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THE PERSISTENCE OF GUNSHOT RESIDUE IN DECOMPOSING TISSUE AND BLOWFLY LARVAE

presented by

LISA MARIE LAGOO

has been accepted towards fulfillment
of the requirements for the

M.S. degree in Forensic Science

Major Professor's Signature

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Date

THE PERSISTENCE OF GUNSHOT RESIDUE IN DECOMPOSING TISSUE AND BLOWFLY LARVAE

By

Lisa Marie LaGoo

A THESIS

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ABSTRACT

THE PERSISTENCE OF GUNSHOT RESIDUE IN DECOMPOSING TISSUE AND BLOWFLY LARVAE

By

Lisa Marie LaGoo

The persistence of gunshot residue (GSR) in porcine tissue in the fall and in the winter was investigated by scanning electron microscopy/energy dispersive spectroscopy (SEM/EDS) and inductively coupled plasma-mass spectrometry (ICP-MS). SEM/EDS was performed on adhesive carbon tabs that had been dabbed on tissue surrounding gunshot wounds in order to look for characteristic GSR particles containing Pb, Ba, and Sb. Such particles were located for up to one day after death. ICP-MS was used to analyze microwave digested tissue from a shot pig and a control pig over time for the presence of Ba, Sb, and Pb. All three elements were detected in the tissue over the entire sampling time frames in both the fall and winter in the shot pig only. The time frame for the fall study encompassed all stages of decomposition and lasted thirty-seven days, while in the winter study, the pigs did not decompose due to low temperatures over the entire sixty day time interval.

In addition, the presence and persistence of GSR was investigated in blowfly larvae that had been feeding on shot tissue. The larvae were analyzed by ICP-MS for the presence of Ba, Sb, and Pb. A controlled study was performed indoors and the characteristic elements were observed for nine days following the exposure of the larvae to the GSR containing tissue. In addition, larvae found naturally feeding in gunshot wounds were analyzed and found to contain the elements on days 3 and 4 after death.

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1. INTRODUCTION

1.1 FIREARMS

Firearms were first developed in China as early as the 1200s and were introduced to Europe by the 1300s.[1] Since then, they have played important roles in every military conflict. Today, firearms still play an important military role, but are also commercially available to the public for hunting, recreation, and self-defense. Unfortunately, firearms are not only used for these purposes. Of the 14,990 murders in 2006 in the United States, 10,177 of them were firearm related.[2] Handguns were the most common firearms used in these crimes, accounting for 7,795 of the cases. Shotguns and rifles accounted for 917 of the cases in roughly equal numbers and the remaining cases involved firearms of an unknown nature.[2]

Handguns are small firearms that are generally of the semiautomatic or revolver varieties. As the name suggests, they are held and fired from the hand. Rifles are generally larger and fired from the shoulder. They generally have an accurate range of approximately fifty meters, as opposed to rifles, which can be accurate for several hundred yards. Both handguns and rifles have rifled barrels. Rifled barrels have grooves cut in a spiral pattern down the barrel to impart spin to the bullet and stabilize its trajectory. Shotguns, like rifles, are fired from the shoulder, but unlike rifles, have smooth barrels. Handguns and rifles fire bullet cartridges with a single projectile known as a bullet, or slug, while shotguns fire cartridges containing shot, which consists of hundreds of packed lead pellets.[3]

Cartridges consist of a casing, primer, propellant, and a bullet[3] [Figure 1.1]. Bullets are typically lead based and can be unjacketed, semi-jacketed, or fully jacketed.

The jackets are a thin layer of another metal, often copper. Bullets can be rounded or pointed, which generally keep their form upon impact; or hollow point, which deform into a mushroom shape upon hitting the target. Mushroomed bullets cause more damage than rounded or pointed bullets. The casing is typically brass and the propellant is a low explosive, such as a smokeless powder. Smokeless powder consists of nitrocellulose (NC, single-base), a mixture of NC and nitroglycerine (NG, double-base), or a mixture of NC, NG, and nitroguanidine (triple-base).[3] The primer compounds are located inside the primer cup, which is positioned in the center of the breach face. The primer is stable but explosive upon impact. It is composed of an explosive, an oxidizer, and a fuel. In the US, the most common explosive used is lead styphnate, the most common oxidizer is barium nitrate, and the most common fuel is antimony sulfide.[3] Shotgun shells are constructed similarly, but with packed shot replacing the bullet. Also, the casing is plastic rather than metal.[3]

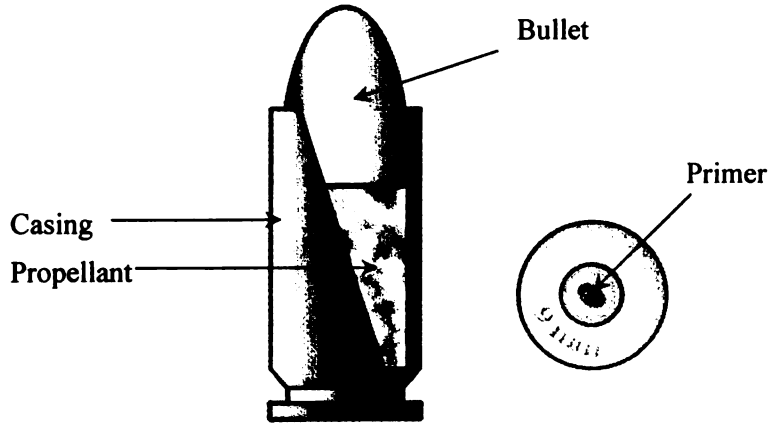

Figure 1.1 – Diagram of a bullet showing a stripped side view (left) and the breach face view (right).

A firearm is discharged when the firing pin strikes the primer. The impact causes the primer to explode and ignite the propellant, which combusts and produces a rapidly expanding gaseous cloud that expels the projectile. The heated primer partially vaporizes and plumes from the gun along with unburned propellant. The vapor condenses to form small droplets with a diameter of approximately ten microns. These droplets harden and are deposited on the hands of the shooter, the surrounding area, and the tissue of the shooting victim. The combination of hardened primer droplets, combustion products, and unburned propellant is known as gunshot residue (GSR).[3] GSR particles are generally spheroid and consist primarily of lead (Pb), barium (Ba), and antimony (Sb), which, as described above, are components of the primer.[4] This information is useful in the chemical identification of GSR.

1.2 FORENSIC ANALYSIS OF GUNSHOT RESIDUE

Historically, GSR has been chemically detected on the hands of suspected shooters and around possible bullet holes using a variety of methods. The most common methods have included various color tests, neutron activation analysis (NAA), atomic absorption spectroscopy (AAS), and scanning electron microscopy with energy dispersive spectroscopy (SEM/EDS). The use of inductively coupled plasma-atomic emission spectroscopy (ICP-AES) and inductively coupled plasma-mass spectrometry (ICP-MS) to detect GSR are currently being investigated.[5]

The most common presumptive tests for GSR are the modified Griess test and the sodium rhodizonate color test. The modified Griess test is a color test that identifies the presence of nitrites, which are produced in the combustion of smokeless powder, through the use of sulfanilic acid, alpha-naphthol and dilute acetic acid. Vaporous lead deposits

are detected through the subsequent addition of sodium rhodizonate, buffer, and dilute hydrochloric acid.[6-7,25] This test is useful to identify GSR from hand swabs and on clothing; however, the technique is limited for dark-colored clothing as the results rely on the visual identification of a color change. The pattern of GSR on clothing can be used to determine the muzzle to target firing distance based on comparisons with patterns made from test fires at known distances.

NAA was the first elemental analysis technique used for GSR analysis. It was first published in 1964 by Ruch *et al.* and was shown to be sensitive enough to identify trace amounts of Sb and Ba on hand swabs.[8] The GSR was collected by swabbing shooters hands with filter paper wetted with 1% nitric acid. This technique was widely used for years because of its sensitivity, which is on the order of parts per billion (ppb, or μg/L), despite the need for a nuclear reactor to generate neutrons.[5] The use of AAS to detect GSR components was introduced by Krishnan in 1971, however, the flame AAS used was only sensitive enough (on the order of parts per million, or mg/L) to detect the Pb from hand swabs, which is typically present in higher concentrations than Sb and Ba.[9] Later, flameless AAS instruments achieved the appropriate sensitivity levels for Sb and Ba analysis of hand swabs, which were approximately 0.02 to 0.06 μg/L,[30] in addition to Pb analysis.[10] AAS, however, is a single element technique, which makes its use for multiple elements quite time consuming. Each GSR sample would have to be analyzed three times: once for Pb, once for Ba, and once for Sb. If each element is analyzed in triplicate, a total of nine runs would be needed per sample, which is not only time consuming but costly as well.

SEM/EDS is currently the most common technique for the detection of GSR because it combines a morphological examination with elemental analysis. The most extensive study on GSR analysis by SEM/EDS was published by Wolten *et al.* in 1979.[11] Four elemental compositions were observed in GSR only and were thus labeled characteristic GSR. The most prominent was the combination of Pb, Ba, and Sb. The other three combinations were as follows: 2. Ba, calcium (Ca), and silicon (Si) with traces of sulfur (S); 3. Ba, Ca, and Si with traces of Pb if copper (Cu) and zinc (Zn) are absent; 4. Sb and Ba. GSR sampling for SEM/EDS is most often utilized by swabbing the hands of shooters with either dilute nitric acid or a solution of ethylene diamine tetraacetic acid (EDTA) or by dabbing with an adhesive tab. The most common method for collecting the particles from hands or surfaces with GSR deposits is dabbing the area suspected to have GSR with an adhesive tab mounted on an aluminum stub.[5,12] This method was shown to be the most efficient collection technique by DeGaetano *et al.* in 1992 when compared with glue lifting and concentrating techniques.[12] SEM is then used to locate spheroid particles of a diameter of approximately ten microns,[4] and if such particles are located, an EDS spectrum is taken in order to determine which elements are present. The presence of Pb, Ba, and Sb is considered characteristic of the presence of GSR on the swab.[11] Torre *et al.* demonstrated that certain types of brake pads contain particles with Pb, Ba, and Sb, however, these particles lack the characteristic spherical morphology of GSR particles and would be excluded based on SEM examination.[13]

Although SEM/EDS is a proven technique, there are several drawbacks for its use. Searching large areas for very small particles by SEM can be quite time consuming, automated programs have been developed to search for particles that fit the description of

GSR. One of the major problems associated with SEM/EDS analysis of GSR is the distinct possibility of false negative results. A lack of GSR particles could indicate that no firearm had been discharged, that the suspected shooter did not discharge a firearm, or that the sampling procedure had simply not collected any of the GSR particles.

In 1998 Koons demonstrated that ICP-MS could be used to detect Pb, Ba, and Sb in extracts from hand swabs of people who had recently fired a firearm.[14] Through the analysis of standard solutions of Pb, Ba, and Sb, it was determined that ICP-MS was fast, accurate, precise, with relative standard deviations (RSDs) of less than 5% for each element. ICP-MS also exhibited very low detection limits of 0.1 μg/L for $^{206+207+208}$Pb, 0.02 μg/L for ^{138}Ba, and 0.05 μg/L for ^{121}Sb. In order to assess the usefulness of ICP-MS of forensically relevant samples, it was then performed on acid extracts of swabs of the hands of a person who had recently discharged a firearm. The GSR components were successfully detected and the concentrations measured were between 0.004 and 0.95 μg/swab.[14] In 2007, a feasibility study was conducted by Santos *et al.* in order to see if shooting distance could be determined by quantifying the amount of gunshot residue deposited on cotton tissue.[15] Test shots of varying distances were fired into the cotton and one cm^2 squares were cut at four radial distances from the hole. The squares were extracted in 10% nitric acid with sonication. The samples were diluted and then analyzed using ICP-MS to quantify Pb, Ba, and Sb. It was found that it was possible to estimate the firing distance when the shots were fired from between twenty to eighty centimeters. The best sampling location was determined to be approximately four centimeters away from the bullet hole as it was the distance that provided the most linear data.[15]

All of the techniques outlined have been used to detect GSR that is deposited on the hands of a shooter or on a nearby surface. Only very recently has work been done to identify GSR that had tattooed into the tissue of a shooting victim. The ability to discriminate wounds from jacketed bullets and unjacketed bullets was investigated by Klintean *et al* in 2007. Fresh skin from gunshot wounds of people who had been shot to death was digested in acid and analyzed by ICP-AES,[16] which has detection limits of approximately 0.3 to 50 μg/L, depending on the instrument used.[30] ICP-AES is significantly less sensitive than ICP-MS, which has detection limits of approximately 0.02 to 0.06 μg/L for Pb, Ba, and Sb. It was found that wounds from jacketed bullets contained high concentrations of iron (Fe) and Zn from the jacket, while wounds from unjacketed bullets contained high concentrations of Pb. The large amount of Pb detected was due to the bullet, which was made of Pb. Ba and Sb were also detected in the tissue from both bullet types,[16] which indicated that they were likely from the GSR.

In 2007, the ability to detect GSR in decomposing tissue was investigated by Schaeffer.[17] It was demonstrated that GSR could be detected in porcine tissue up to two days after death by SEM/EDS where the GSR was collected by dabbing with carbon-coated adhesive tape.[17] The same study demonstrated that GSR could be detected by ICP-MS for at least seven days following death by microwave digesting the skin surrounding the wound in concentrated nitric acid.[17] Schaeffer's work was limited by the number of samples that were tested. The gunshot wounds were only tested for seven days after death and significant levels of GSR components were still detected at the end of the sampling period. Thus, it is not known how long after death GSR could be detected by ICP-MS.

The ability to chemically identify gunshot residue in the tissue of a victim would be useful to aid in the identification of a gunshot wound.

1.3 PATHOLOGY OF GUNSHOT WOUNDS

Gunshot wounds are typically identified by a small, round or ovoid entrance wound and a larger, irregular exit wound. Contact wounds often show the stamp of the muzzle in the skin. They may also exhibit star shaped tearing around the hole due to a build up of gases under the skin that then explodes outwards. With close range gunshot wounds, GSR will often tattoo into the tissue, which results in the phenomenon known as stippling. Stippling is a pattern of tiny abrasions surrounding the wound [Figure 1.2].[26] The pathologist will typically conduct a visual examination of the suspected gunshot wound, often accompanied by a histological examination of the wound edge to look for GSR particles [Figure 1.2]. Radiology is also often employed to visualize internal injuries and any bullets, bullet fragments, or shot that may be present.[18] Besides the identification of a gunshot wound, it is important for the pathologist to also be able to estimate the firing distance based on the amount of stippling, the presence and location of abrasions or tears, etc.[18]

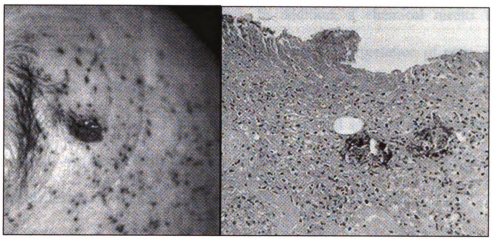

Figure 1.2 – Gunshot wound stippling (left) and histology of gunshot wound showing imbedded GSR particles (right).[19]

Gunshot wounds can vary greatly based on the type of firearm, the distance from the barrel to the victim, the type of ammunition, and the location of the wound. The variability in gunshot wound appearance can make their interpretation extremely difficult for medical personnel. A study conducted by Collins found that 52% of 46 presented gunshot wounds at a Level I trauma center were misinterpreted by trauma specialists.[20] The difficulty in gunshot wound interpretation can be due to many different factors, which may include the level of bodily decomposition, the distance from which the firearm was discharged, whether a body had been buried, and any insect activity in and around the wound tract. Decomposition and burial can obscure any obvious GSR tattooing or stippling and the firing distance affects the amount of GSR deposited. Insects can chew new tracts, obscure existing tracts, and/or change the morphology of the wound. They can even cause an entrance wound to resemble an exit wound.

