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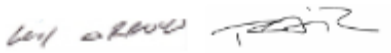

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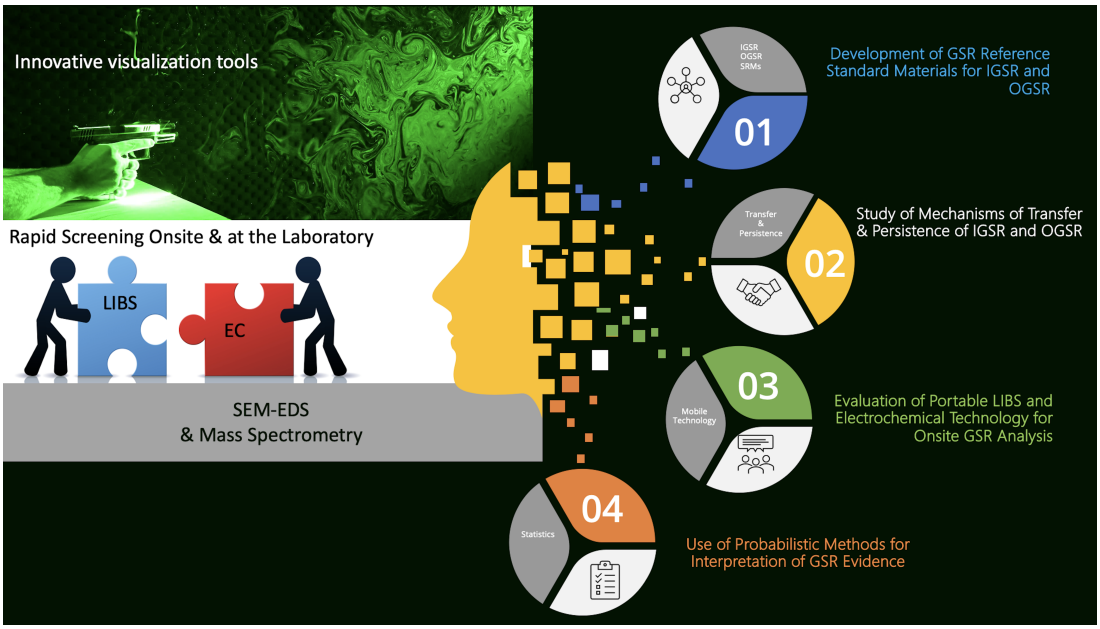

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Comprehensive Assessment of Novel Reference Standard Materials and Analytical Methods for the Analysis and Interpretation of Organic and Inorganic Gunshot Residues

I SUMMARY OF THE PROJECT

1.1. Abstract

Classic inorganic gunshot residue analysis (IGSR) relies on standardized protocols that offer confidence to practitioners when conducting these examinations. However, the evolution of ammunition to greener alternatives and technological advances are changing the identification and interpretation paradigms. Organizations like NIST/OSAC and NIJ/TWG have reported relevant gaps in this field. For instance, the development of technologies that offer extended detection to include non-traditional organic markers (OGSR). Also, access to reference materials can lead to harmonized QC policies across laboratories. Finally, there is a demand for interpretation models that incorporate analytical data, collection, deposition, and persistence information to assess the weight of the evidence. This study aims to address those priorities by developing solutions that increase the reliability and efficiency of GSR examinations.

The overarching goal of this study was to build capacity with emerging methods and standard materials and increase the quality and usage of data. This was accomplished through four specific goals. First, to design characterized organic and inorganic GSR reference standards representative of modern ammunition for research and crime laboratories. This tailor-made standard was created for QC, validation of existing and new methods, and interlaboratory testing. Second, to develop novel routes for studying the transfer and persistence of IGSR and OGSR using our tailor-made standard. To this end, a known number of characterized GSR particles were deposited on over 600 specimens under systematic and controlled conditions to evaluate different factors that affect GSR retention (e.g., activities, time, ammunition, and clothing types).

Third, to compare the performance and cost-efficiency of portable and bench-top LIBS and electrochemical systems, using over 1000 authentic GSR samples and standards, through a strategic collaboration with industry and practitioners. The portable instrumentation expands the utility of the methods to an on-site testing platform currently unavailable in the field while potentially transforming case management and decision-making processes. Finally, this study applied statistical methods for interpreting GSR evidence considering probabilistic approaches and Bayesian networks, using the data from an extensive population database. This work addressed several needs in the field and responded to more than one of the national priorities identified by NIJ: a) educating and training a future workforce, b) transferring technology from laboratory to marketplace, and c) partnering with industry and academia. The findings of this study provide a robust platform for training the next generation of forensic scientists and for future applications to the collection, examination, and interpretation of evidence in the criminal justice system.

1.2. Major Goals and Objectives

This research aims to develop a comprehensive strategy to enhance the reliability of gunshot residue analysis and interpretation. Our approach involves the development of novel laboratory reference materials and accurate, cost-effective, and rapid methods for examining and interpreting inorganic and organic gunshot residues. We proposed to accomplish our overall goal through four interconnected specific objectives:

Objective 1—To develop chemically and morphologically characterized GSR reference standards that can be used for research and in crime laboratories for the examination of IGSR and OGSR. The tailor-made standard will be designed for many applications, including quality control during GSR analysis, validation of existing and novel methods, interlaboratory testing, and systematic transfer and persistence studies.

Objective 2—To develop novel mechanisms for the study of the transfer and persistence of IGSR and OGSR evidence using our tailor-made characterized standards, providing a known number of IGSR particles and OGSR compounds with known composition at the time of deposition.

Objective 3—Evaluate and compare the performance and cost-efficiency of portable and bench-top LIBS and EC units for the detection of GSR, using authentic samples and the in-house GSR standards.

Objective 4—Apply statistical methods for the probabilistic assessment and interpretation of GSR evidence considering information at the source and activity level.

These objectives were accomplished through the following specific tasks:

Task 1 (Objective 1) —To develop, characterize, and validate organic and inorganic GSR reference standards via a multiple-technique approach and interlaboratory studies.
Task 1.1: To develop a mixed microparticle IGSR and OGSR reference standard.
Task 1.2: To test the stability and collection efficiency of the developed IGSR and OGSR standards.
Task 1.3: To evaluate the stability and efficiency of fluorescent taggants on IGSR and OGSR standards.
Task 1.4. Interlaboratory exercises using the newly developed OGSR-IGSR standard mix.

***Task 2 (Objective 2*)—**To develop systematic methods for the transfer and persistence of gunshot residues using in-house OGSR & IGSR microparticle standards.
Task 2.1. To assess the effect of activity on the transfer and persistence of IGSR and OGSR on hands.
Task 2.2: To evaluate the deposition, transfer, and persistence of GSR on hands over time.
Task 2.3: To determine the influence of fiber type on the persistence of IGSR and OGSR on clothing substrates.

Task 3 (Objective 3)— Testing of portable LIBS and EC units for the detection of GSR at the crime scene and comparative studies of cost-efficiency and reliability of bench-top and portable units.
Task 3.1. Comparison of the performance of bench-top LIBS and ECD instruments to the portable version of the instrumentation using the OGSR-IGSR standard mix and authentic samples.
Task 3.2. Testing of the portable and bench-top methods at mock crime scenes.

Task 4 (Objective 4)— To apply data from population datasets and transfer and persistent studies in the assessment of a probabilistic approach for the interpretation of GSR evidence.

DISCLAIMER: This report summarizes the main findings of this research project. Some of these findings have been published in scientific journals,[1-11] thesis, or dissertations. [12-15] Therefore, some content, tables, and figures are an adaptation of published articles. Per journal copyright policies, the authors are entitled to re-use portions, excerpts, and their figures or tables in other works not published commercially without permission or payment (with full acknowledgment of the original article). More detailed information about the methods, data analysis, and results can be found in the published manuscripts listed in Section 3.1 and cited in the respective sections of this document.

1.3. Research Questions and Hypothesis

With over three hundred thousand shooting incidents a year, gun violence affects the United States society with figures just comparable to motor vehicle accidents, suicide, and drug overdose deaths. Assessing relationships between an individual of interest and the activities and circumstances surrounding the case is of utmost importance to these firearm-related cases. The identification of gunshot residues (GSR) can provide valuable leads to this matter. However, despite existing reliable and standardized methods for GSR analysis, the field still faces several challenges. For instance, the turnaround time required to produce a forensic report is lengthy, and unlike most forensic disciplines, there are no screening methods routinely used for rapid field and laboratory GSR testing.

On top of that, the increased occurrence of heavy metal-free (HMF) ammunition in casework requires adjustments to current methodologies. Also, more overarching interpretation approaches are needed to contend with the complex mechanisms of transfer and persistence of GSR. Therefore, it is not surprising that diverse expert groups, such as the NIST/OSAC and NIJ/TWG, have called the forensic community to address priority research needs. In particular, these groups have identified gaps in evaluating complementary analysis methods, establishing adequate quality assurance and interlaboratory testing, and conducting comprehensive studies of GSR background occurrence, transfer, and persistence.

Our ultimate goal is to develop solutions that can respond to these needs, providing valuable data and a streamlined process that leads to fewer errors and delays. We propose to do that by building capacity and using emerging methods and standards that can increase the data quality and, most importantly, the usage of the data for increased impact. Also, we are committed to making those methods cost-

effective and practical so they can be accessible, easy to maintain, and provide rapid feedback to the users.

The central hypothesis of this project is that the combination of versatile characterized reference materials and fast data acquisition methods will produce a breakthrough in knowledge on GSR transfer and persistence and allow for a thorough assessment of the use of organic gunshot residues in the forensic interpretation of evidence. Also, the reference standards are compatible with the sampling methodology and analysis currently used at forensic laboratories (i.e., SEM-EDS collection stubs), and it is anticipated to enhance current capabilities across forensic and research institutions.

Our hypothesis and experimental framework are based upon extensive preliminary work conducted by our team through a first-phase funded effort (NIJ-2018-DU-BX-0186). Our team has developed a comprehensive population dataset of IGSR and OGSR markers found on the hands of individuals who have not fired a gun, including those with a low risk of exhibiting exogenous GSR-like residues and those with higher chances of producing false positives. The dataset also includes results from the hands of individuals who have recently fired typical leaded and heavy-metal-free (HMF) ammunition and those who have done several activities after shooting. This encompassing population contains data from different methods, including Scanning Electron Microscopy-Energy Dispersive X-ray Spectroscopy (SEM-EDS), Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS), Laser-Induced Breakdown Spectroscopy (LIBS), and Electrochemical Sensors (ECD), serving as a critical foundation for this study.

LIBS and EC methods have shown an unprecedented speed of analysis and accuracy without substantially sacrificing the integrity of the sample and, therefore, providing a unique alternative for reliable screening. Moreover, the development of GSR-characterized standards opens unique opportunities to investigate modern ammunition and the composition, behavior, and informative value of inorganic and organic GSR compounds. Additionally, a vital aspect of this research is evaluating the probabilistic interpretation of the weight of GSR evidence.

As a result, this study was designed to answer fundamental questions to build stronger scientific foundations of GSR examinations and provide the criminal justice system with reliable resources to assess the relevance of the evidence.

1.4. Research Design, Methods, Data Analysis

1.4.1. Research design and methods of analysis

Project tasks and methodology

The methodology and experimental design are described below within four major tasks to address the major objectives of this study (See **Figure 1).**

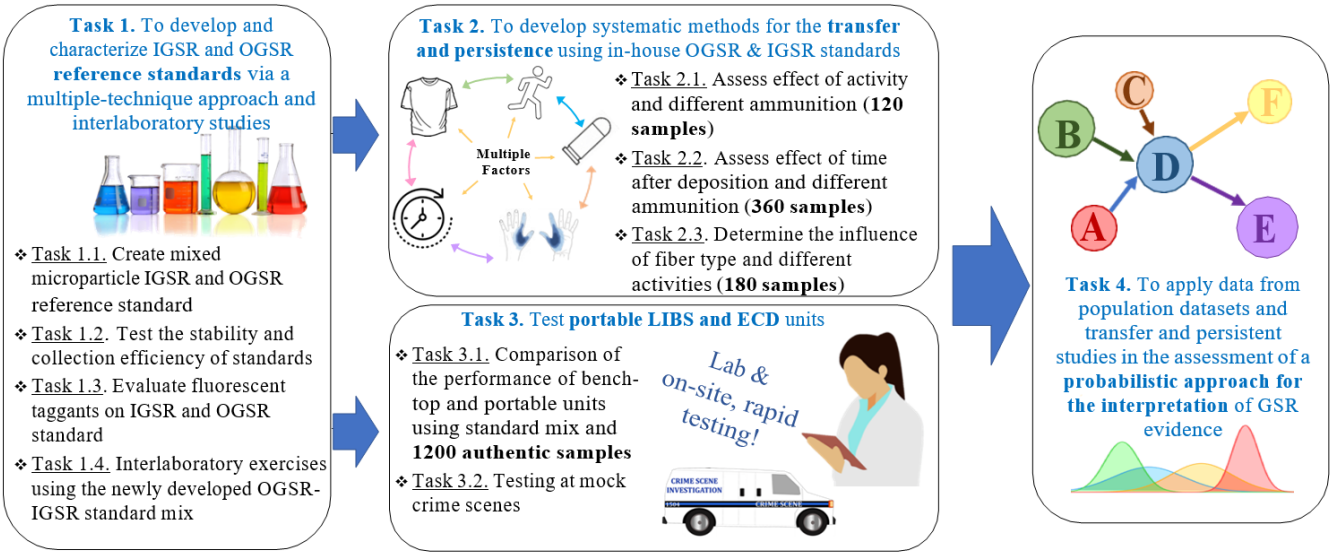


Figure 1. *Summary of the main four experimental research tasks.*

Task 1 (Objective 1)— To develop, characterize, and validate organic and inorganic GSR reference standards via a multiple-technique approach and interlaboratory studies.

This task validated IGSR and OGSR standard materials that served as a basis for other tasks in this study (**Figure 1**). Our research group at WVU developed in-house standards composed of IGSR microparticles (NIJ fellowship # 2018-R2-CX-0009) that are fully characterized by SEM-EDS, ICP-MS, and LIBS.[11] The standards are created by firing a gun under controlled conditions using a cartridge containing only the primer. The micro-particles are collected in a Nalgene container and then suspended in an organic solvent, where they have been shown to remain stable for over a year.

