Intra-individual and Inter-individual Variation in Mercury Concentrations in Modern Human Skeletons: Impact of Dental Amalgam

by

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ABSTRACT

The distribution and transport of mercury in the human body are poorly constrained. For instance, the long-term persistence and intra-individual distribution of mercury in bones from dental amalgams or environmental exposure have not been studied. A robust method validated for accuracy and precision specifically for mercury in human bones would facilitate studies of mercury in anthropological, forensic, and medical studies. I present a highly precise, accurate mercury concentration analytical method targeted to human bone samples. This method uses commercially commonly available and reliable instruments that are not limited to elemental Hg analysis. This method requires significantly lower sample amounts than existing methods because it has a much lower limit of detection compared to the best mercury analyzers on the market and other analytical methods. With the low limit of detection achieved, this mercury concentration protocol is an excellent fit for studies with a limited amount of samples for destructive analysis. I then use this method to analyze the mercury concentration distribution in modern skeletal collections provided by three U.S. anthropological research facilities. Mercury concentration and distribution were analyzed from 35 donors' skeletons with 18 different skeletal elements (bones) per donor to evaluate both the intra-individual and inter-individual variation in mercury concentration. Considered factors include geological differences in decomposition sites and the presence of dental amalgam filling. Geological differences in decomposition sites did not statistically affect the mercury concentration in the donor's skeleton. The presence of dental amalgam significantly affected the inter-individual and intra-individual mercury concentration variation in donors' skeletal samples. Individuals who had dental amalgam had significantly higher mercury

concentration in their skeleton compared to individuals who did not have dental amalgam (p-value <0.01). Mercury concentration in the mandible, occipital bone, patella, and proximal phalanx (foot) was significantly affected by the presence of dental amalgam.

DEDICATION

To this beautiful/fascinating world filled with incredible loving people.

Also, to my guitar. Thank you for the accompany.

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*The IRB determined that human subjects were not involved in this research as defined by DHHS and FDA regulations.

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CHAPTER 1

INTRODUCTION

The pathways of deposition and transport of mercury in humans are important in forensics, health, and anthropology. These fields require mercury (Hg) concentration analyses with high accuracy and high precision on very small sample sizes (<10 mg). Research on the concentration and distribution of mercury in human skeletons is constrained by limits of detection and the challenges of sacrificing material from skeletal collections to destructive analysis.

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is arguably the most versatile, element-specific detection technique (Pröfrock & Prange, 2012). Quadrupole Inductively Coupled Plasma Mass Spectrometers (Q-ICP-MS) are reliable and commonly available instruments for high accuracy and precision elemental concentrations with low limit of detections. Mercury's volatility presents a challenge to routine, accurate, and precise measurement of mercury concentrations in human bone. Successful methods have been developed for measuring mercury concentration in samples with Q-ICP-MS in geochemistry (Zheng et al., 2018). However, these methods are not successfully adapted to measure bone samples, due to the differences in sample preparation and sample availability. Bone samples are generally more porous and covered by organic material, so additional cleaning steps for bone samples are required. Destructive analyses in the field of anthropology and archaeology must be minimized to preserve samples intact, so sample availability is challenging.

In Chapter 2, I present a highly precise and accurate mercury concentration analytical method targeted to human bone samples, circumventing the difficulties

mentioned above. This method develops a sample preparation protocol that allows for not only mercury concentration measurement, but also major and trace element concentrations, and can be additionally prepared for analysis of strontium and lead isotopes – all on 10 mgs of sample powder. This method exploits commercially commonly available and reliable instruments that are not limited to elemental Hg analysis. This method requires significantly less sample amounts than existing methods and has a much lower limit of detection compared to the best mercury analyzers on the market.

Chapter 3 presents the analysis of mercury concentration distribution in modern skeletal collections provided from three U.S. anthropological research facilities. I investigated factors that could affect mercury concentration and distribution of 35 donors' skeletons with 18 different skeletal elements per donor to evaluate both the intra-individual and inter-individual variation in mercury concentration. Considered factors include geological differences in decomposition sites, and dental amalgam filling. Chapter 3 also includes investigations on why certain skeletal elements have higher mercury concentrations than others.

Finally, chapter 4 concludes the results of this study and discusses the results of studying mercury concentration and distribution in the human skeleton. Chapter 4 includes ideas for further studies on investigating the pathways and sources of mercury in human skeletons and how much benefit it can bring to anthropology, forensics, and the medical field.

CHAPTER 2

MERCURY ANALYSIS BY Q-ICP-MS COUPLED WITH A LIQUID-VAPOR SEPARATOR

2.1 Introduction

Mercury measurements in the human skeleton require destructive analysis, which prevents bone samples from being analyzed for anthropological metrics, or any other associated analyses. Typically, samples from archaeological, forensic, or medical studies are sharply limited. The goal of this chapter is to develop a mercury analysis method with high precision and accuracy for human skeleton samples.

In previous studies, Rasmussen et al. (2013) used a mercury analyzer coupled with a Cold Vapor Atomic Absorption Spectrometer (CV-AAS) to measure mercury concentration in bone samples for archaeological studies. Rasmussen measured mercury concentration in the skeleton of two human individuals from medieval Denmark. The results of their mercury concentration analysis were 403 ppm for individual AG93 and 556 ppm for individual AG104. A Cold Vapor Atomic Absorption Spectrometer (CV-AAS) on a dedicated mercury analyzer (FIMS-400) was used for mercury concentration analysis. With this setup, they were able to achieve 1.5 ppb (30 ng of Hg) limit of detection with 20 mg of samples usage per run. In our study, we analyzed modern human bones samples. The mercury concentration of most of our samples was below 1.5 ppb, and we only had 5 mg per sample available for mercury analysis. By using ICP-MS coupled with a liquid-vapor separator we achieved a detection limit of (0.0456 pg of Hg) 0.00912 parts per trillion for 200Hg and (0.08165 pg) 0.01633 ppt for Hg202 for Hg concentration in bone analysis.

There are previous methods developed for mercury isotopic measurement using MC-ICP-MS coupled with a continuous flow cold vapor (CV) generation system, which is similar to a liquid-vapor separator (Chen et al., 2010). Their method was dedicated to mercury isotopic measurements of natural water samples with low mercury concentration, showing the high consistency of this sample introduction system to ICP-MS. However, solid sample mercury concentration measurements require different sample preparation procedures compared to natural water mercury isotopic measurements. For natural water mercury isotopic measurements, samples can be analyzed directly after going through the mercury column, but solid samples require acid dissolution and dilution procedures during sample preparation

2.2 Sample collection and storage

Standards were obtained from multiple sources and required different storage conditions. Rib bone samples from Elton (a two-year old grass-fed steer from Eloy, Arizona) were used as in-house standard because a large number of subsamples could be processed in parallel with samples. These rib bone samples had the majority of the soft tissue removed and stored inside a freezer. Elton's ribs were cut into 1-inch-long sections with a surgical oscillating saw and placed into paper envelopes before mechanical cleaning. Human bone samples were allowed to naturally decompose in one of three outdoor anthropological research facilities, followed by maceration to remove soft tissue. Samples were cut into 1 to 3 gram pieces at the skeletal collections corresponding to the anthropological research facilities. IAEA-086 hair standard, a fine powder, is certified for mercury concentration. IAEA-086 is humidity sensitive and was stored in a desiccator.

JWS, a human cremain standard, and NIST 1400, a powdered ashed bone standard, were also stored in a desiccator.

2.3 Sample cleaning and powdering

Due to the characteristics of human skeleton samples, additional cleaning procedures were required in sample preparation compared to working on other solid samples. Human bone samples and Elton bone standards were mechanically cleaned with a Dremel rotary tool to remove visible dirt, debris, any remaining muscle tissue and the periosteum. The trabecular bone has a large surface area and high porosity and permeability making it susceptible for accumulating contaminants. It was also removed mechanically with the Dremel. Sample powdering was required for improving the homogeneity of samples and increasing surface area for better acid digestion efficiency.

A DREMEL 4000 High-Performance variable speed rotary tool with a flex shaft rotary attachment and diamond DREMEL bits were used in the sample mechanically cleaning process. The flex shaft rotary attachment reduced the weight of the tool held by the operating hand which significantly reduced workload and provide more stable control of the tool. In addition, the flex shaft enabled the motor for the Dremel to be at some distance from the sample, drastically reducing the amount of air turbulence that caused sample powder to be lost. To make the cleaning process easier, weighing papers were used for making sample trays to capture sample powder during the mechanical cleaning process. Each sample was processed on a freshly made paper tray with cleaned diamond rotary bit and a new pair of nitrile gloves to avoid cross-contamination. The paper tray was folded as shown in Figure 2.1. There were “walls” on three sides the paper tray to

capture bone dust. No wall on the right side of the paper tray allowed the tool holding hand to move more freely (no wall on the left side for left-handed users). Bone dust does not go to the right side when using the right hand to operate the tool. Dust-resistant lab suit, closed-toe shoes, nitrile gloves, goggles, and a respirator mask were required as personal protection equipment for mechanically cleaning and powdering samples due to health and safety concerns. Samples were processed in a laminar air-flow exhausted polypropylene biosafety hood to minimize potential risk to the analyst while simultaneously minimizing contamination risk both from the laminar air flow and the all-plastic construction. Ethanol, 18.2 MΩ purified water, and Micro-90 soap were used for hardware and hood cleaning between samples to avoid possible cross-contamination. The SOP of mechanical cleaning bone samples is included in the appendix X.

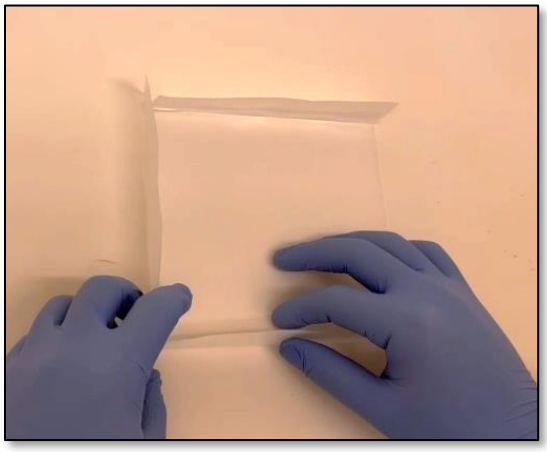

Figure 2.1 Paper tray

Figure 2.2 Tools for mechanical sample cleaning and preparation

After mechanical cleaning, each sample was chemically cleaned to wash out any possible contaminants and dust produced during the mechanical cleaning process. Each sample was placed in 15 mL trace-metal grade centrifuge tube, then washed and sonicated in 18.2 MΩ purified water and ethanol five times each. After the washing process, samples were dried in a forced air-drying oven at 50 °C overnight. The SOP of chemical cleaning bone samples is included in the appendix X.

After the cleaning and drying process, each sample was powdered twice to maximize the surface area of the sample for more efficient acid dissolution. Samples were powdered with the DREMEL rotary tool with diamond rotary bits first, then powdered again with a set of mortar and pestle. Each sample was placed in a 2 mL snap-cap acid-cleaned centrifuge tube after powdering. The additional powdering process maximizes the homogeneity of each sample which benefits sample digestion consistency. This set of human skeleton samples was also used for carbonate analysis; a high homogeneity of the samples was required, which were achieved by the additional powdering process. This powdered bone was then subdivided for Hg concentration

analysis (this thesis), elemental concentrations, strontium isotope composition, phosphate oxygen isotope composition, and carbonate carbon and oxygen isotope composition.

Each sample was mechanically cleaned and powdered on a freshly made weigh paper tray with cleaned diamond rotary bit and a new pair of nitrile gloves to avoid cross-contamination. The powdering hood and the DREMEL rotary tool were wiped with ethanol after processing each sample. The diamond rotary bits were rinsed and sonicated in 18.2 MΩ purified water then air-dried after each use. Mortar and pestle were first rinsed with deionized water, washed with Micro-90 soap, then rinsed again with 18.2 MΩ purified water before each use. Five mL of ethanol was used to speed up the air-drying process of the mortar and pesto. This cleaning procedure was applied thoroughly before processing each sample.

2.4 Sample Dissolution and Sample Dilution

After the initial sample processing, 10 mg of each bone sample and 5 mg of IAEA 086 standard were weighed with a microbalance and placed in a 4 mL screw-top bottle. Samples were then transferred to the trace metal clean lab for acid digestion and dilution. Figure 2.3 shows the workflow of the sample dissolution and sample dilution process. The SOP of bone sample dissolution and sample dilution is included in the appendix Y.