If no bullet is recovered and the wound is obscured by any means, the ability to chemically detect GSR around a suspected gunshot wound would be a valuable tool for

definitively identifying a gunshot wound. In addition, a chemical means for the identification of gunshot wounds could also aid in the interpretation of entry wounds versus exit wounds in situations where one of the wounds has been compromised. The GSR content would be expected to be much higher around an entry wound than around an exit wound. As was described in the previous section, very little work has actually been done to chemically identify gunshot wounds in decomposing tissue.

1.4 BLOWFLY LARVAE

Blowflies are typically the first to arrive at a death scene and lay eggs immediately in mucous membranes and wounds.[21] They proliferate quickly and rapidly fill any exposed orifices. Because they are often present when a body is found in a decomposed state, their interaction with gunshot wounds cannot be ignored.

Blowflies have been of use to forensic scientists for years, particularly in time since death estimations. They develop in a very predictable manner according to the ambient temperature. The eggs hatch small, off-white larvae. These larvae are known as first instars. As they grow, they molt to become second instar larvae. This process happens once more to produce third instar larvae. All of the instars actively feed on dead tissue and accelerate the decompositional process [Figure 1.3]. After a time, third instars finish the feeding stage and leave the body in search of a cool, dry place to pupate. Their outer layer hardens and darkens and they shrink in length. The new blowfly emerges from the puparium after a time and leaves the body to begin the process again.[21]

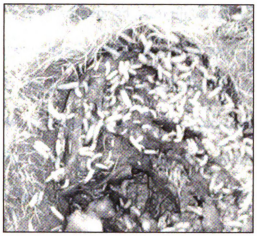

Figure 1.3 – Blowfly larvae of various instars.

Blowfly larvae have been shown to be useful for more than just time since death estimations. Since the larvae feed directly on the tissue of a corpse, they contain some compounds found in the tissues. In 1972, Nuorteva and Hasanen showed that mercury could be detected in blowfly larvae that had been feeding on fish containing high levels of mercury.[22] A year later, Beyer *et al.* detected phenobarbital in trace levels in larvae that had been feeding on a suicide victim.[23] Since then, various other toxicological substances have been detected in larvae. As gunshot residue has been shown to actually tattoo into the skin of shooting victims, the question of whether gunshot residue could be detected in blowfly larvae feeding on said tissue arises. Recently, Roeterdink *et al.* tested whether GSR could be detected in larvae that had been feeding on tissue that had been shot.[24] A one-kilogram block of beef was shot twice for maximum GSR deposition, placed in a controlled environment, and colonized with *Calliphora dubia* larvae. The larvae were allowed to feed on the GSR tissue for up to four days. Larvae, pupae, puparia, and adult flies were collected at various time points over the course of the experiment. The study was conducted twice, however, adult flies were only analyzed in

one study. After collection, they were rinsed, then digested by boiling in nitric acid. The digest solutions were evaporated, reconstituted in dilute nitric acid, and analyzed by ICP-MS for the presence of Pb, Ba, and Sb. Larvae that had been feeding for up to 84 hours on the beef were found to consistently contain the characteristic GSR metals, whereas larvae that had been feeding for 96 hours on the beef, post-feeding larvae, pupae, puparia, and adult flies did not exhibit significant Sb levels and Pb and Ba were only occasionally detected.[24] The data were not normalized to the mass of the larvae and the isotopes that were monitored were not reported, so the significance of the results obtained is difficult to assess.

1.5 RESEARCH OBJECTIVES

Gunshot wounds in decomposing tissue have been shown to be quite difficult to identify with confidence, which makes a means to chemically identify them desirable. As very little work has been done to chemically characterize GSR content in decomposed tissue or in blowfly larvae found naturally occurring in the decomposing tissue, the goals of this research are as follows:

- To analyze Pb, Ba, and Sb in GSR containing blowfly larvae by ICP-MS.
- To investigate the persistence of GSR in decomposing porcine tissue by SEM/EDS of tissue swabs and ICP-MS of digested tissue.
- To determine the feasibility of detecting GSR components in blowfly larvae found naturally feeding in gunshot wounds.

The research presented in this thesis will impact the forensic community by serving as a means to chemically identify gunshot wounds on decomposing victims. This information will be beneficial, for example, in cases where no bullet was recovered and

the body is in a stage of decomposition that makes it difficult to visualize stippling around a wound. The ability to detect GSR in blowfly larvae found feeding in suspected gunshot wounds would be useful because blowfly larvae are easy to collect and are typically quite abundant on decomposing tissue.

2. MATERIALS AND METHODS

2.1 EXPERIMENTAL DESIGN

2.1.1 Introduction

The extraction of gunshot residue (GSR) from blowfly larvae was first investivaged in an indoor study. Blowfly larvae were allowed to feed on beef that had been shot; the larvae were then collected and microwave digested in nitric acid and hydrogen peroxide. The GSR content was subsequently determined by inductively coupled plasma-mass spectrometry (ICP-MS). The persistence of GSR in decomposing porcine tissue and in blowfly larvae found feeding in the tissue was studied in the fall and winter. GSR was confirmed on the tissue by scanning electron microscopy/energy dispersive spectroscopy (SEM/EDS) and the characteristic elements, lead (Pb), barium (Ba), and antimony (Sb), were quantified by ICP-MS.

2.1.2 Indoor study design

A 2.78 pound round bottom roast, purchased from a local grocery store, was shot eight times with a 9mm GLOCK™ handgun with a 4" barrel at a distance of approximately one foot in order to deposit a maximum amount of GSR over the entire surface of the meat. The cartridges used were 115 grain jacketed hollow point American Eagle Brand Federal Cartridge +p+ with 1250 ft/s muzzle velocity (Federal Cartridge Co., Anoka, MN, Lot 430399WI40). The meat was then cut into approximately 1" cubes and placed onto a plastic platform in the bottom of a plastic specimen cup (Premium Plastics, Inc., Chicago, IL). Five specimen cups were prepared containing beef that had been shot and five were prepared containing control beef that had not been shot. Approximately twenty sterile first instar *Phaenicia sericata* medicinal maggots (Monarch

Labs, Irvine, CA) were placed on each piece of beef. These larvae had previously been fed a diet of soy protein and yeast. Wet paper towel was put under the platform containing the meat in an attempt to keep the atmosphere moist and the pieces of meat were sprinkled with water every day to prevent the meat from desiccating. The cups were covered with two layers of Kimwipes® and secured with a rubber band [Figure 2.1]. Approximately twelve larvae from a GSR cup and twelve from a control cup were collected on days 1, 2, 3, 4, and 5 and stored at -80°C. Additional surviving larvae and pupae were collected and frozen on days 7 and 9. Additional larvae were allowed to feed on the excess shot beef in case there was a problem with the larvae in the smaller cups. Larvae and pupae from the excess beef were collected on days 4, 7, and 9.

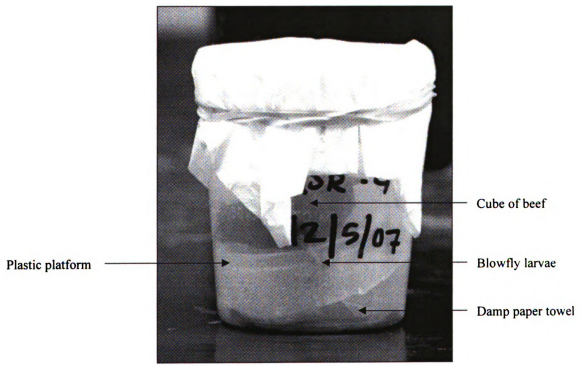

Figure 2.1 - Indoor study setup.

2.1.3 Fall and winter outdoor studies day zero procedures

On September 5, 2007, two approximately 200 pound pigs were obtained from the MSU Swine Facility and euthanized by on-site staff with FATAL-PLUS (Vortech Pharmaceutical Ltd., Dearborn, MI), a solution of pentobarbital sodium at 390 mg/mL, propylene glycol at 0.01 mg/mL, ethanol at 0.25 mg/mL, and benzyl alcohol at 0.2 mg/mL. The pigs were transported to the shooting range where one pig was shot eleven times by the same firearms specialist from the local police department as the indoor study. The spots were marked with an "X" using a Sharpie® marker. The weapon used was a GLOCK™ model 19 with a 4" barrel and polygonal rifling, and was the same weapon that had been used in the indoor study. The ammunition was American Eagle brand 115 grain full metal jacketed cartridges (Federal Cartridge, Model AE9AP, Lot 430399WI40) with a nominal speed of 1250 ft/s at sea level. The muzzle to target distance was consistently five centimeters and shots were spaced at least ten centimeters apart to minimize overlap of GSR between shots. The firearm was cleaned with MP Pro7™ gun cleaner (n-methylpyrrolidone and diethylene glycol) between shots in order to minimize carryover and to make the shots as reproducible as possible. On the day of the shooting, the outside temperature was approximately 80°F with high wind and humidity.

Both pigs were transported to the MSU Entomology Research Field and the second pig was stabbed with a clean scalpel eleven times in corresponding locations as the wounds on the shot pig. Wire cages were placed over the pigs to prevent predation of the carcasses by larger animals while still allowing environmental exposure. The pigs were placed approximately two feet apart.

The procedure described above was repeated with two approximately 160 pound pigs on January 11, 2008. The euthanized pigs were shot and stabbed in the same locations, the same officer performed the shooting using the same weapon, the ammunition was from the same box (Lot 430399WI40), and the shooting distance and spacing were the same as the previous study. The outside temperature was approximately 40°F with wind.

2.1.4 Sample collection and storage

Tissue samples were collected from both the shot and stabbed pigs on eleven days over a thirty-seven day interval after death in the fall [Figure 2.2] and over a sixty day interval in the winter. A circular tissue sample of the skin and underlying fat was excised in an approximately four-centimeter radius around the wound, wrapped loosely in waxed paper, sealed in a plastic freezer bag, and stored at -80°C until analysis.

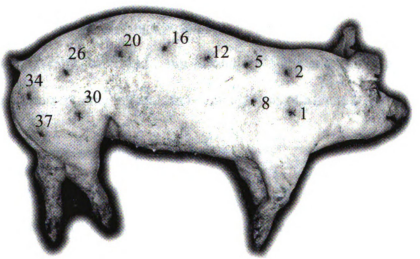

Figure 2.2 - Fresh gunshot wounds showing time interval from death to collection in days for the fall experiment.

Blowfly larvae were collected directly from the wounds on both the stabbed and shot pigs on ten different days between days three and fifteen after death. That is, blowfly larvae were collected on days when no tissue was collected and larvae were present in the wounds. The larvae were sealed in plastic freezer bags and stored in the -80°C freezer until analysis. Blowfly larvae were only present in the wounds in the fall as it was too cold for them to survive in the winter.

2.2 SCANNING ELECTRON MICROSCOPY/ENERGY DISPERSIVE SPECTROSCOPY (SEM/EDS)

2.2.1 Introduction

Scanning electron microscopy/energy dispersive spectroscopy (SEM/EDS) is a versatile technique that is often used in forensic science to combine imaging with qualitative or quantitative elemental analysis. The SEM produces a topographical image of a sample through the use of a high power scanning electron beam and is capable of up to 150,000x magnifying power and 3 nm resolution[28]. The addition of an EDS detector allows for the concurrent imaging and elemental analysis of a sample.

2.2.2 Gunshot residue sample preparation

Gunshot wounds were removed from the -80°C freezer and allowed to thaw. The wounds were dabbed 100 times with a 9 mm diameter carbon adhesive tab (SPI® Supplies, West Chester, PA) on an aluminum stub. Although samples are usually coated with gold or carbon prior to analysis to ensure conductivity, the samples in question were not coated, as the particles of interest are metallic (Pb, Sb, and Ba) and therefore are already conductive.

2.2.3 Scanning electron microscopy

A SEM consists of an electron gun, a series of electromagnetic lenses, scan coils, a final aperture, a scintillator, a detector, a computer, and a cathode-ray tube [Figure 2.3].[28] The instrument is kept under vacuum using a series of pumps and cooling systems.[28]

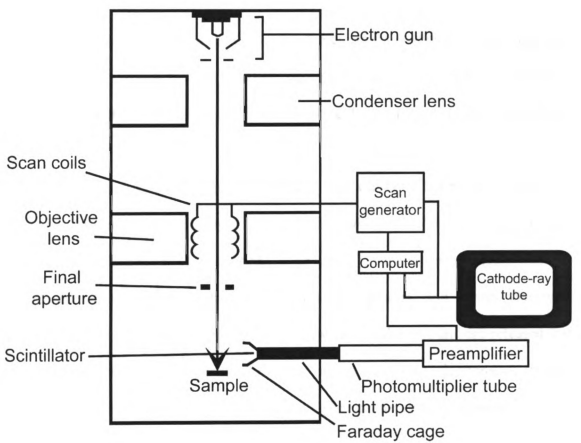

Figure 2.3 - Diagram of an SEM

The electron gun is composed of a high voltage source connected to an electron source, typically a lanthanum hexaboride (LaB_6) crystal or a tungsten wire. This electron

source is the cathode for the electron gun. A negatively charged shield is placed around the cathode with a small aperture for the electron beam to focus the emitted electrons into a beam. The anode of the electron gun is located below the shield. The voltage difference between the cathode and anode is known as the accelerating voltage of the beam and is typically set between 5 kV and 30 kV. After the beam is accelerated it travels through the condenser lens, which is a ring-shaped electromagnetic lens that serves to condense the electron beam. The electrons then travel to the objective lens, where they are focused into a very narrow beam. The scan coils within the objective lens serve to allow the beam to move back and forth in a raster pattern. After the electromagnetic lenses and scan coils, the beam travels through the final aperture, which serves to limit the number of electrons striking the sample. The number of electrons striking the sample influences the brightness and contrast of the final image.

SEM samples must be free from volatile compounds, either by the nature of the sample or by preparation; firmly mounted, and conductive. When the electron beam strikes the sample, a series of interactions occur which has many varied effects, including the release of secondary electrons, backscattered electrons, and x-rays. Secondary electrons are low energy electrons, typically between 3 and 5 eV, and are released from just under the surface of the sample.[28] Because of this, secondary electrons are the most useful for imaging. Backscattered electrons are higher in energy than secondary electrons and are the result of elastic scattering from deeper within the sample.[28] Because they come from a deeper area of the sample, back scattered electrons provide less information about topography. In exchange, however, they provide information about atomic weight. Areas with a higher average atomic weight will appear brighter in backscatter mode.