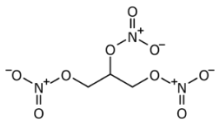
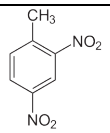
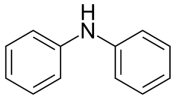
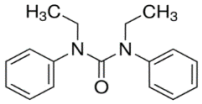
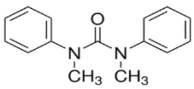
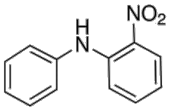
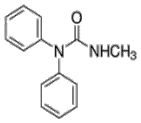
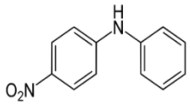
In this study, we expanded the characterization of in-house standards to include inorganic and organic compounds commonly found on modern ammunition (**Figure 2**). These materials can be used as the basis for quality control, method validation, and error rate studies, and they can be used to build knowledge of GSR transfer and persistence and are anticipated to fill a current gap of nonexistent standards. Indeed, the development and characterization of these standards addressed three research needs. First, this study provided QA/QC reference standards with a known number of characterized GSR particulates to evaluate instrument performance and comparability across examiners and laboratories. Second, the standard materials facilitated the comprehensive feasibility of OGSR analysis, including stability studies on sampling media, compatibility with GSR stubs, and transfer and persistence studies. Finally, the characterized standards allowed controlled studies of particle deposition and recovery efficiency and population studies for probabilistic assessments of GSR evidence.

Task 1.1: To develop a mixed microparticle IGSR and OGSR reference standard

The GSR standards were designed to be versatile, as they can be used in solution or dry forms and contain both inorganic and organic compounds representing leaded and HMF formulations. Each

microparticle stock solution is prepared as a 100 mL suspension/solution capable of producing 5,000 individual standards (each of 20 ul). We developed a 1 ppm organic gunshot residue standard containing 8 analytes of interest: including nitroglycerin (NG), 2,4-dinitrotoluene (2,4-DNT), diphenylamine (DPA), ethyl centralite (EC), methyl centralite (MC), 2-nitrodiphenylamine (2-NDPA), 4-nitrodiphenylamine (4-NDPA), and akardite II (AK II). These eight compounds were chosen for their functionality and popularity in modern smokeless powder formulations (See **Table 1**).

Table 1. *List of compounds of interest for this study that are commonly reported in propellant formulations, molecular structures, recorded weight, and primary function.*

Compound	**Molecular Structure**	**Molecular Weight**	**Main Function**
Nitroglycerin (NG)		227 g/mol	Explosive
2,4-Dinitrotoluene (2,4-DNT)		182 g/mol	Explosive/Plasticizer/Flash Suppressor
Diphenylamine (DPA)		169 g/mol	Stabilizer
Ethyl Centralite (EC)		268 g/mol	Stabilizer
Methyl Centralite (MC)		240 g/mol	Stabilizer
2-Nitrodiphenylamine (2-NDPA)		214 g/mol	Stabilizer
Akardite II (AK II)		226 g/mol	Stabilizer
4-Nitrodiphenylamine (4-NDPA)		214 g/mol	Stabilizer

The IGSR and OGSR standards were stored separately due to the chemical and physical properties of the inorganic and organic compounds. The IGSR microparticle standards were stored at room temperature in polypropylene containers, while the OGSR standards were stored at -26 C in glass containers. The standards could not be stored together as organic compounds will volatilize/evaporate

at room temperature over time, and the inorganic particles have been shown to absorb into the sides of the glass containers. However, we developed an approach to combine them in situ. The deposition of IGSR and OGSR standards onto the sampling matrix occurs separately on the analysis day. This is to avoid immiscible interactions between the two solvent types and dilution of organic analyte concentrations when mixing the two standards. Multiple elemental techniques characterized the conventional (leaded) and HMF IGSR microparticle standards, including LIBS, SEM-EDS, and ICP-MS, for elemental composition, particle morphology, and particle count reproducibility (**Figure 2**). OGSR standards in ethanol, methanol, and acetone were characterized by GC/MS and LC/MSMS for the identification of OGSR compounds and the stability of their respective concentrations in each standard.

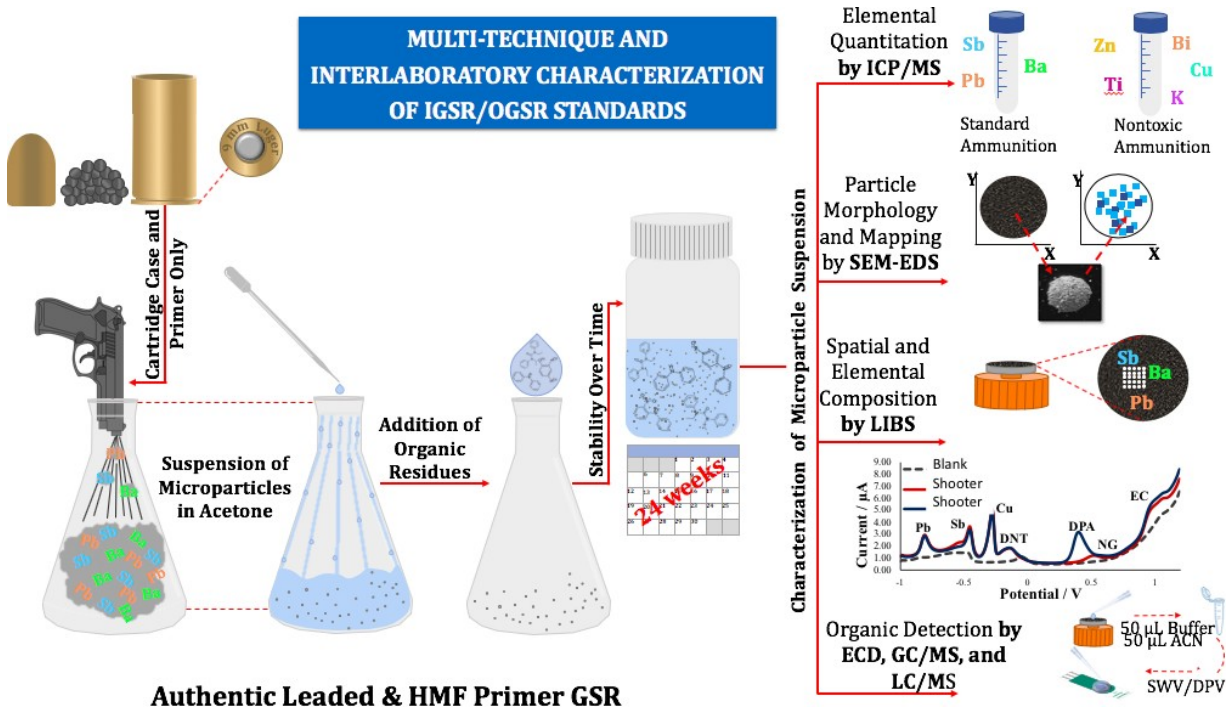


***Figure 2**. Standard IGSR/OGSR characterization approach.*

Task 1.2: To test the stability and collection efficiency of the developed IGSR and OGSR standard

Ruggedness Testing for Storage Conditions and Analysis of OGSR Standard

A ruggedness experiment assessed five factors at two levels each (**Table 2** and **Figure 3**) and their effect on the efficiency of quantifying the OGSR standard analytes over time. The factors were combined 16 times to achieve a statistical power of 94.9% and ensure a valid experimental design. A Plackett-Burman design was utilized, and after the critical factors were identified, the future method was designed to control these variables to improve analysis efficiency. Statistical analysis of the ruggedness experiment was performed using the concentrations of OGSR analytes reported by LC-MSMS as the response of interest. The experiment was repeated on OGSR standards on various solvents dissolved in acetone, ethanol, or methanol. The ruggedness testing was also used to assess

solvent effects on the eight OGSR analytes of interest. Statistical software, including JMP Pro 15.0.0 (SAS Institute Inc., NC), was used to conduct the assessment.

Table 2. *Factors and Levels Chosen for Plackett-Burman Ruggedness Experiment*

Factor	Low Level	High Level
Storage Seal	Regular Seal	PolySeal Cap
Temperature Equilibration time (minutes)	15	30
Dry down volume (µL)	0	50
Sample Reconstitution Method	Weight	Volume
Head Space Percentage (%)	10	25

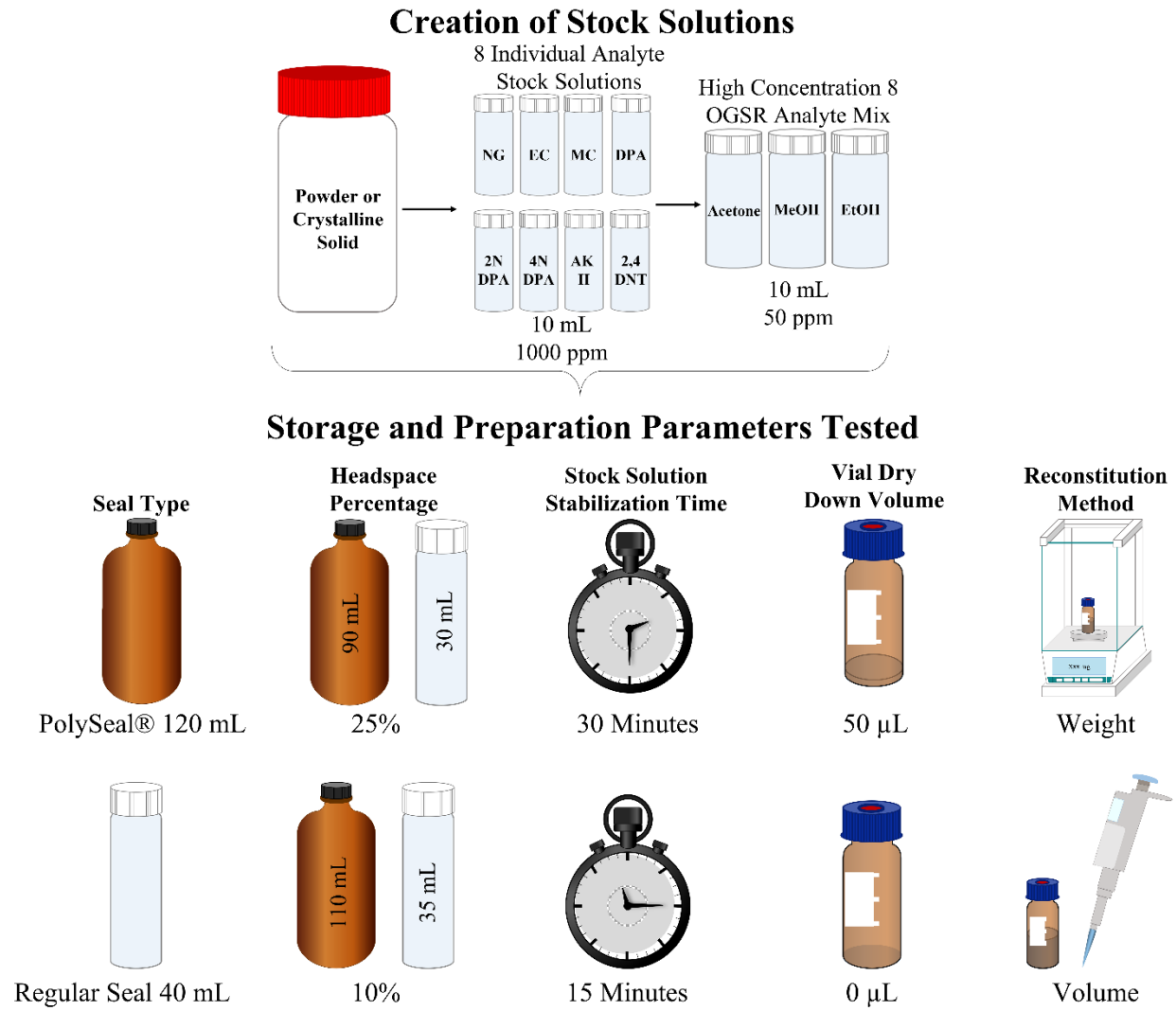


Figure 3. *Dilution scheme to create an eight (8) analyte OGSR standard and testing stability factors (solvent type, container type, free headspace, solution stabilization time to room temperature, dry-down method, and reconstitution method).*

Testing for Recovery Efficiency of OGSR Standard from GSR Carbon Adhesive and Synthetic Skin Membranes

A recovery testing was designed for the methanol-OGSR standard on carbon stubs and synthetic skin membranes. To perform the recovery studies, 100 µl of the 1ppm OGSR standard in methanol was deposited onto the matrix surfaces. Once dried, the residues were then extracted from the collection stub in two manners: (1) a washing procedure that included 6 x 50 µl methanol washes and (2) an exhaustive extraction that included placing the carbon adhesive in 500 µl of methanol and sonicating for 5 minutes.

All extractions were dried completely under a constant stream of N_2 before reconstitution and the addition of internal standard (D_{10}-DPA). This ensured no unintentional dilution of analytes in the sample, leading to more accurate recovery percentages.

Task 1.3: To evaluate the stability and efficiency of fluorescent taggants on IGSR and OGSR standard

In this study, we initially aimed to utilize fluorescent chemical taggants in the IGSR/OGSR standards to aid their visualization after deposition and provide a practical and complementary tool to transfer and persistence studies. An in-depth literature review evaluated multiple types of taggants that may or may not be used to visualize GSR. The focus was on taggants similar in chemical properties to inorganic and organic gunshot residues but did not directly interfere or react with the developed standards.

We explored the addition of fluorescent powders into the ammunition to visualize the post-shooting GSR depositions. Our collaborators at NIST conducted a preliminary test by adding 10 to 20% fluorescent powder to standard propellant. GLO-GERM powder was selected as the fluorescent material because of its non-toxic nature, as it is used as a training aid to determine hand washing, cross-contamination avoidance, and surface cleaning effectiveness, specifically to avoid transmission of microbes. The modified ammunition was then fired using a mount in the ballistic laboratory, and the GSR plume was visualized using a high-speed camera and laser-lighting set-up. However, significant changes in the deflagration and combustion were observed, preventing the cartridge from being ejected from the barrel. This may create safety issues, so a modified strategy was designed.

As an alternative approach, we used particle counters after firing regular ammunition to estimate particle size distribution over time in the vicinity of the firing while using laser sheets and high-speed video cameras to capture particle dynamics.

