

RESIDENTIAL MERCURY CONTAMINATION AND EXPOSURE
IN HUANCAVELICA, PERU

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ABSTRACT

Nicole A. Hagan: Residential Mercury Contamination and Exposure in Huancavelica, Peru
(Under the direction of J. Jason West)

Between 1564 and 1810, nearly 17,000 metric tons of mercury (Hg) vapor were released to the environment during cinnabar refining in the small town of Huancavelica, Peru. Much of this vapor deposited locally. The present study investigated the extent of residential Hg exposure today due to historic Hg contamination by: (1) determining total Hg concentrations in adobe bricks, dirt floors, surface dust, and air in 60 residences and comparing these concentrations across four different neighborhoods; (2) characterizing the species and bioaccessibility of Hg present in residences and evaluating potential exposure risks against international health benchmarks, and (3) characterizing and evaluating personal Hg exposures using total Hg concentrations in hair, total and speciated Hg measurements in residential samples, and self-reported questionnaire data.

Concentrations of total Hg in adobe bricks, dirt floors, and surface dust ranged from 8.00 to 1070 $\mu\text{g/g}$, 3.06 to 926 $\mu\text{g/g}$, and 0.02 to 9.69 $\mu\text{g/wipe}$, respectively, with significant differences across the four neighborhoods. A strong correlation between total Hg concentrations in adobe bricks and dirt floors confirmed that the bricks were likely made on-site.

Although total Hg concentrations in these residential samples were high, less than 10% of the total Hg was found to be soluble and bioaccessible following ingestion. Calculations for various exposure scenarios revealed that bioaccessible Hg concentrations in some households

exceed health benchmarks for soluble Hg. The apparent public health threat is much lower when standards are compared against the soluble Hg present, rather than against total Hg.

Total Hg concentrations in hair ranged from 0.10 to 3.6 $\mu\text{g/g}$, similar to concentrations found in the U.S. and lower than concentrations in other Hg-exposed populations around the world. Total Hg concentrations in hair were significantly related to gender ($p < 0.001$), living in a neighborhood where smelters were previously located ($p = 0.021$), smoking status ($p = 0.003$), frequency of house cleaning ($p = 0.019$), and frequency of fish consumption ($p = 0.046$).

A comprehensive analysis of residential Hg contamination and exposure in Huancavelica will guide the development and implementation of mitigation and remediation strategies in the community to reduce potential health risks from residential Hg exposure.

To the residents of Huancavelica, Peru

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CHAPTER 1. INTRODUCTION

This research presents results of a study that investigated residential mercury contamination and exposure in the city of Huancavelica, Peru. Mercury (Hg) exists in varying chemical forms and is present in all compartments of the environment. Cinnabar, or mercury sulfide (HgS), has historically been mined and refined into liquid Hg, resulting in contamination at these sites around the world. Residents of Huancavelica are exposed to Hg as a result of past cinnabar refining not only from the ambient environment, but also from building adobe brick houses using Hg-contaminated soil. Exposure to Hg from inhalation and ingestion may lead to adverse health outcomes, including neurological and renal effects.

1.1 Common chemical forms of mercury

In the environment mercury (Hg) exists in chemical and physical forms whose properties and chemical behaviors depend strongly on its oxidation state. These three primary oxidation states are elemental (Hg^0), mercurous (Hg_2^{2+}), and mercuric (Hg^{2+}). Elemental Hg exists as a heavy, silvery-white liquid under ambient temperatures and pressures that vaporizes under ambient conditions (Schroeder and Munthe 1998; US EPA 1997).

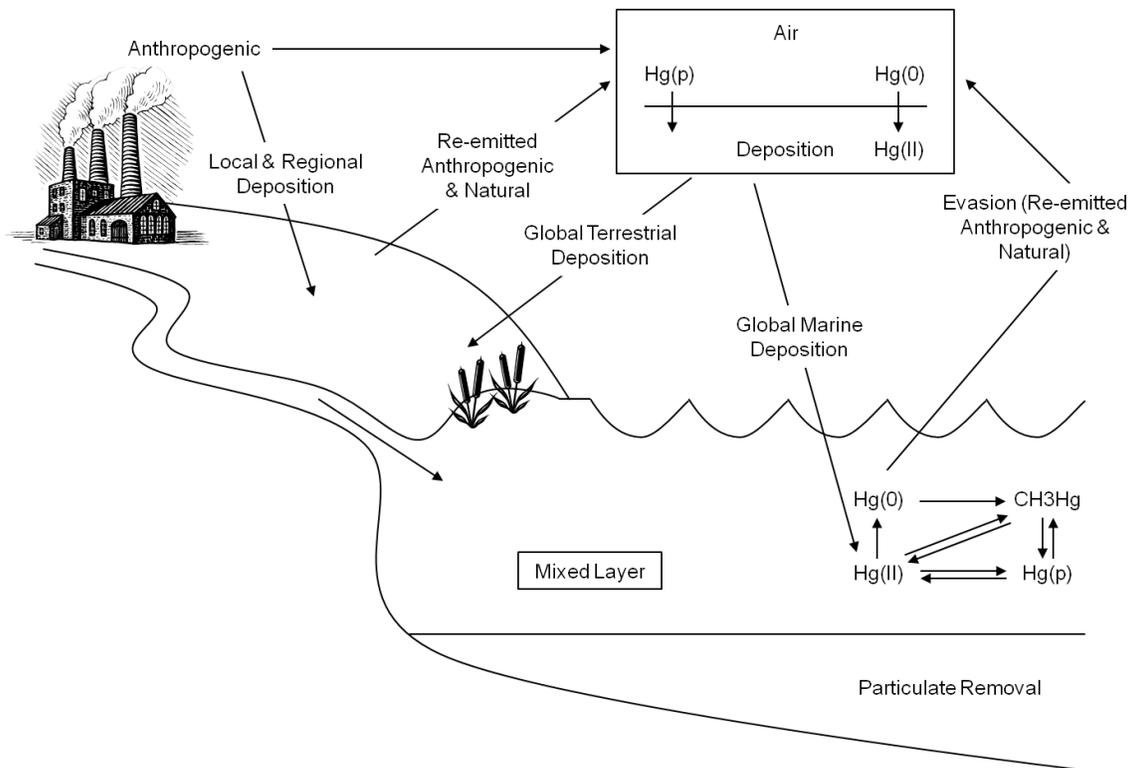
Although mercurous Hg is rarely stable in the environment, both mercurous and mercuric species of Hg can form many organic and inorganic compounds. Unlike other metals, Hg is more likely to form covalent bonds rather than ionic bonds. In the environment, a majority of the Hg found in water, soil, sediment, and biota exists as inorganic mercuric salts and organomercurics. Organomercurics are compounds that possess a covalent carbon-mercury (C-Hg) bond. The most

common Hg-containing compounds found in the environment include mercuric salts (i.e., HgCl_2 , $\text{Hg}(\text{OH})$), cinnabar (HgS), methylmercury compounds (CH_3Hg^+ , CH_3HgCH_3), methylmercuric chloride (CH_3HgCl) and methylmercuric hydroxide (CH_3HgOH) (US EPA 1997).

1.2 Mercury in the environment

Mercury is a naturally occurring element in the earth's crust and is the only metal that exists as a liquid at room temperature. Mercury is emitted into the environment from both natural and anthropogenic sources and is cycled between all compartments of the environment, as shown in Figure 1-1. Mercury can be emitted naturally via emissions from oceans and lakes, biomass burnings, volcanic eruptions, and geothermal activity (US EPA 1997). Anthropogenic emissions are often a result of combustion processes, including coal combustion, smelting operations for other metals (e.g., gold, copper, lead, and zinc), cement production, and waste incineration

Figure 1-1. Global mercury cycle (Source: adapted from US EPA 1997)



(Pirrone et al. 2010; UNEP 2002, 2008). Mercury is transformed and transported in the air, deposited onto the earth's surface, and stored in or emitted by soil, water, and air, being continuously cycled in the environment and oxidized and reduced to different species of Hg.

1.2.1 Mercury in the atmosphere

The four basic processes involved in the fate and transport of Hg in the atmosphere include: emission to the atmosphere, transformation and transport in the atmosphere, deposition from the air, and reemission to the atmosphere. Natural emissions occur primarily as the elemental Hg species. Naturally occurring emissions of Hg are the result of either pre-industrial equilibrium of Hg (e.g., flux from soil) or mobilization of Hg from geologic deposits (e.g., volcanic eruptions). Anthropogenic emissions of Hg include both gaseous and particulate mercury. Gaseous Hg includes elemental and divalent species of Hg, whereas particulate Hg is primarily divalent Hg (US EPA 1997). Present-day global emissions of Hg are estimated at 7527 metric tons per year; approximately 69% of these emissions result from natural sources while 31% are released from anthropogenic sources (Pirrone et al. 2010).

Once emitted to the atmosphere, Hg is subjected to transformation and transport. Elemental Hg has an average atmospheric residence time of one year and is distributed evenly throughout the troposphere. Divalent Hg can be quickly deposited through both wet and dry deposition, resulting in a residence time ranging from hours to months. Some fine particles that contain divalent Hg, however, can have greater residence times and remain in the atmosphere for up to a year (US EPA 1997). Gaseous and particulate divalent Hg dry deposit at significant rates if Hg compounds are present in the atmosphere. The deposition velocity of particulate Hg is strongly dependent on particle size and atmospheric conditions. Divalent Hg has a lower Henry's law constant than elemental Hg, which suggests that divalent Hg more strongly partitions into

the water phase than the air phase. Gaseous and particulate forms of divalent Hg are scavenged by precipitation and washed out via wet deposition. Because of its reactivity and water solubility, the removal of gaseous divalent Hg occurs more readily and effectively than the removal of particulate divalent Hg (Gochfeld 2003; US EPA 1997).

Elemental Hg does not undergo any of the major processes of deposition in the atmosphere because of its high vapor pressure and low water solubility. However, potential removal mechanisms from the atmosphere include plant-leaf uptake and transformations in cloud water. Studies in forest canopies have found that gas exchange at the leaf surface followed by the assimilation of Hg in the interior of the leaf during daylight hours can cause the accumulation of elemental Hg vapor (US EPA 1997). However, this process only occurs if the atmospheric concentration of elemental Hg vapor is greater than the concentration in the local ecosystem. If the concentration of elemental Hg is greater in the ecosystem, the forest canopy acts as a source and releases elemental Hg vapor into the atmosphere (US EPA 1997).

Gaseous elemental Hg can be transformed into divalent Hg (gaseous and particulate forms) in cloud water. This transformation leads to the deposition of divalent Hg onto land and water that can occur far from the original emission source because of the slow uptake of elemental Hg by the cloud water. In terms of Hg pollution, this mechanism has been suggested to be globally important, whereas local pollution is more often the result of direct wet deposition of anthropogenic divalent Hg (US EPA 1997).

Mercury compounds can also be reemitted to the atmosphere after deposition. One significant source of Hg re-emission is from oceans, where anthropogenic divalent Hg emissions are deposited and reduced to volatile Hg vapor and re-released into the atmosphere. Additionally, Hg compounds in soil can be reduced to elemental Hg and released back into the atmosphere

(US EPA 1997). Thus, under certain conditions, sinks of atmospheric Hg become sources of atmospheric Hg.

1.2.2 Mercury in soil

Once deposited from the atmosphere onto the earth's surface, divalent Hg species can undergo many chemical and biological transformations. Soil conditions, including temperature, humic content, and pH, promote the production of inorganic divalent Hg compounds, including HgCl_2 , $\text{Hg}(\text{OH})_2$, and inorganic divalent compounds containing organic anions. In general, inorganic divalent Hg compounds are soluble and mobile. However, in soil, these compounds interact with organic matter, primarily humic and fulvic acids, and mineral colloids and become less soluble and immobile. Most of the Hg present in soil is bound to organic matter and is only released in runoff as a result of being attached to suspended humus or soil present in runoff water. Dissolvable organic ligands and other types of dissolved organic carbon can have Hg absorbed onto their surfaces and in this case, Hg can be released in runoff as the dissolved phase (US EPA 1997).

Although divalent Hg and its compounds account for 97% to 99% of Hg found in soils, elemental Hg and organomercurials can result from reactions in the soil. Elemental Hg can be formed in soil through reduction of divalent species by humic substances and photolysis. Elemental Hg can diffuse through the soil and be reemitted into the atmosphere. In the presence of microbial reactions, methylmercury can form from the transformation of divalent Hg compounds. Methylmercury is bound to organic matter and accounts for approximately 1% to 3% of the total Hg found in soil (US EPA 1997).

1.3 History of cinnabar mining

Cinnabar (or mercury sulfide, HgS) is a bright red crystalline mineral found throughout the earth's crust. Cinnabar, also known as vermilion due to its brilliant red color, has historically been used in paints, felting, and medicine by many cultures around the globe. While mined in limited quantities in pre-Colombian times, it was with the introduction of the process of amalgamation for silver ores in the 1550s that cinnabar ore was mined and refined in large quantities (Lane 2012). In the earth's crust, Hg occurrence is approximately 0.07-0.08 parts per million (Lane 2012) and profitable mining of Hg requires concentrated ore deposits that are typically found as mercury (II) sulfide or cinnabar. Mining and refining of cinnabar and resulting Hg contamination has been best documented at large mines such as Almadén, Spain, and Idrija, Slovenia (Lane 2012).

The Almadén mine was the world's largest Hg mine and occupies nearly 100 km² in Central Spain. Before closing in the early 2000s, the Almadén mine produced 285,000 metric tons of Hg over 2000 years, or nearly 30% of the global production (Millán et al. 2006; Molina et al. 2006). However, estimates suggest that up to 25% of the total Hg produced was released into the environment during the refining process as documented by Hg soil contamination near the Almadén mine (Conde Bueno et al. 2009; Higuera et al. 2006; Llanos et al. 2011; Millán et al. 2006). Studies of the Almadén soil have shown Hg concentrations that range from 0.13 µg/g to nearly 8900 µg/g (Bernaus et al. 2005; Conde Bueno et al. 2009; Higuera et al. 2006; Millán et al. 2006; Molina et al. 2006).

The Idrija Hg mine, located near Ljubljana, Slovenia, was the second largest Hg producing mine in the world. Mining operations began in the early 1490s and continued until 1994, producing nearly 107,000 metric tons of Hg during this period (Gosar et al. 1997; Kocman

et al. 2004). Environmental releases of Hg routinely occurred with an estimated 33,000 metric tons of Hg lost during the refining process (Gosar et al. 1997). Recent studies of Hg concentrations in the soils of Idrija have ranged from 0.39 µg/g to nearly 2800 µg/g (Gnamuš et al. 2000; Kocman et al. 2004; Palinkaš et al. 1995).

The Santa Barbara mine near Huancavelica, Peru, was the largest Hg deposit in the New World and the fourth largest globally. Prior to 1532 the Incas mined the area on a limited basis (Cooke et al. 2009; Wise and Féraud 2005), but the rediscovery of cinnabar ore by Spanish colonists in 1564 initiated large scale mining and refining operations in the nearby city of Huancavelica (Robins 2011; Wise and Féraud 2005).

The refining operations in Huancavelica differed from those in Almadén and Idrija in that the residents of the densely populated town of Huancavelica lived and worked within the shadows of the cinnabar smelters (Robins 2011; Robins and Hagan 2012). Researchers have estimated that 68,000 metric tons of Hg were produced in Huancavelica during the 246 year Spanish colonial period (Robins 2011; Robins et al. 2012). Although legacy soil contamination from historic cinnabar refining has been studied in great detail in Almadén and Idrija, environmental studies in Huancavelica were not undertaken until 2009.

1.4 Mercury contamination in Huancavelica

Mercury refining in Huancavelica involved the extraction and crushing of cinnabar ore, followed by a smelting process in which the Hg was volatilized and collected. The liquid mercury was then shipped to the Andean silver mining centers near Potosí, Bolivia where it was used in an amalgamation process with crushed silver ore to produce high grade silver. Thus, Hg was a critical component in the development and expansion of Spain's global economy. Because the silver amalgamation process required approximately two parts Hg per one part silver

produced, large quantities of liquid Hg were needed (Bakewell 1975; Hagan et al. 2011; Robins and Hagan 2012).