The electrons produced are typically detected with an Everhart-Thornley detector. The detector consists of a Faraday cage with a scintillator, followed by a light pipe, a photomultiplier tube (PMT), and a preamplifier. The Faraday cage is positively charged at approximately 300 V and thus attracts the electrons from the sample into the detector. The electrons come in contact with the scintillator, which is a positively charged metal-coated disc that converts the electrons into photons, which enter the light pipe and are transmitted outside the vacuum.[28] The photons enter the PMT and cause the release of many electrons, which are converted to a voltage, which the preamplifier amplifies.[28] The amount of voltage ultimately determines the brightness of a spot on the cathode ray tube, which produces the final image. Higher voltages result in brighter spots, which correspond to raised areas of the sample.[28]

2.2.4 X-ray emission

X-rays are the result of an inelastic electron scatter that creates a vacancy in one of the inner shells of a particular atom. When this vacancy is created, an electron from an outer shell fills the vacancy [Figure 2.4].[1] The result is an energy difference, which can be resolved in one of two ways: the ejection of an electron from an outer shell, known as an Auger electron, or the production of an X-ray.[28]

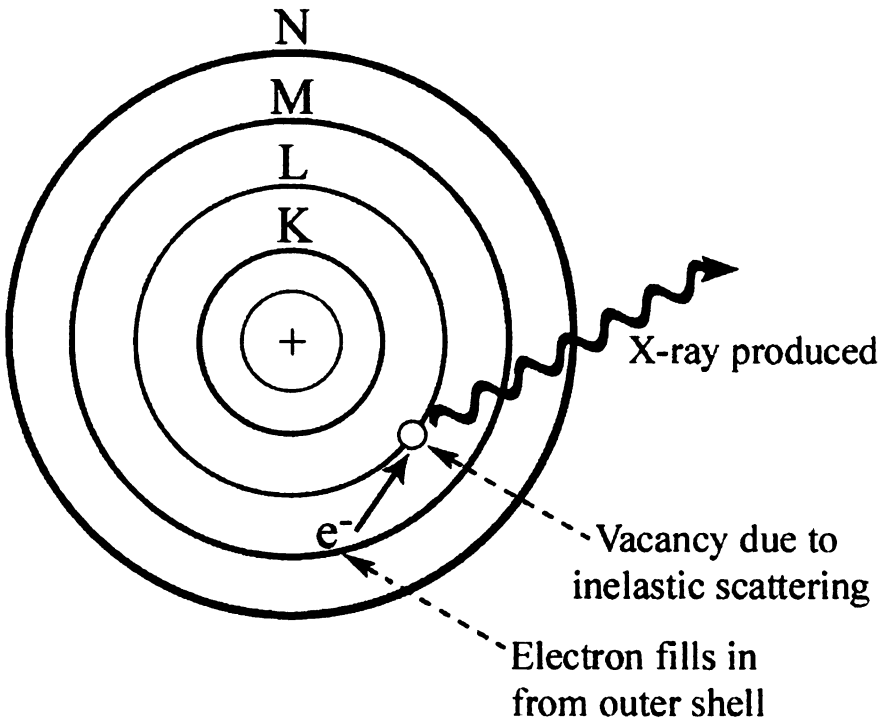

Figure 2.4 - Inelastic scatter resulting in an X-ray. An electron from the M shell fills in a vacancy in the L shell, resulting in a L_α X-ray.

X-rays are named according to the shell (K, L, M, or N) where the vacancy occurred and according to how many energy level jumps were required to fill the vacancy, where a one-shell jump is denoted α, two-shells is β, etc.[28] For example, if a vacancy occurred in the L shell and an electron jumped one shell to fill it in from the M shell, the X-ray produced would be an L_α X-ray. The critical excitation energy is the energy required to eject an electron from a given energy level for an X-ray to be produced.[28] The accelerating voltage of the SEM can be adjusted in order to obtain the intensity needed to produce sufficient X-rays for analysis.

2.2.5 Energy dispersive spectroscopy

EDS uses the X-rays produced by inelastic scattering to determine qualitative and quantitative information about the elements present in a given sample. Each X-ray of a given type (e.g. L_α or K_β, etc) is called a line. If sufficient X-rays of a specific line are produced, they produce a peak on the EDS spectrum at the corresponding energy. The energy, E, of the X-ray is related to its wavelength, λ, according to the equation $\lambda = 1.2398/E$. [28] The resulting EDS spectrum is a plot of intensity versus X-ray energy.

The EDS detector attaches to the column of a SEM and consists of a collimator, a window, a crystal, a field-effect transistor, a pulse processor, an analog-to-digital converter, the multichannel analyzer, and a computer [Figure 2.5].[28]

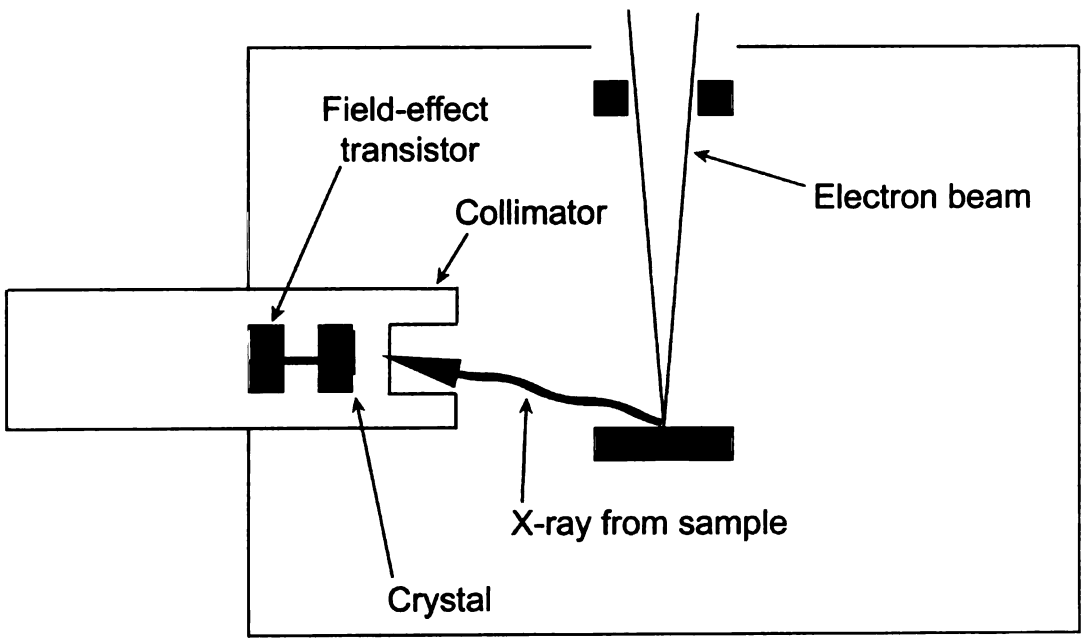

Figure 2.5 - Schematic of EDS detector components.

The collimator blocks stray X-rays and backscattered electrons from entering the detector and producing noise in the spectrum. It is lined with carbon, which acts to absorb

the stray radiation. The window is a wafer that acts as a physical barrier protecting the crystal from the column vacuum and any vapors that may be present. The detector crystal is a silicon wafer with lithium drifted in to produce a semiconductor region. The crystal must be kept at liquid nitrogen temperature to ensure the stability of the lithium region When an X-ray enters the semiconductor region, a vacancy and a free electron are created there. The release of electrons results in a voltage pulse proportional to the energy of the X-ray.[28] The field-effect transistor amplifies the voltage pulse and reduces electronic noise. The pulse processor generates separate voltage pulses from the ramp output from the preamp. These separate pulses are proportional to the energy of the X-rays that produced them. The analog-to-digital converter converts the pulses to a signal that can be recognized by the computer, and the multichannel analyzer sorts its output into channels of varying energy and counts the X-rays that have energies in each channel. The computer then ultimately produces a spectrum containing peaks that correspond to different types of X-rays at the corresponding energy.[28]

2.2.6 SEM/EDS analysis of wound samples

A JEOL 6400V SEM (JEOL Ltd., Tokyo, Japan) with LaB_6 emitter coupled with an INCA EDS detector (Oxford Instruments, Oxfordshire, United Kingdom) located in the Center for Advanced Microscopy at Michigan State University was used for this study. The accelerating voltage used was 20 kV and the working distance was 15 mm. The samples were manually scanned for spheroid particles with a diameter greater than 10 μm. If a particle was located, a qualitative EDS spectrum was collected over the entire particle to qualitatively identify Pb, Ba, and Sb.

2.3 MICROWAVE DIGESTION

2.3.1 Background

Microwave digestion is a versatile technique for the digestion of complex solid matrices into liquid form. The matrix is degraded in order to release the elements of interest into the solution.[29] Although there are many different techniques for accomplishing this, the most common methods have employed a closed system. That is, the sample to be digested is placed in a closed container with digestion agents, sealed tightly, and subjected to microwave irradiation. Digestion agents typically consist of a combination of acids and oxidizing agents, such as concentrated nitric acid, hydrofluoric acid, or hydrogen peroxide. Microwaves rotate polar molecules within a sample. This rotation causes friction between the molecules and consequently releases a significant amount of heat. The advantage of a closed system is that the heat generated increases the amount of vapor in the container, which increases the pressure and raises the boiling point of the reagents, ultimately resulting in a more efficient decomposition of the sample.[29]

Commercial microwave digestion systems are capable of digesting several samples at once while following a specific temperature program. Temperature monitoring is typically achieved through the use of a thermocouple or fiber optic probe.

2.3.2 Procedure

The wounds and blowfly larvae were taken from the -80°C freezer and thawed. The wound skin was separated from the underlying fat and connective tissue using a scalpel. Between 0.30 and 0.90 grams of porcine skin or between 0.03 and 0.50 grams of larvae were placed into acid and peroxide washed quartz vessels. 1.00 mL of 30%

hydrogen peroxide (H_2O_2, CCI, Columbus, WI) and 2.00 mL Optima grade nitric acid (HNO_3, Fluka, Buchs, Switzerland) were added to the vessels, which were then capped and placed inside a larger Teflon® vessel containing 10.0 mL high purity water (Honeywell Burdick & Jackson, Muskegon, MI) and 2.00 mL 30% hydrogen peroxide. These vessels were securely closed and placed into a Milestone Ethos EZ microwave digester (Milestone, Inc., Shelton CT). The microwave program consisted of a fifteen minute ramp to 210°C followed by a ten minute hold at that temperature. The maximum wattage was set at 600W in order to prevent system errors. The Teflon® vessels were allowed to cool to below 95°C before they were opened. Each digest run consisted of nine samples and one procedural blank containing only 2.00 mL HNO_3 and 1.00 mL H_2O_2. Procedural blanks were run in each vial after samples had been run as a cleaning step and then rinsed with deionized water before reuse.

After microwaving, the digest solutions were immediately diluted to 2% nitric acid by adding 0.5 mL of the digest solution to 11.15 mL high purity water (Honeywell Burdick & Jackson). The dilution step was necessary to prevent degradation of the polypropylene tubes used for storage as well as for use with ICP-MS. Both the diluted solutions and original digest solutions were stored in a refrigerator in 15 mL polypropylene tubes (Corning Inc., Corning, NY).

2.4 INDUCTIVELY COUPLED PLASMA-MASS SPECTROMETRY

2.4.1 Introduction

Inductively coupled plasma-mass spectrometry (ICP-MS) is a fast, precise, and sensitive mass spectral technique that allows for the multielement analysis of samples

over a wide range of concentrations. The technique utilizes an argon plasma which is more than twice as hot as a combustion flame to atomize and ionize a sample prior to introduction into a mass spectrometer.[30] The mass spectrometer is capable of determining which elements are present in the sample based on mass-to-charge (m/z) ratio and quantifying the elements using internal and external calibration.

2.4.2 Calibration Standards and Sample Preparation

Thirteen multielement calibration standards were prepared using SPEX CertiPrep standards of 1,000,000 ppb of Pb, Ba, and Sb in 2% HNO_3 (SPEX CertiPrep, Inc., Metuchen, NJ). The solutions were diluted with 2% HNO_3 (Optima grade, Fluka) to concentrations ranging from 0.10 ppb to 500 ppb. Standards were analyzed in order of increasing concentration to minimize error due to memory effects.

Prior to analysis, 2 mL of diluted digest or standard solution was added to 2 mL of an internal standard solution containing 20 ppb SPEX CertiPrep indium (In) and bismuth (Bi) in 2% HNO_3. Digest samples and procedural blanks were analyzed in triplicate and a set of standards was analyzed after every thirty to thirty-three samples in order to minimize error due to instrumental drift.

2.4.3 Instrumental theory

ICP-MS consists of a quartz torch, a series of cones, a high-voltage extraction lens, a hexapole collision cell, a quadrupole mass analyzer, electrostatic deflector plates, and an ion detector [Figure 2.6].[30]

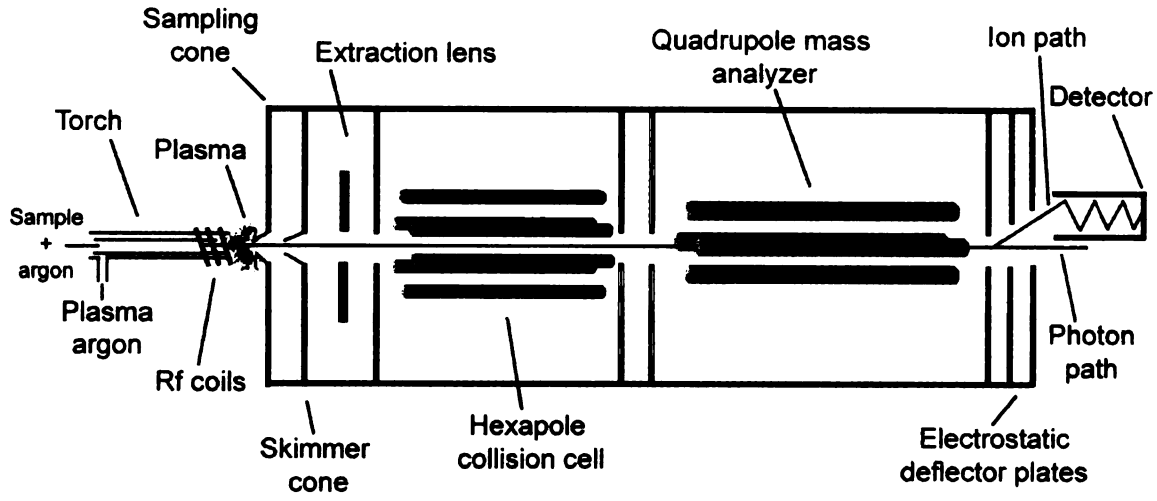

Figure 2.6 - Schematic of ICP-MS components

The torch contains the argon plasma source and consists of three concentric tubes for three flows of argon (Ar), one to carry the sample, one to support the plasma, and one to cool and lift the plasma. A spark from a Tesla coil ionizes argon gas and a radio-frequency field that oscillates around the coil accelerates free electrons. These electrons collide with the argon and transfer their energy to the gas, creating and sustaining the plasma. The sample being analyzed is carried into the plasma by a carrier gas and is immediately atomized and ionized. The sample is then carried through the plasma and onto a sampling cone, which allows only a fraction of sample to enter the mass spectrometer. The sample then passes through the skimmer cone, which is similar to the sampling cone but with a smaller aperture. Since the ICP operates at atmospheric pressure and the mass spectrometer operates under vacuum, the sampling and skimmer cones also act as barriers for differential vacuum zones. The extraction lens sits behind the skimmer cone and is negatively charged in order to attract positive ions from the sample. The ions that successfully passed through the extraction lens enter the collision cell, which contains a gas, typically helium or hydrogen, that causes the dissociation of

polyatomic ions that could interfere with elemental analysis, such as $^{40}Ar_2$, which has an indistinguishable m/z to ^{80}selenium (Se). The collision cell also reduces the spread of ion kinetic energies before mass spectral analysis.[30]

The ions then enter the quadrupole mass analyzer, which consists of four conducting rods with an oscillating voltage and radio frequency. The ratio between the voltage and radio frequency remains constant. The resulting electric field causes ions to travel in a somewhat spiral path along the length of the instrument. At a given dc/RF voltage ratio only ions within a narrow m/z range can pass through the quadrupole to the detector. All ions with other m/z ratios become unstable and are not detected. A range of masses can be scanned or only selected masses may be chosen to be allowed to reach the detector.[30] The latter technique is known as selected ion monitoring (SIM),[30] which increases the sensitivity of the instrument by dwelling longer at selected dc/RF ratios that allow m/z ratios of interest to reach the detector and not spending time scanning the entire available m/z range.

