destructive method to estimate blood sample volume within a DBS card. In this work, we first collected samples from 9 individuals and created hematocrit standards to develop a regression model using the area of the spot and the color code. We then validated the method and model using all the samples from chapters 2 (n=49) and 4 (n=35). The results showed an accurate method for volume estimation and the analysis of MeHg concentrations, that could be applied for future studies. The manuscript will be submitted to Bioanalysis for publication.

CONTRIBUTION OF THE AUTHORS

This thesis is comprised of four original research chapters (Chapters 2 - 5), each which have been prepared for submission, submitted and/or or published to an academic journal. As the author of this thesis, and first author of each manuscript, I was responsible for the development of study objectives and hypotheses, literature review, lab work, data management, statistical analysis, interpretation of findings, and writing. Dr. Niladri Basu, as my primary advisor, provided guidance, feedback, and overall support throughout the thesis. For each manuscript, I developed the study design, methods, and rationale in collaboration with my co-authors.

Manuscript I. Mercury Speciation in Whole Blood and Dried Blood Spots from Capillary and Venous Sources. Published in Analytical Chemistry. 2020, 92(5): 3605–3612. Authors: Andrea Santa-Rios, Benjamin Barst, and Niladri Basu

Andrea Santa-Rios: Conceptualization, methodology, formal analysis, investigation, resources, writing- original draft, writing-review and editing, and visualization.

Benjamin Barst: Writing- review and editing

Niladri Basu: Conceptualization, writing- review and editing, resources, funding acquisition.

Manuscript II. The Use of Dried Blood Spots for Methylmercury Exposure Assessment in Field-Based Research. Submitted to Environmental Toxicology and Chemistry (20/11/2020). Authors: Andrea Santa-Rios, Julius Fobil, and Niladri Basu

Andrea Santa-Rios: Conceptualization, methodology, formal analysis, investigation, writing- original draft, writing-review and editing, and visualization.

Julius Fobil: Resources

Niladri Basu: Conceptualization, writing- review and editing, resources, funding acquisition.

Manuscript III. Dried blood spots to characterize mercury speciation and exposure in a Colombian artisanal and small-scale gold mining community. Published in Chemosphere (19/11/2020). Authors: Andrea Santa-Rios, Benjamin D. Barst, Lesly Tejeda, Yuber Palacios-Torres, and Niladri Basu.

Andrea Santa-Rios: Conceptualization, methodology, formal analysis, investigation, resources, writing- original draft, writing-review and editing, and visualization.

Benjamin Barst: Writing- review and editing

Lesly Tejeda: Resources

Yuber Palacios-Torres: Investigation

Niladri Basu: Conceptualization, investigation, writing- review and editing, resources, funding acquisition.

Manuscript IV. The application of a non-destructive method to estimate sample volume on dried blood spots for the analysis of mercury species. The publication is planned for submission to Bioanalysis. Authors: Andrea Santa-Rios, Jessica Ewald and Niladri Basu.

Andrea Santa-Rios: Conceptualization, methodology, formal analysis, investigation, resources, writing- original draft, writing-review and editing, and visualization.

Jessica Ewald: Software

Niladri Basu: Conceptualization, investigation, writing- review and editing, resources, funding acquisition.

LIST OF ABBREVIATIONS

ASGM	Artisanal and small-scale gold mining
BMI	Body mass index
CDC	Centers for Disease Control and Prevention
CHMS	Canadian Health Measures Survey
CI	Confidence Interval
CNS	Central Nervous System
COPHES	European coordination action on human biomonitoring
CV	Coefficient of Variation
DBS	Dried Blood Spots
DORM	Fish protein certified reference material for trace metals
DTIE	Division of Technology, Industry, and Economics
EPA	Environmental Protection Agency
GC-CVAFS	Gas Chromatography Cold Vapor Atomic Fluorescence Spectrometer
HBM	Human Biomonitoring
Hg	Mercury
Hg0	Elemental Mercury
IDRC	International Development Research Centre
InHg	Inorganic Mercury
INSPQ	Institut national de santé publique du Québec
IPR	Initial Precision Recovery
LOA	Limit of Agreement
MAD	Maximum Allowed Difference
MDL	Method Detection Limit
MeHg	Methylmercury
NHANES	National Health and Nutrition Examination Survey
NIST	National Institute of Standards and Technology
OPR	Ongoing Precision Recovery
PMDL	Practical Method Detection Limit
QC	Quality Control
RBC	Red Blood Cells
RGB	Red Green and Blue
RPD	Relative Percent Difference
SD	Standard Deviation
THg	Total Mercury
TMDL	Theorical Method Detection Limit
UN	United Nations
UNEP	United Nations Environment Program

UNIDO	United Nations Industrial Development Organization
USAID	United States Agency for International Development
WHO	World Health Organization

1. CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

1.1 General introduction

Mercury is a pollutant of concern now recognized by the global community under the UN Minamata Convention on Mercury (UN Environment Programme, 2019). Worldwide 35% of the Hg emitted and released to the environment is estimated to come from ASGM(UN Environment Programme, 2019). Artisanal and Small-scale Gold Mining sites are typically situated in resource-limited settings, and the mining practice tends to use rudimentary techniques to prospect mines and process gold ore (Clifford, 2014; Ha et al., 2016; UN Environment Programme, 2013a). Gold is extracted from the ore by the use of Hg, and thus communities may be exposed to two chemical forms of Hg: 1) elemental Hg [Hg^0], which is released once the gold-Hg amalgam is burned; and 2) MeHg through the consumption of fish procured from Hg-contaminated water bodies (UN Environment Programme, 2019; Veiga and Baker, 2004). The issue is not insignificant as the modern gold rush has involved around 10-15 million artisanal miners worldwide who live within communities with about 100 million people (Veiga and Baker, 2004). As the ASGM activities are generally located where people live, the Hg exposures among the miners and community members are both high. Notably, global synthesis of biomonitoring surveys in these communities show that ASGM miners and community members have the highest Hg exposures worldwide compared to the general population (Gibb and O'Leary, 2014), with a median blood Hg level of 10.9 µg/L (interquartile range (IQR): 6.9 - 21.6 µg/L) for ASGM communities versus 1.5 µg/L (IQR: 1.0 - 2.9 µg/L) for the general background populations (Basu et al., 2018b). Due to the potential health risks in all communities, it is pertinent to develop Hg monitoring programs suitable for resource-limited and remote settings (UN Environment Programme, 2013b).

The accurate assessment of Hg exposure is vital to characterize risk in communities as well as evaluate the success of Hg-reduction efforts such as those listed in the Minamata Convention (Eagles-Smith et al., 2018). The direct measurement of Hg in whole blood is an accepted biomarker for Hg exposure in humans (Horvat et al., 2012). Despite the acceptance of whole blood, there remain challenges to venipuncture thus posing a barrier from wider adoption. Notable challenges include ethical concerns related to sampling blood in many populations, logistical difficulties (e.g.,

need for a clinical setting and trained phlebotomist), and elevated costs associated with aforementioned aspects as well as the need for specialized collection supplies and post-collection sample storage and transport costs.

A potential alternative to sampling whole blood via venipuncture is the use of dried blood spots (DBS) which are drops of whole blood collected from a finger or heel prick onto filter paper (USAID, 2012). The DBS method involves the use of a micro-lancet to deliver a controlled puncture, typically to the finger or heel (USAID, 2012), yielding around five drops of blood (usually ~30-60 µL per drop) that are then collected on filter paper. In contrast to venipuncture, DBS sampling has fewer logistical requirements as highly trained personnel are not needed, DBS can be stored at ambient temperature and shipments involve relatively small packages (Denniff and Spooner, 2014a). Furthermore, sample collection is minimally-invasive with only a small volume of sample collected (Enderle et al., 2016) increasing the feasibility of collecting large numbers of samples in communities of interest (Mcdade, 2013), while also providing a potential cost-effective opportunity for retrospective (e.g., DBS archived from newborn screening programs) and prospective studies.

Several studies have demonstrated that Hg can be measured in DBS. To my knowledge, the first research was a pilot study in 2008 where researchers analyzed THg in 18 newborn samples using inductively coupled plasma mass spectrometry (ICP-MS). In doing so they highlighted the stability and reproducibility of THg measures from DBS while also raising concerns about the method detection limit (Chaudhuri et al., 2009). Subsequent studies evaluated THg in newborn DBS samples using ICP-MS (Funk, 2015; Funk et al., 2013; Nelson et al., n.d.), also noting a relatively elevated method detection limit (only 33-38% of the samples were above the method detection limit). Studies of THg using paired whole blood-DBS (Funk et al., 2013; Elias Nyanza et al., 2019) and cord blood-DBS samples (Funk, 2015) demonstrated a correlation between whole blood and DBS, and lower detection limits for cord blood (Funk, 2015), resulting in a higher percentage of detectable samples for cord blood (62%) compared with DBS (38%). One study validated the method in the field showing low THg concentrations on field blanks (average 0.02 µg/L) (Elias. Nyanza et al., 2019). As part of a collaborative study, I was involved in the first study to characterize MeHg (note, past studies examined THg) in DBS samples in 2017 (Basu et al., 2017).

We analyzed 675 newborn DBS using gas chromatography–cold vapour atomic fluorescence spectrometry (GC-CVAFS). Our results indicated an accurate method (MeHg recovery range from 96% to 115%) though with high variance among lower MeHg concentrations.

While studies have explored measuring Hg in DBS, the methods to date have been challenged technically (e.g., detection limit, accuracy) and nearly all have focused on total Hg measures. Further, few studies have validated the methods (e.g., compared against a range of quality performance criteria, or compared DBS measures against corresponding whole blood values), and applied them in real-world communities. As such, the main goal of this thesis was to develop, validate, and apply an accurate and precise method to use DBS to assess Hg in communities with a range of exposure to Hg.

Aim- 1: To develop an accurate and precise method to quantify Hg speciation (InHg and MeHg) in DBS. While DBS is a novel method to assess total Hg exposure, there exists no reliable established method for quantifying Hg species in DBS. The *objective* here was to develop a GC-CVAFS-based method to determine Hg speciation in DBS using paired blood-DBS samples and assess assay performance against international quality control measures. Artificially created DBS (Whatman 903 protein saver cards) from reference materials from the Institut National de Santé Publique du Québec (INSPQ), and whole blood from a volunteer were used for the experimental method development.

Aim- 2: To validation the Hg speciation method in DBS in a clinical setting. One of the main advantages of using DBS for biomonitoring is that DBS is collected using capillary blood from a finger prick. However, knowledge gaps related to the use of DBS for Hg exposure assessment still need to be covered, including the difference in Hg levels between venous whole blood (the gold standard biomarker on Hg speciation) and capillary whole blood, and the agreement between measures taken in whole blood and DBS. The *aim* here was to validate the method from Aim #1 by comparing Hg species in paired blood-DBS from capillary and whole blood collected from the same individuals. A secondary aim was to determine the differences between blood sources (capillary versus venous blood) and samples matrixes (whole blood versus DBS). A cross-sectional study was conducted with 49 members from McGill University's Macdonald Campus.

Aim- 3: To study the suitability of DBS for real-world settings: To understand how to sample, and use, DBS in resource-limited settings, a case study is necessary to increase understanding of logistical (e.g., storage and transport) and technical (e.g., contamination) issues in the field. *The objective* was to collect DBS in real-world settings and analyze differences in Hg levels in DBS samples collected in the field and created under controlled laboratory conditions. Here, we randomly selected twenty samples (DBS and whole blood) from a contaminated site (e-waste, Ghana) with higher Hg exposure and different field settings than the background population of Aim 2 and created DBS under laboratory conditions to compare them with the DBS collected in the field.

Aim- 4: Application of the DBS method to assess Hg species exposure on an ASGM community: A major goal of this dissertation was to evaluate the application of our developed method in an ASGM community. *The objective* was to assess the feasibility and validity of DBS to characterize and help differentiate among occupational, environmental, and dietary exposures to Hg. We collected samples from 35 community members from an ASGM sector in Colombia, and combined DBS results with a detailed questionnaire to address this objective.

Aim-5: Sample volume estimation: The DBS community is challenged in that the volume of blood on a DBS is not known. Non-destructive methods to overcome the hematocrit effect (differences in the diffusion of blood through the paper due to blood viscosity), have previously used the analysis of the area of the spot, or spot color to estimate sample volume. *The objective* was to create an empirical model function to calculate sample volume in DBS combining both non-destructive methods (absorbance and area of the spot). This method was tested in samples from Aim #2 and 4.

1.2 Literature review

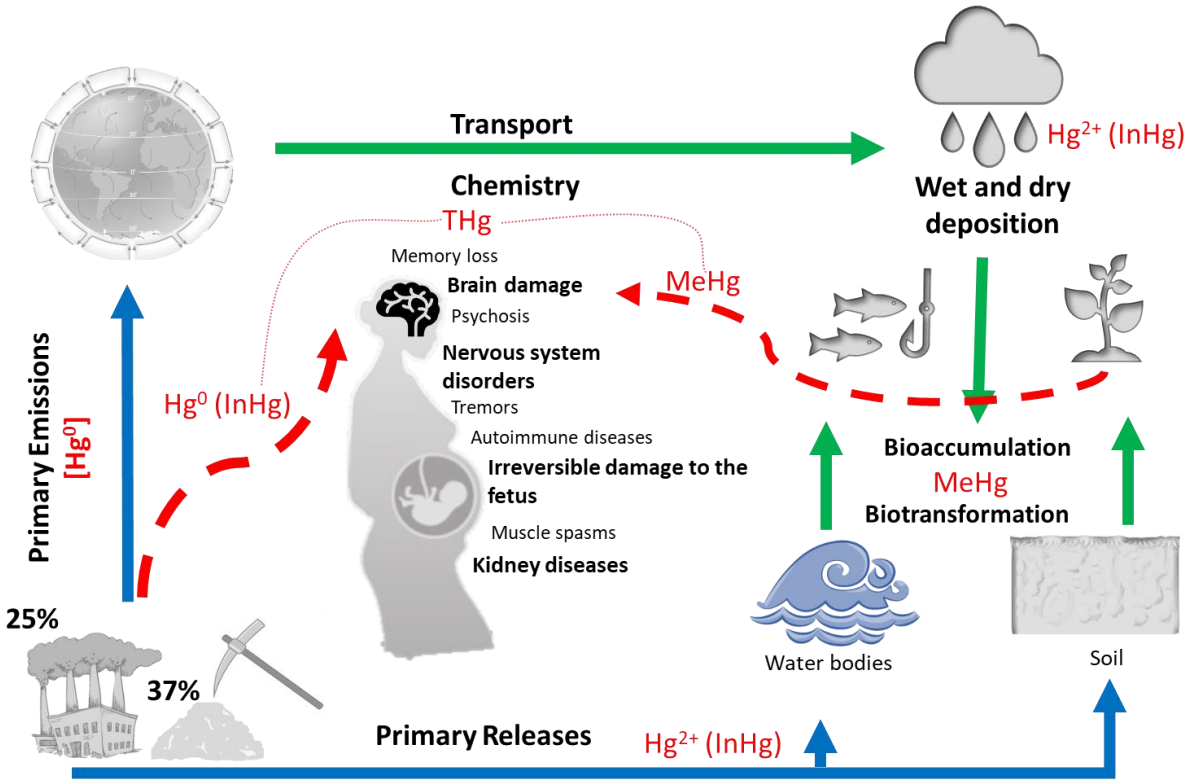
1.2.1 Mercury in the environment

Mercury is a pollutant of global concern recognized by UNEP under the Minamata Convention on Mercury in 2013 (UN Environment Programme, 2013b). Mercury undergoes long-range atmospheric transport, bioaccumulates and biomagnifies in food webs, and has known adverse effects on human health and the environment (Choi et al., 2012; Driscoll et al., 2013; Harris et al., 2007; Selin, 2009; Telmer and Veiga, 2009).

Mercury is a naturally occurring element contained in minerals, non-ferrous metals, and fossil fuels. It is released to the environment from natural sources (natural weathering of Hg-containing rocks, volcanoes, and geothermal activity), and anthropogenic activities (coal burning, mining, smelting, and production of iron and non-ferrous metals, cement production, oil refining and ASGM (UN Environment Programme, 2013a).

The most predominant forms of Hg in the environment are elemental Hg, divalent or InHg, and organic Hg (mainly MeHg) (UN Environment Programme, 2019). The transformations and pathways of Hg are complex and significantly affected by local conditions. Mercury released by natural and anthropogenic activities to the atmosphere is predominantly elemental Hg (≈95%), this relatively insoluble form has a residence time of up to one year allowing it to be transported far from sources (Driscoll et al., 2013; Pirrone et al., 2010, 2010; Selin, 2009; Slemr et al., 2003; Steffen et al., 2008). Gaseous elemental Hg is oxidized, mainly to InHg, which enters the soil, sediment, and aquatic ecosystems by wet and dry deposition. In aquatic environments, InHg is transformed by bacteria to MeHg, the most toxic relevant form of Hg, which bioaccumulates in organisms and biomagnifies in food webs (Driscoll et al., 2013) (Figure 1-1).

Figure 1-1. Fate and transport of Hg in the environment. The blue arrows show emission and releases; green arrows are transport in the environment; red arrows are Hg exposure to humans.



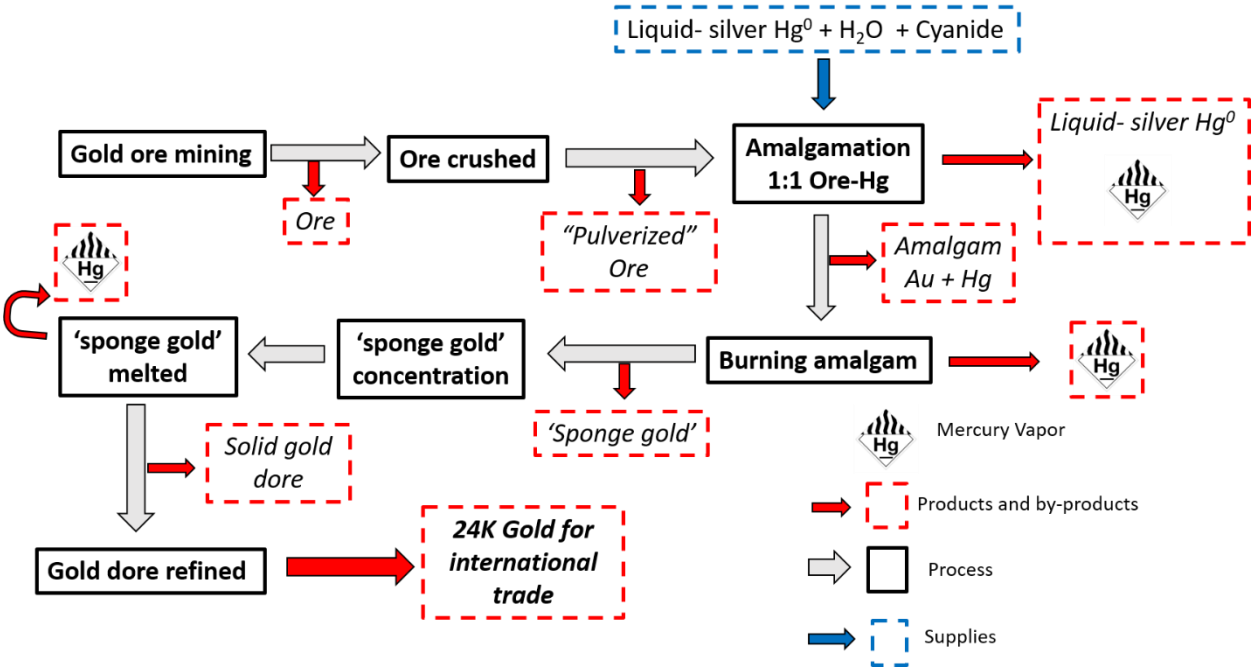
1.2.2 Mercury in Artisanal and Small-Scale Gold Mining (ASGM)

Artisanal and small scale gold mining is estimated to contribute the most significant amount of Hg emissions, contributing ≈37% of all global Hg (UN Environment Programme, 2019, 2013a). Annually, ASGM operations contribute more than ~800 tonnes of Hg to water and land, and ~727 tonnes Hg to the atmosphere (UN Environment Programme, 2019, 2013a). The ASGM sector is undergoing a rapid expansion owing to increasing gold prices and widespread poverty (Clifford, 2014; UN Environment Programme, 2010). Worldwide 10-15 million people are working directly in the ASGM sector, with upwards of 100 million residing in ASGM communities (Council, 2015).

The ASGM sector exists in over 70 countries across Africa, Asia, and South America (mainly in countries with low- and middle-income economies) (UN Environment Programme, 2013a).

The ASGM sector primarily consists of informal mining activities that use rudimentary processes to extract gold from secondary and primary ore bodies (Veiga, 1997). The use of Hg in the mining process, is one of the major environmental and health concerns for ASGM communities (Figure 1-2). In brief, the gold ore is mined and crushed to obtain a pulverized ore, which is then mixed with water and liquid elemental Hg to create an amalgam of gold and Hg. Next, the amalgam is burned to separate the Hg from the gold, which generates a product called "sponge gold" that is further refined to solid gold ore. During amalgam burning and melting of the "sponge gold," a vapor is released to the atmosphere that has high Hg concentrations. For example, Cordy et al. (2011) measured Hg in the air in 5 ASGM municipalities in Colombia. They found the levels to range from 1000 to 200,000 ng Hg/m^3, which is several times higher than the World Health Organization (WHO) limit for public exposure (1000 ng Hg/m^3).

Figure 1-2. Schematic outlining of the typical gold extraction scheme in an Artisanal and Small-Scale Gold Mining site.



1.2.3 Impacts of Hg to human and environmental health

Contemporary awareness related to the public health risks posed by Hg compounds started in the 1950s. Notably, it followed events that began in 1953 in Minamata city (Kumamoto region, Japan) where the Chissso Corporation released industrial wastewater contaminated with MeHg into Minamata Bay. The MeHg was accumulated by local fish and shellfish, which, when eaten by the local human population, resulted in devastating health effects referred to as Minamata Disease (Kurdland et al., 1960). Other prominent Hg poisoning outbreaks occurred in Iraq (1971-72), Pakistan (1969), and Ghana (1969) and collectively these alongside the events at Minamata Bay spurred concerns over Hg pollution but also contributed to the modern environmental movement.

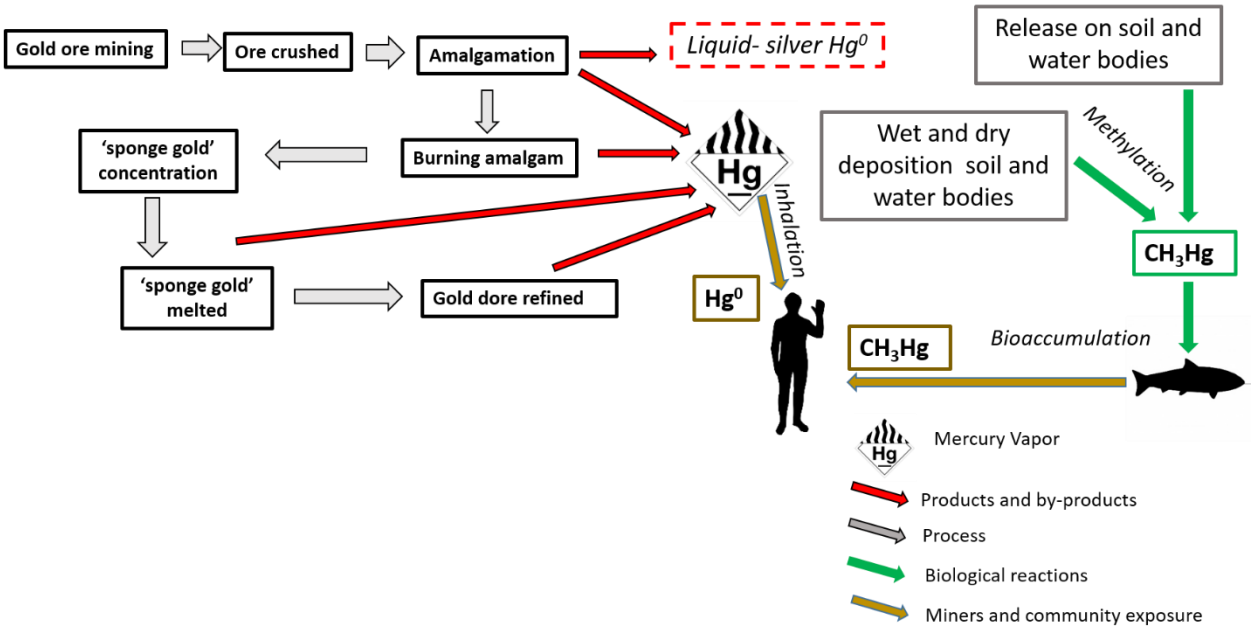
Acute Hg poisoning due to industrial releases is now a rare event. However, humans worldwide are exposed to some amount of elemental Hg, InHg, and MeHg as a result of contaminated food items or occupational exposures that span the world (Clarkson et al., 2003; Ha et al., 2016). Exposure to elemental Hg and InHg may occur from environmental pollution, occupational settings, and contact with specific products, meanwhile dietary intake of contaminated seafood and rice grown in contaminated sites are the primary sources of exposure to MeHg (Basu et al., 2018a).

1.2.4 Mercury exposure in ASGM sites

Of all human populations worldwide, miners and community members living within, or close to, an ASGM site are particularly unique in that they are exposed to the 3 main forms of Hg (elemental Hg, InHg, and MeHg), and that exposures to each form are high (Figure 1-3) (UNEP, 2012). Adverse health effects due to Hg exposure in ASGM communities include kidney dysfunction, autoimmune dysfunction, and neurological symptoms (World Health Organization (WHO), 2013). Vapor inhalation is the primary exposure pathway to elemental Hg. The process of burning the gold/Hg amalgam is often performed within homes and urban areas, increasing the risk of exposure to vulnerable groups such as children and pregnant women (García et al., 2015; UNEP DTIE Chemicals Branch & WHO Department of Food Safety, 2008). The use of Hg in ASGM communities poses a health risk for miners and community members (Basu et al., 2015; Ha et al., 2016; UN Environment Programme, 2012). The elemental Hg emitted from ASGM activities can be inhaled into the lungs and absorbed into red blood cells where it is rapidly oxidized to the divalent inorganic cation (inorganic Hg, InHg). Individuals residing in ASGM communities are also exposed to methylmercury (MeHg) through the consumption of contaminated fish. While all fish are contaminated with some level of MeHg (World Health Organization (WHO), 2019), the use of Hg near ASGM sites can lead to direct contamination of adjacent water bodies (UN Environment Programme, 2019). In aquatic environments, microbes convert inorganic Hg to

methylmercury (MeHg), which bioaccumulates and biomagnifies in food webs (Obrist et al., 2018). Thus, Hg use at ASGM sites has the potential to increase MeHg contamination of nearby aquatic food webs, resulting in fish with elevated MeHg concentrations (Rajaee et al., 2015b; UN Environment Programme, 2012).

Figure 1-3. Typical routes of inorganic and organic Hg exposure in ASGM



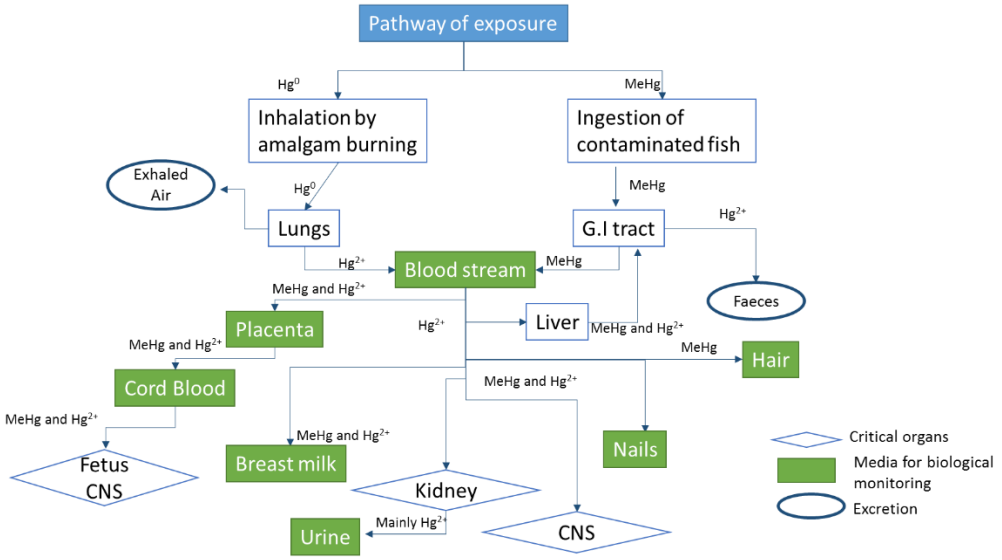
Within ASGM communities some studies have found that Hg biomarker levels in miners and non-miners are not different as mentioned by the review done by Basu et al. (2015). Kristensen et al., (2014) reviewed elemental Hg exposure within ASGM sites which included 26 studies involving a total of 3,005 participants from 14 different developing countries. Across these studies it was concluded that all participants had levels of exposure to elemental Hg that are associated with potentially harmful health effects. The results are consistent with another review on total Hg (THg) exposure in ASGM sites. Levels of THg from approximately 60 studies were above the World Health Organization health guidance values for ASGM workers and their families (Gibb &

O'Leary, 2014). Taken together, these studies demonstrate that ASGM workers and community members worldwide are exposed to some of the highest levels of Hg worldwide, and that these exposures cover the different major forms of Hg.

1.2.5 Toxicokinetics

The toxicological effects of Hg depend on its chemical form along with the route and duration of exposure. Each type of Hg has different toxic properties that are mainly a function of binding interactions that occur between Hg ions with selenols, protein thiols, and nonprotein thiols within the target cells (Berlin et al., 2007; Wang et al., 2013). In the context of this study, the metabolism, and toxic effects of elemental Hg, InHg, and MeHg will be described since they are the primary Hg forms that ASGM communities, as well as the general population are exposed to (Figure 1-4).

Figure 1-4. Mercury toxicokinetic for ASGM communities. Modified from Horvat, Snoj Tratnik, & Miklavčič, 2012. CNS, central nervous system; GI, gastrointestinal.



a. Elemental Mercury

Air Hg concentrations in gold mining communities may be above the WHO limit for public exposure (1000 ng/m^3). As an example, Cordy et al., 2011, found urban air Hg levels of 10,000 ng Hg/m^3 in residential areas and 1 million ng Hg/m^3 inside gold shops in an ASGM town in Colombia. Inhalation is the main route of exposure to elemental Hg, where 80% of the Hg inhaled is retained in the body (World Health Organization (WHO), 2003). After inhalation, elemental Hg enters the respiratory tract as an uncharged monoatomic gas that is highly lipophilic (Horvat et al., 2012), which helps the vapor to penetrate the membranes of the body and to be easily absorbed by alveolar membranes (Berlin et al., 2007). Following absorption, elemental Hg is reduced to Hg ions (InHg) in the red blood cells and lungs (World Health Organization (WHO), 2003). According to a study with human volunteers who inhaled a tracer dose of elemental Hg, the absorption rate of InHg in the blood is 0.2 hours with median half time in the body of 10 days (Sandborgh-Englund et al., 1998).

The majority of InHg is found in erythrocytes or hemoglobin (Cherian et al., 1978). Hemoglobin is an oxygen transport protein, which inadvertently serves to transport Hg throughout the body (Berlin et al., 2007). Although the oxidation process from elemental Hg to InHg takes place very soon after absorption, some elemental Hg stays in the blood for a few minutes. This retention time is enough time for elemental Hg in the blood to reach the blood-brain barrier, where due to the lipid solubility and high difusibility, elemental Hg crosses and is oxidized to InHg in brain tissue (World Health Organization (WHO), 1991). This is supported by in vitro studies, which indicate that elemental Hg arrives unoxidized to the brain due to the short transit time from lungs to the brain (Hursh, D. A., Wendt, S. F., Lee, C. F., 1989). Classic studies in mice (Berlin, M., and Johansson, 1964) and primates (Berlin, M., Fazackerly, J., and Nordberg, 1969), showed that elemental Hg is ten times more retained in the brain than when the animals were exposed to InHg. Short- and long-term exposure to elemental Hg affects the central nervous system. For example, exposed workers from a chloralkali plant had a weakness, paraesthesia, and muscle cramps (Discalzi G, Fabbro D, Meliga F, Mocellini A, 1993). The aforementioned health effects have been also found in dentists that demonstrated impaired performance on several neurobehavioral tests (Sletvold et al., 2012).

Animal studies on exposure to elemental Hg (Berlin, M., and Johansson, 1964) have shown that InHg has a particular affinity with ectodermal and endodermal epithelial cells. Therefore it accumulates in the skin, hair, salivary glands, thyroid, liver, pancreas, sweat glands, kidney, testicles, and prostate, as well as in the grey matter of the brain and some parts of the cerebellum (Berlin, M., Fazackerly, J., and Nordberg, 1969). The half time of InHg differs in each organ, though the most extended retention times are in the brain, kidney, and testicles (Berlin et al., 2007). After exposure to elemental Hg, elimination and excretion occur mainly in the form of InHg. Earlier studies with humans exposed to elemental Hg have shown that a small amount of elemental Hg is exhaled as a result of the reduction of InHg stored in tissues (Dunn, 1978). Most InHg is excreted in urine and feces; animal experiments have shown that excretion is better correlated with the concentration of InHg in blood than in kidneys since InHg is transported from blood to the tubular cells and transferred to the tubular lumen (Berlin and Gibson, 1963). Currently, there is no suitable biological index for the concentration of InHg in the critical organs for humans, however the measurement of InHg in whole blood or urine could be used to measure ongoing exposure to elemental Hg.

b. Methylmercury

Inorganic Hg enters the aquatic environment using two pathways in ASGM communities. After the burning of the amalgam, elemental Hg its transported in the atmosphere and deposited in aquatic ecosystems. Liquid Hg from the tailings are discharged into the ASGM surrounding water bodies (Figure 1-4). Elemental Hg emitted to the atmosphere can be oxidized InHg (Sonke et al., 2013), deposited into water bodies, and then methylated to MeHg by bacteria in soils, freshwater, and saltwater (Agency for Toxic Substances and Disease Registry, 1999). Methylmercury is the most relevant toxic form of Hg (Shi et al., 2005; Wang et al., 2009), it can be bioaccumulated by aquatic organisms and biomagnifies in food webs (Wang et al., 2013). Artisanal and Small Scale Gold Mine communities and surrounding populations are exposed to MeHg by the consumption of food like fish and rice (Akagi et al., 2000; Faial et al., 2015; Guimaraes et al., 2011; Li et al., 2011; Malm et al., 1995). Methylmercury ingested with food is efficiently absorbed through the

intestinal tract (Berlin et al., 2007; Clarkson et al., 2007). Methylmercury ingested through contaminated foods (e.g., fish and in some cases rice) (Akagi et al., 2000; Bradley et al., 2017; Faial et al., 2015; Guimaraes et al., 2011; Malm et al., 1995) may be efficiently transported across the gut tract into the bloodstream from where it may then cross both blood-brain and blood-placental barriers (Clarkson and Magos, 2006; Horvat et al., 2012). The majority of MeHg is associated with RBCs and evidence suggests reversible binding with hemoglobin in the bloodstream MeHg is accumulated in the red cells (Berlin et al., 2007) and distributed from the blood to body tissues, where the equilibrium between blood and the body takes four days (Kershaw TG, Clarkson TW, 1980). MeHg is transported across the blood-brain barrier by L-type amino acid transporters, probably due to its structural resemblance to methionine. Therefore, the brain can accumulate MeHg concentrations that are 3-6 times higher than in blood (Syversen and Kaur, 2012). Other organs that can concentrate MeHg are the liver and kidneys (Berlin et al., 2007). MeHg is also transported through the placenta to the fetus (Reynolds WA, 1975), where the levels of MeHg in the fetal brain are higher than in the mother (Syversen and Kaur, 2012).

Methylmercury is transformed in the body to InHg by demethylation accomplished by OH-radicals (Suda and Takahashi, 1992). After ingestion of MeHg, InHg has been found in the kidney, liver, feces, bile, and urine (Berlin et al., 2007). Data collected from the epidemic poisoning in Iraq demonstrate that the net daily excretion rate of MeHg in humans is 1% with a half-life of about 70% (Bakir et al., 1973).

The brain and the central nervous system are primary target sites where MeHg effects manifest (Agency for Toxic Substances and Disease Registry, 1999; World Health Organization (WHO), 2013). Clinical signs of MeHg intoxications are characterized by disturbances in the sensitivity in extremities, tongue, and around the lips. Additionally, visual and hearing impairment can occur (Berlin et al., 2007). For populations that consume fish, an increase in hair MeHg during pregnancy has been associated with psychomotor test performance in kids (Grandjean et al., 1997).

1.2.6 Biomonitoring

a. Biomarkers of exposure in ASGM

The global concern over Hg toxicity led to the creation of the Minamata Convention on Mercury. This legally binding global treaty aims to regulate anthropogenic emissions and releases of Hg into the environment. The Convention has appointed exclusively for ASGM an Article (Article 7) and an Annex (C) that calls for the development of National Action Plans for the ASGM sector to "reduce and, where feasible, eliminate the use of Hg and Hg compounds in the process." The Convention emphasizes the development of capacities for the monitoring and gathering of data related to health risks as a result of exposure to Hg and Hg compounds (Annex C (h), Article 6 (b), Article 19 (c), Article 22 (UN Environment Programme, 2013b)).

Human exposure to Hg is estimated by the use of human tissues (blood, hair, and urine) known as biomarkers of exposure (Horvat et al., 2012). Article 16 of the Minamata Convention calls to "Establish and strengthen, as appropriate, the institutional and health professional capacities for the prevention, diagnosis, treatment, and monitoring of health risks related to the exposure to Hg and Hg compounds." Even though this legal mechanism is in action for gathering data, per UNEP (United Nations Environmental Program) one of the primary data gaps on developing countries with ASGM are the lack of databases on inventories of Hg consumption, environmental releases, and levels of Hg compounds in various media including human exposure.

Mercury biomarkers have been used in many health biomonitoring studies of ASGM communities. The selection of biomarkers for human biomonitoring requires the consideration of different factors: (1) the Hg compound analyzed (InHg or MeHg); (2) level of exposure; (3) time of sampling after the exposure; (4) cultural characteristics of the population; and (5) available equipment, personnel and resources (UNEP & WHO, 2008). ASGM sites are a challenge for human biomonitoring programs since they are found mainly in remote settings.

Based on my review of the literature, I conclude that the Hg human biomonitoring programs in ASGM communities must include the following:

- The biomarker should be as less invasive as possible to be able to access information of exposure for infants, children, and the elderly (McDade et al., 2012a).
- Due to the double Hg exposure (InHg and MeHg) experienced by people inhabiting ASGM sites, the biomarker has to be viable for Hg speciation (Poulin and Gibb, 2008).
- Minimum expenses and materials for transport and storage due to the remote and resource-limited settings of ASGM sites, and large populations they tend to contain (Veiga & Baker, 2004).
- The analytical methods and the equipment should be reliable for the nature of the sample and, in particular, for the expected concentration levels of Hg in the sample, including a very wide range in concentrations (NRC, 2000; UNEP & WHO, 2008).
- Avoid external contamination of the biomarker (NRC, 2000) given the high levels of different Hg species in diverse media (especially air) within ASGM communities.

For ASGM communities, three main biomarkers have been used for Hg exposure assessment (urine, hair, and blood). Each biomarker offers different information on the type of Hg (InHg or MeHg), and a timeline of the exposure (Table 1-1), nevertheless they all have limitations in terms of their use in ASGM-focused biomonitoring efforts.

Table 1-1. Information related to biomarkers of exposure

Biomarker	Mercury exposure measurement	Timeline / half-lives
Urine	InHg	Recent exposure and past exposure Half-life: 1-2 months
Hair	MeHg	Long-term average exposure Half-life: 102 ±31 days
Blood	MeHg and InHg	Recent or current exposure Half-life: 50 days

b. Hair

The measurement of Hg in scalp hair can correlate well with dietary MeHg intake (Poulin and Gibb, 2008), and the analysis of hair total Hg (THg) is a recommended approach for monitoring MeHg exposures in the general population (Esteban et al., 2015). Exposure over time, including magnitude and peak levels, can be estimated using THg measurements taken at increasing distances from the root given that hair grows at approximately 1 cm/month (NRC, 2000). Hair sampling, transport, and storage are simple and non-invasive (Poulin and Gibb, 2008). Nevertheless, ethical and logistical limitations have been reported for the use of hair in ASGM communities including 1) superstitions about the use of hair for "black magic" (Ikingura JR, 1996); 2) sampling in men with short hair (Veiga & Baker, 2004); 3) the use of soaps that contain Hg (Glahder et al., 1999); and 4) external environmental contamination of hair with elemental Hg (Esteban et al., 2015). Notably, a recent study using Hg stable isotopes found that THg in the hair of ASGM miners is not an accurate measure of exposure to MeHg by fish consumption due to the prevalence of elemental Hg in the local atmosphere. Thus, it is challenging to differentiate Hg in hair as either InHg or MeHg (Sherman et al., 2015).

c. Urine

Total Hg in urine has been associated with recent and cumulative exposure to InHg (from elemental Hg) (Li et al., 2011). The average concentrations of THg in urine samples collected from ASGM community members are 20µg/L for miners who frequently burn amalgams (Veiga and Baker, 2004), and 1,168µg/L for gold shoppers (Malm et al., 1995). These are much higher than urine THg levels from background populations which tend to fall under 3 µg/L (Basu et al. 2018).

Though relatively simple to obtain, urine samples do pose some technical challenges. First, the dilution factor should be corrected by creatinine or specific gravity (Veiga & Baker, 2004); and second, samples require relatively large volumes and bulky materials, and the sample should be frozen shortly after collection and kept frozen during transportation (UNEP & WHO, 2008), which makes urine challenging for biomonitoring Hg in ASGM communities.

d. Whole blood

The concentration of THg in whole blood shows recent exposure to MeHg for the general population. It is often used as a proxy of MeHg exposure through fish consumption as it is assumed that around 90% of the THg in fish is present as MeHg (Bloom, 1992). For communities exposed to elemental Hg and MeHg, whole blood can give useful information of exposure to both types of Hg, especially for areas where the hair is not a suitable biomarker for MeHg, as in ASGM sites (Drasch et al., 2001). For communities exposed to elemental Hg (i.e., ASGM), InHg can be a significant contributor to THg in the blood (Berglund et al., 2005) though surprisingly few studies have well characterized Hg speciation in blood sampled from ASGM community members. Even though whole blood can be used as a biomarker for InHg and MeHg, it requires invasive techniques to obtain (compared to urine and hair), generating ethical considerations on the number of samples according to the health and age status of the person. Whole blood sampling requires clinical settings, and samples need to be centrifuged and preserved in cold temperatures, increasing the cost and requirements for transportation and storage (UNEP & WHO, 2008).

Though blood can supply information about exposure to MeHg and InHg, it is challenging to obtain in ASGM communities. Notable limitations are related to the context of the sites (e.g., large populations, remote settings, high exposures to Hg species, vulnerable populations), and collection requirements (trained personnel/phlebotomists, equipment including specialized syringes and tubes), and the need for "cold chain" for transport and preservation. These limitations call for the need to explore alternative approaches.

Nonetheless, the ability to speciate mercury in blood can potentially yield important information on health risks, assessment of sources of exposure, and policy evaluation. As mentioned in the previous section (1.2.5 toxicokinetics), each mercury species has different toxicokinetics and target organs, and thus the health risks can vary. The primary targets of InHg are the nervous system and the kidneys, though it can cause damage to the respiratory, cardiovascular, digestive and immune system (Clarkson and Magos, 2006). On the other hand, the brain and the central nervous system are primary target sites where MeHg's adverse effects usually manifest (Agency for Toxic Substances and Disease Registry, 1999; World Health Organization (WHO), 2013).

Chemical speciation of Hg in whole blood can help distinguish between occupational (mainly InHg) and dietary (mainly MeHg) exposures, and it is vital to characterize risk in communities as well as evaluate the success of Hg-reduction efforts such as those listed in the Minamata Convention (Eagles-Smith et al., 2018).

1.2.7 Dried Blood Spots (DBS)

Dried blood spots represent an alternative for classical venous blood sampling (Table 1-2). DBS are drops of whole blood collected on a standardized paper from a finger or heel prick (Raju et al., 2016). Contrary to the whole blood samples, the blood to create DBS does not need to be centrifuged, separated, or frozen after collection (McDade et al., 2007). The low burden of sampling is also helpful for studies with infants, children, and the elderly (for which venipuncture can be particularly problematic), increasing the feasibility of collecting large numbers of samples in a community. Accordingly, DBS supplies a minimally invasive method that is conducive for

field-based research in resource limiting settings (such as ASGM sites) as there is no need for specialized equipment, cold-chain handling, and phlebotomists.

Table 1-2. Advantages and limitations of DBS compare with whole blood samples.

Variables	Whole Blood		DBS	
	Advantages	**Disadvantages**	**Advantages**	**Disadvantages**
Sample collection		Invasive	Relative painless and non-invasive	
		Clinical settings	No need for trained personnel or clinical settings	
Sample storage	Sample stability has been studied. Methods and supplies are set up.	Blood needs to be centrifuged, separated, or immediately frozen after collection	No need for particular settings.	After collection DBS are left to dry, cross-contamination during this time can occur. Unknown stability of Hg in the sample.
Sample transport		A necessity for cold chain from the sampling location to the laboratory.	No especially requirements for transport	
Laboratory analysis	It is the gold standard. Most studies have measured it			Requires the development of new analytical methods
	Known sample volume			Unknown sample volume
	Analysis can be done in serum and plasma			Gives only information on whole blood

a. Dried blood spots for Hg exposure assessment

Previous academic research studies, as well as some state health agencies, have reported the use of DBS for Hg in newborn screening projects (Minnesota Department of Health Division of Environmental Health., 2007; Utah Department of Epidemiology, 2014). Key studies are reviewed here with detailed information on these studies presented in Chapter 2 (Supporting Information S2-1): (a) Basu et al. (2017) was the first study to characterize MeHg in DBS samples, and the authors followed US EPA 1630 method using a GC-CVAFS to analyze MeHg levels in DBS from newborns (n = 675) from the Michigan BioTrust for Health program. The results demonstrated an accurate method (range from 96% to 115%) though with high variance at the lower concentration range (Basu et al., 2017); (b) Nelson et al. (2016) analyzed THg in paired cord blood-DBS from 48 individuals by ICP-MS, and their results showed higher limit of detection for DBS than for cord blood with THg detected in 38% of the DBS samples and 62% of the cord blood samples (Nelson et al., 2016); (c) Funk et al. (2015) analyzed THg on paired whole blood-DBS samples from 82 individuals by ICP-MS, and found a correlation between whole blood and DBS Hg levels with a weak-to-moderate R^2 value (0.498) possibly due to a background contamination of the card (Funk, 2015); (d) Funk et al. (2013) analyzed 49 newborn DBS by ICP-MS, though THg was on detected in 33% of the samles (Funk et al., 2013); (e) Chaudhuri et al. (2008) analyzed 18 newborn DBS samples from the U.S. Rocky Mountain region by ICP-MS. While the method highlighted the stability and reproducibility of DBS for the analysis of THg, 33% of the samples were below the limit of detection (Chaudhuri et al., 2009).