Problems developed in Huancavelica because the crushing operations were dusty, and the Hg vapor and liquid routinely escaped from the inefficient smelters. Colonial records of Hg production in Huancavelica, adjusted for contraband (or unreported production), report that approximately 68,000 metric tons of Hg were produced between 1564 and 1810 (Arena 1901; Brown 1988; Caravantes 1989; Fisher 1977; Lohmann Villena 1999; Patiño Paúl Ortíz 2001; Whitaker 1941) and Hg vapor emissions from this period were estimated at 17,000 metric tons (Robins 2011; Robins and Hagan 2012; Robins et al. 2012). The majority of the vaporous and liquid Hg likely deposited in the vicinity of the smelters, ultimately contaminating the entire community of Huancavelica.

This legacy continues to haunt the present 42,000 residents of Huancavelica who are faced with living with over 400 years of Hg contaminated soil (Robins 2011; Robins et al. 2012.) Many of these residents are impoverished and not aware of the high levels of Hg contamination throughout the city; those who are informed lack the resources to alleviate the problem. Results of a recent study of ambient soils from 20 locations in Huancavelica showed that Hg concentrations ranged from 1.75 to 689 $\mu\text{g/g}$ (Robins et al. 2012); these Hg concentrations are among the highest urban soil concentrations in the world. Because over 80% of residents in Huancavelica live in adobe brick homes fabricated on-site from this contaminated soil, concern exists for residential exposure (Mendoza 2012). Problems may arise because the interior walls and floors are often unsealed and uncovered, which allows Hg to emanate in both particulate and vapor form. Inhalation of particulates and Hg vapor and ingestion from settled particles on food

and surfaces can cause serious neurological and other health problems, especially for children and pregnant women.

1.5 Health effects of mercury

As with all compounds, the chemical species, exposure route, and exposure duration are important in determining health effects of Hg. Regulatory efforts to reduce health impacts of Hg in the United States have generally focused on controlling elemental Hg vapor emissions, particularly from power plants and other industrial processes. However, all species of Hg pose a human health risk, with organomercurials thought to be the most toxic species, particularly from consumption of contaminated fish (Revis et al. 1990). Methylmercury (CH_3Hg^+) toxicity has been studied in great detail due to exposures (mainly through dietary consumption of seafood and shellfish) across large segments of the population (NRC 2000). However, in the contaminated residential setting of Huancavelica, inhalation of vapor and ingestion of particulates are the major pathways of Hg exposure for residents.

Following the inhalation of elemental Hg (Hg^0) vapor, up to 80% of inhaled elemental mercury is absorbed by the lungs and rapidly distributed throughout the body (Environment Agency 2009). Once absorbed into the bloodstream, elemental Hg is oxidized in the red blood cells to divalent Hg, a largely non-diffusible form of Hg that binds to globular proteins including albumin and globulins (Environment Agency 2009; Klaassen et al. 2008). Molecules of elemental Hg readily cross placental and blood-brain barriers and can be retained in brain tissue (EPA 1997; Klaassen et al. 2008). Exposure to elemental Hg vapor can cause both physical and psychological symptoms, particularly relating to the central nervous system, that include tremors, excess salivation, irritability, anxiety, and difficulty concentrating (ATSDR 1999). Elemental Hg has a half-life in the body of one to two months before elimination primarily through urine and

feces, although sweat, saliva, and expired air may also excrete some of the Hg (Environment Agency 2009; Klaassen et al. 2008).

Inorganic divalent Hg (Hg^{2+}), often found in abundance in soils, can be ingested, absorbed through the gastrointestinal tract, and distributed to all tissues. Absorption can vary depending on the type of Hg^{2+} mineral phase ingested. Studies have shown that 100% of mercuric chloride can be absorbed by the gastrointestinal tract (Welfringer and Zagury 2009), while absorption of up to 20% has been reported for other forms of Hg^{2+} (EPA 1997; Klaassen et al. 2008). The most critical toxicological endpoint for ingestion of inorganic Hg^{2+} is the kidney, with symptoms that range from pale and swollen kidneys, proteinuria, and decreased urine production to complete renal failure (ATSDR 1999). Inorganic Hg has a half-life of one to two months prior to excretion in urine and feces; the major excretion route following chronic exposure to low doses of inorganic Hg is through urine (Environment Agency 2009; Klaassen et al. 2008).

1.6 Motivation

With the financial support of a Duke Global Health Institute pilot grant, a trans-disciplinary, inter-institutional study was launched in 2010 to investigate residential Hg contamination and exposure in Huancavelica, Peru, and Potosí, Bolivia. Researchers from North Carolina State University, Duke University, The University of North Carolina at Chapel Hill, and the U.S. Environmental Protection Agency began a collaborative study that combined detailed Spanish records of Hg production in Huancavelica and Hg consumption in Potosí with modern science to evaluate: (1) the historical exposures to Hg from the refining processes, and (2) the lasting consequences to current and future residents of these towns. As a part of this much larger

study, my research project was designed to characterize and evaluate residential Hg contamination in Huancavelica, Peru.

1.7 Objectives

The purpose of the research in this thesis was to investigate the extent of residential exposure to Hg contamination from historic cinnabar refining in Huancavelica, Peru, and to assess personal mercury exposures from indoor sources. The overall objective was pursued as three specific research objectives:

- i. Characterize the spatial distribution of Hg in residences by (a) determining the total Hg concentrations from multiple samples of adobe bricks, dirt floors, surface dust, and vapor collected from 60 residences in four distinct neighborhoods, and (b) comparing the concentrations across the 60 residences with respect to the four different neighborhoods studied.
- ii. Characterize the Hg present in residences and determine its bioaccessibility, and evaluate potential exposure risks against international health benchmarks and cleanup values for Hg.
- iii. Characterize and evaluate personal Hg exposure using total Hg concentrations in hair, total and speciated Hg measurements in residential samples, and self-reported questionnaire data to support the development of a risk-based method to prioritize mitigation or intervention strategies in Huancavelica.

Chapter 2 of this thesis details the study of Hg contamination in adobe homes. In this study, total Hg concentrations were measured in adobe brick, dirt floor, surface dust, and air samples from the interior of 60 adobe houses located in four distinct neighborhoods of Huancavelica. Colonial records showed that cinnabar smelters had operated in three of the four

neighborhoods. All samples were compared to control samples taken from Ayacucho, Peru, a non-mining town 150 kilometers southeast of Huancavelica. Concentrations of total Hg in adobe bricks, dirt floors, and surface dust ranged from 8.00 to 1070 $\mu\text{g/g}$, 3.06 to 926 $\mu\text{g/g}$, and 0.02 to 9.69 $\mu\text{g/wipe}$, respectively, with statistically significant differences between the three neighborhoods and the non-smelting neighborhood. Concentrations of Hg in adobe brick and dirt floor samples in Huancavelica were orders of magnitude higher than in the control, non-mining town. The strong correlation between total Hg concentrations in adobe bricks and dirt floors confirmed that adobe bricks were likely made on-site and were not purchased from an off-site, uncontaminated source. A strong correlation between surface dust and adobe bricks and dirt floors indicates that walls and floors serve as indoor sources of Hg contamination. Elemental Hg vapor concentrations were below detection ($<0.5 \mu\text{g/m}^3$) in most homes; however, in homes with detectable levels, concentrations up to $5.1 \mu\text{g/m}^3$ were observed. No statistically significant differences in Hg vapor measurements were observed between neighborhoods. This study demonstrates that the adobe bricks may be a substantial source of residential Hg exposure, especially in historic silver or gold refining communities where Hg was produced or used for amalgamation in artisanal gold or silver production. These results have been presented at the International Conference on Mercury as a Global Pollutant (2011) and the Society of Environmental Toxicology and Chemistry (2012) meetings and published in the peer-reviewed journal PLoS ONE in September 2013.

Chapter 3 describes the speciation and bioaccessibility of Hg in residential samples from adobe brick homes in Huancavelica. In this study, Hg was measured in adobe brick and dirt floor samples from 60 households in Huancavelica using total Hg extractions, simulated gastric fluid (GI) extractions, and sequential selective extractions (SSEs) that provide more detailed data but

are resource intensive. Most Hg present in samples was relatively insoluble, although in some households, soluble Hg species were present in water soluble (F1), 'human stomach acid' soluble (F2), and organo-chelated (F3) fractions of SSEs. The percent of the total Hg extracted in the simulated GI extractions for adobe bricks and dirt floors was up to 7.4% and 6.8%, respectively. While the bioaccessible Hg is only a small fraction of the total Hg present in a sample, there is still potential for concern following ingestion. A strong correlation was identified between simulated GI extractions for adobe bricks and dirt floors and the more soluble fractions of Hg from SSEs. This is an important finding, in that simulated GI extractions have the potential to be used as a screening tool to target time and resources effectively by performing SSEs on samples that have measurable concentrations of bioaccessible Hg. By combining information related to body weights and ingestions rates for children and health benchmarks for oral exposure to Hg, it was possible to calculate a Level of Soil Contamination (LSC) for varying exposure scenarios. These calculations revealed that Hg concentrations in simulated GI extractions for adobe brick and dirt floor samples in some households exceed health benchmarks for soluble Hg, although the magnitude of the potential public health impact is much lower than when comparing total Hg. Combining simulated GI extraction data with health benchmarks permits intervention strategies targeted to households with the greatest potential health threat from ingestion of Hg-contaminated particles. These results have been presented at the Society of Environmental Toxicology and Chemistry (2012, 2013) meetings and were submitted to the peer-reviewed journal *Environmental Geochemistry and Health* in February 2014.

Chapter 4 characterizes individual exposure to mercury using total and speciated Hg from residential samples, total Hg in hair, and self-reported questionnaire data regarding factors influencing exposure (e.g., frequency of fish consumption, occupation). Total Hg concentrations

in hair from 118 participants ranged from 0.10 to 3.6 $\mu\text{g/g}$, similar to concentrations found in the U.S. and lower than concentrations in other Hg-exposed populations around the world. Pearson correlation coefficients for data in this study suggest that, while there is a positive association between concentrations of total Hg in hair and concentrations of total Hg in adobe bricks, dirt floors, and surface dust, these correlations are not statistically significant. Results of a one-way analysis of variance (ANOVA) identified that total Hg concentrations in hair were significantly related to gender ($p < 0.001$), living in a neighborhood where smelters were previously located ($p = 0.021$), smoking status ($p = 0.003$), frequency of house cleaning ($p = 0.019$), and frequency of fish consumption ($p = 0.046$). These results highlight the need for future studies to better characterize Hg exposure in Huancavelica, particularly as related to residential contamination. A comprehensive analysis of residential Hg contamination and exposure in Huancavelica will guide the development and implementation of mitigation and remediation strategies in the community to reduce potential health risks from residential Hg exposure. These results have been presented at the Society of Environmental Toxicology and Chemistry (2013) meeting and are currently under review for submission to the peer-reviewed journal *Environmental Geochemistry and Health* in April 2014.

Chapter 5 discusses the key scientific findings of the research resulting from this dissertation. The future research questions identified by this research are identified. Finally, the implications of the findings of this study in future intervention and remediation strategies to reduce exposure to Hg are identified and discussed.

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CHAPTER 2. RESIDENTIAL MERCURY CONTAMINATION IN ADOBE BRICK HOMES IN HUANCAVELICA, PERU¹

2.1 Introduction

Legacy mercury (Hg) contamination resulting from cinnabar mining and refining, particularly in ambient soils, has been studied in various parts of the world, most notably Almadén, Spain (Higuera et al. 2006; Molina et al. 2006) and Idrija, Slovenia (Gnamuš et al. 2000). In Almadén, Hg concentrations in ambient soil have varied across studies, ranging from less than 1 µg/g (Molina et al. 2006) to over 8800 µg/g (Higuera et al. 2006), while in Idrija measured concentrations have ranged from less than 1 to over 2700 µg/g (Gnamuš et al. 2000). Huancavelica was the site of one of the largest urban cinnabar refining operations, yet legacy contamination was not studied in the city until 2009 (Robins 2011; Robins and Hagan 2012; Robins et al. 2012). In a previous study, we reported Hg concentrations in ambient soil ranging up to 1200 µg/g (Robins et al. 2012), among the highest concentrations noted in the literature. It is important to understand the extent of this contamination and characterize the Hg present to estimate potential risks to the local populations from inhalation of Hg offgassing as vapor or from inhalation or ingestion of contaminated soil particles. This issue is especially important for children who tend to ingest more soil and dust than adults as a result of hand to mouth activity.

¹ This chapter was published in its original form in *PLoS One* and is distributed under the terms of the Creative Commons Attribution License, which permits reproduction as a part of this dissertation.

Hagan N, Robins N, Hsu-Kim H, Halabi S, Espinoza Gonazales RD, Richter D, et al. 2013. Residential mercury contamination in adobe brick homes in Huancavelica, Peru. *PLoS One* 8:e75719.

Huancavelica, Peru is located at an elevation of 3660 m in the Andes, where for over 350 years it served as the primary source of Hg used for amalgamation-based silver production in South America (Cooke et al. 2009; Robins 2011). The 1563 discovery of cinnabar ore (HgS) in the Santa Barbara Hill just outside of Huancavelica provided a vast economic resource for Spain because Hg was essential to silver refining. To refine Hg, extracted cinnabar ore was crushed and smelted, a process in which the Hg was volatilized, collected, and shipped to Andean silver mining centers (Robins 2011). Extensive environmental contamination occurred in Huancavelica because the Hg vapor and liquid would routinely escape from the inefficient smelters located in the city. Based on colonial records, 17,000 metric tons of Hg vapor were emitted in Huancavelica between 1564 and 1810 (Robins 2011; Robins and Hagan 2012). Most of the Hg vapor likely deposited in and around the city, resulting in extremely high levels of urban contamination.

In Huancavelica, exposure to Hg contamination may be intensified compared to other cinnabar refining areas like Almadén and Idrija. Huancavelica is the capital of the most impoverished department of Peru where its 42,000 inhabitants live with the toxic legacy of over 400 years of Hg contamination. Most residents are not aware of the high levels of Hg to which they may be exposed or the health effects of Hg contamination; those who are informed often lack the resources to alleviate the problem. Health effects may be exacerbated as more than 80% of homes in the city are constructed with adobe bricks fabricated on-site from contaminated soil (Mendoza 2012). Problems arise because the interior walls and floors are generally unsealed, allowing Hg to emanate in both particulate and vapor form into what is usually a poorly ventilated space. Unlike residents of Almadén and Idrija, who live in homes constructed using modern materials such as masonry brick, residents in Huancavelica are exposed to Hg not only

from ambient contamination but also from the contaminated interior walls and floors of their homes. This study was undertaken to determine the levels of Hg to which the current residents of adobe brick homes in Huancavelica are exposed. Its goal was to better understand the primary sources of Hg within residences, and the interrelationships among these sources.

The results of this study have broad application to other areas in the world that use Hg for artisanal silver and gold refining and that construct buildings and homes from adobe brick, such as Madre de Dios and Puerto Maldonado, both also in Peru. It is important to identify the primary indoor sources of Hg and the distribution of contamination across the city with respect to historic sources, both to characterize exposure and potential health outcomes, as well as to develop and evaluate mitigation and remediation efforts.