The electrostatic deflector plates direct ions that reach the end of the rods towards the detector, which is not aligned with the rest of the instrument. Because only ions can be deflected electrostatically, any photons from the plasma that enter the mass analyzer travel straight through and miss the dynode detector. Photons would cause an interfering signal if allowed to strike the detector.[30] Ions strike the detector and are converted to an electronic signal. The signal generated from the detector is proportional to the amount of ions of a given m/z hitting the detector. The output is a spectrum with a peak at a particular m/z, which corresponds to the element present.

2.4.4 ICP-MS of standards and digest solutions

The ICP-MS used was a Micromass (now Thermo Fisher Scientific, Inc., Waltham, MA) Platform quadrupole ICP-MS with a hexapole collision cell, a Dynolite™ detector with a -15kV conversion dynode and photomultiplier tube, and a CECTAC ASX-500 autosampler, located in the ICP-MS Laboratory at Michigan State University. Argon gas carried the samples into the argon plasma and hydrogen gas was used in the collision cell. The sample scan time was 1.25 min with a 0.1 sec dwell. The cool gas flow rate was 13.00 L/min and the auxiliary gas flow rate and the sample gas flow rate were 0.72 L/min. The RF power was 1350W. The sample cone was nickel with a copper core and a 1.14 mm diameter orifice and the skimmer cone was nickel with a 0.89 mm diameter orifice.

After a sample injection, the injector was rinsed for three minutes with clean 2% nitric acid. In general, the injection order was as follows: procedural blanks and control blowfly digests, control tissue digests, GSR blowfly digests, and GSR tissue digests. The GSR tissue digests were run from the last day of collection to the first day of collection, as it was hypothesized that wounds collected later would contain less GSR than wounds collected earlier, and would thus have lower concentrations of Pb, Ba, and Sb. A set of standards in order from low concentration to high concentration was run at the beginning of each different set of samples (e.g. control tissue digests) and again at the end of the run. The order was chosen in order to minimize memory effects. Selected ion monitoring of ^{121}Sb, ^{138}Ba, and ^{208}Pb, was performed and the signal was normalized to that of ^{115}In for ^{121}Sb and ^{138}Ba and ^{209}Bi for ^{208}Pb. These isotopes were chosen based on relative abundance and a lack of interference with other elements. The analysis software used was MassLynx v. 3.4 (Thermo).

Concentrations of Sb, Ba, and Pb were reported in parts per billion (ppb), which is equivalent to units of micrograms of analyte per liters of solution (μg/L). The concentrations were used to calculate the amount of each element in the original mass of tissue or blowfly larvae that were digested in units of microgram of element per gram of tissue or larvae (μg/g).

3. RESULTS AND DISCUSSION

3.1 INTRODUCTION

As stated in Chapter 1, the objectives of this research were to extract the characteristic GSR components from blowfly larvae and analyze the extracts by ICP-MS, to investigate the persistence of GSR in decomposing porcine tissue by SEM/EDS and ICP-MS, and to determine the feasibility of detecting GSR elements in blowfly larvae that were found naturally feeding in gunshot wounds.

In order to fulfill these objectives, three separate studies were conducted. The first study involved shooting a piece of beef and allowing blowfly larvae to colonize the tissue for up to nine days. These larvae were then microwave digested and analyzed by ICP-MS in order to determine if Ba, Sb, and Pb could be detected. To assess the persistence of GSR in decomposing porcine tissue, one pig was shot eleven times at close range and tissue samples were collected over the following thirty-seven days. The tissue samples were dabbed with carbon tabs and analyzed by SEM/EDS for characteristic GSR particles in order to confirm the presence of GSR. Tissue samples were then microwave digested and analyzed for the presence of Ba, Sb, and Pb by ICP-MS. This study was originally conducted in the fall and then was repeated in the winter over the course of sixty days in order to assess the effect of temperature on GSR persistence. The feasibility of detecting GSR components in blowfly larvae found in gunshot wounds was also investigated by microwave digesting larvae found in the gunshot wounds from the decomposing pig and then analyzing the digests by ICP-MS. This study was only performed in the fall, as blowflies were not present in the winter.

3.2 DECOMPOSITION OBSERVATIONS

3.2.1 Decomposition during fall sampling period (37 days)

The fresh gunshot wounds, inflicted from a firing distance of 5 cm, exhibited typical characteristics of close range gunshot wounds including blackened wound edges and GSR particle deposition in and around the wound [Figure 3.1]. Since the pigs were euthanized prior to the shooting, no stippling was present, as stippling is an antemortem phenomenon that requires blood flow.[26]

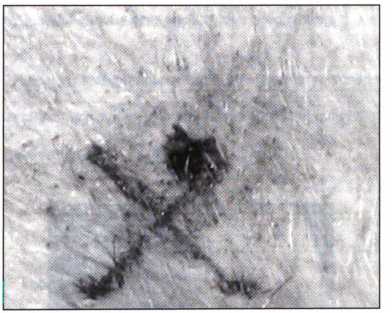

Figure 3.1 – Day 0 gunshot wound exhibiting blackened wound edges and GSR deposition.

On day 1, both the shot pig and stabbed control pig were beginning to bloat and blowflies were actively colonizing the carcasses. Blowfly eggs were observed in the orifices [Figure 3.2], which indicated their early arrival to the carcasses, and flies were observed walking in and around the gunshot and stab wounds.

Figure 3.2 – Blowflies and blowfly eggs one day after the shooting.

By day 2, bloating was pronounced and putrefaction was evident in the blood vessels on the carcasses' undersides. Blowfly larvae filled the mouths, snouts, and eyes while eggs were present in both types of wounds [Figure 3.3].

Figure 3.3 – Day 2 bloating, putrefaction, and blowfly larvae.

Bloating continued through days 3 and 4, along with blowfly larvae colonization of the wounds. Seepage of decompositional fluids was evident by day 4 and pronounced by day 5, which indicated the start of active decay. Skin slippage was also evident beginning on day 4 and pronounced on day 5. There was a large larvae mass adjacent to the carcasses as well as in all orifices and wounds. Active decay began to taper off and was no longer obviously happening by day 11, which indicated the start of desiccation. Blowfly larvae were present in the wounds until day 15, and around the carcass until day 17. By day 37, the end of the collection period, the carcasses were almost fully desiccated. The process is illustrated in Figure 3.4.

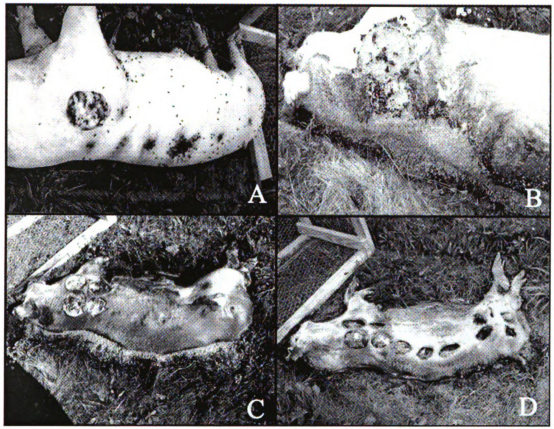

Figure 3.4 – Shot pig stages of decomposition. A) Day 2 bloating and blowflies in wounds. B) Day 5 active decay, skin slippage, and blowfly larvae mass. C) Day 11 beginning of desiccation and diminishing larvae population. D) Day 37 fully desiccated.

The weather over the course of the experiment included a maximum daily high of 88.6°F on day 19 and a minimum daily low of 35.3°F on day 11. The maximum average temperature was 75.4°F on day 0 and the minimum average temperature was 45.3°F on day 37. Higher temperatures generally result in faster decomposition rates, although there are many factors involved. Significant rainfall occurred on days 2, 5, 20, 21, 26, and 35. The details of the weather conditions are located in Appendix 1.[31]

Initially, the stab wounds and gunshot wounds were easily distinguishable. However, over the course of the collection period, the wounds became much more

similar in appearance, as is illustrated in Figure 3.5. GSR was no longer visible on the surface of the tissue after the area around each wound darkened. As the tissue darkened on earlier days closer to the head, this happened later for the thigh wounds.

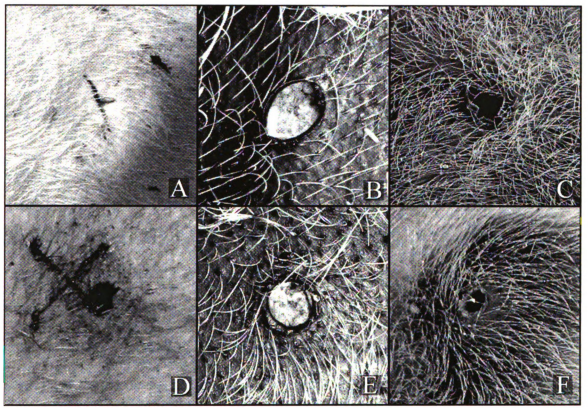

Figure 3.5 – Wound comparisons. A, B, and C show stab wounds on days 0, 7, and 26, where D, E, and F show gunshot wounds on the same days.

3.2.2 Decomposition during winter sampling period (60 days)

As in the fall, the fresh gunshot wounds exhibited typical characteristics of close range gunshot wounds including blackened wound edges and GSR particle deposition in and around the wound edges [Figure 3.6].

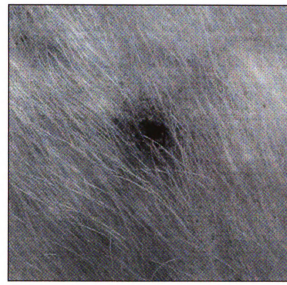

Figure 3.6 – Fresh gunshot wound from winter study.

The primary difference between the fall study and the winter study was that the pigs did not significantly decompose over the course of the study. Even by day 60 bloating was not yet evident, however, the tissue was developing a light dusky green color that was most likely the result of the beginning of putrefaction. That is to say, after sixty days the carcasses were still less decomposed than on day 1 (75°F) of the fall experiment. Figure 3.7 shows a side-by-side comparison of day 0 (39°F) versus day 60 (27°F) for the winter pigs.

GSR was still visible around the gunshot wounds after sixty days; however, it was less prominent than on day 0. After the second day, many of the wounds began secreting a milky, gelatinous substance similar in consistency to petroleum jelly [Figure 3.8].

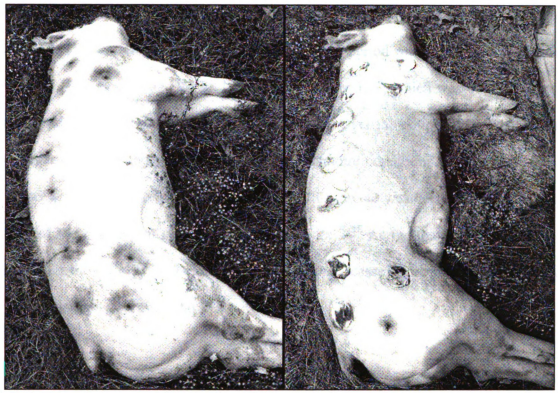

Figure 3.7 – Comparison of images of the shot pig on day 0 (left) and on day 60 (right).

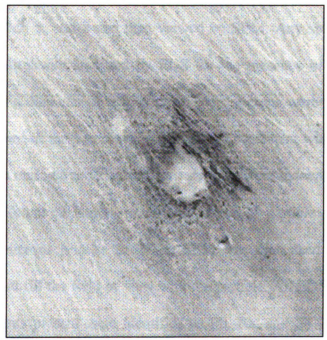

Figure 3.8 – Gunshot wound on day 20 with milky secretion.

The maximum daily high over the collection period was 54.0°F, which occurred on day 52. The minimum daily low over the collection period was -5.4°F, which occurred on day 31. Day 52 also had the highest average temperature, which was 41.3°F, whereas day 31 had the lowest average temperature of 3.2°F. Over the course of the experiments the carcasses were subjected to freeze/thaw temperature conditions. The carcasses were covered with snow on days 5, 8, 12, 16, 26, 30. Complete weather tables for the entire course of the experiment are available in Appendix 2.[31] As the temperature was below freezing for the majority of the sampling period, the carcasses were still in the fresh stage of decomposition after sixty days.

3.3 SEM/EDS RESULTS AND DISCUSSION

3.3.1 Fall SEM/EDS analysis results

Adhesive carbon tabs were dabbed 100 times each on gunshot wound tissue samples from days 1,2, 5, and 8 and then viewed by SEM. Any spheroid particles that were seen were qualitatively analyzed by EDS for the presence of Pb, Ba, and Sb. If spheroid particles of a diameter of approximately ten microns were found to contain all three elements of interest, the particle was determined consistent with GSR according to parameters that were outlined in Chapter 2. All samples were manually searched and the day 1 sample was found to contain a roughly spheroid particle with a diameter of approximately ten microns [Figure 3.9]. The SEM was then used to zoom in on the particle of interest and fill the field of view with the particle. An EDS spectrum was then taken and the elements present were identified as Pb, Ba, and Sb [Figure 3.10]. Other particles were located, however, the shapes of these particles were more crystalline in

nature and contained elements such as silicon, potassium, calcium, and chlorine. These particles were not consistent with GSR.

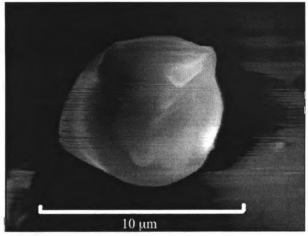

Figure 3.9 – Example spheroid particle from the day 1 gunshot wound.

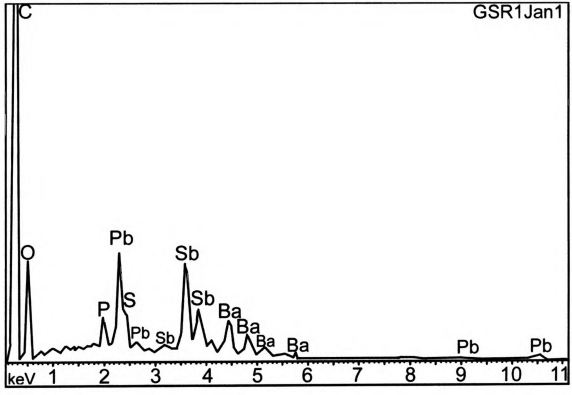

Figure 3.10 – Representative EDS spectrum of particle shown in Figure 3.9 that indicated the presence of Pb, Ba, and Sb in the particle.

The images obtained were not of the highest quality as the samples were uncoated and nonmetallic particles that were present exhibited significant charging. These issues were of little concern, however, as the morphology of the particle was still apparent. Furthermore, determinations are based only partly on morphology, as particle appearance can be somewhat variable, and rely more heavily on the EDS spectrum. The ability to image the sample is not related to the ability to take an accurate EDS spectrum over the area of interest.

No particles that were consistent with GSR were observed for samples analyzed from subsequent time points. This lack of GSR particles could likely be due to the fact that significant rainfall occurred overnight between the day 1 and day 2 samplings. It is thought that the rain likely washed away much of the GSR that was sitting on the surface of the skin and in the hairs. Time points after day 8 were not sampled due to excessive oily discharge from the wounds and the lack of GSR found on days 2, 5, and 8. The discharge most likely prevented any particles that may have been present from sticking to the adhesive tab. Also, for samples to be analyzed by SEM they must be free of volatiles, and the discharge formed a film of oily liquid on the tab; thus, the samples were not compatible with SEM/EDS analysis.