Despite promises in characterizing Hg exposure in past studies, there are notable analytical challenges to overcome for the use of DBS as an accurate and precise method for measuring Hg. In addition, few studies have characterized chemical species of Hg in DBS.

b. Limitations

Here I highlight the challenges faced by the broader community interested in biomarker measurements in DBS.

- **Small and unknown sample volume**

The collection of a DBS sample involves the use of a micro-lancet to deliver a controlled puncture (USAID, 2012), and around five drops of blood of unknown volume (usually ~30-60 µL per drop) are collected onto the filter paper. In contrast to a venipuncture blood draw that generally yields at least 5 ml (i.e., a known and measurable volume) of whole blood (Mcdade, 2013). The small amount of blood in DBS may limit the analysis of THg and Hg species due to detection limit concerns as outlined above in the studies by Chaudhuri et al., (2009) and Funk et al., (2013) (Table 1-3 summarizes the concerns related with method detection limit related with each instrument of analysis).

Table 1-3. Method detection limits for DBS using a variety of instruments.

Equipment	Method detection limit (µg/L)	References
ICP-MS	THg 0.01 – 0.7	(Chaudhuri et al., 2009; Nelson et al., 2016; Elias Nyanza et al., 2019)
DMA	THg 12.0	(Perkins and Basu, 2018)
GC-CVAFS	MeHg 0.3	(Basu et al., 2017)

The blood volume used to saturate the paper is proportional to the hematocrit level of the sample. This may result in changes in the viscosity of blood leading to differences in the diffusion of blood through the paper (Denniff and Spooner, 2010) . The spreadability factor is a potential source of error for an analyte concentration in a DBS (Mei et al., 2001; O'Broin et al., 1995). Previous studies have also found the heterogeneous distribution of metals and analytes (Fan and Lee, 2012; Hempen et al., 2015; Lenk et al., 2015a; O'Mara et al., 2011; Vries et al., 2013), and THg

(Chaudhuri et al., 2009; Perkins and Basu, 2018) within a DBS spot. Studies on the homogeneity of the spots have found higher concentrations on the edge of the spot, which has been referred to as the "coffee-stain" effect. Therefore the location of a sub-sample of a DBS sample can bias the measurement (Lenk et al., 2015a; O'Mara et al., 2011).

- **Dried Blood Spots are not the clinical standard**

Since DBS are taken from capillary blood, comparing DBS results with assays from venous blood samples via venipuncture is not accurate (McDade et al., 2007). Therefore, the development of analytical methods for DBS needs to be validated with the use of a gold-standard method (i.e., compare capillary and venous blood measures). Past studies have reported on differences in hematological parameters (e.g., erythrocyte count, hematocrit and hemoglobin) between venous blood and capillary blood, and also differences in these parameters between continuous drops of blood (Bond & Richards-Kortum, 2015; Daae et al., 1988; Daae et al., 1988). Since MeHg is bound to hemoglobin in the red blood cells (RBC), and InHg is distributed between RBC and plasma (Berglund et al., 2005), there is likely to be variability in the concentration of Hg between the different sources of blood, but this is not yet known.

- **Storage and field conditions**

The chemical stability of an analyte in DBS can be affected by conditions of collection, transportation, and storage. Changes in temperature and humidity over time need consideration when developing a DBS method. Artisanal and small-scale gold mining communities are usually located in tropical and subtropical countries (UN Environment Programme, 2013a), with high humidity and hot temperatures conditions (Rajaee et al., 2015). Past studies examining a variety of time and storage conditions (temperature and humidity) concluded that THg values in DBS are stable (Chaudhuri et al., 2009; Perkins and Basu, 2018). Though similar studies have not examined whether this stability extends to different chemical species of Hg in DBS.

Further, as of now there is no validated technique for the analysis of Hg species in DBS. The development and validation of an assay protocol to use DBS for the study of InHg and MeHg exposure for biomonitoring purposes, especially within ASGM communities, could represent essential tool as it is expected to overcome existing technical, logistical, and ethical barriers associated with traditional biomonitoring approaches. In doing so, a DBS-based method may help meet practical and regulatory needs worldwide.

1.3 References

Agency for Toxic Substances and Disease Registry, 1999. Toxicological Profile for Mercury Department of Health and Human Services 676.

Akagi, H., Castillo, E.S., Cortes-Maramba, N., Francisco-Rivera, A.T., Timbang, T.D., 2000. Health assessment for mercury exposure among schoolchildren residing near a gold processing and refining plant in Apokon, Tagum, Davao del Norte, Philippines. Sci. Total Environ. 259, 31–43. https://doi.org/10.1016/S0048-9697(00)00547-7

Bakir F, Damluji SF, Amin-Zaki L, Murtadha M, Khalidi A, al-Rawi NY, Tikriti S, Dahahir HI, Clarkson TW, Smith JC, D.R., 1973. Methylmercury poisoning in Iraq. Science (80-.). 181, 230–241.

Basu, N., Clarke, E., Green, A., Calys-Tagoe, B., Chan, L., Dzodzomenyo, M., Fobil, J., Long, R., Neitzel, R., Obiri, S., Odei, E., Ovadje, L., Quansah, R., Rajaee, M., Wilson, M., 2015. Integrated Assessment of Artisanal and Small-Scale Gold Mining in Ghana—Part 1: Human Health Review. Int. J. Environ. Res. Public Health 12, 5143–5176. https://doi.org/10.3390/ijerph120505143

Basu, N., Eng, J.W.L., Perkins, M., Santa-Rios, A., Martincevic, G., Carlson, K., Neitzel, R.L., 2017. Development and application of a novel method to characterize methylmercury exposure in newborns using dried blood spots. Environ. Res. 159, 276–282. https://doi.org/10.1016/J.ENVRES.2017.08.021

Basu, N., Horvat, M., Evers, D.C., 2018a. A Systematic Review of Mercury Exposures in Human Populations Worldwide Between 2000 and 2018.

Basu, N., Horvat, M., Evers, D.C., Zastenskaya, I., Weihe, P., Tempowski, J., 2018b. A State-of-the-Science Review of Mercury Biomarkers in Human Populations Worldwide between 2000 and 2018. Environ. Health Perspect. 126, 106001. https://doi.org/10.1289/ehp3904

Berglund, M., Lind, B., Björnberg, K.A., Palm, B., Einarsson, Ö., Vahter, M., 2005. Inter-individual variations of human mercury exposure biomarkers: a cross-sectional assessment.

Berlin, M., and Johansson, L.G., 1964. Mercury in mouse brain after inhalation of mercury vapor and after intravenous injection of mercury salt. Nature 204, 85–86.

Berlin, M., Fazackerly, J., and Nordberg, G., 1969. Mercurial induced brain monoamine oxidase inhibition in the teleostChanna punctatus (bloch). Arch. Environ. Heal. 18, 719–729.

Berlin, M., Zalups, R.K., Fowler, B.A., 2007. Mercury, in: Gunnar F. Nordberg, Bruce A. Fowler, M.N. (Ed.), Handbook on the Toxicology of Metals. Academic Press, 2014, pp. 675–729. https://doi.org/10.1016/B978-0-12-369413-3.50088-4

Bloom, N.S., 1992. On the Chemica Form of Mercury in Edible Fish and nvertebrate Tissue. Fish Aquat. Sci 49, 1010–1017.

Bond, M.M., Richards-Kortum, R.R., 2015. Drop-to-drop variation in the cellular components of fingerprick blood: Implications for point-of-care diagnostic development. Am. J. Clin. Pathol. 144, 885–894. https://doi.org/10.1309/AJCP1L7DKMPCHPEH

Bradley, M., Barst, B., Basu, N., 2017. A Review of Mercury Bioavailability in Humans and Fish. Int. J. Environ. Res. Public Health 14, 169. https://doi.org/10.3390/ijerph14020169

Chaudhuri, S.N., Butala, S.J.M., Ball, R.W., Braniff, C.T., 2009. Pilot study for utilization of dried blood spots for screening of lead, mercury and cadmium in newborns. J. Expo. Sci. Environ. Epidemiol. 19, 298–316. https://doi.org/10.1038/jesee.2008.19

Cherian, M.G., Hursh, J.B., Clarkson, T.W., Allen, J., 1978. Radioactive Mercury Distribution in Biological Fluids and Excretion in Human Subjects after Inhalation of Mercury Vapor. Arch. Environ. Heal. An Int. J. 33, 109–114. https://doi.org/10.1080/00039896.1978.10667318

Choi, A.L., Fitzgerald, W.F., Hammerschmidt, C.R., Lamborg, C.H., Soerensen, A.L., 2012. Mercury biogeochemical cycling in the ocean and policy implications. Environ. Res. 119, 101–117. https://doi.org/10.1016/J.ENVRES.2012.03.013

Clarkson, T.W., Magos, L., 2006. The Toxicology of Mercury and Its Chemical Compounds. Crit. Rev. Toxicol. 36, 609–662. https://doi.org/10.1080/10408440600845619

Clarkson, T.W., Magos, L., Myers, G.J., 2003. The toxicology of mercury current exposures and clinical manifestations. N. Engl. J. Med. 349, 1731–1737. https://doi.org/10.1056/NEJMra022471

Clarkson, T.W., Vyas, J.B., Ballatori, N., 2007. Mechanisms of mercury disposition in the body. Am. J. Ind. Med. 50, 757–764. https://doi.org/10.1002/ajim.20476

Clifford, M.J., 2014. Future strategies for tackling mercury pollution in the artisanal gold mining sector: Making the Minamata Convention work. Futures 62, 106–112. https://doi.org/10.1016/j.futures.2014.05.001

Cordy, P., Veiga, M.M., Salih, I., Al-Saadi, S., Console, S., Garcia, O., Mesa, L.A., Velásquez-López, P.C., Roeser, M., 2011a. Mercury contamination from artisanal gold mining in Antioquia, Colombia: The world's highest per capita mercury pollution. Sci. Total Environ. 410–411, 154–60. https://doi.org/10.1016/j.scitotenv.2011.09.006

Cordy, P., Veiga, M.M., Salih, I., Al-Saadi, S., Console, S., Garcia, O., Mesa, L.A., Velásquez-López, P.C., Roeser, M., 2011b. Mercury contamination from artisanal gold mining in Antioquia, Colombia: The world's highest per capita mercury pollution. Sci. Total Environ. 410–411, 154–160. https://doi.org/10.1016/j.scitotenv.2011.09.006

Council, A.G., 2015. Developing Baseline Estimates of Mercury Use in Artisanal and Small-Scale Gold Mining Communities : A Practical Guide (Version 1.0).

Daae, L.N.W., Halvorsen, S., Mathisen, P.M., Mironska, K., 1988. A comparison between haematological parameters in “capillary” and venous blood from healthy adults. Scand. J. Clin. Lab. Invest. 48, 723–726. https://doi.org/10.3109/00365518809085796

Denniff, P., Spooner, N., 2014. Volumetric Absorptive Microsampling: A Dried Sample Collection Technique for Quantitative Bioanalysis. Anal. Chem 86, 34. https://doi.org/10.1021/ac5022562

Denniff, P., Spooner, N., 2010. The effect of hematocrit on assay bias when using DBS samples for the quantitative bioanalysis of drugs. Bioanalysis 2, 1385–1395. https://doi.org/10.4155/bio.10.103

Discalzi G, Fabbro D, Meliga F, Mocellini A, C.F., 1993. Effects of occupational exposure to mercury and lead on brainstem auditory evoked potentials. Int. J. Psychophysiol. 14, 21–25.

Drasch, G., Böse-O’Reilly, S., Beinhoff, C., Roider, G., Maydl, S., 2001. The Mt. Diwata study on the Philippines 1999--assessing mercury intoxication of the population by small scale gold mining. Sci. Total Environ. 267, 151–68. https://doi.org/10.1016/S0048-9697(00)00806-8

Driscoll, C.T., Mason, R.P., Chan, H.M., Jacob, D.J., Pirrone, N., 2013. Mercury as a Global Pollutant: Sources, Pathways, and Effects. Environ. Sci. Technol. 47, 4967–4983. https://doi.org/10.1021/es305071v

Eagles-Smith, C.A., Silbergeld, E.K., Basu, N., Bustamante, P., Diaz-Barriga, F., Hopkins, W.A., Kidd, K.A., Nyland, J.F., 2018. Modulators of mercury risk to wildlife and humans in the context of rapid global change. Ambio 47, 170–197. https://doi.org/10.1007/s13280-017-1011-x

Enderle, Y., Foerster, K., Burhenne, J., 2016. Clinical feasibility of dried blood spots: Analytics, validation, and applications. J. Pharm. Biomed. Anal. 130, 231–243. https://doi.org/10.1016/j.jpba.2016.06.026

Esteban, M., Schindler, B.K., Jiménez, J.A., Koch, H.M., Angerer, J., Rosado, M., Gómez, S., Casteleyn, L., Kolossa-Gehring, M., Becker, K., Bloemen, L., Schoeters, G., Hond, E. Den, Sepai, O., Exley, K., Horvat, M., Knudsen, L.E., Joas, A., Joas, R., Aerts, D., Biot, P., Borošová, D., Davidson, F., Dumitrascu, I., Fischer, M.E., Grander, M., Janasik, B., Jones, K., Kašparová, L., Larssen, T., Naray, M., Nielsen, F., Hohenblum, P., Pinto, R., Pirard, C., Plateel, G., Tratnik, J.S., Wittsiepe, J., Castaño, A., 2015. Mercury analysis in hair: Comparability and quality assessment within the transnational COPHES/DEMOCOPHES project. Environ. Res. 141, 24–30. https://doi.org/10.1016/j.envres.2014.11.014

Faial, K., Deus, R., Deus, S., Neves, R., Jesus, I., Santos, E., Alves, C.N., Brasil, D., 2015. Mercury levels assessment in hair of riverside inhabitants of the Tapajós River, Pará State, Amazon, Brazil: fish consumption as a possible route of exposure. J. Trace Elem. Med. Biol. 30, 66–76. https://doi.org/10.1016/j.jtemb.2014.10.009

Fan, L., Lee, J. a, 2012. Managing the effect of hematocrit on DBS analysis in a regulated environment. Bioanalysis. https://doi.org/10.4155/bio.11.337

Funk, W.E., 2015. Use of Dried Blood Spots for Estimating Children's Exposures to Heavy Metals in Epidemiological Research. J. Environ. Anal. Toxicol. s7. https://doi.org/10.4172/2161-0525.S7-002

Funk, W.E., McGee, J.K., Olshan, A.F., Ghio, A.J., 2013. Quantification of arsenic, lead, mercury and cadmium in newborn dried blood spots. Biomarkers 18, 174–7. https://doi.org/10.3109/1354750X.2012.750379

Gibb, H., O'Leary, K.G., 2014. Mercury exposure and health impacts among individuals in the artisanal and small-scale gold mining community: A comprehensive review. Environ. Health Perspect. 122, 667–672. https://doi.org/10.1289/ehp.1307864

Glahder, C.M.; Appel, P.; Asmund, G., 1999. Mercury in Soap in Tanzania.

Grandjean, P., Weihe, P., White, R.F., Debes, F., Araki, S., Yokoyama, K., Murata, K., S??rensen, N., Dahl, R., J??rgensen, P.J., 1997. Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. Neurotoxicol. Teratol. 19, 417–428. https://doi.org/10.1016/S0892-0362(97)00097-4

Guimaraes, J.R.D., Betancourt, O., Miranda, M.R., Barriga, R., Cueva, E., Betancourt, S., 2011. Long-range effect of cyanide on mercury methylation in a gold mining area in southern Ecuador. Sci. Total Environ. 409, 5026–33. https://doi.org/10.1016/j.scitotenv.2011.08.021

Ha, E., Basu, N., Bose-O 'reilly, S., Dórea, J.G., Mcsorley, E., Sakamoto, M., Chan, H.M., 2016. Current progress on understanding the impact of mercury on human health. Environ. Res. 1–15. https://doi.org/10.1016/j.envres.2016.06.042

Harris, R.C., Rudd, J.W.M., Amyot, M., Babiarz, C.L., Beaty, K.G., Blanchfield, P.J., Bodaly, R.A., Branfireun, B.A., Gilmour, C.C., Graydon, J.A., Heyes, A., Hintelmann, H., Hurley, J.P., Kelly, C.A., Krabbenhoft, D.P., Lindberg, S.E., Mason, R.P., Paterson, M.J., Podemski, C.L., Robinson, A., Sandilands, K.A., Southworth, G.R., St Louis, V.L., Tate, M.T., 2007. Whole-ecosystem study shows rapid fish-mercury response to changes in mercury deposition. Proc. Natl. Acad. Sci. U. S. A. 104, 16586–91. https://doi.org/10.1073/pnas.0704186104

Hempen, C.M., Koster, E.H.M., Ooms, J.A., 2015. Hematocrit-independent recovery of immunosuppressants from DBS using heated flow-through desorption. Bioanalysis.

Horvat, M., Snoj Tratnik, J., Miklavčič, A., 2012. Mercury: Biomarkers of Exposure and Human Biomonitoring, Biomarkers and Human Biomonitoring Vol.1: Ongoing Programs and Exposures.

Hursh, D. A., Wendt, S. F., Lee, C. F., et al., 1989. No Title. J. Clin. Microbiol 27, 1695–1696.

Ikingura JR, A.H., 1996. Monitoring of fish and human exposure to mercury due to gold mining in the Lake Victoria goldfields, Tanzania. Sci. Total Environ. 191, 59–68.

Kershaw TG, Clarkson TW, D.P., 1980. The relationship between blood levels and dose of methylmercury in man. Arch Env. Heal. 1, 28–36.

Kurdland, L.T., Faro, S.N., Siedler, H., 1960. Minamata disease. The outbreak of a neurologic disorder in Minamata, Japan, and its relationship to the ingestion of seafood contaminated by mercuric compounds. World Neurol. 1, 370–395.

Lenk, G., Hansson, J., Beck, O., Roxhed, N., 2015. The effect of drying on the homogeneity of DBS. Bioanalysis.

Li, P., Feng, X., Shang, L., Qiu, G., Meng, B., Zhang, H., Guo, Y., Liang, P., 2011. Human co-exposure to mercury vapor and methylmercury in artisanal mercury mining areas, Guizhou, China. Ecotoxicol. Environ. Saf. 74, 473–9. https://doi.org/10.1016/j.ecoenv.2010.10.030

Malm, O., Castro, M.B., Bastos, W.R., Branches, F.J.P., Guimarães, J.R.D., Zuffo, C.E., Pfeiffer, W.C., 1995. An assessment of Hg pollution in different goldmining areas, Amazon Brazil. Sci. Total Environ. 175, 127–140. https://doi.org/10.1016/0048-9697(95)04909-6

Mcdade, T., 2013. Development and validation of assay protocols for use with dried blood spot samples. Am. J. Hum. Biol. 9, 1–9. https://doi.org/10.1002/ajhb.22463

McDade, T., Williams, S., Snodgrass, J., 2007. What a drop can do: Dried blood spots as minimally invasive method for integrating biomarkers into population-based research. Demography 44, 899–925.

Mei, J. V, Alexander, J.R., Adam, B.W., Hannon, W.H., 2001. Innovative Non- or Minimally-Invasive Technologies for Monitoring Health and Nutritional Status in Mothers and Young Children Use of Filter Paper for the Collection and Analysis of Human Whole Blood Specimens 1. J. Nutr. 131, 1631–1636.

Minnesota Department of Health Division of Environmental Health., 2007. Mercury Levels in Blood from Newborns in the Lake Superior Basin. (GLNPO ID 2007-942).

Nelson, J.W., Edhlund, B.L., Johnson, J., Rosebush, C.E., Holmquist, Z.S., Swan, S.H., Nguyen, R.H.N., 2016. Assessing a new method for measuring fetal exposure to mercury: Newborn bloodspots. Int. J. Environ. Res. Public Health 13. https://doi.org/10.3390/ijerph13070692

Nelson, J.W., Edhlund, B.L., Johnson, J., Rosebush, C.E., Holmquist, Z.S., Swan, S.H., Nguyen, R.H.N., Theall, K.P., Johnson, C.C., n.d. Assessing a New Method for Measuring Fetal Exposure to Mercury: Newborn Bloodspots. https://doi.org/10.3390/ijerph13070692

NRC, 2000. Risk Characterization and Public Health Implications, in: Toxicological Effects of Methylmercury. National Academies Press, Washington, US, pp. 304–332.

Nyanza, E.C., Bernier, F.P., Manyama, M., Hatfield, J., Martin, J.W., Dewey, D., 2019a. Maternal exposure to arsenic and mercury in small-scale gold mining areas of Northern Tanzania. Environ. Res. 173, 432–442. https://doi.org/10.1016/j.envres.2019.03.031

Nyanza, E.C., Dewey, D., Bernier, F., Manyama, M., Hatfield, J., Martin, J.W., 2019b. Validation of Dried Blood Spots for Maternal Biomonitoring of Nonessential Elements in an Artisanal and Small-Scale Gold Mining Area of Tanzania. Environ. Toxicol. Chem. 38, 1285–1293. https://doi.org/10.1002/etc.4420

O'Broin, S.D., Kelleher, B.P., Gunter, E., 1995. Evaluation of factors influencing precision in the analysis of samples taken from blood spots on filter paper. Clin. Lab. Haematol. 17, 185–8.

O'Mara, M., Hudson-Curtis, B., Olson, K., Yueh, Y., Dunn, J., Spooner, N., 2011. The effect of hematocrit and punch location on assay bias during quantitative bioanalysis of dried blood spot samples. Bioanalysis 3, 2335–47. https://doi.org/10.4155/bio.11.220

Obrist, D., Kirk, J.L., Zhang, L., Sunderland, E.M., Jiskra, M., Selin, N.E., 2018. A review of global environmental mercury processes in response to human and natural perturbations: Changes of emissions, climate, and land use. Ambio 47, 116–140. https://doi.org/10.1007/s13280-017-1004-9

Perkins, M., Basu, N., 2018. Dried blood spots for estimating mercury exposure in birds. Environ. Pollut. 236, 236–246. https://doi.org/10.1016/J.ENVPOL.2018.01.036

Pirrone, N., Cinnirella, S., Feng, X., Finkelman, R.B., Friedli, H.R., Leaner, J., Mason, R., Mukherjee, A.B., Stracher, G.B., Streets, D.G., Telmer, K., 2010. Global mercury emissions to the atmosphere from anthropogenic and natural sources. Atmos. Chem. Phys. 10, 5951–5964. https://doi.org/10.5194/acp-10-5951-2010

Poulin, J., Gibb, H., 2008. Mercury Assessing the environmental burden of disease at national and local levels. WHO Environ. Burd. Dis. 16, 1–42.

Rajaee, M., Long, R., Renne, E., Basu, N., 2015a. Mercury Exposure Assessment and Spatial Distribution in A Ghanaian Small-Scale Gold Mining Community. Int. J. Environ. Res. Public Health 12, 10755–10782. https://doi.org/10.3390/ijerph120910755

Rajaee, M., Obiri, S., Green, A., Long, R., Cobbina, S.J., Nartey, V., Buck, D., Antwi, E., Basu, N., 2015b. Integrated Assessment of Artisanal and Small-Scale Gold Mining In Ghana Part 2: Natural Sciences Review. Int. J. Environ. Res. Public Health 12, 8971–9011. https://doi.org/10.3390/ijerph120808971

Raju, K.S.R., Taneja, I., Rashid, M., Sonkar, A.K., Wahajuddin, M., Singh, S.P., 2016. DBS-platform for biomonitoring and toxicokinetics of toxicants: proof of concept using LC-MS/MS analysis of fipronil and its metabolites in blood. Sci. Rep. 6, 22447. https://doi.org/10.1038/srep22447

Reynolds WA, P.R., 1975. Transplacental passage of methylmercury and its uptake by primate fetal tissues (38576). Proc Soc Exp Biol Med. 148, 523–526.

Sandborgh-Englund, G., Elinder, C.G., Johanson, G., Lind, B., Skare, I., Ekstrand, J., 1998. The absorption, blood levels, and excretion of mercury after a single dose of mercury vapor in humans. Toxicol. Appl. Pharmacol. 150, 146–153. https://doi.org/10.1006/taap.1998.8400

Selin, N.E., 2009. Global Biogeochemical Cycling of Mercury: A Review. Annu. Rev. Environ. Resour. 34, 43–63. https://doi.org/10.1146/annurev.environ.051308.084314

Sherman, L.S., Blum, J.D., Basu, N., Rajaee, M., Evers, D.C., Buck, D.G., Petrlik, J., DiGangi, J., 2015. Assessment of mercury exposure among small-scale gold miners using mercury stable isotopes. Environ. Res. 137, 226–34. https://doi.org/10.1016/j.envres.2014.12.021

Shi, J.B., Liang, L.N., Jiang, G. Bin, Jin, X.L., 2005. The speciation and bioavailability of mercury in sediments of Haihe River, China. Environ. Int. 31, 357–365. https://doi.org/10.1016/j.envint.2004.08.008

Slemr, F., Brunke, E.-G., Ebinghaus, R., Temme, C., Munthe, J., Wängberg, I., Schroeder, W., Steffen, A., Berg, T., 2003. Worldwide trend of atmospheric mercury since 1977. Geophys. Res. Lett. 30, n/a-n/a. https://doi.org/10.1029/2003GL016954

Sletvold, H., Svendsen, K., Aas, O., Syversen, T., Hilt, B., 2012. Neuropsychological function and past exposure to metallic mercury in female dental workers. Scand. J. Psychol. 53, 136–143. https://doi.org/10.1111/j.1467-9450.2011.00929.x

Sonke, J.E., Heimbürger, L.-E., Dommergue, A., 2013. Mercury biogeochemistry: Paradigm shifts, outstanding issues and research needs. Comptes Rendus Geosci. 345, 213–224. https://doi.org/10.1016/j.crte.2013.05.002

Steffen, A., Douglas, T., Amyot, M., Ariya, P., Aspmo, K., Berg, T., Bottenheim, J., Brooks, S., Cobbett, F., Dastoor, A., Dommergue, A., Ebinghaus, R., Ferrari, C., Gardfeldt, K., Goodsite, M.E., Lean, D., Poulain, A.J., Scherz, C., Skov, H., Sommar, J., Temme, C., 2008. A synthesis of atmospheric mercury depletion event chemistry in the atmosphere and snow. Atmos. Chem. Phys. 8, 1445–1482. https://doi.org/10.5194/acp-8-1445-2008

Suda, I., Takahashi, H., 1992. Degradation of methyl and ethyl mercury into inorganic mercury by other reactive oxygen species besides hydroxyl radical. Arch. Toxicol. 66, 34–39. https://doi.org/10.1007/BF02307267

Syversen, T., Kaur, P., 2012. The toxicology of mercury and its compounds. J. Trace Elem. Med. Biol. 26, 215–226. https://doi.org/10.1016/j.jtemb.2012.02.004

Telmer, K.H., Veiga, M.M., 2009. Mercury Fate and Transport in the Global Atmosphere, Mercury Fate and Transport in the Global Atmosphere: Emissions, Measurements and Models. Springer US, Boston, MA. https://doi.org/10.1007/978-0-387-93958-2

UN Environment Programme, 2019. Global Mercury Assessment 2018. Geneva, Switzerland.

UN Environment Programme, 2013a. Global Mercury Assessment 2013: Sources, Emissions, Releases, and Environmental Transport, Narayana Press.

UN Environment Programme, 2013b. Minamata Convention on Mercury - Text and Annexes.

UN Environment Programme, 2012. Reducing Mercury Use in Artisanal and Small-Scale 76 pages.

UN Environment Programme, 2010. Addressing the Social and Financial Challenges of Artisanal and Small Scale Gold Mining: Meeting Summary.

UN Environment Programme, World Health Organization (WHO), 2008. Guidance for Identifying Populations At Risk From Mercury Exposure. Exposure 176.

USAID, 2012. Biomarker field manual: Demographic and Health Surveys Methodology. Calverton, Maryland.

Utah Department of Epidemiology, 2014. Utah Statewide Investigation of Neonatal Blood Lead Levels Using Newborn Blood Spot Specimens.

Veiga, M., 1997. Mercury in artisanal gold mining in Latin America: Facts, fantasies and solutions. Pollut. Deriving from Artis. Gold Min. 1–23.

Veiga, M.M., Baker, R., 2004. Protocols for Environmental and Health Assessment of Mercury Released by Artisanal and Small-Scale Gold Miners, Unido.

Vries, R. de, Barfield, M., Merbel, N. van de, Schmid, B., Siethoff, C., Ortiz, J., Verheij, E., Baar, B. van, Cobb, Z., White, S., Timmerman, P., 2013. The effect of hematocrit on bioanalysis of DBS: results from the EBF DBS-microsampling consortium. Bioanalysis.

Wang, H.S., Xu, W.F., Chen, Z.J., Cheng, Z., Ge, L.C., Man, Y.B., Giesy, J.P., Du, J., Wong, C.K.C., Wong, M.H., 2013. In vitro estimation of exposure of Hong Kong residents to mercury and methylmercury via consumption of market fishes. J. Hazard. Mater. 248–249, 387–393. https://doi.org/10.1016/j.jhazmat.2012.12.060

Wang, Shaofeng, Jia, Y., Wang, Shuying, Wang, X., Wang, H., Zhao, Z., Liu, B., 2009. Total mercury and monomethylmercury in water, sediments, and hydrophytes from the rivers, estuary, and bay along the Bohai Sea coast, northeastern China. Appl. Geochemistry 24, 1702–1711. https://doi.org/10.1016/j.apgeochem.2009.04.037

World Health Organization (WHO), 2019. GEMS/Food Contaminants Mercury [WWW Document]. URL https://extranet.who.int/gemsfood/Search.aspx?Contaminant=mercury (accessed 4.6.20).

World Health Organization (WHO), 2013. Mercury Exposure and Health Impacts among Individuals in the Artisanal and Small-Scale Gold Mining (ASGM) Community. Prev. Dis. through Heal. Environ. 1–7.

World Health Organization (WHO), 2003. Elemental mercury and inorganic mercury compounds: human health aspects. World Heal. Organ. Libr. 68.

World Health Organization (WHO), 1991. Inorganic Mercury.

CONNECTING PARAGRAPH

Chapter two consists of a published manuscript on the development and validation of a method under controlled laboratory conditions for Hg speciation in DBS. The paper covers a series of knowledge gaps related to the use of DBS for Hg exposure assessment, including the difference in Hg levels between venous whole blood (the gold standard biomarker on Hg speciation) and capillary whole blood, and the agreement between whole blood and DBS. Detailed information related to the background literature review on the use of DBS for the analysis of Hg, results of method development, experimental description of the selected method, quality control and results, statistical results, the stability of MeHg over time, and InHg measurements are presented in the Supporting Information.

This chapter was co-authored by the candidate's supervisor, Dr. Niladri Basu, and Dr. Benjamin Barst. The manuscript was published in Analytical Chemistry in 2020 as:

Santa-Rios A, Barst BD, Basu N. 2020. Mercury Speciation in Whole Blood and Dried Blood Spots from Capillary and Venous Sources. Analytical Chemistry. 92(5): 3605-3612. doi: 10.1021/acs.analchem.9b04407

2. CHAPTER 2. MERCURY SPECIATION IN WHOLE BLOOD AND DRIED BLOOD SPOTS FROM CAPILLARY AND VENOUS SOURCES

Andrea Santa-Rios[1], Benjamin D. Barst[2], Niladri Basu[1]

[1]Faculty of Agricultural and Environmental Sciences, McGill University, Montreal, Quebec, Canada. [2]Alaska Stable Isotope Facility, Water and Environmental Research Center, Institute of Northern Engineering, University of Alaska Fairbanks, Fairbanks, AK 99775, USA

2.1 Abstract

There is interest in measuring total mercury (THg) and methylmercury (MeHg) in dried blood spots (DBS) though more research is required to evaluate mercury (Hg) speciation in DBS and to validate the agreement between blood sources (venous vs. capillary) and matrices (whole blood vs. DBS). Therefore, the present study aimed to develop, evaluate, and validate a DBS-based method to assess MeHg and inorganic mercury (InHg) exposure in human population studies. First, we used volume-controlled (40µl) paired DBS-whole blood samples to develop an analytical method that involved the extraction and quantification of MeHg and InHg with gas chromatography–cold vapor atomic fluorescence spectrometry (GC-CVAFS). Next, we carried out a validation study using paired DBS-whole blood samples from venous and capillary sources from 49 volunteers with Hg exposures similar to background populations (i.e., MeHg in venous whole blood ranged from 0.2 to 3 µg/L with a median value of 0.8). The limits of detection were higher for InHg (1.9 and 1.1 µg/L in DBS and whole blood, respectively) than MeHg (0.3 and 0.2 µg/L in DBS and whole blood, respectively). The MeHg concentrations among blood sources and matrices were highly correlated (r ranged from 0.85 to 0.95), with no constant bias (intercept ranged from -0.05 to 0.13 µg/L) and small proportional bias (slopes ranged from 0.92 to 1.08). Bland-Altman plots indicated little bias between MeHg measurements with 82 – 98% of the cases meeting the analytical acceptance criterion of 35% maximum allowed difference. Our results

indicate that measures of MeHg in capillary DBS reflect concentrations in the gold standard (i.e., venous whole blood) and that DBS are a suitable tool for assessing MeHg exposure in human population studies, but that more work is required to assess InHg exposures.

2.2 Introduction

Mercury (Hg) is a contaminant of concern to ecosystem and human health worldwide (UN Environment Programme, 2019, 2013a, 2013b). Human biomonitoring studies of Hg exposure tend to focus on measuring Hg levels in biological samples with many researchers, scholarly works, and institutions favoring whole blood measures (Basu et al., 2018b). Despite the acceptance of whole blood as a biomarker of Hg exposure, there remain challenges to venipuncture thus posing a barrier from wider adoption. This is of particular note since Article 22, for example, of the Minamata Convention on Mercury calls out the need to monitor trends in human populations. Notable challenges to venipuncture include ethical concerns in many populations, logistical difficulties (e.g., need for a clinical setting and trained phlebotomist), and elevated costs associated with aforementioned aspects as well as the need for specialized collection supplies and post-collection sample storage and transport costs. An alternative to venipuncture is dried blood spot (DBS) sampling. This method involves the use of a micro-lancet to deliver a controlled puncture, typically to the finger or heel (USAID, 2012), yielding around five drops of blood (usually ~30-60 µL per drop) that are then collected on filter paper. In contrast to venipuncture, DBS sampling has fewer logistical requirements as highly trained personnel are not needed, DBS can be stored at ambient temperature, and shipments may involve small packages (Denniff and Spooner, 2014a). Furthermore, sample collection is minimally-invasive with only a small volume of sample collected (Enderle et al., 2016) increasing the feasibility of collecting large numbers of samples in communities of interest (Mcdade, 2013), while also providing a potential cost-effective opportunity for retrospective (e.g., DBS archived from newborn screening programs) and prospective studies.

The research community has started to develop and apply methods to measure Hg in human DBS samples, and these are summarized (Supporting Information S2-1. Previous studies). A pilot study in 2008 was the first in this field where researchers analyzed total Hg levels in 18 newborn DBS samples using inductively coupled plasma mass spectrometry (ICP-MS). In doing so they highlighted the stability and reproducibility of total Hg measures from DBS while also raising concerns about the method detection limit (MDL). Subsequent studies evaluated THg in newborn DBS samples using ICP-MS (Funk, 2015; Funk et al., 2013; Nelson et al., 2016), also noting a

relatively high MDL (i.e., only 33-38% of the samples were above the MDL). Studies comparing Hg levels in whole blood (Funk, 2015; Elias Nyanza et al., 2019) and cord blood (Nelson et al., 2016) with their corresponding DBS demonstrated a correlation. One study validated the method in a field setting showing low total Hg concentrations in field blanks (Elias. Nyanza et al., 2019). Our group was the first to characterize methylmercury (MeHg) levels in DBS samples given that this chemical form of Hg in most populations is of greatest health concern (Basu et al., 2017). We analyzed 675 newborn DBS using a GC-CVAFS, and in doing so delivered a method that paid close attention to a range of quality control criteria outlined by US EPA Method 1630.

Although previous studies have started to demonstrate the suitability of DBS for the analysis of THg and MeHg, outstanding gaps and analytical challenges remain, and these pose a barrier for wider adoption of this approach. First, few studies to date have carefully performed, examined, or documented key parameters related to measurement quality such as analytical accuracy, precision, relevant range, or detection limits (see Table S2-1. Previous studies). Even fewer studies have attempted to determine how stable Hg species are in the DBS following prolonged storage periods (seldom would DBS be analyzed immediately upon collection). Second, most DBS studies have focused on characterizing levels of total Hg. While the majority of blood Hg in most populations is in the organic MeHg form (USAID, 2012), the proportion can range from 0 to 100% (Mahaffey et al., 2004). There are notable communities with elevated environmental and occupational exposures to inorganic mercury (InHg, e.g., dental workers, artisanal gold miners) (Agency for Toxic Substances and Disease Registry, 1999; Clarkson and Magos, 2006; UN Environment Programme, 2019, 2013a). These exposures result in differing speciation profiles in whole blood that are not well characterized by simple measures of THg (Ha et al., 2016; World Health Organization (WHO), 2008), and thus there is a need to determine Hg species (MeHg and InHg) in DBS which has yet to be addressed. Finally, DBS are derived from capillary blood and it is not known how well this reflects the gold standard (i.e., venous whole blood). Capillary whole blood is a mixture of arterial and venous whole blood plus interstitial fluid(Enderle et al., 2016); thus, physiologically it differs from venous whole blood. No study has systematically characterized Hg species between venous and capillary blood sources as well as different matrices (DBS versus whole blood).

The objective of the present study was to develop and validate a method to analyze MeHg and InHg in DBS by GC-CVAFS for use in human biomonitoring studies. This study was comprised of two phases (Supporting Information S2-2. Overview of Study). First, a robust method was developed in careful consideration of the quality assurance and control criteria outlined in US EPA Method 1630(U.S. Environmental Protection Agency, 1998) using paired whole blood-DBS samples from reference materials and a volunteer. Second, a validation study was performed on paired whole blood-DBS samples from venous and capillary blood from 49 volunteers to understand if there were significant differences in MeHg and InHg concentrations between capillary and venous whole blood, and between matrices (whole blood versus DBS). Finally, we aimed to evaluate if Hg species in DBS are stable over time given the lengthy delay in most situations between collection and analysis.

2.3 Experimental section

2.3.1 Phase 1. Method development

Method development is detailed in the Supporting Information (S2-3. Method Development). Briefly, we first carried out a literature review of whole blood Hg speciation methods(Chen et al., 2009; De Souza et al., 2013; Rodrigues et al., 2010; Yuliya L Sommer et al., 2014) and carefully reviewed past studies that characterized Hg in DBS (Table S2-1) including our own method that provided the foundation for the current study (Basu et al., 2017; Perkins and Basu, 2018). We developed the method principally with two sources of blood (i.e., human whole blood reference materials from the *Institut National de Santé Publique du Québec* with reported THg and MeHg concentrations; whole blood from a consenting volunteer that was Hg-spiked). Next, we conducted a series of experiments with a variety of chemicals and extraction temperatures and times to develop and optimize an accurate and precise method while also considering background (or matrix) contamination and detection limits. Results from the various tests are presented in the Supporting Information (S2-3 Method Development). For the selected extraction method, detailed

information on the extraction recovery, analytical accuracy and precision, matrix interference, and method detection limit (MDL) are presented in the Supporting Information (S2-4 and S2-5).

2.3.2 Phase 2. Method validation

a. Sampling design

Approval from the McGill University Institutional Review Board (#A05-M26-16B) was received for this work. Whole blood (venous and capillary) was collected from 49 consenting individuals (age range: 19 to 53 years) from the Mary Emily Clinic at McGill University in December 2016. From each individual, 7 ml of venipuncture whole blood was collected into a single trace metal free K_2EDTA tube. Subsamples of this whole blood were pipetted onto Whatman 903 protein saver cards (3 spots were filled with 40µl of venous whole blood, and two spots were filled with 60µl of venous whole blood), and the remaining whole blood was kept frozen at -20 ℃ until analysis.

Capillary blood was also collected from each participant following the protocol outlined by the "USAID Biomarker Manual" for DBS collection(USAID, 2012). Briefly, the side of the second, third or fourth finger was warmed, cleaned with an alcohol wipe, and pricked with a contact activated lancet (BD Microtainer®, High flow, 1.5mm blade, Franklin Lakes, NJ). The initial drop of blood formed at the puncture site was wiped away with sterile gauze due to possible contamination of the blood drop with alcohol, cell debris, and or tissue fluid(Bond and Richards-Kortum, 2015). The next three blood drops were collected with a 40µl microcapillary tube (accuracy ± 0.5% Sigma Aldrich), and transferred to the DBS cards where they filled three distinct spots. Then, the following two drops of blood were collected onto the cards without any volume control to yield two additional spots (this mimics practice in most real-world settings). The DBS cards were left to dry at room temperature for 4 hours, then packed into a clean polypropylene bag with a desiccant pack. Finally, capillary whole blood (~900 µL) was dripped into a K_2EDTA (BD

Microtainer® Tube with MicrogardTM closure, Franklin Lakes), and kept frozen at -20 ℃ until analysis.

b. Mercury analysis

Sample extraction and Hg analysis, as well as quality assurance and quality control steps were included in both phase 1 (development) and 2 (validation) studies. In phase 2, samples were extracted and analyzed in batches according to the matrix (whole blood or DBS) and blood source (venous or capillary) following the Health Canada and European Medicine Agency guidelines on bioanalytical method validation(European Medicines Agency, 2011; Health Canada, 2012).

Sample extraction methods were derived from the results of the studies performed during phase 1 with specific findings presented in the Supporting Information (S2-4) Based on a review of the phase 1 results and in careful consideration of established QC guidelines from the US EPA 1630 method (see Table S2-4), the extraction method for the quantification of Hg species was defined as follows: individual whole blood or DBS samples (both 40 µl) were placed into acid washed (HCl 10%) 4.5 mL polypropylene tubes (Sarstedt) with 600 µl of a solution containing 4M HNO_3 (Omnitrace® Millipore Sigma) and 0.02% M L-Cysteine (≥97%, FG, Sigma-Aldrich). The tubes were capped tightly and placed in an oven at 60 ℃ for 24 hours. Next, a subsample (300 µL) of the cooled digest was added to a borosilicate glass vessel with ultrapure water, adjusted to pH 4.0–4.5 using citrate buffer and ethylated using 1% sodium tetraethyl borate ($NaBEt_4$) (min 98%, Strem) according to the US EPA 1630 method (note that the sample was diluted 1:100 with ultrapure water such that the resulting Hg value would fall within the standard calibration range of the GC-CVAFS, 0.02ng/L to 6ng/L). The digest was introduced to a GC-CVAFS (Tekran® Series 2700, Tekran Instruments Corporation, Toronto, Canada) as we have previously detailed(Basu et al., 2017) where Hg species were separated and quantified. Additional details on the method are provided in the Supporting Information (Figure S2-2) including information of quality assurance and quality control data (Supporting Information S2-6).

c. Data Analyses

All statistical analyses were carried out using Minitab 17 Statistical Software 2010, and XLSTAT 2014(Addinsoft, n.d.; State College, PA: Minitab, 2010). Method precision and accuracy are expressed as the relative standard deviation and percent recovery, respectively. These calculations, and others, were compared against assay performance criteria outlined in US EPA Method 1630. To validate our DBS method, we initially subjected all data to descriptive statistics and made comparisons with other biomonitoring efforts (notably the Canadian Health Measures Survey, CHMS). The core study objectives (i.e., compare Hg measures among different blood sources and matrix types and over-time) were systematically tackled by examining: 1) differences in mean values between samples using Wilcoxon Signed-rank tests; 2) if there was a constant bias or proportional bias through linear regressions; 3) Bland-Altman plots to better understand the differences in agreement; and 4) coefficient of variances to gauge intra-individual variability for a given measure.

2.4 Results and discussion

2.4.1 Method detection limit

The MDLs for MeHg in DBS and whole blood were 0.3 µg/L and 0.2 µg/L, respectively. For InHg in DBS and whole blood, the MDLs were 1.9 µg/L and 1.1 µg/L, respectively. The MDL for MeHg in whole blood was similar to the MDL reported in the CMHS cycle 4(Canadian Health Measures Survey CHMS, 2014) (0.19 µg/L) and U.S. NHANES 2013-2014(National Center for Environmental Health, 2014) (0.12 µg/L). Conversely, the MDL for InHg in whole blood we report here was higher than an ICP-MS method reported by the U.S. CDC and U.S. NHANES(National Center for Environmental Health, 2014; National Health and Nutrition Examination Survey - NHANES, 2017; Yuliya L Sommer et al., 2014) (0.27 µg/L) warranting a need for further improvements to our GC-CVAFS method. For DBS, the MDL for MeHg here was similar to our previous work (0.3 µg/L)(Basu et al., 2017), and to the best of our knowledge this is the first study to report a MDL for InHg in DBS.

For MeHg, the relatively low MDLs reported here in both whole blood and DBS supports the use of our method in population based human biomonitoring studies. The calculated detection limits for both matrices are less than the mean values reported by national biomonitoring programs including the CHMS (0.6 µg/L) and U.S. NHANES (0.5 µg/L).

2.4.2 Quality control

a. Accuracy and precision

Results of the accuracy and precision during method development (phase 1) are presented in the Supporting Information (Table S2-4). Our results showed that our method is both accurate and precise for both whole blood and DBS, with performance values falling well within the US EPA

1630 performance criteria for both reference materials, and the spiked matrix. The accuracy and precision of the spiked blanks indicated no matrix interference.

In the validation portion of this study (phase 2), batch runs were designed to focus on a single matrix type (DBS or whole blood), and each batch run contained a range of quality control samples (i.e., reference materials, blanks, sample replicates). For MeHg, analytical accuracy was within the US EPA 1630 performance criteria limits for the two matrices (Table S2-5). The analytical precision was below 13% for the DBS samples (n=10) and below 5% for whole blood (n=10), and these also fell within the US EPA 1630 performance criteria. However, we note that sample precision was variable across exposure levels: for MeHg levels <1µg/L, the %RSD was <20% for whole blood and <23% for DBS while for blood MeHg values >1µg/L the %RSD was <0.4% for whole blood and <13% for DBS.

b. Background contamination

Whatman 903 protein saver cards are most commonly used in the DBS field, and so we wanted to understand the extent to which these may contain background levels of Hg. The levels of MeHg and InHg in the digestion reagents as well as blank filter paper taken from unused cards and cards with DBS were measured in both phase 1 and 2 studies. The measured amounts of MeHg and InHg in reagents and unused filter paper cards were below the method detection limits. Such has also been reported by most previous studies on Hg in DBS(Basu et al., 2017; Funk et al., 2013; Nelson et al., 2016; Elias Nyanza et al., 2019) (see Table S2-1). For cards with DBS, a full blank spot (i.e., filter paper without blood) was taken from the same card and digested concurrently with the DBS from the same card using the same reagents. This was done for every 10 samples. In the blank spots, the MeHg content averaged 1.39 ± 0.51pg (range: 0.88 – 1.67pg) and InHg averaged 34.22 ± 9.42pg (range: 26.48 - 51.56pg). Considering that a full spot is equivalent to about 60 µl of sample(Funk, 2015), blank concentrations for MeHg were ~0.02 ± 0.01µg/L (range 0.01 to 0.04 µg/L), these values are much less than real-world concentrations of Hg in background populations (e.g. CHMS cycle 4 geomean was 0.59 µg/L) (Canadian Health Measures Survey CHMS, 2014). In contrast, the concentration of InHg in blank DBS was ~0.57 ± 0.16µg/L (range 0.35 to 0.86

µg/L), and this value is near the 90th percentile exposure level from the US NHANES 2011-2014 (0.60 µg/L).