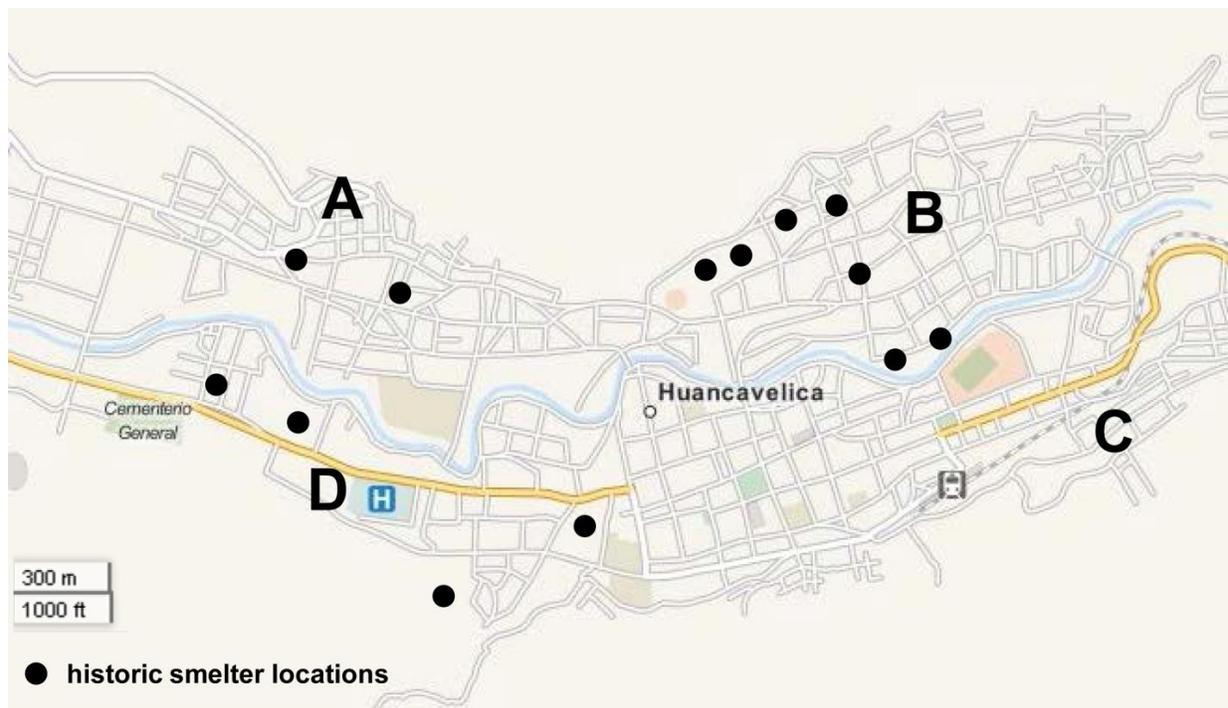
2.2 Methods

Figure 2-1 shows a map of the historic smelter locations and neighborhoods in Huancavelica. We expected to find higher Hg concentrations in samples from homes located in neighborhoods with historic cinnabar smelters (Ascencion (A), San Cristóbal (B), and Yananaco (D), shown in Figure 2-1) as compared to Santa Ana (C), a neighborhood with no historic cinnabar refining. We also expected to find similar concentrations of Hg in adobe bricks and dirt floors, assuming adobe bricks were made on-site. Moreover, we expected Hg concentrations in surface dust to be correlated with concentrations in adobe bricks and dirt floors, as we hypothesized that surface dust was primarily coming from indoor sources. We did not expect to find significant differences in Hg vapor concentrations between neighborhoods because vapor is relatively mobile.

2.2.1 Sample collection

Samples of interior adobe brick, dirt floor, surface dust, and vapor were collected from 60 residences in Huancavelica, Peru in August 2010. These samples were collected from 15 homes in each of the four neighborhoods, Ascención, San Cristóbal, Santa Ana and Yananaco, shown in Figure 2-1. These neighborhoods were selected based on the historical location of cinnabar smelters and the results of previous ambient soil sampling (Robins et al. 2012). Participants were recruited from public community meetings and interactions; verbal consent for participation was given by an adult resident or homeowner. Consent was recorded through the inclusion of the participant on the field sampling log. Institutional review board (IRB) approval for the study and verbal consent was obtained through Duke University.

Figure 2-1. Map of historic smelter locations and neighborhoods sampled in 2010 in Huancavelica, Peru



In each home, samples were collected from the room where residents spent the most time. Triplicate samples were collected from the same room of each house. Adobe brick samples were

obtained by scraping approximately 20 g of surface material to a depth of 2.5 cm from the interior adobe brick walls from three locations in the room. Dirt floor samples were collected by removing approximately 20 g of surface soil from the floor to a depth of 2.5 cm from three locations in the same room. In homes that had solid sealants on floors (e.g., wood or concrete), floor samples were obtained from the dirt patio immediately outside of the home (less than 10% of homes in the study). Triplicate samples of the adobe brick walls and dirt floors were not pooled; each sample was stored individually in a specimen bag. Surface dust samples were obtained by wiping three separate 100 cm² areas of a hard, flat surface (e.g., kitchen table, dresser) with three moistened smear tabs (Whatman Low Ash Grade 50 Filter Paper, Fisher Scientific). Each smear tab was folded with the sample side inward and stored in a separate specimen bag. Once transported, all samples were stored at 4 °C in the laboratory until analysis.

Control samples were collected in Ayacucho, a non-mining town in Peru, located about 150 km southeast of Huancavelica. Triplicate samples of exterior adobe brick scrapings were collected from three homes and duplicate samples of ambient soil were collected from five homes using the same sampling and storage methods used in Huancavelica.

In situ mercury vapor measurements were recorded using a Jerome J405 Mercury Vapor Analyzer (Arizona Instrument LLC, Chandler, AZ). The instrument was purged outside each home prior to collecting measurements indoors. Three measurements were recorded for each household. Measurements below the limit of detection (LOD, 0.5 µg/m³) were set to LOD divided by the square root of 2 (resulting in 0.35 µg/m³ used for below LOD samples) when averaging the data.

2.2.2 Sample preparation and Hg analysis

Adobe brick, dirt floor, and surface dust wipe samples were digested by hot block extraction in 4:1 HCl:HNO₃ at 85°C for five hours. After digestion, the extract was diluted in 1% bromine monochloride and analyzed for total Hg content by stannous chloride reduction, gold amalgamation, cold vapor atomic fluorescence spectrometry (EPA Method 1631) (US EPA 2002) using a Brooks Rand MERX-T (Brooks Ran Labs, LLC, Seattle, WA). Instrument calibrations were performed with an acidified mercuric nitrate stock solution. A NIST-certified standard reference material (2709 San Joaquin soil) was digested in parallel with each batch of samples. The average recovery of total Hg in the standard reference material (SRM) was 107 ± 14% (n=21). Recovery of Hg from the SRM was within the acceptable range of the certified value (1.4 ± 0.08 µg/g).

2.2.3 Statistical analysis

Total Hg concentrations were tested for normality and were found to be non-normally distributed; therefore, the data were log-transformed prior to statistical analyses. Diagnostic tests for fit (scatter plots of residuals, absolute residuals, and observed responses by predicted values; studentized residuals by leverage; Cook's D by observation; a Q-Q plot of residuals; a residual histogram; and a residual-fit spread plot) indicated that there were no true outliers in the data set following log-transformation. Results of a three-way analysis of variance (ANOVA) suggested that differences in Hg concentrations for all sample types were driven by neighborhood and household variability. Statistically significant differences were not found between the triplicate samples; therefore, concentrations of Hg in all triplicate samples were averaged for each sample type within a home.

Results shown in figures and tables are arithmetic means. Descriptive statistics were computed and boxplots were used to summarize Hg concentrations. The values represented in the boxplots are the first quartile, median, and third quartile. The whiskers are the minimum and maximum values and the diamonds represent the arithmetic mean.

Exploratory analyses were performed where log-transformed total Hg concentrations in adobe brick, dirt floor, surface dust, and vapor samples were compared between neighborhoods using one-way ANOVA. Tukey's Studentized Range (HSD) test was used to identify all pairwise comparisons that were statistically significantly different controlling for the experiment wise error rate. In addition, Pearson correlation coefficients, denoted by r , were calculated to estimate the dependence of sample types on a neighborhood level and Huancavelica-wide. No adjustment was made for multiple comparisons in testing the correlations between sample types. All statistical analyses were completed using SAS 9.2 software (SAS Institute Inc., Cary, NC).

2.3 Results

2.3.1 Total Hg in adobe bricks, dirt floors, and surface dust

The total Hg concentrations in the adobe bricks and dirt floors from 15 homes in each of the four neighborhoods are shown in Figure 2-2. The figure shows that total Hg concentrations in the adobe bricks and dirt floors from the 60 households ranged from 8.00 to 1070 $\mu\text{g/g}$ and 3.06 to 926 $\mu\text{g/g}$, respectively. The median (10th and 90th percentile) total Hg concentrations in adobe bricks and dirt floors were 85.5 $\mu\text{g/g}$ (11.3 $\mu\text{g/g}$ and 563 $\mu\text{g/g}$, respectively) and 63.3 $\mu\text{g/g}$ (6.99 $\mu\text{g/g}$ and 369 $\mu\text{g/g}$, respectively). In comparison, the total Hg concentrations in exterior adobe brick and ambient soil samples from the reference site, Ayacucho, ranged from 0.03 to 0.57 $\mu\text{g/g}$ and 0.07 to 0.15 $\mu\text{g/g}$, respectively. The total Hg concentrations in surface dust wipes across the 60 households are shown in Figure 2-3 and ranged from 0.02 to 9.69 $\mu\text{g/wipe}$. The median (10th

and 90th percentile) total Hg concentrations in surface dust were 2.25 µg/g (0.03 µg/g and 1.09 µg/g, respectively).

Figure 2-2. Total Hg concentrations in adobe bricks and dirt floors by neighborhood

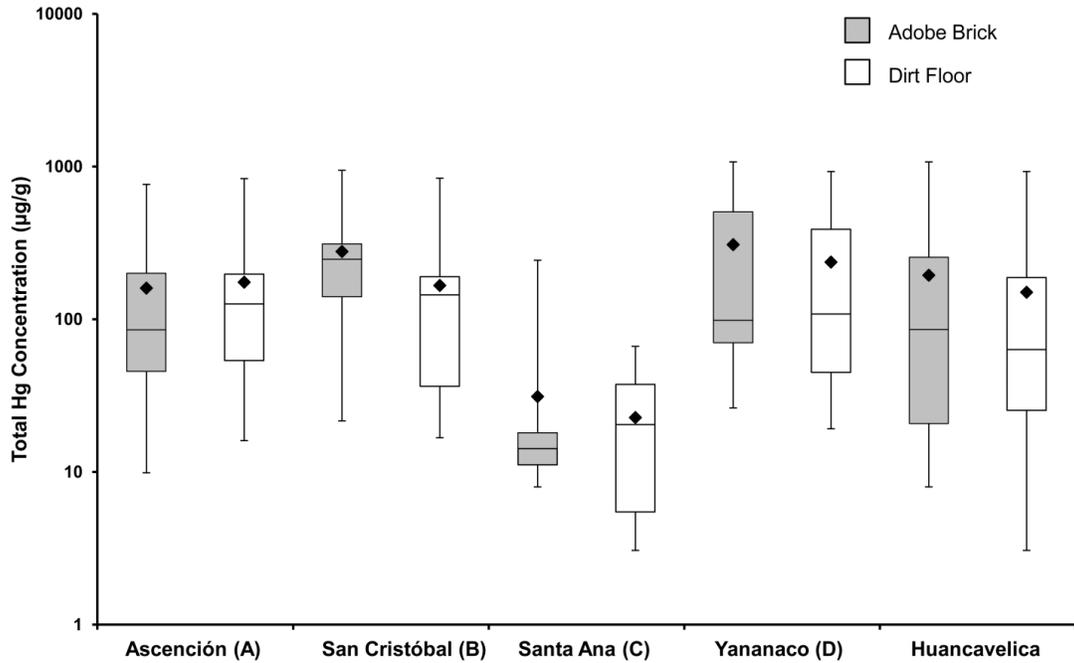
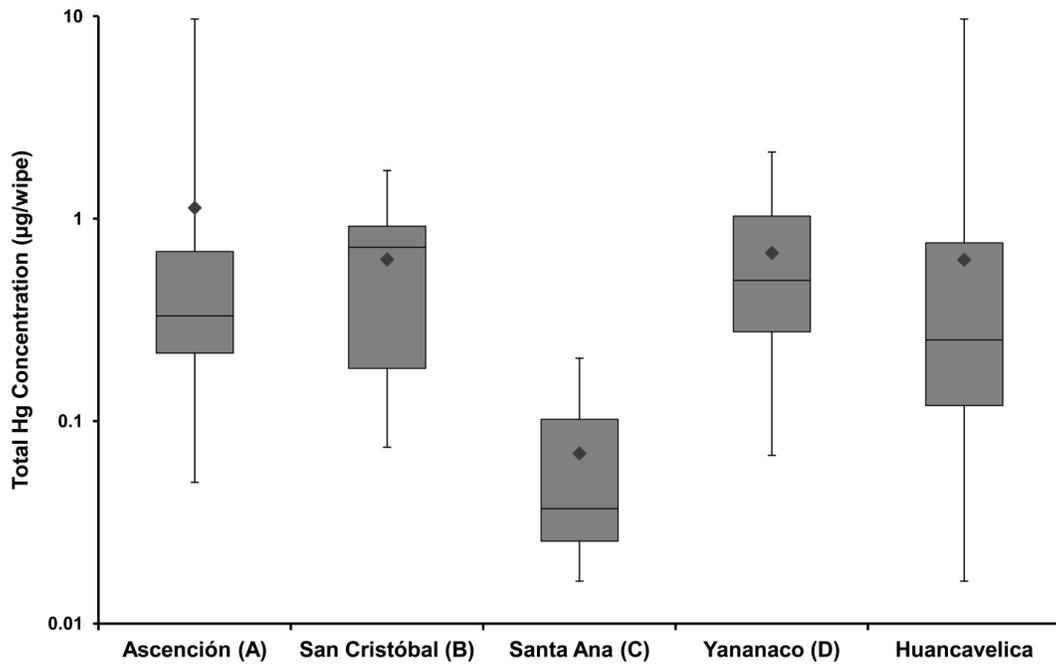


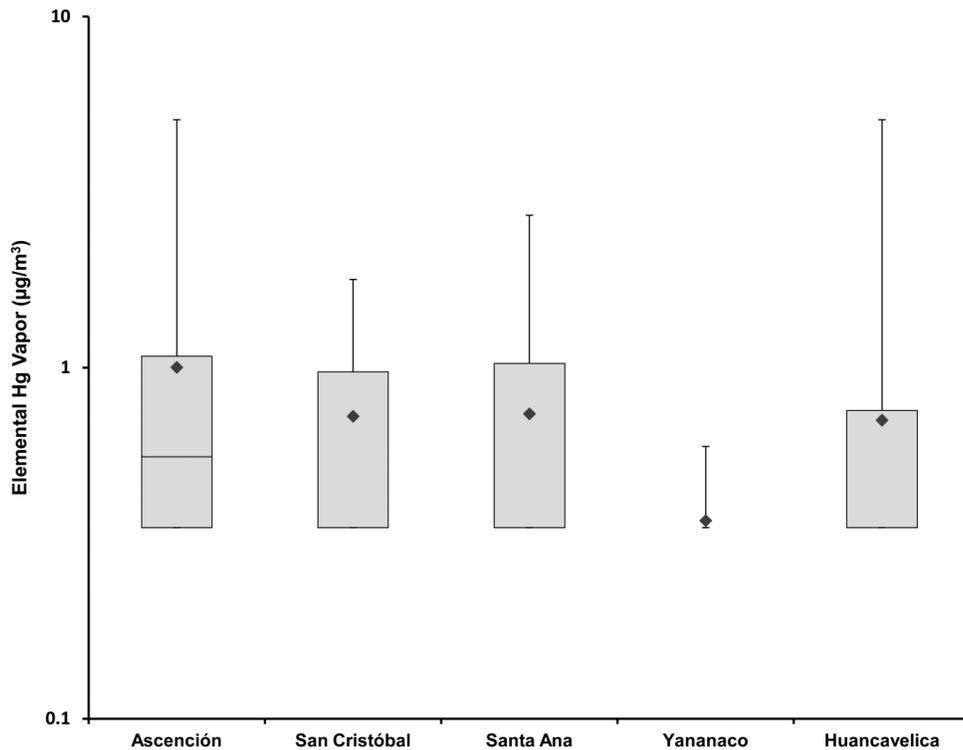
Figure 2-3. Total Hg concentrations in surface dust by neighborhood



2.3.2 Elemental Hg vapor in indoor air

Figure 2-4 shows the elemental Hg vapor concentrations that were measured in 15 homes from each of the four neighborhoods, using the Jerome J405 Mercury Vapor Analyzer. Thirty nine of the 60 households were below the limit of detection of the instrument. Elemental Hg vapor concentrations across the 60 households ranged from 0.35 to 5.1 $\mu\text{g}/\text{m}^3$.