Based on its lack of usefulness for time points after environmental exposure washed away surface GSR or after decompositional secretions made particle collection with an adhesive unachievable, it can be determined that SEM/EDS, while a sufficient technique for the analysis of GSR from swabs of shooters' hands or perhaps even from fresh gunshot wounds, is not a reliable technique for the analysis of GSR on decomposed tissue.

3.3.2 Winter SEM/EDS analysis results

The procedure outlined above was repeated for several time points from the winter study, specifically on the day 1, 2, 5, and 44 gunshot wound samples. As was stated above, the tissue did not progress through the stages of decomposition as with the fall pig, however, many of the wounds secreted a milky, gelatinous substance that was possibly adipocere. This substance became smeared around many of the wounds and prevented the sampling of those wounds for the same reasons as were listed above concerning the oily discharge from the fall pigs.

The day 1 sample yielded several particles which were consistent with GSR particles. The particles were spheroid in nature and the EDS spectrum showed Pb, Ba, and Sb. Similar particles were not observed for day 2, 5, and 44 samples. It is possible that weathering effects such as wind and a slight amount of precipitation could account for the loss of some surface GSR. Also, the tissue was already slightly oily by day 2, which likely inhibited particle adhesion to the carbon tab.

As was seen in the fall, SEM/EDS failed to be useful for the analysis of GSR for time points after environmental exposure would have washed away surface GSR or after decompositional secretions made particle collection with an adhesive unachievable. This indicates that SEM/EDS may not be a reliable technique for the analysis of GSR from wounds that are more than one day old.

3.4 ICP-MS RESULTS AND DISCUSSION

3.4.1 ICP-MS calibration

Multielement calibration standards of Ba, Sb, and Pb were prepared in 2% HNO_3 as described in Chapter 2. The standards were prepared at concentrations ranging from 0 to 500 ppb of each of the elements. The peak areas were normalized to the peak areas of internal standards. The signals from ^{138}Ba and ^{121}Sb were normalized to the signal from ^{115}In, whereas the signal from ^{208}Pb was normalized to the signal from ^{209}Bi. The resulting response factor was then plotted against concentration to generate a calibration curve. All of the theoretical concentrations of the standards were within 15% of the calculated values. Figure 3.11 demonstrates linearity for each element over the interval of 0 to 500 ppb and Figure 3.12 demonstrates linearity even at the low end of the concentration range, from 0 to 5 ppb. The standards were prepared in exactly the same manner and the same concentrations in the fall and winter. Each R^2 value is greater than or equal to 0.9995 for both the entire range and the low concentrations, indicating the wide linear dynamic range of the instrument.

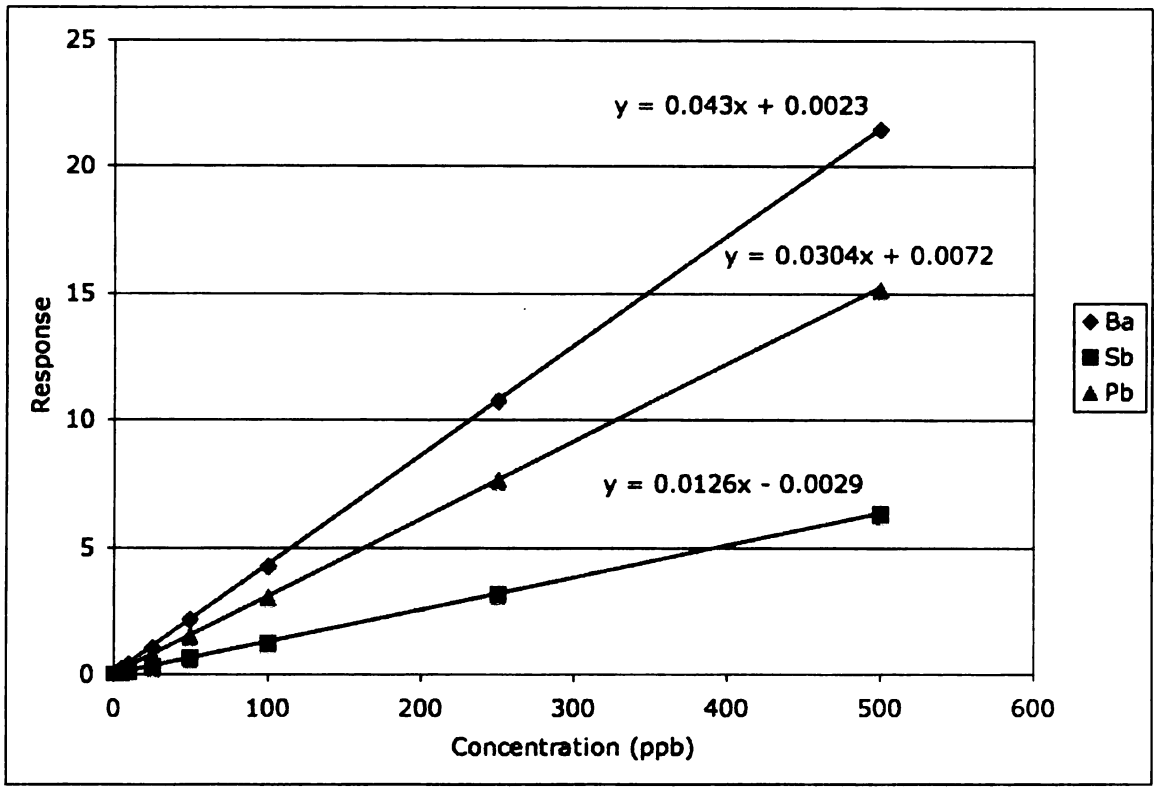

Figure 3.11 – Example calibration curves showing linearity from 0 – 500 ppb for Ba, Sb, and Pb standard solutions.

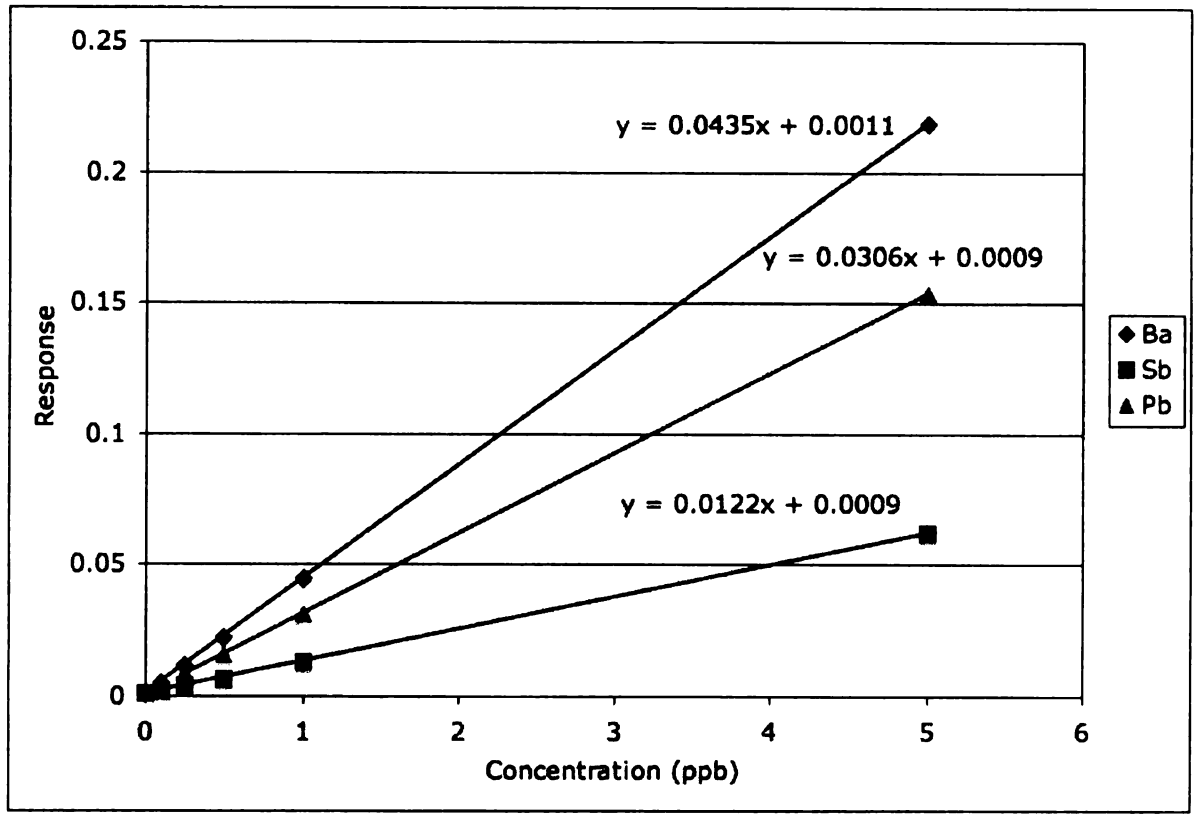

Figure 3.12 – Example calibration curves showing linearity from 0 – 5 ppb for Ba, Sb, and Pb standard solutions.

The limit of quantification (LOQ) is the lowest concentration that could be reported with confidence, and was determined as the lowest standard that was part of the linear range for each set of samples. For Ba, the LOQ ranged from 0.10 ppb to 1.0 ppb; for Sb, the LOQ ranged from 0.10 ppb to 0.25 ppb; and for Pb, the LOQ ranged from 0.10 ppb to 1.0 ppb. For the following sections, no sample with a concentration below the LOQ for that set of samples was reported.

The limit of detection (LOD) was calculated for each element in the same manner as reported by Koons.[14] The LOD was determined by finding the standard deviation (σ) of the response for each element in the 0.0 ppb standards and multiplying by three. This value was then divided by the sensitivity, which is the slope of the standard curve for

each element when element response is plotted against concentration.[14] The average LOD values were calculated to be 0.074, 0.106, and 0.017 ppb for Ba, Sb, and Pb, respectively. These values correspond somewhat to the values reported by Koons, which were 0.02, 0.05, and 0.1 ppb for Ba, Sb, and Pb, respectively.[14] Low LOD values combined with a wide linear dynamic range for Ba, Sb, and Pb, the characteristic elements of GSR, demonstrate that ICP-MS is an excellent technique for the trace elemental analysis of GSR containing samples.

3.4.2 ICP-MS precision study

Five microwave digest solutions were prepared for ICP-MS analysis as described in Chapter 2. The five digests were a sample of control stab wound tissue, two samples of tissue from the fall day 12 GSR tissue samples, a sample of ten blowfly larvae harvested from gunshot wounds on day 7, and a procedural blank. Each digest was analyzed by ICP-MS in replicate (n=5) in order to assess instrumental precision. The concentrations of Ba, Sb, and Pb were determined from the corresponding calibration curve and the reported solution concentrations were converted to microgram of element per gram of sample (tissue or larvae) with the exception of the procedural blank. The average value and relative standard deviation (RSD) were computed for each digest (Table 3.1) based on replicate analyses.

Table 3.1 – ICP-MS precision study results. Each average represents five replicates.

Sample	Average [Ba] (μg/g)	Ba RSD	Average [Sb] (μg/g)	Sb RSD	Average [Pb] (μg/g)	Pb RSD
Day 12 control wound tissue	0.16	1.5%	0.04	6.0%	0.14	1.0%
Day 7 blowfly larvae	2.30	0.8%	*	*	*	*
Day 12 gunshot wound digest 1	0.91	0.4%	0.35	1.2%	1.00	0.3%
Day 12 gunshot wound digest 2	0.80	0.6%	0.34	2.2%	1.08	0.7%

* ICP-MS concentrations were below LOQ and therefore not reported.

The RSD values for each sample type were below 2.5% with the exception of the RSD for Sb from the Day 12 control tissue. The high RSD for this set of replicates is due to the fact that the concentration of Sb was very low, at 0.04 μg/g. The tissue concentrations of Sb for the five injections were 0.05, 0.04, 0.04, 0.04, and 0.04 μg/g, which is quite precise. Each of the element concentrations for all of the procedural blank replicates was below their respective LOQ for the run and consequently were not reported. Acceptable precision, good sensitivity, and a wide linear dynamic range make ICP-MS a good technique for the detection of low concentrations of GSR components.

As this study demonstrated that ICP-MS exhibits excellent precision, the rest of sample digests were analyzed in triplicate. If any of the three injections seemed to be an outlying data point, a statistical Q test was performed to determine if the point was a statistical outlier and could therefore be eliminated.[30]

3.4.3 ICP-MS analysis of procedural blanks

Five procedural blanks were microwave digested in the fall and five procedural blanks were microwave digested in the winter. These blanks were analyzed by ICP-MS in order to see if any significant amounts of the elements of interest were present in the solutions used in the digestion procedure. For the fall set of procedural blanks, the

concentrations of Ba, Sb, and Pb were all below their respective LOQ for the run. The same was true in the winter for Sb and Pb; however, the LOQ for Ba was lower in the winter set of samples and as a result, Ba was quantified. The average concentration was 0.18 ppb with a standard deviation of 0.087 ppb. The concentration of Ba was much lower than any concentration reported for a digest solution of gunshot wound tissue or blowfly larvae that had been feeding on GSR containing tissue. These results indicate that the microwave digestion process and sample preparation procedure contributed no significant amount of Pb, Ba, or Sb to observed concentrations in tissue or larvae digests.

3.4.4 ICP-MS analysis of blowfly larvae from indoor study

Initially, first instar *Phaenicia sericata* blowfly larvae were placed onto shot beef and control beef as described in Chapter 2. By day 2, the larvae were primarily second instars, and by day 3, most were early third instars. Pupae were first observed on day 9. Microwave digests of between 0.03 and 0.5 grams of blowfly larvae from the control beef and from the shot beef were analyzed in triplicate by ICP-MS. The difference in weight was due to the difference in mass of early instar larvae when compared to third instar larvae. Approximately ten larvae were included in each sample. One sample of day 9 pupae from each of the control beef and shot beef were also microwave digested and analyzed by ICP-MS in triplicate. Figure 3.13 shows the average concentrations of Pb, Ba, and Sb in the larvae and pupae in micrograms of element per gram of larvae or pupae. The Ba concentrations in the larvae from the shot beef started at approximately 10.3 μg/g for days one and two after exposure to the GSR beef and then rose to between 50 and 80 μg/g for days 3 through 9. It is possible that the larvae were bioaccumulating Ba over the course of the experiment, as barium exhibits similar chemical properties to

that of calcium, which is necessary for larval development;[24] however, further studies would be needed to investigate this hypothesis.