Analysis of background contamination for DBS cards was reported previously for THg(Chaudhuri et al., 2009; Funk, 2015; Elias Nyanza et al., 2019). Only one study to our knowledge evaluated paired DBS-blanks for all the study participants (83 children)(Funk, 2015), and they reported THg concentrations ranging from 0.04 to 2.86 µg/L in the blanks; however, they did not find visible improvement in the whole blood-DBS relationship after within-card background correction. Another relevant study reported internal blanks with a similar range of THg values (0.73-2.83 µg/L) with the aforementioned work(Chaudhuri et al., 2009). One study compared THg levels between field blanks (mean = 0.06 ±0.02 µg/L) and laboratory blanks (mean = 0.03 ±0.02 µg/L)(Elias Nyanza et al., 2019), and while the levels in the field blanks are two fold higher that the laboratory blanks, the concentrations are extremely low and irrelevant. In general, the THg measurements in blanks from many prior studies are relatively high considering that blood Hg levels in most populations worldwide are below 5 µg/L(Basu et al., 2018b). For MeHg, we are aware of only one study (by our group) to report MeHg levels in DBS blanks, and in that case blank punches were studied from 10% of the used DBS cards (n=78) with a mean MeHg value of 0.16 ± 0.02 µg/L(Basu et al., 2017).

The large spot-to-spot variation presented in THg, MeHg and InHg in blank DBS(Basu et al., 2017; Chaudhuri et al., 2009; Funk, 2015; Elias Nyanza et al., 2019) requires close attention during population exposure studies. For this study, the fold-difference between DBS blanks and Hg concentrations within the same DBS card averaged 9% for MeHg (range: 1–36%) and 54% for InHg (range: 15-100%), indicating a larger heterogeneity in InHg blanks possibly due to external contamination or the high MDL. Even though previous studies do not recommend a blank correction at the individual sample level(Funk, 2015), future studies should carefully perform calculations to determine the necessity of this step. In our case, the MeHg levels were relatively low and not blank-corrected.

2.4.3 Phase 2 validation study

In phase 2, a validation study was performed on 49 volunteers to understand if there were significant differences in MeHg and InHg concentrations between capillary and venous whole blood, and between matrices (whole blood versus DBS). The majority of measured MeHg concentrations (94%) were above the MDL across the matrix-source combinations, and the detection frequencies for MeHg in whole blood compare favorably with large biomonitoring programs (82% in CHMS cycle 4; 84% in US NHANES)(Canadian Health Measures Survey CHMS, 2014; National Health and Nutrition Examination Survey - NHANES, 2017).

For InHg, only 8% of the whole blood samples and 16% of the DBS samples were above the MDL meaning that for most samples a confident measure could not be realized. While the US NHANES reported a lower MDL for InHg (0.27 µg/L)(Yuliya L. Sommer et al., 2014) than the present study, they still reported a low proportion of participants with InHg blood concentrations above their MDL (e.g., for the 2015-2016 cycle only 736 samples (13%) of 5815 were above the MDL)(National Health and Nutrition Examination Survey, 2018). Since most of the InHg data in the current study was below the InHg MDL (as expected for a population with low exposures to InHg), from this point on we only present results for MeHg. Future studies with populations with higher InHg exposure coupled with improvements in the analytical method are required to validate the method for InHg. For those interested, the InHg data are presented in the Supporting Information (S2-7. Mercury levels).

The MeHg concentrations across the four matrix-source combinations are summarized in Supporting Information (S2-7. Mercury levels). No significant differences were found for the 25^{th}, 50^{th}, 75^{th} and 90^{th} percentiles and geometric mean among the four matrix-source combinations. The geometric mean concentrations of MeHg across the four matrix-source combinations (0.78 – 0.86µg/L) were similar to those reported by the CHMS (0.69µg/L in cycle 4) but slightly higher than those from 2013-2014 U.S. NHANES (0.49µg/L). MeHg concentrations for the 90th percentile for the four matrix-source combinations (1.70 – 2.23µg/L) were below those reported

for the CHMS cycle 3 and 4 (3.3 and 2.8µg/L, respectively)(Canadian Health Measures Survey CHMS, 2014; Health Canada, 2015). The median intra-individual variability (%CV) among the four matrix-source combinations was 18% (range 4-52%). The higher variability was found in the lower exposure groups (<0.70µg/L), as we found in our previous work(Basu et al., 2017).

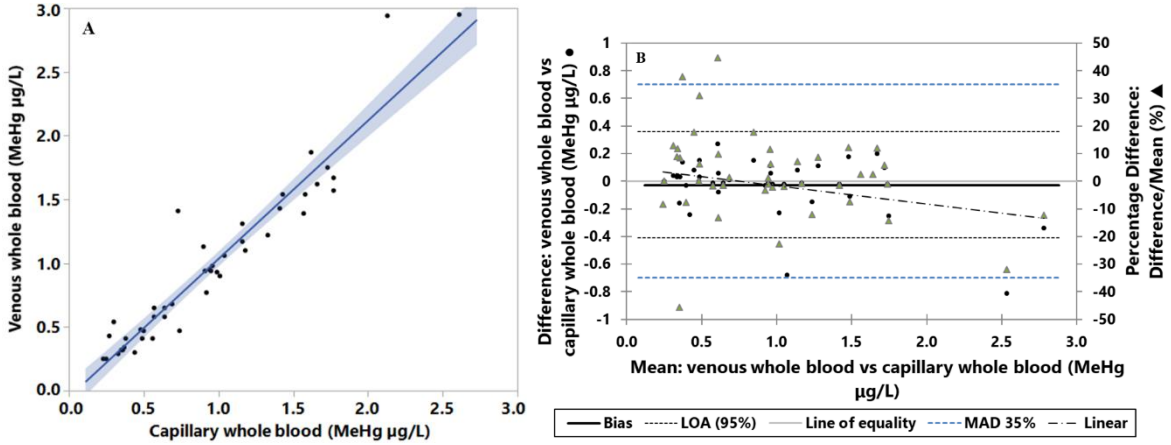
a. Agreement between capillary and venous whole blood

Since DBS are typically produced from capillary whole blood, comparing DBS results with assays from venous whole blood samples may not be accurate(McDade et al., 2007). Capillary whole blood is a mixture of arterial and venous whole blood plus interstitial fluid(Enderle et al., 2016); thus, physiologically it differs from venous whole blood. Previous studies have reported higher levels of hemoglobin, hematocrit, red blood cells, white blood cells, and white blood cells in capillary whole blood than in venous whole blood(Akenzua et al., 1974; de Alarcon et al., 2013; Kayiran et al., 2003). Such differences in blood count parameters between capillary and venous whole blood could have implications for the Hg concentrations especially since MeHg is bound to hemoglobin in the red blood cells, and InHg is distributed between red blood cells and plasma (Sheehan et al., 2014). Thus, variability in the concentration of Hg between the different sources of blood is plausible. To our knowledge this is the first study to characterize Hg levels in capillary blood and then to evaluate differences in Hg concentrations between venous and capillary blood. It is important to note that to avoid the hematocrit effect and volume bias (Denniff and Spooner, 2014a; Holub et al., 2006; Lawson et al., 2016; Velghe et al., 2019) this study analyzed a DBS of a controlled sample volume.

In the current study there was no significant difference (p=0.74) in MeHg concentration between the two blood sources, and a strong linear relationship was found between them (R^2=0.91) (Figure 2-1A). Furthermore, for this relationship there was not a significant constant bias (intercept -0.05 µg/L; 95% CI -0.16 – 0.06) or a proportional bias (slope 1.08; 95% CI 0.98 – 1.18). While we are not aware of another study comparing Hg levels between venous and capillary whole blood, a three-year study focused on blood lead with 499 paired samples also showed no significant differences (Patrick J. Parsons, Andrew A. Reilly, 1997).

In addition to relating MeHg concentrations between the two whole blood sources using a regression-based approach, we constructed a Bland-Altman plot to better understand the differences in agreement (Figure 2-1B; supporting information in Table S2-8). The mean bias difference between the two measures was 2% indicating only a slight discrepancy in MeHg between capillary and venous sources. To better understand the differences between the two measurements, we added to the plot a maximum allowed difference (MAD) of 35% based on analytically relevant criteria as established by US EPA 1630. Three samples were outside the 35% difference criteria and two samples were outside the limits of acceptance (LOA). The 95% confidence interval surrounding the mean bias value was quite narrow, and although there was a significant shift in bias across the exposure range (0 not within the 95% CI of the slope) we conclude that there was very little difference in MeHg concentrations from the two whole blood sources.

Figure 2-1. (A) Regression line between venous whole blood MeHg (µg/L) and capillary whole blood MeHg (µg/L) with the blue band indicating the 95% confidence interval; (B) Bland-Altman plot of differences (left axis; black circles) and percentage differences (right axis; green triangles) of MeHg measures between venous whole blood and capillary whole blood versus the mean of the two measurements.



b. Agreement between paired DBS-Blood samples

The previous section compared MeHg values between venous whole blood and capillary whole blood, and here we transitioned to comparing whole blood measurements with corresponding DBS values (from the same blood source) to identify if there may be matrix interference. First, there was not a significant difference in MeHg concentrations between paired whole blood-DBS measurements in venous (p=0.53) and capillary (p=0.16) blood sources. Second, MeHg levels in capillary whole blood versus capillary DBS presented a close 1:1 relationship (Figure 2-2A) with no constant bias (intercept 0.09µg/L; 95% CI -0.11 – 0.30) and no proportional bias (slope was 1.01; 95% CI 0.81 – 1.20) (Figure 2-2B). For venous blood, a strong linear relationship close to 1:1 was found between the paired DBS-blood samples (Figure 2-3A) with no constant bias (intercept 0.11µg/L; 95% CI -0.06 – 0.29) and no proportional bias (slope was 0.92; 95% CI 0.76 – 1.07) . Third, there was a strong correlation (R^2=0.72) between the paired capillary blood-DBS samples as well as for the paired venous whole blood-DBS samples (R^2=0.77).

The Bland-Altman plot (Figure 2-2B) indicated differences between the venous blood matrix combinations mostly at the lower concentrations (i.e. <1µg/L) with seven samples outside the MAD and three samples outside the LOA. For the capillary blood matrix combinations, three samples were outside of the MAD and one sample was outside of the LOA (Figure 2-3B). A low bias was found for the venous blood matrix combination (4%) with no significant shift in bias (95% CI of slope -0.10 – 0.20). For the capillary blood matrix combination, there was a higher bias (10%) and a significant shift in bias across the range of MeHg concentrations for capillary blood (95% CI of slope 0.03 – 1.02). These indicate that for capillary blood there may be a discrepancy, although limited, in MeHg concentrations between whole blood and DBS. We also note that there was an increase in the LOA range from the comparisons on paired whole blood sources (0.77µg/L) versus paired blood-DBS (venous 1.27µg/L; capillary 1.33µg/L) thus indicating a higher variability possibly due to the DBS matrix.

Figure 2-2. (A) Regression line between capillary whole blood MeHg (µg/L) and capillary DBS MeHg (µg/L) with the blue band indicating the 95% confidence interval; (B) Bland-Altman plot of differences (left axis; black circles) and percentage differences (right axis; green triangles) of MeHg measures between capillary whole blood and capillary DBS versus the mean of the two measurements.

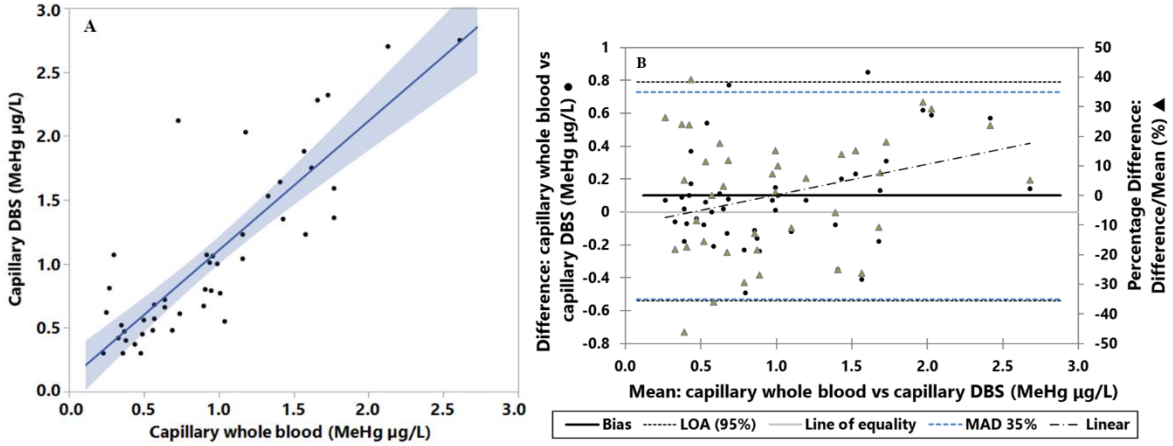
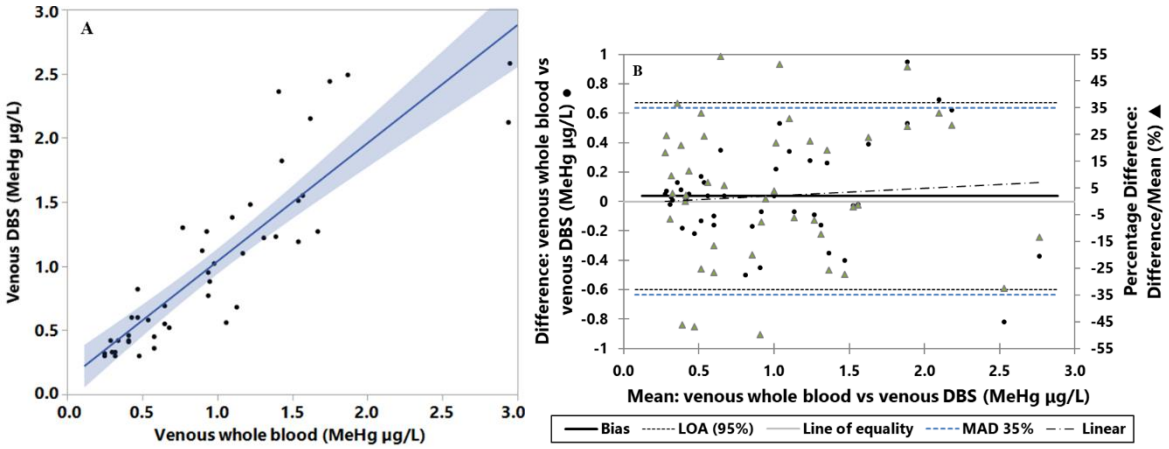


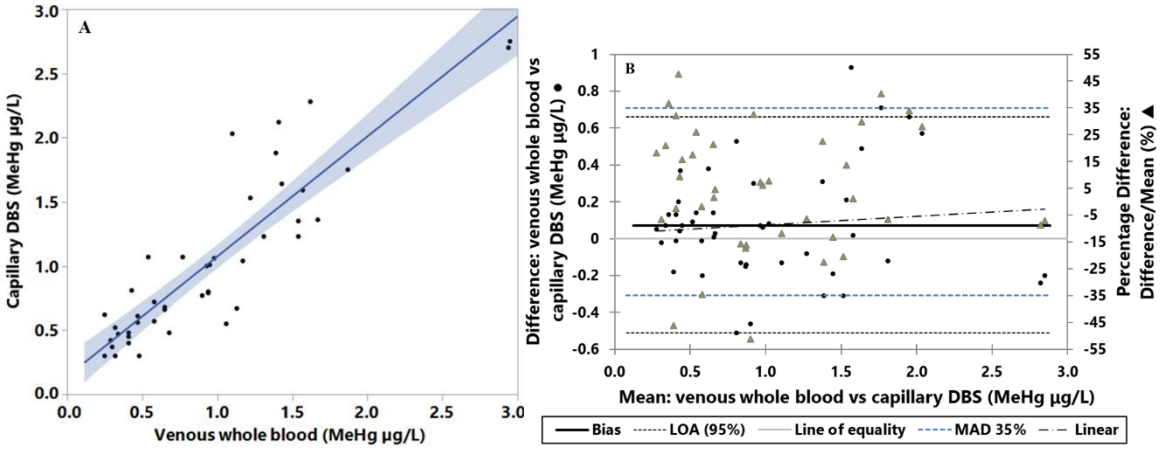
Figure 2-3. (A) Regression line between venous whole blood MeHg (µg/L) and venous DBS MeHg (µg/L) with the blue band indicating the 95% confidence interval; (B) Bland-Altman plot of differences (left axis; dark circles) and percentage differences (right axis; grey triangles) of MeHg measures between venous whole blood and venous DBS versus the mean of the two measurements.



c. Agreement between capillary DBS and the gold standard

The ultimate goal of this paper was to determine if measures of MeHg in capillary DBS reflect the gold standard (i.e., venous whole blood measures), and the overly simplified answer is yes (Figure 2-4A). First, there was not a significant difference between the two measurements (p=0.20). Second, the slope of the linear regression was close to a 1:1 relationship with no constant bias (intercept 0.13µg/L; 95% CI -0.02 – 0.30) and no proportional bias (slope was 0.93; 95% CI 0.79 – 1.07). Third, the coefficient of determination (R^2=0.80) indicated a strong relationship between the paired capillary blood DBS and venous whole blood. Fourth and finally, the Bland-Altman plot (Figure 2-4B) indicated no significant differences between the two measurement types as the bias was consistent across the concentration range. There were five measurements outside the 35% MAD . The range of the LOA was 1.17 µg/L with two measurements outside of the LOA. The relevance of these differences and the variability of the LOA in terms of exposure values are minimal, considering that the concern levels of Hg range above 5 µg/L (e.g., alert level is 5-15 µg/L)(Schulz et al., 2011). In general, this work demonstrates a relatively good agreement between MeHg levels in capillary DBS and the gold standard.

Figure 2-4 (A) Regression line between venous whole blood MeHg (µg/L) and capillary DBS MeHg (µg/L) with the blue band indicating the 95% confidence interval; (B) Bland-Altman plot of differences (left axis; black circles) and percentage differences (right axis; green triangles) of MeHg measures between venous whole blood and capillary DBS versus the mean of the two measurements.



d. Stability of Hg species

Seldom are DBS immediately analyzed after collection and so the stability of Hg in archived samples deserves attention. To the best of our knowledge, the stability of MeHg and InHg in DBS have not been previously reported though past studies have evaluated the stability of THg. The first study analyzed a set of samples stored in a well-lit room at room temperature as well as another set of samples refrigerated in the dark, and for both sets there was little evidence of THg levels changing over a period of 1 – 2 months(Chaudhuri et al., 2009). Another study by our group found that THg concentrations in DBS do not change under a series of storage treatments that included different levels of humidity, temperature, and storage time (1 year)(Perkins and Basu, 2018).

For the current study, the 49 capillary DBS from the validation study were analyzed one year after collection to characterize the stability of MeHg and InHg on samples stored at room temperature

(Supporting Information S2-9. Stability of MeHg over time). Since 16% of the DBS InHg data was above the MDL, we cannot asses the stability of InHg over time. For MeHg, after a one-year storage period 80% of the sample measurements fell within the 35% criterion while six samples were below the MDL. The median concentration was initially 0.88 μg/L and after one year it was measured to be not significantly different at 0.74 μg/L (p=0.08). Second, MeHg levels over the year presented a close to 1:1 relationship with no constant bias (intercept 0.10μg/L; 95% CI -0.09 – 0.31) and no proportional bias (slope was 0.81; 95% CI 0.64 – 0.98) (Figure S2-3). These results indicate that MeHg (like THg) is stable in DBS after one year of storage under room temperature.

2.5 Conclusion

This study increases our understanding of DBS as a potentially novel means to assess Hg exposure in the general population. We established a method to characterize MeHg and InHg in DBS, and carefully validated the work using paired whole blood-DBS samples. To our knowledge, this is the first study to analyze Hg species in DBS, and also the first to analyze differences in Hg levels between blood sources (venous and capillary) and sample matrices. Overall, this study helps validate DBS as a novel tool to assess MeHg exposure in human population studies. Even though this study tackled and resolved key knowledge gaps concerning the use of DBS for Hg exposure assessment, one outstanding question pertains to the high MDL for InHg; even under controlled conditions we were unable to improve this aspect. Future studies focused on populations with higher exposures to InHg are required to validate the use of DBS for InHg.

2.6 References

Addinsoft. (n.d.). XLSTAT- Statistics Package for Excel. Paris. Retrieved from http://www.xlstat.com

Agency for Toxic Substances and Disease Registry. (1999). Toxicological Profile for Mercury Department of Health and Human Services, 676. Retrieved from http://www.atsdr.cdc.gov/toxprofiles/tp46.pdf

Akenzua, G. I., Hui, Y. T., Milner, R., & Zipursky, A. (1974). Neutrophil and Band Counts in the Diagnosis of Neonatal Infections. Pediatrics, 54(1). Retrieved from https://pediatrics.aappublications.org/content/54/1/38.abstract

Basu, N., Eng, J. W. L., Perkins, M., Santa-Rios, A., Martincevic, G., Carlson, K., & Neitzel, R. L. (2017). Development and application of a novel method to characterize methylmercury exposure in newborns using dried blood spots. Environmental Research, 159, 276–282. https://doi.org/10.1016/J.ENVRES.2017.08.021

Basu, N., Horvat, M., Evers, D. C., Zastenskaya, I., Weihe, P., & Tempowski, J. (2018). A State-of-the-Science Review of Mercury Biomarkers in Human Populations Worldwide between 2000 and 2018. Environmental Health Perspectives, 126(10), 106001. https://doi.org/10.1289/ehp3904

Bond, M. M., & Richards-Kortum, R. R. (2015). Drop-to-drop variation in the cellular components of fingerprick blood: Implications for point-of-care diagnostic development. American Journal of Clinical Pathology, 144(6), 885–894. https://doi.org/10.1309/AJCP1L7DKMPCHPEH

Canadian Health Measures Survey CHMS. (2014). Fourth Report on Human Biomonitoring of Environmental Chemicals in Canada. Retrieved from https://www.canada.ca/content/dam/hc-sc/documents/services/environmental-workplace-health/reports-publications/environmental-contaminants/fourth-report-human-

biomonitoring-environmental-chemicals-canada/fourth-report-human-biomonitoring-environmental-chem

Chaudhuri, S. N., Butala, S. J. M., Ball, R. W., & Braniff, C. T. (2009). Pilot study for utilization of dried blood spots for screening of lead, mercury and cadmium in newborns. Journal of Exposure Science & Environmental Epidemiology, 19(3), 298–316. https://doi.org/10.1038/jesee.2008.19

Chen, J., Chen, H., Jin, X., & Chen, H. (2009). Determination of ultra-trace amount methyl-, phenyl-and inorganic mercury in environmental and biological samples by liquid chromatography with inductively coupled plasma mass spectrometry after cloud point extraction preconcentration. Talanta, 77, 1381–1387. https://doi.org/10.1016/j.talanta.2008.09.021

Clarkson, T. W., & Magos, L. (2006). The Toxicology of Mercury and Its Chemical Compounds. Critical Reviews in Toxicology, 36(8), 609–662. https://doi.org/10.1080/10408440600845619

de Alarcon, P. A., Werner, E. J., & Christensen, R. D. (2013). Neonatal Hematology: Pathogenesis, Diagnosis, and Management of Hematologic (Second Edi). New York: Cambridge University Press. Retrieved from https://books.google.ca/books?hl=en&lr=&id=xZJ7AAAAQBAJ&oi=fnd&pg=PR1&dq=Hematologic+problems+of+the+neonate&ots=08Q-lbvoX7&sig=o9ayLIVwLuBYxqSL9SqjS25wu4Q&redir_esc=y#v=onepage&q=Hematologic problems of the neonate&f=false

De Souza, S. S., Campiglia, A. D., & Barbosa, F. (2013). A simple method for methylmercury, inorganic mercury and ethylmercury determination in plasma samples by high performance liquid chromatography–cold-vapor-inductively coupled plasma mass spectrometry. Analytica Chimica Acta, 761, 11–17. https://doi.org/10.1016/j.aca.2012.11.038

Denniff, P., & Spooner, N. (2014). Volumetric Absorptive Microsampling: A Dried Sample Collection Technique for Quantitative Bioanalysis. Anal. Chem, 86, 34. https://doi.org/10.1021/ac5022562

Enderle, Y., Foerster, K., & Burhenne, J. (2016). Clinical feasibility of dried blood spots: Analytics, validation, and applications. Journal of Pharmaceutical and Biomedical Analysis, 130, 231–243. https://doi.org/10.1016/j.jpba.2016.06.026

European Medicines Agency. (2011). Guideline on bioanalytical method validation. Retrieved from www.ema.europa.eu/contact

Funk, W. E. (2015). Use of Dried Blood Spots for Estimating Children's Exposures to Heavy Metals in Epidemiological Research. Journal of Environmental & Analytical Toxicology, s7. https://doi.org/10.4172/2161-0525.S7-002

Funk, W. E., McGee, J. K., Olshan, A. F., & Ghio, A. J. (2013). Quantification of arsenic, lead, mercury and cadmium in newborn dried blood spots. Biomarkers : Biochemical Indicators of Exposure, Response, and Susceptibility to Chemicals, 18(2), 174–177. https://doi.org/10.3109/1354750X.2012.750379

Ha, E., Basu, N., Bose-O 'reilly, S., Dórea, J. G., Mcsorley, E., Sakamoto, M., & Chan, H. M. (2016). Current progress on understanding the impact of mercury on human health. Environmental Research, 1–15. https://doi.org/10.1016/j.envres.2016.06.042

Health Canada. (2012). Guidance Document Conduct and Analysis of Comparative Bioavailability Studies. Ottawa. Retrieved from https://www.canada.ca/content/dam/hc-sc/documents/services/drugs-health-products/drug-products/applications-submissions/guidance-documents/bioavailability-bioequivalence/conduct-analysis-comparative.pdf

Health Canada. (2015). Third report on human biomonitoring of environmental chemicals in Canada: Results of the Canadian Health Measures Survey Cycle 3 (2012-2013) (Vol. 3).

Holub, M., Tuschl, K., Ratschmann, R., Strnadová, K. A., Mühl, A., Heinze, G., … Bodamer, O. A. (2006). Influence of hematocrit and localisation of punch in dried blood spots on levels of amino acids and acylcarnitines measured by tandem mass spectrometry. Clinica Chimica Acta, 373(1–2), 27–31. https://doi.org/10.1016/j.cca.2006.04.013

Kayiran, S. M., Ozbek, N., Turan, M., & Gurakan, B. (2003). Significant differences between capillary and venous complete blood counts in the neonatal period. Clinical and Laboratory Haematology, 25(1), 9–16. https://doi.org/10.1046/j.1365-2257.2003.00484.x

Lawson, A. J., Bernstone, L., & Hall, S. K. (2016). Newborn screening blood spot analysis in the uk: Influence of spot size, punch location and haematocrit. Journal of Medical Screening, 23(1), 7–16. https://doi.org/10.1177/0969141315593571

Mahaffey, K. R., Clickner, R. P., & Bodurow, C. C. (2004). Blood Organic Mercury and Dietary Mercury Intake: National Health and Nutrition Examination Survey, 1999 and 2000. Environmental Health Perspectives, 112(5), 562–570. https://doi.org/10.1289/ehp.6587

Mcdade, T. (2013). Development and validation of assay protocols for use with dried blood spot samples. American Journal of Human Biology, 9(August 2013), 1–9. https://doi.org/10.1002/ajhb.22463

McDade, T., Williams, S., & Snodgrass, J. (2007). What a drop can do: Dried blood spots as minimally invasive method for integrating biomarkers into population-based research. Demography, 44(4), 899–925. Retrieved from http://link.springer.com/article/10.1353/dem.2007.0038

National Center for Environmental Health, C. (2014). Laboratory Procedure Manual Inorganic Mercury, Methyl Mercury, Ethyl Mercury Blood mercury speciation. Retrieved from https://wwwn.cdc.gov/nchs/data/nhanes/2013-2014/labmethods/IHgEM_H_MET.pdf

National Health and Nutrition Examination Survey. (2018). NHANES 2015-2016: Mercury - Inorganic, Ethyl & Methyl - Blood Data Documentation, Codebook, and Frequencies.

National Health and Nutrition Examination Survey - NHANES. (2017). Fourth national report on human exposure to environmental chemicals-. Department of Health and Human Services, Volume One.

Nelson, J. W., Edhlund, B. L., Johnson, J., Rosebush, C. E., Holmquist, Z. S., Swan, S. H., & Nguyen, R. H. N. (2016). Assessing a new method for measuring fetal exposure to mercury: Newborn bloodspots. International Journal of Environmental Research and Public Health, 13(7). https://doi.org/10.3390/ijerph13070692

Nyanza, E. C., Bernier, F. P., Manyama, M., Hatfield, J., Martin, J. W., & Dewey, D. (2019). Maternal exposure to arsenic and mercury in small-scale gold mining areas of Northern Tanzania. Environmental Research, 173(March), 432–442. https://doi.org/10.1016/j.envres.2019.03.031

Nyanza, E. C., Dewey, D., Bernier, F., Manyama, M., Hatfield, J., & Martin, J. W. (2019). Validation of Dried Blood Spots for Maternal Biomonitoring of Nonessential Elements in an Artisanal and Small-Scale Gold Mining Area of Tanzania. Environmental Toxicology and Chemistry, 38(6), 1285–1293. https://doi.org/10.1002/etc.4420

Patrick J. Parsons, Andrew A. Reilly, and D. E.-J. (1997). Screening children exposed to lead: an assessment of the capillary blood lead fingerstick test. Drug Monitoring and Toxicology, 2(4). Retrieved from http://clinchem.aaccjnls.org/content/clinchem/43/2/302.full.pdf

Perkins, M., & Basu, N. (2018). Dried blood spots for estimating mercury exposure in birds. Environmental Pollution, 236, 236–246. https://doi.org/10.1016/J.ENVPOL.2018.01.036

Rodrigues, J. L., de Souza, S. S., de Oliveira Souza, V. C., & Barbosa, F. (2010). Methylmercury and inorganic mercury determination in blood by using liquid chromatography with inductively coupled plasma mass spectrometry and a fast sample preparation procedure. Talanta, 80(3), 1158–1163. https://doi.org/10.1016/j.talanta.2009.09.001

Schulz, C., Wilhelm, M., Heudorf, U., & Kolossa-Gehring, M. (2011). Update of the reference and HBM values derived by the German Human Biomonitoring Commission. International Journal of Hygiene and Environmental Health, 215(1), 26–35. https://doi.org/10.1016/j.ijheh.2011.06.007

Sheehan, M. C., Burke, T. A., Navas-Acien, A., Breysse, P. N., McGready, J., & Fox, M. A. (2014). Global methylmercury exposure from seafood consumption and risk of developmental neurotoxicity: a systematic review. Bulletin of the World Health Organization, 92(4), 254-269F. https://doi.org/10.2471/BLT.12.116152

Sommer, Y. L., Verdon, C. P., Fresquez, M. R., Ward, C. D., B., E. W., Yi Pan., … Robert, J. (2014). Measurement of Mercury Species in Human Blood using Triple Spike Isotope Dilution with SPME-GC-ICP-DRC-MS. Anal Bioanal Chem, 406(20), 5039–5047. https://doi.org/10.1530/ERC-14-0411.Persistent

Sommer, Y. L., Verdon, C. P., Fresquez, M. R., Ward, C. D., Wood, E. B., Pan, Y., … Jones, R. L. (2014). Measurement of mercury species in human blood using triple spike isotope dilution with SPME-GC-ICP-DRC-MS. Analytical and Bioanalytical Chemistry, 406(20), 5039–5047.

State College, PA: Minitab, I. (www. minitab. com. (2010). Minitab 17 Statistical Software.

U.S. Environmental Protection Agency. (1998). Method 1630: Methyl Mercury in Water by Distillation, Aqueous Ethylation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry. Retrieved from https://www.epa.gov/sites/production/files/2015-08/documents/method_1630_1998.pdf

UN Environment Programme. (2013a). Global Mercury Assessment 2013: Sources, Emissions, Releases, and Environmental Transport. Narayana Press.

UN Environment Programme. (2013b). Minamata Convention on Mercury - Text and Annexes.

UN Environment Programme. (2019). Global Mercury Assessment 2018. Geneva, Switzerland. Retrieved from https://wedocs.unep.org/bitstream/handle/20.500.11822/27579/GMA2018.pdf?sequence=1&isAllowed=y

USAID. (2012). Biomarker field manual: Demographic and Health Surveys Methodology. Calverton, Maryland. Retrieved from http://www.measuredhs.com/publications/publication-dhsm7-dhs-questionnaires-and-manuals.cfm

Velghe, S., Delahaye, L., & Stove, C. P. (2019, January 30). Is the hematocrit still an issue in quantitative dried blood spot analysis? Journal of Pharmaceutical and Biomedical Analysis. Elsevier B.V. https://doi.org/10.1016/j.jpba.2018.10.010

World Health Organization (WHO). (2008). Methylmercury. Environmental Health Criteria. No. 101.

2.7 Supporting information

2.7.1 S.1. Previous studies on mercury in human DBS

Table S 2-1 Summary of past studies that analyzed mercury (Hg) in human dried bloodspots (DBS) alongside the findings of the current study (last column); adapted and updated from Basu et al., 2017

Reference:	Chaudhuri et al. 2008	Funk et al., 2013	Funk et al., 2015	Nelson et al., 2016	Basu et al., 2017	Nyanza et al., 2019	Nyanza et al., 2019	This study
Study location and year	Utah, New Mexico, Montana, Wyoming (year not mentioned)	North Carolina (2003-2009)	Chicago (year not mentioned)	Minnesota (2010-2012)	Michigan (2003-2015)	Tanzania (year not mentioned)	Tanzania (2015-2017)	Montreal (2016)
Sample size	18	49	82	48	675	44	1056	49
DBS sample studied	Two ¼" punch (estimate 11.5 µl blood)	Half-spot (~30µl blood)	One spot (~60µl blood)	Two 3 mm punches (estimate 3.1µl/punch)	Two 3mm punches (estimate 6.2 µl blood)	One 8 mm punch 45-55 µl	One spot 50 – 55 µl	One spot 40 µl
Instrument	ICP-MS	ICP-MS	ICP-MS	ICP-MS	GC-CVAFS	ICP-MS	ICP-MS	GC-CVAFS
Detection limit	THg: 0.65 µg/L	Not mentioned	Not mentioned	THg: 0.7 µg/L	MeHg: 0.3 µg/L	THg: 0.012 µg/L	THg: 0.02 µg/L	MeHg: 0.3 µg/L

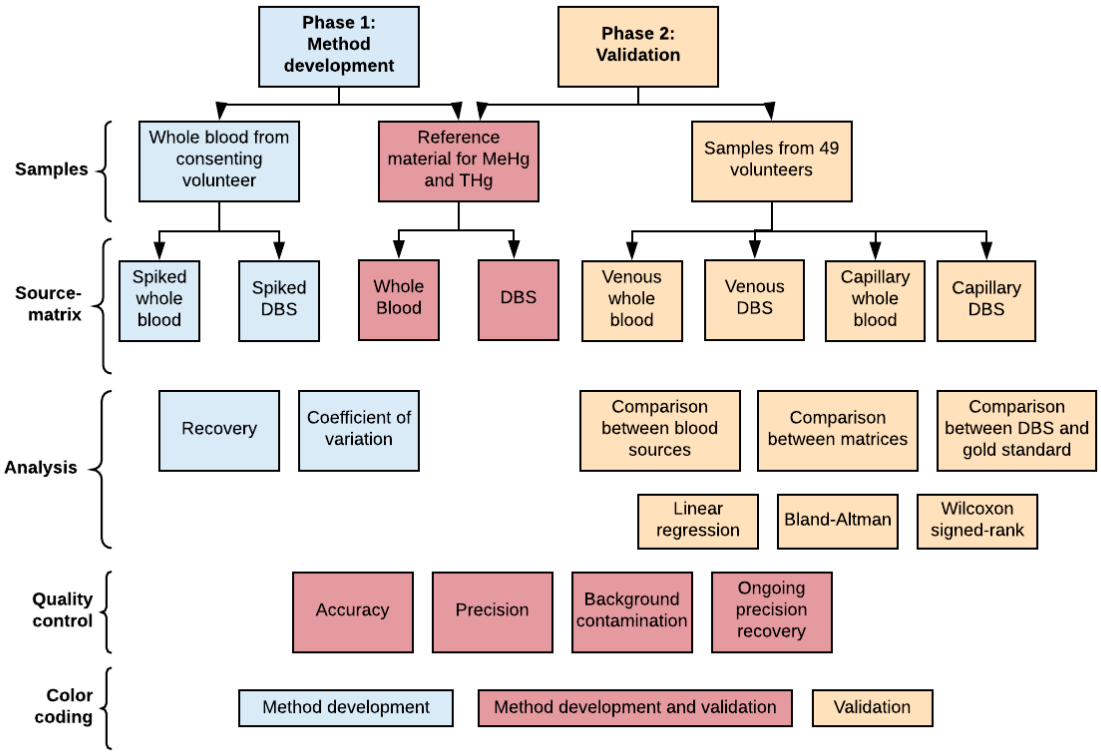
								InHg: 1.9 µg/L
DBS Blanks	THg: < 0.65 µg/L	THg: 0.00 µg/L	0.10 µg/L	THg: < 0.7 µg/L	MeHg: 0.16 µg/L	THg: 0.003 µg/L	Not mentioned	MeHg: 0.02 µg/L InHg: 0.57 µg/L
% non-detect	28%	67%	Not mentioned	62%	0%	0%	Not mentioned	MeHg: 4% InHg:84%
Median value	Not provided (we estimated THg: 0.37 µg/L, calculated from data in Table 15b	THg: 0 µg/L indicated in Table 1 (we estimated to be THg: 0.3 µg/L based on a visual inspection of Figure 2)	THg: 0.36 µg/L	Not provided (estimated at THg: 0.5 based on a median THg: 0.6 µg/L in cord blood and a reported 0.85 ratio of DBS: cord blood)	MeHg: 1.46 µg/L	THg: 1.16 µg/L	ASGM* workers: THg: 1.2 µg/L Non ASGM workers: THg: 0.66 µg/L	MeHg: 0.74 µg/L InHg: 1.67 µg/L

Upper measurement value	THg: 6.5 µg/l (90^{tho}%), calculated from data in Table 15b	THg: 1.9 µg/L (90th %)	THg: 1.11 µg/L (95th %)	THg: 2.6 µg/L (95th %)	MeHg: 3.0 µg/L (95th %)	THg: 2.86 µg/L (maximum value)	ASGM workers: THg: 28 µg/L Non ASGM workers: THg: 2.8 µg/L Calculated from figure 2	MeHg: 2.30 µg/L InHg: 2.39 µg/L
Linearity and Range	Not addressed	Not addressed	Not addressed	Not addressed	0.999 (0.02–2 ng/L)	Not addressed	Not addressed	>0.996 (0.02–6 ng/L)
Precision	9.2 – 36.9%	Not addressed	Not addressed	Not addressed	20%	<10%	<10%	MeHg: 17% InHg: 25%
Accuracy	Within 13% of expected using various reference materials (Utah Labs; INSPQ)	48-230% for spiked samples	Not addressed	Within 24% of expected value using spiked samples and NIST 966	96-115%	95.9%	Not addressed	MeHg 72-128% InHg: 132-25%

Hg species	THg	THg	THg	THg	MeHg	THg	THg	MeHg and InHg
Paired Whole blood -DBS	No	No	Yes	Paired Cord blood- DBS	No	Yes	No	Yes
Differences between capillary and venous blood	No	No	No	No	No	No	No	Yes
Field Validation	No	No	No	No	No	Yes	Yes	No

2.7.2 S2. Overview of study

Figure S 2-1 Diagrammatic overview of the study



2.7.3 S3. Method development

The method was developed using three different sample types:

1) Certified reference material of a different matrix with known MeHg and InHg concentrations (DORM-4, Fish protein certified reference material for trace metals, National Research Council of Canada) was used in the very early stages of methods development.

2) Human whole blood reference materials (n=4) from the *Institut National de Santé Publique du Québec* (INSPQ) with reported THg and MeHg concentrations were used to create DBS samples in the laboratory (PC-B-M1506, THg = 1.66 ± 0.07 µg/L; PC-B-M1404, THg = 1.96 ± 0.08 µg/L; PC-B-M1510, MeHg = 2.56 ± 1.25 µg/L; PC-B-M1112, MeHg = 8.68 ± 0.26 µg/L). Specifically, with a calibrated pipet, 40 µl of whole blood was spotted onto Whatman 903 protein saver cards (GE Healthcare, Dassel). The protein saver cards were dried at room temperature for 4 hours and then stored in individual plastic bags containing a desiccant pack at room temperature. While we recognize the limitations of these available blood reference materials (i.e., do not speciate both MeHg and InHg; Hg concentrations below 1 µg/L lacking), the assigned levels largely lie under 5 µg/L which represents a total Hg concentration found in background populations worldwide[2].

3) Venous whole blood from a consenting volunteer was spiked with Hg. The blood was collected into trace metal-free dipotassium ethylenediaminetetraacetic acid K_2EDTA tubes (BD Vacutainer®, Specialty Tubes, K_2EDTA 10.8 mg, Franklin Lakes), and then spiked with 2µg/L (which is within background populations worldwide) of standards of MeHg (methylmercury (II) chloride 1000 mg/L standard, VWR) and InHg ($HgCl_2$ in 0.1 mol/L HNO_3 1003 mg/L, Wako). These whole blood samples, with known additions of MeHg and InHg, were pipetted onto protein saver cards as described above for the reference materials.

Initial efforts focused on characterizing a control volume of 40µl for both whole blood and DBS (i.e., 40 µl of whole blood spotted onto the filter paper). A minimum of three replicates of paired whole blood-DBS samples (from both the reference materials and the matrix spikes) were analyzed throughout each stage of method development. The samples were digested using a variety of chemicals, times, and temperatures until we found the most accurate and precise method. Even

though a series of experiments were done with different extraction chemicals, temperatures, and times; here we only present the most relevant results of our experiments (see Tables S2-2 and S2-3). Second, we applied the methods from the first step to our in-house reference materials of DBS (Table S2-3).

Table S 2-2Accuracy and precision results from the most relevant experiments done during the first phase of method development

Method	Dorm – 4		Whole Blood		Spiked Sample	
	MeHg	**InHg**	**MeHg**	**InHg**	**MeHg**	**InHg**
Digestion with 1% Tetramethylammonium hydroxide solution 25 wt. % in methanol, 24 hours at 80 ˚C	Accuracy: 100.10% Precision: 4.95%	Accuracy: 84.45% Precision: 30.54%	Accuracy: 99.58% Precision: 19.34%	NA	Accuracy: 89.24% Precision: 31.59%	Accuracy: 14.92% Precision: 34.03%
Digestion with hydrochloric acid 11.6M, 24 hours at 80 ˚C	Samples did not digest					
Digestion with nitric acid 15.8M, 24 hours at 80 ˚C	Accuracy: 82.40% Precision: 6.73%	Accuracy: 125.85% Precision: 2.25%	Accuracy: 98.21% Precision: 16.69%	NA	Accuracy: 112.40% Precision: 2.66%	Accuracy: 109.23% Precision: 6.28%
Digestion with nitric acid 4M, 0.02% M L-Cysteine, 24 hours at 60 ˚C	Accuracy: 105.08% Precision: 6.93%	Accuracy: 118.79% Precision: 31.8%	Accuracy: 121.04% Precision: 14.74%	NA	Accuracy: 97.75% Precision: 2.66%	Accuracy: 100.95% Precision: 4.92%

Table S 2-3. Accuracy and precision results from the most relevant experiments done for DBS.

Method	Reference material DBS		Spiked DBS Sample	
	MeHg	**InHg**	**MeHg**	**InHg**
Digestion with nitric acid 15.8M, 24 hours at 80 ˚C	Accuracy: 142.04% Precision: 66.93%	NA	Accuracy: 82.65% Precision: 5.89%	Accuracy: 29.62% Precision: 20.62%
Digestion with nitric acid 4M, 0.02% M L-Cysteine, 24 hours at 60 ˚C	Accuracy: 111.89% Precision: 15.40%	NA	Accuracy: 97.99% Precision: 2.25%	Accuracy: 80.76% Precision: 3.73%

2.7.4 S4. Experimental description of the selected method

Figure S 2-2 Analysis of mercury species with a GC-CVAFS. More information of the detection method can be found in the Series 2700, Tekran Analytical Guide for US EPA Method 1630, Tekran Corporation, 2011.

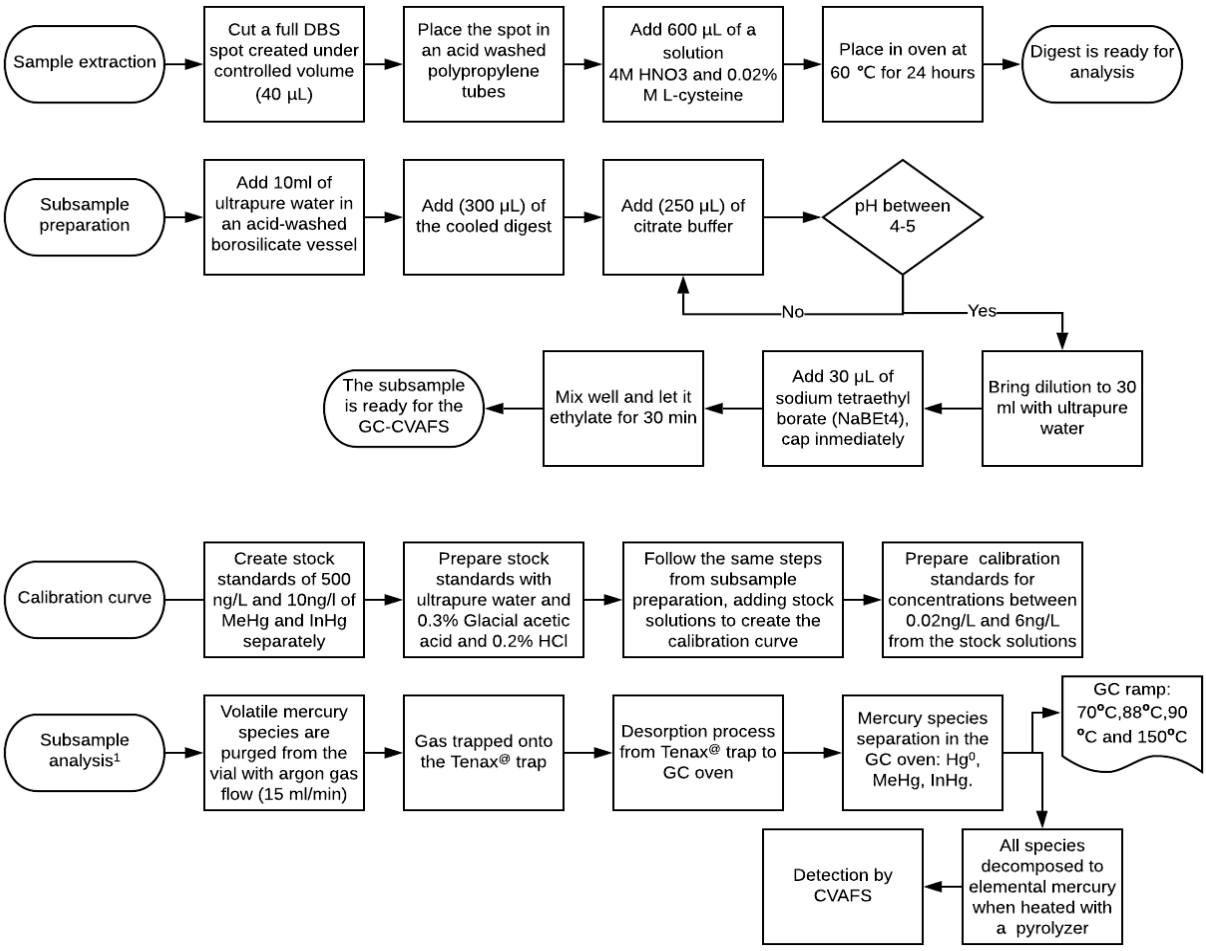


Table S 2-4. Accuracy and precision of seven replicates of reference material PC-B-M1510 (MeHg = 2.56 ± 1.25 µg/L) and PC-B-M1112 (MeHg = 8.68 ± 0.26 µg/L), as well as a spiked matrix with 2µg/L of MeHg and InHg during method development.