Instrumentation

Custom-made particle counters were built from a model PMSA003 (Plantower Technology, Jiangxi Provence, China) atmospheric sampler (designed by Matt Staymates, NIST). Each was attached to an Arduino microcontroller (Arduino, MA, USA) to allow for communication with a computer, as shown in **Figure 4.** An APS 3321 aerodynamic particle sizer (TSI Incorporated, Shoreview, MN, USA) was used to provide additional information regarding particle counts and sizes. An in-house made laser sheet was created by attaching a refractive element before a green laser beam. This served to spread the beam in the vertical direction while constraining its width. Combined with a dark ballistics room, this allowed for vision only in a thin slice of the room's area. A high-speed camera, Photron Fastcam NOVA-S9 (Photron, Tokyo, Japan) was used to record video at 3000 frames per second. A standard video camera was also used to record lower refresh rate video with color.

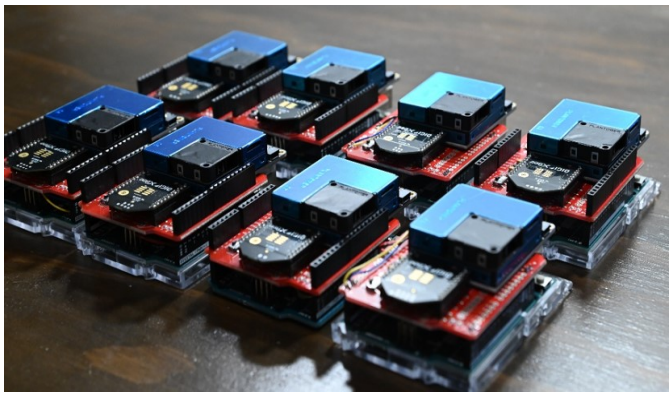


Figure 4. Custom-made particle counting devices. Photograph courtesy of Matthew Staymates

Ammunition, Firearms, and Setup

All shooting experiments took place at WVU's indoor ballistics facility. Firearms include a Springfield® XD-9 9 mm pistol (SP) and a Taurus® 905 9 mm revolver (RV). In this study, only factory-loaded Winchester® 9 mm ammunition was used. A shooting rest 56 inches high was constructed to provide a repeatable position from which the operator could fire. The positions of the particle counters and APS are shown in **Figure 5**.

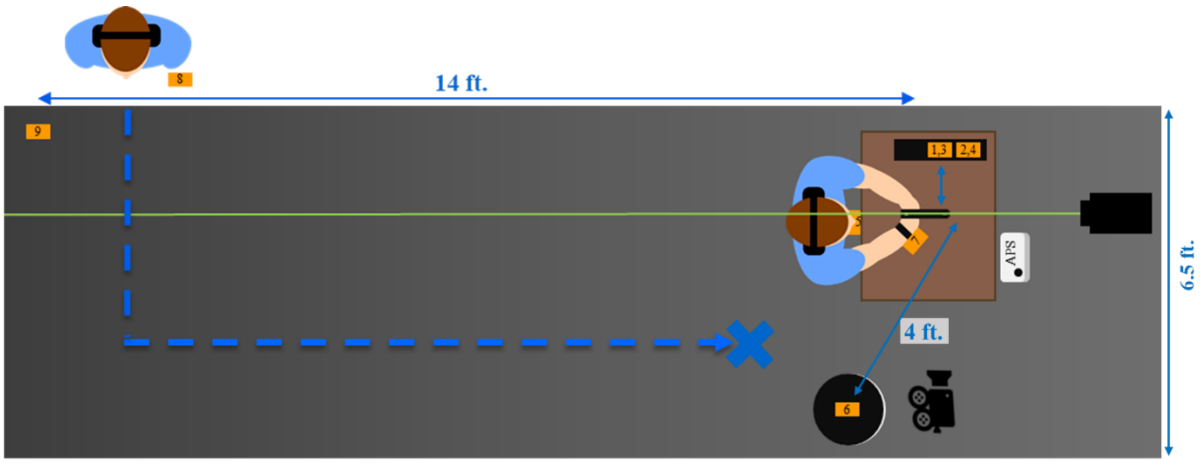


Figure 5. A diagram of the top-view of the experimental setup utilized within WVU's indoor ballistics facility, indicating the position of the nine custom-made sensors, the APS system, the video cameras, and the laser. Each number in orange square indicates the location of one particle sensor.

Figure 6. *A diagram showing the positions of each type of collection device in this study.*

The collection apparatus shown in **Figure 6** was constructed to allow users to carry multiple collection equipment simultaneously. SEM stubs with carbon adhesive tape were placed into holders in four locations on the apparatus. These stubs would remain in place following a shooting event, allowing for passive collection (deposition) of both IGSR and OGSR. LC-MS/MS and SEM-EDS analyzed the stubs. One stub (analyzed by LC-MS/MS) had a small section of STRAT-M synthetic skin adhered to the carbon tape to mimic deposition on skin. In addition to the passively collecting stubs, three carbon adhesive stubs were placed 2mm away from the outlet of the custom-made particle counters. These are designated preconcentrated stubs and allow for capturing particles that exit from the particle counters. This aimed to ensure that the particles counted within the counters were GSR by confirming their elemental profiles with SEM-EDS.

Experimental Methods

Four types of samples were collected in this pilot study. These include one shot fired from the pistol (SP1), one shot fired from the revolver (RV1), and five shots fired from the pistol (SP5). To collect a sample, it was ensured that the air-cleaning ventilation system in the range was turned off and remained off for at least two minutes. The substantial effects of the ventilation system can be seen in **Figure 7.** Then, the custom-made particle counters were turned on, and data collection began. At this point, the shooter entered the shooting position and aligned the firearm with the laser sheet, and an operator began recording with the camera and APS software. Once ready, the shooter was instructed to fire.

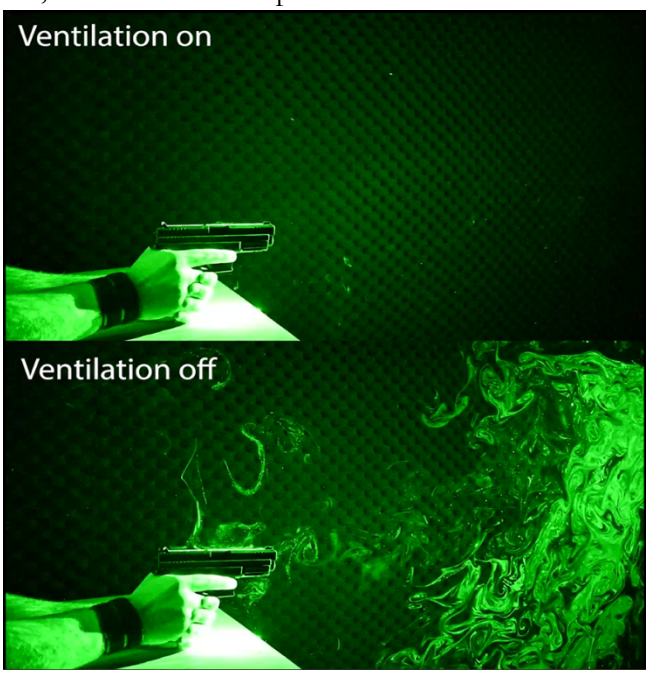


Figure 7. A comparison between particles observed 5 seconds

After firing the corresponding number of shots to the sample type, the shooter remained in place for 2 minutes, at which time the bystander entered the room with sensor 8. The bystander remained in place behind the shooter for approximately 60 seconds. The recording was stopped, and the air purification system was turned back on to purge the room of the suspended GSR. Six replicates were performed for SP1 analysis. Three replicates were performed for both RV1 and SP5 analyses.

Additionally, the utility of the custom-made atmospheric samplers for preconcentration was tested. By attaching carbon adhesive

traditionally used in SEM-EDS analysis to the outlet of the atmospheric sampler, we can capture escaping particles via adhesion to the surface. This was then placed onto an aluminum stub and analyzed by SEM-EDS.

Experiment 1: GSR Settling

Three types of experiments were performed in this study. First, an extended sampling experiment was conducted to determine the settling rates of GSR particles following a shooting event. In this experiment, a wooden tower with sensor holders separated at equal distances was set up. The APS was positioned directly beside the shooting position (within 6 inches of the firearm barrel). The APS was programmed to record sections of data in one-minute intervals for five hours. The particle counters were activated and allowed to record for the same length. The shooter fired one shot, began recording data, and immediately left the range. The door was closed and locked, and the range was undisturbed for five hours. Two trials were performed with particle counter data collection, but only one included APS data collection, as the APS encountered an error during collection.

Experiment 2: Link Between Sensors and Particles

The setups shown in **Figures 5** and **6** were used in the second type of experiment. Upon firing by the shooter, data collection with the APS and particle counters began. After five minutes, the door to the range was slowly opened to allow light and passive airflow to simulate a scenario in which a passerby opens the door to enter a room. After an additional five minutes, the passerby entered the room and remained for five minutes. Finally, the air purification system in the range was turned on to prepare for the next sample by removing airborne GSR. A total of five trials were performed in this experiment.

Experiment 3: OGSR Deposition

In the third type of experiment, the passive collection of OGSR was repeated. This study included sampling from five locations: the vicinity of a shooter directly beside the firearm, the shooter's hand, a bystander, and a passerby. Two collection devices were used: an SEM stub with carbon adhesive and STRAT-M synthetic skin. In addition to the passive collection stubs, hand samples were taken from the shooter, bystander, and passerby. A timer was initiated upon firing. After 5 minutes had passed, the door to the range was opened, and a passerby entered after another five minutes. Finally, the collection was concluded after a total of 15 minutes had passed. A total of five trials were performed in this experiment. Samples from this experiment were analyzed by LC-MSMS and SEM-EDS.

Sample Processing

Aluminum stubs with carbon adhesive designated for LC-MS/MS analysis were extracted by depositing six aliquots of 50 μL MeOH onto the stub. Each aliquot was deposited and withdrawn six times to ensure effective extraction of the stub. This extract was filtered through a 0.22 μm microcentrifuge filter and dried under a steady stream of N_2. Finally, this was reconstituted with 50 μL of MeOH with 0.1% FA and 150 ppb D_{10}-DPA (internal standard) for analysis. Aluminum stubs with synthetic skin were extracted by removing the synthetic skin with a clean pair of tweezers. This was cut into 10 small sections and submerged in 500 μL of methanol. The exhaustive extraction was finished by sonicating the mixture for five minutes. This extract was removed, dried under a steady stream of N_2, and reconstituted with 50 μL of MeOH with 0.1% FA and 150 ppb D_{10}-DPA (internal standard) for analysis.

Data Analysis Methods

Particle counter data was recorded with an in-house R code and exported as a Microsoft Excel® file. APS data was recorded using Aerosol Instrument Manager® software (TSI Incorporated, Shoreview, MN, USA). Particle counts and sizes were then exported as a text file to Microsoft Excel® for further analysis. After processing, statistical analysis was performed on various values using JMP Pro® statistical software version 16.0.0. LC-MS/MS data analysis was performed using MassHunter Qualitative Analysis 10.0.

Task 1.4. Interlaboratory exercises using the newly developed OGSR-IGSR standard mix

Interlaboratory study for an in-house IGSR standard by SEM-EDS

An interlaboratory study was conducted with the WVU research group and the Sacramento County District Attorney's Crime Laboratory (SCDACL) to assess the reproducibility of deposited particles from the pGSR standard. This study aimed to evaluate the sources of variability that could be introduced into the particle counts of the standard deposits on SEM-EDS stubs. First, the deposition and drying process required small volumes. Two microliters of the pGSR standard were deposited on carbon stubs, then dried down and reconstituted. Although these steps were carefully controlled, the random suspension of particles in small volumes can produce a variation in the number of deposited GSR particles. Second, the instrumental parameters that map the particles under SEM-EDS can affect the particle counts. Thus, we aimed to assess the uncertainty associated with our standards when analyzed at different labs. Third, SEM-EDS is often optimized at crime labs for standard leaded ammunition, with heavy elements such as Pb, Ba, Sb, so we wanted to explore how reproducible particle counts on standard pGSR are compared to more challenging lead-free formulations.

The interlaboratory study consisted of SEM-EDS particle count analysis on two pGSR standards, INC and WIN (ID 18MAY21), each deposited in three locations within a carbon stub. Each laboratory was asked to measure each spot three times in a single day and repeat the experiment on three different days. Although there are inherent differences among the instrumental configurations, participants were given specific parameters for the instrument settings, such as probe current, magnification, and recipe setup. Intraday variability was evaluated when the triplicate deposits on the same stub were run in one day, and interday variability assessed the reproducibility of the same deposited spot (either A, B, or C) on different days.

Interlaboratory study for an in-house IGSR standard by LC/MSMS

An interlaboratory study was conducted between West Virginia University (WVU) and the National Institute of Standards and Technology (NIST). The purpose of the inter-lab study was to evaluate the reproducibility of the in-house OGSR standard. Reproducibility was evaluated by comparable composition and concentration of organic analytes in a 1 ppm standard mix suspended in methanol. The analytes of interest were akardite II, diphenylamine, 2-nitrodiphenylamine, 4-nitrodiphenylamine, ethyl centralite, and methyl centralite. This inter-laboratory study aimed to determine if concentrations were reproducible between different analysts, laboratories, and instruments. An instruction sheet was provided to NIST indicating the samples that needed to be run, WVU's ESI source conditions, and mass spectrometer parameters. The OGSR standard was run in triplicate, along with a nine-point calibration curve, within the same day.

The instrument at WVU was an Agilent 1290 Infinity II equipped with a pentafluorophenyl (PFP) Poroshell® 120 column (2.7 mm 2.1 x 50 mm) and coupled to a 6470-triple quadrupole (QQQ) mass analyzer. Mobile phase solvents consisted of H_2O with 0.1% FA (A) and ACN with 0.1% FA (B), with a flow rate of 0.350 mL/min. Initial mobile phase conditions were set to A-80%/B-20% and

transitioned to A-5%/B-95% over 10 minutes. Optimized source conditions and collision cell conditions are listed below in **Tables 3 and 4**.