In order to ensure data quality control and comparability across both time and multiple batches of samples processed and analytical sessions, samples were divided into 15 batches with 49 samples per batch. For each batch, there were three process blanks. Within each batch of 49 samples, there were three samples of the Elton cow bone standard, processed as unknowns by assigning a randomized laboratory identification

number. Ten percent of all samples were divided in two and processed in replicate. Finally, all samples, including replicates, were randomized prior to assigning a laboratory identification number. This ensured that any variation between individuals, sites, or skeletal element (occipital, rib, femur…) did not correlate with batch of samples processed or analytical session.

For each batch of 40 samples processed, there were three process blanks, a sample of NIST 1400, IAEA 086, JWS in-house cremains, and three of the samples were digested in triplicate. The samples selected for triplicate digestion were the first three samples of the batch that had sufficient powdered material to digest in triplicate and leave sufficient material for carbonate and phosphate isotope preparation. Because the Elton in-house standards had been randomized during the bone cleaning process, and the analyst was blind to their identity at this point in sample processing, they may have been used as one of the samples processed in triplicate. In addition, a subset of samples were processed in duplicate if there was any question of the accuracy of the weighing process.

0.125 mL of 12M trace metal grade hydrochloric acid (HCl) and 0.375 mL of 16M trace metal grade nitric acid (HNO_3; creating reverse aqua regia) were added to the human bone samples, in-house bone standards, bone ash standards, IAEA 086 hair standards, 10 pg Hg samples and process blanks during the acid digestion process. Samples were sonicated for an hour, then placed in a ventilated hood for 24-72 hours, allowing it to fully digest. After all solids were dissolved, 3.5 mL of 18.2 MΩ purified water were added to each sample to make 4 mL of stock digest solution at 0.375 M HCl and 1.5 M HNO_3. This lower molarity solution was more appropriate for long-term

storage for future strontium isotope sample preparation while still maintaining sufficient chlorine to stabilize mercury in solution.

Two mL of stock solution was pipetted to a weighed glass vial that was pre-cleaned with 1% BrCl solution to reduce the mercury blank. Due to supply chain issues during the covid-19 pandemic, glass vials were not available for processing all samples. Hence, later batches were processed in trace-metal grade 15 mL polypropylene centrifuge tubes. The trace-metal tubes were verified to be low in mercury and were not cleaned with 1% BrCl prior to use. In order to prevent mercury for adhering to the plastic walls of the tubes, samples were analyzed within 72 hours of being placed in the tube, and within five days of the beginning of the digest.

To each sample aliquot, 0.25ml BrCl was added, bringing the final intended ICP solution volume to 5% BrCl. 0.75 mL of BrCl was added to the IAEA 086 samples because the final ICP volume for IAEA samples was 15 mLs because IAEA 086 has a higher expected Hg concentration. 3.25 mL of Hg matrix solution (0.5% BrCl, 0.1% hydroxylamine-hydrochloride, 0.5% HCl) was added to the samples except for IAEA 086 samples, which were diluted with 13.75 mL of Hg matrix solution. Because the matrix solution is intended to neutralize the BrCl, the matrix solution was added within 48 hours of analysis to prevent volatilization and loss of mercury prior to analysis.

Mercury in samples becomes highly volatile during the acid digestion process. BrCl solution (5% to total diluted sample volume) was added to the Hg samples to stabilize Hg in solution, which minimizes Hg loss. BrCl solution oxidizes elemental Hg(0) to Hg(2+), which is the stable form of Hg in the solution. Samples do not have to be analyzed right

away after adding BrCl solution, which creates more flexibility across the samples analysis process.

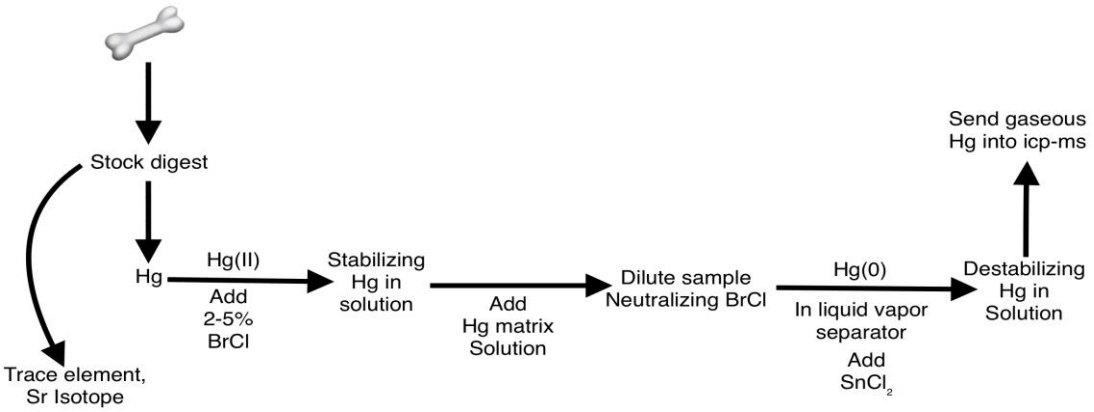

Figure 2.3 Sample dissolution and dilution workflow.

2.5 Instrumental Analysis

The instrumental configuration used in this work was a liquid-vapor separator coupled directly to a quadrupole ICP-MS. An iCAP-Q™ quadrupole mass spectrometer (ThermoFisher Scientific) was used for detection and quantification of mercury. A glass liquid-vapor separator was used to volatilize the mercury from samples in liquid form to gaseous species. A liquid-vapor separator only allows gaseous species to enter the instrument, dramatically reducing the matrix effects. An SC-4DX auto-sampler (Elemental Scientific, Inc.), accommodating up to 240 15 mL and 10 50 mL centrifuge tubes, allowed more samples in a sequence to maximize analytical efficiency.

Hg in samples were converted from liquid form to gaseous form through liquid-vapor separator which extraordinarily enhanced the accuracy and precision of Hg measurements with ICPMS. Gaseous samples are relatively clean to the plasma compared to liquid samples, which result in less sediment buildup on the cone, expand the lifetime

of the cone, minimize instrumental drifts throughout measurements, and enhance the reliability of the instrument. Gaseous samples require less energy from ICP due to the absence of the desolation process and vaporization process, which enhance the ionization efficiency, resulting in a higher sensitivity of elemental Hg analysis (Chan et al., 2021).

With the use of a liquid-vapor separator, the instrument analyzed Hg samples in gaseous form. One consequence of this configuration is internal standard, typically used in solution mode ICP-MS for monitoring instrumental sensitivity drift, is not co-aspirated with sample. Because the liquid-vapor separator prevents the sample matrix from entering the instrument, it eliminates the primary cause of changing instrumental sensitivity. The cones remain clean for extended periods, as minimal salts as aspirated into the instrument. In theory, data could have been corrected for changes in sensitivity using the frequent check standards. In practice, this was not done since the precision and accuracy of the check standards across multiple days of runs and throughout long sequences was very good (see Tables 2.2 and 2.3)

A low Hg blank Hg rinse solution was made with 5% nitric acid, and 5% hydrochloric acid in 18.2 MΩ purified water for rinsing out carryover Hg in the system between each sample analysis. The Hg rinse solution was not only made using nitric acid because the nitrate ion is not a strong enough oxidizer to maintain Hg in an ionic form that is water-soluble. Nitric acid catalyzes the reduction of mercury, which will make it couple with anything else in the solution that can be oxidized, so neutral, hydrophobic mercury will bind anything hydrophobic such as Teflon and PVC pump tubing. Thus, hydrochloric acid was added to the Hg rinse solution because chloride is a soft base anion

ligand that will coordinate soft acid cation Hg and help it remain unoxidized and soluble resulting a cleaner rinse.

The liquid-vapor separator come with two parts, a glass liquid-vapor separator housing and a frosted glass rod, both needs to be cleaned and conditioned prior to connecting to the inlet of the iCAP-Q™. The frosted glass rod was preserved in 10% KOH solution when not in use. The 10% KOH solution is an alkaline solution used to keep the surface of the frosted glass rod from drying out during the setup procedure. A dried frosted glass rod could cause liquid samples not evenly distributed on the glass rod while reacting with $SnCl_2$ resulting an unstable analysis. The frosted glass rod was rinsed with 18.2 MΩ purified water then gently placed into the housing of the gas-vapor separator. High vacuum grease and a plastic clamp sealed and secured the connection of the frosted glass rod and the liquid-vapor separator housing. The liquid-vapor separator was then filled with 10% nitric acid for conditioning. Ten percent nitric acid neutralizes possible remining KOH solution on the glass rod. The conditioning process of the liquid-vapor separator took about 20 minutes. The conditioning solution was drained shortly before the starting of instrumental tuning process to prevent the frosted glass rod from drying.

After conditioning, the liquid-vapor separator was connected to the iCAP-Q™ nebulizer inlet as a reaction vessel for the reduction reaction of Hg(2+). When Hg(2+) is reduced on the high surface area of the frosted glass rod to Hg(0), it volatilizes the Hg from the liquid sample. A lower opening of the liquid vapor separator connected to the additional gas 2 argon supply of the iCAP-Q™ for pushing gaseous Hg samples upward toward the outlet leading to the nebulizer of the instrument. An upper opening of the gas

vapor separator was connected to the Neb gas argon supply of the iCAP-Q™ for pushing gaseous Hg samples into the nebulizer of iCAP-Q™. The flow rate of additional gas 2, and neb gas is included in appendix Z performance report. Another lower opening of the liquid vapor separator was connected to the waste line for waste removal. A Y-connector was then placed 1 mm above the tip of the frosted glass rod inside the liquid-vapor separator housing. It was used to deliver a mixture of sample solution (or rinse solution) with $SnCl_2$ solution (purged with argon to release trace Hg) to the frosted glass rod with a 1:1 ratio for starting the reduction reaction of Hg(2+). One inlet of the Y-connector was connected to the auto sampler for transporting sample solution and rinse solution and the other side connected to argon-purged $SnCl_2$ solution. Figure 2.5 demonstrates the configuration of the gas vapor separator after the conditioning process.

In order to make Hg in liquid samples fully vaporize in the liquid-vapor separator, the Hg matrix solution contains hydroxylamine-hydrochloride which neutralizes the access of BrCl added to samples for preservation, creating a clear path for the reduction reaction of Hg(2+).

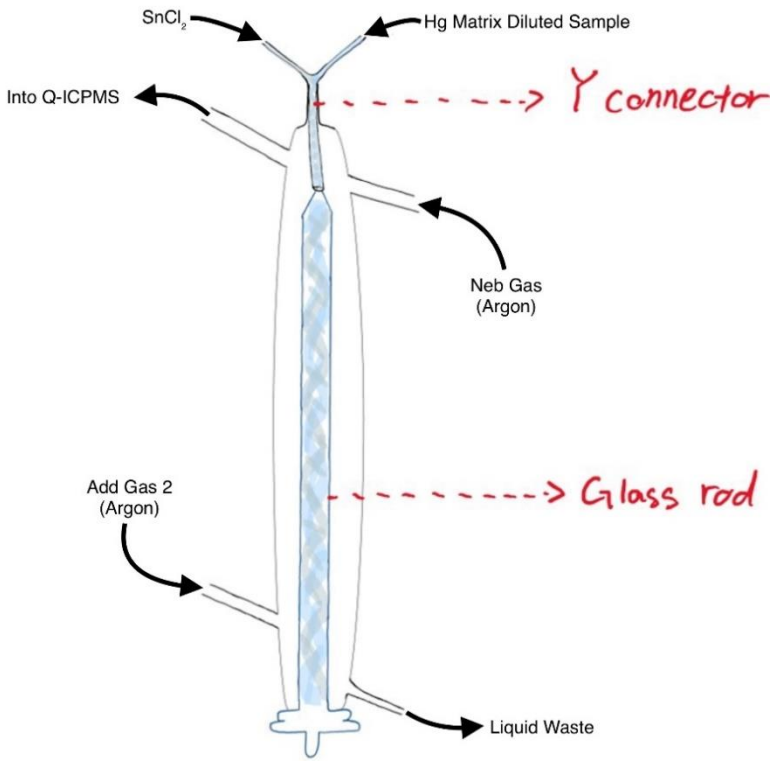

Figure 2.4, Liquid-vapor separator

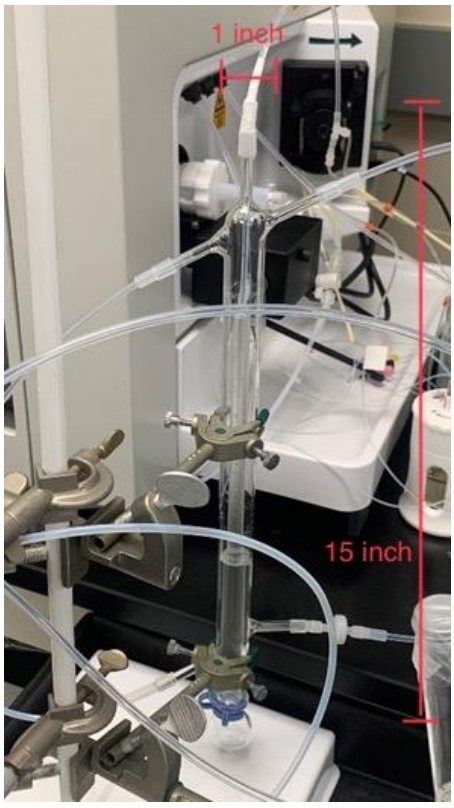

Figure 2.5, Liquid-vapor separator discarding conditioning solution

2.6 Software setup and Calibration

Qtegra is the default iCAP-Q™ software used for instrumental calibration, control, and analytical sequence setup. It helped to setup the best performance of the iCAP-Q™ when paring with the liquid vapor separator. The autotune feature in Qtegra was set to achieve the sensitivity of at least 100,000 counts per second for a 100 ppb tune solution, but analysis proceeded only when it performs over 300,000 cps. Instrumental sensitivity can be affected by many variables, and the running configurations is included in appendix Y. Sample analytical sequence was programmed through Qtegra.