		MeHg				EPA 1630	InHg			
Matrix		**DBS**	**Whole Blood**	**DBS blank**	**Reagents**	**Criteria**	**DBS**	**Whole Blood**	**DBS blank**	**Reagents**
Reference Material	**Accuracy**	93 – 128 %	96 - 127%	NA	NA	65- 135%	*	NA	NA	NA
	Precision	0.3-5%	5-14%	NA	NA	<35%	*	NA	NA	NA
Spiked Matrix	**Accuracy**	72 – 102%	81 – 111%	125 - 129%	108 – 122%	65- 135%	80 - 132%	71- 135%	77 – 101%	93 -105%
	Precision	17 %	15 %	2%	11%	<35%	25 %	31 %	14%	6%

2.7.5 S5. Method detection limit

The method detection limit (MDL) of MeHg and InHg was calculated at the beginning of the study to determine the minimum concentration above which sample values will be reported. It was calculated following the 40 Code of Federal Regulations (CFR) Appendix B Part 136 procedure (US EPA, 2016) from US EPA 1630.

In brief, nine replicates of a spiked sample (whole blood and DBS), and nine method blanks were analyzed on three separate calendar dates. We selected a spike concentration for MeHg and InHg of 0.02 ng/L, that was similar to the suggested detection limit provided by the equipment manufacturer (Tekran, see further below). Method detection limits were calculated for each matrix (whole blood and DBS) using the following equation:

$$MDL = t_{(n-1,\alpha=0.99)}S$$

Where: t(n-1,1-α=.99) = the students' t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom, and S = standard deviation of the replicate analyses.

To assess extraction recovery, precision, and matrix interference of the developed method, we analyzed seven replicates of reference material and blood from a consenting volunteer spiked with MeHg and InHg (2 µg/L), for a series of different matrices, including DBS, DBS blanks, whole blood, and method blanks (Table S2-4). The selected MeHg and InHg spike of 2 µg/L was applied to the samples to maintain the same spiked level for each Hg species and avoid interference with the MDL.

2.7.6 S6. Quality accuracy and quality control

To derive a robust method, we continually evaluated our results against key performance criteria described in US EPA Method 1630. All calibration standards and spikes were prepared using certified MeHg (II) chloride and InHg standards (1000 mg/L). Calibration curves were prepared daily using five standards and three blanks. The curve's linearity was calculated using least squares linear regression and compared with the reference value of linearity criteria (R^2=0.99-1.01). Our results are similar our previous study on MeHg (Basu et al., 2017). Standards showed good linearity in the range of 0.02 ng/L to 6 ng/L for MeHg and 0.5 to 6 ng/L for InHg. The correlation coefficient values were above 0.996 for both InHg and MeHg. Individual accuracy of the standards was in the range of 82-96% for MeHg and 72-118% for InHg.

Initial Precision Recovery (IPR) and Ongoing Precision Recovery (OPR) consisted of repeated analysis of standards (0.5 ng/L of MeHg and InHg). IPR samples were analyzed every day at the start of the day, and OPRs were included in each analytical batch (i.e., every ten samples) and at the end of each batch. Ethylation blanks (n=33) consisting of all reagents except the sample were run with each IPR (n=5) and OPR (n=28) sample to evaluate potential background contamination. Calibration blanks, OPRs, IPRs and blanks were all prepared in the same matrix than the samples (Supporting Information Figure S2-1).

OPRs and IPRs were measured every ten samples during phase 1 and phase 2 studies (n=32). The calculated recovery and precision of OPRs and IPRs for MeHg and InHg were within the performance criteria for MeHg and for most of the InHg measurements (92%) thus indicating good inter- and intra-day performance (Table S2-5). Equipment blanks were measured every ten samples (n=32) with reported values for MeHg (0.004-0.016 ng/L) and InHg (0.06-0.18 ng/L) falling below the US EPA method 1630 criteria of <0.05 ng/L.

Method blanks (n=20) including a full spot of unused filter paper (an area of paper that would correspond to a volume of ~60µl)(Funk, 2015), sample replicates (n=20), and reference material for DBS and whole blood (n=20) were used to calculate, respectively, background contamination, precision (%RSD, relative standard deviation) and accuracy (% bias); and these were measured in

every batch of 10 samples. Paired whole blood – DBS produced from the human whole blood reference materials and matrix spiked samples were analyzed seven times to calculate accuracy and precision during method development.

Table S 2-5. Accuracy and precision measures taken during the phase 2-validation study. Accuracy and precision of reference material PC-B-M1510 (MeHg = 2.56 ± 1.25 µg/L) (n=5) and PC-B-M1112 (MeHg = 8.68 ± 0.26 µg/L) (n=5). A sample replicate was analyzed every 10 samples.

	MeHg		US. EPA 1630 Criteria		InHg	
	Accuracy	**Precision**	**Accuracy**	**Precision**	**Accuracy**	**Precision**
OPR (n=32)	83- 116%	<8%	67 – 133%	NA	97-141%	<22%
Reference Material DBS (n=10)	90 – 134%	1-5%	65 – 135%	35%	No reference material for InHg	
Reference Material Whole Blood (n=10)	91 – 135%	0.3 - 14%				
Samples DBS (n=10)	NA	<13%			NA	<13%
Samples Whole Blood (n=10)	NA	<5%			NA	<5%

2.7.7 S7. Mercury levels

In this supporting information, we present descriptive statistics for the whole blood and DBS MeHg and the InHg concentrations from our phase 2 validation study. Table S2-6 and S2-7 present the concentration data for the four matrix-source combinations for MeHg and InHg, respectively. Since most of the InHg data were below the detection limit (1.9µg/L for DBS and 1.1 µg/L for whole blood), the results are not reliable for a method validation.

Table S 2-6. MeHg concentrations in whole blood and DBS (Dried Blood Spots) from venous and capillary blood sources. All units are expressed in µg/L.

Blood Source	Matrix	Geometric Mean (SD)	Range	% <MDL	10th	25th	50th	75th	90th
Venous	Whole blood	0.79 (0.63)	0.25 – 2.95	0	0.31	0.45	0.93	1.40	1.70
	DBS	0.81 (0.67)	<MDL - 2.58	4	0.32	0.43	0.82	1.34	2.23
Capillary	Whole blood	0.78 (0.56)	0.23 - 2.61	0	0.32	0.48	0.91	1.37	1.74
	DBS	0.86 (0.66)	<MDL – 2.75	4	0.38	0.53	0.80	1.44	2.18

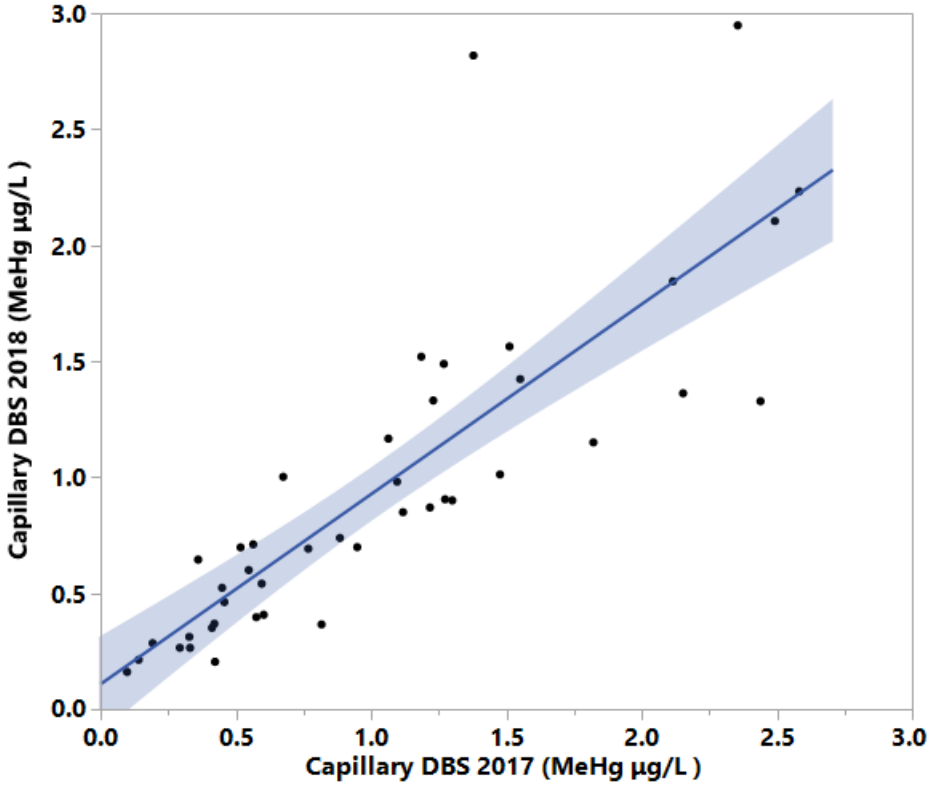
Table S 2-7 Inorganic Hg concentrations in whole blood and DBS (Dried Blood Spots) from venous and capillary blood sources. All units are expressed in µg/L.

Blood Source	Matrix	Geometric Mean (SD)	Range	% <MDL	10th	25th	50th	75th	90th
Venous	Whole blood	0.68 (0.32) (<MDL)	0.13–1.59	90	0.34 (<MDL)	0.49 (<MDL)	0.58 (<MDL)	0.83 (<MDL)	1.18
	DBS	1.25 (0.65) (<MDL)	0.06 -2.56	84	0.16 (<MDL)	0.81 (<MDL)	1.37 (<MDL)	1.69 (<MDL)	2.09
Capillary	Whole blood	0.64 (0.21) (<MDL)	0.08-1.10	98	0.43 (<MDL)	0.52 (<MDL)	0.65 (<MDL)	0.81 (<MDL)	0.85 (<MDL)
	DBS	1.38 (0.60) (<MDL)	0.16 -3.03	84	0.35 (<MDL)	1.11 (<MDL)	1.41 (<MDL)	1.79 (<MDL)	2.07

Table S 2-8. Bland-Altman plot statistics. Concentrations are in µg/L.

Matrix-source combination	Capillary whole blood vs venous whole blood	Capillary DBS vs capillary whole blood	Venous DBS vs venous whole blood	Capillary DBS vs venous whole blood
Bias	-0.02	0.10	0.04	0.07
CI Bias	-0.08 – 0.31	-0.001 – 0.21	-0.06 – 0.13	-0.01 – 0.16
SD	0.19	0.35	0.32	0.30
LOA	-0.41 – 0.36	-0.54 – 0.79	-0.60 – 0.67	-0.51 – 0.66
Linear regression				
Slope	-0.13	0.52	0.05	0.04
CI slope	-0.22 – -0.03	0.03 – 1.02	-0.10 - 0.20	-0.09 – 0.19

Figure S 2-3. MeHg levels in capillary DBS immediately after collection versus after one-year of storage at room temperature.



2.7.8 References

Basu, N., Eng, J. W. L., Perkins, M., Santa-Rios, A., Martincevic, G., Carlson, K., & Neitzel, R. L. (2017). Development and application of a novel method to characterize methylmercury exposure in newborns using dried blood spots. Environmental Research, 159, 276–282. https://doi.org/10.1016/J.ENVRES.2017.08.021

US EPA. (2016). Definition and Procedure for the Determination of the Method Detection Limit—Revision 1.11. Code of Federal Regulations, Title 40, Part 136, Appendix B, (December). Retrieved from https://www.law.cornell.edu/cfr/text/40/part-136/appendix-B%5Cnhttp://www.epa.gov/region9/qa/pdfs/40cfr136_03.pdf

CONNECTING PARAGRAPH

Chapter two presented the development and validation of a laboratory-based method to measure MeHg and InHg in DBS. To our knowledge the study on chapter two was the first one to validate the DBS method for Hg speciation using different whole blood sources and matrices. Limitations concerning the earlier chapter were the application of the method in field-based research, and the high method detection limit for InHg.

The present chapter aims to validate the method under real world-settings. The manuscript is a short communication on the analysis of DBS taken in the field compared to DBS artificially created in the laboratory under controlled conditions; this paper was done with 20 individuals from an contaminated site in Ghana, that presents different levels of exposure and field settings than the population of chapter 2. The main aim of this manuscript was to identify differences in MeHg concentrations and background contamination between laboratory DBS and DBS collected in the field. The samples were obtained as part of a collaboration project with the West Africa GEOHealth network. This manuscript is co-authored by the candidate's supervisor Dr. Niladri Basu and Julius Fobil. It is planned for submission to PeerJ Analytical Chemistry.

Aside, this chapter is complemented by a collaborative study done to analyze the use of DBS for estimating Hg exposure in landlocked Arctic Char that compares total Hg concentrations in DBS with total Hg in fish tissues:

Barst, B. D., Wooller, M. J., O'Brien, D. M., **Santa-Rios, A.,** Basu, N., Köck, G., … Muir, D. C. G. (2020). Dried blood spot sampling of landlocked Arctic char (*Salvelinus alpinus*) for estimating mercury exposure and stable carbon isotope fingerprinting of essential amino acids. Environmental Toxicology and Chemistry, 39(4): 893-903

3. CHAPTER 3. THE USE OF DRIED BLOOD SPOTS FOR METHYLMERCURY EXPOSURE ASSESSMENTS IN FIELD-BASED RESEARCH

Andrea Santa-Rios[1], Julius Fobil[2], and Niladri Basu[1]

[1] Faculty of Agricultural and Environmental Sciences, McGill University, Montreal, Quebec, Canada. [2] Department of Biological, Environmental & Occupational Health Sciences, School of Public Health, University of Ghana, Legon, Accra, Ghana

3.1 Abstract

Biomonitoring methylmercury (MeHg) exposure is problematic in resource-limited settings and difficult-to-access populations where the use of traditional biomarker approaches poses 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. DBS and whole blood samples were collected from electronic waste workers (n=20) from Agbogbloshie (Ghana), and DBS were also artificially created in the laboratory using the field-collected blood. Blood MeHg averaged 0.84µg/L, and this was not different from levels measured in the DBS (field-collected or artificial-created). Linear regressions revealed no differences in MeHg concentrations between whole blood samples and field-collected DBS (slope 0.89, R2=0.94), and between field- and laboratory-DBS (slope 0.89, R2=0.96). Bland-Altman plots showed a small bias between comparisons (range -0.02 – 0.01 µg/L). And finally, no significant differences were found between the blanks. Our results indicated negligible absolute differences between MeHg concentrations in DBS collected in the field, laboratory-created DBS, and whole blood. Indicating that DBS are a suitable tool for assessing MeHg exposure in human population studies under real-world settings.

3.2 Introduction

Mercury is a toxic chemical of global concern that is found in the environment in different chemical forms (UN Environment Programme, 2019). All peoples worldwide are exposed to some amount of mercury though most human populations are exposed to the methylmercury (MeHg) form, and this is realized through fish and seafood consumption (Mergler et al., 2007; Sheehan et al., 2014; UN Environment Programme and World Health Organization (WHO), 2008). 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 biomonitor MeHg levels in people. 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 to identify and protect populations at risk of high exposures (UN Environment Programme, 2013b).

Human exposures to MeHg are best realized through biomonitoring efforts that make use of biomarkers, and specifically measures of total Hg content in blood and hair (Horvat et al., 2012; UN Environment Programme and World Health Organization (WHO), 2008). However, these measures remain challenging for application in many populations particularly those situated in resource-limited settings or remote locations (Basu et al., 2018b), as well as those communities with high exposure to elemental Hg (e.g., dental workers and artisanal gold miners). The collection of venous whole blood is invasive, requires trained medical personnel along with bulky supplies and cold chain for sample storage and transportation (Lee and Li, 2014). The collection of hair in some communities is objectionable due to cultural concerns (Basu et al., 2018b). While 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, as speciation profiles in whole blood can differ from the general

population, and hair can be contaminated by exogenously adsorbed inorganic Hg (Akagi et al., 2000, 1995; 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, and particularly in communities located in resource-limited settings or remote locations. 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, convenient, and overcomes restrictions related to field-based research cutting the need for specialized equipment, cold-chain handling, and phlebotomists (Lee and Li, 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 (Basu et al., 2017; Chaudhuri et al., 2009; Funk, 2015; Funk et al., 2013; Nelson et al., 2016; Elias Nyanza et al., 2019; Elias. Nyanza et al., 2019). In recent years, our team has extended upon this line of research to specifically measure MeHg levels in DBS (Basu et al., 2017; Santa-Rios et al., 2020), though these studies were focused on DBS obtained in clinical and well-resourced environments with population groups that largely have background exposures to Hg. These methods have yet to be validated in real-world settings that may be confounded by logistical and technical issues (e.g., sampling, transport of materials, contamination). Therefore, the present study aimed to validate our previously developed method (Santa-Rios et al., 2020) for the analysis of MeHg in DBS under field-based conditions. This was realized by studying DBS and whole blood collected from electronic waste (e-waste) workers from Agbogbloshie, Ghana.

3.3 Methods

3.3.1 Sampling

Venous whole blood and DBS were collected from a subset of randomly selected e-waste workers (n=20) from Agbogbloshie (Accra, Ghana) participating in the larger GEOHealth study described elsewhere (Nti et al., 2020; Takyi et al., 2020). Agbogbloshie is widely recognized as one of the world's most chemically contaminated locations (Basu et al., 2016; Landrigan et al., 2018) and thus 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). The IRB 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 vial 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 (USAID, 2012). Five drops of capillary blood were collected onto Whatman 903 protein saver cards (Whatman 903 protein saver cards, G.E. Healthcare, Dassel, Germany) and left to dry on the site at room temperature. In addition to these samples obtained from the field, we also created DBS artificially in the laboratory by pipetting 40µL 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 four hours and then packed into a clean polypropylene bag with a desiccant pack.

3.3.2 Methylmercury 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 20µL of the sample, and for the field-DBS and laboratory-DBS we took three punches (each being 3mm in diameter) from each DBS assuming these to contain a total blood volume of 9.3 µL (Lee and Li, 2014). In brief, samples were extracted with 200 µL of a solution containing 4M HNO_3 (Omnitrace® Millipore Sigma, NX0407-4) and 0.02% M L-Cysteine (≥97%, F.G., Sigma-Aldrich, W326305) by placing them into a pre-acid washed (HCl 10%) 4.5 mL polypropylene tubes (Sarstedt, 60.557) and in an oven at 60 ℃ for 24 hours. 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 ng/L to 6 ng/L). The analysis was carried out using a gas chromatography cold vapor atomic fluorescent spectrometer (GC-CVAFS) (Tekran® Series 2700, Tekran Instruments Corporation, Toronto, Canada).

The quality analysis was carried out using a standard of MeHg (0.5ng/L) at the beginning of the study (IPR=initial precision recovery) and every ten samples (OPR=ongoing precision recovery), combined with method blanks according to the U.S. EPA Method 1630 (U.S. Environmental Protection Agency, 1998). For quality control, we used laboratory-created DBS with 40µL of human blood reference material PC-B-M1112 (MeHg = 8.68µg/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 ten samples.

3.3.3 Data analysis

Data was analyzed using JMP (JMP®, Version 14. SAS Institute Inc., Cary, NC, 1989-2019). For quality analysis, we calculated the accuracy and precision of the IPR and OPR samples as percent recovery and the relative standard deviation, 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 US EPA Method 1630.

To validate DBS measures taken in the field, we focused our study on three main comparisons. First, we compared MeHg concentration results from the field-DBS against those from the whole blood (i.e., the gold standard). 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 potential for sample contamination by analyzing differences between field-DBS blanks and laboratory-DBS blanks. In each of these three 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 intra-individual differences.

3.4 Results

The main results from quality analysis and quality control are shown in Table 3-1. All of our analyses resulted in data that fell within parameters established by US EPA Method 1630. Only one individual had DBS MeHg values that fell below the method detection limit (MDL) we established as 0.3 µg/L (Santa-Rios et al., 2020). The MeHg concentrations across the sample types are summarized in Table 3-2. No significant differences were found between mean (± SD in brackets) levels of MeHg in whole blood (0.84 µg/L ± 0.54), field-DBS (0.84 µg/L ± 0.54), and laboratory-DBS (0.83 µg/L ± 0.45). The median intra-individual coefficient of variation was 9% and it ranged from 1% to 20%.

Table 3-1. Quality analysis and quality control following the U.S EPA Method 1630

Parameter	Unit	Value	EPA 1630
Method Blanks	Average MeHg (µg/L)	0.02	<0.05 ng/L
IPR/OPR	Precision (%)	3.66	<35%
	Recovery (%)	108	65-135%
Sample Replicates	Precision (%)	1-24	<35%
Reference Material	Recovery (%)	79-121	65-135%

Table 3-2 Concentrations of MeHg (µg/L) among e-waste workers for samples DBS samples collected in the field (n=20) , whole blood (n=20), artificially created DBS under laboratory conditions (n=20), and field and laboratory blanks (n=20). IQR= interquartile range (25^{th},75^{th}). MDL refers to method detection limit (0.3 for µg/L DBS samples and 0.2 µg/L for whole blood).

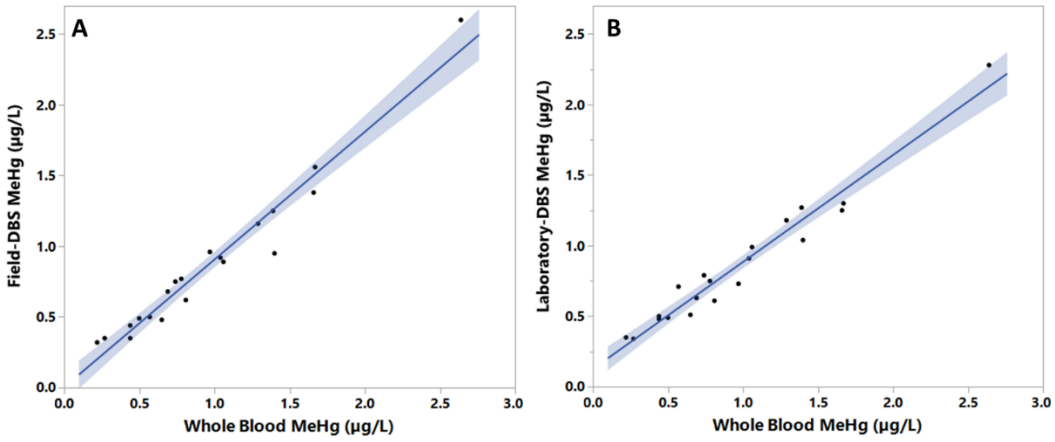
Variables	Field-DBS	Whole blood	Laboratory DBS
Median (IQR)	0.75 (0.48 – 0.96)	0.70 (0.45-1.16)	0.73 (0.50 – 1.00)
Mean ±SD	0.84 ±0.54	0.84 ±0.54	0.83 ±0.45
Geometric mean	0.72	0.72	0.74
Minimum	0.31	0.24	0.34
Maximum	2.60	2.37	2.28
Blank range	(0.06 – 0.28)		(0.02 – 0.26)
Samples below the MDL.	1	0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

3.4.1 Agreement between DBS and whole blood

All the statistical results from our validation tests are presented in Supporting Information Table S3-1. We compared the results from field and laboratory DBS with those from whole blood. The Wilcoxon Signed-rank tests showed no significant differences in MeHg concentrations between field-DBS and whole blood (p=0.81), as well as between the laboratory-DBS and whole blood (p=1.00). Through analysis of linear regression relationships (Figure 3.1A and 3.1B), 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% CI= -0.03 – 0.17 for field-DBS, and intercept -0.05 µg/L, 95% CI= -0.16 – 0.06 for laboratory-DBS), or proportional bias (slope of 0.89, 95% CI 0.78 – 1.00 for field-DBS, and slope of 1.05, 95% CI 0.94 – 1.17 for laboratory-DBS).

When analyzing the Bland-Altman plots (Supporting Information Figure S3-1 and S3-2), 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 (LOA) ranged from -0.25 to 0.21 µg/L, and one sample fell outside the LOA range. Further, all measured samples fell within the maximum allowed difference (MAD) of 35% based on analytically relevant criteria established by US EPA method 1630.

Figure 3-1 (A) Regression line between venous whole blood MeHg (µg/L) and Field-DBS MeHg (µg/L); (B) Regression line between venous whole blood MeHg (µg/L) and laboratory-DBS MeHg (µg/L). The blue bands indicate the 95% confidence interval.



3.4.2 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 two 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 (Supporting Information, Figure S3-3) showed no differences in MeHg measures between field and laboratory DBS, with a mean bias of -0.01 µg/L and a narrow 95% confidence interval (CI= -0.07 – 0.05). A small shift in bias across the exposure range (CI of the slope -0.26 – -0.06) was calculated. One sample fell outside of the LOA (which ranged between -0.26 and 0.24 µg/L), and all samples were between the (MAD).

3.4.3 Background contamination

A key objective of this study is to validate DBS for field settings in a contaminated site, therefore 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. Field-DBS blanks MeHg content averaged 0.86 ± 0.41 pg (range: 0.14 − 1.59 pg) and laboratory-DBS blanks averaged 0.86 ± 0.39 pg (range: 0.36 − 1.73 pg). Considering that 3 punches are equivalent to 9.3 µL (Lee and Li, 2014), the MeHg concentrations in blanks (after taking into account dilution calculations) were all below the MDL ranging between 0.02 and 0.28 µg/L.

3.5 Discussion

The objective of the current study was to determine if a DBS-based method to evaluate MeHg exposure previously outlined in laboratory- and clinical-based settings could be utilized in the real-world for application in a contaminated and resource-limited site. Our results here demonstrate such a method could be used in a field setting with negligible differences found between MeHg measures taken from the gold standard (whole blood) and field-collected DBS, as well as between field-collected DBS with artificially created DBS in the laboratory.

The quality analysis and quality control results were similar to our previous study (Santa-Rios et al., 2020), and the calculated values were within US EPA 1630 method performance criteria. Methylmercury levels reported here (e.g., in field-DBS the median was 0.7 µg/L, IQR 0.5-1.0) were similar to total Hg levels reported in a past study at the same site in Ghana (median whole blood total Hg 0.9 µg/L, IQR 0.7-1.3 (Srigboh et al., 2016)), and in the range of blood Hg values expected for populations sampled from across Africa (median whole blood total Hg 1.3 µg/L, IQR 0.7–2.8 (Basu et al., 2018b)). Though, and as expected, the MeHg levels reported here were higher than the general population of the U.S. (geomean 0.4µg/L for 2015 – 2016 (U.S. Department of Health and Human Services, 2019)) and Canada (geomean 0.6µg/L for 2014 – 2015 (Canadian Health Measures Survey CHMS, 2014)) based on national biomonitoring survey results.

There are concerns about potential contamination of filter paper during its manufacturing (Resano et al., 2018), as well as external contamination of these 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 finding that THg concentrations in field-DBS blanks were two-fold higher (mean = 0.06 ± 0.02 µg/L) than the laboratory-DBS blanks (mean = 0.03 ± 0.02 µg/L) (Elias Nyanza et al., 2019). For Hg, concerns over external contamination may be minimal as levels in the air can range from 0.87 – 1.91 ng/m^3 in rural areas (UN Environment Programme, 2018) and as high as 1 million ng Hg/m^3 in heavily contaminated sites (Cordy et al., 2011a), but the chemical form of Hg in the air is in an

inorganic or elemental state and not in the organic MeHg form. Nonetheless, it is still important to demonstrate background levels of MeHg in the cards (from manufacturing processes), and also in blank cards taken to field locations (to account for exogenous contamination during use). Here, 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 ± 0.06μg/L) were similar to values previously reported from our group though in a study set in a "clean" environment (mean 0.16 ± 0.02 μg/L) (Basu et al., 2017). As the study site of Agbogbloshie is considered to be one of the most contaminated worldwide, such a finding increases confidence that DBS cards can be sampled in field settings. Our study, coupled with previous studies (Basu et al., 2017; Santa-Rios et al., 2020) indicate that MeHg levels in blank filter cards are low and not significant in the time of measuring MeHg concentrations in real-world and laboratory settings.

The agreement in 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 the gold standard. This extends on a past study of ours involving 49 volunteers in which a similar observation was made (Santa-Rios et al., 2020). While both populations presented similar blood MeHg levels, the current study was performed in a resource-limited field setting that is notorious for being contaminated while our past study was performed in a well-resourced clinical setting. Therefore, 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 current 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). In our past work, we derived a similar estimate (Basu et al., 2017) and thus applied it into the current study. To help understand the legitimacy of this assumption, here we compared blood MeHg values between the field-collected DBS (in which the sample volume is not known) with DBS

samples artificially created in the lab 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 two 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 our earlier validation study provides 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) (Elias Nyanza et al., 2019) 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, and so future studies are needed to apply the method outlined here to study populations with relatively high exposures.

3.6 Conclusion

Even though there are several validation studies for the use of DBS for total Hg and MeHg exposure, to our knowledge, this is the first study that validates the use of DBS for the analysis of MeHg exposure under real-world settings by taking the approach into a resource-limited and contaminated site. Here, we showed the analytical accuracy, precision, and feasibility of the DBS-method compares well with the gold standard (whole blood).

3.7 References

Asampong, E., Dwuma-Badu, K., Stephens, J., Srigboh, R., Neitzel, R., Basu, N., & Fobil, J. N. (2015). Health seeking behaviours among electronic waste workers in Ghana Environmental health. BMC Public Health, 15(1), 1–9. https://doi.org/10.1186/s12889-015-2376-z

Basu, N., Eng, J. W. L., Perkins, M., Santa-Rios, A., Martincevic, G., Carlson, K., & Neitzel, R. L. (2017). Development and application of a novel method to characterize methylmercury exposure in newborns using dried blood spots. Environmental Research, 159, 276–282. https://doi.org/10.1016/J.ENVRES.2017.08.021

Basu, N., Horvat, M., Evers, D. C., Zastenskaya, I., Weihe, P., & Tempowski, J. (2018). A State-of-the-Science Review of Mercury Biomarkers in Human Populations Worldwide between 2000 and 2018. Environmental Health Perspectives, 126(10), 106001. https://doi.org/10.1289/ehp3904

Basu, N., Pawly, J., & Neitzel, R. L. (n.d.). Analysis of copper, selenium, and zinc in newborn dried bloodspots using total reflection X-ray fluorescence (TXRF) spectroscopy. https://doi.org/10.7717/peerj-achem.1

Burns, K., Sun, K., Fobil, J., & Neitzel, R. (2016). Heart Rate, Stress, and Occupational Noise Exposure among Electronic Waste Recycling Workers. International Journal of Environmental Research and Public Health, 13(1), 140. https://doi.org/10.3390/ijerph13010140

Canadian Health Measures Survey CHMS. (2014). Fourth Report on Human Biomonitoring of Environmental Chemicals in Canada. Retrieved from https://www.canada.ca/content/dam/hc-sc/documents/services/environmental-workplace-health/reports-publications/environmental-contaminants/fourth-report-human-biomonitoring-environmental-chemicals-canada/fourth-report-human-biomonitoring-environmental-chem

Clarkson, T. W., & Magos, L. (2006). The Toxicology of Mercury and Its Chemical Compounds. Critical Reviews in Toxicology, 36(8), 609–662. https://doi.org/10.1080/10408440600845619

Horvat, M., Snoj Tratnik, J., & Miklavčič, A. (2012). Mercury: Biomarkers of Exposure and Human Biomonitoring. Biomarkers and Human Biomonitoring Vol.1: Ongoing Programs and Exposures (Vol. 1).

Karagas, M. R., Choi, A. L., Oken, E., Horvat, M., Schoeny, R., & Kamai, E. (2012). Review Evidence on the Human Health Effects of Low-Level Methylmercury Exposure. Environmental Health Perspectives, 120(6), 799–806.

Lee, M. S., & Li, W. (2014). Dried blood spots : applications and techniques. Retrieved from https://www.wiley.com/en-ca/Dried+Blood+Spots:+Applications+and+Techniques-p-9781118054697

Nyanza, E. C., Dewey, D., Bernier, F., Manyama, M., Hatfield, J., & Martin, J. W. (2019). Validation of Dried Blood Spots for Maternal Biomonitoring of Nonessential Elements in an Artisanal and Small-Scale Gold Mining Area of Tanzania. Environmental Toxicology and Chemistry, 38(6), 1285–1293. https://doi.org/10.1002/etc.4420

Santa-Rios, A., Barst, B. D., & Basu, N. (2020). Mercury Speciation in Whole Blood and Dried Blood Spots from Capillary and Venous Sources. ANALYTICAL CHEMISTRY. https://doi.org/10.1021/acs.analchem.9b04407

Sherman, L. S., Blum, J. D., Basu, N., Rajaee, M., Evers, D. C., Buck, D. G., … DiGangi, J. (2015). Assessment of mercury exposure among small-scale gold miners using mercury stable isotopes. Environmental Research, 137, 226–234. https://doi.org/10.1016/j.envres.2014.12.021

Srigboh, R. K., Basu, N., Stephens, J., Asampong, E., Perkins, M., Neitzel, R. L., & Fobil, J. (2016). Multiple elemental exposures amongst workers at the Agbogbloshie electronic waste (e-waste) site in Ghana. https://doi.org/10.1016/j.chemosphere.2016.08.089

U.S. Department of Health and Human Services. (2019). Fourth National Report on Human Exposure to Environmental Chemicals. Updated Tables, January 2019, Volume One. Fourth National Report on Human Exposure to Environmental Chemicals, (January), 1–529.

U.S. Environmental Protection Agency. (1998). Method 1630: Methyl Mercury in Water by Distillation, Aqueous Ethylation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry. Retrieved from https://www.epa.gov/sites/production/files/2015-08/documents/method_1630_1998.pdf

USAID. (2012). Biomarker field manual: Demographic and Health Surveys Methodology. Calverton, Maryland. Retrieved from http://www.measuredhs.com/publications/publication-dhsm7-dhs-questionnaires-and-manuals.cfm

Wilson, S. H., & Suk, W. A. (2002). Biomarkers of Environmentally Associated Disease (1st edition). Boca Raton: CRC Press.

3.8 Supporting information

Table S 3-1 Statistical results from field-DBS vs Whole blood (n=19), laboratory DBS vs whole blood (n=19) and field-DBS vs laboratory-DBS (n=19)

Statistical Analysis	Field-DBS vs Whole blood	Laboratory DBS vs Whole blood	Field DBS vs Laboratory DBS
Wilcoxon Signed Rank Test			
Test Statistic S	-7.00	0.00	13.5
p	0.81	1.00	0.61
Linear Regression			
Slope (CI)	0.89 (-0.03 – 0.17)	1.05 (0.94 – 1.17) -0.05	0.89 (0.75 – 0.92)*
Intercept (CI)	0.07 (0.78 – 1.00)	(-0.16 – 0.06)	0.12 (0.04 - 0.20)*
R2	0.94	0.95	0.96
Bland-Altman			
Bias	-0.02	0.01	-0.01
CI Bias	-0.07 – 0.04	-0.04 - 0.06	-0.07 – 0.05
SD	0.12	0.12	0.13
LOA	-0.25– 0.21	-0.23 – 0.25	-0.26 – 0.24
Slope	-0.08	0.08	-0.16
CI slope	-0.18 – 0.03	-0.04 – 0.20	-0.26 – -0.06*

Figure S 3-1. Bland-Altman plot of differences (left axis; black ●) and percentage differences (right axis; gray ▲) of MeHg measures between whole blood and field-DBS versus the mean of the two measurements.

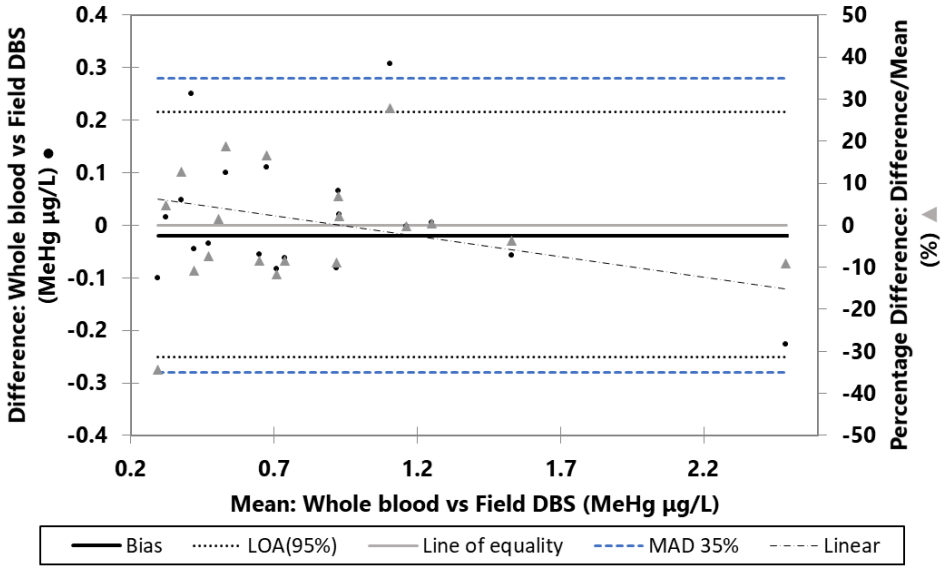


Figure S 3-2. Bland-Altman plot of differences (left axis; black ●) and percentage differences (right axis; gray ▲) of MeHg measures between whole blood and laboratory-DBS versus the mean of the two measurements.

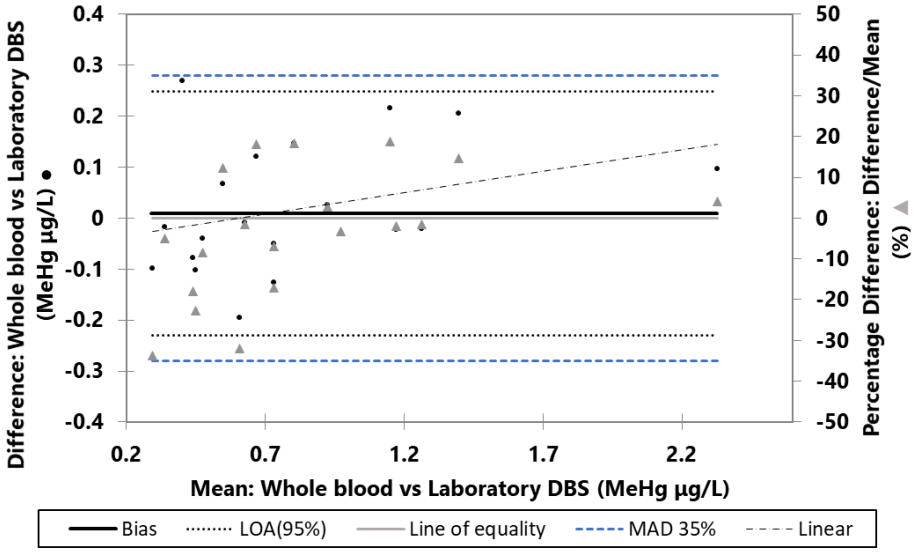
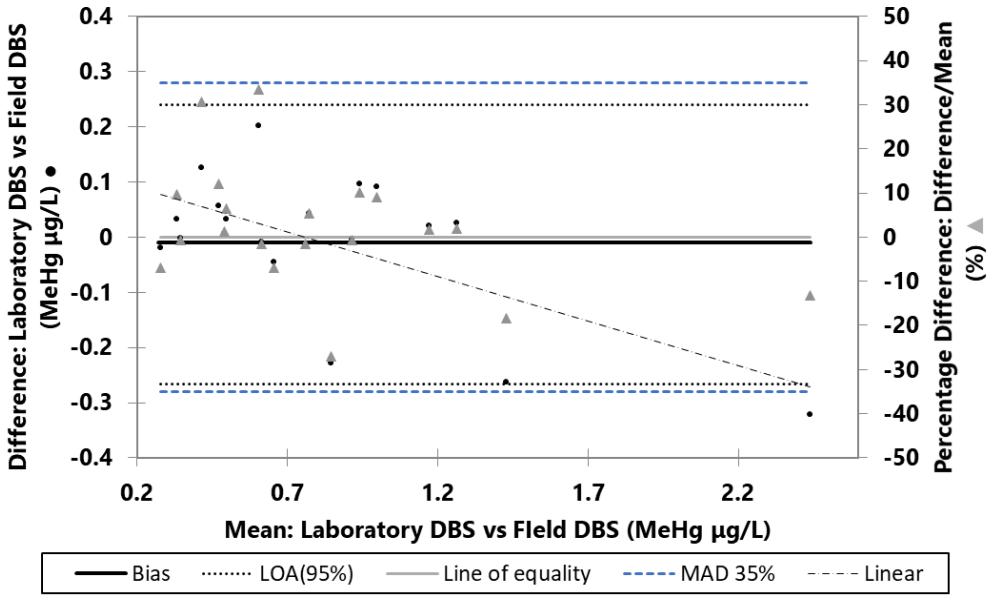


Figure S 3-3. Bland-Altman plot of differences (left axis; black ●) and percentage differences (right axis; gray ▲) of MeHg measures between field-DBS and laboratory-DBS versus the mean of the two measurements.



CONNECTING PARAGRAPH

The main aim of this dissertation was to develop and validate a DBS-method for the analysis of Hg exposure in a variety of communities aiming for vulnerable communities in difficult-to-access locations. During the last two chapters, I presented the development and validation of a laboratory-based and field-based method to measure MeHg and InHg in DBS from communities with background exposures to Hg (Chapter 2) as well as one located in a resource-limited setting with multiple contamination concerns (Chapter 3) . In the present chapter I aimed to use the DBS-based method to analyze the exposure to Hg species in an ASGM community. I collected DBS samples from 35 community members of an ASGM site in Colombia in conjunction with an exposure questionnaire and urine samples. I analyzed Hg species and their associations with occupational, dietary habits and health outcomes.

This manuscript is co-authored by the candidate's supervisor Dr. Niladri Basu, Dr. Benjamin Barst, Benjamin D. Barst, Dr, Lesly Tejeda, and Dr. Yuber Palacios-Torres, and it is under revision at Chemosphere (Submitted (07/12/2020), Revision email (08/10/2020).

4. CHAPTER 4. DRIED BLOODSPOTS TO CHARACTERIZE MERCURY SPECIATION AND EXPOSURE IN A COLOMBIAN ARTISANAL AND SMALL-SCALE GOLD MINING COMMUNITY

Andrea Santa-Rios[1], Benjamin D. Barst[2], Lesly Tejeda[3], Yuber Palacios-Torres[4], and Niladri Basu[1]

[1] Faculty of Agricultural and Environmental Sciences, McGill University, Montreal, Quebec H9X 3V9, Canada. [2] Water and Environmental Research Center, Institute of Northern Engineering, University of Alaska Fairbanks, Fairbanks, Alaska 99775, United States. [3] Grupo de investigación en Ciencias Biomédicas, Toxicológicas y Ambientales, Campus de Zaragocilla, Universidad de Cartagena, 130015, Colombia. [4] Grupo de Toxicología Ambiental y Recursos Naturales, Universidad Tecnológica del Choco "Diego Luis Cordoba", Quibdo, Choco, A.A. 292, Colombia.

4.1 Abstract

The artisanal and small-scale gold mining (ASGM) sector uses the most mercury (Hg) worldwide. Despite health concerns associated with high Hg exposures in these communities, ASGM sites are often situated in resource limited and remote regions which challenge traditional human biomonitoring approaches. To help overcome such challenges, here we report on the development of a high-quality method to characterize chemical speciation of Hg in dried blood spots (DBS), and then apply this method to assess Hg exposures in people sampled from an ASGM community (Pueblito Mejia) and a nearby town (Barranco de Loba) in Colombia. We collected DBS and urine samples from 35 individuals in 2018, and used these to assess occupational (DBS inorganic Hg (InHg) and urine total Hg (THg) measures) and environmental (DBS methylmercury (MeHg) measures) exposure of participants to different forms of Hg. The accuracy and precision of the

DBS-based measures generally met assay performance guideline. In study participants, the mean concentrations of DBS MeHg, InHg, and THg, and urine THg were 1.9, 4.1, 6.0, and 3.1 µg/L, respectively. For 37% of the participants, DBS THg values exceeded the 5 µg/L 'alert level' proposed by the German HBM Commission. About 60% of the blood Hg was in the InHg form thus exemplifying a need to speciate Hg in blood sampled from ASGM sites to better understand the contributions of environmental and occupational exposure sources. This study demonstrates the feasibility of using DBS for Hg speciation exposure assessments in remote and resource-limited areas such as ASGM communities.

4.2 Introduction

Exposure to Hg from artisanal and small-scale gold mining (ASGM) is a pervasive environmental exposure impacting hundreds of millions of people in low- and middle-income communities (U.N. Environment Programme, 2019; Clifford, 2014; Ha et al., 2016). In the process of ASGM, miners add liquid elemental mercury to extract gold from ore or sediment which forms an Hg-gold amalgam. The Hg-gold amalgam is subsequently heated to volatilize Hg, leaving behind the extracted gold (UN Environment Programme, 2013). The sector emits an estimated 1,220 tons of Hg into water and land and 838 tons of Hg into the air (U.N. Environment Programme, 2019). Average yearly exposures in ASGM communities routinely surpass the World Health Organization guideline (average yearly indoor concentration of 1 µg/m3) (World Health Organization (WHO), 2000).

Exposures to Hg in ASGM communities pose serious health risks for miners and non-miners (Basu et al., 2015; Ha et al., 2016; UN Environment Programme, 2012). Elemental Hg emitted from mining activities can be inhaled into the lungs and absorbed into red blood cells where it is rapidly oxidized to the divalent inorganic cation (inorganic Hg, InHg). Elevated elemental Hg exposures

can cause damage to the respiratory, cardiovascular, digestive, and immune systems (Clarkson and Magos, 2006), though the primary targets are the nervous system and the kidneys where InHg preferentially accumulates following inhalation of elemental Hg vapor (Agency for Toxic Substances and Disease Registry, 1999). Inorganic Hg is subsequently excreted in urine, which has previously served as a biological marker in exposure assessments (Horvat et al., 2012; UN Environment Programme and World Health Organization (WHO), 2008)

Residents of ASGM communities may also be exposed to MeHg through the consumption of contaminated fish and other foods. While all fish worldwide are contaminated with some level of MeHg (World Health Organization (WHO), 2019), Hg emissions at ASGM sites can directly contaminate nearby water bodies (UN Environment Programme, 2019). In aquatic environments, microbes convert InHg to MeHg and the resulting MeHg can bioaccumulate and biomagnify in food webs (Obrist et al., 2018). Thus, Hg use at ASGM sites has the potential to increase MeHg contamination of nearby aquatic food webs, resulting in fish with elevated MeHg concentrations (Rajaee et al., 2015b; UN Environment Programme, 2012). Methylmercury ingested through contaminated foods (e.g., fish and in some cases rice) (Akagi et al., 2000; Bradley et al., 2017; Faial et al., 2015; Guimaraes et al., 2011; Malm et al., 1995) may be efficiently transported across the gut tract into the bloodstream from where it may then cross both blood-brain and blood-placental barriers (Clarkson and Magos, 2006; Horvat et al., 2012). Total Hg analyses of human blood and/or hair are typically used to gauge MeHg exposures (Horvat et al., 2012; UN Environment Programme and World Health Organization (WHO), 2008).