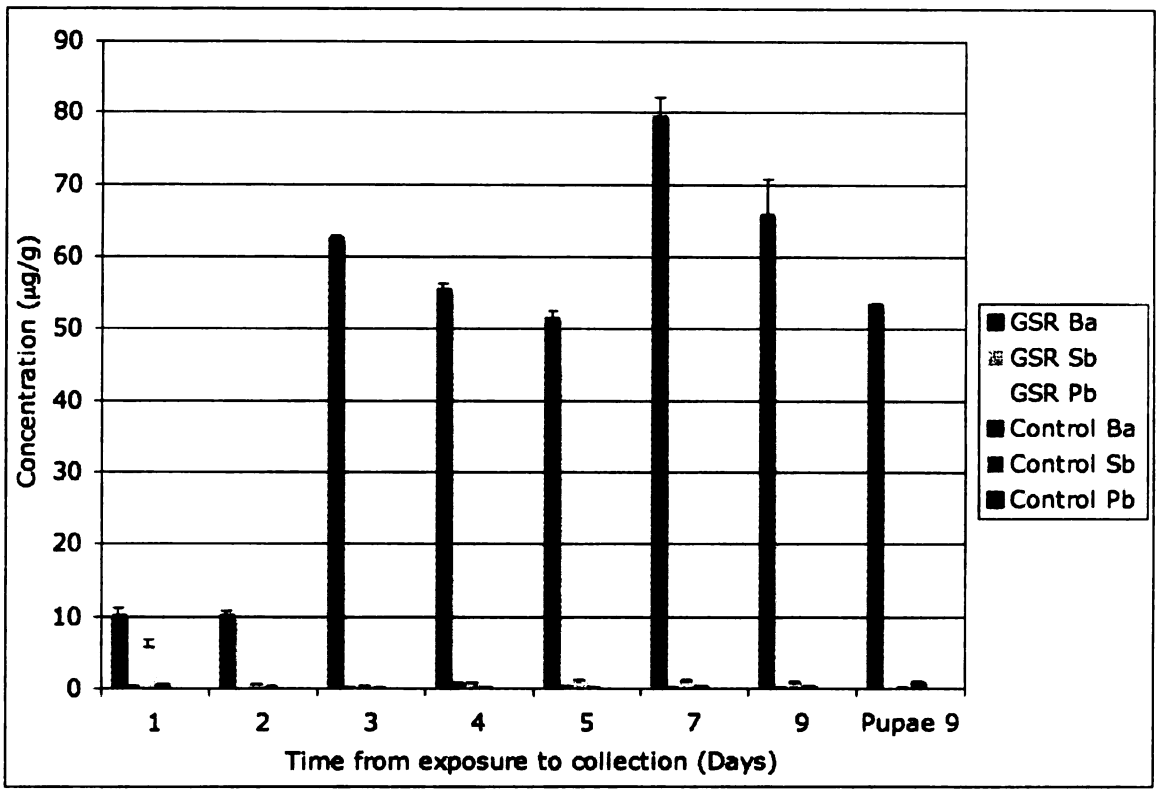

Figure 3.13 – Graph showing average Pb, Ba, and Sb concentrations in blowfly larvae and pupae. "GSR" indicates blowfly larvae that had been feeding on shot beef. "Control" indicates blowfly larvae that had been feeding on control beef.

Ba was also observed in the control larvae; however, the concentrations were much lower and were all below 1.0 µg/g. The low concentrations are visible in Figure 3.14, which shows the same data as Figure 3.13 on a decreased scale. As there is no apparent trend in the control Ba concentrations, it is thought that blowfly larvae could contain a certain amount of endogenous barium; however, many more blowfly larvae would need to be analyze in order to test this hypothesis. Sb and Pb were not observed in any of the larvae that had been feeding on the control beef. Sb was observed in the day 1 larvae from the

shot beef, as well as in the larvae from days 3, 4, 5, 7, and 9 in concentrations ranging from 0.15 to 0.75 μg/g. Sb concentrations in the day 2 and day 9 pupae samples were not above the LOQ and are therefore not reported here. The lack of Sb in the day 2 sample when it was present on days 1 and 3 would need to be investigated further, however, it is likely that the larvae collected on that day simply did not consume enough GSR particles for Sb to be detected.

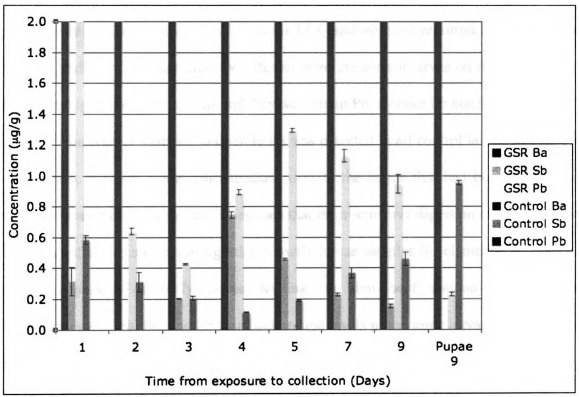

Figure 3.14 – Decreased scale version of the graph showing Pb, Ba, and Sb concentrations in blowfly larvae and pupae.

The concentration of Pb was 6.26 μg/g on day 1 and then decreased to between 0.42 and 1.29 μg/g for subsequent time points. Further testing would be needed to explain the large amount of lead in the day 1 sample. As this study was conducted indoors with no

environmental exposure, all of the larvae were feeding pieces of the same shot steak, and all of the larvae were received in a batch from the same company, it can be assumed that the Pb in the day 1 sample is indeed from the gunshot residue rather than external contamination.

All of the characteristic metals of GSR were detected in *P. sericata* larvae that had been feeding on shot beef on days 1, 3, 4, 5, 7, and 9, where only Pb and Ba were detected in day 2 larvae and the day 9 pupae. Even though the concentrations of Sb in the day 2 larvae and day 9 pupae were below the LOQ and were not reported, the larvae from the shot beef were still significantly different from the control larvae on the same day as they have high Ba concentrations and they do contain Pb. Neither Pb nor Sb was detected in any of the control samples, and while Ba was detected in all control larvae samples, it was present at much lower concentrations than in the larvae that had been feeding on GSR containing beef. These results indicate that the microwave digestion protocol used is indeed an effective means for digesting blowfly larvae samples for elemental analysis of GSR components. Blowfly larvae feeding on shot beef contain much higher concentrations of Pb, Ba, and Sb than background levels in the same larvae, which makes the analysis of these elements for GSR detection in blowfly larvae feasible.

3.4.5 ICP-MS analysis of blowfly larvae found feeding in gunshot wounds (Fall)

Blowfly larvae were harvested from the gunshot and stab wounds from the fall set of pigs. Between 0.03 and 0.50 grams of blowfly larvae were digested in the same manner as the larvae from the indoor study and were then analyzed by ICP-MS in triplicate for the characteristic elements of GSR. Ba, Sb, and Pb were detected in day 3 and 4 larvae samples from the gunshot wounds and were not detected in the larvae

samples from the stab wounds [Figure 3.15]. For subsequent days, Ba was present in all larvae and the levels were comparable between the larvae from gunshot wounds and stab wounds. Low levels of Sb was detected in the day 9, 10, and 15 larvae from gunshot wounds, along with the day 15 larvae from the stab wound at a similar concentration [Figure 3.16]. Similarly, Pb was detected in the day 10 and 11 samples of larvae from gunshot wounds and in the day 11 larvae from the stab wound [Figure 3.16]. As the procedural blanks have shown to contain no significant concentrations of these elements, it can be assumed that the observed concentrations of the elements of interest are indeed from the larvae.

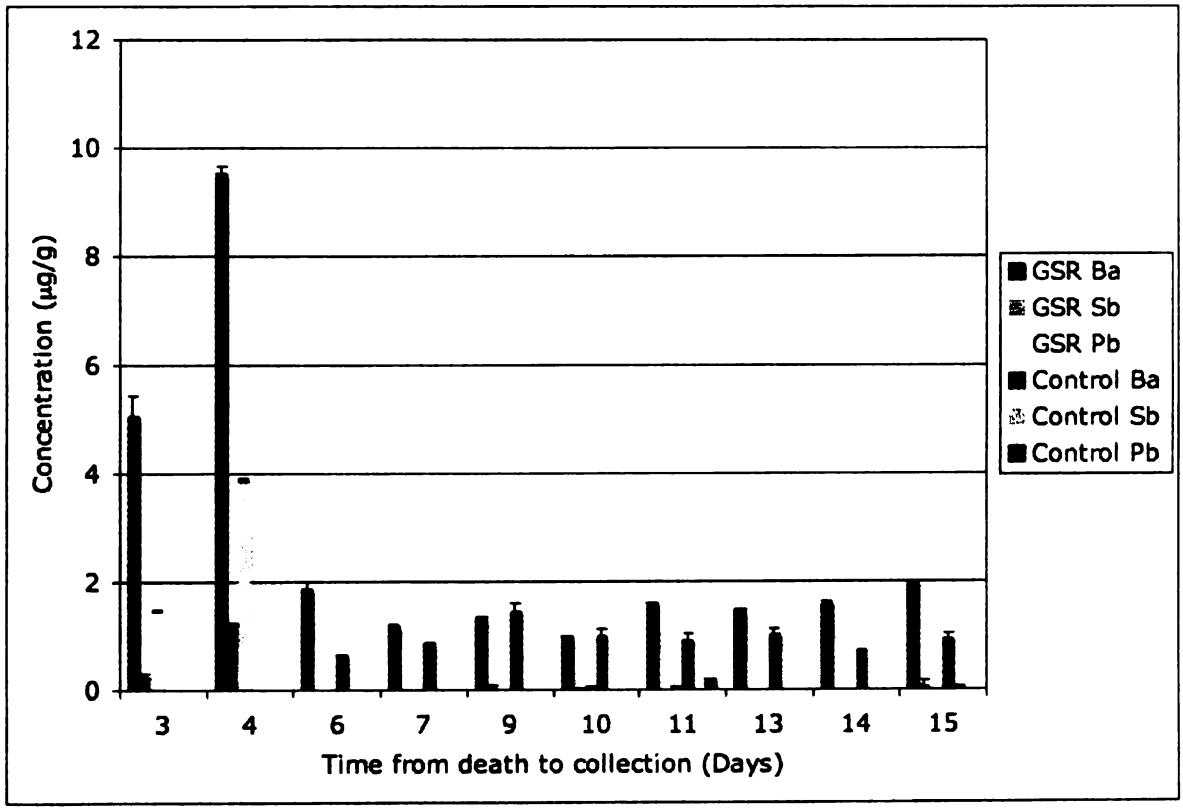

Figure 3.15 – Graph of concentrations of Ba, Sb, and Pb in blowfly larvae from gunshot and stab wounds over time.

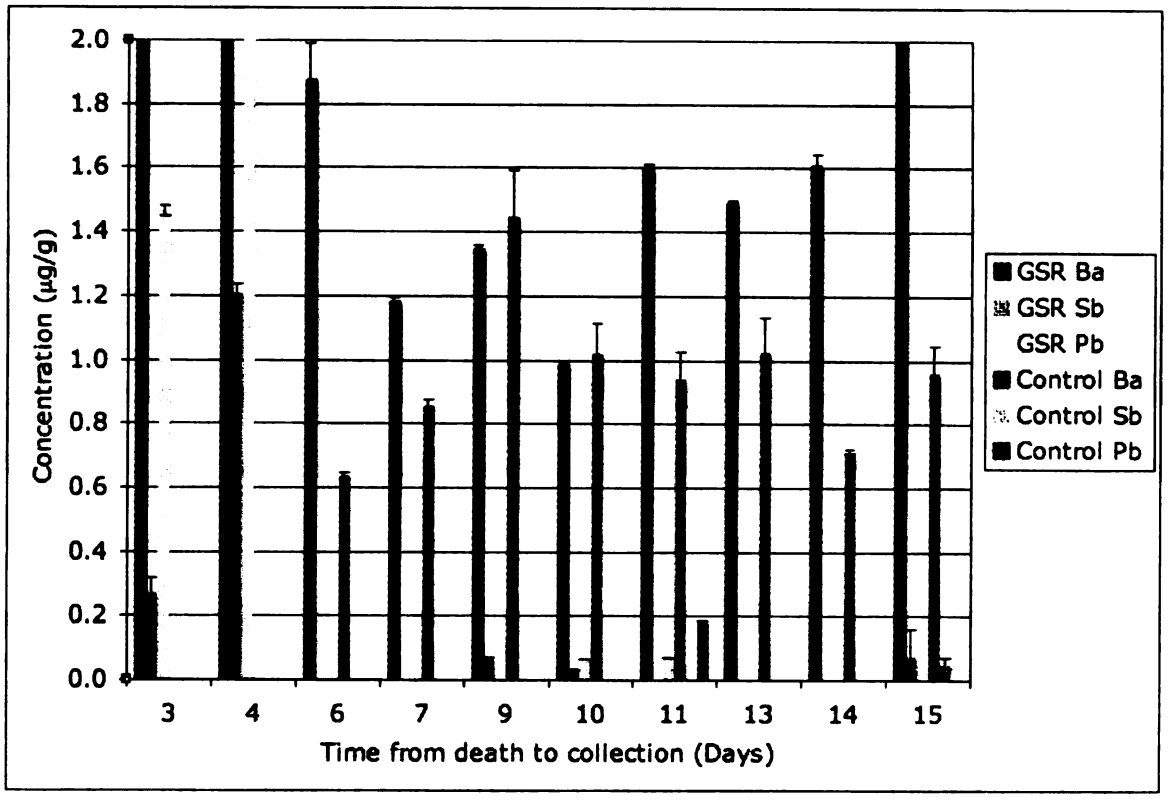

Figure 3.16 – Decreased scale version of the graph of concentrations of Ba, Sb, and Pb in blowfly larvae from gunshot and stab wounds over time showing low concentrations at later time points.

These results indicate that the blowfly larvae from gunshot wounds tested on days 3 and 4 contained GSR components, whereas the larvae from stab wounds did not. On subsequent days, the larvae from gunshot wounds did not contain significantly different concentrations of the characteristic GSR metals than the larvae from the stab wounds. It is possible that this is due to sampling technique. The larvae had most likely been feeding on the tissue of the wound tract rather than the skin, where much of the GSR had deposited. Each day the larvae were harvested from just inside the gunshot wounds. As the larvae from days 3 and 4 were smaller and appeared to be generally less mobile, they had most likely been feeding on the wound tract tissue the earliest, when more GSR had

been present. Later, the larvae were larger and could cover more area than before. Also, the tissue containing the most GSR from the upper one to two centimeters of the wound tract would have been consumed. Therefore, when the larvae were harvested from that area, they had not been feeding on GSR containing tissue and would therefore not contain the characteristic GSR elements in significant concentrations. Further studies would need to be performed to lend more weight to this hypothesis, but it is thought that if larvae had been collected from further in the wound tract, larvae samples from later time points would have yielded characteristic GSR components. Nevertheless, this study demonstrates the feasibility of detecting characteristic GSR components in blowfly larvae found feeding naturally in gunshot wounds.

3.4.6 ICP-MS analysis of decomposing porcine tissue for GSR components (Fall)

Microwave digests of tissue samples from control stab wounds and gunshot wounds were analyzed by ICP-MS in triplicate for the presence of Ba, Sb, and Pb. All three elements were found in digests of gunshot wound tissue [Figure 3.17]. The overall trend shows a decrease in metal content consistent with GSR over the first three time points with a leveling off by day 8. The highest Ba concentration observed was on the first day after death, at 163.63 μg/g of tissue. Day 1 also exhibited the highest Sb and Pb concentrations, at 56.50 and 122.56 μg/g, respectively. Between days 8 and 37 the concentrations of Ba ranged from 1.21 μg/g on day 8 and 7.54 μg/g on day 34. Sb ranged from 0.59 μg/g on day 37 to 3.64 μg/g on day 34, while Pb ranged from 1.09 μg/g on day 16 to 5.20 μg/g on day 34. With the exception of the lowest Sb concentrations, all of the values were in the same order of magnitude.