Each sample, blank, and standard analysis was comprised of fifteen main runs to monitor solution washing in and washing out of the sample. Each main run had 100 sweeps of 10ms for ^{202}Hg and 50ms for ^{200}Hg. Each analysis was proceeded by five 9.6 second survey scans for potential interferents and checking signal stability before each analysis. However, survey scans were only spot checked and were not routinely examined.

A low concentration check standard of 5 parts per trillion (ppt) was analyzed every six samples to monitor for any drifts in the instrument's background and measurement accuracy as well as sample washout. A check standard of 33 ppt was measured after every third 5 ppt check standard to monitor accuracy and precision at higher concentrations. The washout time between sample analyses was 210 seconds, and instrument blanks were measured at least every six samples to monitor for varying background and carryover between samples.

The iCAP-Q™ converts counts per second to a concentration unit using the slope of the calibration curve. Therefore, different brands of standards were used to make the

calibration solutions and the check standards to construct trusted analyses. A pre-diluted 9.932 ppb JT Baker Hg standard (containing 5% BrCl) was used to construct the calibration curve after diluted with Hg matrix solution with targeted range of 1 part per trillion to 100 parts per trillion (1ppt, 2.5ppt, 10ppt, 25ppt, 50ppt, 100ppt). 5ppt and 33ppt check standards were made with NIST-3133 Hg standard (contained 5% BrCl) and diluted with Hg matrix solutions. Due to the volatility of mercury, calibration and check standards were not used more than 72 hours after dilution for analysis.

2.7 Method Validation

This method of measuring Hg concentration in bone samples was tested and developed with a variety of in-house standards, certified standards, check standards and process blanks. Certified standards and in-house standards were prepared and analyzed in parallel with the human bone samples to monitor the accuracy and reproducibility of the entire procedure. Multiple standards were used because there are no known matrix-matched bone samples with reported mercury concentrations. The certified standards include NIST-1400 cow bone ash standard and IAEA-086 hair standard. In-house standards include JWS human cremains, a grass-fed cow named Elton, and 10 picogram (pg) Hg samples (made from JT Baker Hg standard). All the certified and in-house standards went through the same acid digestion and dilution process with the human bone samples.

Sample contamination is a primary concern when working in a lab with other ongoing projects covering many different sample types. Process blanks were made in

parallel with samples during the sample preparation processes to monitor the Hg blank of the reagents, containers, and facility.

2.8 Results

2.8.1 Limits of detection, quantitation, and performance of check standards

Table 2.1 shows the achieved limit of detection (LOD) and limit of quantitation (LOQ) for this mercury analysis method. LOD and LOQ were calculated with the equation LOD=3σ/k, and LOQ=10σ/k, where k was the sensitivity of the instrument which was calculated as the slope of the calibration curve, and σ was the standard deviation of the instrumental response in cps from 10 analytical blank measurements.

Table 2.1, Limit of detection (LOD) and limit of quantitation (LOQ) of Hg concentration analysis in bone samples. Sample batch #15

	^{200}Hg (ppt)	^{202}Hg (ppt)
LOD	0.00912	0.0163
LOQ	0.0304	0.0545

To evaluate the accuracy of the analyses, several check standards were made from a gravimetrically prepared stock solution of NIST 3133. These check standards were independent of the calibration solution, made from a gravimetrically prepared Hg ICP standard from JT Baker. This also allowed evaluation of the long-term stability of the two stocks; they had slightly different matrices and were unlikely to have lost mercury at the same rate. To monitor background stability for low concentration samples, a 5 ppt check standard was measured 305 times. The average percentage recovery for 5 ppt check standards was 96.5 ± 7.5 % for ^{200}Hg and 96.2 ± 7.5 % for ^{202}Hg (n= 305, 1s; Table 2.2). The 5 ppt check standard was run most frequently because it was a better match to the

concentrations of the samples. In addition, the accuracy and precision of the 5 ppt check standard was a more rigorous test of the method compared to a higher concentration check standard. The average percentage recovery of ^{200}Hg for 33 ppt check standards was 100.5 ±5.5 %) and 100.5 ±5.5 % for ^{202}Hg (n=169, 1s; Table 2.3). The 33 ppt check standards had a higher percent of recovery and a lower standard deviation in both ^{200}Hg and ^{202}Hg compared to the 5 ppt check standards.

A total of 57 process blanks were analyzed across 15 batches of samples resulting in an average of 10.08 pg of Hg blank with a 6.61 pg standard deviation. Data for samples and processed certified standards was corrected for the process blank for each specific batch. This enabled correction of slightly different backgrounds due to different reagent batches.

Table 2.2, Summary of 5 ppt check standards measurement after process *blank correction*

	^{200}Hg Concentration	^{202}Hg Concentration	^{200}Hg % Recovery	^{202}Hg % Recovery
Average	4.79 ppt	4.74 ppt	96.52%	96.20%
Standard deviation (1σ)	0.39 ppt	0.39 ppt	7.52%	7.49%
n	305			

Table 2.3, Summary of 33 ppt check standards measurement after process *blank correction*

	^{200}Hg Concentration	^{202}Hg Concentration	^{200}Hg % Recovery	^{202}Hg % Recovery
Average	32.68 ppt	32.68 ppt	100.52%	100.53%
Standard deviation (1σ)	2.01 ppt	2.01 ppt	5.48%	5.49%
n	169			

2.8.2 Results of processed bone standards

As mentioned above, there are no known bone standards with certified mercury concentrations. However, several bone standards were processed in parallel with samples despite the anticipated low mercury concentrations. Aliquots of all samples discussed here were also measured for major and trace element concentrations, as well as strontium isotopic composition. Those results are outside the scope of this thesis. Two of the bone standards processed in parallel with samples, NIST 1400 Bone Ash and JWS (human cremains), were processed at cremation temperatures for sample stability and ease of storage. However, high temperature in an oxidizing atmosphere is likely to volatilize mercury, so these samples were anticipated to have low mercury concentrations. The third bone standard (Elton) was a grass-fed two-year old steer from Eloy, Arizona. Although the mercury concentration was not known *a priori*, from independent knowledge of the area and the husbandry practices of the rancher, this sample was also expected to be low. However, these bone samples were useful for constraining the accuracy and precision of the other measurements, as well as evaluating the efficiency of the acid digestion protocol to solubilize the bone. Many traditional methods of processing anthropological bone ash bone samples prior to acid digestion to remove organic matter (cf, Marsteller et al 2017).

Table 2.5-2.7 are data summaries of NIST-1400, JWS and Elton samples. As expected, they all have low Hg concentrations. The high standard deviations were caused by low Hg concentration (<1 ppt) which shows that higher amount of sample usage were required for a more precise and accurate measurement of NIST-1400, JWS and Elton samples. Equation for dilution calculation shown in Equation (1).

Table 2.5, NIST-1400 Hg concentration measurement

	ppb of Hg in sample	ppb of Hg with averaged replicate measurements
Average	0.41	0.45
Standard deviation (1σ)	0.37	0.39
n	18	15

Table 2.6, JWS Hg concentration measurement

	ppb of Hg in sample	ppb of Hg with averaged replicate measurements
Average	0.43	0.46
Standard deviation (1σ)	0.33	0.34
n	17	15

Table 2.7, Elton Hg concentration measurement

	ppb of Hg in sample
Average	0.99
Standard deviation (1σ)	1.33
n	40

$$\frac{\left(\frac{Final\ Dilution\ Weight\ (g) \times Instrument\ Readout\ (ppt)}{1000}\right)}{\left(\frac{Weight\ of\ Hg\ Aliquot\ (g)}{Weight\ of\ Stock\ Digest\ (g)} \times Sample\ Weight\ (g)\right)} = \text{Hg Concentration in Samples (ppb)} \quad (1)$$

2.8.3 Results for biological standard certified for mercury

Because the matrix-matched bone standards were all expected to be low in mercury concentration, an alternate biological samples certified for mercury was sought. IAEA-086 is a hair standard certified for mercury. Although it is not a good matrix-match for bone, it was anticipated to digest in the same acid protocol as the bone digestion method. This allowed the use of an identical acid digestion procedure for all samples. A different sample type, such as fish muscle, would be expected to require hydrogen peroxide to ensure successful digestion of lipids and other biological molecules.

A total of 24 IAEA-086 samples were analyzed, and 7 of them had replicate measurements. After process blank correction, the percent recovery of IAEA-086 samples was 98 ±16 % (*n* =x, 1s). The percent recovery calculated with averaged

replicate measurements after the process blank correction was 99%, with a standard deviation of 18%. Table 2.8 shows the data summary of IAEA-086 samples. The higher standard deviation of IAEA-086 hair standard samples was possibly caused by human error during the weighing process. IAEA-086 hair standard came in as a powder and was stored in a desiccator to avoid moisture. During the sample weighing process, the sudden humidity change from the container stored in a desiccator to the lab environment could result in electric charge build-up in IAEA-086 hair standard samples. IAEA-086 samples would stick to the weighing paper and the outside of the thread of the screw-top bottles due to the electrostatic effect causing inconsistency in the sample weighing process for the IAEA-086 hair standard. Another factor contributing to the higher standard deviation was that 5 mg of IAEA-086 hair standard was used for each sample digestion compared to 10 mg used for other bone samples, so the inconsistency caused by the electrostatic effect was further magnified.

A total of fourteen 10 pg Hg samples were analyzed to monitor the reproducibility of sample dissolution and dilution procedures. and 5 of 10 pg Hg samples had replicate measurements. The 10 pg Hg samples were made from the JT Baker Hg standard and went through the same digestion and dissolution process with other samples. After process blank correction, the percent recovery of 10 pg Hg samples was 97.1%, with a 6.6% standard deviation. The percent recovery calculated with the average replicate measurements was 97.7%, with a 5.8% standard deviation. Table 2.9 shows the data summary of 10 pg Hg samples.

Table 2.8, IAEA-086 Hg concentration measurement

	ppb of Hg in sample	ppb of Hg with averaged replicate measurements	% Recovery	% Recovery with averaged replicate measurements
Average	560.80	567.09	98%	99%
Standard deviation (1σ)	93.56	101.09	16%	18%
n	31	24	31	24

Table 2.9, 10 pg Hg samples Hg concentration measurement

	ppb of Hg in sample	% Recovery	% Recovery with averaged replicate measurements
Average	107.88	97.1%	97.7%
Standard deviation (1σ)	36.46	6.6%	5.8%
N	19	19	24

2.8 Discussion

The low limit of detection achieved by this method results less samples usage for precise and accurate Hg concentration analysis. With the extraordinarily low limit of detection, and high sample reproducibility achieved, this mercury analyzing method is a perfect fit for studies with limited amount of sample usage for destructive analysis, which could benefit archaeological, forensic, and medical field.

CHAPTER 3

INCORPORATION OF DENTAL AMALGAM INTO HUMAN BONES

3.1 Introduction

The goal of this chapter was to investigate the intra-individual and inter-individual variation in mercury concentrations in modern human skeletons caused by dental amalgam.