Table 3. *Source Parameters for LC-MS/MS Detection of Organic Gunshot Residue Compounds at West Virginia University*

Parameter	Measure
Source Type	ESI
Dry Gas Flow	10 L/min
Dry Gas Temperature	300°C
Sheath Gas Flow	7 L/min
Sheath Gas Temperature	250°C
Nebulizer Pressure	20 psi

Table 4. *Collision Cell Parameters for LC-MS/MS Detection of Organic Gunshot Residue Compounds at West Virginia University*

Compound	Precursor Ion	Product Ion	Dwell	Fragmentor	Collision Energy	Accelerator Voltage
EC	269	148	50	96	12	3
EC	269	120	50	96	25	3
MC	241	134	50	100	16	3
MC	241	106	50	100	32	3
AK II	227	170	50	96	16	3
AK II	227	168	50	96	24	3
AK II	227	92	50	96	32	3
4NDPA	215	198	50	90	10	3
4NDPA	215	167	50	90	50	3
2NDPA	215	180	50	100	16	3
DPA	170	98	50	120	32	3
DPA	170	66	50	120	56	3
DPA	170	65	50	120	32	3
D_{10}-DPA	180	98	50	120	52	3
D_{10}-DPA	180	71	50	120	60	3

The instrument at NIST was a Thermo ABSciex 4000 equipped with a pentafluorophenyl (PFP) Poroshell® 120 column (2.7 mm 2.1 x 50 mm). Mobile phase conditions and intervals were also kept the same as WVU. However, the ABSciex operates with a different model of ESI source. The Optimized source and collision cell conditions used at NIST are seen below in **Tables 5 and 6**.

Table 5. *Source Parameters for LC-MS/MS Detection of Organic Gunshot Residue Compounds at NIST*

Parameter	Measure
Source Type	ESI
ESI Voltage	(+)5000
Desolvation Temp (∘C)	150
Nebulizer Gas (psig)	30
Turbo Gas (psig)	30
Curtain Gas (psig)	10

Table 6. *Collision Cell Parameters for LC-MS/MS Detection of Organic Gunshot Residue Compounds at WVU*

Compound	Precursor Ion	Product Ion	Dwell	Declustering Potential	Collision Energy	Accelerator Voltage
EC	269.2	148	200	70	20	4
		120	200		33	4
MC	241.2	134	200	70	24	4
		106	200		36	4
AK II	227.1	170	200	90	27	4
		92	200		36	4
4NDPA	215.1	198	200	60	18	4
		167	200		47	4
2NDPA	215.1	197	200	80	14	4
		180	200		23	4
DPA	170.1	93	200	100	32	4
		66	200		58	4
D_{10}-DPA	180.1	98	200	110	32	4
		70	200		58	4

Interlaboratory Study for the Electrochemistry Method

An interlaboratory study was conducted between West Virginia University and the New Jersey State Police Office of Forensic Sciences (NJSP OFS). WVU Graduate Research Assistant Kourtney Dalzell visited the NJSP OFS for two weeks to perform on-site training and validation on the portable electrochemical unit, including training on electrochemistry theory, sample preparation, software, and data analysis. Validation was completed by analyzing calibration curves, quality control mixtures, pGSR/OGSR standards, and authentic shooter and background population collected at the NJSP OFS. Calibration curves were completed using individual stock solutions to create 5-point curves in triplicate on screen-printed carbon electrodes.

Task 2 (Objective 2)—To develop systematic methods for the transfer and persistence of gunshot residues, using in-house OGSR & IGSR microparticle standards

Existing transfer and persistence methods rely on the identification of IGSR particles after firing a gun. Most of these studies use SEM-EDS methods to count the number of particles present on the matrix of interest. However, once these particles are collected on a carbon stub, they cannot be redeposited on the hands or material under study. As such, these studies don't have a ground truth of the number of particles or compounds deposited at time zero and how many persist (e.g., once the shooting occurs). For example, the percent loss of particles is often estimated from comparing particle counts from repeated firings at time zero versus collections done after the individual is engaged in a post-shooting activity. The challenge with these types of studies is that the deposition of GSR is a highly variable process, and uncertainty in these estimations are considerable. Moreover, only a limited number of research studies have investigated the transfer and persistence of OGSR. In contrast, the in-house IGSR and OGSR microparticle standards developed here can provide a unique opportunity

to conduct transfer and persistence studies of inorganic and organic compounds in a controlled and systematic fashion.

The transfer and persistence of inorganic and organic gunshot residues were evaluated in two ways: (1) using authentic shooter samples on human skin from the shooters and (2) using in-house pGSR and OGSR standard solutions on a synthetic skin membrane (StratM®). A synthetic skin membrane was used in this study as a skin substitute to avoid potential safety risks associated with applying organic materials to human skin. The StratM®, initially developed for dermatology and pharmaceutical applications, had three layers. The top layer of the membrane was a tightly packed polyether sulfone (PES) that mimicked the texture and absorption properties of the stratum corneum. The "dermis" layer was less compact PES, and the final layer was non-woven polypropylene fibers that simulated the hypodermis and subcutaneous fat layer. Although the thickness of the synthetic membrane was not identical to human skin, the layer structure, ratio, and composition provided realistic interactions with organic and inorganic substances. All shootings for transfer and persistence were performed at West Virginia University Ballistics lab with standard and non-toxic ammunition.

Materials, methods, and experimental design

The pGSR standard was created by discharging a cartridge case containing only the primer into a polypropylene Erlenmeyer flask. The authentic gunshot residue particles were collected from the flask through suspension in acetone and characterized by various analytical methods. This study's pGSR standard of choice was created from leaded primers containing major inorganic gunshot residue elements of Pb, Ba, and Sb. The OGSR standard contained eight analytes previously described in methanol at a concentration of 5 ppm. It should be noted that the two standards were stored separately due to constraining conditions. For example, the pGSR standard could not be stored in glass containers due to the leaching and adsorption of the metals. In contrast, the OGSR standard could be stored in polypropylene containers because of potential evaporation. The OGSR standard was kept below 0°C to prevent evaporation, while the pGSR standard was stored at room temperature. Therefore, both standards were stored separately and only combined upon deposition onto the substrate.

Authentic shooter samples were collected at West Virginia University's ballistics laboratory and shooting range using a 9mm Springfield XD9 pistol. Ammunition used in this study consisted of Starline brass cartridge cases loaded with CCI Magnum leaded primers, Winchester 231 smokeless powder, and Speer (9 mm) total metal jacketed bullets. Participants discharged five consecutive shots and held their shooting position for two minutes after the final firing to achieve a consistent settling time of gunshot residues. During this time, airflow in the range was turned off to simulate a more realistic enclosed environment. After two minutes, the participants were escorted to the laboratory area, where they were seated at a designated location previously sanitized and layered with protective paper coverings. Three post-firing wait times were chosen to evaluate residue persistence: 0hr, 1hr, and 3hr. Zero-hour samples served as baseline concentrations of pGSR and OGSR. In contrast, samples collected after one hour and three hours were used to assess the loss of inorganic particles and evaporation of organic compounds over time. After the designated times, individuals were sampled from five areas: hands, nose, ears, forehead, and hair. Different-sized carbon SEM-EDS stubs were used depending on the collection location. Smaller 9 mm stubs were used for ears (1 stub for both ears; 10 dabs in each) and noses (1 stub for both nostrils; 10 dabs surrounding each nostril). Larger 12 mm stubs were used for hands (1 stub for both hands; ~15 stubs per side of the hand), foreheads (1 stub; 20 dabs over the area), and hair (1 stub; 20 dabs covering up to 2 inches behind the hair line). Designated collectors sampled the participant's hands, and then the participants were

responsible for sampling the other locations using a mirror for reference. After complete sampling, participants were asked to wash their hands with soap and water and remove residues from other areas using DI water, disposable tissues, cotton pads, and cotton swabs. This study collected and analyzed a total of 107 hand samples and 240 nose, ear, forehead, and hair samples (60 each).

Samples were either analyzed for organic GSR by LC-MS/MS or inorganic GSR by SEM-EDS. For organic analysis, extractions were performed using 300 µl of methanol for 12 mm stubs and 150 µl for 9 mm stubs. Washes were performed consecutively on the stub surface in increments of 50 µl, with each aliquot transferred to an LC-MS/MS vial. Extracts were entirely dried under a constant stream of nitrogen before reconstitution with methanol (0.1% formic acid) and an internal standard of D_{10}-DPA. No additional sample preparation was needed for samples analyzed by SEM-EDS. Due to the lengthy SEM-EDS analysis times, only a subset of samples was analyzed by this technique.

Transfer and Persistence Studies using StratM® Membranes and Fabric Samples

The standard solutions were deposited on substrates similarly—first, 20 µl of the 5 ppm OGSR standard on the sample. Once dried, 50 µl of the pGSR standard suspended in acetone was deposited on the same area. Persistence of pGSR and OGSR was evaluated on the StratM® at four different post-deposition times (0hr, 1hr, 3hr, 6hr). Each time segment was run on separate days, with a subset of 0 hr samples to test inter-day variability of the deposition and recovery methods. After depositing the GSR standards, the membranes were left static in a clean room until the designated time had lapsed. One hundred twenty samples (30 samples per time) were collected for this portion of the study. Transfer studies were performed on both StratM® and fabric samples (405 total samples). A diagram outlining each of the activities performed can be seen in **Figure 8** below.

Transfer and Persistence Experimental Design using StratM® Membranes and Various Activities

The StratM® was subjected to one of three activities after depositing the GSR standards: rubbing hands, shaking hands, and washing hands. Additionally, 30 samples had no activity performed as baseline levels of particle counts and analyte concentrations.

(1) **Rubbing hands (30 sample sets)**: While applying pressure, two pieces of the StratM® pieces were rubbed together for 30 seconds. One membrane contained deposited OSGR/pGSR, while the other was pristine. Membranes containing GSR standards were assessed for loss of residues, while clean membranes were used to determine any secondary transfer of GSR constituents.

(2) **Shaking Hands (30 sample sets):** Membranes were attached to the webbed portion of two individuals' hands (gloved) with double-sided adhesive. While lining up the membranes, three firm handshakes were performed. The two membranes were used to assess loss and secondary transfer. Between sets, participants changed gloves to avoid cross-contamination.

(3) **Washing Hands (30 samples):** Membranes were washed by rubbing 30 µL of a 1:1 hand soap-to-water solution onto the surface for 30 seconds. After washing, the membranes were held at an approximate 30° angle and rinsed using 1000 µL of DI water. Before sampling, Kim Wipes were then gently dabbed onto the surface to remove excess water, like drying hands with a paper towel.

Transfer and Persistence Experimental Design using Fabric Samples

Three fabric types were tested to determine how fiber composition and construction affected the retention of gunshot residues after running, struggling, or fabric washing. The three fabric compositions tested were 100% cotton (weave), 100% polyester (knit), and a 35% cotton/65% polyester blend (weave). Fifteen sample sets were collected for each combination of fabric composition and activity. Like the membranes, 45 inactive samples (15/material) were collected to

serve as baseline controls.

(1) **Running (45 samples):** Fabric squares (2 cm x 2 cm) were attached to a piece of clean butcher paper using binder clips and adhered to the front and back of a participant's shirt. Once secured, the individual jogged around the lab for 60 seconds before sampling. The butcher paper was replaced between jogging sets to prevent contamination between samples.

(2) **Struggle (45 sample sets):** Two pieces of the same fabric composition were rubbed together for 30 seconds to simulate struggling during an arrest. One fabric contained the spiked pGSR/OGSR, while the other was pristine. Secondary transfer to the clean piece of cloth was also assessed during this study.

(3) **Fabric Washing (45 samples):** A washing solution was prepared by mixing 2.7 mL of Tide® free & gently laundry detergent with 2.1 L of DI water. This ratio was concurrent with recommended proportions for standard washing machines. The fabric pieces were placed into a beaker containing 40 mL of the detergent and agitated for thirty seconds by spinning a traditional cooking whisk. The fabric was then transferred to a second beaker containing clean DI water to represent a rinse cycle. Finally, the fabric was placed in a salad spinner for sixty seconds for the "dry" process. Between samples, washing liquids were replaced, and all materials were cleaned.

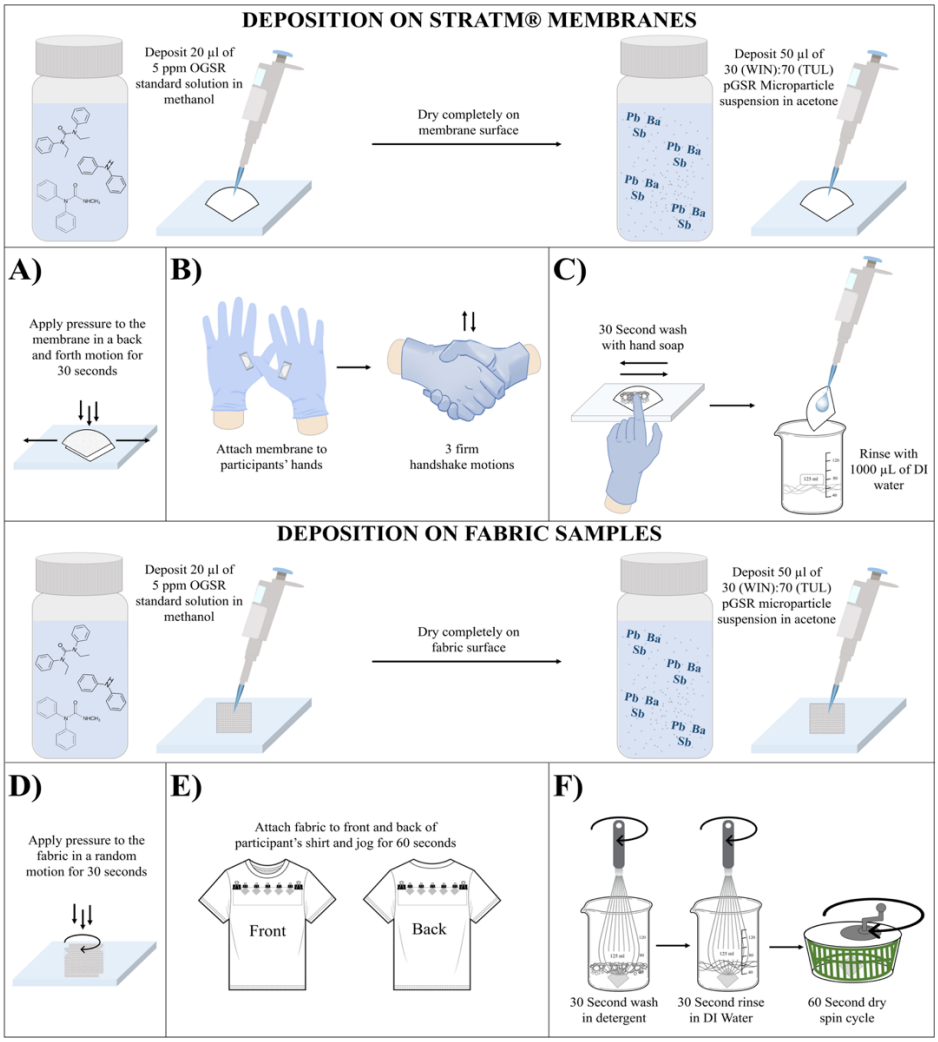


Figure 8. *Schematics showing procedures for simulated transfer studies for A) rubbing hands, B) shaking hands, C) washing hands, D) simulating struggle, E) jogging, and F) washing clothing. Samples were analyzed by SEM-EDS, LC/MSMS, ECD and LIBS.*

Extraction and Analysis Procedures of Transfer and Persistence Samples

All substrates collected during this portion of the study were sequentially extracted to gain inorganic and organic information from a single sample. The first step collected pGSR from the substrate's surface using 12 mm carbon adhesive stubs. Each substrate surface area was dabbed five times to collect pGSR particles. Next, the substrates were cut and transferred to glass test tubes containing 500 µL of methanol to extract OGSR constituents exhaustively. After a 5-minute sonication, the 500 µL aliquot was transferred to a 0.2 µm filtration tube (Millipore®, MA) and centrifuged for five minutes at 3000 rpm to remove any unwanted debris. Following filtration, the 500 µl were transferred to an LC-MS/MS vial and diluted to a final concentration of ~100 ppb using MeOH (0.1% formic acid) to fall within the validated LC-MS/MS calibration curve and spiked with D_{10}-DPA at a final concentration of 150 ppb.