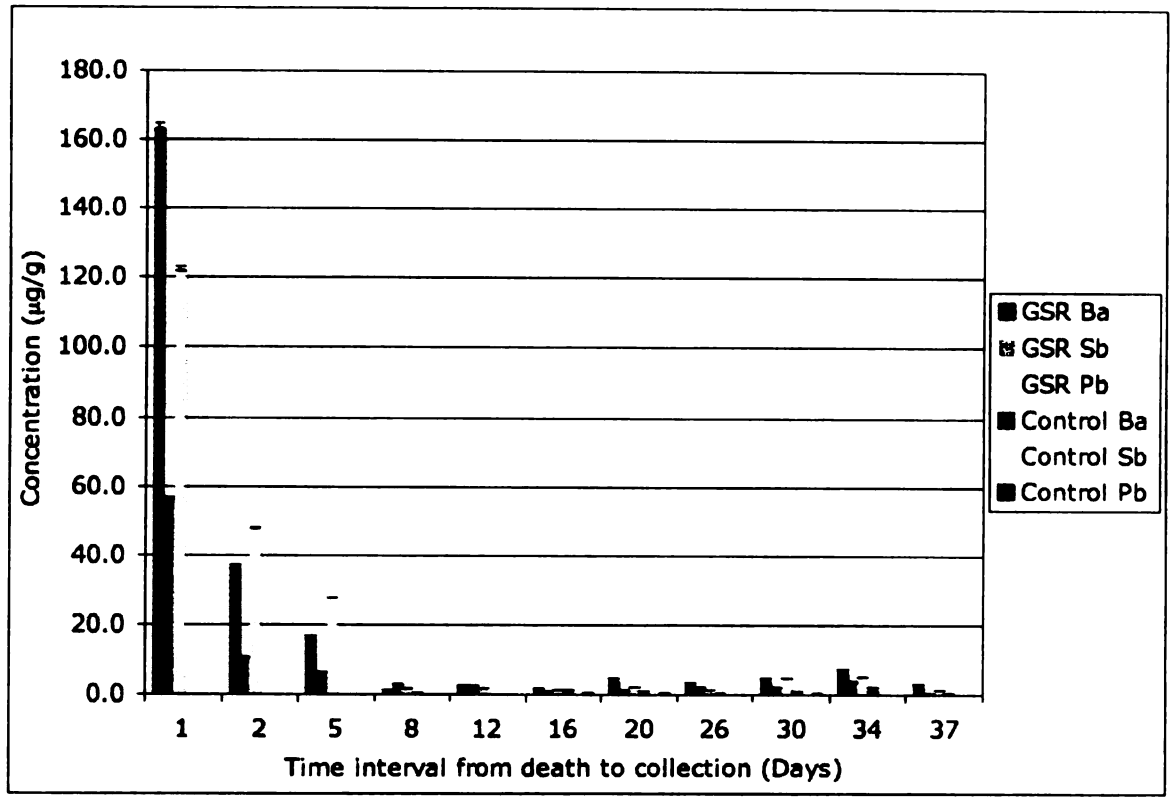

Figure 3.17 – Graph of concentrations of Ba, Sb, and Pb in tissue from gunshot and stab wounds over thirty-seven days in the fall.

Even the lowest Sb concentrations are almost an order of magnitude higher than the highest Sb concentrations observed in the control wound digests [Figure 3.18]. Sb was only observed in the day 16 and 30 control wound digests at concentrations of 0.08 µg/g and 0.05 µg/g, respectively. Pb was observed in the control wound digests for all time points with the exception of days 1 and 2. The concentrations ranged from 0.04 µg/g on day 5 to 0.28 µg/g on day 20. These values are at least an order of magnitude lower than the amount of Pb found in all time points of the gunshot wound digests. Ba was observed in the control wound digests for all time points with the exception of days 2 and 5. The concentrations ranged from 0.12 µg/g on day 1 to 1.99 µg/g on day 34. With the

exception of day 34, the concentration of Ba in the control wound digests is almost an order of magnitude lower than in the gunshot wound digests.

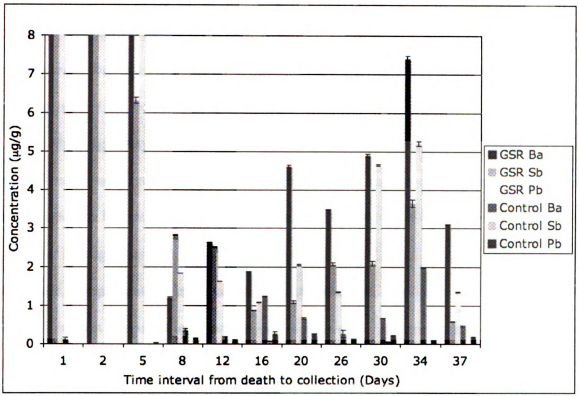

Figure 3.18 – Decreased scale version of the graph of concentrations of Ba, Sb, and Pb in tissue from gunshot and stab wounds over thirty-seven days in the fall.

Although the difference between the control wound and gunshot wound Ba concentration on day 34 may not be significant, the difference in Sb and Pb is significant. This indicates that there is still a pronounced difference between the GSR components in the gunshot wound digest versus the control wound digest.

The decrease in Ba, Sb, and Pb in the gunshot wound tissue after day 1 is likely due to a partial loss of surface GSR. Significant rainfall occurred between the collection time on days 1 and 2. The particles that had lightly adhered to the hairs and skin may

have been washed away in the rain. Between the second and fifth days, additional surface GSR likely was lost due to wind and decompositional fluid seepage. Additional significant rainfall occurred on the evening of the fifth day. Between the wind, rain, skin slippage, and active decay processes, it can be assumed that only the GSR that had tattooed into the skin remained by the eighth day. This would account for the apparent leveling off of GSR component levels in the tissue.

The slight day-to-day variations of the element concentrations in the gunshot wound tissue samples are most likely the result of the sample collection procedure. As can be seen in Figures 3.1, 3.6, and 3.7, the distribution of GSR around the bullet hole was not homogenous. As it was not visually apparent where most of the GSR had deposited, sampling was simply conducted approximately one centimeter away from the bullet hole. If it happened to be on the side of the wound that contained the most GSR, a higher metal content would be observed than if the sampling happened to occur on the opposite side. By the end of the collection the skin had leathered to the point that tissue excision by scalpel had become extremely difficult. Also, despite every precaution taken to ensure that the gunshot wounds were as similar as possible, the wounds most likely differed somewhat due to slight variations in wind speed, angle of shot, and individual bullet cartridge characteristics. Quantification of element concentrations spatially was not a goal of this project, however, as GSR determination is primarily based on a qualitative identification of the elements of interest. The quantitative aspect was employed to distinguish the amount of Pb, Ba, and Sb in the gunshot wound tissue from the control wound tissue.

Overall, these results indicate that microwave digestion of small samples of wound tissue, coupled with ICP-MS analysis of Ba, Sb, and Pb, is an effective means for determining GSR in decomposing tissue for at least thirty-seven days after death, even after several significant rainfalls. The time frame studied encompassed many stages of decomposition and the skin was quite leathered by the end of the sampling process.

3.4.7 ICP-MS analysis of decomposing porcine tissue for GSR components (Winter)

The fall study described above was repeated in the winter with tissue samples taken at the same time points until thirty days after death, with the final two time points taking the study out farther, to days 44 and 60 after death. Microwave digests of tissue samples from control stab wounds and gunshot wounds were analyzed by ICP-MS in triplicate for the presence of Ba, Sb, and Pb. All three elements were found in digests of gunshot wound tissue [Figure 3.19]. The highest Ba concentration observed in gunshot wound tissue was on day 44, at 125.90 μg/g of tissue, whereas the lowest concentration observed was on day 16, at 34.91 μg/g. Day 5 exhibited the highest Sb concentration, at 35.6 μg/g, while the lowest concentration was observed on day 12, at 5.23 μg/g. The amount of Pb in the day 5 digest solution saturated the detector, while the concentration of Pb in the day 60 digest was significantly above the range of standards. Therefore, while the actual values of the tissue concentrations in these samples are not reliable, their approximate values are represented in Figure 3.19 in order to illustrate their concentrations relative to the other concentrations. Regardless of their actual values, the Pb concentrations on days 5 and 60 were the highest observed over the course of the

experiment. The lowest Pb tissue concentration of the gunshot wound samples was on day 30, at 81.27 μg/g.

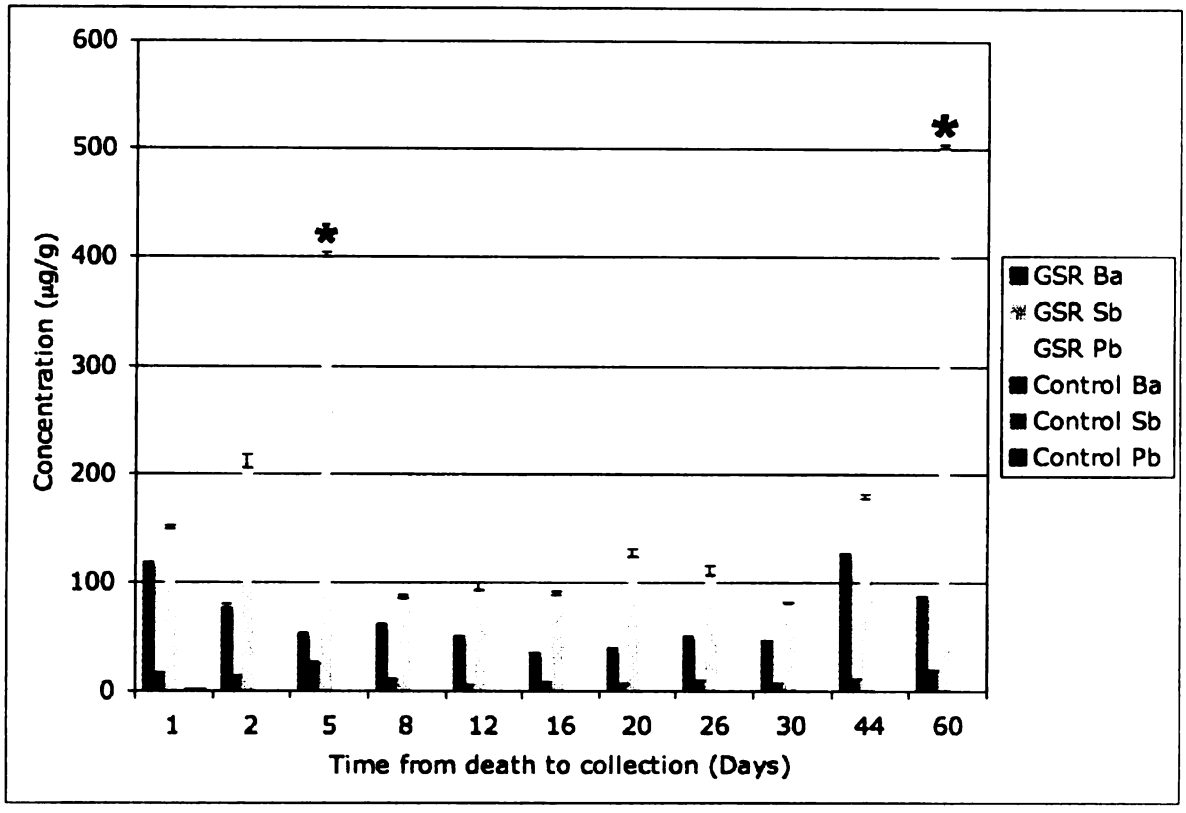

Figure 3.19 – Graph of concentrations of Ba, Sb, and Pb in tissue from gunshot and stab wounds over sixty days in the winter. *The concentration of Pb in the gunshot wound digest saturated the detector on days 5 and was above the upper LOQ on day 60. These concentrations are thus only included as approximations of the tissue concentrations.

Ba was present in all control wound samples in concentrations below 0.50 μg/g, which is two orders of magnitude lower than the lowest concentrations observed in the gunshot wound tissue samples. Sb was detected in all control tissue samples with the exception of the day 26 and 60 samples. The concentrations reported were all below 0.60 μg/g, which is at least one order of magnitude lower than the tissue concentrations

reported for the gunshot wound tissue samples. Pb was reported in the day 1, 2, and 16 samples at concentrations of 1.20, 0.27, and 0.07 μg/g, respectively. Again, these concentrations are one to two orders of magnitude lower than the Pb levels observed in the gunshot wound samples. The low concentrations are visible graphically in Figure 3.20, which is the decreased scale version of Figure 3.19.

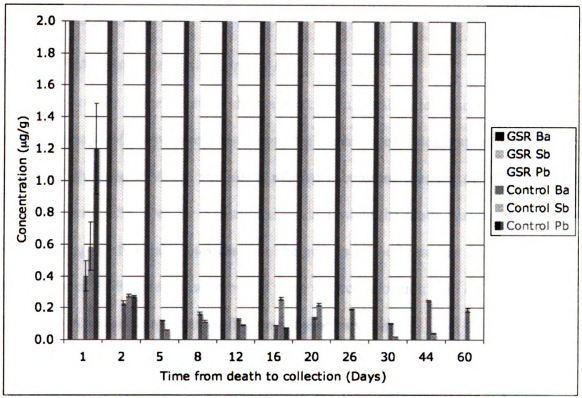

Figure 3.20 – Decreased scale version of the graph of concentrations of Ba, Sb, and Pb in tissue from gunshot and stab wounds over sixty days in the winter.

The consistent results over the entire range of collection time points indicate that any significant weathering effects on the surface GSR likely occurred before the first collection point. However, the high concentrations compared to the concentrations measured in the fall study indicate that a less significant amount of weathering occurred.

The amount of GSR that was visible on the tissue originally was slightly reduced by day 1 but appeared consistent throughout the rest of the collection period despite the snow and sleet. As was likely the case for the later time points in the fall study, the daily variation in metal concentration is likely due to the non-homogenous distribution of GSR around the bullet wound. These results indicate that the concentrations of Ba, Sb, and Pb remain somewhat stable over time when tissue decomposition does not occur, even when exposed to snow and sleet.

3.4.8 ICP-MS tissue analysis summary

The results of the fall study show a decrease in metal content consistent with GSR over the course of thirty-seven days, from the fresh stage to desiccation, whereas the results of the winter study yielded a relatively constant amount of metal content consistent with GSR over the course of sixty days when the tissue was prevented from decomposing by freezing temperatures. The results of both studies indicate that the persistence of GSR depends more on the stage of decomposition than on the amount of time that has passed from wound infliction to tissue harvesting.

It is interesting to note that the general elemental distribution were quite variable. The elemental distributions were calculated in the form of percentages and compared between the studies. These results are shown in Table 3.2.

Table 3.2 – Elemental distribution in gunshot wound digest samples: Fall and Winter.

Fall	Mass Percent (μg/g)		
Day	[Ba]	[Sb]	[Pb]
1	47.75%	16.49%	35.76%
2	38.91%	11.12%	49.96%
5	33.04%	12.37%	54.58%
8	20.52%	47.97%	31.51%
12	35.61%	33.94%	30.45%
16	48.90%	22.78%	28.32%
20	59.32%	14.13%	26.54%
26	50.33%	30.05%	19.62%
30	42.01%	18.09%	39.90%
34	45.57%	22.41%	32.03%
37	61.45%	11.61%	26.93%
Average:	43.95%	21.91%	34.15%
RSD:	**27%**	**52%**	**30%**

Winter	Mass Percent (μg/g)		
Day	[Ba]	[Sb]	[Pb]
1	41.27%	5.83%	52.91%
2	25.71%	4.53%	69.77%
5	*	*	*
8	38.51%	6.61%	54.88%
12	32.60%	3.48%	63.92%
16	26.06%	6.52%	67.42%
20	22.42%	3.59%	73.99%
26	28.87%	5.86%	65.27%
30	34.58%	5.45%	59.97%
44	39.91%	3.48%	56.61%
60	*	*	*
Average:	32.21%	5.04%	62.75%
RSD:	**21%**	**26%**	**11%**

*These data were not reported because the Pb concentrations were above the upper LOQ.