Figure 2-4. Elemental Hg vapor concentrations in indoor air by neighborhood



2.4 Discussion

2.4.1 Differences in total Hg in adobe bricks, dirt floors, and surface dust between neighborhoods

Results of the one-way ANOVA for adobe bricks, dirt floors, and surface dust suggest that a significant difference exists in the total Hg concentrations according to the neighborhood ($p < 0.0001$). Results of the Tukey's HSD test for adobe brick samples, dirt floor samples and for surface dust samples indicate that Ascensión (A), San Cristóbal (B), and Yananaco (D) were not

significantly different from one another; however, Santa Ana (C) had significantly lower Hg concentrations in adobe bricks, dirt floors, and surface dust than the other three neighborhoods ($p < 0.0001$).

As shown in Figure 2-1, the neighborhoods of Ascensión (A), San Cristóbal (B), and Yananaco (D) historically had smelters operating throughout the neighborhood (Robins 2011; Robins et al. 2012). Santa Ana (C), in contrast, did not have any smelters during the colonial refining period. The lower concentrations of total Hg in adobe bricks, dirt floors, and surface dust in Santa Ana may be due to the absence of historic cinnabar refining in this section of the city.

2.4.2 Relationships between total Hg in adobe bricks, dirt floors, and surface dust

Pearson correlation coefficients were calculated for total Hg concentrations in adobe bricks, dirt floors, and surface dust on a neighborhood and Huancavelica-wide basis, as shown in Table 2-1. A strong correlation was observed in Yananaco (D) between total Hg concentrations in adobe bricks and dirt floors ($r = 0.766$, $p < 0.001$). Moderate correlation was observed between these sample types in Ascensión (A) ($r = 0.545$, $p = 0.036$) and San Cristóbal (B) ($r = 0.500$, $p = 0.058$). These results suggest a significant correlation between total Hg concentrations in adobe bricks and dirt floors in Ascensión (A) and Yananaco (D). Moderate correlation was observed between total Hg concentrations in surface dust and dirt floors in San Cristóbal (B) ($r = 0.539$, $p = 0.038$) and Yananaco (D) ($r = 0.593$, $p = 0.020$). A statistically significant correlation was not found between total Hg concentrations in surface dust and adobe bricks in any of the individual neighborhoods.

Table 2-1 shows that the correlations between all sample types become statistically significant ($p < 0.001$) when Pearson correlation coefficients are calculated for total Hg

concentrations in adobe bricks, dirt floors, and surface dust across all 60 residences in Huancavelica. The strong correlation between total Hg concentrations in adobe bricks and dirt floors ($r=0.720$, $p<0.001$) confirms what the residents of Huancavelica reported: that they build adobe bricks using the materials present on their property rather than purchase manufactured adobe bricks made from materials external to their property. Moreover, the moderate correlation coefficients for total Hg concentrations in surface dust and adobe bricks ($r=0.589$, $p<0.001$) and surface dust and dirt floors ($r=0.685$, $p<0.001$) across the entire community suggest that adobe bricks and dirt floors are the primary source of Hg in surface dust inside the residences. Because many homes in Huancavelica are built from on-site adobe, these results indicate the potential for widespread Hg contamination throughout several neighborhoods.

Pearson correlation coefficients were also calculated city-wide after excluding Santa Ana (not shown in Table 2-1) because total Hg concentrations in adobe brick, dirt floors, and surface dust were found to be significantly lower in Santa Ana than in the other four neighborhoods. When Santa Ana is excluded, moderate correlation was observed for the remaining households ($n=45$) for adobe bricks and dirt floors ($r=0.591$, $p<0.001$), surface dust and adobe bricks ($r=0.349$, $p<0.001$), and surface dust and dirt floors ($r=0.529$, $p<0.001$). Although the correlation coefficients are lower when excluding Santa Ana, the correlations between sample types are still very significant.

Previous studies of Hg concentrations in residential surface dust have primarily focused on the contribution of outdoor sources of Hg in more developed parts of the world. Lemus et al. (1996) measured Hg concentrations in household dust and outdoor soil in urban and rural homes in south Louisiana. Concentrations of Hg in household dust ranged up to 157 $\mu\text{g/g}$ and 175 $\mu\text{g/g}$ for urban and rural houses, respectively. For outdoor soil, concentrations of Hg ranged up to 125

$\mu\text{g/g}$ and $82.2 \mu\text{g/g}$ for urban and rural houses, respectively. The authors estimated an indoor to outdoor mean ratio for Hg of 0.44 based on arithmetic means, which suggested that Hg in household dust was coming from an outside source.

Table 2-1. Pearson correlation coefficients, sample size, and p-value for log-transformed total Hg concentrations in adobe bricks, dirt floors, and surface dust by neighborhood

Neighborhood	Sample Size	Sample Type	Adobe Brick	Dirt Floor	Surface Dust
Ascención (A)	15	Adobe Brick	1.00		
		Dirt Floor	0.545 (p=0.036)*	1.00	
		Surface Dust	0.214 (p=0.443)	0.477 (p=0.072)	1.00
San Cristóbal (B)	15	Adobe Brick	1.00		
		Dirt Floor	0.500 (p=0.058)	1.00	
		Surface Dust	0.510 (p=0.052)	0.539 (p=0.038)*	1.00
Santa Ana (C)	15	Adobe Brick	1.00		
		Dirt Floor	0.402 (p=0.138)	1.00	
		Surface Dust	-0.006 (p=0.982)	0.122 (p=0.666)	1.00
Yananaco (D)	15	Adobe Brick	1.00		
		Dirt Floor	0.766 (p<0.001)*	1.00	
		Surface Dust	0.461 (p=0.084)	0.593 (p=0.020)*	1.00
Huancavelica	60	Adobe Brick	1.00		
		Dirt Floor	0.720 (p<0.001)*	1.00	
		Surface Dust	0.589 (p<0.001)*	0.685 (p<0.001)*	1.00

*indicates statistical significance at $\alpha=0.05$; p-values not corrected for multiple comparisons

In areas close to historical Hg mining locations such as Idrija, Slovenia, elevated Hg levels in dust have also been observed (Gosar et al. 2006). In that study, the median Hg concentrations in attic dust and soils near homes in Idrija were $129 \mu\text{g/g}$ and $47 \mu\text{g/g}$, respectively, for the closest and most contaminated area (Area 1). The authors found that Hg concentrations decreased with distance from the source of contamination, with median Hg concentrations in attic dust (Area 2: $17 \mu\text{g/g}$, Area 3: $6.1 \mu\text{g/g}$) and soils (Area 2: $3.2 \mu\text{g/g}$, Area 3: $1.0 \mu\text{g/g}$) well below Area 1. The authors found a strong relationship between Hg

concentrations in attic dust and soils, suggesting outdoor sources of Hg influenced indoor dust concentrations.

Likewise, in a study in Ottawa, Canada, Rasmussen et al. (2001) measured Hg in indoor dust and exterior soils and dust in Ottawa, Canada. Average Hg concentrations in garden soil and street dust were 0.107 $\mu\text{g/g}$ and 0.029 $\mu\text{g/g}$, respectively, while average house dust Hg concentrations were 3.633 $\mu\text{g/g}$. The authors found that indoor sources contributed to Hg concentrations in house dust more than outdoor sources.

Rasmussen et al. (2001) also suggested that, because of higher concentrations of biogenic particles and organic matter in indoor dust compared to outdoor soil and dust, there is potential for indoor dust to accumulate higher concentrations of Hg and other metals. The authors suggest that this accumulation, a result of poor air exchange particularly in damp homes, can lead to an increase in fungi in indoor dust, which can accumulate greater concentrations of Hg. This finding is particularly important in Huancavelica, where homes are often damp and poorly ventilated. Because the adobe brick walls and dirt floors in Huancavelica are typically unsealed and subjected to dampness and inadequate air exchange, there is potential for walls and floors, in addition to surface dust, to accumulate greater concentrations of Hg than a home with sealants applied to walls and floors.

2.4.3 Differences in elemental Hg vapor between neighborhoods

Results of the one-way ANOVA for elemental Hg vapor suggest that there is not a significant difference in elemental Hg vapor concentrations based on neighborhood ($p=0.126$). The Tukey's HSD test for elemental Hg vapor confirms that there are not significant differences between neighborhoods at a significance level of $\alpha=0.05$. Hg vapor is relatively mobile, so may move more readily than dust or dirt. This finding may also be a reflection of the large number of

measurements that were below the limit of detection of the instrument (39 of 60 homes) and suggests that, while some individual households appear to have elevated elemental Hg vapor concentrations, these variations cannot be attributed to differences in characteristics of the four neighborhoods.

2.4.4 Implications for potential health effects from exposure

The semi-arid climate of Huancavelica results in a very dusty ambient environment stemming from many unpaved roads and outdoor surfaces. Results of a previous study have identified ambient total Hg concentrations in surface soil in Huancavelica ranging between 1.75 and 698 $\mu\text{g/g}$ (Robins et al. 2012). Results of the present study found similar or greater concentrations of total Hg in adobe bricks, dirt floors, and surface dust. While health effects resulting from Hg exposure depend on the exposure pathway and the type of Hg present, it is clear that the residents of Huancavelica can be exposed to Hg through both outdoor and indoor environments. Robins et al. (2012) suggest that, in ambient soil, a majority of the Hg present is a sulfur-bound mineral phase (e.g., cinnabar or metacinnabar). While other forms of Hg, such as organic and elemental Hg, have more immediate and severe health implications, all species of Hg pose a potential health risk to those exposed.

In the dusty, widely contaminated community of Huancavelica, it is important to recognize that ingestion of particle-bound Hg may affect health. A previous study demonstrated that children are especially vulnerable due to their hand-to-mouth activity (Hagan et al. 2011). An 11 kg, 1 year old toddler with a median ingestion rate of 100 mg of dirt and dust from hand-to-mouth activity per day (US EPA 2008) would exceed EPA's Reference Dose (RfD) for inorganic mercury ($0.3 \text{ mg kg}^{-1} \text{ day}^{-1}$; US EPA 1995) if the soil contained more than 33 $\mu\text{g Hg/g}$ soil. For the 60 households studied in Huancavelica, 40 adobe brick, 42 dirt floor, and 30 surface

dust samples had total Hg concentrations above 33 $\mu\text{g/g}$. The dirt and dust ingestion rate used in the EPA study were representative of children in the United States. It is likely that children in Huancavelica, and the Andean region in general, are smaller than children in the United States and, because adobe walls and dirt floors are typically unsealed and the community is generally dusty, children in Huancavelica could potentially ingest more dirt and dust from hand-to-mouth activity than the average child in the United States. If so, then the calculations above associated with ingesting dirt and dust for children in Huancavelica may underestimate the potential risks.

This study demonstrates that building materials used widely in the developing world, such as adobe bricks, may be a substantial source of residential Hg exposure in contaminated communities. In Huancavelica, Hg in adobe brick homes is a result of legacy contamination that primarily occurred hundreds of years ago during the Spanish colonial period. Understanding exposure to Hg from such building materials, particularly adobe bricks made in the community, has broader relevance to evaluating risks in communities that currently produce or refine silver and gold, such as Madre de Dios and Puerto Maldonado, both also in Peru. In these communities, gold shops and other buildings used for refining are often constructed from adobe brick that has the potential to act as a sink for elemental Hg vapor released from current refining processes and may be a source of Hg exposure in the future.

2.5 Conclusions

The purpose of this study was to determine the current levels of mercury to which residents of adobe brick homes in Huancavelica are exposed. Results from this study suggest that total Hg concentrations in residential samples are similar to or greater than ambient soil samples from previous studies conducted in Huancavelica, and that widespread residential exposure to Hg is presently occurring there. Total Hg concentrations ranged from 8.00 to 1070 $\mu\text{g/g}$ in adobe

bricks, 3.06 to 926 $\mu\text{g/g}$ in dirt floors, and 0.02 to 9.69 $\mu\text{g/wipe}$ in surface dust. These results indicate that total Hg concentrations vary by neighborhood, with Santa Ana (C) having consistently lower Hg concentrations than the other three neighborhoods. This finding may suggest that the present-day spatial distribution of total Hg contamination in residences is influenced by the location of historical cinnabar smelters.

These results also point to a strong relationship between total Hg concentrations in adobe bricks and dirt floors. This result confirms that residents of Huancavelica are using materials on their property to construct adobe bricks for their homes, rather than purchasing manufactured adobe bricks. Additionally, the strong relationship between total Hg concentrations in surface dust and adobe bricks and surface dust and dirt floors suggests that adobe bricks and dirt floors are the primary source of Hg in indoor dust, rather than an outdoor source.

Elemental Hg vapor concentrations from 39 of the 60 residences in Huancavelica were below the limit of detection. For residences with measurements above the limit of detection, concentrations of elemental Hg vapor ranged up to 5.1 $\mu\text{g/m}^3$. While individual homes vary in Hg vapor concentrations, no significant difference in these concentrations was found between neighborhoods.

The results of this study demonstrate that adobe bricks and other building materials used in developing communities may be a substantial source of residential Hg exposure, particularly in communities with past or present mercury production and where silver or gold refining utilizes Hg for amalgamation. Adobe bricks are used to construct buildings around the world, from the southwest United States to South America to Africa. As the first study of adobe brick contamination anywhere in the world, the present study draws attention to a previously unidentified source of potential exposure to Hg and possibly to other contaminants. To better

understand potential health effects from exposure, it is important to quantify levels of contaminants, identify the chemical species and bioaccessibility of contaminants in the building materials, and to identify possible mitigation strategies to reduce exposure within these homes.

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CHAPTER 3. MERCURY SPECIATION AND BIOACCESSIBILITY FROM ADOBE BRICKS AND DIRT FLOORS IN HUANCVELICA, PERU

3.1 Introduction

Mercury (Hg) is emitted from natural and anthropogenic sources and is measured in all compartments of the environment. Hg is transformed and transported in air, deposited onto the earth's surface, and can be stored in or emitted by soil, water, and air, continuously being cycled in the environment and being oxidized and reduced to different species. Once deposited from the atmosphere onto the surface, Hg compounds in soil can undergo in situ reactions resulting in the formation of elemental Hg and organomercurials (US EPA 1997). While all species of Hg are toxic, some species are less mobile and bioaccessible (Gochfield 2003; Revis et al. 1990). Bioaccessibility refers to the fraction of the total Hg that is desorbed from contaminated particles and dissolved in the gastrointestinal tract (Zagury et al. 2009).

In Huancavelica, Peru, a previous study measured levels of total Hg in residential samples and identified the primary sources of Hg in adobe brick homes (Hagan et al. 2013). Based on the results of that study, the inadvertent ingestion of Hg-contaminated soil particles by children was identified as an exposure pathway of interest. Health benchmarks are available for ingestion of Hg-contaminated soil and are often cited in the literature when evaluating total Hg measurements (Hagan et al. 2011; Li et al. 2013; Li 2013; Robins et al. 2012). Health benchmarks, such as those shown in Table 3-1 issued by the European Union Environment

Agency (EA), Agency for Toxic Substances and Disease Registry (ATSDR), US Environmental Protection Agency (US EPA), and Joint FAO/WHO Committee on Food Additives (JECFA) are all based on mercuric chloride, which is up to 100% soluble following ingestion (ATSDR 1999; Environment Agency 2009; JECFA 2010; US EPA 1995). Total Hg measurements do not distinguish between soluble and insoluble forms of Hg so that comparisons between total Hg measurements and existing health benchmarks may overestimate the potential risks from exposure. In a site-specific situation, additional chemical analyses (e.g., sequential selective extractions or simple bioaccessibility extraction tests) can be used to further classify the species of Hg present in soils and to provide more detailed information for evaluating potential risks from ingestion.