Worldwide, ASGM workers and community members have some of the highest Hg exposures. For example, in support of the 2018 UN Global Mercury Assessment, a systematic review of Hg biomarkers in human populations worldwide demonstrated that urine Hg levels sampled from the highest exposed ASGM worker or community member was 188-times greater than members of the general background population (Basu et al., 2018). Further, this review effort drew information from 7,800 ASGM members though this represents a tiny fraction of the >100 million miners and community members estimated worldwide (UN Environment Programme, 2019). A deeper

analysis of this aforementioned systematic review dataset demonstrated that biomonitoring in ASGM sites was largely based on measures of THg in hair (51%), urine (32%), and blood (17%) (Basu et al., 2018). Unfortunately, there are shortcomings associated with the use of these biomarkers for exposure assessments at ASGM sites. First, in communities exposed to high levels of InHg, the use of THg measures have been shown to overestimate true MeHg exposure. For example, a study using Hg stable isotopes showed that THg in the hair of ASGM miners is derived from a mixture of ingested MeHg from fish as well as exogenously adsorbed InHg (Sherman et al., 2015). Second, people exposed to high levels of elemental Hg can have varying proportions of different chemical species of Hg in their blood (Akagi et al., 2000, 1995), and that these proportions are not distinguished by THg measures. Finally, urinary Hg is an accepted biomarker of exposure to InHg but not MeHg.

Assessment of occupational and environmental exposures to Hg in ASGM communities is a major barrier to large-scale evaluation of Hg-related policies and programs, and for conducting epidemiologic studies. Chemical speciation of Hg in whole blood can better distinguish between occupational (mainly InHg) and dietary (mainly MeHg) exposures , but its collection, transport, and storage poses considerable costs and both cultural and logistical challenges, including the need for specialized supplies and trained phlebotomists and cold chain custody (Lee and Li, 2014). These difficulties are exacerbated in remote and resource-limited areas like those where most ASGM communities are located.

The collection and analysis of dried blood spots (DBS) for biomarker assessment is an attractive alternative to whole blood due to its relative ease of collection and transport and the relatively low costs of storage (Edelbroek et al., 2009; McDade et al., 2007; Sharma et al., 2014). This micro-blood sampling technique was used to assess THg exposure in groups across North America (Chaudhuri et al., 2009; Funk, 2015; Funk et al., 2013; Nelson et al., 2016), and two recent studies measured THg in DBS collected from people residing and working within ASGM communities (Nyanza et al., 2019a;Nyanza et al., 2019b). However, to our knowledge, no studies have chemically speciated Hg in human DBS from ASGM sites which is particularly needed since these

communities are contaminated by both organic and inorganic Hg sources. We recently developed and validated methods to quantify exposures to MeHg and InHg in DBS in human populations with relatively low Hg exposures (Basu et al., 2017; Santa-Rios et al., 2020). In the present study, we aimed to further refine and apply these methods for high-exposure populations with the following objectives: (1) to determine if high-quality Hg speciation data could be obtained from DBS obtained from individuals remote and rural ASGM communities, and (2) to quantify and compare Hg exposures among residents of an ASGM mining community and a nearby community without ASGM activity. To our knowledge, this is the first study to chemically speciate Hg in DBSs from residents in ASGM sites, which is particularly needed since these communities are contaminated by both organic and inorganic Hg sources.

4.3 Methods

4.3.1 Study areas

We conducted our study in a ASGM mining community (Pueblito Mejia) and a nearby town (Barranco de Loba) in the Department of Bolivar in northern Colombia (Figure 4-1). We selected these sites for study to compare Hg exposures between people living in a mining village and those living in a larger city located further from the mining center. Pueblito Mejia is a remote and rural community with a population of 1011 residents (Alcaldía Municipio Barranco de Loba, 2013), most of whom work in the ASGM sector. In addition to its remoteness, the village is difficult to access due to the control of gold mining activities by criminal organizations (Wagner, 2016). While previous studies have characterized Hg exposures in the region (Carranza-Lopez et al., 2019; Díaz et al., 2018; Olivero-Verbel et al., 2015), to our knowledge, and according to information provided by community members, , this is the first study to address Hg exposures directly in Pueblito Mejia.

Figure 4-1. . Map of the study area in the Department of Bolivar (main map) within Colombia (inset). The town locations are indicated with the two solid black circles.

Barranco de Loba's principal economic activity is artisanal fishing, with a total population of 5722 in 2011 (Ministerio de Minas y Energía and Universidad de Córdoba, 2014). Even though Barranco de Loba is not a mining community, the town is surrounded by two mines (Santa Cruz and San Martin de Loba) so most adult residents are previous or current ASGM workers.

Mercury concentrations above the safety limit have been reported in the near environment and community, e.g., air Hg levels up to 45 µg/m3 in Santa Cruz and 27 µg/m3 in San Martin de Loba (Olivero-Verbel et al., 2014) exceeding the WHO guideline of 1 µg Hg/m3 (World Health Organization (WHO), 2000). An urban gold processing center in Santa Cruz (located in the outskirts of Barranco de Loba) presented the highest air Hg concentrations (45 µg/m3) and is expected that processing and smelting of gold likely occurs in Barranco de Loba.

4.3.2 Study design

We conducted a cross-sectional study in February 2018 in which convenience sampling was used to recruit participants (n=35). Adults interested in participation were introduced to the study by local field staff in collaboration with community leaders, and were asked to read and sign a Spanish informed consent document before completing a questionnaire (UN Environment Programme and World Health Organization (WHO), 2008; Veiga and Baker, 2004) and providing a DBS and spot urine sample. Our research protocol was approved by the Research Ethics Board of the University of Cartagena (REB File #4341022017) and McGill University (REB File #127-0818).

4.3.3 Collection of dried blood spots and urine specimens

Dried blood spots samples were obtained via finger prick by a health professional from the University of Cartagena following protocols described in the USAID Biomarker Manual (USAID, 2012). For each participant, the side of the second, third, or fourth finger was cleaned with an alcohol wipe and pricked with a contact activated lancet (BD Microtainer®, High flow, 1.5 mm blade, Franklin Lakes, NJ). The initial drop of blood formed at the puncture site was wiped away with sterile gauze. The next five drops of blood were collected within the individual outlined circles of specialized filter paper cards (Whatman 903 protein saver cards, G.E. Healthcare, Dassel,

Germany). Following collection, samples were air-dried on-site and placed in individual plastic bags with a silica gel desiccant sachet for transportation at ambient temperature. We collected 8 field blanks (19% of total samples) using blank filter paper cards that were subject to the same field and drying conditions to account for potential field contamination. In addition to these field blank cards, from each participant's filter card Hg measurements were taken of the dried blood sample as well as a nearby blank spot.

Participants also supplied a 30-50 mL spot urine sample into a urine specimen cup. Urine samples were kept at ambient temperature before and during transport to the laboratories at the University of Cartagena, after which they were then stored at 4ºC.

All specimens were transported to McGill University 8 months after collection, where they were stored frozen (-20ºC) until analysis.

4.3.4 Mercury analysis

Mercury analyses from DBS specimen was carried out using published methods (Santa-Rios et al., 2020), with minor modifications. In brief, three punches of 3 mm were taken from each DBS and blank area of each filter paper card. We assumed that the three punches were equivalent to a total blood volume of 9.3 µl (Lee and Li, 2014). Mercury species (MeHg and InHg) were extracted from the dried blood by placing the filter paper punches into pre-acid washed (HCl 10%) 4.5 ml polypropylene tubes (Sarstedt, 60.557) with 200 µl of a solution containing 4M HNO3 (Omnitrace® Millipore Sigma, NX0407-4) and 0.02% M L-Cysteine (≥97%, F.G., Sigma-Aldrich, W326305). Tubes were capped tightly, placed in an oven at 60 ℃ for 24 hours, and then allowed to cool before analysis. A 100µl subsample of the cooled digest was added to a borosilicate glass vessel with ultrapure water. The pH was adjusted to 4.0–4.5 using citrate buffer, and then 1% sodium tetraethyl borate (NaBEt4) (>98%, Strem, 110575) was added as an ethylating reagent.

Mercury species were separated and quantified using a gas chromatography cold vapor atomic fluorescent spectrometer (GC-CVAFS) (Tekran® Series 2700, Tekran Instruments Corporation, Toronto, Canada) according to US EPA Method 1630 (U.S. Environmental Protection Agency, 1998).

Quality assurance and quality control measures included the analysis of initial and ongoing precision recovery (IPR and OPR, both of 0.5 ng/L of MeHg and InHg) samples (n=7), sample duplicates (n=5), method blanks (n=3), field blanks (n=8), and artificially created DBS samples with human whole blood reference material (n=9). For these artificially created DBS cards, 40µL of human whole blood reference material [PC-B-M1112 (MeHg = 8.68µg/L) and QME-B-Q1313 (THg = 6.11µg/L) from the Institut National de Santé Publique du Québec] were spotted onto cards. For each individual, sample blanks (as three 3mm punches from the same card) were analyzed alongside the DBS samples.

For the analysis of THg in urine, a 100 µl subsample of urine was placed into a quartz vessel and then introduced into a direct THg analyzer (Nippon Model MA-3000, Nippon Instruments Corporation, Japan). Sample replicates (n=3), blanks (n=3), and human urine reference material (n=3) QM-U-Q1109 (THg=5.05µg/L from the Institut National de Santé Publique du Québec) were measured every ten samples for quality control (Perkins and Basu, 2018).

4.3.5 Questionnaire

Field staff administered a standardized health assessment questionnaire from the United Nations Industrial Development Organization (UNIDO) (Veiga and Baker, 2004) to obtain information on their demographic and occupational characteristics of the participants, along with their self-reported dietary habits and potential symptoms of Hg intoxication. A health professional (holding a Doctor of Medicine with specialization in toxicology from the University of Cartagena) read the questions in Spanish to participants and recorded their responses directly on the questionnaire. No information on participant education or income were obtained due to cultural limitations.

Information on occupational exposure included questions related to the number of years lived in the study area, occupation (miner or nonminer), Hg use and storage practices, use of personal protection, and their frequency and duration of exposure to Hg. The dietary habits portion of the survey collected basic information on food intake and fish consumption including the source of the fish. Data were transformed into categorical variables (0-3) according to the score recommended by the Guidance for Identifying Populations at Risk from Mercury Exposure (UN Environment Program and World Health Organization (WHO), 2008). The health professional collected anamnestic data (including the analysis of symptoms like metallic taste, tremors at work, excessive salivation amongst others), clinical data (ataxia and tremors), and conducted neuropsychological tests to identify tremors and memory symptoms. The list of questions from the health information questionnaire is presented in Table S4-1 of the Appendix. The health information, in combination with the results of Hg levels in the biomarkers, was applied to a decision algorithm for chronic Hg intoxication (Table S4-2 Appendix) (Veiga and Baker, 2004).

4.3.6 Data analyses

To assess quality control, we calculated the precision of samples replicates (%RPD, relative percent difference for sample replicates), the accuracy and precision of reference materials, method blanks, and sample blanks (% bias and %RSD, relative standard deviation). Note that for DBS, we calculated THg concentrations as the sum of MeHg and InHg concentrations.

Data analyses were performed using JMP (JMP®, Version 14. SAS Institute Inc., Cary, NC, 1989-2019). Continuous variables (including concentrations of MeHg, InHg, and THg in DBS and THg concentrations in urine) were summarized using median and interquartile ranges, and categorical variables derived from the questionnaire were summarized using counts and percentages. For comparisons between occupation and areas of residence, a 2-sided F-test was applied to test homogeneity of variance; for non-equal variances, we used Welch's ANOVA test, and one-way

ANOVA test for equal variances. Crude (unadjusted) associations between occupational activities and dietary habits were assessed through Chi-square tests. An alpha level of 0.05 was used for all analyses.

4.4 Results

4.4.1 Quality control results of mercury analyses

The method detection limits (MDLs) for MeHg and InHg analyses of DBS by GC-CVAFS were previously calculated by our group following the 40 Code of Federal Regulations (CFR) Appendix B Part 136 procedure from US EPA 1630. The MDLs, calculated from nine replicates of spiked DBS samples were 0.3µg/L and 1.9 µg/L for MeHg and InHg, respectively (Santa-Rios et al., 2020). In the present study, one DBS sample for MeHg (3%) and four DBS samples for InHg (11%) were below the MDLs. These samples with Hg concentrations below the MDL were not used for data analysis.

For THg measures in urine using the direct Hg analyzer, the theoretical method detection limit (TMDL=$\bar{X}$+3*SD) and the practical method detection limit (PMDL=$\bar{X}$+5*SD) were calculated with the method blanks (TMDL=0.03 ng and PMDL=0.04ng). One urine THg value was below the TMDL and PMDL.

For duplicate measures of DBS MeHg, the average relative percentage difference (RPD) was 17%. The RPD for InHg from duplicate DBS samples was 36%, and for duplicate urine samples the RPD for THg was 11%. Percent recoveries of THg (as the sum of MeHg and InHg quantified with the GC-CVAFS) and MeHg from DBS created with human whole blood reference materials were within the US EPA 1630 and 1631 guidelines (i.e., accuracy 75-125% and precision 35% for MeHg; accuracy 71-121% and precision 21% for THg) (U.S. Environmental Protection Agency, 2002, 1998). For THg and MeHg in DBS created with human whole blood reference materials, the

accuracy ranged from 83% to 109% with associated RSDs of 15% for THg and 5% for MeHg. The average percent recovery of THg from the urine reference material was 94% (range = 90-99%), with an RSD of 3%.

4.4.2 Background contamination

One of the main concerns of using DBS in the field (especially in an ASGM community) is the potential for contamination of the filter paper with externally deposited Hg. To address this, in the current study we took to the field site eight separate filter paper cards to serve as field blanks (i.e., these were kept outside during the human biomonitoring sampling campaign). In 3 mm punches from field blank filter cards, the MeHg amount averaged 0.30 ± 0.64 pg (range: -0.57 to 0.96 pg), and for InHg the measured amount averaged 10.32 ± 7.24 pg (range: -3.76 to 19 pg). Considering that three 3mm punches from DBS cards were pooled for the human biomonitoring component of this work and that the total blood volume in these three punches samples is estimated to be 9.3 µl, the theoretical concentrations of MeHg and InHg derived from background conditions were estimated to average ~0.07 ± 0.15 µg/L and ~1.16 ± 0.79 µg/L, respectively. For each MeHg and InHg measurement, only one blank sample returned values that were above the MDL and the calculations were performed on values returned by the machine.

In addition to the eight field blank filter cards we also analyzed sample blanks for each participant. Thus, on a participant's filter card onto which their dried blood was collected, we took punches of both the dried blood as well as a piece of the same filter card without any blood. In these participant blank samples, the MeHg amount averaged 1.66 ± 2.05 pg (range: -0.98 to 8.50 pg) for MeHg, and for InHg the measured amount averaged 17.76 ± 10.43 pg (range: 0.76 to 36.57pg). Using the same assumptions detailed in the preceding paragraph, the concentrations of Hg derived from background conditions in a participant's filter card were calculated to average 0.15 ± 0.19µg/L (range 0.10 to 0.70 µg/L) for MeHg, and 1.77 ± 4.06µg/L (range 0.08 to 3.1 µg/L) for InHg. To

avoid bias related to external contamination, all samples were individually blank corrected for InHg and MeHg.

4.4.3 Demographic characteristics

Table 4-1 presents the demographic information of the 35 study participants. Participants ranged in age from 17 to 88 years and were 69% male. Females had slightly higher BMI than males (27 versus 25 kg/m2). Approximately two-thirds (n=23) self-identified as current miners while the rest identified as nonminers. Most of the study participants (24 of 35) lived in the mining area of Pueblito Mejia; of these individuals, most were men and active miners. Only eight percent of the participants reported either current or previous smoking and 54% reported drinking alcohol.

Table 4-1. Demographic information of study participants from Pueblito Mejia and Barranco de Loba

		All	Pueblito Mejia (mine)	Barranco de Loba (town)
n participants		35	24	11
Sex	Males (%)	69	62	82
Age, years	Females	49 ± 17	51 ± 19	43 ± 2
(Mean ± SD)	Males	43 ± 15	40 ± 13	47 ±18
BMI, kg/m^2	Females	27 ± 4	27 ± 4	27 ± 3
(Mean ± SD)	Males	25 ± 3	25 ± 3	25 ± 3
Occupation				
Current Miner		23 (66%)	18 (51%)	5 (14%)
Nonminer		12 (34%)	6 (17%)	6 (17%)
Smoking				
Never		30 (86%)	22 (63%)	8 (23%)
Current smoker		2 (5%)	-	2 (5%)
Ex-smoker		1 (3%)	1 (3%)	-
N/A		2 (6%)	1 (3%)	1 (3%)
Alcohol				
Never		14 (40%)	11 (32%)	3 (8%)
At least once a month		16 (46%)	9 (26%)	7 (20%)
At least once a week		3 (8%)	3 (8%)	-
N/A		2 (6%)	1 (3%)	1 (3%)

4.4.4 Mercury concentrations in biomarkers

Table 4-2 summarizes the Hg concentrations in DBS (THg, MeHg, and InHg) along with the urine THg values stratified by sex, residence, and occupation. For all study participants, THg levels in the DBS varied considerably from 1.1 to 22.4, with an average of 6.0 µg/L. For InHg in the DBS, concentrations ranged from below the MDL (1.9 µg/L) to 19.8, with an average of 4.1 µg/L. The distribution of MeHg levels in DBS ranged from below the MDL (0.3 µg/L) to 5.9 µg/L (mean: 1.9). The MeHg values in DBS (as a percent of THg) ranged from 5% to 100% (mean: 39%). There was a wide distribution in urine THg concentrations, which varied from below the MDL (0.03) to 19.4 µg/L (mean: 3.1).

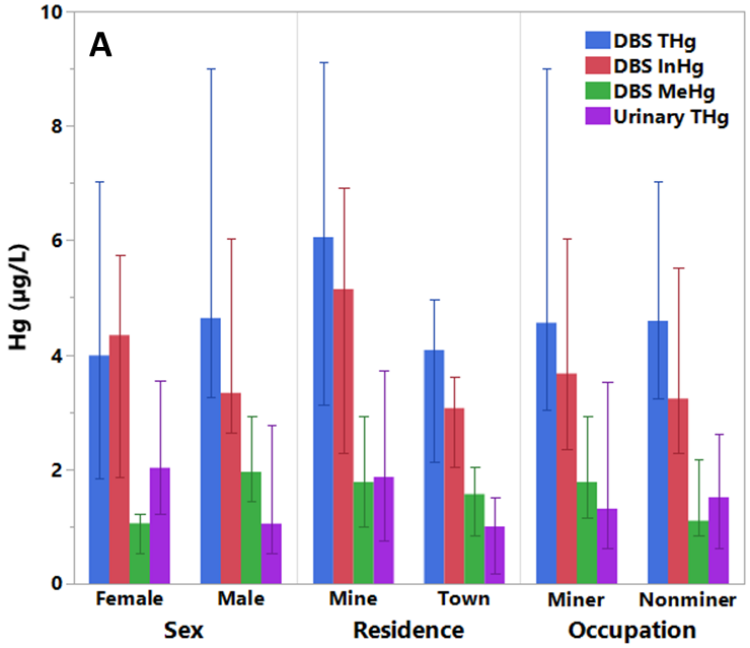
Significant differences between DBS MeHg concentrations were found between males and females (Figure 4-2A) with higher mean levels in males (2.2±1.2 µg/L) than in females (1.3±1.1 µg/L). Conversely, DBS THg, InHg, urine THg, and percent MeHg in DBS did not differ between males and females. We observed low correlations between measures of MeHg and InHg in the DBS (Spearman $\rho = 0.3$, $p=0.1$), between urine THg with DBS MeHg ($\rho = 0.09$, $p=0.6$), and between urine THg with DBS InHg ($\rho = 0.3$, $p=0.1$).

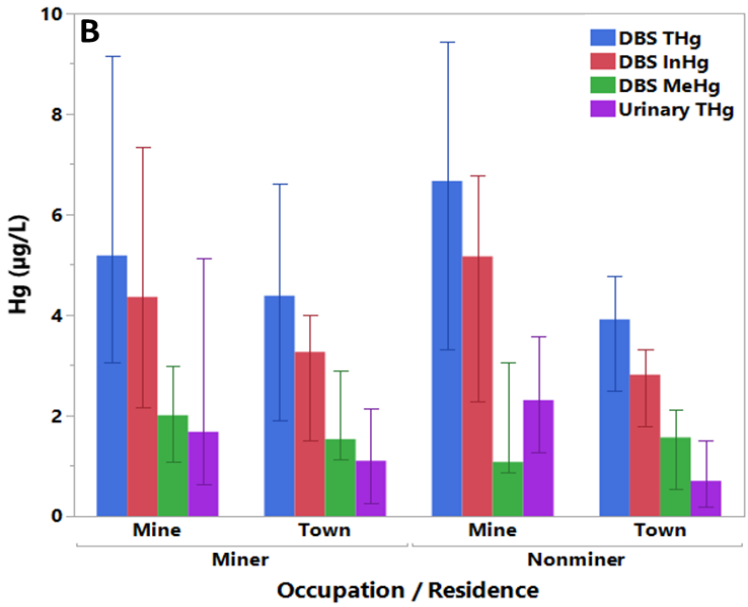
Table 4-2. Descriptive summary of mercury concentrations (µg/L) in DBS and urine from all the participants, and split according to sex, residential location, and occupation.

	DBS THg (MeHg + InHg)			DBS InHg				DBS MeHg				Urine THg		
	Mean (SD)	**Median**	**Range**	**Mean (SD)**	**Median**	**Range**	**%THg (SD)**	**Mean (SD)**	**Median**	**Range**	**%THg (SD)**	**Mean (SD)**	**Median**	**Range**
All	6.0 ±4.7	4.6	1.1 – 22.4	4.1 ±4.3	3.1	<MDL – 19.8	60 ±27	1.9 ±1.2	1.7	<MDL - 5.9	39 ±27	3.1 ±4.5	1.7	<MDL - 19.4
Gender														
Female	4.8 ±3.2	4.0	1.1 - 10.4	3.6 ±2.6	3.5	<MDL – 7.6	68 ±28	1.3 ±1.1	1.0	<MDL – 4.0	32 ±27	2.4 ±1.1	2.3	<MDL – 4.0
Male	6.5 ±5.3	4.6	1.4 - 22.4	4.4 ±5.0	3.0	<MDL – 19.8	57 ±27	2.2 ±1.2	2.0	<MDL - 5.9	43 ±27	3.4 ±5.3	1.1	0.03 – 19.4
Residence														
Pueblito Mejia	6.9 ±5.3	6.0	1.1 – 22.4	4.9 ±4.9	4.0	<MDL - 19.8	63.±26	1.9 ±1.1	1.8	<MDL – 4.0	37 ±26	3.9 ±5.2	2.0	0.1 - 19.4
Barranco de Loba	4.0 ±2.4	4.1	1.2 – 9.8	2.2 ±1.6	3.0	<MDL – 4.2	54 ±29	1.8 ±1.5	1.5	<MDL - 5.9	46 ±29	1.0 ±0.8	1.0	<MDL- 2.4
Occupation														
Miner	6.5 ±5.5	4.1	1.1 – 22.4	4.4 ±5.1	3.3	<MDL - 19.8	56 ±30	2.0 ±1.3	1.8	<MDL – 5.9	44 ±30	3.7 ±5.2	1.5	<MDL - 19.4
Nonminer	5.1 ±2.7	4.6	1.5 – 10.4	3.5 ±2.1	3.9	<MDL – 7.6	63±16	1.6 ±1.1	1.6	<MDL - 4.0	31 ±16	1.6 ±1.2	1.5	0.1 – 3.6

Concentrations of THg and InHg in DBS differed between the two study sites (Figure 4-2A). On average, people residing in Pueblito Mejia had higher DBS THg levels than people from Barranco de Loba, independent of occupation. Similarly, mean DBS InHg levels were almost two-fold higher in residents of Pueblito Mejia (4.9 µg/L) compared with those in Barranco de Loba (2.2 µg/L). Conversely, MeHg DBS concentrations were not different between these communities (mean levels of 1.9 µg/L in Pueblito Mejia versus 1.8 µg/L in Barranco de Loba). Urine THg concentrations were higher in residents of Pueblito Mejia than from Barranco de Loba. There was not a difference in DBS Hg (including THg, MeHg, InHg, and %MeHg) and urine THg concentrations by self-reported occupation (Table 4-2, Figure 4-2B) .

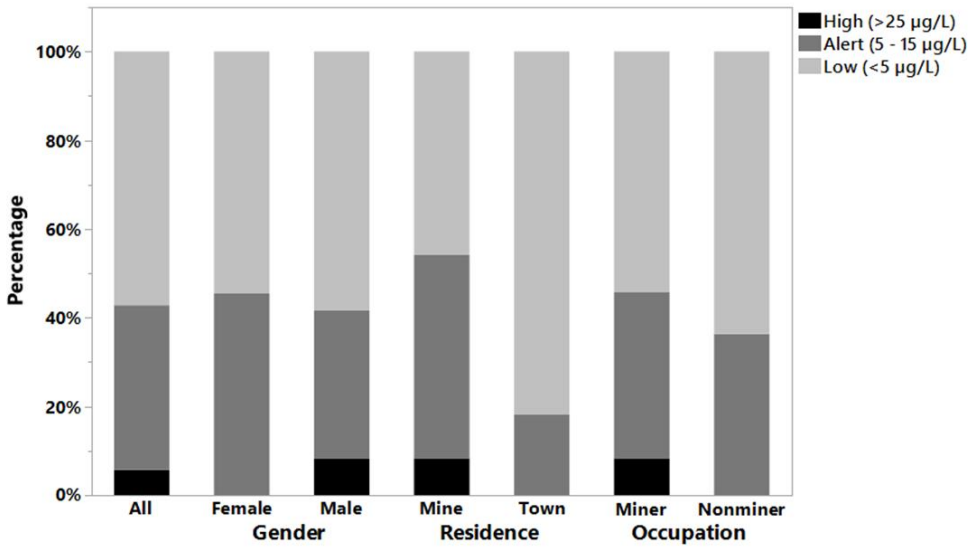
Figure 4-2. A) Concentrations of total mercury (THg), inorganic mercury (InHg), and methylmercury (MeHg) in DBS as well as THg in urine stratified by sex, residence (mine - Pueblito Mejia; town - Barranco de Loba) and occupation. Bars represent the median values with the interquartile range (25th percentile, 75th percentile) indicated as error bars, and B. Concentrations of total mercury (THg), inorganic mercury (InHg), and methylmercury (MeHg) in DBS as well as THg in urine stratified by residence location (mine - Pueblito Mejia; town - Barranco de Loba) and occupation. Bars represent median values with the interquartile range (25th percentile, 75th percentile) indicated as error bars.





Focusing on guidelines derived from the German HBM program (see Appendix, Table A.3), for DBS THg, most of the study population (57%) presented low levels of exposure (HBM-I = <5 µg/L; concentrations associated with no risk of adverse health effects) (Figure 3). For 37% of the participants, the DBS THg values were in the alert level (HBM-II = 5 to 15µg/L; concentrations at which adverse health effects are possible) and six percent (2 participants, both miners from Pueblito Mejia) presented values at the high level (DBS THg 18-22 µg/L). All the nonminers living in Barranco de Loba had DBS THg levels below HBM-I. In contrast, 67% of the nonminers living in Pueblito Mejia presented levels of exposure consistent with the alert level of exposure (i.e., HBM-II).

Figure 4-3. Percentage of participants with DBS THg concentrations in the low (<5 µg/L), alert (5–10 µg/L), and high (>25 µg/L) level of risk based on Human Biomonitoring guidelines established by the German Federal Environmental Agency (Schulz et al., 2011).



4.4.5 Survey Outcomes

a. Occupational questionnaire

Responses to the occupational questionnaire are presented in Table S4-4 of the Appendix. Participants had lived in their study area for an average of 19 years. Most (86%) reported current or previous work as an ASGM miner, with a range of 2 to 36 years of time working in the mines. All current miners worked with elemental Hg and burned the amalgams in open pans. Over half (57%) reported storing flasks with Hg at home and/or at work, and 63% reported keeping their work clothes at home (63%). Among nonminers, 33% previously worked as a miner and reported direct contact with elemental Hg, and 17% reported burning amalgams in open pans.

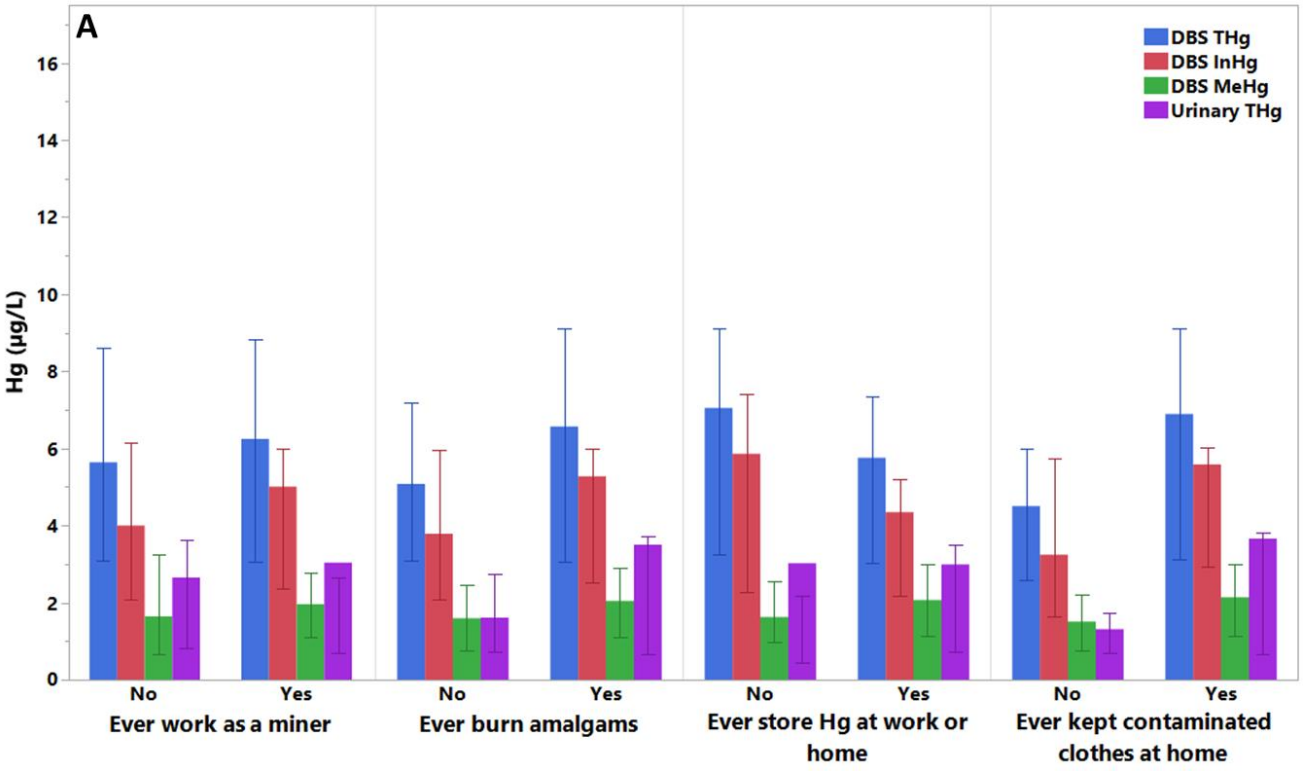
We did not observe large differences in exposure between the key comparison groups. When biomarker concentrations were compared with survey responses (Figure S4-4 and Table S4-4 of the Appendix), no significant associations were found between Hg concentrations in biomarkers and responses. Regardless of the lack of statistically significant associations there were some notable trends in the data. For example, higher levels of DBS THg, InHg, and MeHg were found in people that answered "yes" to previously or currently worked as a miner, ever done amalgam burning, and ever kept clothes contaminated with Hg at home than people that answer "no". For urine THg, we found an almost two-fold difference between people that reported to work in amalgam burning ("yes" 3.5 ±5.1µg/L, "no" 1.6 ±1.2µg/L), and people that kept contaminated clothes at home ("yes" 3.6 ±5.1µg/L, "no" 1.3 ±1.1µg/L).

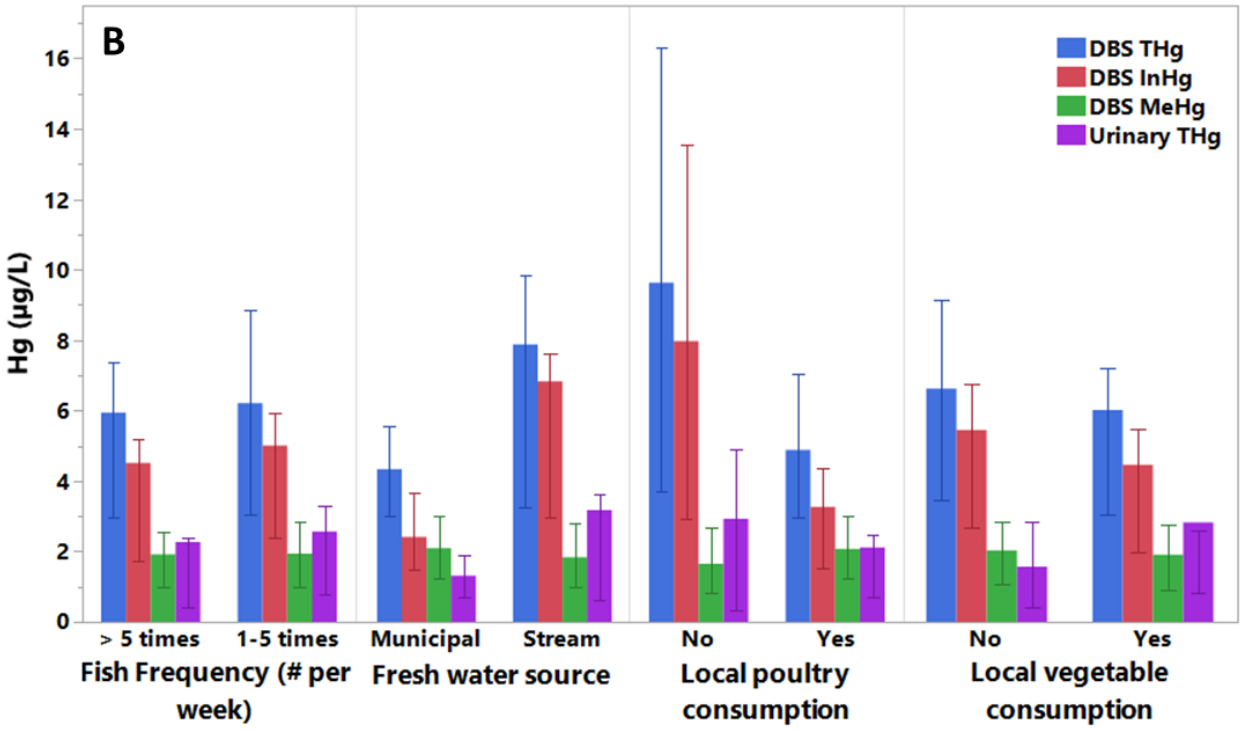
b Dietary habits questionnaire

Information on basic dietary habits was obtained from the health assessment questionnaire and summarized in Table S4-5 of the Appendix. All participants reported consuming fish as part of their diet, ranging from once per week to 21 times per week. Average fish consumption was higher among participants from Barranco de Loba (14 times per week) compared with those from Pueblito Mejia (9 times per week). All participants reported consumption of endemic fish, with Bocachico Prochilodus magdalenae (91%) being most commonly consumed followed by Bagre Tigre Pseudoplatystoma magdaleniatum (51 %), and Mojarra Caquetaia kraussii (37%). Fifteen participants reported obtaining drinking water from the public system and seventeen obtained it from the local ravine. All the participants reported consuming local beef, 66% reported consuming domestic poultry, and 60% percent reported consuming local vegetables and fruits.

Associations between the Hg biomarker results, and dietary habits are presented in Figure 4-4B. There were no significant associations between self-reported fish consumption and Hg concentrations in DBS or urine. When other dietary habits were related to biomarkers, there was a significant difference in DBS THg and DBS InHg concentrations with the source of drinking water and consumption of local poultry.

Figure 4-4. Concentrations of DBS total mercury (THg), inorganic mercury (InHg), and methylmercury (MeHg) and THg in urine in relationship with A) occupational activities, and B) dietary habits. Bars represent median values with the interquartile range (25th percentile, 75th percentile) indicated as error bars.





c Mercury intoxication

Because age is a strong confounding variable for the tested neurological symptoms, five participants older than 59 years were excluded from this analysis (Bose-O'Reilly et al., 2010a). Responses to the section of the questionnaire related to health symptoms were obtained by a health professional, and the results of the neuropsychological test were analyzed by a neuropsychologist.

Results from the health surveys are presented in Table S4-6 of the Appendix. No associations of statistical significance were found between Hg exposure biomarkers and signs of Hg intoxication in this study population though some expected trends were observed. For example, a higher median DBS THg was found in participants who self-reported a metallic taste, finger to nose tremors, eyelid tremors, and orientation problems. For DBS InHg, higher median concentrations were reported for people who self-reported a metallic taste, finger to nose tremors, and eyelid tremors.

Median DBS MeHg were higher for participants who self-reported a metallic taste and finger to nose tremors, and finally for urinary THg difference were found for people who reported metallic taste.

Self-reported responses for metallic taste, excessive salivation, tremors at work, sleeping problems at night, and health problems worsening since exposure to Hg were more prominent for miners (average positive responses 72%) than nonminers (27%). Three miners (one from Barranco de Loba and two from Pueblito Mejia) were positive for the finger to nose tremor test, and of those three miners, two had DBS THg above the HBM I threshold limit. For eyelid tremors, seven miners and two nonminer presented symptoms of which five had DBS THg above the HBM I threshold limit. For the neuropsychological tests, a significant difference was found by residence but not by occupation. People living in Pueblito Mejia had more positive results for the tests than people living in Barranco de Loba.

None of the participants presented a medical score above 10, with an average of 3 and a range of 0-9. Most had a score between 0 and 4 (n=24, 80%) and most of these were miners from Pueblito Mejia. There was no difference in Hg biomarker values between those with lower and higher medical score sums. The results of the UNIDO algorithm that combines the information from the medical tests and biological markers estimated no intoxication in any of the study participants.

4.5 Discussion

To our knowledge, this is the first study to use DBS to characterize exposure to chemical species of Hg in an ASGM community. We were able to quantify, with relatively good accuracy and precision, MeHg and InHg levels in blood samples in nearly all of the 35 participants. By collecting DBS specimens from individuals from remote ASGM sites in Colombia, we demonstrate how these methods may help overcome barriers (e.g., sample collection, storage, and transportation logistics) associated with biomonitoring in resource-limited settings. Specifically, biological samples were collected without the need of a phlebotomist, and sample transportation and storage was relatively easy due to the small space required by DBS samples and the elimination of cold chain transport. In our discussion with study participants, their acceptability of the sample collection method was high since the use of finger-prick blood sampling in the area is routinely used for malaria surveillance (World Health Organization (WHO), 2015). In past studies, Hg concentrations in DBS were shown to be stable under varying storage conditions (Chaudhuri et al., 2009; Perkins and Basu, 2018; Santa-Rios et al., 2020).

In addition to demonstrating the feasibility of this DBS-based method, the application of this novel method to characterize Hg speciation in blood deepened our understanding of environmental and occupational exposures in a Colombian ASGM region. The work shows that Hg exposures were high for participants who not only worked but lived in the mine (Pueblito Mejia) but also in the nearby town (Barranco de Loba). Notably, many individuals in this nearby town have previously been involved in the mining sector, and there is active smelting operations in the town which can release Hg into the environment. The results also demonstrated that the THg in blood was not principally in the organic MeHg form (mean MeHg 40%) thus supporting the need for Hg speciation among individuals with high environmental and occupational exposures, such as those found in ASGM communities, to avoid overestimation when using blood THg as a proxy of MeHg exposure.

4.5.1 Mercury species in biomarkers

In the current study, the THg levels measured in the DBS (median 4.6 µg/L interquartile range (IQR): 2.9 – 8.7 µg/L) were similar to those previously reported by the Colombian Ministry of Health for people residing in the department of Bolivar (median blood THg 4.5 µg/L (IQR): 3.4 – 5.5 µg/L) (Ministerio de Salud y Protección Social, 2016), as well as a population living near an ASGM community in Bolivar (median blood THg 5.5 µg/L (IQR): 2.7–11.2 µg/L) (Díaz et al., 2018). Concentrations of InHg and THg in DBS were higher in participants living in the extraction area (Pueblito Mejia) compared with those living in the town (Barranco de Loba), independent of occupation, suggesting that elemental Hg exposure is elevated in the mining area. Median DBS THg levels in Pueblito Mejia were lower than the median THg concentrations in blood from a compilation of ASGM studies worldwide (THg= 10.9 µg/L) (Basu et al., 2018). These results of blood THg are similar to other studies where THg in whole blood can be as 1.5 fold higher for mining areas residents than for nearby control areas (Bose-O'Reilly et al., 2010d, 2010b; Drake et al., 2001; Drasch et al., 2001; Hurtado et al., 2006; Yard et al., 2012). Most of the population from Pueblito Mejia (68%) had DBS THg levels >HBM I as reported by previous studies in the area (Díaz et al., 2018), where 72% of the people living in the mining areas of Colombia presented blood THg levels above the HBMI I(Ministerio de Salud y Protección Social, 2016).

Our chemical speciation of Hg in blood samples demonstrated that the largest proportion of THg was comprised of InHg (averaged 60% of the THg), which is expected in a population with occupational exposures to Hg vapor (UN Environment Programme and World Health Organization (WHO), 2008). The relatively high percentage of InHg (of THg) in blood exemplifies a need to speciate Hg in blood sampled from ASGM workers and community members to better understand the contributions of both MeHg and InHg (more generally environmental and occupational, respectively) to overall exposures. In these communities, the measure of THg in blood (or DBS) does not serve as strong proxy for MeHg exposure, and in making such an assumption one may overestimate MeHg exposures from dietary habits such as fish consumption. Mercury speciation can give information of not only source and exposure pathways but also clues into possible health

outcomes. Exposure of adults to MeHg (through the consumption of contaminated food) is usually linked with central nervous system impacts while exposure to InHg (through inhalation in occupational settings) is usually linked with effects on the peripheral nervous system and kidneys (Clarkson et al., 2003). To be able to properly ascribe health outcomes in ASGM communities with Hg exposures, it is important to conduct exposure studies that differentiate chemical forms of Hg.

Exposure to elemental Hg is usually gauged by the presence of Hg in urine. Urine THg concentrations in our study (median value of 1.7 μg/L) were lower than those previously reported in a nearby ASGM community (8.2 μg/L; Díaz et al., 2018) and than values reported for the department of Bolivar (median 9.8 μg/L; Ministerio de Salud y Protección Social, 2016), though there was overlap in the ranges (e.g., <MDL - 19.5 μg/L in our study versus 0.4 – 47.5 μg/L from the aforementioned study concerning the department of Bolivar). Urine THg concentrations in our study were almost two-fold higher in participants from Pueblito Mejia than from Barranco de Loba, likely because the former is more directly exposed to mining activities.

4.5.2 Associations between Hg in biomarkers and occupational and dietary habits

There were no significant associations between participants' occupation (miners or nonminers) and Hg biomarker concentrations, though other ASGM studies have previously reported associations between these variables (Paruchuri et al., 2010; Rajaee et al., 2015a). The current study was not principally designed to characterize this relationship and the lack of association here could be due to the relatively small sample size coupled with the variable nature of the data collected using the survey instrument (e.g., self-reported measures and recall bias, transient nature of work activities). Nonetheless, similar to past studies here we found higher concentrations of THg (blood and urine) in people who burned amalgam and kept work clothes at home (Bose-O'Reilly et al., 2010e, 2010c; Yard et al., 2012).

No significant differences in DBS MeHg concentrations were found in relation to fish consumption and this is likely related to the simple dietary survey utilized here and the small sample size coupled with recall bias. Concentrations of DBS THg and InHg were elevated for people that obtained water from the local stream compared with the municipal system, and similar associations have been reported previously among ASGM community members in Colombia and Peru (Díaz et al., 2018; Yard et al., 2012). These results could be associated with high concentrations of Hg in the drinking water. For example, a study on the water quality of San Martin de Loba (located 16 km from Pueblito Mejia), reported water THg concentrations up to 73.2 µg/L which is much higher than the US EPA's maximum Hg contaminant level for drinking water of 2.0 µg/L (U.S. Environmental Protection Agency, 2009).

4.5.3 Mercury intoxication

In the current study no associations were found between Hg biomarker measures and potential health symptoms though past studies involving ASGM communities using similar survey instruments have found Hg-associated health (Doering et al., 2016). However, regardless of the Hg biomarkers we do note that people living in the mining site (Pueblito Mejia) had a higher recurrence of symptoms than residents from the town (Barranco de Loba). On a group level, such findings are similar to past studies using the same questionnaire that documented fewer symptoms in the control group versus the exposed group (Bose-O'Reilly et al., 2010a, 2010b; Drasch et al., 2001).

4.5.4 Limitations and advantages of the study

To our knowledge, this study is the first to report blood MeHg and InHg in an ASGM site using DBS. We demonstrated that DBS are particularly well suited for these remote locations, and in doing so we were able to increase the understanding of Hg exposures in vulnerable populations living in or near mining sites. Another strength of this study concerns the DBS method reported upon. In our previous study on Hg speciation in DBS, only 16% of the samples were above the MDL (Santa-Rios et al., 2020). While such a detection frequency was similar to values reported by large biomonitoring initiatives such as the US NHANES (e.g., in the 2015-2016 cycle they reported 13% of the measures were above the MDL) (National Health and Nutrition Examination Survey, 2018). However, in the current study involving a highly exposed population, we demonstrate that the DBS method was suitable for detecting a concentration in nearly all participant samples.

Our study is not without limitations. While a random sampling strategy was envisioned, challenges faced by our research team in accessing the remote site coupled with security concerns in the region drove our decision to pivot to a convenience sampling strategy. Such a decision does not impair the primary objective of the study, which was to determine if high-quality Hg speciation data could be obtained from DBS from individuals in these remote and rural ASGM communities. Our relatively small sample size of 35 participants limited our power for statistical analysis, though sufficient data was obtained to address the study's primary objective and the secondary objective (Hg exposure assessment) yielded new and unique data as detailed in the preceding paragraph.