LC-MS/MS analysis was completed using an Agilent 1290 Infinity II equipped with a pentafluorophenyl (PFP) Poroshell® 120 column (2.7 mm 2.1 x 50 mm) and coupled to a 6470-triple quadrupole (QQQ) mass analyzer. Mobile phases consisted of water with 0.1% FA for the aqueous portion and acetonitrile with 0.1% FA for the organic portion. Starting mobile phase conditions were set to aqueous-80%/organic-20% and transitioned to aqueous-5%/organic-95% over 10 minutes using a constant flow rate of 0.30 mL/min. Source conditions included a dry gas temperature of 300°C at 10 L/min, a sheath gas temperature of 250°C at 7 L/min, and a nebulizer pressure of 20 psi.

SEM-EDS analysis was conducted on a JEOL 6490LV (Peabody, MA) following ASTM E1588-20 standard for GSR analysis. The SEM user interface software was version 8.14. The instrumental parameters used during analysis and the spectral collection consisted of an accelerating voltage of 25 kV, a spot size of 60 µm, a working distance of approximately 18 mm, and a magnification of 500x. A backscatter and a secondary electron detector were used to image particles, while an Oxford Instrument Xplore 30 (EDS, England) detector collected elemental information about the particles of interest. Each sample was mapped using automated software for feature analysis. LIBS and ECD methods were performed on specimens following validated protocols previously described.

Task 3 (Objective 3) — Testing of portable LIBS and EC units for the detection of GSR at crime scenes and laboratories, and comparative studies of cost-efficiency and reliability of bench-top and portable units

This task focuses on developing and evaluating the methods for the portable configurations and comparing the performance between the portable instruments and the bench-top versions. The results are published in two manuscripts[2, 6]

Task 3.1. Comparison of the performance of bench-top LIBS and ECD instruments to the portable version of the instrumentation using the OGSR-IGSR standard mix and authentic samples

The benchtop LIBS system in this study is an ns-Nd:YAG 266nm laser with a 6-channel CCD detector (J200, Applied Spectra, US). The LIBS laser can be fired at 25 discrete locations within each carbon stub, creating simultaneous chemical information from dozens of multi-elemental emissions in under one minute. The laser pattern allows the collection of 25 spectra with their respective spatial

information (100 μm resolution). The portable LIBS uses a ns-Nd:YAG 1064nm laser with a CMOS detector, an adapted micro-camera, and a custom-made stage to accommodate the SEM carbon stub holders without altering the evidence. The portable LIBS allows for particle search and single-particle analysis.

The electrochemical analysis is performed by a Metrohm Autolab 128N (Methohm, FL, USA) workstation. The electrochemical workstation has advanced software for graphical display and data analysis. The use of disposable carbon electrodes will allow for testing small volumes of samples (up to 50uL). Sampling was possible on the same carbon stub used for LIBS, ECD, and SEM-EDS analysis. Reliable and cheap electrochemical units are already available in the market. We used a portable EC unit (PalmSens 4 potentiostat, BASi, IN, US) with USB and battery power and housing of 16x10x3.5cm^3. The software PSTrace was used on laptops and Android devices with communication via USB and Bluetooth.

Method optimization of the portable units was performed using a Box-Behnken surface response design with three to five different factors at three levels each, using experimental models tested for the respective benchtop systems. The overall goal for the optimization was to maximize signal-to-noise ratios for GSR analytes and reproducibility. All collections of authentic shooters were performed at the WVU Ballistics lab with a variety of firearms and ammunition[2] (Background samples will be collected from willing participants from West Virginia University's campus.

Task 3.2. Testing of the portable and bench-top methods at mock crime scenes

Materials and Methods

Reagents, Standards, and Collection Devices

The solvents utilized in the analysis included acetate buffer prepared to a pH of 4.0 using sodium acetate anhydrous and glacial acetic acid (HPLC grade), acetonitrile (Optima®), and methanol (Optima®) purchased from Fisher Scientific (Fair Lawn, NJ). The pH measurements were taken using a Mettler Toledo FiveEasy pH meter (Columbus, OH). A Millipore Direct- Q® UV water purification system (Billerica, MA) provided ultrapure water (≥18.2 MΩ). Analyte standards for electrochemical and liquid-chromatography were purchased as follows: 1,3- diethyl- 1,3- diphenyl urea 99% (ethyl centralite) from Sigma- Aldrich (St. Louis, MO); diphenylamine from SPEX Certiprep® (Metuchen, NJ); d_{10}-diphenylamine from Toronto Research Chemical (Toronto, Canada); lead, copper, and antimony from Ultra Scientific® (Kingstown, RI); and nitroglycerin and 2,4- dinitro toluene from AccuStandard® (New Haven, CT). Nitrogen was purchased from Matheson Tri-Gas, Inc. (Irving, TX).

This study utilized three different devices for GSR collection, including the traditional carbon adhesive stubs and two in-house created stubs. The traditional carbon adhesive used a double-layer carbon adhesive tab purchased from Ted Pella, Inc. (Redding, CA) onto 12mm aluminum SEM mounts. For mobile LIBS analysis, the carbon adhesive increases the background noise due to the coupling of the laser wavelength and the carbon, which absorbs light. For this reason, two in-house collection devices for GSR sampling were created using Scotch permanent double-sided tape purchased from local office supply stores and Whatman-42 filter paper obtained from Millipore Sigma (Burlington, MA). The white stubs were created by layering filter paper between two layers of permanent double-sided tape on the aluminum SEM mounts and removing the excess with a scalpel. Half-carbon, half-white stubs used the same procedure; however, the aluminum SEM mount was covered halfway by the carbon

adhesive tab and half with the double-sided tape and filter paper. The procedure for creating these stubs is demonstrated in **Figure 9**.

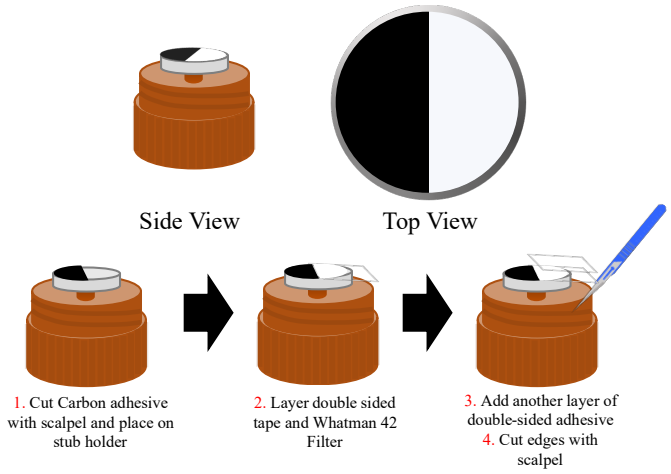


Figure 9. *Procedure for creating the half-carbon half-white in-house GSR collection stubs.*

Instrumentation

Microscopy and Fourier-Transform Infrared Spectroscopy

The physical and chemical properties of the adhesives were analyzed using microscopic examination and Attenuated Total Reflection-Fourier Transform Infrared Spectroscopy (ATR-FTIR). Microscopy examination used a Leica FS 4000 polarized light microscopy (PLM) with Olympus DP71 C-mount 70X camera attachment to take measurements of the cross sections of the adhesive layers at 10X and 20X magnifications using the Olympus DP 2-BSW software. ATR-FTIR analysis was completed using a Perkin Elmer Spectrum Two with UATR Two attachment, where each adhesive was analyzed in 3 replicates. The parameters for spectra collection used a sample scan type over a wavelength range from 4000-400cm^{-1} with a resolution of 4cm^{-1}, and 8 scans were accumulated per replicate. A library search was performed through the Perkin Elmer Spectrum software (version 10.5.4) using a match score threshold of 0.8000 and above.

Mobile Electrochemical Analysis

Electrochemical data acquisition was performed using a PalmSens4 Potentiostat (PalmSens) and a square-wave anodic stripping voltammetry method (SWASV) for the detection of IGSR and OGSR compounds in the PSTrace (version 5.9) software. Measurements were carried out using screen-printed carbon electrodes (SPCEs) purchased from Metrohm, USA, in the DRP-110 configuration. The SPCEs contain carbon working and auxiliary electrodes and a pseudo-silver reference electrode. **Table 7** describes the parameters used for the SWASV method.

Quality control samples were prepared before authentic sample analysis using two mixtures of the IGSR and OGSR analytes in acetate buffer, where the first was a solution consisting of 2 ppm Pb, 0.2 ppm Cu, 8 ppm Sb, and 10 ppm of OGSR (2,4- DNT, DPA or NG, and EC). The second solution was the same; however, DPA was replaced with NG to evaluate their peak potential since peak resolution is difficult to achieve when DPA and NG are in solution together. These two solutions were called the 10 ppm NG QC and 10 ppm DPA QC. Then, 1:4 dilutions were made to generate a

mixture of 2.5 ppm of OGSR analytes and 0.5 ppm Pb, 0.05 ppm Cu, and 2 ppm Sb for the IGSR analytes. Other controls run before analysis were a tailor-made pGSR standard, negative substrate control, and reagent control to ensure the quality performance of the instruments.[11]

Table 7. *Parameters for the square-wave anodic stripping voltammetry method for electrochemical analysis of mock crime scene samples using the PalmSens4 potentiostat.*

Parameter	Value
Deposition Time	120 s
Deposition Potential	-0.95 V
Start Potential	-1.0 V
End Potential	1.2 V
Potential Step	0.005 V
Amplitude	0.025 V
Frequency	11 Hz

Extraction of carbon or white stubs followed the procedure described in Ott et al.[16] Extraction of the half-carbon half-white stubs followed a modified extraction procedure to perform electrochemical and LC-MS/MS analysis on the samples. The extraction was performed on the carbon surface side, where 50 µL of acetate buffer was placed to perform a surface washing and then set aside in a microcentrifuge tube. Then, two aliquots of 50 µL acetonitrile were used to wash the surface of the stub and placed in a separate microcentrifuge tube. From the acetonitrile extraction, 50 µL would be placed in an LC vial for LC-MS/MS analysis. The 50 µL of acetonitrile left for EC analysis would then be dried under nitrogen and reconstituted with the acetate buffer fraction. Once reconstituted, the sample would be vortexed for 15 seconds before performing the electrochemical measurements. The 50 µL of acetonitrile for LC analysis would also be dried down under a stream of nitrogen and reconstituted with 42.5 µL of methanol with 0.1% formic acid and 7.5 µL of 5 ppm d_{10}-diphenylamine internal standard. The sample would then be analyzed using the LC-MS/MS.

Data analysis was performed in PSTrace software to perform peak integration and export the voltammetric and peak integrations to Microsoft Excel (Version 16.56, Microsoft Corporation). Analyte detection was positive if an analyte was above the critical thresholds for lead, copper, or nitroglycerin 1.59×10^{-8} A × V, 3.33×10^{-8} A × V, 4.28×10^{-9} A × V, respectively. A positive sample was determined by containing two or more IGSR, OGSR, or combinations of analytes above the critical threshold.

Mobile Laser-Induced Breakdown Spectroscopy Analysis

Mobile LIBS analysis followed the same operating procedure described in the Vander Pyl et. al.[2] The samples were placed in the mobile unit 6-sample chamber, and the enhanced magnification allowed the user to perform a "particle search" on the stub surface to visualize 1–20-micron potential IGSR particles. Ten particulates of various sizes were imaged and analyzed for each sample using the parameters shown in **Table 8**. For any control or background samples, five particles resembling GSR were targeted, photo-documented, and analyzed for comparison to the known GSR-containing samples.

Identification of the presence of GSR utilized emission lines, including Sb 259.8 nm (I), Pb 405.8 nm (I), and Ba 493.4 nm (II) as target emission lines for characteristic classification of IGSR elements. Data pre-processing of LIBS spectra used Clarity-Next (Applied Spectra, version 1.28.06.22) software to perform background subtraction and peak integration. Microsoft Excel (Version 16.56) was used to calculate signal-to-noise ratios to determine the presence of elements over an SNR of 3 to call an element present. A positive spot was determined by having one or more elements above an SNR of three. A sample was considered positive for GSR if at least two elements were detected from the ten particles or spots.

Table 8. *Mobile LIBS unit parameters for data acquisition of mock crime scene samples.*

Parameter	Value
Laser Wavelength	1064 nm
Laser Output Energy	85% (~7.5 mJ)
Laser Repetition Rate	7 Hz
Spot Size	30 μm (fixed)
Gate Delay	0.7 μs
Argon Flow in Chamber	1.3 mL/min
Detector	CMOS

Confirmatory OGSR Analysis by Liquid-Chromatography Tandem Mass Spectrometry
Samples were analyzed on an Agilent 1290 Infinity II as explained in the previous section. After analysis, analyte concentrations in the OGSR standard were calculated using equations produced by nine-level calibration curves ranging from 0 – 200 ppb.