Table 3-1. Current health benchmarks for oral exposure to inorganic Hg

Agency	Description	Benchmark Value ($\mu\text{g Hg/kg bw/day}$)	Reference
EA	Mean Daily Intake (MDI)		Environment Agency 2009
	70-kg adult	0.014	
	20-kg child	0.037	
ATSDR	Minimal Risk Level (MRL)	2	ATSDR 1999
EPA	Reference Dose (RfD)	0.3	US EPA 1995
JECFA	Provisional Tolerable Weekly Intake (PTWI)	0.57 ^a	JECFA 2010

^a adjusted from units of $\mu\text{g Hg/kg bw/week}$ to $\mu\text{g Hg/kg bw/day}$

The objectives of this study were to characterize the species of Hg present in adobe brick and dirt floor samples from adobe brick homes in Huancavelica, Peru, and to evaluate Hg bioaccessibility following ingestion of contaminated particles. The study's goal was to evaluate the potential health implications of ingestion, particularly for children, based on the solubility of

Hg in adobe bricks and dirt floors in order to support effective development, implementation, and evaluation of future intervention, mitigation, and remediation strategies in the community.

3.2 Materials and Methods

3.2.1 Study area

Huancavelica, located in the Andean region of Peru, was the primary source of Hg used in amalgamation-based silver refining in South America for over 350 years. Beginning in 1563, Spanish colonists used the cinnabar ore in the Santa Barbara Hill outside of the city to produce liquid Hg in Huancavelica, which was then shipped to Andean silver refining centers. As a result of the cinnabar refining process, the local community was extensively contaminated by routine releases of Hg vapor and liquid, a legacy that still haunts the residents of Huancavelica decades after refining ceased in the 1970s (Robins 2011; Robins and Hagan 2012; Robins et al. 2012).

More than 80% of homes in Huancavelica are built from adobe bricks (Mendoza 2012). A previous study demonstrated elevated levels of total Hg in adobe bricks and dirt floors collected in Huancavelica, with concentrations ranging up 1070 $\mu\text{g/g}$ and 926 $\mu\text{g/g}$, respectively (Hagan et al. 2013). Because the walls and floors of adobe brick homes are generally uncovered and unsealed, residents are exposed to Hg-bound particles and vapor from these surfaces along with ambient exposure to Hg-contaminated soils (Robins et al. 2012).

3.2.2 Sample collection

Triplicate samples of adobe bricks and dirt floors were collected from 60 residences in Huancavelica in 2010 as described by Hagan et al. (2013). Composite samples for this study were obtained by weighing and combining approximately 1 g of each triplicate sample and removing any particles, rocks, debris, etc. greater than 3 mm at the widest diameter. Composite samples were created so each of the 60 residences had one adobe brick and one dirt floor sample.

3.2.3 Analytical methods

Households were assigned to batches using computer-generated randomization. Total Hg extractions, sequential selective extractions (SSEs), and simulated gastric fluid (GI) extractions were performed on composite samples of adobe brick and dirt floor samples from each household. All solutions were prepared using reagent-grade chemicals and all procedures were performed using ultra-clean sample handling to avoid low-level Hg contamination.

Total Hg extractions were performed according to EPA Method 1631 (US EPA 2002). Adobe brick and dirt floor samples were digested in 4:1 HCl:HNO₃ at 85°C for five hours. Following digestion, samples were diluted to a final volume of 40 mL with deionized (DI) water and oxidized using 1% bromine monochloride.

SSEs were performed according to the methodology developed by Bloom et al. (2003) and verified with complementary spectroscopic methods (Kim et al. 2003). The SSE method utilizes five extractant solutions that separate Hg compounds based on chemical behavioral classes. The five Hg fractions included in the SSE procedure, in sequential order, are: deionized water (F1, water soluble), 0.2 M acetic acid, pH 2 (F2, ‘human stomach acid’ soluble), 1 M KOH (F3, organo-chelated), 12 M HNO₃ (F4, elemental Hg), and aqua regia (F5, mercuric sulfide). All extractions were performed using 0.2 ± 0.02 g of composite sample and 20 mL of the extractant fluid, yielding a 1:100 solid:liquid ratio. In each extraction step, the solid-liquid slurry was mixed end-over-end for 18 ± 4 hours. After this period, the liquid phase was separated from the solid by centrifuge and decanted into trace clean 40 mL glass vials. The solid pellet was rinsed, suspended, and separated from a rinse of the extractant fluid, which was added to the initial extraction solution. All fractions of were preserved for analysis with bromine monochloride.

Simulated GI extractions (pH 1.5) were performed using a simple bioaccessibility extraction test (SBET) that has often been used for trace metals other than Hg, but has increasingly been used to simulate Hg bioaccessibility (Schaidler et al. 2007). *In vivo* absorption of metals typically occurs in the epithelium of the small intestine which has circumneutral pH; however, absorption rates can be limited by the dissolution of particulate metals in the acidic gastric fluids. Therefore, the SBET utilized a simulated gastric fluid is a solution comprised of 0.4 M glycine and adjusted to pH 1.5 using concentrated HCl. A 1:100 solid:liquid mass ratio was used (same as the SSEs). The liquid:solid slurry was mixed for 1 hour at 37°C and separated by centrifuge prior to decanting the liquid phase into trace clean 40 mL glass vials and oxidation with bromine monochloride.

All samples were analyzed for Hg using a RA-915+ Lumex Mercury Vapor Analyzer equipped with a RP-M324 attachment (Ohio Lumex Co., Twinsburg, OH). For total Hg, SSEs, and simulated GI extractions for adobe bricks and dirt floors, aliquots up to 100 µL of each sample were analyzed for total Hg following EPA Method 30B (US EPA 2008a). Instrument calibrations were performed using mercuric chloride standards (SCP Science, Champlain, NY & Inorganic Ventures, Christiansburg, VA). Method blanks (n=11) were all below the limit of detection of the instrument (~1 ng Hg). A NIST-certified SRM (2709 San Joaquin soil) was digested with each batch of samples. The average recovery of total Hg in the SRM was 1.17 ± 1.03 ug/g (n=6). Recovery of Hg from the SRM was within the acceptable range of the certified value (1.4 ± 0.08 ug/g).

3.2.4 Statistical methods

Mass-based Hg concentrations for total Hg, SSEs, and GI extractions were tested for normality and were found to be non-normally distributed; therefore, mass-based data were log-

transformed prior to statistical analyses. Prior to log-transformation, the raw data were adjusted for values below calibration and below the limit of detection of the instrument. Measurements below the lowest calibration point but with area counts greater than 200 were adjusted using a response factor (US EPA 2008a). For SSEs, samples with a recovery less than 60% or greater than 140% of the total Hg extraction were excluded. In the reporting of summary statistics only, measurements below the LOD of ~1 ng Hg (equivalent to an area count of 200) were set to 0.00125 µg/g Hg (½LOD mass adjusted to 0.2 g of sample); all other analyses were performed without substitution. Method duplicates that met inclusion criteria were included in the analysis. Statistical analyses were completed using SAS 9.2 software (SAS Institute Inc., Cary, NC).

3.3 Results

3.3.1 Recovery of Hg in SSEs and GI extractions

Summary statistics for extractable Hg in each fraction of the SSEs are shown in Table 3-2. The percent of Hg recovered in each fraction is consistent with other studies that have performed SSEs on refining-related soil contamination, with lower amounts recovered in F1, F2, and F3, and a majority of the Hg present in the F4 and F5 fractions (Hagan et al. 2011; Male et al. 2013; Zagury et al. 2009).

The percent bioaccessible Hg in simulated GI extractions for adobe bricks and dirt floors is also shown in Table 3-2. The percent of bioaccessible Hg in adobe brick samples ranged from below the limit of detection to 7.4% of total Hg. The percent of bioaccessible Hg in dirt floor samples ranged from below the limit of detection to 6.8%.

Table 3-2. Extractable Hg in adobe bricks and dirt floors expressed on a mass basis ($\mu\text{g/g}$) and as a percentage of total Hg (%) from residences in Huancavelica, Peru

		Fraction 1 (F1): deionized water	Fraction 2 (F2): 0.2 M acetic acid (pH 2)	Fraction 3 (F3): 1 M KOH	Fraction 4 (F4): 12 M HNO ₃	Fraction 5 (F5): aqua regia (HCl + HNO ₃)	Simulated GI
Adobe Bricks							
n		61	61	61	61	61	61
mean \pm 1 sd	$\mu\text{g/g}$	1.9 \pm 4.5	1.0 \pm 2.7	4.2 \pm 6.6	17 \pm 17	150 \pm 210	1.6 \pm 2.7
	%	0.73 \pm 1.7	0.30 \pm 0.77	1.7 \pm 2.1	13 \pm 10	69 \pm 17	0.81 \pm 1.4
min, max	$\mu\text{g/g}$	0.0013, 20	0.0013, 17	0.0013, 25	0.0013, 80	2.9, 1100	0.0013, 11
	%	0.00013, 8.8	0.00013, 3.8	0.00052, 9.2	0.0067, 45	33, 120	0.00013, 7.4
Dirt Floors							
n		49	49	49	49	49	49
mean \pm 1 sd	$\mu\text{g/g}$	1.8 \pm 3.9	1.2 \pm 3.3	5.0 \pm 9.6	20 \pm 19	120 \pm 190	1.9 \pm 3.7
	%	0.57 \pm 1.0	0.73 \pm 2.1	3.2 \pm 4.4	16 \pm 9.6	76 \pm 25	0.89 \pm 1.5
min, max	$\mu\text{g/g}$	0.0013, 17	0.0013, 17	0.0013, 49	0.0013, 67	2.1, 1100	0.0013, 20
	%	0.00058, 4.2	0.00023, 12	0.00058, 21	0.016, 41	37, 130	0.00023, 6.8

3.3.2 Comparisons between extraction methods

Pearson correlation coefficients were calculated for extractable Hg in SSE and simulated GI extractions, as shown in Table 3-3. For these calculations, the log-transformed mass of extractable Hg in the simulated GI extractions was compared to individual and combined fractions from the sequential selective extraction procedure that would represent the more soluble forms of Hg following ingestion (water soluble (F1), ‘human stomach acid’ soluble (F2), organo-chelated (F3)).

Table 3-3. Pearson correlation coefficients, p-values, and sample sizes for log-transformed mass of extractable Hg in adobe bricks and dirt floors

	F1	F2	F3	F1+F2+F3
Simulated GI adobe bricks	0.546 <0.001* 47	0.559 <0.001* 44	0.782 <0.001* 49	0.780 <0.001* 49
Simulated GI dirt floors	0.738 <0.001* 35	0.478 0.005* 33	0.565 <0.001* 40	0.728 <0.001* 40

* indicates statistical significance at $\alpha=0.05$

The mass of extractable Hg in the individual SSE fractions was significantly correlated with the simulated GI extractions. Although statistically significant, the simulated GI extractions were not as strongly correlated with the F2 fraction as expected, given the similar fluids used in the F2 extraction and the simulated GI extraction. The mass of extractable Hg in the simulated GI extractions was strongly correlated with the organo-chelated Hg (F3) for adobe bricks, also unexpected given the very different chemical compositions of the F3 and simulated GI extraction fluids. When the mass of extractable Hg in the F1, F2, and F3 fractions was summed and compared with that from the simulated GI extractions, the bioaccessible fractions were strongly correlated for both adobe brick and dirt floor samples.

3.3.3 Comparisons between adobe brick and dirt floor samples

The relationship between the sum of the more soluble fractions of Hg from SSEs (F1 (water soluble), F2 ('human stomach acid' soluble), and F3 (organo-chelated)) in adobe bricks and dirt floors was explored using the 38 residences that had paired SSE data for both sample types. A moderate correlation was observed between the F1+F2+F3 fractions of the adobe bricks and dirt floors ($r=0.437$, $p=0.006$). The relationship between simulated GI extractable Hg in adobe bricks and dirt floors was explored using the 32 households that had paired simulated GI data for both sample types. The Pearson correlation coefficient, r , was 0.866 ($p<0.0001$), suggesting a very strong and statistically significant correlation between GI extractable Hg concentrations in adobe bricks and dirt floors. These results are consistent with a previous study where total Hg concentrations were found to be statistically correlated between adobe bricks and dirt floors within a residence (Hagan et al. 2013).

3.4 Discussion

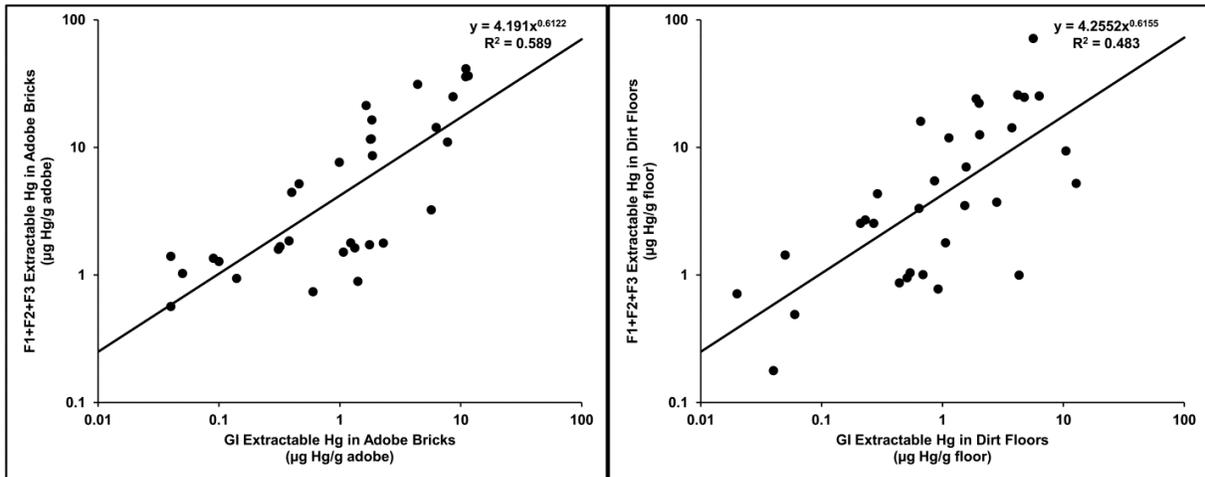
3.4.1 Comparisons between extraction methods

The Bloom et al. (2003) SSE method is capable of providing detailed information regarding the speciation of Hg in contaminated soil, including the more soluble forms of Hg. However, this procedure is very time and resource intensive. If the first three fractions were utilized as a proxy for bioaccessibility in an ingestion scenario, then the SSE method would require approximately three days of tumbling and three different extraction fluids to measure the different fractions. In comparison, the simulated GI extraction requires only one hour and one extraction fluid to measure the more bioaccessible forms of Hg following ingestion. The strong correlation between F1+F2+F3 and simulated GI extractions (Table 3-3) suggests that the simulated GI extraction could be applied as a first screen of the bioaccessibility of Hg at

contaminated sites, followed by the Bloom et al. (2003) sequential selective extraction procedure as warranted by simulated GI extraction results if necessary for more detailed information.

To further explore the relationship between F1+F2+F3 and simulated GI extractions, a least square regression was fitted for households with complementary SSE and simulated GI extraction data (n=32) and data power regression was applied to the data as shown in Figure 3-1. The regressions for adobe bricks and dirt floors suggest a moderate relationship between F1+F2+F3 extractable Hg and simulated GI extractable Hg (adobe: $r^2 = 0.589$, floors: $r^2 = 0.483$). The average ratio of F1+F2+F3 extractable Hg to simulated GI extractable Hg is 6.9 for adobe bricks and 7.9 for dirt floors. This suggests that while the correlation between the two sample types is very strong, the mass of Hg recovered in the simulated GI extractions is proportional to the mass of Hg recovered in the more bioaccessible fractions of the SSEs and has the potential to serve as a screening tool for additional analyses.