4.6 Conclusion

To our knowledge, this is the first study to characterize Hg speciation in DBS from an ASGM community. We demonstrate that coupling minimally invasive DBS sampling with a simple health questionnaire, researchers and practitioners can improve their understanding of occupational and environmental Hg exposure in remote and resource limited ASGM communities. This approach will facilitate a greater number of exposure assessments in Colombia, the world's third largest source of Hg emissions from ASGM (Telmer and Veiga, 2009) and the country with the highest per capita Hg pollution levels (Cordy et al., 2011).

4.7 References

Agency for Toxic Substances and Disease Registry, 1999. Toxicological Profile for Mercury Department of Health and Human Services 676.

Akagi, H., Castillo, E.S., Cortes-Maramba, N., Francisco-Rivera, A.T., Timbang, T.D., 2000. Health assessment for mercury exposure among schoolchildren residing near a gold processing and refining plant in Apokon, Tagum, Davao del Norte, Philippines. Sci. Total Environ. 259, 31–43. https://doi.org/10.1016/S0048-9697(00)00547-7

Akagi, H., Malm, O., Branches, F.J.P., Kinjo, Y., Kashima, Y., Guimaraes, J.R.D., Oliveira, R.B., Haraguchi, K., Pfeiffer, W.C., Takizawa, Y., Kato, H., 1995. Human exposure to mercury due to goldmining in the Tapajos River Basin, Amazon, Brazil: speciation of mercury in human hair, blood and urine. Water. Air. Soil Pollut. 80, 85–94.

Alcaldía Municipio Barranco de Loba, 2013. Mantenimiento y mejoramiento de la via que comunica al corregimiento de Pueblito mejia, Vereda Pueblo Bello y Pital con el municipio.

Basu, N., Clarke, E., Green, A., Calys-Tagoe, B., Chan, L., Dzodzomenyo, M., Fobil, J., Long, R., Neitzel, R., Obiri, S., Odei, E., Ovadje, L., Quansah, R., Rajaee, M., Wilson, M., 2015. Integrated Assessment of Artisanal and Small-Scale Gold Mining in Ghana—Part 1: Human Health Review. Int. J. Environ. Res. Public Health 12, 5143–5176. https://doi.org/10.3390/ijerph120505143

Basu, N., Eng, J.W.L., Perkins, M., Santa-Rios, A., Martincevic, G., Carlson, K., Neitzel, R.L., 2017. Development and application of a novel method to characterize methylmercury exposure in newborns using dried blood spots. Environ. Res. 159, 276–282. https://doi.org/10.1016/J.ENVRES.2017.08.021

Basu, N., Horvat, M., Evers, D.C., Zastenskaya, I., Weihe, P., Tempowski, J., 2018. A State-of-the-Science Review of Mercury Biomarkers in Human Populations Worldwide between 2000 and 2018. Environ. Health Perspect. 126, 106001. https://doi.org/10.1289/ehp3904

Bose-O'Reilly, S., Drasch, G., Beinhoff, C., Rodrigues-Filho, S., Roider, G., Lettmeier, B., Maydl, A., Maydl, S., Siebert, U., 2010a. Health assessment of artisanal gold miners in Indonesia. Sci. Total Environ. 408, 713–725. https://doi.org/10.1016/j.scitotenv.2009.10.070

Bose-O'Reilly, S., Drasch, G., Beinhoff, C., Tesha, A., Drasch, K., Roider, G., Taylor, H., Appleton, D., Siebert, U., 2010b. Health assessment of artisanal gold miners in Tanzania. Sci. Total Environ. 408, 796–805. https://doi.org/10.1016/j.scitotenv.2009.10.051

Bradley, M., Barst, B., Basu, N., 2017. A Review of Mercury Bioavailability in Humans and Fish. Int. J. Environ. Res. Public Health 14, 169. https://doi.org/10.3390/ijerph14020169

Chaudhuri, S.N., Butala, S.J.M., Ball, R.W., Braniff, C.T., 2009. Pilot study for utilization of dried blood spots for screening of lead, mercury and cadmium in newborns. J. Expo. Sci. Environ. Epidemiol. 19, 298–316. https://doi.org/10.1038/jesee.2008.19

Clarkson, T.W., Magos, L., 2006. The Toxicology of Mercury and Its Chemical Compounds. Crit. Rev. Toxicol. 36, 609–662. https://doi.org/10.1080/10408440600845619

Clarkson, T.W., Magos, L., Myers, G.J., 2003. The toxicology of mercury current exposures and clinical manifestations. N. Engl. J. Med. 349, 1731–1737. https://doi.org/10.1056/NEJMra022471

Clifford, M.J., 2014. Future strategies for tackling mercury pollution in the artisanal gold mining sector: Making the Minamata Convention work. Futures 62, 106–112. https://doi.org/10.1016/j.futures.2014.05.001

Cordy, P., Veiga, M.M., Salih, I., Al-Saadi, S., Console, S., Garcia, O., Mesa, L.A., Velásquez-López, P.C., Roeser, M., 2011. Mercury contamination from artisanal gold mining in Antioquia, Colombia: The world's highest per capita mercury pollution. Sci. Total Environ. 410–411, 154–160. https://doi.org/10.1016/j.scitotenv.2011.09.006

Díaz, S.M., Muñoz-Guerrero, M.N., Palma-Parra, M., Becerra-Arias, C., Fernández-Niño, J.A., 2018. Exposure to Mercury in Workers and the Population Surrounding Gold Mining Areas in the Mojana Region, Colombia. Int. J. Environ. Res. Public Health 15. https://doi.org/10.3390/IJERPH15112337

Doering, S., Bose-O'reilly, S., Berger, U., 2016. Essential Indicators Identifying Chronic Inorganic Mercury Intoxication: Pooled Analysis across Multiple Cross-Sectional Studies. https://doi.org/10.1371/journal.pone.0160323

Drake, P.L., Rojas, M., Reh, C.M., Mueller, C.A., Jenkins, F.M., 2001. Occupational exposure to airborne mercury during gold mining operations near El Callao, Venezuela. Int. Arch. Occup. Environ. Health 74, 206–212. https://doi.org/10.1007/s004200000206

Drasch, G., Böse-O'Reilly, S., Beinhoff, C., Roider, G., Maydl, S., 2001. The Mt. Diwata study on the Philippines 1999--assessing mercury intoxication of the population by small scale gold mining. Sci. Total Environ. 267, 151–68. https://doi.org/10.1016/S0048-9697(00)00806-8

Edelbroek, P.M., van der Heijden, J., Stolk, L.M.L., 2009. Dried blood spot methods in therapeutic drug monitoring: methods, assays, and pitfalls. Ther. Drug Monit. 31, 327–36.

Faial, K., Deus, R., Deus, S., Neves, R., Jesus, I., Santos, E., Alves, C.N., Brasil, D., 2015. Mercury levels assessment in hair of riverside inhabitants of the Tapajós River, Pará State, Amazon, Brazil: fish consumption as a possible route of exposure. J. Trace Elem. Med. Biol. 30, 66–76. https://doi.org/10.1016/j.jtemb.2014.10.009

Funk, W.E., 2015. Use of Dried Blood Spots for Estimating Children's Exposures to Heavy Metals in Epidemiological Research. J. Environ. Anal. Toxicol. s7. https://doi.org/10.4172/2161-0525.S7-002

Funk, W.E., McGee, J.K., Olshan, A.F., Ghio, A.J., 2013. Quantification of arsenic, lead, mercury and cadmium in newborn dried blood spots. Biomarkers 18, 174–7. https://doi.org/10.3109/1354750X.2012.750379

Guimaraes, J.R.D., Betancourt, O., Miranda, M.R., Barriga, R., Cueva, E., Betancourt, S., 2011. Long-range effect of cyanide on mercury methylation in a gold mining area in southern Ecuador. Sci. Total Environ. 409, 5026–33. https://doi.org/10.1016/j.scitotenv.2011.08.021

Ha, E., Basu, N., Bose-O 'reilly, S., Dórea, J.G., Mcsorley, E., Sakamoto, M., Chan, H.M., 2016. Current progress on understanding the impact of mercury on human health. Environ. Res. 1–15. https://doi.org/10.1016/j.envres.2016.06.042

Horvat, M., Snoj Tratnik, J., Miklavčič, A., 2012. Mercury: Biomarkers of Exposure and Human Biomonitoring, Biomarkers and Human Biomonitoring Vol.1: Ongoing Programs and Exposures.

Hurtado, J., Gonzales, G.F., Steenland, K., 2006. Mercury exposures in informal gold miners and relatives in southern Peru. Int. J. Occup. Environ. Health 12, 340–345. https://doi.org/10.1179/oeh.2006.12.4.340

Lee, M.S., Li, W., 2014. Dried blood spots : applications and techniques.

Malm, O., Castro, M.B., Bastos, W.R., Branches, F.J.P., Guimarães, J.R.D., Zuffo, C.E., Pfeiffer, W.C., 1995. An assessment of Hg pollution in different goldmining areas, Amazon Brazil. Sci. Total Environ. 175, 127–140. https://doi.org/10.1016/0048-9697(95)04909-6

McDade, T., Williams, S., Snodgrass, J., 2007. What a drop can do: Dried blood spots as minimally invasive method for integrating biomarkers into population-based research. Demography 44, 899–925.

Ministerio de Minas y Energia, 2012. Por la cual se delimita el Area de Reserva Especial Pueblito Mejia, ubicada en el Municipio de San Martin de Loba, Departamento de Bolivar. Republica de Colombia.

Ministerio de Minas y Energía, Universidad de Córdoba, 2014. Estudio de la cadena del mercurio en Colombia con énfasis en la actividad minera de oro. Bogota.

Ministerio de Salud y Protección Social, 2016. Informe Quincenal Epidemilógico Nacional. Vigilancia epidemiológica y evaluación del impacto en salud por exposición ocupacional y ambiental a mercurio en los departamentos de la zona de la Mojana, Colombia, 2014 - 2015., Iqen.

National Health and Nutrition Examination Survey, 2018. NHANES 2015-2016: Mercury - Inorganic, Ethyl & Methyl - Blood Data Documentation, Codebook, and Frequencies [WWW Document].

Nelson, J.W., Edhlund, B.L., Johnson, J., Rosebush, C.E., Holmquist, Z.S., Swan, S.H., Nguyen, R.H.N., 2016. Assessing a new method for measuring fetal exposure to mercury: Newborn bloodspots. Int. J. Environ. Res. Public Health 13. https://doi.org/10.3390/ijerph13070692

Nyanza, E.C., Bernier, F.P., Manyama, M., Hatfield, J., Martin, J.W., Dewey, D., 2019a. Maternal exposure to arsenic and mercury in small-scale gold mining areas of Northern Tanzania. Environ. Res. 173, 432–442. https://doi.org/10.1016/j.envres.2019.03.031

Nyanza, E.C., Dewey, D., Bernier, F., Manyama, M., Hatfield, J., Martin, J.W., 2019b. Validation of Dried Blood Spots for Maternal Biomonitoring of Nonessential Elements in an Artisanal

and Small-Scale Gold Mining Area of Tanzania. Environ. Toxicol. Chem. 38, 1285–1293. https://doi.org/10.1002/etc.4420

Obrist, D., Kirk, J.L., Zhang, L., Sunderland, E.M., Jiskra, M., Selin, N.E., 2018. A review of global environmental mercury processes in response to human and natural perturbations: Changes of emissions, climate, and land use. Ambio 47, 116–140. https://doi.org/10.1007/s13280-017-1004-9

Olivero-Verbel, J., Young-Castro, F., Caballero-Gallardo, K., 2014. Contaminación por mercurio en aire del distrito minero de San Martín de Loba en el departamento de Bolívar, Colombia. Rev. Int. Contam. Ambient. 30, 07–13.

Paruchuri, Y., Siuniak, A., Johnson, N., Levin, E., Mitchell, K., Goodrich, J.M., Renne, E.P., Basu, N., 2010. Occupational and environmental mercury exposure among small-scale gold miners in the Talensi-Nabdam District of Ghana's Upper East region. Sci. Total Environ. 408, 6079–6085. https://doi.org/10.1016/j.scitotenv.2010.08.022

Perkins, M., Basu, N., 2018. Dried blood spots for estimating mercury exposure in birds. Environ. Pollut. 236, 236–246. https://doi.org/10.1016/J.ENVPOL.2018.01.036

Rajaee, M., Long, R.N., Renne, E.P., Basu, N., 2015a. Mercury Exposure Assessment and Spatial Distribution in A Ghanaian Small-Scale Gold Mining Community. Int. J. Environ. Res. Public Health 12, 10755–82. https://doi.org/10.3390/ijerph120910755

Rajaee, M., Obiri, S., Green, A., Long, R., Cobbina, S.J., Nartey, V., Buck, D., Antwi, E., Basu, N., 2015b. Integrated Assessment of Artisanal and Small-Scale Gold Mining In Ghana Part 2: Natural Sciences Review. Int. J. Environ. Res. Public Health 12, 8971–9011. https://doi.org/10.3390/ijerph120808971

Santa-Rios, A., Barst, B.D., Basu, N., 2020. Mercury Speciation in Whole Blood and Dried Blood Spots from Capillary and Venous Sources. Anal. Chem. https://doi.org/10.1021/acs.analchem.9b04407

Schulz, C., Wilhelm, M., Heudorf, U., Kolossa-Gehring, M., 2011. Update of the reference and HBM values derived by the German Human Biomonitoring Commission. Int. J. Hyg. Environ. Health 215, 26–35. https://doi.org/10.1016/j.ijheh.2011.06.007

Sharma, A., Jaiswal, S., Shukla, M., Lal, J., 2014. Dried blood spots: Concepts, present status, and future perspectives in bioanalysis. Drug Test. Anal. 6, 399–414. https://doi.org/10.1002/dta.1646

Sherman, L.S., Blum, J.D., Basu, N., Rajaee, M., Evers, D.C., Buck, D.G., Petrlik, J., DiGangi, J., 2015. Assessment of mercury exposure among small-scale gold miners using mercury stable isotopes. Environ. Res. 137, 226–34. https://doi.org/10.1016/j.envres.2014.12.021

Telmer, K.H., Veiga, M.M., 2009. Mercury Fate and Transport in the Global Atmosphere, Mercury Fate and Transport in the Global Atmosphere: Emissions, Measurements and Models. Springer US, Boston, MA. https://doi.org/10.1007/978-0-387-93958-2

U.S. Environmental Protection Agency, 2009. National Primary Drinking Water Regulations.

U.S. Environmental Protection Agency, 2002. Method 1631, Revision E: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry Method 1631, Revision E: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry Method 1631, Revision E.

U.S. Environmental Protection Agency, 1998. Method 1630: Methyl Mercury in Water by Distillation, Aqueous Ethylation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry.

UN Environment Programme, 2019. Global Mercury Assessment 2018. Geneva, Switzerland.

UN Environment Programme, 2013. Global Mercury Assessment 2013: Sources, Emissions, Releases, and Environmental Transport, Narayana Press.

UN Environment Programme, 2012. Reducing Mercury Use in Artisanal and Small-Scale 76 pages.

UN Environment Programme, World Health Organization (WHO), 2008. Guidance for Identifying Populations At Risk From Mercury Exposure. Exposure 176.

USAID, 2012. Biomarker field manual: Demographic and Health Surveys Methodology. Calverton, Maryland.

Veiga, M.M., Baker, R., 2004. Protocols for Environmental and Health Assessment of Mercury Released by Artisanal and Small-Scale Gold Miners, Unido.

Wagner, L., 2016. Organized Crime and Illegally Mined Gold in Latin America. Geneva.

World Health Organization (WHO), 2019. GEMS/Food Contaminants Mercury [WWW Document]. URL https://extranet.who.int/gemsfood/Search.aspx?Contaminant=mercury (accessed 4.6.20).

World Health Organization (WHO), 2015. Guidelines for the Treatment of Malaria. 3rd edition. Geneva: World Health Organization; 2015. Annex 4, Grading of Recommendations Assessment, Development and Evaluation (GRADE) for assessing the quality of evidence., in: Guidelines for the Treatment of Malaria. 3rd Edition. World Health Organization.

World Health Organization (WHO), 2000. Air Quality Guidelines for Europe Second Edition.

Yard, E.E., Horton, J., Schier, J.G., Caldwell, K., Sanchez, C., Lewis, L., Gastañaga, C., 2012. Mercury Exposure Among Artisanal Gold Miners in Madre de Dios, Peru: A Cross-sectional Study. J. Med. Toxicol. 8, 441–448. https://doi.org/10.1007/s13181-012-0252-0

4.8 Supporting Information

Table S 4-1. Parameters to test the medical score sum from Veiga MM, Baker RF. Protocols for environmental and health assessment of mercury released by artisanal and small-scale miners. Global Mercury Project. 2004

Test	Score Points
Anamnestic data	
Metallic taste	0/1
Excessive salivation	0/1
Tremor at work	0/1
Sleeping problems at night	0/1
Health problems worsened since Hg exposed	0/1
Clinical data	
Bluish coloration of the gingiva	0/1
Ataxia of gait	0/1
Finger to nose tremor	0/1
Dysdiadochokinesia	0/1
Heel to knee ataxia	0/1
Heel to knee tremor	0/1
Mento-labial-reflex	0/1
Proteinuria	0/1
Neuropsychological tests	
Memory test	0/1/2
Matchbox test	0/1/2
Frosting test	0/1/2
Pencil tapping test	0/1/2
Maximum	**21**

Table S 4-2. Algorithm for the diagnosis of chronic mercury intoxication: HBM = Human Bio-Monitoring (Commission Human-Biomonitoring of the Federal Environmental Agency Berlin, 1999), BAT = Biological Tolerance Value (German Research Foundation, 1999).

		Medical score sum		
		0-4	**5-9**	**10-21**
Hg in all bio-monitors	**<HBM I**	No intoxication	No intoxication	No intoxication
Hg in at least one bio-monitor	**>HBM I**	No intoxication	No intoxication	Intoxication
	>> HBM II	No intoxication	Intoxication	Intoxication
	>BAT	Intoxication	Intoxication	Intoxication

Table S 4-3 Toxicologically established threshold limits for Hg in different biomarkers. HBM= Human Biomonitoring from the Commission Human-Biomonitoring of the Federal Environmental Agency Berlin, 1999 (Schulz et al., 2011).

Threshold limit	THg-urine (µg/L)	THg-blood (µg/L)
Below HBM I (low level)	0-7	0-5
Between HBM I and HBM II (alert level)	7-25	5-15
Over HBM II (High level)	>25	>25

Table S 4-4. Results from the occupational exposure questionnaire and mean (SD) concentration of mercury species in biomarkers. For the entire study population ("All" columns), and then by occupation and residence.

Variables		Occupation		Residence		Mercury in biomarkers for all participants			
	All (n=35)	Miners (n=23)	Nonminers (n=12)	Pueblito Mejia (Mine) (n=24)	Barranco de Loba (Town) (n=11)	DBS THg (µg/L)	DBS InHg (µg/L)	DBS MeHg (µg/L)	Urine THg (µg/L)
Occupational exposure: continuous variables (mean (range)), non-continuous (count (percentage))									
Years living in the area									
	22 (2-88)	19 (2-59)	30 (4-88)	23 (2-88)	21 (4-41)				
Ever worked as a miner?									
Yes	30 (86%)	23 (100%)	7 (58%)	20 (83%)	10 (91%)	6.2 ±4.9	5.0 ±4.6	2.0 ±1.2	3.0 ±4.6
No	4 (11%)	-	4 (33%)	4 (17%)	-	5.6 ±2.9	4.0 ±2.2	1.6 ±1.6	2.6 ±1.6
Years working as a miner									
	14 (2-36)	14 (2-36)	17 (10-25)	14 (2-36)	14 (6-25)				
Have you ever worked in direct contact with Hg?									
Yes	27 (77%)	23 (100%)	4 (33%)	19 (79%)	8 (73%)	6.3 ±5.1	5.1 ±4.8	2.0 ±1.2	3.3 ±4.9
No	8 (23%)	-	8 (67%)	5 (21%)	3 (27%)	5.4 ±6.3	3.8 ±2.3	1.5 ±1.3	1.6 ±1.4
Years working with Hg									
	14 (2-36)	14 (2-36)	8 (7-10)	15 (2-36)	11 (6-17)				

Have you ever burned amalgams in open pans?									
Yes	25 (71%)	23 (100%)	2 (17%)	19 (79%)	6 (55%)	6.6 ±5.1	5.3 ±4.9	2.0 ±1.3	3.5 ±5.1
No	9 (26%)	-	9 (75%)	5 (21%)	4 (36%)	5.1 ±2.7	3.8 ±2.2	1.6 ±1.0	1.6 ±1.2
Years working burning amalgams in open pans									
	14 (2-36)	14 (2-36)	8 (6-10)	14 (2-36)	12 (6-17)				
Have you ever stored mercury flasks at home or work?									
Yes	23 (66%)	20 (57%)	3 (25%)	16 (67%)	7 (64%)	5.8 ±4.2	4.3 ±3.7	2.1 ±1.3	3.0 ±3.7
No	11 (31%)	3 (9%)	7 (58%)	8 (33%)	3 (27%)	7.1 ±5.8	5.9 ±5.4	1.5 ±0.8	3.0 ±5.8
Have you kept your dirty work clothes at home?									
Yes	24 (69%)	22 (63%)	2 (17%)	18 (75%)	6 (55%)	6.9 ±4.5	5.6 ±4.9	2.1 ±1.3	3.6 ±5.1
No	9 (26%)	1 (3%)	8 (67%)	5 (21%)	4 (36%)	4.5 ±2.7	3.2 ±2.6	1.5 ±1.3	1.3 ±1.0

Table S 4-5. Results from the dietary habits exposure questionnaire and mean (SD) concentration of mercury species in biomarkers. For the entire study population ("All" columns), and then separated into the two sites.

Variables	Residence			Mercury in biomarkers for all participants			
	All (n=35)	Pueblito Mejia (Mine) (n=24)	Barranco de Loba (Town) (n=11)	DBS THg (µg/L)	DBS InHg (µg/L)	DBS MeHg (µg/L)	Urine THg (µg/L)
Dietary habits exposure: continuous variables (mean (range)), non-continuous (count (percentage))							
How frequently do you eat fish? (times per week)	11 (1-21)	9 (1-21)	14 (1-21)				
How many grams per serving?	321 (200-700)	348 (200-700)	280 (200-700)				
Fish name							
Bocachico *Prochilodus magdalenae*	32 (91%)	22 (92%)	10 (91%)				
Bagre *Pseudoplatystoma magdaleniatum*	18 (51%)	12 (50%)	6 (55%)				
Mojarra *Catfi Caquetaia kraussii*	13 (37%)	8 (33%)	5 (45%)				
Do you know where the fish come from?							
Areas distant from mining	6 (17%)	5 (21%)	1 (9%)	7.5 ±6.7	5.2 ±6.2	2.3 ±1.1	2.4 ±1.3
Areas affected by mining	17 (49%)	12 (50%)	5 (45%)	6.1 ±5.3	4.6 ±4.8	1.5 ±1.0	2.4 ±1.3
No	5 (14%)	4 (17%)	1 (9%)	3.9 ±1.4	1.7 ±1.6	2.2 ±1.3	1.8 ±1.0

Does the river have dark water (Coca-Cola color)?							
Yes	18 (51%)	11 (46%)	7 (64%)	6.3 ±5.0	4.5 ±4.4	1.8 ±1.4	2.6 ±3.9
No	10 (29%)	7 (29%)	3 (27%)	5.0 ±2.8	2.8 ±2.5	2.2 ±1.1	2.4 ±1.0
Name the place where you obtain drinking water							
Municipal system	15 (89%)	7 (29%)	8 (73%)	4.2 ±2.3	2.1 ±1.6	2.1 ±1.5	1.3 ±0.9
Local stream	16 (18%)	14 (58%)	2 (18%)	7.8 ±5.6	6.0 ±5.3	1.8 ±1.1	3.4 ±4.2
Do you consume local chicken, ducks, or eggs?							
Yes	23 (66%)	13 (54%)	10 (91%)	4.8 ±2.8	2.7 ±2.3	2.1 ±1.3	2.1 ±3.2
No	9 (26%)	9 (37%)	-	9.6 ±7.1	8.0 ±6.3	1.6 ±1.1	3.3 ±3.6
Do you consume local beef and pork?							
Yes	31 (88%)	21 (87%)	10 (91%)	5.9 ±4.7	4.0 ±4.3	1.9 ±1.3	2.3 ±3.3
Do you consume local vegetables and fruit?							
Yes	21 (60%)	11 (46%)	10 (91%)	5.9 ±4.9	4.0 ±4.4	1.9 ±1.4	2.8 ±3.9
No	11 (31%)	11 (46%)	-	6.5 ±5.1	4.5 ±4.7	2.0 ±1.1	1.7 ±1.4

Table S 4-6. Results from the health symptoms of exposure questionnaire and mean (SD) concentration of mercury species in biomarkers. Results of symptoms for the entire study population ("All" columns), and then by occupation and residence.

	Occupation			Residence		Mercury in biomarkers for all participants			
Test	Total (n=30)	Miner (n=22)	Nonminer (n=8)	Pueblito Mejia (Mine) (n=20)	Barranco de Loba (town) (n=10)	DBS THg (µg/L)	DBS InHg (µg/L)	DBS MeHg (µg/L)	Urine THg (µg/L)
Responses to health symptoms of exposure: continuous variables (mean (range)), non-continuous (count (percentage))									
Anamnestic data									
Metallic taste									
Yes	5 (17%)	3 (14%)	2 (25%)	4 (20%)	1 (10%)	7.6 ±4.1	4.9 ±3.6	2.7 ±0.9	2.7 ±2.5
No	25 (83%)	19 (86%)	6 (75%)	16 (80%)	9 (90%)	5.8 ±5.2	3.9 ±4.8	1.8 ±1.3	2.7 ±4.4
Excessive salivation									
Yes	7 (23%)	5 (23%)	2 (25%)	3 (15%)	4 (40%)	4.2 ±2.3	2.8 ±2.1	1.4 ±0.8	1.7 ±1.5
No	23 (77%)	17 (77%)	6 (75%)	17 (85%)	6 (60%)	6.7 ±5.5	4.6 ±5.1	2.1 ±1.3	3.0 ±4.6
Tremor at work									
Yes	4 (13%)	3 (14%)	1 (13%)	2 (10%)	2 (20%)	5.5 ±3.3	3.1 ±2.3	2.4 ±2.4	1.2 ±.07
No	26 (87%)	19 (86%)	7 (88%)	18 (90%)	8 (80%)	6.2 ±5.3	4.3 ±4.9	1.9 ±1.0	3.0 ±4.4
Sleeping problems at night									

Yes	6 (20%)	5 (23%)	1 (13%)	6 (30%)	0 (0%)	4.6 ±2.7	3.1 ±2.1	1.5 ±1.3	2.1 ±1.6
No	24 (80%)	17 (77%)	7 (88%)	14 (70%)	10 (100%)	6.5 ±5.5	4.4 ±5.1	2.1 ±1.2	2.9 ±4.6
Health problems worsened									
Yes	7 (23%)	5 (23%)	2 (25%)	6 (30%)	1 (10%)	4.9 ±2.6	2.7 ±2.2	2.2 ±1.4	2.3 ±1.6
No	23 (77%)	17 (77%)	6 (75%)	14 (70%)	9 (90%)	6.5 ±5.6	4.6 ±5.1	1.9 ±1.2	2.8 ±4.5
Clinical data									
Bluish coloration of gingiva									
Yes	-	-	-	-	-	-	-	-	-
No	30 (100%)	22 (100%)	8 (100%)	20 (100%)	10 (100%)	6.1 ±5.1	4.1 ±4.6	2.0 ±1.3	2.7 ±4.1
Ataxia of gait									
Yes	1 (3%)	-	1 (13%)	-	1 (10%)	2.2 ±0	-	2.2±0	1.7 ±0
No	29 (97%)	22(100%)	7 (88%)	20 (100%)	9 (90%)	6.3 ±5.1	4.3 ±4.6	2.0 ±1.3	2.8 ±4.2
Finger to nose tremor									
Yes	3 (10%)	3 (14%)	-	2 (10%)	1 (10%)	6.6 ±2.9	4.1 ±1.3	2.5 ±3.0	1.0 ±0.8
No	27 (90%)	19 (86%)	8 (100%)	18 (90%)	9 (90%)	6.1 ±5.3	4.1 ±4.9	1.9 ±1.0	3.0 ±4.3
Dysdiadochokinesia									
Yes	-	-	-	-	-	-	-	-	-
No	30 (100%)	22 (100%)	8(100%)	20 (100%)	10 (100%)	6.1 ±5.1	4.1 ±4.6	2.0 ±1.3	2.7 ±4.1
Tremors eyelid									

Yes	9 (30%)	7 (32%)	2 (25%)	6 (30%)	3 (30%)	6.7 ±5.4	4.5 ±4.8	2.2 ±1.7	1.8 ±1.6
No	21 (70%)	15 (68%)	6 (75%)	14 (70%)	7 (70%)	5.8 ±5.0	4.0 ±4.6	1.8 ±1.0	3.0 ±4.6
Dysmetria									
Yes	-	-	-	-	-	-	-	-	-
No	30 (100%)	22 (100%)	8 (100%)	20 (100%)	10 (100%)	6.1 ±5.1	4.1 ±4.6	2.0 ±1.3	2.7 ±4.1
Neuropsychological tests									
Memory test									
Yes	-	-	-	-	-	-	-	-	-
No	30 (100%)	22 (100%)	8 (100%)	20 (100%)	10 (100%)	6.1 ±5.1	4.1 ±4.6	2.0 ±1.3	2.7 ±4.1
Orientation									
Yes	4 (13%)	3 (14%)	1 (13%)	2 (10%)	2 (20%)	7.2 ±5.5	4.0 ±4.8	3.2 ±1.8	2.5±2.5
No	26 (87%)	19 (86%)	7 (87%)	18 (90%)	8 (80%)	5.9 ±5.1	4.1 ±4.7	1.8 ±1.0	2.7 ±4.4
Frosting test									
Yes	6(20%)	5 (23%)	1 (13%)	2 (10%)	4 (40%)	3.1 ±1.3	1.7 ±1.5	1.4 ±0.5	0.8 ±1.0
No	24 (80%)	17 (77%)	7 (88%)	18 (90%)	6 (60%)	6.9 ±5.4	4.8 ±5.0	2.1 ±1.4	3.3 ±4.5
Pencil tapping test									
Yes	10 (33%)	7 (32%)	3 (38%)	7 (35%)	3 (30%)	5.7 ±4.0	3.3 ±3.2	2.4 ±1.6	1.6 ±2.0
No	20 (67%)	15 (68%)	5 (63%)	13 (65%)	7 (70%)	6.3 ±5.6	4.5 ±5.2	1.8 ±1.1	3.4 ±4.9
Medical score sum									
0-4	24 (80%)	17 (77%)	7 (88%)	15 (75%)	9 (90%)	6.3 ±5.4	4.4 ±5.1	1.9 ±1.0	3.0 ±4.6
5-9	6 (20%)	5 (23%)	1 (13%)	5 (25%)	1 (10%)	5.6 ±3.4	3.2 ±2.1	2.4 ±2.1	1.9 ±1.6

CONECTING PARAGRAPH

Over the last three chapters I developed and validated the use of DBS for MeHg assessment in the general population sampled in a clinical environment (Chapter 2), a population situated in a contaminated and resource-limited setting (Chapter 3), and for the simultaneous analysis of both MeHg and InHg in a population with relatively high exposures to different forms of Hg (Chapter 4). A major issue with DBS is the lack of knowledge of the volume of blood on the filter paper as well as uneven spreading driven by the hematocrit (Hct) effect. The studies in my thesis used a variety of techniques to measure Hg, including analysis of an entire DBS created with controlled volume to the use of a standardized 3mm punch as a subsampling method to avoid the hematocrit (Hct) bias. Notably in Chapter 3 I demonstrated that while there is a relatively good agreement between DBS sampled in the field (in which blood volumes are not known) and those created artificially in the lab using whole blood from the same individuals (in which DBS volumes can be controlled), there were slight biases recorded. In this final chapter, I aimed to create a non-destructive method to analyze sample volume in an entire DBS spot. This manuscript is co-authored by the candidate's supervisor Dr. Niladri Basu and Jessica Ewald and is planned for submission to Bioanalysis.

5. CHAPTER 5. THE APPLICATION AND VALIDATION OF A NON-DESTRUCTIVE METHOD TO ESTIMATE SAMPLE VOLUME ON DRIED BLOOD SPOTS FOR THE ANALYSIS OF MERCURY SPECIES

Andrea Santa-Rios, Jessica, Nil Basu

Faculty of Agricultural and Environmental Sciences, McGill University, Montreal, Quebec H9X 3V9, Canada

5.1 Abstract

Dried blood spots (DBS) are made with a drop of blood collected into a standardized filter paper. Advantages related to DBS sampling have a wide range of application in fields spanning clinical practice to drug discovery to public health. A limitation preventing wider adoption of DBS in many cases concerns the lack of knowledge of the blood volume sampled onto a card coupled with the hematocrit (Hct) effect in which variations in blood viscosity result in different spot sizes. A range of methods and devices have been proposed to overcome the Hct bias, including the analysis of the area of the DBS to estimate sample volume, knowledge gaps regarding the use of these methods include the use of Hct standards and the application for samples created under a variety of conditions. Here, to estimate sample volume we combine two approaches (quantify sample area and sample color), and then validate this method in two populations. We generated Hct DBS standards with different Hct values (30-60) and sample volumes (20-60µl) from nine volunteers, and then scanned the samples and calculated area and color codes (RGB) to create a regression model with an R^2=0.9. Method validation included samples from two populations:1. Samples (49) taken with controlled volume, 2. Samples (35) taken under field-conditions without any volume control). For the first population, we found high mean volume recovery 114 ± 9.4%, and high

association between methylmercury (MeHg) in DBS from the estimated volume and the gold standard (venous blood) (slope 0.93 ; 95% CI = 0.76−1.09; R^2 = 0.8). For the other population, we also found an association between samples from a standardized 3mm punch and entire spot samples with estimated sample volume for MeHg (slope 0.93; 95% CI = 0.81−1.08; R^2 = 0.9), but not for inorganic mercury (InHg) warranting the need for further investigation. In conclusion, our study was able to develop and validate a method for volume estimation that can be applied for the analysis of MeHg when the sample volume control is not possible.

5.2 Introduction

Dried blood spots (DBS) represent an alternative for classical venous blood sampling, The collection of a DBS sample involves the use of a micro-lancet to deliver a controlled puncture (USAID, 2012), and around five drops of blood of unknown volume (usually ~30-60 µL per drop) are collected onto the filter paper. In contrast to a venipuncture blood draw that generally yields at least 5 ml (i.e., a known and measurable volume) of whole blood (Mcdade, 2013). Dried blood spots are commonly used in newborn screening, therapeutic drug monitoring and has become popular in population studies for the quantification and identification of a wide range of analytes (Malsagova et al., 2020; Mcdade, 2013; Resano et al., 2018). Contrary to the whole blood samples, the blood to create DBS does not need to be centrifuged, separated, or frozen after collection (McDade et al., 2007). The low burden of sampling is also helpful for studies with infants, children, and the elderly (for which venipuncture can be particularly problematic), increasing the feasibility of collecting large numbers of samples in a community (Lee and Li, 2014). Accordingly, DBS offer a minimally invasive method that is conducive for field-based research in resource limiting settings as there is no need for specialized equipment, cold-chain handling, and phlebotomists.

Although DBS are a valuable alternative, hematocrit (Hct) related issues that result in an unknown volume of blood and unequal blood spreading remain a significant barrier for the acceptance of this sampling technique (Velghe et al., 2019). The Hct-based bias can be divided into Hct-area bias, Hct-based recovery bias, and Hct-based matrix effect bias (Abu-Rabie et al., 2015). The blood volume used to saturate the paper is proportional to the Hct level of the sample. This may result in changes in the viscosity of blood leading to differences in the diffusion of blood through the paper (Denniff and Spooner, 2010) . The spreadability factor is a potential source of error for an analyte concentration in a DBS (Mei et al., 2001; O'Broin et al., 1995). Previous studies have also found the heterogeneous distribution of metals and analytes within a DBS spot (Chaudhuri et al., 2008; Fan and Lee, 2012; Hempen et al., 2015; Lenk et al., 2015a; O'Mara et al., 2011; Vries et al., 2013). Studies on the homogeneity of the spots have found higher concentrations on the edge of the spot, which has been referred to as the "coffee-stain" effect. Therefore the location of a sub-sample of a DBS card can also bias the measurement (Lenk et al., 2015a; O'Mara et al., 2011).

Numerous strategies to solve hematocrit-related issues have been proposed and these have largely focused on three aspects: the control of sample volume, the control of the Hct-bias, and the estimation of sample volume. The majority of the studies have focused on the control of sample volume created with a device or technique (Table 5-1). Three main approaches have been reported to minimize the Hct-bias: 1. design of a special filter, or filter materials, such as the HemaSpot^{TM} -HF (Spot on Sciences, Austin, Texas) or the Qyntest cards (Qynion, Geelen, The Netherlands) (Mengerink et al., 2015; Spot on Sciences, 2020); 2. directly measuring, or estimating the Hct of the blood contained on a DBS by the use of destructive and non-destructive methods (Capiau et al., 2017, 2016; Liao et al., 2018; Miller IV, 2013; Richardson et al., 2018); and 3. the analysis of potassium as a marker of Hct (Bloem et al., 2018; Capiau et al., 2013; Den Burger et al., 2015; Rufail et al., 2017). Two main approaches have been reported to measure or estimate the volume of blood: 1. the use of specialized equipment by the analysis of the electoral conductivity (Kadjo et al., 2016), and liquid chromatography-electrospray ionization-mass spectrometry for the estimation of HCT markers (Liao et al., 2016); and 2. a non-destructive analysis of sample volume

by different techniques, including internal standard spray prior to analysis (Abu-Rabie et al., 2015), using images to calculate the area of the spot (Alsous et al., 2020; Dana F. El-Hajjar et al., 2007; Perkins and Basu, 2018). Some of these approaches involve the study of the area using ImageJ® software (image processing program, National Institute of Health, USA) and Visual Basic (Microsoft Corporation, USA).

Even though the studies mentioned above found important information related to non-destructive methods to calculate the volume of the spot, knowledge gaps remain. First, the regression model to determine the area of the spot needs to consider both the different Hct levels and the color intensity of the sample using Hct standards, and second, the regression model needs to be validated with real-world DBS samples.

Therefore, the present study aims to develop and validate a non-destructive method to quantify sample volume in an entire spot with a desktop scanner. This was realized using blood hematocrit standards and DBS collected under laboratory and field conditions from two different populations. We hypothesized that the color and area measurements of the DBS could be used as a non-destructive method to estimate sample volume of an entire spot, and further tested this hypothesis through the study of DBS samples (with both known and unknown volumes, and in some cases corresponding whole blood samples) from human population studies in which we previously measured concentrations of mercury (Hg) species (methylmercury MeHg and inorganic mercury InHg). For method development, we artificially created DBS from whole blood samples of nine volunteers to realize a range of hematocrit levels and blood spot volumes. We scanned the samples and calculated the area and color of the spot to create a simple multivariate model to quantify sample volume. To validate this method, we calculated the blood volume in DBS samples from two different populations in which we have previously measured Hg concentrations. First, we compared the model-computed blood volume with the actual spotted blood volume from DBS obtained from 49 individuals that were created under controlled conditions(Santa-Rios et al., 2020). In this group we evaluated differences in Hg species concentrations using the computed blood volume and the known blood volume, and also evaluated the DBS-based Hg concentrations

with values measured in corresponding venous whole blood. In a second population group, DBS samples (with no volume control) were obtained from 35 individuals collected under field conditions. In this group we calculated the blood volume and used this information to determine MeHg and InHg concentrations in samples which in turn were compared with the concentrations calculated using a default and assumed blood volume in a standard 3mm punch.

Table 5-1. **Devices and techniques to cope with the Hct issue, Data from (Velghe et al., 2019)**

Name	Pros	Contras	Reference
Anticoagulant-coated microcapillaries or accurate pipetting	Accurate and precise volume of blood	Need for experienced nurse or trained laboratory personnel	(De Kesel et al., 2013a)
HemaPen® (Trajan Scientific and Medical, Australia)	Dispense a fixed volume of 2.74 µL	Transfer velocity changes with Hct level No real-life applications	(Neto et al., 2018)
Mitra® (Neoteryx)	Collection of an accurate and precise volume (10, 20, 30 µL)	Variation of blood retained on the tips for device misuse	(Denniff and Spooner, 2014b; Neoteryx, 2019; Spooner et al., 2015)
VAMS™	Able to minimize the volumetric Hct-effect independently of the blood Hct	Does not overcomes the Hct-depended recovery	(Velghe et al., 2019)
Volumetric absorptive paper disk (VAPD) and mini disc (VAPDmini)	Accurate sample volume Analytical recovery Hct independent	Only applied to limited compounds	(Nakahara et al., 2018)
Capitainer-B (KTH, Stockholm, Sweden)	Fixed volume of 13.5 µL Analytical recovery Hct independent	No real-life applications	(Lenk et al., 2015b; Velghe and Stove, 2018)
HemaXis DB device (DBS System SA, Gland, Switzerland)	Fixed volume (5.5 to 10 µL) Analytical recovery Hct independent Easy for patient self-sampling	Not known	(Alexis Leuthold et al., 2015; Verplaetse and Henion, 2016)

5.3 Methods

5.3.1 Method development

For method development, approval from the McGill University Institutional Review Board (#A09-M31-17B) was obtained before sample collection. Two K_2EDTA tubes of whole venous blood were collected from consenting participants (n=9) by a trained health professional at the Mary Emily Clinical Nutrition Research Unit at McGill University. Samples with different hematocrit values (30, 40, 50, 55, 60%) were prepared by centrifuging whole blood and removing or adding a certain amount of plasma (Koster et al., 2015). Per individual, we created triplicate DBS with different blood volumes (20, 30, 40, 50, 60µL) and hematocrit values (30, 40, 50, 55, 60%) by spotting the sample onto Whatman 903 filter paper (GE Healthcare, Dassel, Germany) using a calibrated pipette. The spotted DBS was left out to dry for 4 hours at room temperature. Samples were scanned with a desktop scanner (CanoScan LiDE 90, Canon Canada Inc., Mississauga, ON, Canada) at 300 dpi between the first 48 hours of sample preparation. To avoid sample contamination within the scanner, we covered the dried DBS card with a translucid slide previously cleaned with 2% HNO_3. A MATLAB® based program was created to measure the area of the spot (in pixels) and the color codes (Red, Green, and Blue in nm).

5.3.2 Validation

For the validation step, we used two populations, with different sample collection techniques:

1. Capillary DBS samples of 49 consenting individuals from McGill University were collected with approval from the McGill University Institutional Review Board (#A09-M26-16B). Samples were collected following the USAID Biomarker Manual for DBS Collection (USAID, 2012). The

volume of the spot was controlled using a 40µl microcapillary tube (accuracy ± 0.5% Sigma Aldrich) and transferred to the DBS cards where they filled three distinct spots. The spotted DBS cards were left to dry at room temperature for 4 hours and were scanned between the first 48 hours after collection. Also, 7 mL of venipuncture whole blood was collected into a single trace metal free K2EDTA tube from each individual. For this population, mercury species (methylmercury MeHg and inorganic mercury InHg) were analyzed using the entire spot to avoid volume bias following the method previously described by our group (Santa-Rios et al., 2020).

2. We collected capillary DBS samples from 35 individuals from an artisanal and small-scale gold mining community in Colombia, after approval from the Research Ethics Board of the University of Cartagena (REB File #4341022017) and McGill University (REB File #127-0818). Samples were collected in a remote field setting, and thus were collected without volume control following the USAID Biomarker Manual for DBS Collection (USAID, 2012) and air-dried on-site. For this population, we analyzed the entire spot and three 3mm punches. The three punches were estimated to contain a total blood volume of 9.3 µL (Lee and Li, 2014). Here we compared the Hg species concentrations from a sample punch and the entire spot with the estimated volume from phase 1.

5.3.3 Data analysis

In the method develop phase a series of models were constructed. The primary multivariate linear regression model included all the variables for volume estimation: area (pixels), the colors (Red, Green, and Blue in nm), the sum of the RGB colors (nm), and the color intensity (Average RGB (nm) as follows:

$$Volume\ \mu l = area\ (pixels) + Red\ (nm) + Green\ (G) + Blue\ (B) + sum\ RGB\ (nm) + intensity\ (average\ RGB\ (nm))$$

We assessed the significance of the variables for the model according to the t-ratio and the p-value and evaluate the contribution to the models by single regression analysis. For the significant variables, we also identified the differences between hematocrit levels and the variables using the one-way Anova test. We created a second model with only the significant variables to estimate the sample volume.

For the second phase of the study (validation), we estimated the accuracy of the multivariate model by calculating the estimated volume accuracy (volume estimated/know volume), and the precision of the estimated volume as the coefficient of variation (CV) of sample triplicates per individual. For both populations, we measured MeHg and InHg concentrations in an entire spot using the estimated volume from the linear regression analysis. We compared the Hg species levels with the values obtained using a controlled volume entire spot, whole blood (for the first population), and a standardized 3mm punch (for the second population) using linear regression analysis.

5.4 Results and discussion

5.4.1 Phase 1. Method development

For the first phase, after DBS samples were prepared and scanned, we ran the MATLAB® code that calculated the area of the spot (pixels) and the RGB (nm) with key descriptive statistics provided in Table 5-2. Next, we identified the implications of different Hct levels with each of the variables (area and color). First, when comparing the Hct value with spot area (Figure 5-1A), significant differences were found according to a one-way ANOVA with lower Hct levels associated with higher spot areas. Such associations between Hct levels and spot areas are well documented, and reported as the Hct-area bias (Abu-Rabie et al., 2015; Denniff and Spooner, 2010; Vries et al., 2015).

Second, for the colors (RGB) and the color intensity (Figure 5-1B), we found a negative association with the hematocrit values. For specific colors, we calculated negative associations between Hct levels and the red color (R) (slope = -1.0, R^2 =0.49), the green color (G) (slope = -1.2, R^2 =0.46), and the blue color (B) (slope =1., R^2 =0.46) levels. The intensity of the color (Average RGB) also showed a negative association with the Hct (slope = -1.2, R^2 =0.49). Previous studies have also reported negative associations between the color intensity and the blood area in DBS samples (Dana F El-Hajjar et al., 2007).