Data acquisition was completed using the Agilent MassHunter Workstation Data Acquisition (version B.08.02). Data analysis was completed using the Agilent MassHunter QQQ Quantitative Analysis (version 10.0) software. Individual analytes were determined to be positive if the calculated concentration was over the detection limits. A sample was determined to be positive if the sample contained at least one category I OGSR compound and one category II OGSR compound by the OSAC Standard Practice Guidelines.

GSR Workflow Study Sample Collection and Preparation

An experiment was conducted to evaluate if the argon flow in the portable LIBS affects the recovery of OGSR by LC-MS/MS. The airflow testing was completed by spiking stub with a known concentration of 1000ppb OGSR standard mixture solution with the following analytes: akardite II, methyl centralite, ethyl centralite, 2-nitrodiphenylamine, diphenylamine, and 4-nitrodiphenylamine. A total of 42 white and carbon stubs were divided into two treatment groups. The first group did not experience any argon flow, while the second treatment group was exposed to argon flow (1.3 $Lmin^{-1}$) for 1.5 hours. The duration of exposure to the argon flow was to replicate the analysis time for particle search 6 stubs in the mobile LIBS system. The stubs were spiked with 10 μL of the 1000ppb OGSR mixture solution as ground truth. A total of 13 stubs per adhesive were extracted immediately after the standard was air-dried on the stub for approximately 5 minutes. A total of 8 stubs per adhesive were placed in the mobile LIBS sample chamber after air drying for 1.5 hours, with the argon flow set to 1.3 $Lmin^{-1}$.

All stubs were extracted using the following procedure. Six aliquots of 50µL methanol were used to wash the surface of the stub and placed into 0.22-micron filters. The filters were centrifuged, and the sample was transferred into an LC vial to be dried down under nitrogen to completion. After drying, the samples were reconstituted with 90µL of methanol 0.1% formic acid and 10µL of 1ppm deuterated diphenylamine as internal standard. The target concentration of analytes was 100ppb. Additionally, spiked samples were also analyzed. These samples were made by spiking 10µL of the 1000ppb OGSR mixture into an LC vial and following the same dry down and reconstitution procedure to act as the ground truth concentration of the OGSR solution.

Mock Crime Scene Sample Collection and Preparation

Mock crime scene samples were collected at the WVU Ballistics Laboratory. A Springfield XD9 firearm was used to collect shooter samples where individuals discharged 1, 2, or 5 consecutive shots of American Eagle Federal 9 mm ammunition. For this study, 17 samples were collected in four different mock crime scene scenarios. The scenarios included two arrests, a suicide, and a homicide. Samples were collected using the half-carbon, half-white stubs where one stub would be used to stub the left and right hands of the individual, except for the suicide case.

Precautions were taken at the ballistics laboratory to limit environmental contamination. The individual acting as sample collector wore appropriate PPE and was sampled before and after the collection of mock crime scene samples. Disposable lab coats were also worn by individuals entering the ballistic range for the simulated suicide scenario. The arrest area was lined with painters' paper to protect from environmental contamination on the laboratory floor, and the sampling area was cleaned and lined with butcher paper. Additionally, six positive control samples were collected from the hands of a shooter, which included three replicates of firing one round and three replicates of firing five rounds. All control and mock crime scene samples were analyzed using the same analytical scheme. The samples were analyzed first by the mobile LIBS, then electrochemistry, and finally by LC-MS/MS analysis.

Arrest Scenario 1

The first arrest scenario investigated the potential for secondary GSR transfer from a person of interest who handled an unfired firearm to the arresting officer. To evaluate this transfer mechanism, the person of interest would handle an unfired firearm for one minute, simulating the unloading and loading of a cartridge (**Figure 10**). The officer would also perform the firearm handling procedure to simulate daily firearm checks performed by the officer. After handling the firearm, the officer would wash their hands, followed by simulating driving for 5 minutes. Then, the officer conducts the arrest procedure with the suspect (**Figure 11**). Samples were collected before and after the arrest by the officer and only after the suspect's arrest. **Figure 12** provides a graphical depiction of the steps for arrest scenario 1.

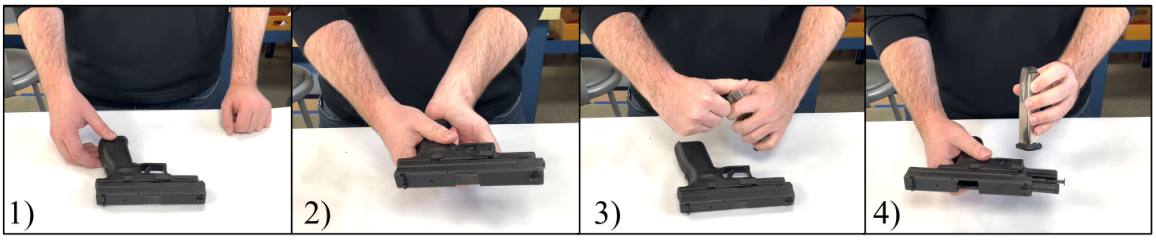


Figure 10. *Photographs of mock crime scene handling of the firearm procedure: 1) firearm in hand, 2) release magazine and rack slide and repeat, 3) load ammunition, and 4) insert magazine.*

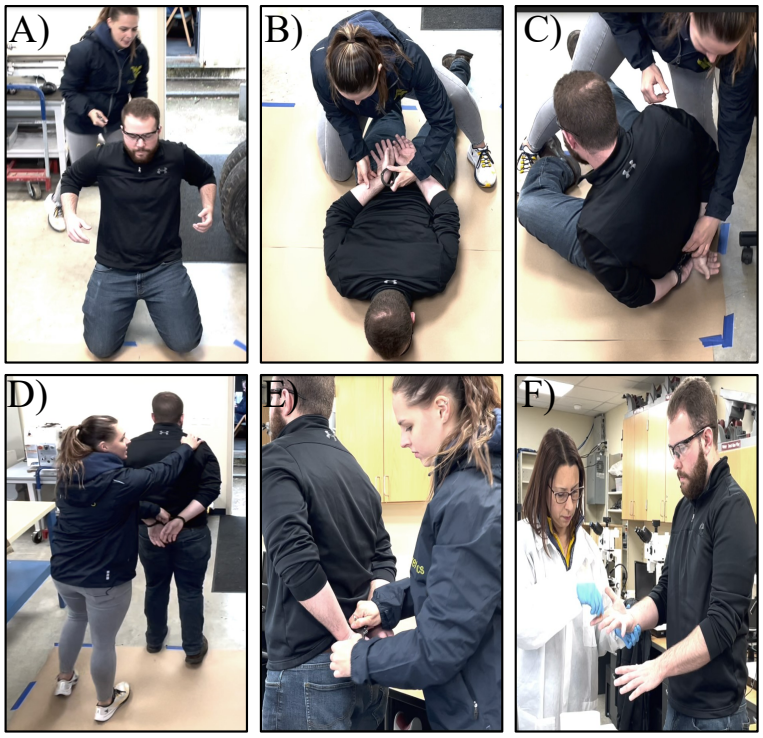


Figure 11. *Photographs of mock crime scene arrest procedure A) suspect gets down on the ground, B) suspect puts a hand behind back, C) officer handcuffs them, D) reading their rights and guide to sampling area, E) uncuffing at sampling area, and F) sampling for GSR.*

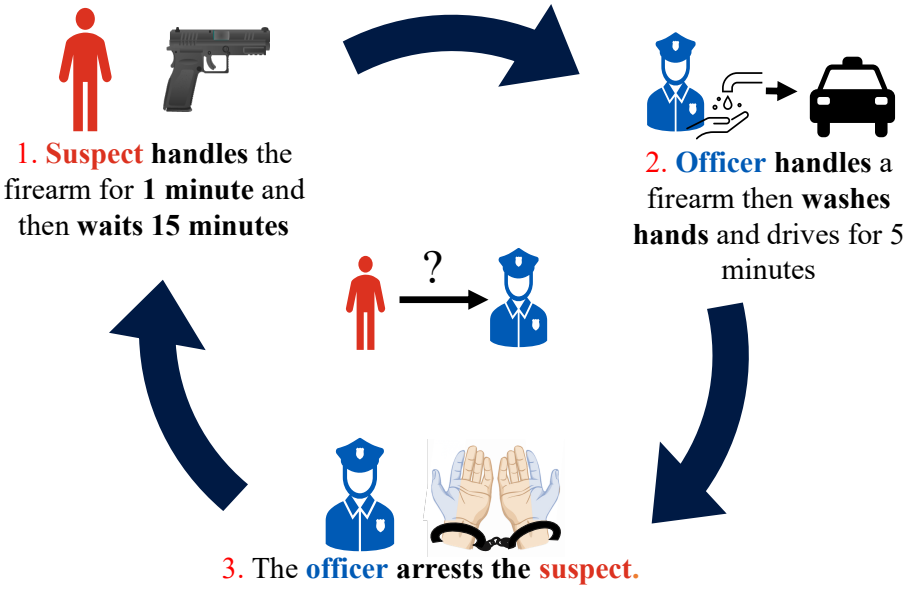


Figure 12. *Graphical depiction of the individuals and actions performed to collect arrest scenario 1 samples.*

Arrest Scenario 2

Two mechanisms for a potential secondary transfer were evaluated in the secondary arrest scenario. The first mechanism mocks the potential for GSR transfer between a person of interest who has not been near a firearm and an officer who recently handled their firearm without firing. The officer (blue in **Figure 13**) would handle the gun following the procedure in **Figure 13** and then drive for 15 minutes before performing the arrest on the person of interest (yellow in **Figure 13**) who had not been near a firearm.

In this scenario, the second mechanism for potential secondary transfer of GSR was assessed from a person of interest who has recently fired a gun to the arresting officer. A second person of interest (red in **Figure 13**) fired five rounds of ammunition before waiting 30 minutes before being arrested. During this time, the second officer (green in **Figure 13**) handled the firearm, washed their hands, and performed 15 minutes of simulated driving. The officer would arrest the suspect; samples were collected like the other arrest scenarios.

Figure 13. *Graphical depiction of the individuals and actions performed to collect arrest scenario 2 samples.*

Suicide Scenario

For the simulated suicide scenario, the mock crime performed investigated the distribution of GSR on the hands of the individual acting as the victim and the potential for secondary GSR transfer to an individual that finds the victim, such as a family member, friend, or first responder. The scenario involved an individual acting as the victim who would fire a single round of ammunition and then be seated in a chair for 30 minutes. After 30 minutes, an individual acting as the first person on the scene would discover the victim, shake their shoulder, check for a pulse, and then simulate a 911 call. The victim's hands were bagged before moving to a clean sampling area to simulate collection at a morgue. The collector sampled the victim's left and right hands individually. The family member was sampled before and after interacting with the victim. **Figure 14** outlines the steps performed during this mock scenario.

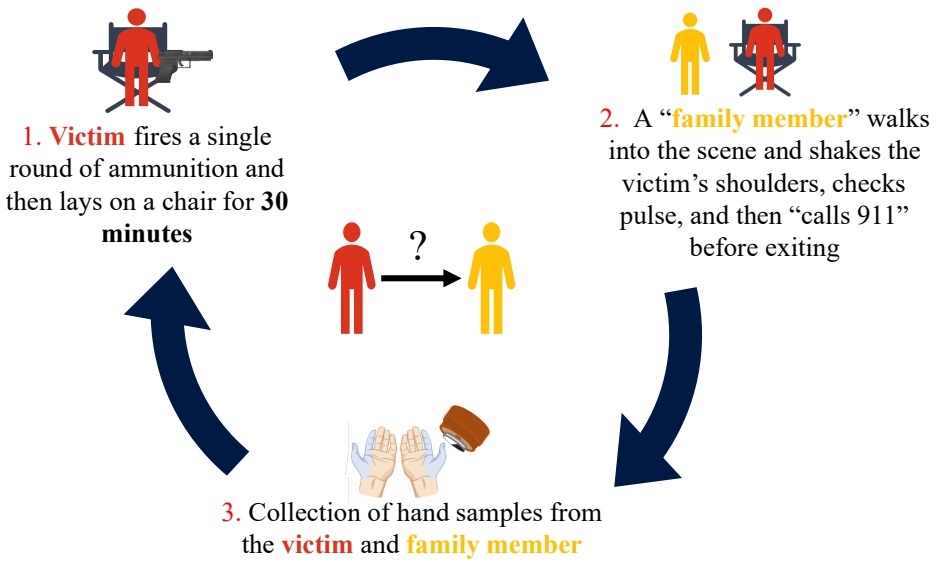


Figure 14. *Graphical depiction of the individuals and actions performed for collection of suicide scenario samples.*

Homicide Scenario

The homicide scenario evaluated a suspect shooting at a cloth substrate representing a t-shirt from the victim or other fabric material at the crime scene. This scenario assessed the capability of the mobile instrumentation for analyzing GSR samples from substrates. The suspect fired two rounds at the cloth substrate at a close range (12 inches measuring muzzle to target) and was immediately sampled for GSR (**Figure 15**). The cloth substrate was removed and packaged for sampling back at the laboratory. Each bullet entrance hole was sampled individually using the half-carbon, half-white stubs. The sampling area included stubbing the bullet wipe, including a 2cm radius around the bullet wipe approximately 8-10 times.