3.1.1 Mercury toxicity and health risks

Mercury is a heavy metal with a wide variety of industrial and dental uses, and it is highly toxic to humans (Tchounwou et al., 2012). Ingestion and inhalation of mercury can cause neurological and behavioral disorders, such as tremors, emotional instability, insomnia, and memory loss (Yilmaz et al., 2014; Anglen et al., 2015; ROSSINI et al., 2000; Siblerud et al., 2019). Overdose of mercury can cause significant organ malfunctions such as kidney failure and thyroid inflammation (Kester et al., 2012; Pamphlett et al., 2021).

3.1.2 Mercury cycling in the environment

Elemental mercury enters the environment naturally through volcanism. However, exposure to modern humans primarily occurs through industrial pollution and amalgamation in the gold mining process. When elemental mercury enters the ocean, microorganisms transform elemental mercury into methylmercury through methylation. Methylmercury builds up in fish through bioaccumulation, resulting commonly consumed aquatic animals such as tuna, king mackerel, shark, and swordfish, which are placed in a higher food chain to have higher mercury concentration.

3.1.3 Sources of mercury to humans: diet

Mercury is a toxic element, yet we still consume mercury daily from unexpected sources (Håkanson et al., 1988; Storelli et al., 2007; Eggleston & Nylander, 1987) One of the common mercury intakes is seafood consumption (Birke et al., 1972). The average mercury intake for males was 4.74 μg/day and 3.07 μg/day for females. Seafood was the food group that contributes the most. Fish, and shellfish accounting for 77.8% of mercury intake from food (Kim et al., 2016).

3.1.4 The cycling of mercury in humans

There are various types of mercury intake to the human body, and they have different pathways. In the human body, mercury occurs in forms of inorganic mercury, elemental mercury (Hg0) and organic mercury compound such as methylmercury (Park & Zheng, 2012). When ingested methylmercury, it will be absorbed by the intestine and then deposited in soft organs like the kidney, whereas the brain is the main reservoir for inorganic mercury, which is absorbed by the lungs through inhalation (Rodríguez & Mandalunis, 2018). The half-life of mercury in the human body is about 70 days, after which 90% is excreted (Parfitt, 2002).

3.1.5 Sources of mercury to humans: dental fillings

Mercury-silver metal amalgam is one of the most common materials used as a dental filling for treating cavities and it is made mostly out of mercury (Spencer, 2000). Dental amalgam usually contains about 50% mercury, 22-32% silver, ~14% tin, and ~8% zinc and other trace elements (Ferracane, 2001). Due to its high mercury concentration, dental amalgam has been banned in Sweden, Denmark, and Norway since 2008 (Kopperud et al., 2016). However, the US Food and Drug Administration (FDA) still considers dental

amalgam safe for adults and children over six years old (Center for Devices and Radiological Health, 2020; Levy et al., 2004). There have been many discussions about the health risks and environmental effects of dental amalgam in the United States and Europe for many years (Mutter, 2011).

For many years, the primary source of mercury in our bodies was believed to come from seafood consumption (Kim et al., 2016). However, with the change in modern human dietary habits, 92% of adults have dental cavities from age 20 to 64 (National Institute of Dental and Craniofacial Research, 2018). American Dental Association reported that 51.5% of restored teeth were done with dental amalgam (Estrich et al., 2021), which raises awareness of human mercury intake through dental amalgam, because dental amalgam stays in the human oral cavity constantly. The average daily absorption of mercury from dental amalgam is estimated at 0.2 to 0.4 μg per day per amalgam-filled tooth surface, or 0.5 to 1 μg per day per amalgam-filled tooth, age and other factors can cause variation (Richardson et al., 2011). Mercury from dental amalgam fillings have become the dominant source of mercury uptake in the human body and central nervous system of the general population in industrialized countries (Nordberg et al., 2007).

Previous studies suggest two ways mercury is absorbed from dental amalgam. One way is that 80% of mercury vapor (Hg0) released from dental amalgam is absorbed through inhalation, and less than 10% of mercury from ingested amalgam particles oxidized to Hg+2 and absorbed by the human intestine (Nordberg et al., 2007; Pant et al., 2012). Because elemental mercury is fat-soluble and can also be absorbed through the central nerve system, red blood cells, and bone marrow (Dantzig, 2003; Haddad et al.,

1990), other pathways of mercury absorption from dental amalgam could have been overlooked.

The human tooth is placed directly onto the alveolar bone with fibrous tissue called the periodontal ligament in between. The pulp chamber inside the tooth has nerve endings and blood vessels constantly supplying nutrients to the tooth (Ghannam, 2021). Dental amalgam contains mostly elemental mercury, and it is highly soluble in lipids and easily crosses cell membranes (Gossel & Bricker, 1994). Once in the blood, elemental mercury can distribute throughout the body, as well as penetrate the blood-brain barrier accumulating in the brain (Berlin et al., 1969). Bone marrow is in charge of producing red blood cells and there is constant nutrient exchange between blood and bone (Marenzana et al., 2013; Cooper, 2011). It is possible that amalgam could leach mercury directly into the blood vessels in the pulp chamber cause a drastic increase in mercury intake in the human skeleton and the human brain.

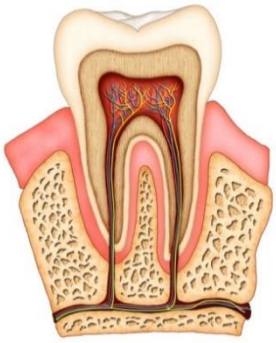

Figure 3.1 Human tooth cross-section (Tooth Cross-Section, 2022)

To this date, there are no studies on the effects of dental amalgam to human skeleton, which can be very useful for understanding how amalgam affects mercury concentration and distribution in the human body. Since there are few studies on mercury

in human skeletons in general, research on how dental amalgam affects mercury concentration distribution in the human skeleton can open a new area of study on mercury transfer in the human body and mercury accumulation in different skeletal elements.

3.1.6 Mercury in anthropology

The study of mercury concentration distribution in modern human skeleton is missing. There are previous studies on mercury concentration in ancient human bones (Emslie et al., 2019; Emslie et al., 2015; Rasmussen et al., 2013). However, they were all conducted on ancient human skeletons which are not direct reflections of mercury concentration and distribution in modern human skeleton. Emslie et al., 2019 measured mercury concentration in human bone samples from archaeological sites in the Iberian Peninsula, a region with a history of using cinnabar as a pigment and as a preservative in burial practices during the 4th to 2nd millennia cal BC. Cinnabar has also been documented for medicinal use in ancient cultures throughout broad geographic regions. In ancient China, Cinnabar was used as a sedative to treat various ailments, including insomnia, inflammations, strokes, and epilepsy, all with limited impact from Hg exposure (Emslie et al., 2015). Previous studies have shown that ancient human bone samples from Neolithic and Chalcolithic sites have a high mercury concentration. However, there are still questions about the source of mercury in these human bone samples (Emslie et al., 2019). Mercury can enter bone samples diagenetically from the soil or cinnabar paint associated with burial procedures, or it can enter bones through biogenic pathways such as consuming mercury-enriched medicine (Emslie et al., 2019).

3.1.7 Study samples

In this chapter, mercury concentration of various skeletal elements from 35 individuals were analyzed to investigate the intra-individual and inter-individual variation in mercury concentration caused by dental amalgam. The 35 individuals selected for this analysis had lived for at least the last 20 years at the same location which should isolate the differences in mercury concentration between skeletal elements to biological parameters. The human skeletal samples used for mercury analysis came from three anthropological facilities. Donors 1-20 came from the Forensic Anthropology Research Facility at Texas State University, donors 21-30 came from the Forensic Anthropology Center at the University of Tennessee, and donors 31-35 came from Colorado Mesa University. Each individual had about 18 skeletal elements across their skeleton, which integrates into 734 human bone samples. Figures 3.2-3.3 show the skeletal elements obtained for this study.

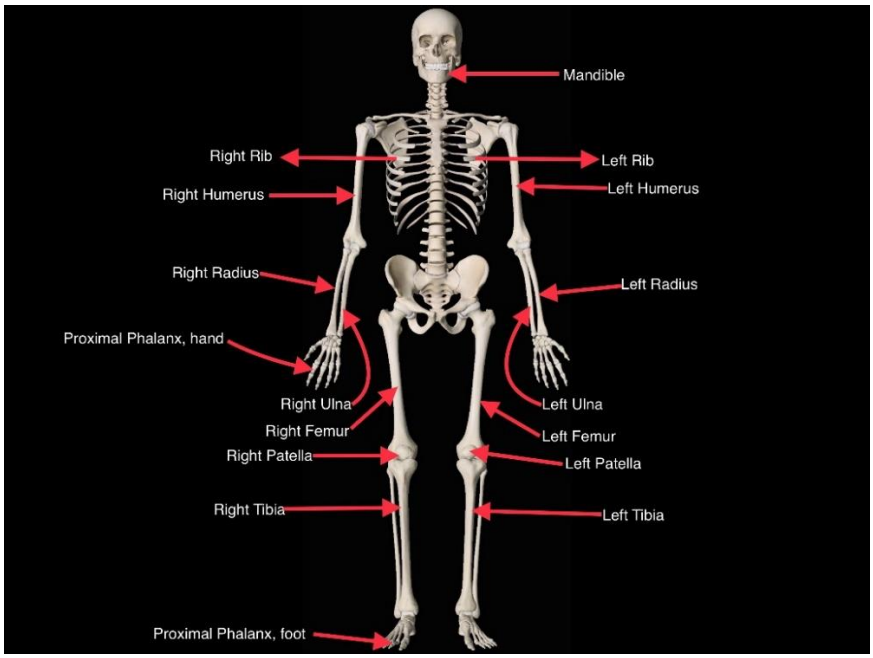

Figure 3.2 Analyzed skeletal elements. Anterior.
Image modified from Essential Skeleton 4

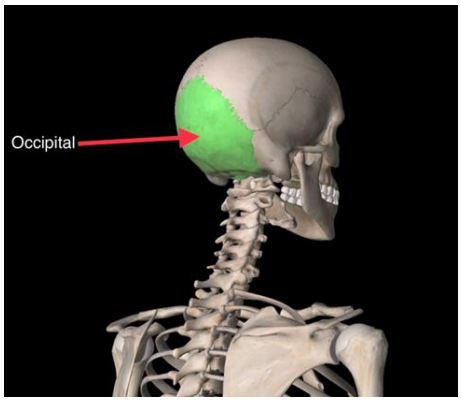

Figure 3.3 Analyzed skeletal elements. Posterior.
Image crated modified from Essential Skeleton 4

3.1.8 Study sites

These three decomposition sites have different geological features, and all 35 donors were left outside in a natural environment during the decomposition process which raised concerns on mercury gain or lose during the natural decomposition process caused by differences in decomposition sites. The University of Tennessee is located in Knoxville, where it rains about 50 inches per year which is a lot more than the other two sites. Humidity is a factor in the decomposition rate of donors and the alteration of their skeleton. Colorado Mesa University is located at Grand Junction, where the elevation is more than 4,500 feet. Due to high elevation, donors from Colorado Mesa University were exposed to high UV radiation, which caused visual effects on the skeletal samples. Texas State University is located in San Marcos. It has a higher average temperature all year round compared to the other two sites, which could be another factor in the decomposition rate and bone alteration. Thus, bulk comparisons were made regarding mercury concentration in these donors from these three anthropological facilities to investigate if geological differences in decomposition sites affect mercury concentration in donors' skeletons.

3.2 Methods

The methods used for preparing samples and measuring mercury are described in detail in Chapter 2.

3.2.1 Quality Control

Analytical studies involving large sample counts would take many days to finish instrumental analysis. Ideally, all the samples would be analyzed nonstop under a constant instrumental performance. However, due to the reliability and availability of the instrument, this ideal situation is less likely to be achieved. In this analysis, we had samples from 35 donors. Each donor has about 18 skeletal elements, and for some of them, we had to make replicate samples which add up to 733 human bone samples. Our samples were divided into 15 sets, with 49 human bone samples per set. Owing to the large number of individual samples we had, the instrumental analysis could not be finished in one sitting. Thus, we completely randomized our samples before organizing them into sets to eliminate analytical biases for each individual and each skeletal element. Some samples were too big to fit inside the 15 ml centrifuge tubes, so they were cut into equal-sized portions and made sample replicates. These sample replicates had their own digestion vessels, which is helpful for monitoring sample digestion reproducibility and the homogeneity of samples.