Figure 3-1. Least squares regression for mass of F1+F2+F3 extractable Hg ($\mu\text{g/g}$) versus simulated GI extractable Hg ($\mu\text{g/g}$)



In the case of Huancavelica, using the simulated GI extractions as a preliminary measure of bioaccessible Hg would allow for time and resources for more detailed analyses as well as intervention, mitigation, or remediation strategies to be allocated first to households with the

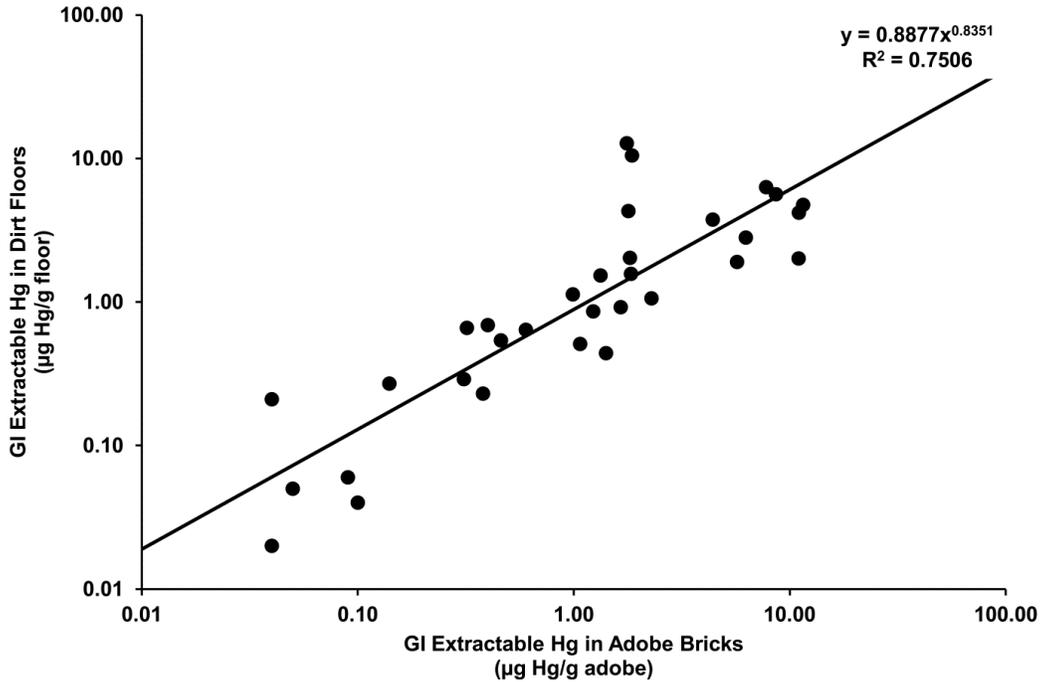
greatest potential for health implications from ingestion of soil containing a higher fraction of soluble Hg.

3.4.2 Relationship between sample types

In this study, total Hg, SSEs and simulated GI extractions were performed on adobe brick and dirt floor samples from all participants' residences (n=60). In order to perform the extractions, samples were collected in the field and transported to a laboratory in the US, requiring significant cost, time, and resources to obtain additional information. Details regarding speciation and bioaccessibility of Hg are helpful in prioritizing households for future phases of research, and identifying a sampling protocol that minimizes cost, time, and resources would be useful in providing this information. Because of the strong correlation between Hg in adobe bricks and dirt floors in a previous study (Hagan et al. 2013) and in the current study, it was expected that this relationship would also exist for Hg concentrations from the simulated GI extractions.

The relationship between Hg concentrations from the simulated GI extractions in adobe bricks and dirt floors was further investigated by performing a least squares regression between households with complementary simulated GI extraction data for both sample types (n=32). As shown in Figure 3-2, the power regression shows a strong relationship between sample types ($r^2=0.75$) and the average ratio of Hg concentrations from simulated GI extractions between the sample types is 1.4. This finding suggests that, in future research efforts, it may be possible to use either an adobe brick or dirt floor sample in order to develop a prioritization scheme for intervention, mitigation, and remediation strategies among the households.

Figure 3-2. Least squares regression for mass of Hg ($\mu\text{g/g}$) from simulated GI extractions in adobe bricks and dirt floors



3.4.3 Implications for risks from soil ingestion

The EPA Child-Specific Exposure Factor Handbook assumes that a one-year old has a median body weight of 11 kg and a corresponding ingestion rate of 100 mg dirt and dust per day from hand-to-mouth activity (US EPA 2008b). While these values are descriptive of children in the United States, application to children in other parts of the world may result in an underestimation of risk from ingestion of contaminated soil. In Huancavelica, children are often smaller in size, with a one-year old having an average body weight of 9 kg (Ecos E, Hagan N, Robins N. Personal communication.). For the purpose of this discussion, we chose to use a body weight of 10 kg for a 1 year old toddler. Additionally, the ingestion rate derived in the Child-Specific Exposure Factor Handbook is not representative of soil and dust ingestion in a home with unsealed dirt walls and floors, as is the case in Huancavelica. While an ingestion rate is not currently available in the published literature for exposure within adobe brick homes, it is not

unreasonable to assume a doubling or tripling of the US-based median ingestion rate in a very dusty environment like Huancavelica.

To identify households at greatest potential risk from ingestion of Hg-contaminated soil in relation to the health benchmarks, a 10 kg body weight and varying ingestion rates were used in Equation 1 to calculate a Level of Soil Contamination (LSC). The LSC is a concentration of Hg in adobe bricks or dirt floors that may result in a child with the given characteristics to exceed the health benchmark.

$$LSC = \frac{BM \times BW}{IR \times AF} \quad (\text{Equation 3-1})$$

where

LSC = level of soil contamination ($\mu\text{g Hg/g soil}$) that may result in a health benchmark value exceedance

BM = health benchmark ($\mu\text{g Hg/kg bw/day}$)

BW = body weight (kg)

IR = ingestion rate (g soil/day)

AF = adjustment factor of 10 to adjust for the proportionality of the Hg concentrations in the F1+F2+F3 and simulated GI extractions

As shown in Table 3-4, the LSC varies greatly between health benchmarks, with the ATSDR MRL of $2 \mu\text{g Hg/kg bw/day}$ (ATSDR 1999) yielding the highest LSCs and the Environment Agency MDI of $0.037 \mu\text{g Hg/kg bw/day}$ (Environment Agency 2009) producing the lowest LSCs.

Table 3-4. Level of soil contamination (LSC) that may result in health benchmark value exceedance for a 10 kg child with varying ingestion rates

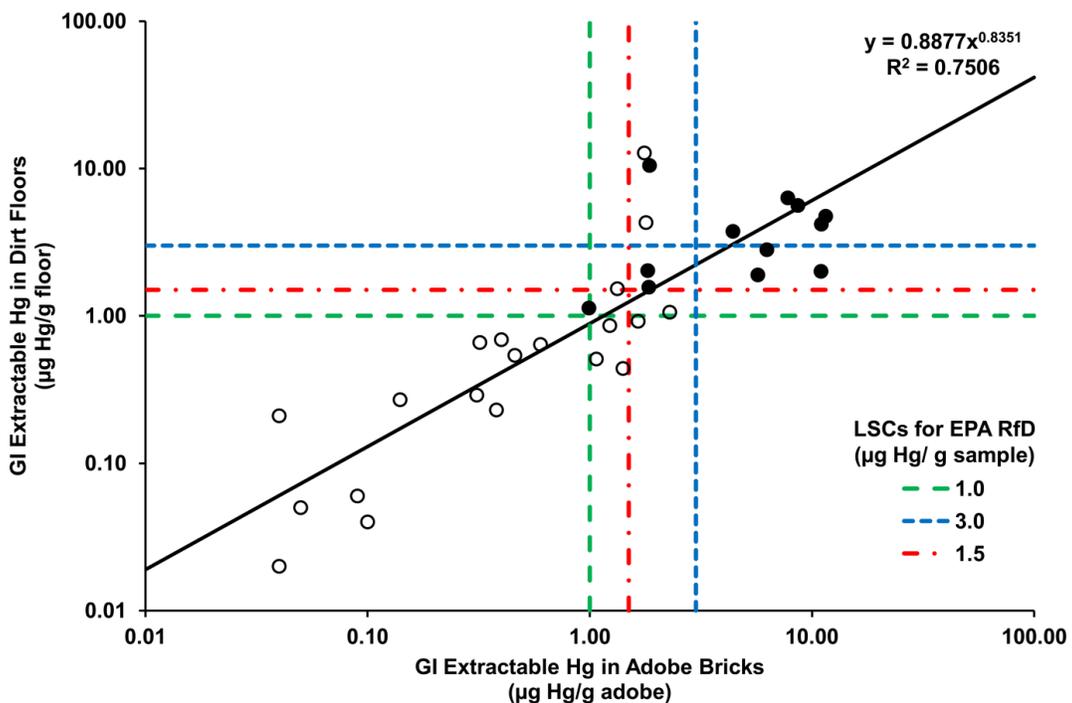
Agency	Benchmark Value (µg Hg/kg bw/day)	Ingestion Rate (g soil/day)	LSC (µg Hg/g soil)
EA	0.037	0.1	0.37
		0.2	0.19
		0.3	0.12
ATSDR	2	0.1	20
		0.2	10
		0.3	6.7
EPA	0.3	0.1	3
		0.2	1.5
		0.3	1
JECFA	0.57	0.1	5.7
		0.2	2.9
		0.3	1.9

In previous studies, total Hg concentrations were compared to the EPA Reference Dose (RfD) and suggested that the potential public health problem from Hg exposure in Huancavelica was widespread (Hagan et al. 2013; Robins et al. 2012), with up to 75% of the 60 households exceeding the RfD. However, by comparing the simulated GI extraction data with the LSCs calculated for the EPA RfD, the number of households identified as being at potential risk from ingestion is substantially reduced.

For example, comparing the simulated GI extraction data with the LSCs calculated for the EPA RfD (after application of adjustment factor of 10), as shown in Figure 3-3, identifies households with GI extractable Hg in adobe and floors above the LSC (upper right quadrant). These households would have a higher priority for additional analyses and mitigation than those households with GI extractable Hg in adobe and floors below the LSC. Assuming a worst case scenario of 0.3 g per day soil ingestion rate (LSC for EPA RfD, large dashed (green) line in

Figure 3-3), the simulated GI extraction data indicate that 15 of the sample households would exceed the LSC. A similar analysis comparing F1+F2+F3 extractable Hg in adobe bricks and dirt floors with the LSCs for the EPA RfD was done to ensure the simulated GI extractions identified the same households exceeding the LSCs as the SSEs. The solid black data points in Figure 3-3 represent the 12 households that would also exceed the LSC when using the SSE procedure.

Figure 3-3. Least squares regression for mass of Hg ($\mu\text{g/g}$) from simulated GI extractions in adobe bricks and dirt floors with Level of Soil Contamination (LSCs) for EPA Reference Dose (RfD)



While traditional application of health benchmarks is intended to be protective by assuming a worst-case scenario, time and resources should be targeted towards those households with the greatest potential risk from ingestion of Hg-contaminated soil. Through minimal calculations and utilizing more detailed information from additional Hg analyses, the households exceeding the LSCs have been identified and can be prioritized for future intervention, mitigation, and remediation strategies, particularly for those households with small children.

3.5 Conclusions

The purpose of this study was to characterize the species of Hg present in adobe brick and dirt floor samples and to evaluate bioaccessibility of Hg in simulated gastric fluid. Results of this study suggest, as is typical in soils, a majority of Hg in adobe bricks and dirt floors was bound with sulfur and relatively insoluble in weakly acidic and strong base solutions. However, in some households, soluble Hg species were present in water soluble (F1), ‘human stomach acid’ soluble (F2), and organo-chelated (F3) fractions of SSEs. The percent of the total Hg extracted in the simulated GI extractions for adobe bricks and dirt floors was up to 7.4% and 6.8%, respectively. While the bioaccessible Hg is only a small fraction of the total Hg present in a sample, there is still potential for concern following ingestion.

The results of this study demonstrate a strong correlation between the sum of the more soluble fractions of the SSE method (F1+F2+F3) and simulated GI extractions for both sample types. This is an important finding, in that simulated GI extractions have the potential to be used as a screening tool to most effectively target time and resources by performing SSEs on samples that have measurable concentrations of bioaccessible Hg.

By combining information related to body weights and ingestions rates for children and health benchmarks for oral exposure to Hg, it was possible to calculate a Level of Soil Contamination (LSC) for varying exposure scenarios. These calculations revealed that Hg concentrations in simulated GI extractions for adobe brick and dirt floor samples in some households exceed health benchmarks for soluble Hg, although the magnitude of the potential public health impact is much lower than when comparing total Hg. Combining simulated GI extraction data within the home with LSCs will allow for more targeted intervention, mitigation,

and remediation strategies in households with the greatest potential health implications from ingestion of contaminated particles.

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CHAPTER 4. MERCURY HAIR LEVELS AND FACTORS THAT INFLUENCE EXPOSURE FOR RESIDENTS OF HUANCVELICA, PERU

4.1 Introduction

Huancavelica is located in the Andean highlands of Peru and was the site of large-scale cinnabar (HgS) refining from 1564 to 1810 (Robins 2011; Robins and Hagan 2012). Cinnabar refining ended in the 1970s but mercury (Hg) contamination persists in the community today. A previous study has reported total Hg concentrations in ambient soils up to 1200 $\mu\text{g/g}$, among the highest concentrations measured in urban areas around the world (Robins et al. 2012). Residents in Huancavelica build their adobe brick houses from contaminated soil and often leave interior walls and floors uncovered and unsealed. Total Hg concentrations in adobe bricks, dirt floors, and surface dust were found to be strongly correlated with one another and ranged from 8.00 to 1070 $\mu\text{g/g}$, 3.06 to 926 $\mu\text{g/g}$, and 0.02 to 9.69 $\mu\text{g/wipe}$, respectively (Hagan et al. 2013). Speciation and bioaccessibility analyses of adobe brick and dirt floor samples suggest that most Hg is immobile, although a small percentage (<10%) of Hg may be bioaccessible following ingestion. While the percentage of the total Hg in adobe bricks and dirt floors that is bioaccessible is small, concerns remain for potential health impacts following ingestion, particularly for small children (Hagan et al. *submitted*).

Hair is routinely used as a biomarker for Hg exposure, particularly for methylmercury exposure from fish consumption (Díez et al. 2011; Nuttall 2006). Mercury is incorporated into hair follicles during growth and arises from the presence of Hg in blood (Díez et al. 2011). Hair grows at a rate of 0.6 to 3.36 cm per month, but is often reported as 1 cm per month in the

literature for convenience (Nutall 2006). Although whole blood and urine may be more reliable biomarkers for Hg exposure than hair, there are advantages to using hair specimens. Mercury has a relatively long half-life in hair of 1.5 to 2 months, which allows for the evaluation of past exposures (Díez et al. 2011; Nutall 2006). The stability of Hg in hair samples makes their transport and storage easier than blood and urine (Nutall 2006). Moreover, higher concentrations of Hg accumulate in hair compared with blood and urine, making measurement less complicated (Nutall 2006).

The purpose of this study was to evaluate individual exposures to Hg in Huancavelica, Peru. The objectives of this study were to: (1) quantify total Hg in hair samples from 118 participants in Huancavelica and compare the concentrations with other Hg-exposed populations, and (2) identify factors that influence exposure as represented by total Hg concentrations in hair, including residential Hg contamination and environmental factors. The results of this study will be used to prioritize future research efforts in Huancavelica for evaluating and reducing exposure.

4.2 Methods

4.2.1 Residential samples

Samples of adobe brick, dirt floor, surface dust, and vapor were collected from 60 residences in four distinct neighborhoods (Ascención, San Cristóbal, Santa Ana, and Yananaco) in August 2010, as described by Hagan et al. (2013). Verbal consent for participation was obtained from an adult resident at each household and was recorded in the field log. Institutional review board (IRB) approval for the study was obtained through Duke University.

Residential samples were analyzed for total Hg concentrations according to EPA Method 1631 (US EPA 2002) as described by Hagan et al. (2013). Adobe brick and dirt floor samples

underwent additional analyses for Hg-speciation (Bloom et al. 2003) and bioaccessibility (Schaider et al. 2007) as described in Hagan et al. (*submitted*).

4.2.2 Hair samples and questionnaire data

Hair samples and self-reported questionnaire data were collected from 118 participants in five distinct neighborhoods (Ascencion, San Cristobal, Santa Ana, Santa Barbara, Yananaco) in July 2012. Written consent for participation was obtained from an adult resident; IRB approval for the study was obtained through Duke University and the Ethics Committee at the National University of Huancavelica.