The overall model fit of the first multivariate linear regression model was R^2=0.87, with the t-ratio and the p-value showing that the area and the intensity (average RGB) were the only significant ($p<0.001$) variables for estimating blood volume (Table 5-3).The final model had an association of R^2= 0.9, when using only the two significant variables (area and color intensity (RGB). Here, we applied both area and intensity for sample volume calculation. The predicted volume of blood in a DBS was calculated as follows:

Volume predicted µL = 41.24 + 0.008 Area (pixels) – 0.18 intensity (R,G,B) (nm)

Table 5-2. Descriptive statistics from method development

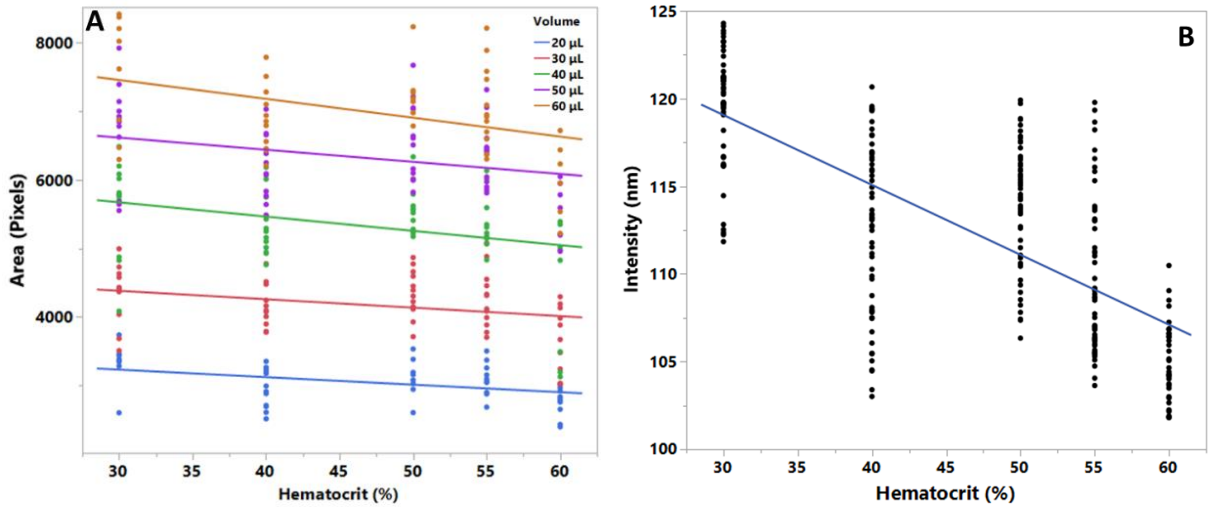
Hct	Volume (µl)	Area (pixels) (mean±SD)	R (nm) (mean±SD)	G (nm) (mean±SD)	B (nm) (mean±SD)	Intensity RGB (nm) (mean±SD)	Sum RGB(nm) (mean±SD)
30	20	3144.3 ±473.9	150.9 ±2.4	108.8 ±2.5	106.6 ±2.3	122.1 ±2.2	366.4 ±6.7
	30	4281.4 ±470.4	149.6 ±2.2	105.7 ±2.4	105.7 ±2.4	121.2 ±2.2	363.7 ±6.7
	40	5616.2 ±681.9	148.4 ±3.5	106.9 ±2.2	104.2 ±2.1	119.8 ±2.5	359.6 ±7.5
	50	6698.2 ±728.9	146.1 ±4.4	106.0 ±3.9	103.6 ±3.5	118.6 ±3.7	355.7 ±11.2
	60	7962.6 ±965.9	144.5 ±6.2	104.7 ±4.1	102.3 ±3.9	117.2 ±3.9	351.6 ±13.9
40	20	2918.8 ±281.6	139.7 ±6.4	101.6 ±4.6	100.1 ±4.0	113.8 ±5.0	341.5 ±15.0
	30	4157.7 ±302.3	140.3 ±7.0	102.2 ±4.8	100.5 ±4.2	114.3 ±5.2	343.0 ±15.6
	40	5317.1 ±446.8	138.8 ±6.7	99.6 ±4.8	97.9 ±4.3	112.1 ±5.2	336.3 ±15.6
	50	6113.1 ±499.8	139.3 ±5.9	100.7 ±4.6	98.9 ±4.0	113.0 ±4.7	338.9 ±14.1
	60	7017.8 ±620.4	136.7 ±4.4	98.0 ±2.9	96.2 ±2.5	110.3 ±3.2	331.0 ±9.6
50	20	3132.6 ±240.1	142.6 ±3.9	103.3 ±2.3	101.5 ±2.2	115.8 ±2.6	347.5 ±7.9
	30	4346.8 ±345.2	141.9 ±5.1	102.6 ±3.8	100.6 ±3.4	115.0 ±4.0	345.1 ±12.0
	40	5562.0 ±347.7	140.3 ±3.8	101.1 ±3.0	99.2 ±2.7	113.5 ±3.1	340.6 ±9.3
	50	6565.3 ±578.3	139.0 ±3.5	99.9 ±2.7	98.1 ±2.4	112.3 ±2.7	337.0 ±8.1
	60	7517.2 ±780.0	138.5 ±3.7	99.9 ±3.7	97.8 ±3.2	112.1 ±3.5	336.2 ±10.6

Hct	Volume (µl)	Area (pixels) (mean±SD)	R (nm) (mean±SD)	G (nm) (mean±SD)	B (nm) (mean±SD)	Intensity RGB (nm) (mean±SD)	Sum RGB(nm) (mean±SD)
55	20	3087.3 ±219.9	136.3 ±6.0	98.8 ±5.0	97.3 ±4.6	110.8 ±5.1	332.4 ±15.4
	30	4263.6 ±425.6	136.9 ±5.8	99.9 ±5.2	98.4 ±4.7	111.8 ±5.1	335.3 ±15.4
	40	5446.0 ±478.6	135.6 ±4.4	97.6 ±3.8	96.0 ±3.4	109.7 ±3.8	329.1 ±11.4
	50	6358.4 ±478.4	133.7 ±3.9	97.1 ±3.3	95.6 ±2.9	108.8 ±3.3	326.5 ±10.0
	60	7075.7 ±596.8	132.7 ±2.4	95.8 ±2.9	94.2 ±2.7	107.5 ±2.6	322.6 ±7.8
60	20	2610.9 ±391.6	129.9 ±2.8	92.9 ±3.0	92.1 ±3.1	105.0 ±2.9	314.9 ±8.8
	30	3488.5 ±815.3	129.9 ±2.0	93.9 ±2.7	93.1 ±2.6	105.6 ±2.2	316.9 ±6.7
	40	4509.3 ±949.9	129.3 ±1.0	92.3 ±1.6	91.5 ±1.7	104.4 ±1.4	313.2 ±4.2
	50	4891.7 ±1878.6	128.0 ±1.4	90.6 ±1.3	89.6 ±1.3	102.7 ±1.0	308.2 ±3.1
	60	6014.5 ±563.0	129.1 ±1.4	92.7 ±1.1	91.4 ±1.0	104.4 ±09	313.2 ±2.8

Table 5-3 Regression model results

	Estimate	t Ratio	p
Area (pixels)	0.008	43.13	<0.001
Red (nm)	-0.12	-0.84	0.39
Green (nm)	-0.49	-0.65	0.51
Blue (nm)	0.25	0.31	0.75
Intensity RGB	-0.39	-8.03	<0.001
Sum RGB	0	0	-

Figure 5-1A) Differences between area in pixels per hematocrit levels (%) at each sample volume (µL). B) Associations between intensity and hematocrit levels (%)



5.4.2 Phase 2. Method validation

a. Population 1. Comparison between Hg species concentrations from samples obtained under controlled volume conditions.

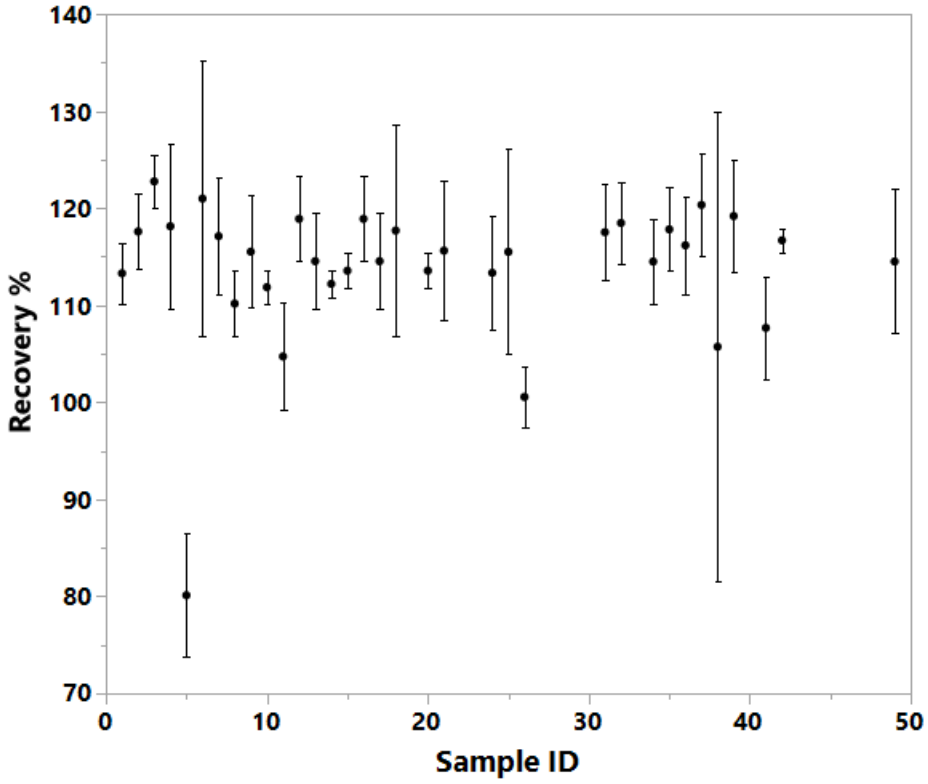
Samples of DBS from 49 individuals created with a control volume of 40µL of whole blood were scanned 48 hours after collection. The sample volume was estimated using the multiple regression model from phase 1, with triplicates per individual. Initially, our MATLAB method could not detect 31% (n = 15) of the samples, due to the quality of the DBS (some of them were overlapping as shown in Figure 5-2). The quality of the spots can be an essential limitation of our method and calls for better coding to define spot boundaries. Also, it is essential to keep an excellent blood-spotting practice to ensure the quality of the blood spot (Timmerman et al., 2011). For the samples that could be detected, the average estimated volume of the full spot was 45.6 ± 3.8µL, with an accuracy average of 114 ± 9.4%. The mean precision (CV) of the triplicate samples was 5.08 ± 3.9% (Figure 5-3). Previous studies have reported similar accuracy using a hematocrit-insensitive device to collect accurate volumes of dried blood spots in a range of hematocrits (30-60%) with a range of 75-118% accuracy (Nakahara et al., 2018).

Figure 5-2 Example of a DBS with overlapping spots.

When using the model-derived volumes to estimate the MeHg concentrations in the DBS, 31% (n = 15) of the samples were below the method detection limit (MDL) (0.3 µg/L as previously

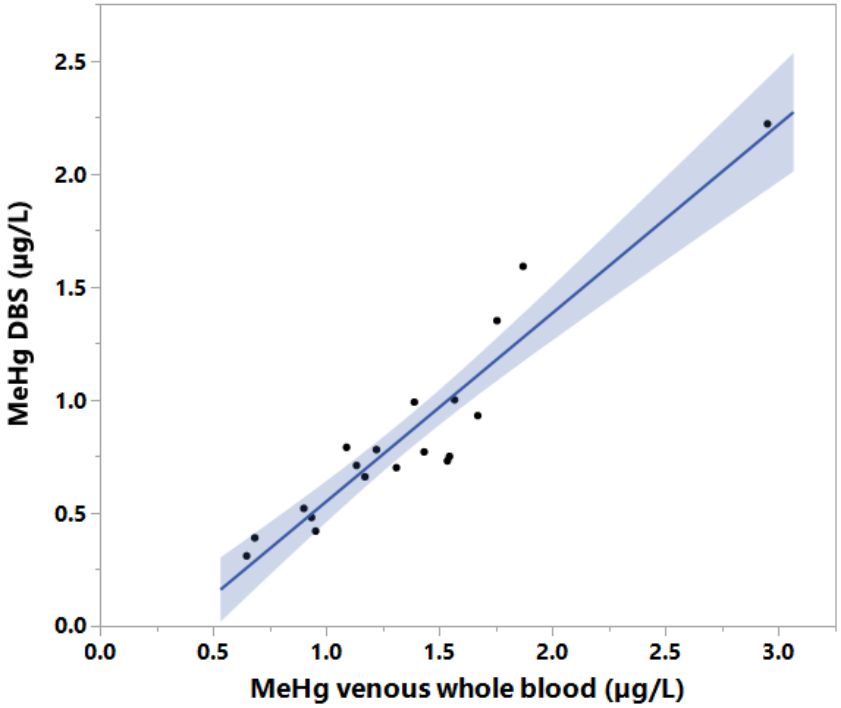
reported) (Santa-Rios et al., 2020). This was higher than the number of samples below the MDL reported before for the same set of samples with known volume 6% (n = 3). The increase of samples below the MDL is due to the low concentrations of MeHg in this population and an under estimation due to the higher calculated volume. Therefore, it is important to evaluate the method on samples with higher concentrations. The mean concentration of MeHg for samples with known volume (mean = 0.8 ± 0.5µg/L) was lower than the mean MeHg for calculated volume (mean = 1.1 ± 0.6µg/L).

Figure 5-3 Recovery of volume calculated using the regression model from samples created with a control volume (40µL). Each error bar is constructed using one standard deviation from the mean



We previously reported a significant association in blood MeHg levels between DBS with known blood volume and venous whole blood (the gold standard) (slope 0.93; 95% CI = 0.79−1.07; R^2 = 0.8) (Santa-Rios et al., 2020). When we re-ran these associations using DBS MeHg concentrations based on the estimated volume from our volume predicted equation, the resulting slope was 0.93 (95% CI = 0.76−1.09) with a R^2 = 0.8 (Figure 5-4). Therefore, the method for volume estimation can be used as an alternative when sample volume cannot be controlled, though a key limitation is with DBS that have overlapping spots and/or unclear boundaries.

Figure 5-4 Regression line between venous whole blood MeHg (µg/L) and DBS MeHg (µg/L) analyzed with the estimated volume from our regression model. The blue band indicates the 95% confidence interval.



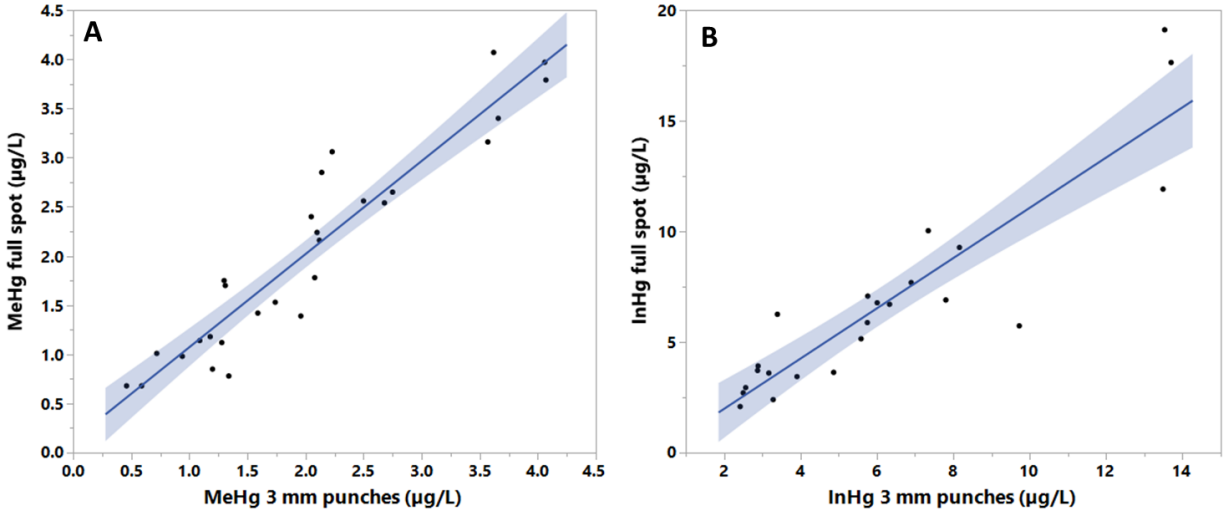
b. Population 2. Comparison between Hg species concentrations from samples obtained under field conditions without volume control.

For the second population group, DBS samples were taken under field conditions and so the volumes were not known. In this field study, there were several limitations concerning sample collection due to the nature of the study site and type of work (i.e., mining community in a resource-limited and remote region). The volunteer's hands were cold, decreasing the blood flow making difficult the capillary blood collection. Second, when we tried to use microcapillary tubes, as with the first population, the tubes did not work under extreme heat conditions and low blood flow. Therefore, samples were taken without any volume control, and such may be expected in many resource-limited settings in studies involving vulnerable populations.

In this case, we did not find differences on the number of samples below the MDL with the volume correction compared to the estimated volume using the 3 mm punches. For MeHg, only one sample (3%) was below the MDL for MeHg, and five (14%) for InHg (MDL =1.9 µg/L)(Santa-Rios et al., 2020). For this validation study we compared the MeHg and InHg concentrations obtained using the standardized 3 mm punches (as concentrations calculated with a known volume) with the entire spot (with the concentration calculated using the volume estimation), to identify the application of the model to samples collected in the field. Significant associations were found for MeHg concentrations between the punches and the entire spot (slope 0.93; 95% CI = 0.81−1.08; R^2 = 0.9) (Figure 5-5A), and lower associations were found for the InHg data with no significant proportional bias (slope 1.13; 95% CI = 0.89 – 1.37; R^2 = 0.9) (Figure 5-5B). The mean concentration of MeHg for the three 3mm punches (mean = 2.0 ± 1.0µg/L) was similar to values from the entire spot with estimated volume (mean = 2.0 ± 1.0µg/L). Unlike MeHg, lower mean concentrations of InHg were found for the three 3mm punches (mean = 6.2 ± 3.6µg/L) than the entire spot with estimated volume (mean = 6.7 ± 4.5µg/L). The concentration overestimation was previously reported for total Hg, the study found higher total Hg (as MeHg + InHg) concentrations when sample volume estimation was applied, and therefore higher recoveries, due to small sample estimation (Perkins and Basu, 2018).

The advantage of analyzing an entire spot (versus punches) has been reported previously, mainly because the Hct-based bias can be avoided when an entire spot is analyzed combined with a strategy to measure sample volume (Abu-Rabie et al., 2015). Another advantage is to be able to analyze more sample volume and therefore avoid the MDL that can be a limitation for populations with low levels of certain analytes.

Figure 5-5 A) Regression line between DBS MeHg (µg/L) calculated from standardized 3mm punches, and DBS MeHg (µg/L) from a entire spot with volume estimated using the regression model, with the blue band indicating the 95% confidence interval; (B) Regression line between DBS InHg (µg/L) calculated from standardized 3mm punches, and DBS InHg (µg/L) from a entire spot with volume estimated using the regression model, with the blue band indicating the 95% confidence interval.



5.5 Limitations

This study is not without limitations. The quality of the DBS can limit the use of the MATLAB® code to analyze sample volume, and a future code must include a method to analyze overlapping samples. This study does not consider that hemoglobin is converted from oxyhemoglobin to methemoglobin upon aging resulting in color change (De Kesel et al., 2013). All the analyses were done using fresh DBS, where samples were scanned after the first 48 hours of collection, therefore this study does not consider the aging of the samples.

5.6 Conclusion

The present study combines two non-destructive methodologies to estimate sample volume in an entire spot and avoid the Hct-area bias using a simple desktop scanner. Our model was created with Hct standards from nine volunteers, and all the significant variables were affected by the Hct levels. The model proved high volume accuracy when compared with samples created under control volume from a population with different Hct levels. The validation of the model showed that the model generates accurate results for the analysis of the entire spot to estimate MeHg and InHg concentrations in two diverse populations. Limitations about the quality of the DBS, and MATLAB® accessibility will need to be further solved for the application of the method for Hg speciation analysis.

5.7 References

Abu-Rabie, P., Denniff, P., Spooner, N., Chowdhry, B. Z., & Pullen, F. S. (2015). Investigation of Different Approaches to Incorporating Internal Standard in DBS Quantitative Bioanalytical Workflows and Their Effect on Nullifying Hematocrit-Based Assay Bias. Anal. Chem, 87, 8. https://doi.org/10.1021/acs.analchem.5b00908

Alexis Leuthold, L., Heudi, O., De, J., Raccuglia, M., Augsburger, M., Picard, F., … Thomas, A. (2015). New Microfluidic-Based Sampling Procedure for Overcoming the Hematocrit Problem Associated with Dried Blood Spot Analysis. Analytical Chemistry. https://doi.org/10.1021/ac503931g

Alsous, M. M., Hawwa, A. F., & McElnay, J. C. (2020). Hematocrit, blood volume, and surface area of dried blood spots – a quantitative model. Drug Testing and Analysis, 12(4), 555–560. https://doi.org/10.1002/dta.2776

Beck, O., Kenan Modén, N., Seferaj, S., Lenk, G., & Helander, A. (2018). Study of measurement of the alcohol biomarker phosphatidylethanol (PEth) in dried blood spot (DBS) samples and application of a volumetric DBS device. Clinica Chimica Acta, 479, 38–42. https://doi.org/10.1016/j.cca.2018.01.008

Bloem, K., Schaap, T., Boshuizen, R., Kneepkens, E. L., Wolbink, G. J., Vries, A. De, & Rispens, T. (2018). Capillary blood microsampling to determine serum biopharmaceutical concentration: Mitra® microsampler vs dried blood spot. Bioanalysis, 10(11), 815–823. https://doi.org/10.4155/bio-2018-0010

Capiau, S., Stove, V. V, Lambert, W. E., & Stove, C. P. (2013). Prediction of the Hematocrit of Dried Blood Spots via Potassium Measurement on a Routine Clinical Chemistry Analyzer. Anal. Chem, 85. https://doi.org/10.1021/ac303014b

Capiau, S., Wilk, L. S., Aalders, M. C. G., & Stove, C. P. (2016). A Novel, Nondestructive, Dried Blood Spot-Based Hematocrit Prediction Method Using Noncontact Diffuse Reflectance Spectroscopy. Anal. Chem, 88, 8. https://doi.org/10.1021/acs.analchem.6b01321

Capiau, S., Wilk, L. S., De Kesel, P. M. M., Aalders, M. C. G., & Stove, C. P. (2017). Correction for the Hematocrit Bias in Dried Blood Spot Analysis Using a Nondestructive, Single-Wavelength Reflectance-Based Hematocrit Prediction Method. Analytical Chemistry, 90, 1795–1804. https://doi.org/10.1021/acs.analchem.7b03784

Chieh Chao, T., Trybala, A., Starov, V., & Das, D. B. (2014). Influence of haematocrit level on the kinetics of blood spreading on thin porous medium during dried blood spot sampling. Physicochem. Eng. Aspects, 451, 38–47. https://doi.org/10.1016/j.colsurfa.2014.03.033

De Kesel, P. M. M., Sadones, N., Capiau, S., Lambert, W. E., & Stove, C. P. (2013, August 13). Hemato-critical issues in quantitative analysis of dried blood spots: Challenges and solutions. Bioanalysis. Future Science Ltd London, UK . https://doi.org/10.4155/bio.13.156

Den Burger, J. C. G., Wilhelm, A. J., Chahbouni, A. C., Vos, R. M., Sinjewel, A., & Swart, E. L. (2015). Haematocrit corrected analysis of creatinine in dried blood spots through potassium measurement. Anal Bioanal Chem, 407, 621–627. https://doi.org/10.1007/s00216-014-8291-9

Denniff, P., & Spooner, N. (2010). The effect of hematocrit on assay bias when using DBS samples for the quantitative bioanalysis of drugs. Bioanalysis, 2(8), 1385–1395. https://doi.org/10.4155/bio.10.103

Denniff, P., & Spooner, N. (2014). Volumetric Absorptive Microsampling: A Dried Sample Collection Technique for Quantitative Bioanalysis. Anal. Chem, 86. https://doi.org/10.1021/ac5022562

El-Hajjar, D. F., Swanson, K. H., Landmark, J. D., & Stickle, D. F. (2007). Validation of use of annular once-punched filter paper bloodspot samples for repeat lead testing. Clinica Chimica Acta, 377(1–2), 179–184. https://doi.org/10.1016/j.cca.2006.09.020

Erik, L., Eibak, E., Hegge, A. B., Rasmussen, K. E., Pedersen-Bjergaard, S., & Gjelstad, A. (2012). Alginate and Chitosan Foam Combined with Electromembrane Extraction for Dried Blood Spot Analysis. Analytical Chemistry, 84, 8783–8789. https://doi.org/10.1021/ac301996n

Kadjo, A. F., Stamos, B. N., Shelor, C. P., Berg, J. M., Blount, B. C., & Dasgupta, P. K. (2016). Evaluation of Amount of Blood in Dry Blood Spots: Ring-Disk Electrode Conductometry. Analytical Chemistry, 88, 6531–6537. https://doi.org/10.1021/acs.analchem.6b01280

Koster, R. A., Alffenaar, J. W., Botma, R., Greijdanus, B., Touw, D. J., Uges, D. R., & Kosterink, J. G. (2015). What is the right blood hematocrit preparation procedure for standards and quality control samples for dried blood spot analysis? Bioanalysis, 7(3), 345–351. https://doi.org/10.4155/bio.14.298 [doi]

Lee, M. S., & Li, W. (2014). Dried blood spots : applications and techniques. Retrieved from https://www.wiley.com/en-ca/Dried+Blood+Spots:+Applications+and+Techniques-p-9781118054697

Lenk, G., Hansson, J., Beck, O., & Roxhed, N. (2015). The effect of drying on the homogeneity of DBS. Bioanalysis.

Lenk, G., Sandkvist, S., Pohanka, A., Stemme, G., Beck, O., & Roxhed, N. (2015). A disposable sampling device to collect volume-measured DBS directly from a fingerprick onto DBS paper. Bioanalysis, 7(16), 2085–2094. https://doi.org/10.4155/bio.15.134

Liao, H.-W., Lin, S.-W., Chen, G.-Y., & Kuo, C.-H. (2016). Estimation and Correction of the Blood Volume Variations of Dried Blood Spots Using a Postcolumn Infused-Internal

Standard Strategy with LC-Electrospray Ionization-MS. Anal. Chem, 88. https://doi.org/10.1021/acs.analchem.6b01145

Liao, H. W., Lin, S. W., Lin, Y. T., Lee, C. H., & Kuo, C. H. (2018). Identification of potential sphingomyelin markers for the estimation of hematocrit in dried blood spots via a lipidomic strategy. Analytica Chimica Acta, 1003(33), 34–41. https://doi.org/10.1016/j.aca.2017.11.041

Malsagova, K., Kopylov, A., Stepanov, A., Butkova, T., Izotov, A., & Kaysheva, A. (2020, April 1). Dried blood spot in laboratory: Directions and prospects. Diagnostics. MDPI AG. https://doi.org/10.3390/diagnostics10040248

Mcdade, T. (2013). Development and validation of assay protocols for use with dried blood spot samples. American Journal of Human Biology, 9(August 2013), 1–9. https://doi.org/10.1002/ajhb.22463

McDade, T., Williams, S., & Snodgrass, J. (2007). What a drop can do: Dried blood spots as minimally invasive method for integrating biomarkers into population-based research. Demography, 44(4), 899–925. Retrieved from http://link.springer.com/article/10.1353/dem.2007.0038

Mengerink, Y., Mommers, J., Qiu, J., Mengerink, J., Steijger, O., & Honing, M. (2015). A new DBS card with spot sizes independent of the hematocrit value of blood. Bioanalysis, 7(16), 2095–2104. https://doi.org/10.4155/bio.15.133

Miller IV, J. H. (2013). An On-card Approach for Assessment of Hematocrit on Dried Blood Spots which Allows for Correction of Sample Volume. Journal of Analytical & Bioanalytical Techniques, 04(01), 1–8. https://doi.org/10.4172/2155-9872.1000162

Nakahara, T., Otani, N., Ueno, T., & Hashimoto, K. (2018). Development of a hematocrit-insensitive device to collect accurate volumes of dried blood spots without specialized skills for measuring clozapine and its metabolites as model analytes. Journal of Chromatography B, 1087–1088, 70–79. https://doi.org/10.1016/j.jchromb.2018.04.019

Neoteryx. (2019). Mitra Microsampling devices. Retrieved June 2, 2020, from https://www.neoteryx.com/micro-sampling-capillary-blood-collection-devices

Neto, R., Gooley, A., Breadmore, M. C., Hilder, E. F., & Lapierre, F. (2018). Precise, accurate and user-independent blood collection system for dried blood spot sample preparation. Analytical and Bioanalytical Chemistry. https://doi.org/10.1007/s00216-018-0993-y

Perkins, M., & Basu, N. (2018). Dried blood spots for estimating mercury exposure in birds. Environmental Pollution, 236, 236–246. https://doi.org/10.1016/J.ENVPOL.2018.01.036

Resano, M., Belarra, M. A., García-Ruiz, E., Aramendía, M., & Rello, L. (2018). Dried matrix spots and clinical elemental analysis. Current status, difficulties, and opportunities. https://doi.org/10.1016/j.trac.2017.12.004

Richardson, G., Marshall, D., & Keevil, B. G. (2018). Prediction of haematocrit in dried blood spots from the measurement of haemoglobin using commercially available sodium lauryl sulphate. Annals of Clinical Biochemistry 2018, 55(3), 363–367. https://doi.org/10.1177/0004563217726809

Rufail, M. L., McCloskey, L. J., & Stickle, D. F. (2017). Estimation of hematocrit in filter paper dried bloodspots by potassium measurement: Advantage of use of perimeter ring samples over circular center sub-punch samples. Clinical Chemistry and Laboratory Medicine, 55(1), 53–57. https://doi.org/10.1515/cclm-2016-0161

Santa-Rios, A., Barst, B. D., & Basu, N. (2020). Mercury Speciation in Whole Blood and Dried Blood Spots from Capillary and Venous Sources. ANALYTICAL CHEMISTRY. https://doi.org/10.1021/acs.analchem.9b04407

Spooner, N., Dennif, P., Michielsen, R. de V., Ji, Q. C., Arnold, M. E., Woods, K., … Rudge, J. B. (2015). A device for dried blood microsampling in quantitative bioanalysis: overcoming the issues associated with blood hematocrit. Bioanalysis, 7(6), 653–659. https://doi.org/10.4155/BIO.14.310

Spot on Sciences. (2020). Hemaspot HF Capillary Blood Collection Device | No Punching Required. Retrieved June 3, 2020, from https://www.spotonsciences.com/hemaspot-hf

Timmerman, P., White, S., Globig, S., Lüdtke, S., Brunet, L., & Smeraglia, J. (2011). EBF recommendation on the validation of bioanalytical methods for dried blood spots. Bioanalysis, 3(14), 1567–1575. https://doi.org/10.4155/bio.11.132

USAID. (2012). Biomarker field manual: Demographic and Health Surveys Methodology. Calverton, Maryland. Retrieved from http://www.measuredhs.com/publications/publication-dhsm7-dhs-questionnaires-and-manuals.cfm

Velghe, S., Delahaye, L., & Stove, C. P. (2019, January 30). Is the hematocrit still an issue in quantitative dried blood spot analysis? Journal of Pharmaceutical and Biomedical Analysis. Elsevier B.V. https://doi.org/10.1016/j.jpba.2018.10.010

Velghe, S., & Stove, C. P. (2018). Evaluation of the Capitainer-B Microfluidic Device as a New Hematocrit-Independent Alternative for Dried Blood Spot Collection. Analytical Chemistry, 90, 12893–12899. https://doi.org/10.1021/acs.analchem.8b03512

Verplaetse, R., & Henion, J. (2016). Hematocrit-Independent Quantitation of Stimulants in Dried Blood Spots: Pipet versus Microfluidic-Based Volumetric Sampling Coupled with

Automated Flow-Through Desorption and Online Solid Phase Extraction-LC-MS/MS Bioanalysis. Anal. Chem, 88, 16. https://doi.org/10.1021/acs.analchem.6b01190

Vries, R. De, Qin, C., Boutet, V., Kushon, S., & Rudge, J. B. (2015). A device for dried blood microsampling in quantitative bioanalysis. Bioanalysis, 7(6), 653–659. https://doi.org/10.4155/BIO.14.310

6. GENERAL DISCUSSION

Due to the potential health risks of exposure to Hg species in many populations worldwide (Basu et al., 2018b), it is pertinent to develop monitoring programs suitable for all populations and locations, including resource-limited and remote settings (UN Environment Programme, 2013b). This is further emphasized now with the UN Minamata Convention on Mercury's entry into force in 2017. The human health impacts of Hg vary according to which chemical species of Hg one is exposed to, and thus there is a need for species-specific exposure methods (Table 6-1). The direct measurement of total Hg levels in whole blood is an accepted biomarker for Hg exposure in humans (Horvat et al., 2012) though challenges regarding venipuncture practices represent a barrier for wider adoption (e.g., logistic requirements, external contamination, costs, ethical considerations) (McDade et al., 2007). Dried blood spot sampling is a potential alternative to venipuncture since it has fewer logistical requirements as highly trained personnel are not needed. Also, DBS can be stored at ambient temperature, and shipments involved small packages (Denniff and Spooner, 2014a). Additionally, sample collection is minimally-invasive, with only a small volume of sample collected (Enderle et al., 2016), increasing the feasibility of collecting large numbers of samples in communities of interest (Mcdade, 2013).

6.1 Quality control advancements

The use of DBS for Hg exposure assessment has been previously reported (Basu et al., 2017; Chaudhuri et al., 2009; Funk, 2015; Funk et al., 2013; Nelson et al., 2016; Elias Nyanza et al., 2019; Elias. Nyanza et al., 2019). While studies have explored measuring Hg in DBS, the methods to date have been challenged technically (e.g., detection limit, accuracy), and nearly all have focused on total Hg measures. Further, few studies have validated the methods (e.g., compared against a range of quality performance criteria, or compared DBS measures against corresponding

whole blood values), and even fewer have applied these methods in real-world communities. Therefore, this doctoral thesis followed a structured method to develop, validate, and apply an accurate and precise method to characterize exposure to Hg species through DBS for application in diverse communities (from clinical environments in high-income settings to contaminated field sites in low-income countries) with a range of Hg exposures (from background levels to high).

This thesis developed and validated a new DBS method for Hg speciation with careful attention to quality control during all the studies (see Table 6-2). Figure 6-1 presents a flow chart of the studies that I developed to validate the method using different sampling settings and populations with a range of exposure to Hg species.

The first manuscript resulted from the first aim (Santa-Rios et al., 2020) was a breakthrough as it carefully looked at all quality control aspects taking into account the European Bioanalysis Forum and US EPA Method 1630 to fulfill the gaps presented by previous studies (see Table S2-1. Summary of past studies that analyzed mercury (Hg) in human DBS). The study was the first to explore the use of DBS for the analysis of MeHg and InHg. Previous studies have only focused in the measurement of THg DBS (Chaudhuri et al., 2009; Funk, 2015; Funk et al., 2013; Nelson et al., 2016; Elias Nyanza et al., 2019; Elias. Nyanza et al., 2019) and only one study analyzed the use of DBS for the analysis of MeHg (Basu et al., 2017). This study was the first to report a MDL for InHg in DBS (1.9 µg/L), and the MDL for MeHg was similar to our previous work (Basu et al., 2017). For MeHg, the relatively low MDLs reported in this aim in both whole blood and DBS supported the use of the method in population-based human biomonitoring studies. The calculated detection limits for both matrices were less than the mean values reported by national biomonitoring programs, including the CHMS (0.6 µg/L) (Canadian Health Measures Survey CHMS, 2014) and U.S. NHANES (0.5 µg/L) (National Center for Environmental Health, 2014).

Figure 6-1. Diagrammatic overview of the study

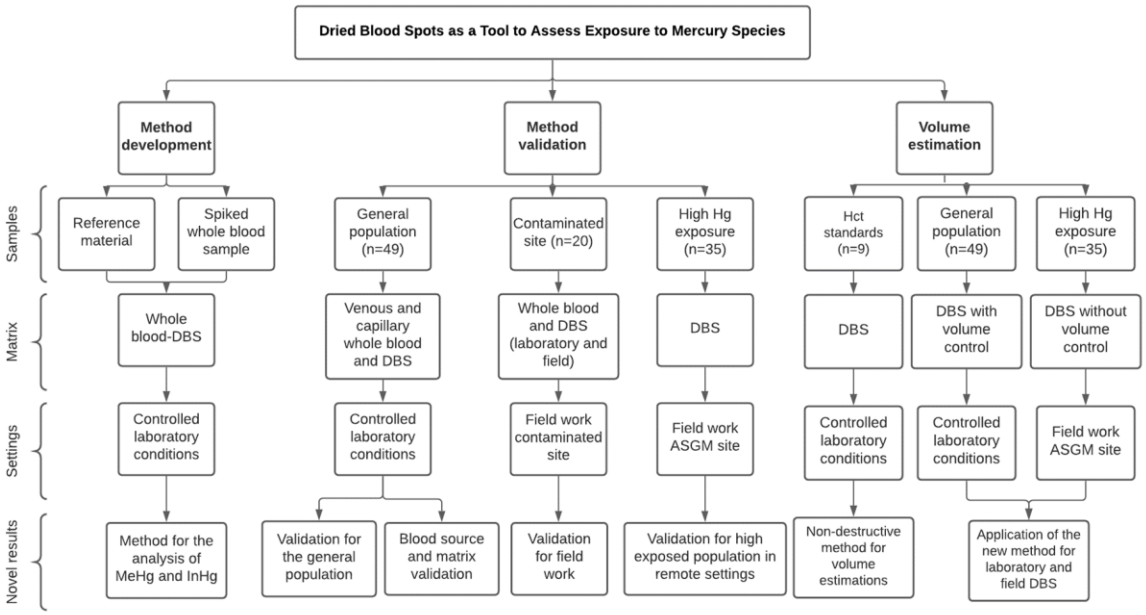


Table 6-1. Important factors of Hg speciation

	MeHg	InHg
Health effects	**Brain and the central nervous system**	**Nervous system and the kidneys**
Exposure and risk assessment	**Dietary**	**Occupational**
Policy	**Potential advice regarding fish consumption**	**Evaluate Hg-reduction efforts**

Table 6-2. Quality control analysis for all the aims

Parameter	Unit	Aim 1	Aim 2	Aim 3	EPA 1630
Blanks	Average (µg/L)	MeHg = 0.02 InHg = 0.57	MeHg = 0.02	MeHg = 0.14 InHg = 1.43	**<0.5 ng/L**
IPR/OPR	Accuracy (%)	MeHg = 83 -116 InHg = 97 – 141	MeHg = 102 -118	MeHg = 92-130 InHg = 72 – 144	**65-135%**
	Precision (%)	MeHg= < 8 InHg < 22	MeHg = <4	MeHg= < 9 InHg < 23	**<35%**
Reference material	Accuracy (%)	MeHg = 91 – 135%	MeHg = 79-121	MeHg = 83- 109	**65-135%**
Sample replicate	Precision (%)	MeHg = <13 InHg = <13	MeHg =<24	MeHg = <17 InHg = <36	**<35%**

6.2 Mercury in different blood types

Venipuncture approaches represent the gold standard in clinical environments and exposure science studies in which venous whole blood is obtained. However, DBS are typically produced from capillary whole blood, and thus comparing DBS results with assays from venous whole blood samples may not be accurate (McDade et al., 2007). Capillary whole blood differs from venous whole blood (Enderle et al., 2016), with higher levels of hemoglobin, hematocrit, red blood cells, white blood cells, and white blood cells in capillary whole blood than in venous whole blood (Akenzua et al., 1974; de Alarcon et al., 2013; Kayiran et al., 2003). In my thesis, there was no significant difference ($p=0.74$) in MeHg concentration between the two blood sources, and a strong linear relationship was found between them ($R^2=0.91$) (Figure 2-1A). To my knowledge, my work represents the first to comparing Hg levels between venous and capillary whole blood. In addition, I also studied potential for matrix interference by comparing whole blood measurements with corresponding DBS values (from the same blood source). MeHg levels in capillary whole blood versus capillary DBS presented a close 1:1 relationship (Figure 2-2A) with no constant bias (intercept 0.09μg/L; 95% CI -0.11 – 0.30) and no proportional bias (slope was 1.01; 95% CI 0.81 – 1.20) (Figure 2-2B). Such a finding suggests that there is no matrix effect due to the paper filter, and thus helps support the use of DBS-based approaches as a proxy for whole blood measures.

6.3 Applications in the real-world

As mentioned earlier, my review of past studies in this area found that most were situated in clinical environments with very few applications in communities that are situated in remote and/or resource-limited settings. After demonstrating a DBS-based method to evaluate MeHg exposure in laboratory- and clinical-based settings involving populations with low background exposures situated in a high-income country (Chapter 2), I next wanted to demonstrate how this method could

be utilized in the real-world for application in a contaminated and resource-limited site which tend to experience the highest Hg exposures worldwide. To my knowledge, only one study has previously studied the application of a DBS-based approach in the field for the analysis of THg (Elias. Nyanza et al., 2019) but not MeHg. Initially, we evaluated concerns about potential contamination of filter paper during its manufacturing (Resano et al., 2018) and the external contamination of these cards, particularly if they are to be used in field locations that may be polluted. The study site of Chapter 3 (Agbogbloshie, Ghana) is considered to be one of the most contaminated worldwide. The background level of MeHg in the blank samples from DBS cards used in this field site (mean 0.86 ± 0.40 pg or equivalent to 0.14 ± 0.06μg/L) were similar to values previously reported from our group though in a study set in a "clean" environment (mean 0.16 ± 0.02 μg/L) (Basu et al., 2017). This 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 not significant when measuring MeHg concentrations in real-world settings that span sterile clinical environments to highly contaminated field sites. Therefore, this study, coupled with the results from Chapter 2, demonstrated the range of settings in which human biomonitoring of Hg exposures can be performed using DBS.

Chapter 4 validated the DBS-method for Hg exposure in an ASGM community in Colombia that is both vulnerable to Hg exposure and remote com. To my knowledge, this is the first study to characterize Hg speciation in DBS from an ASGM community. Here, I demonstrated that DBS are particularly well suited in studies posing logistical challenges exemplified by my own experiences working in the remote location of the community and relatively unsafe conditions of the study area. In this study involving a highly exposed community, I demonstrated that the DBS method was suitable for detecting MeHg and InHg exposures in nearly all participants; such would not have been possible if we sampled whole blood which would have necessitated cold chain custody and bulky supplies that could not be transported by motorcycle. This study also demonstrates the need to speciate Hg in blood samples from ASGM workers and community members to understand better the contributions of both MeHg and InHg (more generally environmental and occupational,

respectively) to overall exposures high exposed communities. The relatively high percentage of InHg (averaged 60% InHg of THg), exemplifies that the measurement of THg in blood (or DBS) does not serve as valid proxy for MeHg exposure in these communities, and in making such an assumption one may overestimate MeHg exposures from dietary habits such as fish consumption.

6.4 Blood volume

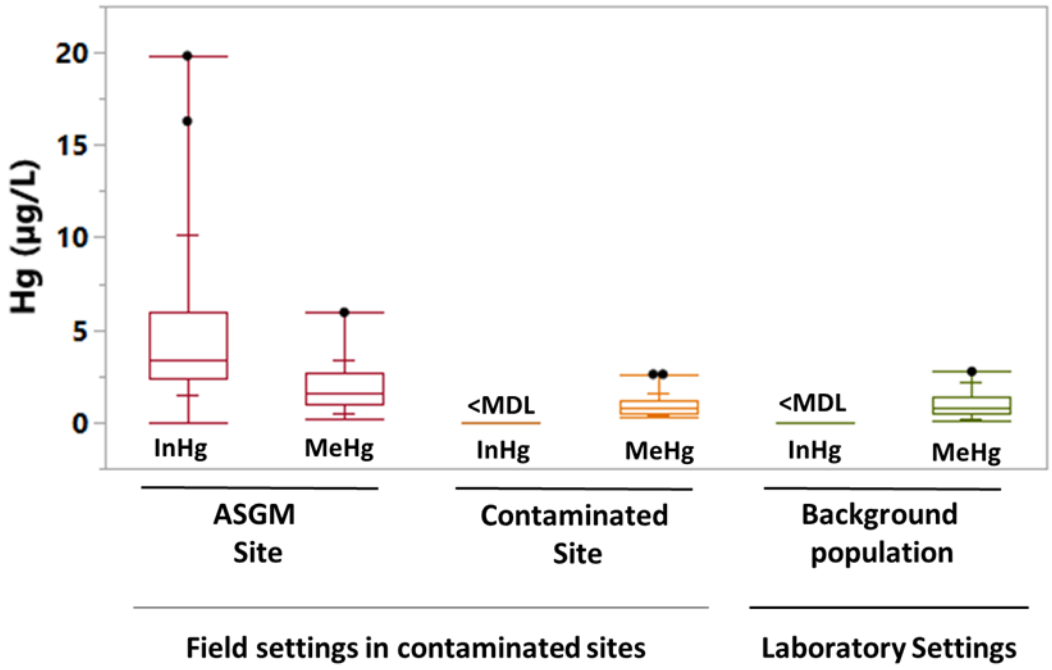
Chapter 5 aimed to create a non-destructive method to calculate sample volume in an entire DBS spot to help overcome a significant issue with DBS, which is the lack of knowledge of the volume of blood on the filter paper. The studies in my thesis used various techniques to spot blood onto filter cards and to measure Hg, including analysis of an entire DBS created with controlled volume to the use of a standardized 3mm punch as a subsampling method to estimate the sample volume. As such, these represented good case studies to build and test a model around. Notably, in Chapter 3, I demonstrated that while there is a relatively good agreement between DBS sampled in the field (in which blood volumes are not known) and those created artificially in the lab using whole blood from the same individuals (in which DBS volumes can be controlled), there were slight biases recorded. Here I developed and used a non-destructive method (i.e., scans of DBS cards), and applied both area and intensity measures for sample volume calculation, combining the results reported by other studies (Alsous et al., 2020; El-Hajjar et al., 2007; Perkins and Basu, 2018). Compared to other studies, the innovation of this method was the application to DBS created under controlled volume conditions as well as DBS collected in the field. The validation of the model indicated that the model generates accurate results for the entire spot's analysis to estimate MeHg and InHg concentrations in two diverse populations. The advantage of analyzing an entire spot has been reported previously, mainly because the Hct-based bias can be avoided when an entire spot is analyzed combined with a strategy to measure sample volume (Abu-Rabie et al., 2015). Another

advantage is to be able to analyze more sample volume and therefore avoid the MDL that can be a limitation for populations with low exposure to analytes.

7. GENERAL CONCLUSION

Overall, this thesis validates DBS as a novel tool to assess MeHg and InHg exposure in human populations with a range of Hg exposure and different field settings (Figure 7-1). This thesis provides a novel method to characterize MeHg and InHg in DBS and validated the work with very careful attention to quality control (Figure 6-1). The study investigated knowledge gaps presented in previous applications of DBS for the analysis of exposure to Hg, including a careful quality control during all stages of the studies, the identification of differences in Hg levels between blood sources (venous and capillary) and sample matrices, the application of the method under real-world conditions compared to laboratory conditions, the use of DBS for an ASGM community, and the use of simple method to estimate sample volume. I proved that coupling minimally invasive DBS sampling with a simple health questionnaire can be used to assess occupational, environmental, and dietary exposure to Hg species in a remote ASGM community. Future researchers can use this method to overcome logistic limitations for exposure assessment in vulnerable populations and accomplish the Article 22 of the Minamata Convention on Mercury that calls out the need to monitor Hg trends in human populations.

Figure 7-1 Summary of Hg concentrations in capillary DBS across study populations. Bars represent the median values with the interquartile range (25th percentile, 75th percentile) indicated as error bars. MDL: Method detection limits



8. MASTER REFERENCE LIST

Abu-Rabie, P., Denniff, P., Spooner, N., Chowdhry, B.Z., Pullen, F.S., 2015. Investigation of Different Approaches to Incorporating Internal Standard in DBS Quantitative Bioanalytical Workflows and Their Effect on Nullifying Hematocrit-Based Assay Bias. Anal. Chem 87, 8. https://doi.org/10.1021/acs.analchem.5b00908

Addinsoft, n.d. XLSTAT- Statistics Package for Excel.