A total of 65 human samples had sample replicates which were cleaned, powdered, digested, and analyzed separately to monitor the sample cleaning process and monitor the acid digestion reproducibility. The median of the standard deviation of sample replicates was 0.53 ppb, and the median of the average Hg concentration across the 18 skeletal

elements was 12.42 ppb which shows the excellent consistency of the sample cleaning, powdering, and acid digesting process.

3.2.2 Statistical Calculation

Parametric tests like the T-test were preferred for testing the statistical differences between data sets. However, in order to perform a t-test, data sets must meet two requirements. The first requirement was the data points must be normally distributed, which were tested with the Shapiro Wilks test. The second requirement was that comparing data sets must have equal variances, which were tested with Levene's test. Wilcoxon tests were used to show statistical differences between data sets when one of the requirements for performing a t-test was not reached. All statistical calculations were done on RStudio, and the CRAN package was used for performing Levene's test.

3.3 Results

The results of analyzing mercury concentration in 35 individuals with 18 skeletal elements per individual are presented in this section. Mercury concentration data from three facilities were compared to see if differences in decomposition sites cause significant mercury concentration variations. Mercury concentrations between skeletal halves were compared to evaluate the reliability and reproducibility of the data. Mercury concentrations in various skeletal elements were compared to investigate the variation of mercury concentration in different skeletal elements. Intra-individual and inter-individual mercury concentration comparisons were made to show the impact of dental amalgam on the mercury concentration and distribution in the human skeleton.

3.3.1 Hg Concentration Variation Within Individuals

Hg concentration in 18 skeletal elements of 35 donors from three anthropological facilities was measured in this analysis. Donors 1-20 come from the Forensic Anthropology Research Facility at Texas State University (TS), donors 21-30 come from the Forensic Anthropology Center at the University of Tennessee (UT), and donors 31-35 come from Colorado Mesa University (CMU).

Figure 3.4 shows the average Hg concentration variations of each donor for a better understanding of the range of Hg concentration in these 35 individuals, and the full data is listed in table 3.5. The y-axis of figure 3.4 is log (Hg concentration of each donor), and the donor number on the x-axis. The average Hg concentration for individual #19 was the lowest, which was 0.89 ppb with a 0.62 ppb standard deviation. Individual #26 has the highest average Hg concentration, which was 1626.5 ppb with a 4720.63 ppb standard

deviation. The average Hg concertation of Donor #21 was 2.24, which was the median of the total 35 donors. Across 35 donors, the average Hg concentration was 59.51 ppb with a 273.49 ppb standard deviation. The average Hg concentration measured across all 35 individuals was 59.51 ppb with 273.49 ppb standard deviation and a median of 6.42 ppb.

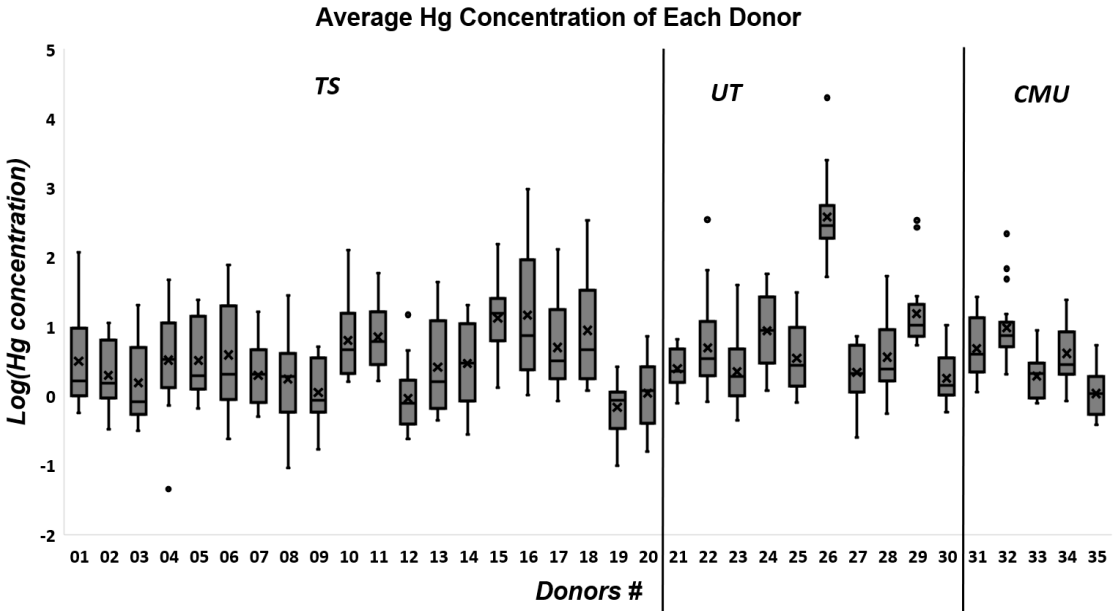

Figure 3.4 Average Hg concentration of 35 donors.

3.3.2 Hg Concentration Variation Between Facilities

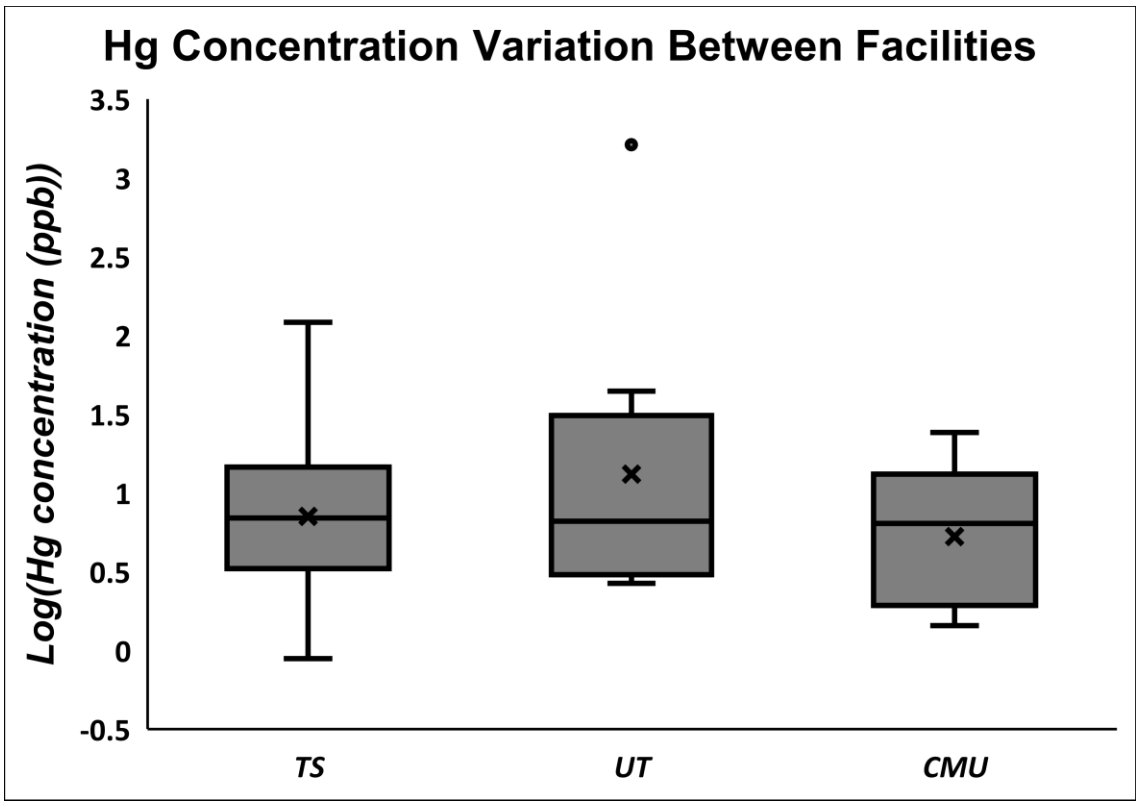

Figure 3.5 Hg concentration variation between three facilities. Differences between facilities were not statistically significant in pair-wise comparison (Table 3.3).

Figure 3.5, Table 3.1, and Table 3.2 show if Hg concentration in the donor's skeleton was affected by the geological difference of deposition sites. Figure 3.5 shows Hg concentration variation between three facilities which were constructed with the average Hg concentration of each donor. Twenty donors out of the 35 donors were from the Forensic Anthropology Research Facility at Texas State University (TS). The average Hg concentration of donors from TS was 15.03 ppb with a standard deviation of 26.52 ppb. Ten donors out of the 35 donors were from the Forensic Anthropology Center at the University of Tennessee (UT). The average Hg concentration of donors from UT was 12.68 ppb with a standard deviation of 14.35 ppb. Five donors out of the 35 donors were from Colorado Mesa University (CMU)). The average Hg concentration of donors from

Texas State University (TS) was 8.35 ppb with a standard deviation of 9.17 ppb.

T-test and Wilcoxon tests were done and shown in table 3.2 to show if there are significant differences between data analyzed from these three anthropological facilities. When p-value is greater than 0.05, the null hypothesis of no existing significant differences of measured Hg concentration between facilities is accepted. Donor #26 from UT has a much higher Hg concentration than others which is shown as an outlier in figure 3.5. This caused data from UT not passing the Shapiro Wilks test, thus Wilcoxon tests were done on statistical calculations involving UT data set.

Table 3.1 Hg concentration between three facilities.

	TS Hg Concentration (ppb)	**UT Hg Concentration (ppb)**	**CMU Hg Concentration (ppb)**
Max	121.15	1626.45	24.16
Min	0.89	2.67	1.44
Median	6.94	6.17	6.39
Mean	15.03	12.68	8.35
n	20	10	5

Table 3.2 Statistical tests between facilities.

	TS, UT (Wilcoxon)	**TS, CMU (t-test)**	**UT, CMU (Wilcoxon)**
***p*-value**	0.59	0.62	0.44
Significant differences	No	No	No

3.3.3 Hg Concentration Variation Withing Skeletal Halves (Left vs. Right)

The purpose of this section was to evaluate the reliability and reproducibility of the data. Figure 3.6 shows Hg concentration variation within skeletal halves, where the y-axis is the Hg concentration offset between skeletal halves, and the name of each skeletal element on the x-axis. Wilcoxon tests were done and shown in table 3.3 to show if there are significant differences between skeletal halves. When p-value is greater than 0.05, the null hypothesis of no existing significant differences of measured Hg concentration

between skeletal halves is accepted.

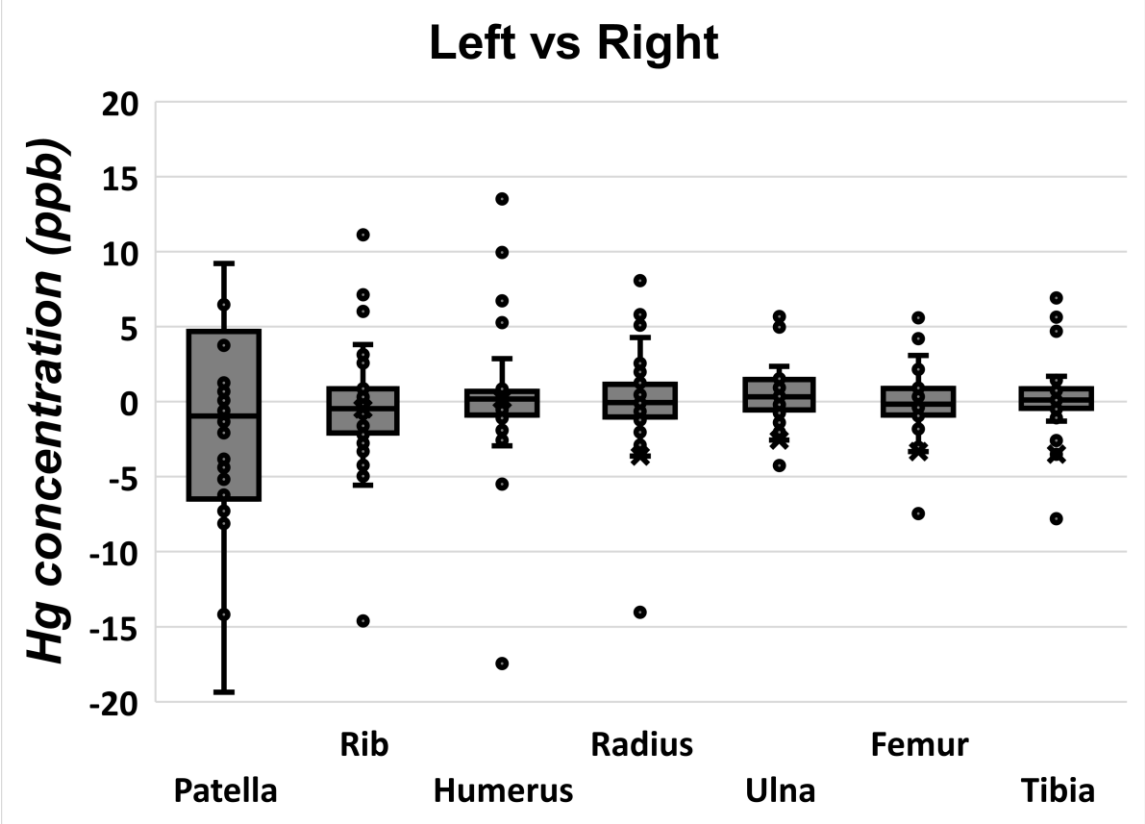

Figure 3.6 Hg concentration variation within skeletal halves

Table 3.3 Wilcoxon test between skeletal halves.