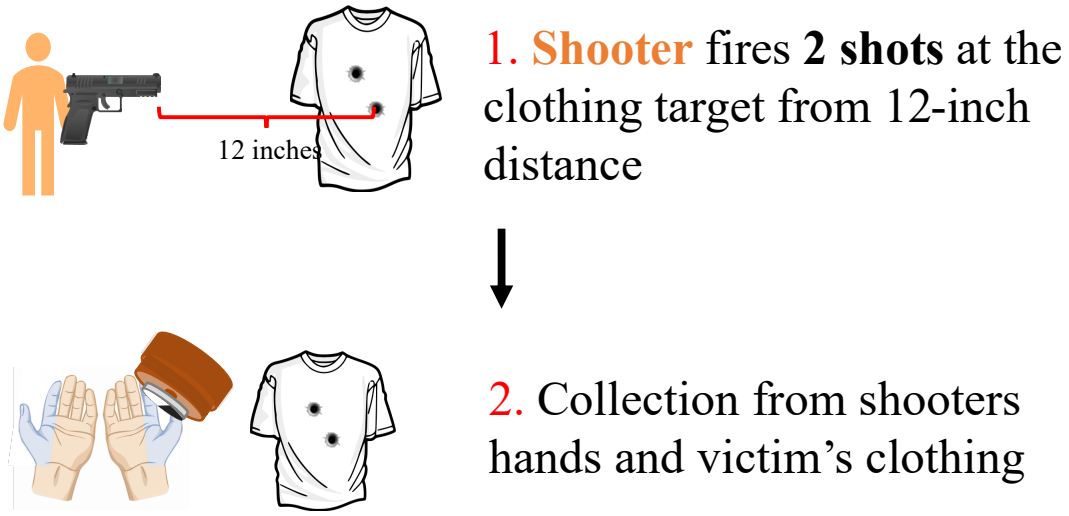


Figure 15. *Graphical depiction of the individuals and actions performed to collect homicide scenario samples.*

Task 4 (Objective 4) — To apply data from population datasets and transfer and persistent studies in the assessment of a probabilistic approach for the interpretation of GSR evidence

This task focuses on developing and validating statistical methods to evaluate data obtained from multiple sensors and under different activities. We are constructing a Bayesian framework to assess the weight of the evidence. The odds form of the Bayes equation is given as:

$$\underbrace{\frac{p(H_p|E)}{p(H_d|E)}}_{\text{Posterior Odds}} = \underbrace{\frac{p(E|H_p)}{p(E|H_d)}}_{\text{Likelihood Ratio}} \text{ x } \underbrace{\frac{p(H_p)}{p(H_d)}}_{\text{Prior Odds}}$$

where the two hypotheses are formulated in terms of the prosecutor hypothesis (Hp or H1) and the defense hypothesis (Hd or H2), which are mutually exclusive, the odds form of the Bayes equation allows for the updating of prior "beliefs" as evidence is assessed. Of most interest to forensic science is the likelihood ratio, or the ratio of the probabilities of the evidence given each hypothesis. The interpretation of forensic evidence generally takes three levels following the hierarchy of propositions: offense, activity, and source. Due to the nature of our current study and the resulting data obtained from our analytical methods, we speak here of the source and activity levels.

Materials and Methods

Data Sets

To develop the Bayesian Networks (BN), retrospective data analysis was performed on two data sets that our research group had collected and reported previously. The datasets used for the BN utilize only the portion of the data analyzed by SEM-EDS because of the capability of this method to do single particle analysis and to monitor the number of particles per stub via automated searching routines. Also, SEM-EDS was chosen as it is the standard method used for GSR analysis in forensic laboratories. Nonetheless, the data acquired by other methods provides complementary information and will be used in the future to expand this model.

The first data set (referred here as Menking-Hoggatt dataset I) was completed in 2022 by Menking-Hoggatt *et. al.* [7] The study observed over 1,000 samples by exploratory screening methods laser-induced breakdown spectrometry (LIBS) and electrochemistry (ECD), where a subset of this data was analyzed by confirmatory analysis method SEM-EDS. The samples were comprised of authentic shooter and non-shooter samples. The shooter dataset was subdivided into the type of ammunition (*i.e.*, leaded and lead-free ammunition), and by post shooting activity. The subset of shooter samples analyzed by SEM consisted of 52 leaded ammunition samples, 52 lead-free ammunition samples, and 54 activity samples.

The post-shooting activities performed in this study included running, rubbing hands, and using hand sanitizer. The non-shooter samples were collected from individuals categorized as low-risk (*i.e.*, individuals who have not been near or fired a firearm in 24 hours) and high-risk (*i.e.*, individuals who have not been near or fired a gun, but their professions can lead to potential risks of false positives). The low-risk background consisted of 54 samples, and the high-risk background consisted of 25 samples analyzed by SEM-EDS. This SEM-EDS dataset of 237 samples followed an automated search recipe that used a ten-characteristic or 20-characteristic particle cutoff limit for leaded

ammunition/post-shooting activities and lead-free ammunition, respectively. Thus, once the cutoff limit was reached, the SEM-EDS completed the analysis of that sample and move on to the following sample in the sequence. These samples were chosen as ground truth as they were known to originate from hands of shooters or non-shooters, respectively.

The second dataset (referred here as the Vander Pyl dataset, dataset II) was completed in 2023 by Vander Pyl *et al.*[3] The samples from this study of interest to the Bayesian networks include the transfer and persistence specimens collected from authentic hand samples after shooting. The study also used synthetic skin membranes to model the activity mechanisms using reference standard materials of known particle count and composition. Samples for this study were subject to analysis by multiple methods, including laser induced breakdown spectroscopy (LIBS), electrochemical detection (ECD), liquid chromatography tandem mass spectrometry (LC-MS/MS), and SEM-EDS for evaluating both inorganic gunshot residue (IGSR) and organic gunshot residue (OGSR). In this study, the amount of gunshot residue deposited on the skin membrane was controlled by using a characterized primer gunshot residue standard to establish a ground truth of the number of characteristic particles on the surface at time zero. For our purpose, only the samples analyzed by SEM-EDS are utilized in the Bayes networks as the ground truth for transfer and persistence mechanisms from this dataset

The authentic persistence samples in the Vander Pyl dataset include the collection from the hands of individuals at three post-shooting times *viz.*, 0, 1, and 3 hours. A total of 107 hand samples were collected; however, only six samples were analyzed by SEM-EDS per post-shooting time for a total of 18 samples. The synthetic skin model was used for comparison and extended the study of post-shooting times to include 0, 1, 3, and 6 hours for a total of six SEM-EDS samples per time (24 samples). Investigation of transfer mechanisms were studied using the skin model to evaluate post-shooting activities such as, handshaking, hand rubbing, and washing hands. A dataset where no activity was performed and immediately collected was used as a control. Each subset completed by SEM-EDS analysis on six samples per activity. The SEM-EDS analysis for the skin model persistence and transfer hand studies estimate the total number of particles by mapping a random selection of 25% of the surface to reduce the total analysis time.

Data Preprocessing

Due to the difference in the SEM-EDS searching recipes between the two datasets, preprocessing for BN performed normalization of the data. The scaling of the datasets was completed using min-max scaling to create two normalized datasets. The first scaled dataset was done to a maximum of 10 characteristic particles to scale the transfer and persistence dataset to the population study dataset. This was done to explore BN with data inputs similar to those laboratories that utilize a 10-particle cutoff. The second scaled dataset was achieved by scaling to 1000, approximately the average number of characteristic particles found immediately after deposition from the primer gunshot residue (pGSR) standard. Normalizing the data to 1000 was meant to mimic results found when completing partial or full mapping of samples.

Background Activities Survey

A survey was created to understand common activities performed by the average person that could influence the transfer and persistence of gunshot residue and how often they are performed. The survey consisted of thirteen questions regarding an individual's environment, activities, and hygiene habits. **Table 9** provides the summary of questions on the survey.

Table 9. *Table summarizing the questions asked on the background activities survey.*

Type of Question	Question Number	Question
Daily Environment	1	What is your typical work environment? Options: Work from home, office, laboratory, Car/vehicle, Ballistic Range/Lab, outdoors, other
	2	How do you get to work? Options: Walk, personal vehicle or carpool, Bus, Bike, other
	3	Approximately, how many times do you use your car a day? Options: None or N/A, 1-2 times, 3-4 times, 5+ times
	4	Approximately, how many hours a day do you use your computer? Options: None, 1-2 hours, 2-4 hours, 5+ hours
Daily Activities	5	Approximately, how many times a day do you rub your hands together? Options: None, 1-2 times, 2-4 times, 5+ times
	6	Approximately, how many times a week do you shake hands with someone else? Options: None, 1-2 times, 2-4 times, 5+ times
	7	Approximately, how many steps do you take daily? Options: 0-2500 steps, 2500-5000 steps, 5000-7500 steps, 7500-1000 steps
	8	Approximately, how many days do you run or exercise a week? Options: none, 1-2 times, 3-5 times, 5-7 times
	9	When you run or exercise, approximately how far is your average distance? Options: None or N/A, 1-2 miles, 3-4 miles, 5-7 miles, 10+ miles
Hygiene Habits	10	Approximately, how many times a day do you wash your hands? Options: None, 1-2 times, 2-4 times, 5+ times
	11	Approximately, how many wears do you allow between washes before you clean your clothes specifically upper garments? Options: 1 wear, 2 wears, 3 wears, if other or it depends, explain.
	12	Approximately, how many times a day do you wash your face? Options: Once a day, twice a day, other
	13	Approximately, how many times a week do you wash your hair? Options: 1-2 times, 3-4 times, 5-6 times, everyday, other

A total of 98 responses were collected using the Qualtrics^{XM} (Seattle, WA) online real-time reporting dashboard. The survey, taking approximately 5-minutes, was voluntary and sent out to individuals within the Department of Forensic and Investigative Science at West Virginia University and the Morgantown area.

Data Visualization and Analysis
Data visualization was performed using R (version 4.1.1) and RStudio (version 2023.09.1, Posit Software, PBC). The ggplot2 package was used to create various plotting methods for the data. The Bayesian networks were designed and computed using the Netica software (Norsys Software Corporation). This software package allows for the development of Bayesian belief networks, decision networks, and influence diagrams. The software can input raw or model data by defining the respective nodes and edges, which can be modified through equations and probability distributions.

1.4.2. Data Analysis

Data analysis in this project required using metadata (descriptive nominal) and numerical data (concentrations, peak areas, particle counts, SNRs, probability outputs, etc.) and spectral raw data. To assess performance, false-positive rates, false-negative rates, sensitivity, specificity, and accuracy are reported for the datasets where ground truth is known for the datasets. The data are also analyzed using various graphical methods (box plots, heat maps, histograms, kernel density distribution plots, ROC curves, and Tippett plots). Performance rates and statistical analysis are performed in Microsoft Excel (Version 19.08), JMP Pro 16 (v.2021, SAS Institute Inc., NC), and mathematical and statistical algorithms created in open access R (version 4.2.2, R studio version 2022.07.2+576). Neural network and Bayesian Network analysis were conducted using JMP, R, or Netica software ((Norsys Software Corporation).

To maintain traceability of the data, files are named with pre-determined nomenclature. An inventory master list was created for this database, containing the ID number and the respective metadata and descriptors associated with each sample. The required datasets and associated documentation are submitted to the funding agency at the end of the project for archiving and availability to any government laboratory that requests it; however, the rights for publication of results derived from the data are retained by WVU investigators.

1.5. Expected applicability of the research

The proposed research is anticipated to substantially impact the forensic community and criminal justice, as this study will provide a leap in the capabilities currently available in this field. These include information about organic and inorganic GSR derived from traditional and non-toxic ammunition, assessing methods now in practice, and emerging techniques.

The forensic significance of novel methods was thoroughly evaluated in this project, providing the forensic community with substantial results about the validity of the methods and their potential for adoption. Since the methods proposed here can be applied to laboratory or portable settings, they could significantly decrease backlogs and increase the efficiency in decision-making processes at the crime laboratory and the investigative reconstruction of events.

This research overall framework and interconnected objectives addressed several needs in our field in a single project. Also, this project contributes to more than one of the NIJ priority practices to leverage research opportunities: a) educating and training a future workforce, b) transferring technology from laboratory to marketplace, and c) partnering with industry and academia. In particular, the strategic partnership of researchers, practitioners, and statisticians, the development of novel and practical GSR standards and emerging technologies, the systematic study of transfer and persistence of IGSR and

OGSR, and the proposed comprehensive interpretation approach are anticipated to have a synergetic effect for the advancement of the body of knowledge in the field.

This research has generated a large dataset of IGSR and OGSR data collected from diverse populations and substrates using a multitude of analytical tools and provide, for the first time:

1) Innovative screening methods of analysis, using LIBS and ECD, that have demonstrated to provide fast and accurate analysis in laboratory settings and have shown to fit for purpose for onsite testing. This technology provides unprecedented rapid response, almost in real-time, allowing quick decision-making processes that are otherwise unavailable. For example, the LIBS/ECD analysis can provide data input to decision-makers in under 5 minutes, while the typical SEM-EDS analysis requires between 2-8 hours per sample, not including the time it takes for the sample to arrive at the laboratory from the collection at the scene and time it takes to do manual inspection of the data and interpretation.
2) A solid scientific foundation of the novel methods' performance through rigorous inter-instrument and inter-laboratory testing, both at the laboratory and the scene.
3) Methods that are compatible with current collection and analysis protocols and, therefore, can be relatively easily adopted.
4) One-of-a-kind microparticle IGSR and OGSR reference standards that have been thoroughly characterized and evaluated for stability over time and collection efficiency in various substrates and analytical sensors. These standards permit the performance of known particle count and chemical composition depositions for controlled experimental conditions not available for GSR until now.
5) A novel approach to visualize the flow dynamics of GSR (both in particulate and vaporous forms) using laser scattering, high-speed videography, portable particle counters, and analytical sensors to evaluate the deposition, transfer, and persistence of GSR in a high dimension. This approach opens new venues to understand the risk of exposure and secondary transfer of IGSR and OGSR.
6) A pioneering approach for using synthetic skin models to understand mechanisms of IGSR and OGSR transfer and persistence.
7) Proof-of-concept studies that use machine learning, probabilistic assessments, and Bayesian Networks for interpreting GSR at the source and activity levels.

Therefore, this research brings several benefits to the criminal justice system by increasing the capacity to complement and modernize current practice, to support other research endeavors, and boost multidisciplinary collaborations across practitioners, researchers, managers, and industry. Ultimately, this research aims to assists practitioners in supporting and informing their opinions with protocols and interpretation approaches that strengthen the scientific validity in this field

II OUTCOMES

2.1. Activities/accomplishments

Executive Summary

One of the main goals of this project was to contribute to the preparation of a specialized future workforce in STEM. This project provided unique opportunities for students and faculty to network across several disciplines, including forensic science, mathematics, management, and statistics.

The graduate students' essential milestones in their program directly impacted the advancement of this research. Of the 12 students and one post-doc who have worked in this research, all 7 students who have graduated achieved job placement in STEM largely due to the learning opportunities provided through this grant. For example, Kourtney Dalzell defended her master's thesis in the summer of 2022 and was admitted to the WVU doctoral program in forensic science in the fall of 2022. Korina Menking-Hoggatt graduated with a Ph.D. in Forensic Science, continued as a post-doc for this award, and then joined the workforce. Also, two other students, Courtney Vander Pyl and Bill Feeney, completed their doctoral degrees and immediately occupied high-end jobs in the market. Three of our six undergraduates either joined graduate school or entered the workforce in the forensic field. The remaining undergraduate and graduate students are still completing their degrees and have gained unique networking skills through this research.