Hair samples were collected from the occipital region of the scalp by isolating a 0.75-1 cm diameter bundle of hair, cutting the hair as close to the scalp as possible, then securing the scalp end of the hair in a folded piece of paper with a paper clip prior to labeling with the sample identification number (UNEP 2008). Hair within 1-2 cm of the scalp region of the sample (mass of 0.01-0.02 g) was analyzed for total Hg by thermal decomposition using a RA-915+ Lumex Mercury Vapor Analyzer equipped with a RP-M324 attachment (Ohio Lumex Co., Twinsburg, OH) according to EPA Method 30B (US EPA 2008).

Health assessment questionnaires were adapted from UNEP (2008) and are provided in the Appendix. Information was collected through a face-to-face interview with an adult resident at the same time as collection of the hair sample. Information was collected related to occupational exposure, diet, confounders, and health effects (e.g., sleep disturbances, physical and mental fatigue, and well-being).

4.2.3 Statistical analyses

Data for concentrations of Hg in residential samples and in hair samples were tested for normality prior to statistical analysis, and both were found to be log-normally distributed.

Relationships between total Hg concentrations in hair samples and for total, speciated, and bioaccessible Hg concentrations in residential samples were evaluated with Pearson correlation coefficients. Correlations were calculated for households with paired hair and residential data (n=34). One-way analysis of variance (ANOVA) was performed between total Hg concentrations in hair and responses from questionnaires to identify associations between factors other than residential concentrations that might influence exposure and body burden. One-way ANOVAs were performed on the 118 participants with hair and questionnaire data, although sample size varied if a participant did not respond to a particular question on the questionnaire. The values represented in the boxplots are the first quartile, median, and third quartile. The whiskers are the minimum and the maximum and the diamonds represent the arithmetic means. Statistical analyses were completed using SAS 9.2 software (SAS Institute Inc., Cary, NC).

4.3 Results

4.3.1 Correlations between Hg in hair and Hg in residential samples

Total Hg concentrations in hair ranged from 0.10 to 3.6 µg/g. Pearson correlation coefficients were calculated for total Hg concentrations in participants' hair and total Hg concentrations in residential samples for these participants, as shown in Table 4-1. The associations between total Hg concentrations in hair and total Hg concentrations in adobe bricks, dirt floors, and surface dust were positive but not statistically significant. Similarly, but not shown in Table 1, Pearson correlation coefficients were calculated for total Hg concentrations in hair and speciated or bioaccessible fractions of Hg in residential samples. Again, no significant correlations were found, with the exception of the “elemental” fraction of Hg in adobe bricks (r=0.493, p=0.023).

Table 4-1. Pearson correlation coefficients (r), p-values (p), and sample sizes (n) for log-transformed mass of total Hg in hair and log-transformed mass of total Hg in residential samples

		Adobe Bricks	Dirt Floors	Surface Dust	Indoor Air
Hair	r	0.226	0.332	0.203	-0.089
	p	0.198	0.055	0.250	0.617
	n	34	34	34	34

4.3.2 Relationships between Hg in hair and other factors

The relationships between total Hg concentrations in hair and questionnaire responses related to occupational exposure, diet issues, confounders, and health effects were evaluated using one-way ANOVA. Responses that were statistically significantly related to total Hg concentrations in hair included: gender ($p < 0.001$) as shown in Figure 4-1; living in a neighborhood where smelters were previously located ($p = 0.021$), Figure 4-2; smoking status ($p = 0.003$), Figure 4-3; frequency of house cleaning ($p = 0.019$), Figure 4-4; and frequency of fish consumption ($p = 0.046$), in Figure 4-5.

Figure 4-1. Total Hg concentrations in hair based on gender (n=116)

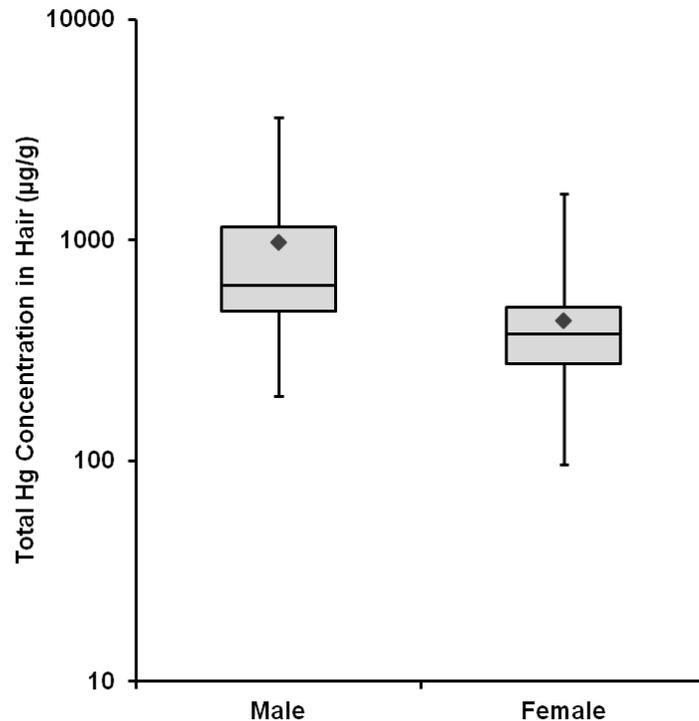


Figure 4-2. Total Hg concentrations in hair based on living in a neighborhood with historical cinnabar smelter activity (n=117)

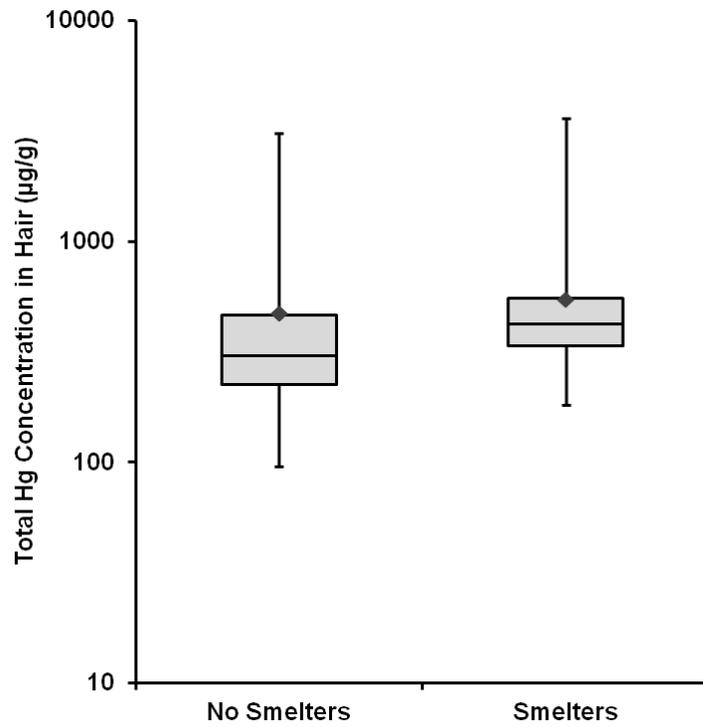


Figure 4-3. Total Hg concentrations in hair based on smoking status (n=117)

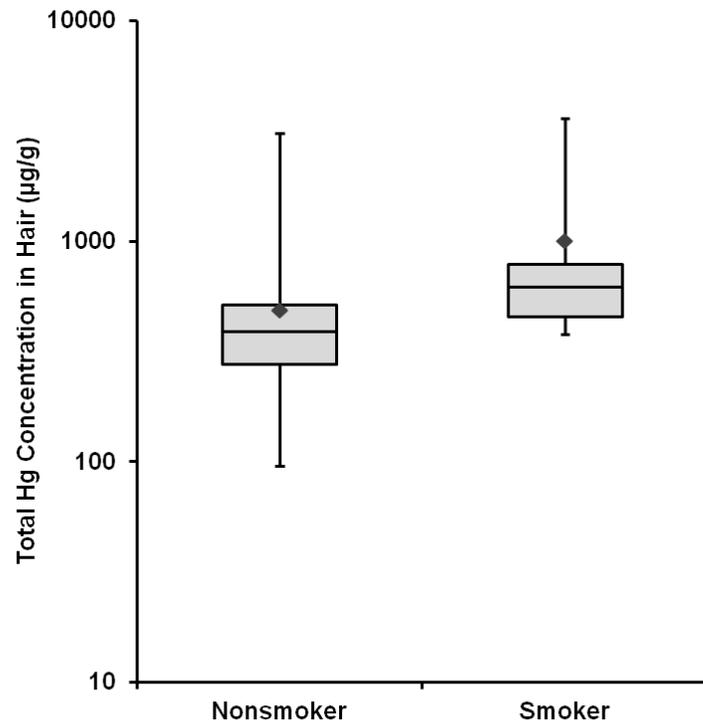


Figure 4-4. Total Hg concentrations in hair based on frequency of house cleaning (n=109)

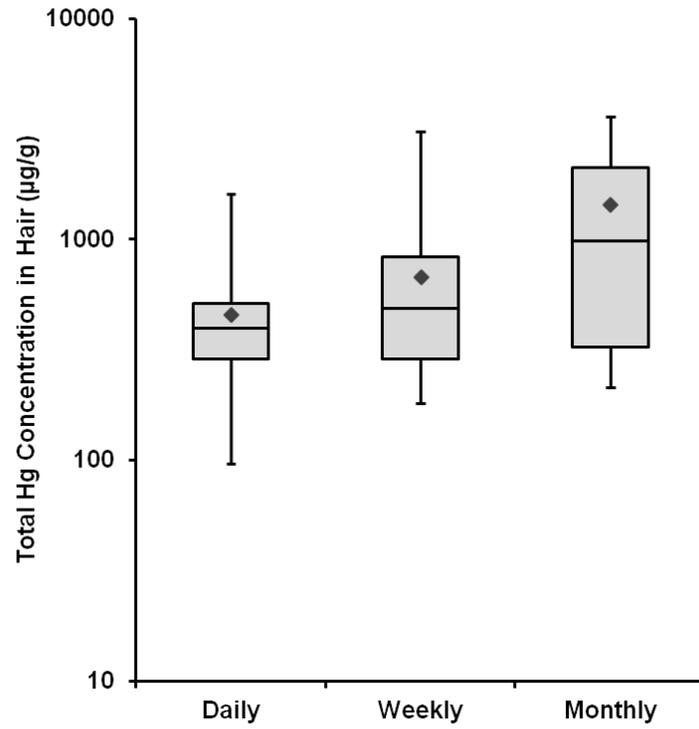
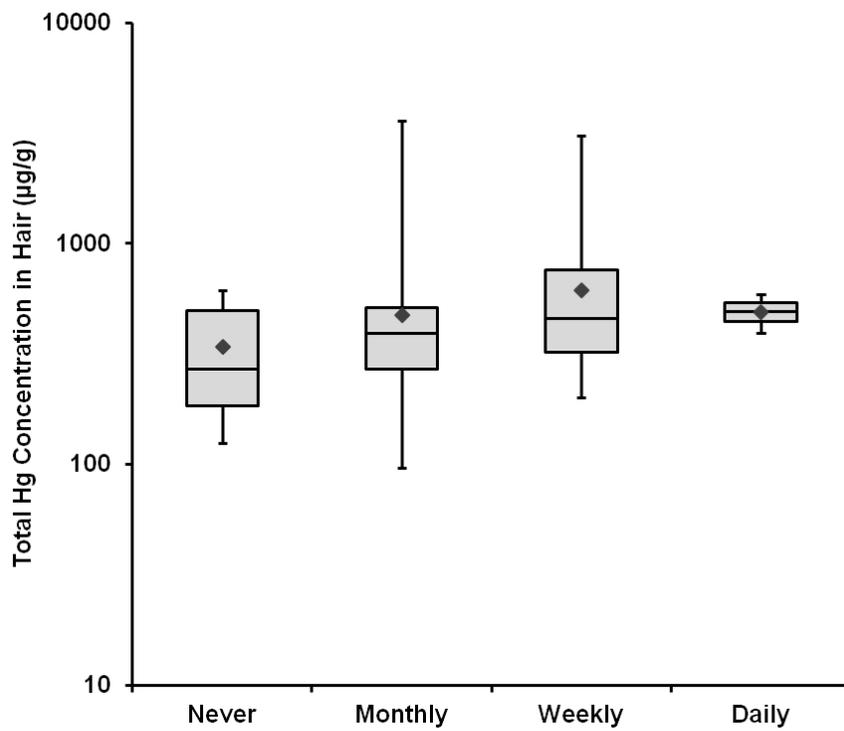


Figure 4-5. Total Hg concentrations in hair based on frequency of fish consumption (n=117)



4.4 Discussion

4.4.1 Relationship between Hg in hair and Hg in residential samples

Total Hg concentrations are relatively high in adobe brick, dirt floor, and surface dust samples in residences in Huancavelica. High residential Hg contamination could be related to high Hg concentrations in hair for the 34 participants who had paired hair and residential data. On the other hand, a previous study found that only a small percentage of the total Hg in residential samples was bioaccessible (Hagan et al. *submitted*). Pearson correlation coefficients for data in the present study suggest that, while there is a positive association between concentrations of total Hg in hair and concentrations of total Hg in adobe bricks, dirt floors, and surface dust, these correlations are not statistically significant. A larger sample size might drive these positive correlations into significance.

4.4.2 Relationships between Hg in hair and other factors

The relationship between gender and total Hg concentrations in hair was highly significant ($p < 0.001$) as shown in Figure 4-1. Males ($n=21$) were found to have significantly higher total Hg concentrations in hair than females ($n=95$). This finding may reflect different exposures between males and females. In Huancavelica, males are more likely to be employed directly in mining or in other occupations that would likely increase exposure (e.g., construction projects that would increase exposure to contaminated dirt and dust in the ambient environment). Díez et al. (2011) also found greater Hg concentrations in hair for males and attributed the difference to males consuming larger portions of fish.

The presence of previous cinnabar refining in the neighborhood was significantly related to total Hg concentrations in hair ($p=0.021$), as shown in Figure 4-2. Participants living in a neighborhood that historically had smelters operating (Ascención, San Cristóbal, Yananaco;

n=87) had significantly higher total Hg concentrations in hair than those living in a neighborhood that did not historically have cinnabar refining operations (Santa Ana, Santa Barbara; n=30). A previous study found statistically significant differences in total Hg concentrations in adobe bricks, dirt floors, and surface dust by neighborhood (Hagan et al. 2013), which would suggest that higher total Hg concentrations in hair are related to legacy contamination from historic cinnabar refining.

Smoking status was also significantly related to total Hg concentrations in hair ($p=0.003$), as shown in Figure 4-3. Smokers (n=9) had significantly higher total Hg concentrations in hair than nonsmokers (n=108). The reason for the positive relationship between smoking and higher total Hg concentrations in hair is not clear. Perhaps an unknown, physiological reaction occurs as a result of smoking that increases the body burden of Hg. Mucociliary clearance in a smoker's respiratory tract tends to be impaired, thereby allowing more Hg-contaminated particles to reach a smoker's lungs. This result may also be a consequence of the hand-to-mouth activity that occurs when smoking.

The frequency of house cleaning was found to be significantly related to total Hg concentrations in hair ($p=0.019$), as shown in Figure 4-4. Participants who cleaned their houses daily (n=86) had lower total Hg concentrations in hair than participants who cleaned their houses monthly (n=4); there was no significant difference in Hg hair concentrations between those who cleaned their houses weekly (n=19) and those who cleaned more or less frequently. The difference between more and less frequent house cleaning is likely a result of having less Hg-contaminated dust on surfaces within the home, thus reducing potential ingestion from hand-to-mouth activity or from food surfaces.

Hair is typically used as a measurement of exposure to methyl mercury from fish consumption. In Huancavelica, total Hg concentrations in hair were also significantly related to the frequency of fish consumption, as shown in Figure 4-5. Residents of Huancavelica consume fish less frequently than other populations, with a majority of participants reporting monthly fish consumption.