Bone	*p*-value
patella	0.94
rib	0.41
humerus	0.88
radius	1.0
ulna	0.16
femur	0.51
tibia	0.73

3.3.4 Hg Concentration Variation Within Skeletal Elements

The purpose of this section was to investigate the variation of Hg concentration in various skeletal elements. Figure 3.7 shows the Hg concentration variation within skeletal elements, where the y-axis is log (Average Hg concentration of each skeletal element),

and the name of each skeletal element on the x-axis. The left patella has the highest Hg concentration across all 18 skeletal elements, which was heavily driven by individual #26. The left radius has the lowest Hg concentration across all 18 skeletal elements.

Table 3.4 is a data summary of Hg concentration analysis in terms of skeletal elements. It provides average Hg concentrations of each skeletal element with standard deviation, median, minimum, and maximum Hg concentrations of each skeletal element across 35 individuals.

Table 3.4 Hg concentration analysis skeletal element summary.

skeletal element	average (ppb)	st dev (ppb)	Median (ppb)	n	Max (ppb)	Min (ppb)
Occipital	23.03	46.73	6.85	35	266.60	0.54
Mandible	59.85	121.65	10.67	35	584.35	0.56
Left rib	21.20	87.30	3.34	35	520.74	0.17
Right rib	21.70	89.67	4.20	35	535.35	0.56
Left humerus	9.35	37.95	1.70	35	226.13	0.24
Right humerus	9.17	40.84	1.69	35	243.58	0.27
Left radius	3.85	8.73	1.29	35	51.65	0.24
Right radius	7.47	31.06	1.46	35	185.05	0.10
Left ulna	7.38	26.00	1.59	35	154.47	0.24
Right ulna	9.93	47.62	1.24	35	283.34	0.05
prox. Phalanx hand	98.49	418.56	10.26	35	2,465.97	0.53
Left femur	9.70	35.48	2.20	35	211.68	0.16
Right femur	13.03	58.81	1.75	35	350.31	0.28
Left patella	666.72	3486.85	14.10	32	19,752.75	0.97
Right patella	73.23	261.89	15.10	34	1,515.67	0.77
Left tibia	8.31	32.04	1.58	35	191.31	0.30
Right tibia	11.81	54.15	1.59	35	322.36	0.31
prox. Phalanx foot	31.25	90.32	9.59	33	516.51	0.49

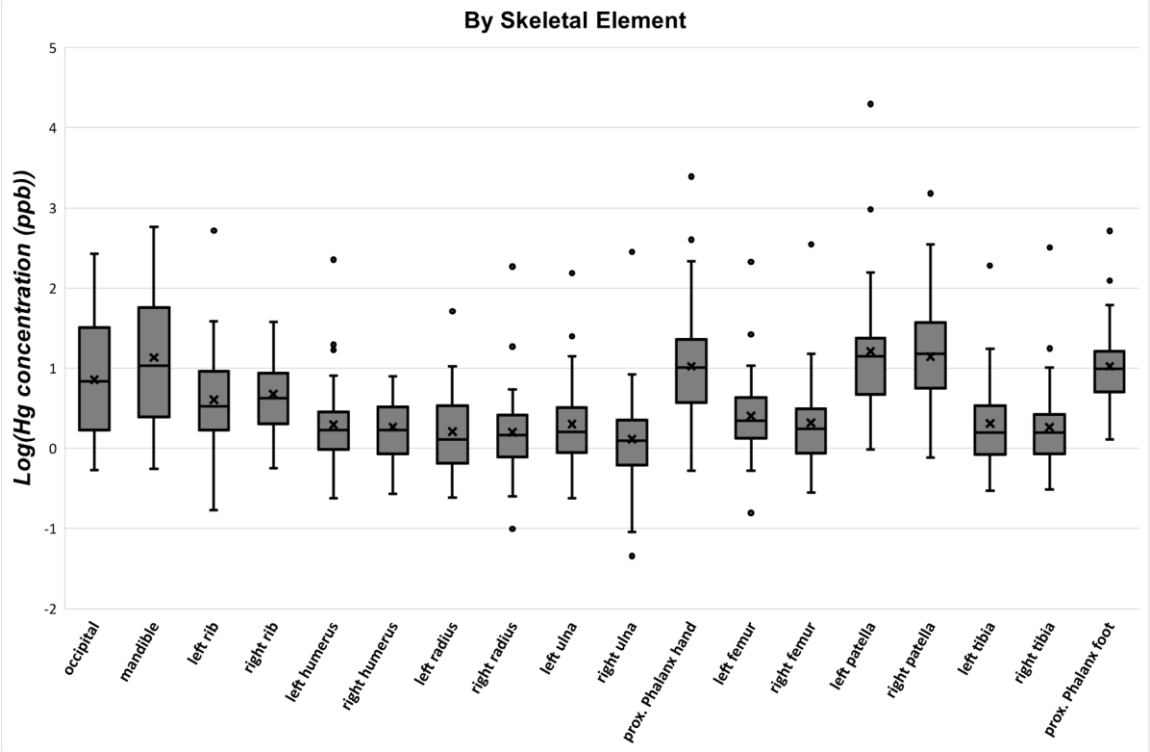

Figure 3.7 Hg concentration variation within skeletal elements

3.3.5 Dental

The purpose of this result section was to show if dental amalgam affects Hg concentration in the human skeleton and how it affects the distribution of Hg in the human skeleton. The technique used for determining the existence of dental amalgam for each individual was done by examining photo archives of their skeletal oral cavity. The problem with this technique was that I do not have information on whether teeth with ceramic fillings have been replacements for dental amalgam, and some individuals had teeth missing or no teeth. Individuals who do not have visible dental amalgam, including individuals who had teeth missing or no teeth, were listed as individuals without dental amalgam. Table 3.5 summarizes the average Hg concentration of each donor with and without dental amalgam reflected from all skeletal elements obtained. Table 3.6 includes the average Hg concentration of each donor across all skeletal elements with standard

deviation, the max, median, and minimum Hg concentration of each donor across all skeletal elements, and the number of skeletal elements included per individual. Out of 35 individuals, 22 individuals have dental amalgam, and 13 individuals do not have dental amalgam, which is indicated in table 3.5 as tinted.

Figure 3.8 shows if there is a significant difference between individuals with and without dental amalgam in terms of Hg concentration in their skeleton. Wilcoxon tests were done and presented in figure 3.8. When p-value is <0.05, the null hypothesis of no existing significant differences between the Hg concentration in individuals who have dental amalgam to individuals who do not have dental amalgam is rejected. Individual #26 was shown as an outlier in figure 3.8 due to the high Hg concentration in all skeletal elements.

Figure 3.9 shows how much dental amalgam affects Hg concentration in different skeletal elements. The x-axis of figure 3.9 was skeletal elements from donors with or without dental amalgam. Where the starred letters are skeletal elements from donors with dental amalgam. (Example: “A*” represents Hg concentration in occipital of donors who have dental amalgam. “A” represents Hg concentration in occipital of donors who do not have dental amalgam.).

T-tests and Wilcoxon tests were done and shown in Tables 3.6 to calculate if there are significant differences in the Hg concentration of skeletal elements from donors with and without dental amalgam. When p-value is <0.05, the null hypothesis of no existing significant differences between the Hg concentration in skeletal element from individuals who have dental amalgam to skeletal element from individuals who do not have dental amalgam is rejected. Data of Hg in the mandible of donors with and without dental

amalgam has the lowest p-value, which indicates a large difference exists between the two sample sets. Tibia has the largest p-value, which indicates no significant difference between the two sample sets.

Table 3.5 Hg analysis individuals' summary.

Donor	Average (ppb)	St dev (ppb)	Median (ppb)	Min (ppb)	Max (ppb)	n
01	13.16	28.47	1.65	0.57	117.29	18
02	3.30	3.51	1.53	0.33	11.25	18
03	3.74	5.61	0.83	0.31	20.10	18
04	7.52	10.85	3.36	0.05	46.65	18
05	6.42	7.44	1.93	0.65	24.07	18
06	14.35	22.67	2.06	0.24	77.52	18
07	3.30	3.94	2.07	0.50	16.05	18
08	3.89	6.43	1.90	0.09	28.04	18
09	1.80	1.68	0.86	0.17	5.10	18
10	14.66	28.49	4.66	1.59	124.50	18
11	11.92	14.40	6.07	1.63	57.95	18
12	1.84	3.36	0.78	0.24	14.66	18
13	7.45	11.47	1.59	0.44	43.93	18
14	6.08	6.77	3.00	0.28	20.11	18
15	25.84	37.06	15.44	1.30	155.09	18
16	121.15	254.26	7.29	1.03	956.49	18
17	15.38	30.30	3.22	0.83	127.85	18
18	36.20	79.23	4.57	1.20	337.82	18
19	0.89	0.62	0.86	0.10	2.59	17
20	1.77	1.82	1.21	0.16	7.12	16
21	3.00	1.87	2.24	0.78	6.48	18
22	27.52	81.64	3.42	0.81	348.98	18
23	4.90	9.35	1.92	0.45	39.86	17
24	15.41	16.04	8.75	1.18	56.84	18
25	6.17	7.57	2.82	0.80	30.88	18
26	1626.45	4710.63	283.34	51.65	19,752.75	17
27	3.03	2.28	2.12	0.25	7.16	18
28	7.11	11.95	2.42	0.55	52.61	18
29	44.30	94.43	10.36	5.32	335.75	18
30	2.67	2.96	1.42	0.58	10.40	18
31	7.20	6.99	3.96	1.12	26.44	18

32	24.16	50.88	7.35	2.03	216.28	18
33	2.58	2.29	2.07	0.78	8.67	17
34	6.39	6.84	2.82	0.85	24.00	18
35	1.44	1.25	1.10	0.38	5.34	18

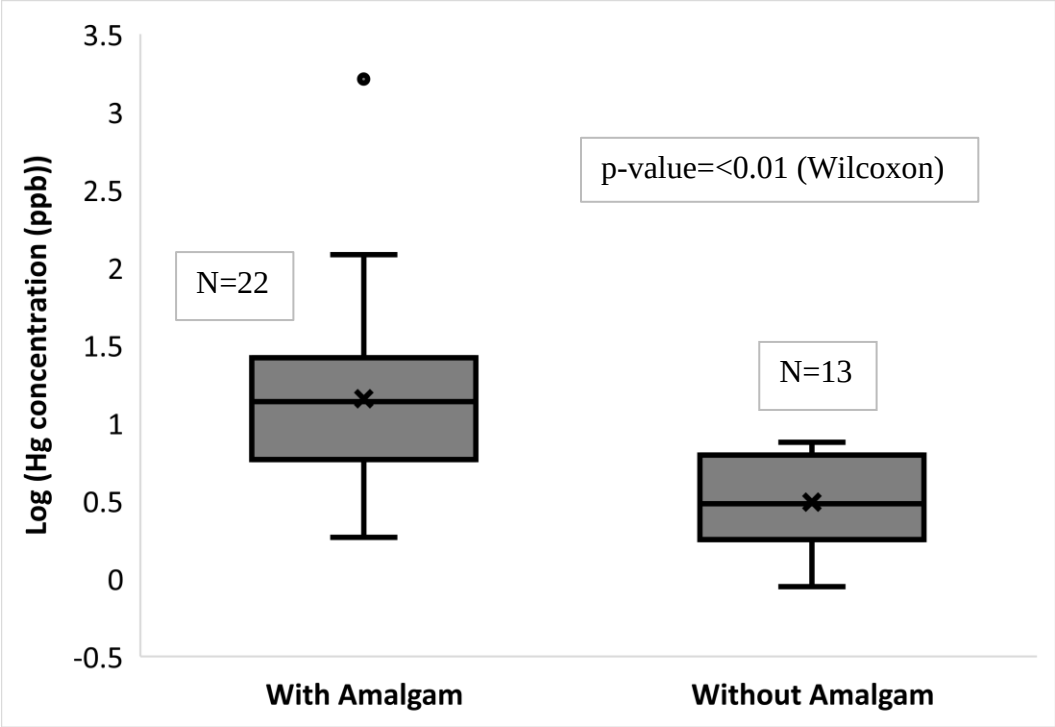

Figure 3.8 Average Hg concentration of donors with dental amalgam vs without dental amalgam