The distributions were not reported for days 5 and 60 of the winter study as the Pb saturated the detector on day 5 and the concentration was significantly above the highest calibration standard on day 60. The data for Ba and Sb were within the standard range for those time points, but without reliable data for all three elements, the ratios are not meaningful. In the winter, the concentration of Pb was always the highest, followed by Ba. Sb always accounted for less than 10% of the total metal detected. The fall studies were much more variable. Since ICP-MS has shown to be a very precise technique, the high RSD values are most likely the result of a heterogeneous elemental distribution in the tissue or cartridge-to-cartridge differences. Further studies would be needed to determine the origins of these differences.

Haag *et al.*[27] has demonstrated that porcine tissue is a suitable model for human skin. The correlation suggests that the technique of microwave digesting tissue and subsequent ICP-MS analysis that has been shown to be useful in the identification of

GSR components in porcine skin would likely also be useful for the chemical identification of gunshot wounds in decomposing human individuals. The ability to use these techniques to identify human gunshot wounds could be useful to pathologists in determining the cause of death when decomposition, insect predation, or other circumstances have precluded conventional means of identifying the wounds.

4. CONCLUSIONS AND FUTURE WORK

4.1 CONCLUSIONS

The results of this study indicated that the extraction of Pb, Ba, and Sb from *Phaenicia sericata* larvae by microwave digestion was successfully achieved from larvae that had been feeding on beef containing GSR. The digests were analyzed by ICP-MS and the concentrations of GSR components, Ba, Sb, and Pb, were determined. It was found that the larvae that had been feeding on the shot beef contained each of the characteristic GSR components. The concentration of Ba was approximately 10 μg/g for the first two days after exposure, then rose to between 50 and 80 μg/g for days 3-9. These results indicated a possible bioaccumulation of Ba in blowfly larvae, although the reason for this was not investigated further during this research. Sb was observed for all time points except for day 2 and the day 9 pupae. The concentrations of Sb ranged from 0.15 to 0.75 μg/g when it was observed. Pb was observed for all time points, beginning at 6.26 μg/g on day 1 and ranging between 0.42 and 1.29 μg/g for later time points. A sample of pupae was found to contain Ba and Pb, but no Sb was detected. More pupae samples would be needed to investigate the statistical significance of these findings. These results were compared to larvae that had been feeding on beef that was not shot. It was found that although the elements were detected for some time points, their levels were significantly lower than in the blowfly larvae that had been feeding on shot beef.

The method developed using *P. sericata* larvae was used to analyze blowfly larvae found feeding in decomposing porcine gunshot wounds for the presence of GSR components. It was found that the larvae contained these components on days 3 and 4 after death. The concentrations of Ba were between 5.0 and 10.0 μg/g, where the

concentrations of Sb were between 0.2 and 1.2 μg/g and the concentrations of Pb were between 1.4 and 4.0 μg/g. For all subsequent time points there was no difference in the element content of the larvae from gunshot wounds versus the control larvae from stab wounds. It is suspected that more time points could have been found to contain GSR if larvae had been sampled from deeper within the wound tract.

SEM/EDS and ICP-MS of the decomposing porcine tissue digests were also employed to determine the persistence of GSR in decomposing tissue in both fall and winter climates. For SEM/EDS analysis, potential GSR particles were collected by dabbing the wound 100 times with a carbon adhesive tab mounted on an aluminum stub. In the fall, it was found that SEM/EDS was useful to detect characteristic GSR particles from the area near the gunshot wounds for up to one day after death. The particles were collected by dabbing the wound 100 times with a carbon adhesive tab mounted on an aluminum stub. It is thought that the weather played a role in washing away GSR particles that were deposited on the surface of the skin between the day 1 and day 2 collection times. No GSR was detected on wounds from later time points. In the winter, characteristic GSR particles were also detected for up to one day after death. No GSR particles were detected on wounds from later time points. It is possible that the lack of GSR for later time points was due to a combination of wind, light precipitation, and the slightly oily nature of the tissue.

SEM/EDS has not been shown to be a reliable technique for the detection of GSR on decomposing tissue as many factors such as rain and decompositional secretions can make its collection with adhesive tabs extremely impractical if not impossible. It may be

a viable technique for fresh gunshot wounds, however, it is likely that the wound would be identifiable by an expert performing a visual and histological examination at that time.

ICP-MS was shown to be a precise multielement technique with a wide linear dynamic range through linearity and precision studies. Multielement calibration standards indicated that the upper LOQ was at or above 500 ppb for each of Ba, Sb, and Pb and that the lower LOQ was between 0.1 and 1.0 ppb for each element. The average 3σ LOD values were calculated to be 0.074, 0.106, and 0.017 ppb for Ba, Sb, and Pb, respectively. Relative standard deviations for replicate injections were less than 3% for each element.

The fall set of pigs decomposed to a desiccated state over the course of the thirty-seven day collection interval. Tissue samples were microwave digested and analyzed by ICP-MS for the presence of GSR components. It was found that all three characteristic elements could be detected in the tissue digests over the course of the entire collection interval. The tissue concentrations of each element decreased over the first three collection points and then leveled off for the rest of the time points. The Ba, Sb, and Pb concentrations were 163.63, 56.50, and 122.56 μg/g on day 1, respectively. By day 8, the concentrations had decreased to between 0.5 and 8.0 μg/g for all elements and remained in this range for the duration of the experiments.

In the winter, the pigs did not decompose over the sixty day collection interval. ICP-MS analysis of tissue digests revealed that all three characteristic elements could be detected for each time point over the sixty days. Moreover, the concentrations of the elements remained relatively constant over the course of the collection. The concentrations of the elements were between 35 and 126 μg/g for Ba and between 5 and 36 μg/g for Sb. The lowest Pb concentration observed was 81.27 μg/g and the highest

concentrations could not be quantitated as they were above the upper LOQ. These results indicate that ICP-MS of tissue digests can provide a chemical means of identifying gunshot wounds and that the concentrations of GSR components are more reliant on the stage of decomposition rather than the amount of time between death and analysis. Unlike SEM/EDS, which proved unreliable after more than a day after death, the ability to detect GSR components by ICP-MS was not hindered by weathering effects on the carcasses. ICP-MS of tissue digests may be useful to a pathologist in the identification of suspected gunshot wounds when decomposition has precluded conventional means of wound identification.

4.2 FUTURE WORK

Now that it is established that GSR components can be detected in decomposing tissue through all stages of decomposition, there are a great many possibilities for future projects. In general, more studies should be conducted to expand the sample population and thus provide some information as to the statistical significance of the findings previously presented. Also, it is necessary to perform the same study over a longer sampling time frame as the latest time points in the studies discussed in this work still exhibited detectable levels of GSR components. It is thought that GSR remains as long as skin is present with the remains, and so leaving some remains with gunshot wounds outside and sampling until the tissue is gone could test this theory.

The usefulness of ICP-MS to determine the firing range could also be investigated by firing shots of known distances and quantifying the GSR components in the tissue in order to see how their concentration varies with firing distance. This study could be conducted preliminarily with fresh gunshot wounds and then again with decomposed

wounds to see if the results differ with decomposed tissue. Additionally, a sampling study should be conducted on fresh gunshot wounds to see how much variability exists around a wound and from wound to wound. This information would be useful in order to determine an optimal sampling scheme from the wounds and could be used to help provide statistical information about the technique itself and about the precision of the firing distance determinations method.

Additionally, it would be useful to perform similar studies using pigs that had been shot then buried. When bodies are recovered after burial, it is often the case that the tissue has decomposed to a point that makes a visual and histological assessment of gunshot wounds extremely difficult. The skin of the victim may have a great deal of dirt embedded in it, which could affect the background concentrations of the metals. The samples could still be microwave digested and analyzed for the characteristic GSR components by ICP-MS.

A simple study could demonstrate the ability of ICP-MS digested tissue to discriminate entrance wounds from exit wounds in decomposed tissue. In such a study, a euthanized pig could be shot so as to incur through and through wounds and tissue could be sampled over time from both the entrance and exit wounds to determine if the concentration of GSR components varies from entrance to exit, and, if the variation exists, the extent to which it is exhibited.

Other studies could investigate different brands of ammunition. It is possible that different types and brands of ammunition would deposit different amounts of GSR with different elemental distribution ratios. The concentrations of Pb, Sb, and Ba could be measured in the tissue surrounding the wound along with other trace elements to see if a

statistical difference exists between different brands of jacketed ammunition along with unjacketed and semi-jacketed types of ammunition. It would also be beneficial to see if the trace elemental profile of various types of ammunition changes with decomposition, for example, if the concentration of zinc decreases over time.

Finally, further work should be done to optimize the collection of blowfly larvae from gunshot wounds for ICP-MS analysis. Larvae could be collected from different wounds from various depths inside the wound tract in order to see if the sampling depth affects the number of days after death that blowfly larvae might be useful for GSR component detection and subsequent gunshot wound identification.

The research presented in this thesis has shown the ability to use ICP-MS to chemically identify GSR components in decomposing porcine tissue over many stages of decomposition and for at least sixty days following death. The similarities between porcine and human tissue make it likely that the techniques outlined in Chapter 2 could be applied to gunshot wounds in decomposing human remains. It was also shown that analyzing blowfly larvae in suspected gunshot wounds for the presence of GSR components using ICP-MS could also be useful to identify gunshot wounds while the larvae are still in early developmental stages.

APPENDIX 1

Weather data for the fall collection period:[31]

2007		Temperature (°F)			Rainfall (in.)
Day	*Date*	*Min*	*Max*	*Average*	*Today*
Wed	**5-Sep**	62.4	88.4	75.4	--
Thu	**6-Sep**	67.0	83.6	75.3	--
Fri	**7-Sep**	68.2	79.5	73.9	0.61
Sat	**8-Sep**	59.5	78.5	69.0	--
Sun	**9-Sep**	60.3	80.3	70.3	--
Mon	**10-Sep**	53.3	66.7	60.0	0.46
Tues	**11-Sep**	52.9	67.2	60.0	0.07
Wed	**12-Sep**	45.9	60.9	53.4	--
Thu	**13-Sep**	42.8	73.1	58.0	--
Fri	**14-Sep**	48.8	63.3	56.1	0.02
Sat	**15-Sep**	41.1	58.8	49.9	--
Sun	**16-Sep**	35.3	65.8	50.6	--
Mon	**17-Sep**	43.0	72.3	57.6	--
Tues	**18-Sep**	54.4	82.1	68.2	--
Wed	**19-Sep**	58.0	82.5	70.3	--
Thu	**20-Sep**	56.8	80.6	68.7	--
Fri	**21-Sep**	57.7	85.5	71.6	--
Sat	**22-Sep**	54.2	74.2	64.2	0.05
Sun	**23-Sep**	42.5	77.3	59.9	--
Mon	**24-Sep**	52.6	88.6	70.6	--
Tues	**25-Sep**	66.3	81.5	73.9	0.59
Wed	**26-Sep**	57.3	70.8	64.0	0.13
Thu	**27-Sep**	50.4	70.1	60.3	--
Fri	**28-Sep**	47.9	70.1	59.0	--
Sat	**29-Sep**	40.7	73.3	57.0	--
Sun	**30-Sep**	50.3	77.0	63.6	--
Mon	**1-Oct**	55.8	69.7	62.7	0.93
Tues	**2-Oct**	55.1	73.4	64.2	--
Wed	**3-Oct**	47.0	72.3	59.7	0.04
Thu	**4-Oct**	42.0	79.4	60.7	--
Fri	**5-Oct**	60.8	86.6	73.7	--
Sat	**6-Oct**	64.4	85.2	74.8	--
Sun	**7-Oct**	63.6	87.1	75.4	--
Mon	**8-Oct**	66.2	86.4	76.3	--
Tues	**9-Oct**	51.5	73.1	62.3	0.07
Wed	**10-Oct**	43.3	53.0	48.2	0.12
Thu	**11-Oct**	43.7	51.6	47.6	0.01
Fri	**12-Oct**	39.9	50.8	45.3	--

APPENDIX 2

Weather data for the winter collection period:[31]

2008		Temperature (F)			Rainfall (in.)
Day	*Date*	*Min*	*Max*	*Average*	*Today*
Fri	11-Jan	33.6	44.4	39.0	0.03
Sat	12-Jan	31.4	38.3	34.9	--
Sun	13-Jan	31.1	40.1	35.6	0.05
Mon	14-Jan	26.3	32.3	29.3	--
Tues	15-Jan	24.7	29.5	27.1	0.01
Wed	16-Jan	14.7	33.0	23.8	--
Thu	17-Jan	20.0	34.7	27.3	0.02
Fri	18-Jan	16.0	25.7	20.8	--
Sat	19-Jan	2.7	20.3	11.5	--
Sun	20-Jan	1.7	12.4	7.1	--
Mon	21-Jan	4.2	23.1	13.6	--
Tues	22-Jan	16.0	28.4	22.2	--
Wed	23-Jan	11.0	17.9	14.5	--
Thu	24-Jan	5.4	19.6	12.5	--
Fri	25-Jan	2.0	18.9	10.4	--
Sat	26-Jan	13.9	24.5	19.2	--
Sun	27-Jan	14.8	28.9	21.9	--
Mon	28-Jan	14.0	44.5	29.2	0.18
Tues	29-Jan	27.8	46.6	37.2	0.40
Wed	30-Jan	6.7	27.9	17.3	0.01
Thu	31-Jan	8.6	24.2	16.4	--
Fri	1-Feb	20.0	28.7	24.3	--
Sat	2-Feb	24.4	28.8	26.6	--
Sun	3-Feb	27.3	30.4	28.9	0.06
Mon	4-Feb	27.7	40.5	34.1	0.20
Tues	5-Feb	31.7	45.7	38.7	0.03
Wed	6-Feb	24.0	32.2	28.1	--
Thu	7-Feb	18.3	28.9	23.6	--
Fri	8-Feb	24.7	32.0	28.4	--
Sat	9-Feb	26	34.6	30.3	0.04
Sun	10-Feb	-4.3	26.7	11.2	--
Mon	11-Feb	-5.4	11.7	3.2	--
Tues	12-Feb	0.3	14.9	7.6	--
Wed	13-Feb	0.3	23.5	11.9	0.01
Thu	14-Feb	13.4	33.2	23.3	0.01
Fri	15-Feb	13.1	28.8	20.9	--
Sat	16-Feb	8.5	28.4	18.5	--
Sun	17-Feb	20.4	46.3	33.3	0.74
Mon	18-Feb	13.1	33.8	23.5	--
Tues	19-Feb	7.8	18.9	13.3	--
Wed	20-Feb	4.4	18.8	11.6	--

Thu	21-Feb	-0.9	21.2	10.1	--
Fri	22-Feb	10.3	29.0	19.7	0.01
Sat	23-Feb	3.3	30.5	16.9	--
Sun	24-Feb	10.1	34.6	22.3	--
Mon	25-Feb	23.6	31.1	27.4	--
Tues	26-Feb	13.7	30.8	22.3	--
Wed	27-Feb	8.0	21.1	14.5	--
Thu	28-Feb	10.2	24.1	17.1	--
Fri	29-Feb	15.8	34.2	25.0	0.13
Sat	1-Mar	15.8	31.7	23.7	0.01
Sun	2-Mar	23.0	41.9	32.5	--
Mon	3-Mar	28.5	54.0	41.3	0.09
Tues	4-Mar	20.6	29.0	24.8	--
Wed	5-Mar	18.8	36.6	27.7	0.06
Thu	6-Mar	23.4	35.1	29.2	--
Fri	7-Mar	18.8	25.8	22.3	--
Sat	8-Mar	11.2	25.5	18.4	--
Sun	9-Mar	11.7	31.4	21.5	--
Mon	10-Mar	19.3	35.3	27.3	--

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