Agency for Toxic Substances and Disease Registry, 1999. Toxicological Profile for Mercury Department of Health and Human Services 676.

Akagi, H., Castillo, E.S., Cortes-Maramba, N., Francisco-Rivera, A.T., Timbang, T.D., 2000. Health assessment for mercury exposure among schoolchildren residing near a gold processing and refining plant in Apokon, Tagum, Davao del Norte, Philippines. Sci. Total Environ. 259, 31–43. https://doi.org/10.1016/S0048-9697(00)00547-7

Akagi, H., Malm, O., Branches, F.J.P., Kinjo, Y., Kashima, Y., Guimaraes, J.R.D., Oliveira, R.B., Haraguchi, K., Pfeiffer, W.C., Takizawa, Y., Kato, H., 1995. Human exposure to mercury due to goldmining in the Tapajos River Basin, Amazon, Brazil: speciation of mercury in human hair, blood and urine. Water. Air. Soil Pollut. 80, 85–94.

Akenzua, G.I., Hui, Y.T., Milner, R., Zipursky, A., 1974. Neutrophil and Band Counts in the Diagnosis of Neonatal Infections. Pediatrics 54.

Alexis Leuthold, L., Heudi, O., De, J., Raccuglia, M., Augsburger, M., Picard, F., Kretz, O., Thomas, A., 2015. New Microfluidic-Based Sampling Procedure for Overcoming the Hematocrit Problem Associated with Dried Blood Spot Analysis. Anal. Chem. https://doi.org/10.1021/ac503931g

Alsous, M.M., Hawwa, A.F., McElnay, J.C., 2020. Hematocrit, blood volume, and surface area of

dried blood spots – a quantitative model. Drug Test. Anal. 12, 555–560. https://doi.org/10.1002/dta.2776

Bakir F, Damluji SF, Amin-Zaki L, Murtadha M, Khalidi A, al-Rawi NY, Tikriti S, Dahahir HI, Clarkson TW, Smith JC, D.R., 1973. Methylmercury poisoning in Iraq. Science (80-.). 181, 230–241.

Basu, N., Ayelo, P.A., Djogbénou, L.S., Kedoté, M., Lawin, H., Tohon, H., Oloruntoba, E.O., Adebisi, N.A., Cazabon, D., Fobil, J., Robins, T., Fayomi, B., 2016. Occupational and Environmental Health Risks Associated with Informal Sector Activities—Selected Case Studies from West Africa. NEW Solut. A J. Environ. Occup. Heal. Policy 26, 253–270. https://doi.org/10.1177/1048291116651726

Basu, N., Clarke, E., Green, A., Calys-Tagoe, B., Chan, L., Dzodzomenyo, M., Fobil, J., Long, R., Neitzel, R., Obiri, S., Odei, E., Ovadje, L., Quansah, R., Rajaee, M., Wilson, M., 2015. Integrated Assessment of Artisanal and Small-Scale Gold Mining in Ghana—Part 1: Human Health Review. Int. J. Environ. Res. Public Health 12, 5143–5176. https://doi.org/10.3390/ijerph120505143

Basu, N., Eng, J.W.L., Perkins, M., Santa-Rios, A., Martincevic, G., Carlson, K., Neitzel, R.L., 2017. Development and application of a novel method to characterize methylmercury exposure in newborns using dried blood spots. Environ. Res. 159, 276–282. https://doi.org/10.1016/J.ENVRES.2017.08.021

Basu, N., Horvat, M., Evers, D.C., 2018a. A Systematic Review of Mercury Exposures in Human Populations Worldwide Between 2000 and 2018.

Basu, N., Horvat, M., Evers, D.C., Zastenskaya, I., Weihe, P., Tempowski, J., 2018b. A State-of-the-Science Review of Mercury Biomarkers in Human Populations Worldwide between 2000 and 2018. Environ. Health Perspect. 126, 106001. https://doi.org/10.1289/ehp3904

Beck, O., Kenan Modén, N., Seferaj, S., Lenk, G., Helander, A., 2018. Study of measurement of the alcohol biomarker phosphatidylethanol (PEth) in dried blood spot (DBS) samples and application of a volumetric DBS device. Clin. Chim. Acta 479, 38–42. https://doi.org/10.1016/j.cca.2018.01.008

Berglund, M., Lind, B., Björnberg, K.A., Palm, B., Einarsson, Ö., Vahter, M., 2005. Inter-individual variations of human mercury exposure biomarkers: a cross-sectional assessment.

Berlin, M., and Johansson, L.G., 1964. Mercury in mouse brain after inhalation of mercury vapor and after intravenous injection of mercury salt. Nature 204, 85–86.

Berlin, M., Fazackerly, J., and Nordberg, G., 1969. Mercurial induced brain monoamine oxidase inhibition in the teleostChanna punctatus (bloch). Arch. Environ. Heal. 18, 719–729.

Berlin, M., Zalups, R.K., Fowler, B.A., 2007. Mercury, in: Gunnar F. Nordberg, Bruce A. Fowler, M.N. (Ed.), Handbook on the Toxicology of Metals. Academic Press, 2014, pp. 675–729. https://doi.org/10.1016/B978-0-12-369413-3.50088-4

Bloem, K., Schaap, T., Boshuizen, R., Kneepkens, E.L., Wolbink, G.J., Vries, A. De, Rispens, T., 2018. Capillary blood microsampling to determine serum biopharmaceutical concentration: Mitra® microsampler vs dried blood spot. Bioanalysis 10, 815–823. https://doi.org/10.4155/bio-2018-0010

Bloom, N.S., 1992. On the Chemica Form of Mercury in Edible Fish and invertebrate Tissue. Fish Aquat. Sci 49, 1010–1017.

Bond, M.M., Richards-Kortum, R.R., 2015. Drop-to-drop variation in the cellular components of fingerprick blood: Implications for point-of-care diagnostic development. Am. J. Clin. Pathol. 144, 885–894. https://doi.org/10.1309/AJCP1L7DKMPCHPEH

Bradley, M., Barst, B., Basu, N., 2017. A Review of Mercury Bioavailability in Humans and Fish.

Int. J. Environ. Res. Public Health 14, 169. https://doi.org/10.3390/ijerph14020169

Canadian Health Measures Survey CHMS, 2014. Fourth Report on Human Biomonitoring of Environmental Chemicals in Canada.

Capiau, S., Stove, V. V, Lambert, W.E., Stove, C.P., 2013. Prediction of the Hematocrit of Dried Blood Spots via Potassium Measurement on a Routine Clinical Chemistry Analyzer. Anal. Chem 85. https://doi.org/10.1021/ac303014b

Capiau, S., Wilk, L.S., Aalders, M.C.G., Stove, C.P., 2016. A Novel, Nondestructive, Dried Blood Spot-Based Hematocrit Prediction Method Using Noncontact Diffuse Reflectance Spectroscopy. Anal. Chem 88, 8. https://doi.org/10.1021/acs.analchem.6b01321

Capiau, S., Wilk, L.S., De Kesel, P.M.M., Aalders, M.C.G., Stove, C.P., 2017. Correction for the Hematocrit Bias in Dried Blood Spot Analysis Using a Nondestructive, Single-Wavelength Reflectance-Based Hematocrit Prediction Method. Anal. Chem. 90, 1795–1804. https://doi.org/10.1021/acs.analchem.7b03784

Chaudhuri, S.N., Butala, S.J.M., Ball, R.W., Braniff, C.T., 2009. Pilot study for utilization of dried blood spots for screening of lead, mercury and cadmium in newborns. J. Expo. Sci. Environ. Epidemiol. 19, 298–316. https://doi.org/10.1038/jesee.2008.19

Chaudhuri, S.N., Butala, S.J.M., Ball, R.W., Braniff, C.T., 2008. Pilot study for utilization of dried blood spots for screening of lead, mercury and cadmium in newborns. J. Expo. Sci. Environ. Epidemiol. 19, 298–316.

Chen, J., Chen, Hengwu, Jin, X., Chen, Haiting, 2009. Determination of ultra-trace amount methyl-, phenyl-and inorganic mercury in environmental and biological samples by liquid chromatography with inductively coupled plasma mass spectrometry after cloud point extraction preconcentration. Talanta 77, 1381–1387.

https://doi.org/10.1016/j.talanta.2008.09.021

Cherian, M.G., Hursh, J.B., Clarkson, T.W., Allen, J., 1978. Radioactive Mercury Distribution in Biological Fluids and Excretion in Human Subjects after Inhalation of Mercury Vapor. Arch. Environ. Heal. An Int. J. 33, 109–114. https://doi.org/10.1080/00039896.1978.10667318

Chieh Chao, T., Trybala, A., Starov, V., Das, D.B., 2014. Influence of haematocrit level on the kinetics of blood spreading on thin porous medium during dried blood spot sampling. Physicochem. Eng. Asp. 451, 38–47. https://doi.org/10.1016/j.colsurfa.2014.03.033

Choi, A.L., Fitzgerald, W.F., Hammerschmidt, C.R., Lamborg, C.H., Soerensen, A.L., 2012. Mercury biogeochemical cycling in the ocean and policy implications. Environ. Res. 119, 101–117. https://doi.org/10.1016/J.ENVRES.2012.03.013

Clarkson, T.W., Magos, L., 2006. The Toxicology of Mercury and Its Chemical Compounds. Crit. Rev. Toxicol. 36, 609–662. https://doi.org/10.1080/10408440600845619

Clarkson, T.W., Magos, L., Myers, G.J., 2003. The toxicology of mercury current exposures and clinical manifestations. N. Engl. J. Med. 349, 1731–1737. https://doi.org/10.1056/NEJMra022471

Clarkson, T.W., Vyas, J.B., Ballatori, N., 2007. Mechanisms of mercury disposition in the body. Am. J. Ind. Med. 50, 757–764. https://doi.org/10.1002/ajim.20476

Clifford, M.J., 2014. Future strategies for tackling mercury pollution in the artisanal gold mining sector: Making the Minamata Convention work. Futures 62, 106–112. https://doi.org/10.1016/j.futures.2014.05.001

Cordy, P., Veiga, M.M., Salih, I., Al-Saadi, S., Console, S., Garcia, O., Mesa, L.A., Velásquez-López, P.C., Roeser, M., 2011a. Mercury contamination from artisanal gold mining in Antioquia, Colombia: The world's highest per capita mercury pollution. Sci. Total Environ.

410–411, 154–60. https://doi.org/10.1016/j.scitotenv.2011.09.006

Cordy, P., Veiga, M.M., Salih, I., Al-Saadi, S., Console, S., Garcia, O., Mesa, L.A., Velásquez-López, P.C., Roeser, M., 2011b. Mercury contamination from artisanal gold mining in Antioquia, Colombia: The world's highest per capita mercury pollution. Sci. Total Environ. 410–411, 154–160. https://doi.org/10.1016/j.scitotenv.2011.09.006

Council, A.G., 2015. Developing Baseline Estimates of Mercury Use in Artisanal and Small-Scale Gold Mining Communities : A Practical Guide (Version 1.0).

Daae, L.N.W., Halvorsen, S., Mathisen, P.M., Mironska, K., 1988. A comparison between haematological parameters in "capillary" and venous blood from healthy adults. Scand. J. Clin. Lab. Invest. 48, 723–726. https://doi.org/10.3109/00365518809085796

de Alarcon, P.A., Werner, E.J., Christensen, R.D., 2013. Neonatal Hematology: Pathogenesis, Diagnosis, and Management of Hematologic, Second Edi. ed. Cambridge University Press, New York.

De Kesel, P.M.M., Sadones, N., Capiau, S., Lambert, W.E., Stove, C.P., 2013a. Hemato-critical issues in quantitative analysis of dried blood spots: Challenges and solutions. Bioanalysis. https://doi.org/10.4155/bio.13.156

De Souza, S.S., Campiglia, A.D., Barbosa, F., 2013. A simple method for methylmercury, inorganic mercury and ethylmercury determination in plasma samples by high performance liquid chromatography–cold-vapor-inductively coupled plasma mass spectrometry. Anal. Chim. Acta 761, 11–17. https://doi.org/10.1016/j.aca.2012.11.038

Den Burger, J.C.G., Wilhelm, A.J., Chahbouni, A.C., Vos, R.M., Sinjewel, A., Swart, E.L., 2015. Haematocrit corrected analysis of creatinine in dried blood spots through potassium measurement. Anal Bioanal Chem 407, 621–627. https://doi.org/10.1007/s00216-014-8291-

Denniff, P., Spooner, N., 2014a. Volumetric Absorptive Microsampling: A Dried Sample Collection Technique for Quantitative Bioanalysis. Anal. Chem 86, 34. https://doi.org/10.1021/ac5022562

Denniff, P., Spooner, N., 2014b. Volumetric Absorptive Microsampling: A Dried Sample Collection Technique for Quantitative Bioanalysis. Anal. Chem 86. https://doi.org/10.1021/ac5022562

Denniff, P., Spooner, N., 2010. The effect of hematocrit on assay bias when using DBS samples for the quantitative bioanalysis of drugs. Bioanalysis 2, 1385–1395. https://doi.org/10.4155/bio.10.103

Discalzi G, Fabbro D, Meliga F, Mocellini A, C.F., 1993. Effects of occupational exposure to mercury and lead on brainstem auditory evoked potentials. Int. J. Psychophysiol. 14, 21–25.

Drasch, G., Böse-O'Reilly, S., Beinhoff, C., Roider, G., Maydl, S., 2001. The Mt. Diwata study on the Philippines 1999--assessing mercury intoxication of the population by small scale gold mining. Sci. Total Environ. 267, 151–68. https://doi.org/10.1016/S0048-9697(00)00806-8

Driscoll, C.T., Mason, R.P., Chan, H.M., Jacob, D.J., Pirrone, N., 2013. Mercury as a Global Pollutant: Sources, Pathways, and Effects. Environ. Sci. Technol. 47, 4967–4983. https://doi.org/10.1021/es305071v

Eagles-Smith, C.A., Silbergeld, E.K., Basu, N., Bustamante, P., Diaz-Barriga, F., Hopkins, W.A., Kidd, K.A., Nyland, J.F., 2018. Modulators of mercury risk to wildlife and humans in the context of rapid global change. Ambio 47, 170–197. https://doi.org/10.1007/s13280-017-1011-x

El-Hajjar, Dana F., Swanson, K.H., Landmark, J.D., Stickle, D.F., 2007. Validation of use of

annular once-punched filter paper bloodspot samples for repeat lead testing. Clin. Chim. Acta 377, 179–184. https://doi.org/10.1016/j.cca.2006.09.020

El-Hajjar, Dana F, Swanson, K.H., Landmark, J.D., Stickle, D.F., 2007. Validation of use of annular once-punched filter paper bloodspot samples for repeat lead testing. Clin. Chim. Acta 377, 179–84.

Enderle, Y., Foerster, K., Burhenne, J., 2016. Clinical feasibility of dried blood spots: Analytics, validation, and applications. J. Pharm. Biomed. Anal. 130, 231–243. https://doi.org/10.1016/j.jpba.2016.06.026

Erik, L., Eibak, E., Hegge, A.B., Rasmussen, K.E., Pedersen-Bjergaard, S., Gjelstad, A., 2012. Alginate and Chitosan Foam Combined with Electromembrane Extraction for Dried Blood Spot Analysis. Anal. Chem. 84, 8783–8789. https://doi.org/10.1021/ac301996n

Esteban, M., Schindler, B.K., Jiménez, J.A., Koch, H.M., Angerer, J., Rosado, M., Gómez, S., Casteleyn, L., Kolossa-Gehring, M., Becker, K., Bloemen, L., Schoeters, G., Hond, E. Den, Sepai, O., Exley, K., Horvat, M., Knudsen, L.E., Joas, A., Joas, R., Aerts, D., Biot, P., Borošová, D., Davidson, F., Dumitrascu, I., Fischer, M.E., Grander, M., Janasik, B., Jones, K., Kašparová, L., Larssen, T., Naray, M., Nielsen, F., Hohenblum, P., Pinto, R., Pirard, C., Plateel, G., Tratnik, J.S., Wittsiepe, J., Castaño, A., 2015. Mercury analysis in hair: Comparability and quality assessment within the transnational COPHES/DEMOCOPHES project. Environ. Res. 141, 24–30. https://doi.org/10.1016/j.envres.2014.11.014

European Medicines Agency, 2011. Guideline on bioanalytical method validation.

Faial, K., Deus, R., Deus, S., Neves, R., Jesus, I., Santos, E., Alves, C.N., Brasil, D., 2015. Mercury levels assessment in hair of riverside inhabitants of the Tapajós River, Pará State, Amazon, Brazil: fish consumption as a possible route of exposure. J. Trace Elem. Med. Biol. 30, 66–76. https://doi.org/10.1016/j.jtemb.2014.10.009

Fan, L., Lee, J. a, 2012. Managing the effect of hematocrit on DBS analysis in a regulated environment. Bioanalysis. https://doi.org/10.4155/bio.11.337

Funk, W.E., 2015. Use of Dried Blood Spots for Estimating Children's Exposures to Heavy Metals in Epidemiological Research. J. Environ. Anal. Toxicol. s7. https://doi.org/10.4172/2161-0525.S7-002

Funk, W.E., McGee, J.K., Olshan, A.F., Ghio, A.J., 2013. Quantification of arsenic, lead, mercury and cadmium in newborn dried blood spots. Biomarkers 18, 174–7. https://doi.org/10.3109/1354750X.2012.750379

Gibb, H., O'Leary, K.G., 2014. Mercury exposure and health impacts among individuals in the artisanal and small-scale gold mining community: A comprehensive review. Environ. Health Perspect. 122, 667–672. https://doi.org/10.1289/ehp.1307864

Glahder, C.M.; Appel, P.; Asmund, G., 1999. Mercury in Soap in Tanzania.

Grandjean, P., Weihe, P., White, R.F., Debes, F., Araki, S., Yokoyama, K., Murata, K., S??rensen, N., Dahl, R., J??rgensen, P.J., 1997. Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. Neurotoxicol. Teratol. 19, 417–428. https://doi.org/10.1016/S0892-0362(97)00097-4

Guimaraes, J.R.D., Betancourt, O., Miranda, M.R., Barriga, R., Cueva, E., Betancourt, S., 2011. Long-range effect of cyanide on mercury methylation in a gold mining area in southern Ecuador. Sci. Total Environ. 409, 5026–33. https://doi.org/10.1016/j.scitotenv.2011.08.021

Ha, E., Basu, N., Bose-O 'reilly, S., Dórea, J.G., Mcsorley, E., Sakamoto, M., Chan, H.M., 2016. Current progress on understanding the impact of mercury on human health. Environ. Res. 1–15. https://doi.org/10.1016/j.envres.2016.06.042

Harris, R.C., Rudd, J.W.M., Amyot, M., Babiarz, C.L., Beaty, K.G., Blanchfield, P.J., Bodaly,

R.A., Branfireun, B.A., Gilmour, C.C., Graydon, J.A., Heyes, A., Hintelmann, H., Hurley, J.P., Kelly, C.A., Krabbenhoft, D.P., Lindberg, S.E., Mason, R.P., Paterson, M.J., Podemski, C.L., Robinson, A., Sandilands, K.A., Southworth, G.R., St Louis, V.L., Tate, M.T., 2007. Whole-ecosystem study shows rapid fish-mercury response to changes in mercury deposition. Proc. Natl. Acad. Sci. U. S. A. 104, 16586–91. https://doi.org/10.1073/pnas.0704186104

Health Canada, 2015. Third report on human biomonitoring of environmental chemicals in Canada: Results of the Canadian Health Measures Survey Cycle 3 (2012-2013).

Health Canada, 2012. Guidance Document Conduct and Analysis of Comparative Bioavailability Studies. Ottawa.

Hempen, C.M., Koster, E.H.M., Ooms, J.A., 2015. Hematocrit-independent recovery of immunosuppressants from DBS using heated flow-through desorption. Bioanalysis.

Holub, M., Tuschl, K., Ratschmann, R., Strnadová, K.A., Mühl, A., Heinze, G., Sperl, W., Bodamer, O.A., 2006. Influence of hematocrit and localisation of punch in dried blood spots on levels of amino acids and acylcarnitines measured by tandem mass spectrometry. Clin. Chim. Acta 373, 27–31. https://doi.org/10.1016/j.cca.2006.04.013

Horvat, M., Snoj Tratnik, J., Miklavčič, A., 2012. Mercury: Biomarkers of Exposure and Human Biomonitoring, Biomarkers and Human Biomonitoring Vol.1: Ongoing Programs and Exposures.

Hursh, D. A., Wendt, S. F., Lee, C. F., et al., 1989. No Title. J. Clin. Microbiol 27, 1695–1696.

Ikingura JR, A.H., 1996. Monitoring of fish and human exposure to mercury due to gold mining in the Lake Victoria goldfields, Tanzania. Sci. Total Environ. 191, 59–68.

Kadjo, A.F., Stamos, B.N., Shelor, C.P., Berg, J.M., Blount, B.C., Dasgupta, P.K., 2016. Evaluation of Amount of Blood in Dry Blood Spots: Ring-Disk Electrode Conductometry.

Anal. Chem. 88, 6531–6537. https://doi.org/10.1021/acs.analchem.6b01280

Karagas, M.R., Choi, A.L., Oken, E., Horvat, M., Schoeny, R., Kamai, E., 2012. Review Evidence on the Human Health Effects of Low-Level Methylmercury Exposure. Environ. Health Perspect. 120, 799–806.

Kayiran, S.M., Ozbek, N., Turan, M., Gurakan, B., 2003. Significant differences between capillary and venous complete blood counts in the neonatal period. Clin. Lab. Haematol. 25, 9–16. https://doi.org/10.1046/j.1365-2257.2003.00484.x

Kershaw TG, Clarkson TW, D.P., 1980. The relationship between blood levels and dose of methylmercury in man. Arch Env. Heal. 1, 28–36.

Koster, R.A., Alffenaar, J.W., Botma, R., Greijdanus, B., Touw, D.J., Uges, D.R., Kosterink, J.G., 2015. What is the right blood hematocrit preparation procedure for standards and quality control samples for dried blood spot analysis? Bioanalysis 7, 345–351. https://doi.org/10.4155/bio.14.298 [doi]

Kurdland, L.T., Faro, S.N., Siedler, H., 1960. Minamata disease. The outbreak of a neurologic disorder in Minamata, Japan, and its relationship to the ingestion of seafood contaminated by mercuric compounds. World Neurol. 1, 370–395.

Landrigan, P.J., Fuller, R., Acosta, N.J.R., Adeyi, O., Arnold, R., Basu, N. (Nil), Baldé, A.B., Bertollini, R., Bose-O'Reilly, S., Boufford, J.I., Breysse, P.N., Chiles, T., Mahidol, C., Coll-Seck, A.M., Cropper, M.L., Fobil, J., Fuster, V., Greenstone, M., Haines, A., Hanrahan, D., Hunter, D., Khare, M., Krupnick, A., Lanphear, B., Lohani, B., Martin, K., Mathiasen, K. V., McTeer, M.A., Murray, C.J.L., Ndahimananjara, J.D., Perera, F., Potočnik, J., Preker, A.S., Ramesh, J., Rockström, J., Salinas, C., Samson, L.D., Sandilya, K., Sly, P.D., Smith, K.R., Steiner, A., Stewart, R.B., Suk, W.A., van Schayck, O.C.P., Yadama, G.N., Yumkella, K., Zhong, M., 2018. The Lancet Commission on pollution and health. Lancet.

https://doi.org/10.1016/S0140-6736(17)32345-0

Lawson, A.J., Bernstone, L., Hall, S.K., 2016. Newborn screening blood spot analysis in the uk: Influence of spot size, punch location and haematocrit. J. Med. Screen. 23, 7–16. https://doi.org/10.1177/0969141315593571

Lee, M.S., Li, W., 2014. Dried blood spots : applications and techniques.

Lenk, G., Hansson, J., Beck, O., Roxhed, N., 2015a. The effect of drying on the homogeneity of DBS. Bioanalysis.

Lenk, G., Sandkvist, S., Pohanka, A., Stemme, G., Beck, O., Roxhed, N., 2015b. A disposable sampling device to collect volume-measured DBS directly from a fingerprick onto DBS paper. Bioanalysis 7, 2085–2094. https://doi.org/10.4155/bio.15.134

Li, P., Feng, X., Shang, L., Qiu, G., Meng, B., Zhang, H., Guo, Y., Liang, P., 2011. Human co-exposure to mercury vapor and methylmercury in artisanal mercury mining areas, Guizhou, China. Ecotoxicol. Environ. Saf. 74, 473–9. https://doi.org/10.1016/j.ecoenv.2010.10.030

Li, W., Lee, M.S., 2014. Dried Blood Spots Wiley Series on Pharmaceutical Science and Biotechnology : Practices, Dried Blood Spots - Applications and Techniques. John Wiley & Sons, Hoboken, New Jersey.

Liao, H.-W., Lin, S.-W., Chen, G.-Y., Kuo, C.-H., 2016. Estimation and Correction of the Blood Volume Variations of Dried Blood Spots Using a Postcolumn Infused-Internal Standard Strategy with LC-Electrospray Ionization-MS. Anal. Chem 88. https://doi.org/10.1021/acs.analchem.6b01145

Liao, H.W., Lin, S.W., Lin, Y.T., Lee, C.H., Kuo, C.H., 2018. Identification of potential sphingomyelin markers for the estimation of hematocrit in dried blood spots via a lipidomic strategy. Anal. Chim. Acta 1003, 34–41. https://doi.org/10.1016/j.aca.2017.11.041

Mahaffey, K.R., Clickner, R.P., Bodurow, C.C., 2004. Blood Organic Mercury and Dietary Mercury Intake: National Health and Nutrition Examination Survey, 1999 and 2000. Environ. Health Perspect. 112, 562–570. https://doi.org/10.1289/ehp.6587

Malm, O., Castro, M.B., Bastos, W.R., Branches, F.J.P., Guimarães, J.R.D., Zuffo, C.E., Pfeiffer, W.C., 1995. An assessment of Hg pollution in different goldmining areas, Amazon Brazil. Sci. Total Environ. 175, 127–140. https://doi.org/10.1016/0048-9697(95)04909-6

Malsagova, K., Kopylov, A., Stepanov, A., Butkova, T., Izotov, A., Kaysheva, A., 2020. Dried blood spot in laboratory: Directions and prospects. Diagnostics. https://doi.org/10.3390/diagnostics10040248

Mcdade, T., 2013. Development and validation of assay protocols for use with dried blood spot samples. Am. J. Hum. Biol. 9, 1–9. https://doi.org/10.1002/ajhb.22463

McDade, T., Williams, S., Snodgrass, J., 2007. What a drop can do: Dried blood spots as minimally invasive method for integrating biomarkers into population-based research. Demography 44, 899–925.

Mei, J. V, Alexander, J.R., Adam, B.W., Hannon, W.H., 2001. Innovative Non- or Minimally-Invasive Technologies for Monitoring Health and Nutritional Status in Mothers and Young Children Use of Filter Paper for the Collection and Analysis of Human Whole Blood Specimens 1. J. Nutr. 131, 1631–1636.

Mengerink, Y., Mommers, J., Qiu, J., Mengerink, J., Steijger, O., Honing, M., 2015. A new DBS card with spot sizes independent of the hematocrit value of blood. Bioanalysis 7, 2095–2104. https://doi.org/10.4155/bio.15.133

Mergler, D., Anderson, H. a, Chan, L.H.M., Mahaffey, K.R., Murray, M., Sakamoto, M., Stern, A.H., 2007. Methylmercury exposure and health effects in humans: a worldwide concern.

Ambio 36, 3–11.

Miller IV, J.H., 2013. An On-card Approach for Assessment of Hematocrit on Dried Blood Spots which Allows for Correction of Sample Volume. J. Anal. Bioanal. Tech. 04, 1–8. https://doi.org/10.4172/2155-9872.1000162

Minnesota Department of Health Division of Environmental Health., 2007. Mercury Levels in Blood from Newborns in the Lake Superior Basin. (GLNPO ID 2007-942).

Nakahara, T., Otani, N., Ueno, T., Hashimoto, K., 2018. Development of a hematocrit-insensitive device to collect accurate volumes of dried blood spots without specialized skills for measuring clozapine and its metabolites as model analytes. J. Chromatogr. B 1087–1088, 70–79. https://doi.org/10.1016/j.jchromb.2018.04.019

National Center for Environmental Health, C., 2014. Laboratory Procedure Manual Inorganic Mercury, Methyl Mercury, Ethyl Mercury Blood mercury speciation.

National Health and Nutrition Examination Survey, 2018. NHANES 2015-2016: Mercury - Inorganic, Ethyl & Methyl - Blood Data Documentation, Codebook, and Frequencies [WWW Document].

National Health and Nutrition Examination Survey - NHANES, 2017. Fourth national report on human exposure to environmental chemicals-. Dep. Heal. Hum. Serv. Volume One.

Nelson, J.W., Edhlund, B.L., Johnson, J., Rosebush, C.E., Holmquist, Z.S., Swan, S.H., Nguyen, R.H.N., 2016. Assessing a new method for measuring fetal exposure to mercury: Newborn bloodspots. Int. J. Environ. Res. Public Health 13. https://doi.org/10.3390/ijerph13070692

Nelson, J.W., Edhlund, B.L., Johnson, J., Rosebush, C.E., Holmquist, Z.S., Swan, S.H., Nguyen, R.H.N., Theall, K.P., Johnson, C.C., n.d. Assessing a New Method for Measuring Fetal Exposure to Mercury: Newborn Bloodspots. https://doi.org/10.3390/ijerph13070692

Neoteryx, 2019. Mitra Microsampling devices [WWW Document]. URL https://www.neoteryx.com/micro-sampling-capillary-blood-collection-devices (accessed 6.2.20).

Neto, R., Gooley, A., Breadmore, M.C., Hilder, E.F., Lapierre, F., 2018. Precise, accurate and user-independent blood collection system for dried blood spot sample preparation. Anal. Bioanal. Chem. https://doi.org/10.1007/s00216-018-0993-y

NRC, 2000. Risk Characterization and Public Health Implications, in: Toxicological Effects of Methylmercury. National Academies Press, Washington, US, pp. 304–332.

Nti, A.A.A., Arko-Mensah, J., Botwe, P.K., Dwomoh, D., Kwarteng, L., Takyi, S.A., Acquah, A.A., Tettey, P., Basu, N., Batterman, S., Robins, T.G., Fobil, J.N., 2020. Effect of particulate matter exposure on respiratory health of e-waste workers at agbogbloshie, Accra, Ghana. Int. J. Environ. Res. Public Health 17. https://doi.org/10.3390/ijerph17093042

Nyanza, Elias., Bernier, F.P., Manyama, M., Hatfield, J., Martin, J.W., Dewey, D., 2019. Maternal exposure to arsenic and mercury in small-scale gold mining areas of Northern Tanzania. Environ. Res. 173, 432–442. https://doi.org/10.1016/j.envres.2019.03.031

Nyanza, Elias, Dewey, D., Bernier, F., Manyama, M., Hatfield, J., Martin, J.W., 2019. Validation of Dried Blood Spots for Maternal Biomonitoring of Nonessential Elements in an Artisanal and Small-Scale Gold Mining Area of Tanzania. Environ. Toxicol. Chem. 38, 1285–1293. https://doi.org/10.1002/etc.4420

O'Broin, S.D., Kelleher, B.P., Gunter, E., 1995. Evaluation of factors influencing precision in the analysis of samples taken from blood spots on filter paper. Clin. Lab. Haematol. 17, 185–8.

O'Mara, M., Hudson-Curtis, B., Olson, K., Yueh, Y., Dunn, J., Spooner, N., 2011. The effect of hematocrit and punch location on assay bias during quantitative bioanalysis of dried blood

spot samples. Bioanalysis 3, 2335–47. https://doi.org/10.4155/bio.11.220

Obrist, D., Kirk, J.L., Zhang, L., Sunderland, E.M., Jiskra, M., Selin, N.E., 2018. A review of global environmental mercury processes in response to human and natural perturbations: Changes of emissions, climate, and land use. Ambio 47, 116–140. https://doi.org/10.1007/s13280-017-1004-9

Patrick J. Parsons, Andrew A. Reilly, and D.E.-J., 1997. Screening children exposed to lead: an assessment of the capillary blood lead fingerstick test. Drug Monit. Toxicol. 2.

Pawly, J., Neitzel, R.L., Basu, N., 2019. Analysis of copper, selenium, and zinc in newborn dried bloodspots using total reflection X-ray fluorescence (TXRF) spectroscopy. PeerJ Anal. Chem. https://doi.org/10.7717/peerj-achem.1

Perkins, M., Basu, N., 2018. Dried blood spots for estimating mercury exposure in birds. Environ. Pollut. 236, 236–246. https://doi.org/10.1016/J.ENVPOL.2018.01.036

Pirrone, N., Cinnirella, S., Feng, X., Finkelman, R.B., Friedli, H.R., Leaner, J., Mason, R., Mukherjee, A.B., Stracher, G.B., Streets, D.G., Telmer, K., 2010. Global mercury emissions to the atmosphere from anthropogenic and natural sources. Atmos. Chem. Phys. 10, 5951–5964. https://doi.org/10.5194/acp-10-5951-2010

Poulin, J., Gibb, H., 2008. Mercury Assessing the environmental burden of disease at national and local levels. WHO Environ. Burd. Dis. 16, 1–42.

Rajaee, M., Long, R., Renne, E., Basu, N., 2015a. Mercury Exposure Assessment and Spatial Distribution in A Ghanaian Small-Scale Gold Mining Community. Int. J. Environ. Res. Public Health 12, 10755–10782. https://doi.org/10.3390/ijerph120910755

Rajaee, M., Obiri, S., Green, A., Long, R., Cobbina, S.J., Nartey, V., Buck, D., Antwi, E., Basu, N., 2015b. Integrated Assessment of Artisanal and Small-Scale Gold Mining In Ghana Part

2: Natural Sciences Review. Int. J. Environ. Res. Public Health 12, 8971–9011. https://doi.org/10.3390/ijerph120808971

Raju, K.S.R., Taneja, I., Rashid, M., Sonkar, A.K., Wahajuddin, M., Singh, S.P., 2016. DBS-platform for biomonitoring and toxicokinetics of toxicants: proof of concept using LC-MS/MS analysis of fipronil and its metabolites in blood. Sci. Rep. 6, 22447. https://doi.org/10.1038/srep22447

Resano, M., Belarra, M.A., García-Ruiz, E., Aramendía, M., Rello, L., 2018. Dried matrix spots and clinical elemental analysis. Current status, difficulties, and opportunities. https://doi.org/10.1016/j.trac.2017.12.004

Reynolds WA, P.R., 1975. Transplacental passage of methylmercury and its uptake by primate fetal tissues (38576). Proc Soc Exp Biol Med. 148, 523–526.

Richardson, G., Marshall, D., Keevil, B.G., 2018. Prediction of haematocrit in dried blood spots from the measurement of haemoglobin using commercially available sodium lauryl sulphate. Ann. Clin. Biochem. 2018, 55, 363–367. https://doi.org/10.1177/0004563217726809

Rodrigues, J.L., de Souza, S.S., de Oliveira Souza, V.C., Barbosa, F., 2010. Methylmercury and inorganic mercury determination in blood by using liquid chromatography with inductively coupled plasma mass spectrometry and a fast sample preparation procedure. Talanta 80, 1158–1163. https://doi.org/10.1016/j.talanta.2009.09.001

Rufail, M.L., McCloskey, L.J., Stickle, D.F., 2017. Estimation of hematocrit in filter paper dried bloodspots by potassium measurement: Advantage of use of perimeter ring samples over circular center sub-punch samples. Clin. Chem. Lab. Med. 55, 53–57. https://doi.org/10.1515/cclm-2016-0161

Sandborgh-Englund, G., Elinder, C.G., Johanson, G., Lind, B., Skare, I., Ekstrand, J., 1998. The

absorption, blood levels, and excretion of mercury after a single dose of mercury vapor in humans. Toxicol. Appl. Pharmacol. 150, 146–153. https://doi.org/10.1006/taap.1998.8400

Santa-Rios, A., Barst, B.D., Basu, N., 2020. Mercury Speciation in Whole Blood and Dried Blood Spots from Capillary and Venous Sources. Anal. Chem. https://doi.org/10.1021/acs.analchem.9b04407

Schulz, C., Wilhelm, M., Heudorf, U., Kolossa-Gehring, M., 2011. Update of the reference and HBM values derived by the German Human Biomonitoring Commission. Int. J. Hyg. Environ. Health 215, 26–35. https://doi.org/10.1016/j.ijheh.2011.06.007

Selin, N.E., 2009. Global Biogeochemical Cycling of Mercury: A Review. Annu. Rev. Environ. Resour. 34, 43–63. https://doi.org/10.1146/annurev.environ.051308.084314

Sheehan, M.C., Burke, T.A., Navas-Acien, A., Breysse, P.N., McGready, J., Fox, M.A., 2014. Global methylmercury exposure from seafood consumption and risk of developmental neurotoxicity: a systematic review. Bull. World Health Organ. 92, 254-269F. https://doi.org/10.2471/BLT.12.116152

Sherman, L.S., Blum, J.D., Basu, N., Rajaee, M., Evers, D.C., Buck, D.G., Petrlik, J., DiGangi, J., 2015. Assessment of mercury exposure among small-scale gold miners using mercury stable isotopes. Environ. Res. 137, 226–34. https://doi.org/10.1016/j.envres.2014.12.021

Shi, J.B., Liang, L.N., Jiang, G. Bin, Jin, X.L., 2005. The speciation and bioavailability of mercury in sediments of Haihe River, China. Environ. Int. 31, 357–365. https://doi.org/10.1016/j.envint.2004.08.008

Slemr, F., Brunke, E.-G., Ebinghaus, R., Temme, C., Munthe, J., Wängberg, I., Schroeder, W., Steffen, A., Berg, T., 2003. Worldwide trend of atmospheric mercury since 1977. Geophys. Res. Lett. 30, n/a-n/a. https://doi.org/10.1029/2003GL016954

Sletvold, H., Svendsen, K., Aas, O., Syversen, T., Hilt, B., 2012. Neuropsychological function and past exposure to metallic mercury in female dental workers. Scand. J. Psychol. 53, 136–143. https://doi.org/10.1111/j.1467-9450.2011.00929.x

Sommer, Yuliya L., Verdon, C.P., Fresquez, M.R., Ward, C.D., B., E.W., Yi Pan., Kathleen L, C., Robert, J., 2014. Measurement of Mercury Species in Human Blood using Triple Spike Isotope Dilution with SPME-GC-ICP-DRC-MS. Anal Bioanal Chem 406, 5039–5047. https://doi.org/10.1530/ERC-14-0411.Persistent

Sommer, Yuliya L, Verdon, C.P., Fresquez, M.R., Ward, C.D., Wood, E.B., Pan, Y., Caldwell, K.L., Jones, R.L., 2014. Measurement of mercury species in human blood using triple spike isotope dilution with SPME-GC-ICP-DRC-MS. Anal. Bioanal. Chem. 406, 5039–5047.

Sonke, J.E., Heimbürger, L.-E., Dommergue, A., 2013. Mercury biogeochemistry: Paradigm shifts, outstanding issues and research needs. Comptes Rendus Geosci. 345, 213–224. https://doi.org/10.1016/j.crte.2013.05.002

Spooner, N., Dennif, P., Michielsen, R. de V., Ji, Q.C., Arnold, M.E., Woods, K., Woolf, E.J., Xu, Y., Boutet, V., Zane, P., Kushon, S., Rudge, J.B., 2015. A device for dried blood microsampling in quantitative bioanalysis: overcoming the issues associated with blood hematocrit. Bioanalysis 7, 653–659. https://doi.org/10.4155/BIO.14.310

Spot on Sciences, 2020. Hemaspot HF Capillary Blood Collection Device | No Punching Required [WWW Document]. URL https://www.spotonsciences.com/hemaspot-hf (accessed 6.3.20).

Srigboh, R.K., Basu, N., Stephens, J., Asampong, E., Perkins, M., Neitzel, R.L., Fobil, J., 2016. Multiple elemental exposures amongst workers at the Agbogbloshie electronic waste (e-waste) site in Ghana. https://doi.org/10.1016/j.chemosphere.2016.08.089

State College, PA: Minitab, I. (www. minitab. com., 2010. Minitab 17 Statistical Software.

Steffen, A., Douglas, T., Amyot, M., Ariya, P., Aspmo, K., Berg, T., Bottenheim, J., Brooks, S., Cobbett, F., Dastoor, A., Dommergue, A., Ebinghaus, R., Ferrari, C., Gardfeldt, K., Goodsite, M.E., Lean, D., Poulain, A.J., Scherz, C., Skov, H., Sommar, J., Temme, C., 2008. A synthesis of atmospheric mercury depletion event chemistry in the atmosphere and snow. Atmos. Chem. Phys. 8, 1445–1482. https://doi.org/10.5194/acp-8-1445-2008

Suda, I., Takahashi, H., 1992. Degradation of methyl and ethyl mercury into inorganic mercury by other reactive oxygen species besides hydroxyl radical. Arch. Toxicol. 66, 34–39. https://doi.org/10.1007/BF02307267

Syversen, T., Kaur, P., 2012. The toxicology of mercury and its compounds. J. Trace Elem. Med. Biol. 26, 215–226. https://doi.org/10.1016/j.jtemb.2012.02.004

Takyi, S.A., Basu, N., Arko-Mensah, J., Botwe, P., Asabea, A., Nti, A., Kwarteng, L., Acquah, A., Tettey, P., Dwomoh, D., Batterman, S., Robins, T., Fobil, J.N., 2020. Micronutrient-rich dietary intake is associated with a reduction in the effects of particulate matter on blood pressure among electronic waste recyclers at Agbogbloshie, Ghana. BMC Public Health 20. https://doi.org/10.1186/s12889-020-09173-8

Telmer, K.H., Veiga, M.M., 2009. Mercury Fate and Transport in the Global Atmosphere, Mercury Fate and Transport in the Global Atmosphere: Emissions, Measurements and Models. Springer US, Boston, MA. https://doi.org/10.1007/978-0-387-93958-2

Timmerman, P., White, S., Globig, S., Lüdtke, S., Brunet, L., Smeraglia, J., 2011. EBF recommendation on the validation of bioanalytical methods for dried blood spots. Bioanalysis 3, 1567–1575. https://doi.org/10.4155/bio.11.132

U.S. Department of Health and Human Services, 2019. Fourth National Report on Human Exposure to Environmental Chemicals. Updated Tables, January 2019, Volume One. Fourth Natl. Rep. Hum. Expo. to Environ. Chem. 1–529.

U.S. Environmental Protection Agency, 1998. Method 1630: Methyl Mercury in Water by Distillation, Aqueous Ethylation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry.

UN Environment Programme, 2019. Global Mercury Assessment 2018. Geneva, Switzerland.

UN Environment Programme, 2018. Technical background report to the global mercury assessment 2018. Odder.

UN Environment Programme, 2013a. Global Mercury Assessment 2013: Sources, Emissions, Releases, and Environmental Transport, Narayana Press.

UN Environment Programme, 2013b. Minamata Convention on Mercury - Text and Annexes.

UN Environment Programme, 2012. Reducing Mercury Use in Artisanal and Small-Scale 76 pages.

UN Environment Programme, 2010. Addressing the Social and Financial Challenges of Artisanal and Small Scale Gold Mining: Meeting Summary.

UN Environment Programme, World Health Organization (WHO), 2008. Guidance for Identifying Populations At Risk From Mercury Exposure. Exposure 176.

US EPA, 2016. Definition and Procedure for the Determination of the Method Detection Limit—Revision 1.11. Code Fed. Regul. Title 40, Part 136, Append. B.

USAID, 2012. Biomarker field manual: Demographic and Health Surveys Methodology. Calverton, Maryland.

Utah Department of Epidemiology, 2014. Utah Statewide Investigation of Neonatal Blood Lead Levels Using Newborn Blood Spot Specimens.

Veiga, M., 1997. Mercury in artisanal gold mining in Latin America: Facts, fantasies and solutions. Pollut. Deriving from Artis. Gold Min. 1–23.

Veiga, M.M., Baker, R., 2004. Protocols for Environmental and Health Assessment of Mercury Released by Artisanal and Small-Scale Gold Miners, Unido.

Velghe, S., Delahaye, L., Stove, C.P., 2019. Is the hematocrit still an issue in quantitative dried blood spot analysis? J. Pharm. Biomed. Anal. https://doi.org/10.1016/j.jpba.2018.10.010

Velghe, S., Stove, C.P., 2018. Evaluation of the Capitainer-B Microfluidic Device as a New Hematocrit-Independent Alternative for Dried Blood Spot Collection. Anal. Chem. 90, 12893–12899. https://doi.org/10.1021/acs.analchem.8b03512

Verplaetse, R., Henion, J., 2016. Hematocrit-Independent Quantitation of Stimulants in Dried Blood Spots: Pipet versus Microfluidic-Based Volumetric Sampling Coupled with Automated Flow-Through Desorption and Online Solid Phase Extraction-LC-MS/MS Bioanalysis. Anal. Chem 88, 16. https://doi.org/10.1021/acs.analchem.6b01190

Vries, R. de, Barfield, M., Merbel, N. van de, Schmid, B., Siethoff, C., Ortiz, J., Verheij, E., Baar, B. van, Cobb, Z., White, S., Timmerman, P., 2013. The effect of hematocrit on bioanalysis of DBS: results from the EBF DBS-microsampling consortium. Bioanalysis.

Vries, R. De, Qin, C., Boutet, V., Kushon, S., Rudge, J.B., 2015. A device for dried blood microsampling in quantitative bioanalysis. Bioanalysis 7, 653–659. https://doi.org/10.4155/BIO.14.310

Wang, H.S., Xu, W.F., Chen, Z.J., Cheng, Z., Ge, L.C., Man, Y.B., Giesy, J.P., Du, J., Wong, C.K.C., Wong, M.H., 2013. In vitro estimation of exposure of Hong Kong residents to mercury and methylmercury via consumption of market fishes. J. Hazard. Mater. 248–249, 387–393. https://doi.org/10.1016/j.jhazmat.2012.12.060

Wang, Shaofeng, Jia, Y., Wang, Shuying, Wang, X., Wang, H., Zhao, Z., Liu, B., 2009. Total mercury and monomethylmercury in water, sediments, and hydrophytes from the rivers, estuary, and bay along the Bohai Sea coast, northeastern China. Appl. Geochemistry 24, 1702–1711. https://doi.org/10.1016/j.apgeochem.2009.04.037

World Health Organization (WHO), 2019. GEMS/Food Contaminants Mercury [WWW Document]. URL https://extranet.who.int/gemsfood/Search.aspx?Contaminant=mercury (accessed 4.6.20).

World Health Organization (WHO), 2013. Mercury Exposure and Health Impacts among Individuals in the Artisanal and Small-Scale Gold Mining (ASGM) Community. Prev. Dis. through Heal. Environ. 1–7.

World Health Organization (WHO), 2008. Methylmercury. Environmental Health Criteria. No. 101.

World Health Organization (WHO), 2003. Elemental mercury and inorganic mercury compounds: human health aspects. World Heal. Organ. Libr. 68.

World Health Organization (WHO), 1991. Inorganic Mercury.