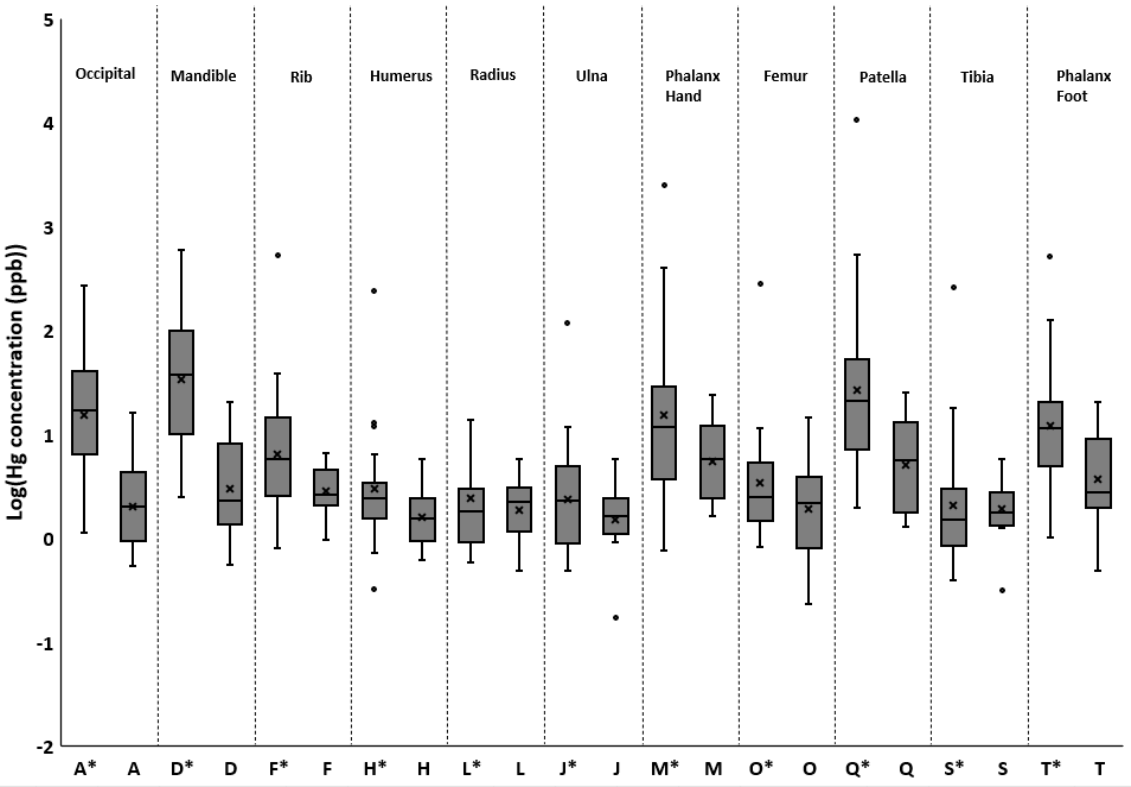

Figure 3.9, Hg concentration of skeletal elements of donors with amalgam vs without amalgam

Table 3.6 Statistical tests of skeletal elements of donors with amalgam vs without amalgam

Skeletal elements	p-value all 35 individuals	p-value excluding individual #26
Occipital (t-test)	<0.01*	<0.01*
Mandible (t-test)	<0.01*	<0.01*
Rib (Wilcoxon)	0.053	0.076
Humerus (Wilcoxon)	0.053	0.14
Radius (Wilcoxon)	0.56	0.43
Ulna (Wilcoxon)	0.91	0.867
Proximal Phalanx hand (t-test)	0.068	0.093
Femur (Wilcoxon)	0.26	0.26
Patella (Wilcoxon)	<0.01*	<0.01*
Tibia (Wilcoxon)	0.45	0.34
Proximal Phalanx foot (t-test)	<0.05*	<0.05*

3.4 Discussion

3.4.1 Comparison with Previous Studies

Studying the risk and health effects of mercury in bone tissue is important because the remodeling process of human bone is continuous throughout the lifespan. Thus, Hg accumulation in bone tissue can be an indicator of long-term Hg exposure.

Hg concentration analysis was conducted on all obtained samples, and Hg was detected in all analyzed samples. Due to the lack of previous studies on modern human skeleton Hg concentration, results from Rasmussen et al. 2013 were used as literature value on Hg concentration in the human skeleton, where Hg concentration analyses on two ancient human skeletons were conducted. The average Hg concentration of 35 individuals in our study was 59.51 ppb, which was much less than the average Hg concentration of the two individuals analyzed by Rasmussen et al. 2013.

The difference in mercury concentration can be caused by the nature of the samples. Our samples were modern human samples decomposed in the natural environment without being buried. Both individuals from Rasmussen et al. 2013 were buried for centuries with the possibility of Hg preservatives involved in the burial process or mercury ingested during life.

3.4.2 Skeletal Sample Analysis

Human bone turnover rate depends on skeletal site and bone type (Lerebours et al. 2020), thus the same skeletal elements left, and right should not have a significant difference in terms of Hg concentration. Statistical tests were done to support this hypothesis, showing no significant differences in Hg concentration between the same

skeletal elements left and right. The p-value for patella was lower than other skeletal elements due to individual #26 having more than ten times more Hg in its left patella than its right patella. P-value was low for radius compared to other skeletal elements, which was also heavily driven by the difference between the left and right radius of individual #26.

Since dental amalgam will cause a rising Hg intake in the human body, a bulk comparison was made to show if dental amalgam affects Hg concentration in the human skeleton. Figure 3.8 shows individuals with dental amalgam (n=23) have significantly higher Hg concentration in their examined skeletal elements than individuals without dental amalgam (n=12) p=0.000189.

Results of Hg concentration examined from all skeletal elements obtained in 35 individuals shows mandible, occipital, patella, and both proximal phalanx have higher Hg concentration than other examined skeletal elements shown in figure 3.7. Some individuals have significantly higher Hg concentration in certain skeletal elements than others. Thus, figure 3.9 and table 3.6 were made to show how much dental amalgam affects Hg concentration distribution in all examined skeletal elements(n=18). Figure 3.9 shows the high Hg concentration of mandible and occipital shown in figure 3.4 bulk comparison was heavily driven by individuals with dental amalgam.

3.5 Hypothesis

Four hypotheses can be presented to investigate why dental amalgam affects certain skeletal elements more than others. The first hypothesis is that skeletal elements with lower bone density (total skeletal element density) have a higher rate of Hg accumulation. The second hypothesis is that mercury is preferentially incorporated into bones with rapid

turnover resulting in weight-bearing bones having higher mercury accumulation. The third hypothesis is that Hg accumulates more in skeletal elements with more direct blood circulation from teeth that went through amalgam dental filling procedure. The fourth hypothesis is that Hg accumulates more in skeletal elements closer to a joint that has direct contact with synovial fluid and articular cartilage.

The first hypothesis is that skeletal elements with lower bone density (total skeletal element density) have a higher rate of Hg accumulation. Trabecular bone has a lower density than cortical bone (Nordin, 2021), so skeletal elements with lower density could have a higher trabecular/cortical bone ratio. Trabecular bone has a large surface area in contact with bone marrow and blood, and it has a higher turnover rate than cortical bone (Parfitt, 2002). Elemental Hg from amalgam is in an uncharged monoatomic form, which is highly diffusible and lipid-soluble. When elemental Hg enters the human body, an oxidation reaction of Hg(0) to Hg(2+) takes place in red blood cells and tissues. When Hg from dental amalgam enters the pulp chamber in a tooth, it could quickly diffuses into human blood through the inferior alveolar vein and inferior alveolar artery and transports to different parts of the human body through blood circulation. There is active blood flow and nutrient exchanges in human bone tissue which cause Hg from blood to accumulate on the collagen portion of bone tissue. Thus, skeletal elements with a lower density would have a higher trabecular/cortical bone ratio and larger surface exposed to blood and bone marrow resulting in a higher Hg accumulation.

This hypothesis was supported by the result of the patella having a higher mercury concentration than most analyzed skeletal elements (Table 3.4). However, the assumption that the patella has a lower density and higher trabecular/cortical bone ratio was

completely based on the observations of this study. There are studies done on the bone mineral density of different skeletal elements but not on the density of each skeletal element as a whole (Atici, 2014). To investigate this hypothesis, the density of each skeletal element (whole bone) had to be determined prior to the analysis. However, we were only able to receive a few skeletal elements as a whole skeletal element. The density of whole skeletal elements for all skeletal samples obtained was not able to be determined because most samples were precut at the anthropological facilities, and only small sections of these skeletal elements were received for this analysis. Further studies on whole skeletal element densities would be helpful for substantiating this hypothesis.

The second hypothesis is that mercury is preferentially incorporated into bones with rapid turnover resulting in weight-bearing bones having higher mercury accumulation. The rate of bone turnover is mainly affected by cytokines, mechanical stimuli, hormones, and growth factors that influence the differentiation, recruitment, and activity of osteoblasts and osteoclasts (Yu, 2019). Individuals who put more mechanical stress on their skeleton, such as athletes and individuals who do weight-bearing activities would have a higher bone turnover rate caused by mechanical stimuli (Sale and Elliott-Sale, 2019; Kohrt, 2009; Humphries, 2000). Thus, skeletal elements placed closer to feet (phalanx and femur) should have higher turnover rates caused by more weight-bearing. Mercury should accumulate more in skeletal elements with more weight-bearing because heavy metals accumulation in bone tissue is related to altered bone metabolism (Scimeca et al., 2016).

To prove this hypothesis, skeletal elements such as the femur and tibia should have higher mercury concentrations than ribs or mandibles. However, mercury concentration

data measured in this study does not support this hypothesis. Rib and mandible have much higher mercury concentrations compared to femur and tibia (Table 3.4), showing that weight bearing difference in skeletal elements was not a factor to cause increase in mercury accumulation in skeletal element.

The third hypothesis is that mercucry accumulates more in skeletal elements with more direct blood circulation from teeth that went through amalgam dental filling procedure. As mentioned in Section 3.1.4, elemental mercury in dental amalgam is fat-soluble and can be absorbed by red blood cells (Dantzig, 2003). Blood vessels in teeth are connected with the inferior alveolar artery which is the main blood supply to the mandible (Nguyen, 2022). Mercury from dental amalgam could enter the pulp chamber of teeth, then the inferior alveolar artery in the mandible, cause rising mercury concentration in the mandible of individuals who have dental amalgam.

The occipital area receives its blood supply from the occipital artery (Germann, 2022). There is blood circulation between inferior alveolar artery, and occipital artery because they are all part of the maxillary artery (Sethi, 2022). Mercury from the inferior alveolar artery in mandible could transfer to the occipital artery through blood circulation, causing an increase in mercury concentration in the occipital bone.

While the mercury concentration in the human brain tissue does correlate with the presence of dental amalgam (Eggleston & Nylander, 1987), there are lack of previous studies supporting mercury from amalgam can be directly transferred into the human brain through the inferior alveolar artery. Our data shows that mercury from dental amalgam does affect the mercury concentration in the mandible the most (p-

value=0.00001) and followed by occipital (p-value=0.00002) which supports this hypothesis (Table 3.6).

The fourth hypothesis is that mercury accumulates more in skeletal elements closer to a joint that has direct contact with synovial fluid and articular cartilage. Autoradiographic studies suggest Hg is taken up and retained by joints and vertebrate bones (Berlin et al., 1966), and uptake of Hg has also been found in articular chondrocytes and synovial cells (Pamphlett & Kum Jew, 2019). Skeletal samples obtained with joint connecting surfaces should have higher mercury concentrations due to they had some surface area in contact with synovial fluid and articular cartilage.