4.4.3 Comparison of total Hg concentrations in hair in Huancavelica and elsewhere

Hair has been used as a biological indicator in other studies evaluating varying routes of exposure to Hg, as shown in Table 4-2. Díez et al. (2011) measured total Hg concentrations in hair in Almadén and Castilla-La Mancha, Spain, for those exposed as a result of historic cinnabar mining and refining. The range of total Hg concentrations for hair in Almadén and Castilla-La Mancha is similar to the range in Huancavelica. However, the mean concentrations in the two Spanish sites are 3 and 4.5 times greater than the mean concentration in Huancavelica. Populations exposed to Hg from gold mining and refining in the Philippines (Drasch et al. 2001) and Bolivia (Barbieri et al. 2009), had hair concentrations orders of magnitude higher than those in Huancavelica. Mezghani-Chaari et al. (2010) measured total Hg concentrations in hair for frequent fish consumers in Tunisia and found a mean concentration of 6.5 µg/g, over 12 times greater than the mean total Hg concentration in hair in Huancavelica. As a comparison, non-fish consumers in Japan (Endo and Haraguchi 2010) had total Hg concentrations in hair nearly 5 times greater than participants in Huancavelica. In the U.S., total Hg concentrations in hair measured as a part of the National Health and Nutrition Examination Survey (NHANES) found a lower range of hair concentrations than in the present study, but the mean concentration of 0.47 µg/g was comparable to the mean concentration in Huancavelica (McDowell et al. 2004).

4.4.4 Limitations of the present study

Although positive associations were found for the relationship between total Hg concentration in participants' hair and Hg concentrations in samples of adobe bricks, dirt floors, and surface dust taken in participants' residences, these relationships were not statistically significant. One reason for the lack of significance for these positive relationships may be that the number of participants with paired hair and residential data was low (n=34).

This study measured total Hg concentrations in hair from adults only. Adults in general have lower hand-to-mouth activity than children and as such would likely be exposed less frequently to Hg-contaminated particles within the home. Children have special health risks due to exposure to Hg that could not be evaluated in the present study.

While hair is routinely used as a biomarker for exposure to methylmercury, other chemical forms of Hg may not be represented adequately in hair concentrations. Inhalation of elemental Hg vapor and ingestion of Hg-contaminated particles might be more accurately characterized using other biomarkers, such as whole blood or urine. Elemental and inorganic forms of Hg are incorporated into hair much less efficiently than methylmercury (Nuttall 2006) so that exposure to these forms is potentially underestimated by using hair samples alone.

Table 4-2. Concentrations of total Hg in hair for populations with different exposures

Location	Sample Size (n)	Mean (µg/g)	Range (µg/g)	Remarks	Reference
Huancavelica, Peru	118	0.53	0.10 – 3.6	Cinnabar mining region	Current study
Almadén, Spain	108	2.4	0.67 – 4.8	Cinnabar mining region	Díez et al. 2011
Castilla-La Mancha, Spain	62	1.7	0.20 – 5.6	Cinnabar mining region	Díez et al. 2011
Diwalwal, Philippines	316	2.7	0.03 – 38	Gold mining region	Drasch et al. 2001
Beni river, Bolivia	150	3.8	0.42 – 16	Gold mining region	Barbieri et al. 2009
Gulf of Gabes, Tunisia	55	6.5	1.3 – 14	Frequent fish consumers	Mezghani-Chaari et al. 2010
Taiji, Japan	50	2.5	0.4 – 6.1	Non-fish consumers	Endo and Haraguchi 2010
United States	1726	0.47	0.35 – 0.58	NHANES study, 16-49 year old women	McDowell et al. 2004

4.5 Conclusions

Total Hg concentrations were measured in hair samples from 118 participants in Huancavelica, Peru, a site of historic cinnabar refining. Total Hg concentrations in hair averaged 0.53 $\mu\text{g/g}$ (range: 0.10 to 3.6 $\mu\text{g/g}$) similar to those found in the U.S. but lower than other Hg-exposed populations around the world. Although a positive relationship was found between total Hg concentrations in hair and Hg-contaminated residential samples, the relationship was not statistically significant. Total Hg concentrations in hair were significantly related to gender, living in a neighborhood with smelters, smoking status, frequency of house cleaning, and frequency of fish consumption.

Further studies are needed to better characterize Hg exposure in Huancavelica, particularly as related to residential contamination. Whole blood and urine can be used in parallel with hair samples to measure recent exposures to other forms of Hg. Future research efforts may require larger sample sizes and evaluation of children's exposures; these studies would require additional IRB approvals. A more complete understanding of exposure will assist future efforts to reduce individual exposure to Hg contamination in Huancavelica, Peru.

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CHAPTER 5. CONCLUDING REMARKS

This chapter summarizes the key conclusions from this dissertation. The first section briefly summarizes the main scientific findings produced by this study. Secondly, future research questions generated from this research are identified. Finally, the implications of the key scientific findings of this study are considered to help address future intervention and remediation strategies to reduce Hg exposure.

5.1 Scientific findings

Chapter 2 was designed to characterize the spatial distribution of Hg in residences by (a) determining the total Hg concentrations in residential samples from 60 homes in four distinct neighborhoods, and (b) compare the concentrations with respect to the neighborhoods. The key scientific findings resulting from this study include:

- Total mercury (Hg) concentrations in adobe bricks, dirt floors, surface dust, and air are elevated and concern exists for potential health effects resulting from exposure.
- Strong relationships exist between total Hg concentrations in adobe bricks, dirt floors, and surface dust, confirming that residents use on-site materials to build their adobe brick houses.

In Chapter 3, the Hg present in residential samples was characterized based on species and bioaccessibility. Moreover, the potential health risks from ingestion of contaminated Hg-particles were evaluated against international health benchmarks. The key scientific findings

from this study include:

- Although total Hg concentrations in residential samples are high, the percent of Hg in adobe bricks and dirt floors that is bioaccessible in simulated gastric fluid is low.
- Concentrations of Hg in simulated gastric fluid and the more soluble fractions of the sequential selective extractions were strongly correlated, suggesting that simulated gastric fluid can be used as a screening tool to effectively target research time and resources.
- The percent of bioaccessible Hg in adobe bricks and dirt floors, although low compared to total Hg concentrations, are of potential concern for small children who may ingest more Hg-contaminated particles from hand-to-mouth activity.

Chapter 4 aimed to characterize and evaluate individual exposure to Hg using total Hg concentrations in hair, total and speciated Hg measurements in residential samples, and self-reported questionnaire data. The key scientific findings from this study include:

- Total Hg concentrations in hair from residents of Huancavelica were similar to those measured in the United States and lower than in other populations around the world.
- Positive correlations existed between total Hg concentrations in hair and total Hg concentrations in residential samples, but statistical significance was lacking.
- Self-reported questionnaire data identified gender, living in a neighborhood with smelters, smoking status, frequency of house cleaning, and frequency of fish consumption as factors that are related to total Hg concentrations in hair.

5.2 Future research questions

The primary objectives of this dissertation were met, as detailed above. However, the studies identified additional areas of potential interest for future studies.

- One of the primary limitations to this study is the use of hair as a biomarker of exposure to total Hg in residential samples. Future research efforts would benefit from the use of whole blood and urine to measure recent Hg exposure. Further characterization of individual exposure could be useful in identifying significant exposure factors that would lead to a refinement of questionnaires in future exposure studies.
- This study included only adult participants in the evaluation of exposure through total Hg concentrations in hair and self-reported questionnaire data. Children likely have different exposures than adults, particularly given the increased hand-to-mouth activity exhibited by young children. Future research efforts to evaluate individual exposure to residential Hg contamination in Huancavelica may benefit from focusing on children's exposure as a particularly susceptible population.

5.3 Implications for intervention and remediation

The research in this dissertation will be used to inform study participants and the broader community about Hg contamination and exposure in Huancavelica and will guide future intervention and remediation efforts in the city.

- Participants should be informed about not only total Hg concentrations in hair and residential samples, but be educated about the species and bioaccessibility of Hg in adobe bricks and dirt floors. By providing a thorough understanding of these measurements, participants will be better informed about ways to reduce residential exposure, particularly for households with small children that would have increased hand-to-mouth activity within the home.
- Total Hg concentrations in residential samples should be combined with total Hg concentrations in hair and speciation and bioaccessibility data to identify households with

the greatest potential risks from Hg exposure. Future efforts to focus on reducing exposure through intervention or remediation strategies, while beneficial to all households, should be targeted towards households with more bioaccessible Hg in adobe bricks and dirt floors and with small children living in the home.

In risk assessment, estimators of exposure range from broad and general data (e.g., demographic information, emissions inventories) to more refined and detailed data (e.g., measured and modeled internal doses, personal measurements) as described by Sexton et al. (1992) and shown in Figure 5-1. With increasing detail and accuracy, the cost associated with exposure estimators increases. This research, from the inception of the larger project as well as the detailed measurement and exposure studies as a part of this dissertation, has followed this paradigm and as a result has allowed for a more refined estimate of exposure for residents of Huancavelica. While initial measurements of total Hg in ambient soil supported concerns for a widespread public health problem, more detailed data suggest that the magnitude of the concern may not be as great as initially thought and that time and resources should be focused on refining estimates of internal dose and populations at greater risk for exposure (e.g., children).

Table 5-1. Traditional exposure estimates in risk assessment and application in this research

Exposure Estimators (Sexton et al. 1992)	Generally Lower Cost and Limited Accuracy	Exposure Estimators (This research project)
Production Volumes	↓	Archival documentation of Hg production and use during Spanish colonial period (Robins 2011; Robins and Hagan 2011)
Emission Inventories		Ambient soil total Hg concentrations (Robins et al. 2012)
Environmental Concentrations - Models - Measurements		Residential total Hg concentrations (Hagan et al. 2013)
Microenvironmental Concentrations - Models - Measurements		Estimating ingestion exposure using speciated and bioaccessible fractions of Hg (Hagan et al. <i>submitted</i>)
Human Contact - Models - Measurements		Generally Higher Cost and Better Accuracy
Internal Dose - Models - Measurements		

The purpose of this research was to investigate the extent of residential exposure Hg contamination in Huancavelica and to evaluate personal exposure to Hg from indoor sources. While total Hg concentrations were demonstrated to be elevated, additional chemical analyses allowed for the identification of households with the greatest potential risk from ingestion of Hg-contaminated particles. Total Hg concentrations in hair were low and should be analyzed in parallel with other biomarkers of exposure (e.g., whole blood, urine) to more fully characterize personal exposure. The combination of total and speciated Hg measurements with biomarkers of

exposure and self-reported questionnaire data can be used to inform residents of Huancavelica and to effectively target future intervention and remediation efforts to reduce Hg exposure.

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APPENDIX: HEALTH ASSESSMENT QUESTIONNAIRE

Structured in accordance with the “Guide for the identification of populations of high risk due to mercury exposure” WHO-UNEP (IOMC-August 2008-Geneva-Switzerland) and reviewed and approved by Duke University, United States.

Health Assessment Questionnaire
Huancavelica, Peru

1. PERSONAL DATA

Participant ID Number: _____

Family Name: _____

First Name: _____

Date of Birth: _____ Age: _____(years)

Gender: ____ Female ____ Male

Address: _____

Telephone for contact: _____

2. GENERAL QUESTIONNAIRE

Date of interview: _____

Name of the interviewer: _____ Code of the interviewer _____

2.1. Residential Exposure

Number of children that live in the house? _____

How old are the children that live in the house? _____

How often do you clean or dust your house? _____

How often do you wash your hands? _____

What are the inside walls of your home made of? _____

What is the floor of your home made of? _____

Where do you wash your clothes? _____

Where do you dry your clothes? _____

2.2. Occupational Exposure

How long have you been living in this area? _____ year(s)

Occupation (Detailed description of the job)

Have you ever worked in cinnabar mining?

____ No ____ Yes

If yes, for how many year(s) and in what capacity?

2.3. Diet Issues

How frequently do you eat fish?

____ Never

____ At least once a **month**

____ At least once a **week**

____ At least once a **day**

Do you know where the fish come from? ____ No ____ Yes

If yes, from where does the fish come? _____

Name the place where you obtain drinking water:

2.4. Confounders

Severe neurological disease such as Parkinson, stroke, severe accident (brain injury), birth trauma, tetanus, polio, hyperthyroidism, epilepsy, malaria or any acute severe disease, etc. may introduce too many factors that could confuse this illness with Hg intoxication symptoms.

Have you ever had any neurological disorders (epilepsy, stroke, Parkinsons, etc.) or mental disorders (schizophrenia, bi-polar disorder, etc.)?

____ No ____ Yes

Which disease (problem)? _____

Have you ever contracted malaria, leprosy, AIDs, Hepatitis, Tuberculosis or any other major infectious disease? No Yes

If yes, which disease?

Did you have any serious accidents (did you have to go to hospital)? Mark with an X

No Yes, but not severe Yes, and it was severe (over 1hr. unconsciousness)

When did this happen? _____

Have you been constantly handling gasoline and kerosene? (this can develop tremors) No Yes

If yes, how many years you have been doing this? _____ (years)

Have you been constantly handling insecticides or pesticides?

No Yes

If yes, how many years you have been doing this? _____ (years)

Do you smoke? Mark with an X

- Never
- Rarely (0-10 cigarettes per day)
- Medium (10-20 cigarettes per day)
- Lots (more then 20 cigarettes per day)

Do you drink alcohol?

- Never
- at least once a month
- at least once a week
- at least once a day

Have you been using whitening soap (for lightening the skin)?

No Yes

3. HEALTH QUESTIONNAIRE

Date of interview: _____

Name of the interviewer: _____ Code of the interviewer _____

Do you feel a metallic taste in your mouth? Mark with an X

Never
 at least once a **month**
 at least once a **week**
 at least once a **day**

Do you suffer from excessive salivation?
 Never
 at least once a **month**
 at least once a **week**
 at least once a **day**

How is your appetite?
 Good Fair Poor

Did you loose weight within the last year?
 No Yes

Did you loose hair within the last year?
 No or only rarely Yes, slight to moderate Yes, marked to severe

Have you been coughing within the last year for more than for 3 months?
 No Yes

Have you ever had kidney disease except urinary tract infection?
 No Yes

Which disease (problem)? _____
Have you ever had severe respiratory problems (asthma, pneumonia)?

No Yes
Which disease (problem)? _____

Are you healthy now?
 Yes No
Why not? _____

TREMORS

Have you had any problems with tremors? Mark with an X

(Clinical Tremor Rating Scale)

- No
- I have some tremors but they do not interfere with my job or responsibilities
- I am able to work, but I need to be more careful than the average person
- I am able to do everything, but with errors; poorer than usual performance because of tremor
- I am unable to do a regular job, I may have changed to a different job due to tremor; it limits some housework, such as ironing
- I am unable to do any outside job; housework very limited

SLEEP DISTURBANCES

How do you feel after a usual night of sleep?
 Good Fair Poor

FATIGUE

Score to estimate the state of fatigue (Wessely S, Powell R: Fatigue syndrome)

Do you get tired easily?
 No Yes

Do you feel sleepy or drowsy?

No Yes

Do you always lack energy?

No Yes

Do you feel weak?

No Yes

Do you suffer from insomnia?

No Yes. If yes, how often? _____

MENTAL FATIGUE Mark with an X

Do you have problems concentrating?

No Yes

Do you have problems thinking clearly?

No Yes

Do you have problems to find correct words when you speak?

No Yes

Do you have problems with eyestrain?

No Yes

Do you have problems with memory?

No Yes

WELL BEING Mark with an X

Do you feel nervous?

Never

at least once a **month**

at least once a **week**

at least once a **day**

Do you feel sad?

Never

at least once a **month**

at least once a **week**

at least once a **day**

Do you lose your patience easily?

No Yes

Do you feel shy?

No Yes. If yes, how often? _____

Do you have palpitations, ie feeling the heart beating rapidly?

Never

at least once a **month**

at least once a **week**

at least once a **day**

Do you have a headache?

Never

at least once a **month**

at least once a **week**

at least once a **day**

Do you have nausea?

Never

at least once a **month**

at least once a **week**

at least once a **day**

Do you feel numbness, prickling, aching at any location of your body?

Never

at least once a **month**

at least once a **week**

at least once a **day**

Do you have difficulty walking steadily?

No Yes

Do you have bluish discoloration of the gums

No

Slight

Yes, obvious

4. SAMPLES

Date and time of sample collection:

Name of the specimen taker: _____ Code _____

Hair

Yes, sample collected

No

Urine

Yes, sample collected

No

Blood

Yes, sample collected

No