Unlike other skeletal elements, patella and both hand and foot proximal phalanx samples were received as a whole bone for this analysis instead of a section cut from the middle of the bone. Thus, patella and both hand and foot proximal phalanx samples are the only skeletal elements obtained for this analysis with joint connecting surfaces, and the entire bone was powdered.

Mercury in synovial fluid and articular cartilage could diffuse into the joint connecting surfaces of the patella and phalanx, causing a higher Hg concentration. The result of high mercury concentration measured in the patella and both proximal phalanx supports this hypothesis (Table 3.4). However, synovial fluid supplies nutrients to the joint, but no previous studies clearly state that there are nutrients exchange between cortical bones and synovial fluid or articular cartilage. Future studies on nutrient transfer between cortical bones and synovial fluid or articular cartilage would be helpful for substantiating this hypothesis.

From all four hypotheses, the hypothesis of that mercury is preferentially incorporated into bones with rapid turnover resulting in weight-bearing bones having higher mercury accumulation was not a preferred hypothesis because it was not supported by analytical results. Hypotheses 1, 3, and 4 are preferred hypotheses because they were supported by analytical results.

CHAPTER 4

CONCLUSION

The Hg analysis method described in Chapter 2 provides outstanding accuracy and precision with a limit of detection lower than any standalone Hg analyzers in the market. With the use of a liquid-vapor separator, Hg samples were efficiently converted into gaseous form, before been analyzed by quadrupole mass spectrometer. Gaseous samples have higher ionization efficiency and minimizes matrix solution introduction to the instrument. This sample introduction technique leads to less sediment buildup on the cone resulting a more stable measurement and lower instrumental drift, enabling analysis of large sample sequences. With the advantage of measuring gaseous samples, less instrumental maintenance was required, which increased the reliability of the instrument. The low limit of detection achieved by this method requires less sample for precise and accurate Hg concentration analysis. With the high reproducibility proven by the results, this method is a great fit for analyzing Hg concentration in skeletal samples in anthropological, archaeological, and medical field where samples are of very limited availability for destructive analysis.

As discussed in Chapter 3, modern human skeletal samples from three different anthropological decomposition facilities with widely varying climate and geological locations did not affect the concentrations of Hg concentration. In addition, there were no significant Hg concentration differences between skeletal halves, indicating that the mercury distributions were symmetric and increasing confidence that the measurements were reliable.

Results from Chapter 3 also clearly shows that the presence of dental amalgam in donor's teeth increases the average Hg concentration in their skeleton significantly. P-values calculated from Hg concentration of obtained skeletal elements show mandible, occipital, and phalanx have significantly high Hg concentration differences between donors who have dental amalgam to donors who do not, which shows Hg concentration in some skeletal elements were affected by dental amalgam more than others. The results of this study with the fact that Hg does tremendous harms to major human organs should raise awareness on how much dental amalgam affects human bone health and encourage future research regard to Hg toxicity to human skeleton.

For complete understanding on Hg distribution in human skeleton, future studies on the rate of absorption of Hg in different parts of human body through circulatory system seems crucial. However, large amount of Hg needs to be introduced to donor's body before death for research purposes which is not humane. This could be done in a different approach. Study on trabecular bone to cortical bone ratio for all skeletal elements across the human body would be helpful, since trabecular bone has higher turnover rate and more surface area in contact with bone merrow and blood which might result a higher Hg accumulation.

Hg concentration in mandible was the most affected by dental amalgam followed by occipital bone, which show that large amount of Hg from dental amalgam could been transferred directly to occipital bone and human brain though occipital artery. However, no previous studies done on the pathway of Hg from dental amalgam to human brain which could be helpful to prove this hypothesis.

The two primary sources of Hg in human body are fish consumption and dental amalgam. To investigate the source of Hg in human skeleton, future studies on Hg isotopes in human skeleton would be a helpful tool. It will be useful for forensic field, providing possible dietary and dental information for unidentifiable skeletons. It will be beneficial for archaeology field, providing information on source of Hg consumption for ancient societies.

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APPENDIX X

CLEANING SOP

Gordon Group
July 2019
Modified by Julianne Sarancha from methods by Christine France and Kelly Knudson

Consistencies in SOP (unless noted otherwise in steps):

Water	Always use Millipore water.
Rinse	Add 10 mL water, centrifuge each rinse, then discard water.
Centrifuge	2000 RPM for 5 min.
Dry	Remove all caps and place in glass petri dish or create foil boat. Loosely cover uncapped tubes and caps with foil. Dry at 60℃.

1. Mechanically clean samples to remove any debris and soft tissue.
    a. Bone: use a Dremel with diamond studded bit. Also remove trabecular bone from cortical bone.
    b. Teeth: use a Dremel with a “polishing bit.”

2. Weigh an empty labeled 15 mL capped tube

3. Weigh amount of sample specified in sample prep SOP and put in empty weighed tube

4. Wash sample with water
    a. Rinse (4x) (do not need to sonicate or centrifuge these rinses)
    b. Add water
    c. Sonicate for 10 min.
    d. Centrifuge

e. Repeat

5. Wash sample with 100% EtOH
    a. Add 10 mL of 100% EtOH to tube
    b. Sonicate 5 min.
    c. Centrifuge
    d. Rinse (5x)

6. Dry overnight

NOTE: Samples must be completely dry. Some may need more than one night.

7. Recap and let cool to room temperature

APPENDIX Y

BONE / ENAMEL TRACE ELEMENT AND HG SAMPLE PREP SOP

Gordon Group

December 2021

Version 2.1 – 2022Mar05 GG

Digestion

1. Take bone that has been mechanically and chemically cleaned according to appropriate protocols. Bone should have also been powdered in a mortar and pestle according to SOP.
2. Take either a 4ml screw-top beaker or a 7 mL Teflon vial that has been acid-cleaned. Add appropriate sample label.
3. Record weight of bottle or vial with cap and label. Write it in the column labelled "weight of labelled 4 mL bottle (g)" in the printed spreadsheet (column C in the Hg-TE tracker file).
4. Take a small square of weigh paper, approximately 1-2 cm across. Fold it in an "x" shape.
5. Tare it on the microbalance.
6. Add approximately 10 mg of powdered bone.
7. Tare the weigh paper.
8. Tap the powdered bone into the pre-weighed 4 mL bottle or 7 mL vial.
9. Put the weigh paper back on the microbalance.
10. Record the mass lost in the column "Weight of sample (mg)" (column D in the Hg-TE tracker file).
11. Put a dot on the lid of the 4 mL bottle to indicate that sample has been added.
12. When you are ready to digest the sample, add 0.125 mL of 12 M trace metal grade hydrochloric acid and 0.375 mL of 16 M trace metal grade nitric acid.
13. Close the lid and shake thoroughly.
14. Put the bottle with a little bit of water into a plastic beaker. Place it in the sonicator and sonicate for one to two hours.
15. Let it sit overnight. All solid should be gone. If it is not, then consult Gwyn.
16. The digestion should be done no more than a few days before the mercury will be measured. Within 72 hours, BrCl should be added to the mercury aliquot to stabilize the Hg. Do NOT add BrCl to the total sample, as it has an extremely high trace element blank, including potassium in particular.

For Hg

1. Clean all Teflon or glass bottles that will be used for mercury analysis with 1% BrCl and rinse three times with distilled water before use. (NOTE: New trace element grade centrifuge tubes do not have to be cleaned.)
2. Dry the cleaned tubes before use, but do not put directly on drying trays due to high potassium blank. Use a sample transportation tray and use an empty laminar air-flow exhaust hood.
3. Label tube and weigh the empty glass tube weight. Record the column "glass vial (g)" (column F in Hg-TE tracker file).
4. Add 3.5 mLs of water to digested bone powder solution. Shake thoroughly.
5. Pipette 2 mls of the digested solution into the pre-weighed glass tube.
6. Weigh tube with digest and record in column "glass vial + Hg aliquot (g)" (column G in Hg-TE tracker file).
7. Add 0.1 ml BrCl, to bring the solution to 5% BrCl.

8. Within 72 hours of measurement, add 2.9 mLs of Hg matrix solution (0.5% BrCl, 0.1% H-H, 0.5% HCl) for a final volume of 5 mLs. For IAEA 086, add 13.75 mLs of Hg matrix solution for a final volume of 15 mLs.
9. Weigh tube with matrix solution, BrCl, Hg aliquot, and glass tube. Record in "glass vial + Hg + BrCl + matrix solution" (column H in Hg-TE tracker file).
10. Analyze for concentration, using the mercury calibration standards. Refer to HgStandardsWeights.xlsx template in folder 2018-DU-BX-0217 / Analytical Data / iCAP data / Hg data / Hg weight sheets.

For trace element analysis (TE)

11. Take a labelled centrifuge tube and tare it on the balance.
12. Pipette 225 uLs of stock solution into the centrifuge tube. Note that some samples may require different dilution factor. In particular, cremains, which have had the organic material burned away, require only 100 microliters of stock.
13. Record weight of aliquot in "aliquot of stock for TE (g)" (column K in Hg-TE tracker file).
14. Bring up to 10 mLs with 0.32 M HNO_3. Record final weight in "final weight for iCAP (g)" (column L in Hg-TE tracker file).
15. Analyze for concentration, using the Penny Anthro calibration standards.

APPENDIX Z

PERFERMANCE REPORT

Performance Report

QTEGRA

System

Start time:	12/17/2021 10:48:27 PM
Instrument:	iCAP Q
Operator:	OKED2644\iCAP Q
Template:	Hg_100ppt_TuningSoln
Instrument Serial Number:	02497R
Last Autotune:	Autotune-Hg_Std_Tune_2-20211217-175243713.imatdat
Solution:	100 ppt Tune solution

Sensitivity & Stability Test

Result	Runs	Sweeps
Passed	5	60

Sensitivity

Analyte	Result	Value	Condition	Limit
Bkg6.5	Passed	0.8 CPS	Less than	5.0 CPS
Bkg220.7	Passed	0.17 CPS	Less than	3.0 CPS
207Pb	Passed	13,456.0 CPS	Less than	100,000.0 CPS
202Hg/207Pb	Passed	41.2	Greater than	10.0
202Hg/200Hg	Passed	1.299	Greater than	0.1
202Hg	Passed	551,911.0 CPS	Greater than	100,000.0 CPS
200Hg	Passed	424,780.0 CPS	Greater than	100,000.0 CPS

Stability

Analyte	Value	Limit
202Hg	0.6%	2
200Hg	0.5%	2

Mass Calibration Test

Result	Channels	Dwell	MeasureWidth	PointSpacing	Sweeps
Passed	75	0.04	1.5	0.02	5

Analyte	Result	Centroid Mass [u]	Offset	Peak width [u]	Peak width min [u]	Peak width max [u]
202Hg	Passed	201.9815	0.0109	0.675	0.650	0.850

Tune Settings

Parameter	Value
Additional Gas Flow 1	0.00
Additional Gas Flow 2	10.00
Additional Gas Flow 3	0.00
Angular Deflection	-390.00
Auxilliary Flow	0.80
CCT Bias	-2.00
CCT Entry Lens	-103.33
CCT Exit Lens	-160.00
CCT Focus Lens	2.40
CCT1 Flow	0.00
CCT1 Shut-Off Valve	0.00
CCT2 Flow	0.00
CCT2 Shut-Off Valve	0.00
Cool Flow	14.00
D1 Lens	-195.20
D2 Lens	-80.00
Deflection Entry Lens	-35.00
Dry Pump Speed	0.00
Extraction Lens 1 Negative	0.00
Extraction Lens 1 Polarity	0.00
Extraction Lens 1 Positive	0.00
Extraction Lens 2	-250.00
Focus Lens	19.60
Nebulizer Flow	0.95
Peristaltic Pump Speed	40.00
Plasma Power	1550.00
Pole Bias	-1.00
Quad Entry Lens	-18.20
Sampling Depth	5.04
Spray Chamber Temperature	20.00
Torch Horizontal Position	-0.28
Torch Vertical Position	0.12
Virtual CCT Mass Maximum Dac Limit Set	4095.00
Virtual CCT Mass parameter b	0.65
Virtual CCT Mass to Dac Factor	130.00
Virtual CCT Mass to Dac Offset	-250.00

Vacuum Check

Parameter	Result	Value
Analyzer Pressure		5.050e-7
Interface Pressure		1.691e+0

Detector Voltages

Analog	Counting
-2612.50	1662.50

Mass Calibration Peaks

Analyte: 202Hg