This project developed and validated innovative standard materials and approaches to evaluate relevant aspects of the transfer and persistence of inorganic and organic gunshot residues that advance the understanding and interpretation of GSR evidence. The standards were developed for universal applicability. They can be used in many matrices and examined by traditional methods such as SEM-EDS and mass spectrometry (GC-MS, LC-MS) and novel technology such as LIBS, LA-ICPMS, and ECD. Along with these unique IGSR/OGSR standards, this study also implemented novel synthetic skin substitutes to study deposition, transfer, and persistence mechanisms.

We investigated the capabilities of a novel invention that combines custom-made atmospheric samplers with high-speed videography and laser sheet scattering to gather information about the flow of GSR plumes and particles under various controlled experimental conditions. Although this was not initially proposed in this study, the ideas evolved from discussions with NIST collaborators and our researchers and provided a unique opportunity to boost the impact of this research. This approach also provided a feasible alternative to using fluorescent taggants for the ammunition, which proved challenging from a safety perspective.

Finally, the fast, selective, and sensitive GSR screening tools developed in phase I (LIBS and ECD) were tested at another level in this grant by collaborating with industry and forensic laboratories to assess pilot portable devices that can bring the technology to the crime scene. Through these partnerships, we have recently received a continuation award from NIJ to continue working with industry, forensic laboratories, and economists to establish technology transition plans for the innovations developed in this grant.

Altogether, these approaches can transform our discipline by providing a leap of knowledge in understanding and interpreting GSR under contexts typically found in cases involving firearm-related incidents. We aim to facilitate using this knowledge through collaboration with practitioners, statisticians, and researchers. **Figure 16** illustrates the main accomplishments and a summary of the main contributions.

Figure 16. *Overview of the main products of this award, such as publications, presentations at scientific meetings, creation of the largest collection set on physical fits, and collaborative activities with practitioners to evaluate the utility and reliability of the method.*

2.2. Results and findings

2.2.1. Executive summary of the main findings of the research

This project developed novel, effective, and practical approaches that enhanced the scientific reliability and knowledge base on gunshot residue. We have accomplished this through four main accomplishments:

1) ***Development of novel organic and inorganic GSR reference standards and visualization approaches***

We developed an OGSR reference standard containing eight (8) top OGSR analytes commonly used in the formulation of smokeless powders or generated through typical combustion and deflagration mechanisms during gun firing. As part of the optimization, we determined the optimal solvent, storage conditions, and sample preparation methods using a Plackett-Burman screening design. LC/MSMS and electrochemistry testing assessed the standards' stability over several weeks. The stability of the

microparticle IGSR (pGSR) standards was also tested over time and evaluated via ICP-MS and SEM-EDS.

An approach was developed and optimized to apply microparticle IGSR and OGSR standards mixtures on various matrices, such as carbon stubs, adhesives, fabrics, and synthetic skin, providing first-time availability to versatile GSR standard materials. Analysts, laboratories, and analytical methods assessed these IGSR/OGSR standards.

To validate these reference materials, we partnered with the Sacramento District Attorney Crime Laboratory in California, the Department of Chemistry at Iowa State University, and NIST to conduct interlaboratory testing. Also, we collaborated with NIST to use airborne particle sensors and laser sheet scattering as an alternative approach to studying and visualizing GSR evolution, from GSR creation to its movement and deposition.

The results of these studies have been published in doctoral dissertations and peer-reviewed manuscripts:

1. Korina Menking Hoggatt, Ph.D, Spring 2021. WVU Department of Forensic and Investigative Science, Characterization of modern ammunition and background profiles: A novel approach and probabilistic interpretation of inorganic gunshot residue. Graduate Theses, Dissertations, and Problem Reports. 8336. https://researchrepository.wvu.edu/etd/8336
2. Courtney Vander Pyl, Ph.D., Fall 2022. WVU Department of Forensic and Investigative Science, Expanding the capabilities of firearm investigations: Novel sampling and analytical methods for gunshot residue detection. Graduate Theses, Dissertations, and Problem Reports. 11509. https://researchrepository.wvu.edu/etd/11509
3. C Vander Pyl, K Dalzell, K Menking-Hoggatt, T Ledergerber, L Arroyo, T Trejos. Transfer and persistence studies of inorganic and organic gunshot residues using synthetic skin membranes. Forensic Chemistry, 34, 2023, 100498, https://doi.org/10.1016/j.forc.2023.100498
4. Sarah Szakas, Korina Menking-Hoggatt, Tatiana Trejos, Alexander Gundlach-Graham. Elemental Characterization of Leaded and Lead-Free Inorganic Primer Gunshot Residue Standards by spICP-TOFMS. Applied Spectroscopy. 2022. DOI: 10.1177/00037028221142624
5. K Menking-Hoggatt, C Martinez, C Vander Pyl, E Heller, E Pollock, L Arroyo, and T. Trejos. Development of Tailor-Made Inorganic Gunshot Residue (IGSR) Microparticle Standards and Characterization with a Multi-technique Approach. *Talanta.* April 2021, 225, https://doi.org/10.1016/j.talanta.2020.121984

2) Development of systematic methods for the study of the transfer and persistence of gunshot residues.

This research conducted an extensive transfer and persistence study on IGSR and OGSR, consisting of over 800 samples collected from multiple matrices (e.g., authentic shooters, fabric samples, and synthetic skin membranes) and exposed to diverse activities and conditions. The utility of novel synthetic skin membranes (StratM®) models for understanding the post-deposition behavior of gunshot residues is demonstrated for the first time.

The results of the studies described in 1) and 2) above have been published and listed below.

1. Courtney Vander Pyl, Kourtney Dalzell, Korina Menking-Hoggatt, Thomas Ledergerber, Luis Arroyo, Tatiana Trejos. "Transfer and Persistence Studies of Inorganic and Organic Gunshot Residues using Synthetic Skin Membranes." Forensic Chemistry. April 2023. 100498. https://doi.org/10.1016/j.forc.2023.100498
2. Courtney Vander Pyl, William Feeney, Luis E. Arroyo, Tatiana Trejos. "Capabilities and Limitations of GC-MS and LC-MS/MS for Trace Detection of Organic Gunshot Residues from Skin Specimens. Forensic Chemistry. 100471. January 2023. https://doi.org/10.1016/j.forc.20

3) *Demonstrated the feasibility of portable LIBS and ECD units for the detection of GSR at the laboratory and at the crime scene.*

The portable LIBS demonstrated improved detection capabilities over benchtop systems due to enhanced CMOS detector technologies, an ablation cell custom-made for GSR examinations, microscopic visualization of particle morphology, and unique capabilities for single-particle analysis. These custom-made improvements were feasible thanks to a partnership with industry (Applied Spectra). A significant novelty of the LIBS portable instrument is its image magnification, which allows quick searching and visualization of GSR particle morphology. The single-particle imaging and elemental composition capability is one of a kind and offers superior confirmatory features for GSR. The mobile LIBS performance was evaluated for residues collected from the hands of shooters (100 samples) and non-shooters (200 background samples) and analyzed sequentially by the mobile instrument and then the laboratory instrument. Both instruments obtained accuracy better than 98.8%, demonstrating their suitability for trace IGSR detection from skin specimens. Implementation of this methodology is anticipated to drastically speed up response times (i.e., from several hours per sample by standard SEM-EDS practice to a few minutes by LIBS). The study shows that screening portable methods can be easily incorporated into workflows to improve decision-making processes at the crime scene and laboratory settings, reduce backlogs and improve case management.

A similar study was conducted to compare the capabilities of ECD portable and benchtop units for IGSR/OGSR detection. The evaluation included 350 hand specimens collected from 200 background individuals (non-shooters), and shooters who fired leaded ammunition (100) and lead-free ammunition (50). Each specimen has evaluated by the benchtop and portable instruments sequentially, and yielded were accuracies of 95.7% and 96.5%, respectively. Figures of merit such as limit of detection, limit of quantitation, precision, linear dynamic range and accuracy were also comparable when using standards for four OGSR compounds and three IGSR elements of interest. The equivalent performance of the portable ECD device indicates that is feasible to transition this methodology for onsite detection at crime scenes for faster decision strategies.

Two manuscripts and one thesis report the main findings of LIBS and ECD portable instrumentation:

1. Courtney Vander Pyl, Korina Menking-Hoggatt, Luis Arroyo, Jhanis Gonzalez, Chunyi Liu, Jong Yoo, Richard Russo, Tatiana Trejos. Evolution of LIBS Technology to Mobile Instrumentation for Expediting Firearm-Related Investigations at the Laboratory and the Crime Scene. Spectrochimica Acta Part B: Atomic Spectroscopy.106741 July 2023. https://doi.org/10.1016/j.sab.2023.106741

2. K Dalzell, C Ott, T Trejos, L Arroyo. Comparison of portable and benchtop electrochemical instruments for detection of inorganic and organic gunshot residues in authentic shooter samples, *J Forensic Sci*, 2022, https://doi.org/10.1111/1556-4029.15049
3. Dalzell KA. Electrochemical and mass spectrometry methods for identification of gunshot residues (GSR) in forensic investigations. Graduate Theses, Dissertations, and Problem Reports. 11354. https://researchrepository.wvu.edu/etd/11354/

4) Development of probabilistic approaches for the interpretation of GSR evidence that includes activity-level assessments.

With the advice of our statistician collaborator, we developed statistical methods to evaluate data obtained from multiple sensors, improve the objectivity of the determinations, and provide a probabilistic output that allows a numerical assessment of the value of the evidence. We have validated the data preprocessing and are using Neural Networks to interpret GSR data derived from our extensive database (>80,000 data files). The machine learning probabilistic outputs were used to calculate log10 likelihood ratios (LR) and evaluate their distribution on the subgroups. The log10 LR was typically between -2.5 to -5 for non-shooters and >5 for shooters, demonstrating good discrimination between the overall populations and the viability of using LR as a metric for reporting the weight of the evidence. Tippet plots were used to evaluate LR and the rates of misleading evidence (RME <3.7%). In addition, we incorporated the new transfer and persistence data to build a Bayesian Network framework that provides a means by which to assess the weight of the evidence at the activity level.

The lessons learned in the studies serve as important benchmarks to provide criteria that assist with the standardization and modernization of the examination and interpretation of GSR evidence. These findings are anticipated to offer a path forward to the forensic community for more comprehensive and streamlined processes. The proposed methods align with ongoing standard guides and research needs identified in the field and can be adapted to current workflows.

In the following sections, we summarize the main findings of this study, organized by main objectives and tasks. Nonetheless, most of this work has been published in various manuscripts, and the reader is directed to these references for additional information.

2.2.2. Development of innovative approaches for GSR analysis, visualization, and interpretation: description of major findings by tasks and milestones.

Results of Task 1 (Objective 1) —To develop, characterize and validate organic and inorganic GSR reference standards via a multiple-technique approach and interlaboratory studies.

Under this task, there are two major milestones. First, the development of one-of-a-kind IGSR and OGSR standard materials, their validation through stability studies and interlaboratory testing, and assessment of their utility on multiple applications and techniques. Second, the development of novel visualization and onsite testing approaches to study the dynamics of GSR after firearm discharge. The sections below describe the main results.

Task 1.1: Results for the development of a mixed microparticle IGSR and OGSR reference standard

The IGSR and OGSR standards proved to be versatile and were utilized for the validation of methods to detect inorganic particles (LIBS, ECD, and IGSR) [7,10,11] and OGSR (ECD, GC/MS, LC-MS-MS, DART-MS).[1,4,8]
To illustrate its use for OGSR analyses, we summarize here an example of its use to assess the limitations and capabilities of liquid chromatography-tandem mass spectrometry (LC-MS/MS) and gas chromatography-mass spectrometry (GC-MS) for the trace detection of organic gunshot residue (OGSR) at trace levels, at a time when forensic scientific organizations in the U.S are developing standards to facilitate their adoption. Two manuscripts discuss using these standards as quality control and during validation. Limits of detection (LODs), limits of quantitation (LOQs), and measurement variability are reported for the top eight analytes of interest (see **Table 10**). Both techniques detected the targeted analytes with LODs as low as 0.3 ppb for LC-MS/MS and 40 ppb for GC-MS. Also, the performance rates of the methods were evaluated using 50 shooter sets, including four different ammunition types. Background specimens were collected from individuals who had not fired a gun in the last 24 hours. The solvent extraction from the carbon stubs was split in half to permit a direct comparison of the methods on the same specimens.

For the ammunition used in this study, enhanced sensitivity was observed for the triple quadrupole configuration (LC-MS/MS), with accuracies as high as 80%. However, under the extraction and EI-quadrupole instrumental configuration used in this study, GC-MS could not detect enough characteristic compounds to lead to the identification of OGSR, especially in lower calibers. As a result, the GC-MS analysis displayed accuracies only as high as 35%, raising a flag that GC-MS alone is not recommended for OGSR detection unless combined with LC-MS/MS. A follow-up study comparing the capabilities of GC-MS with tandem GC-QqQ and GC-MS-FID configurations showed, however, that the performance rates of GC methods vary widely with the type of ammunition. For instance, in a second study that used different ammunition, the accuracy of GC-MS increased to 63-80%. The results indicate that the performance for characterization of OGSR at trace levels in hand specimens increases as follows: GC-Q < GC-QqQ, GC-MS-FID < LC-QqQ. One advantage of GC-QqQ vs LC-QqQ is the detection of NG, a category I compound successfully detected in GC. Moreover, GC-QqQ and LC-MS/<S offered complementary information when measured on the same sample, improving accuracy (100% with characteristic OGSR criteria).

Table 11 shows the quantitative results obtained by LCMS/MS for the first dataset, indicating some differences in the ammunition formulations. Results of the study showed that LC/MSMS analysis yields superior performance rates compared to GC/MS analysis. This is attributed to the low concentrations of OGSR analytes detected on authentic shooter samples collected from the hands of an individual. Most detected analytes in this set were below the detection limits of the GC/MS instrument but above those of the LC/MSMS method.

These findings had several implications. From a research perspective, LC/MSMS shows fit for purpose. Therefore, to ensure accurate analysis in the remaining of our project, only LC/MSMS was used to analyze authentic shooter samples and all transfer and persistence samples using the OGSR standard mixture. Although LC/MSMS cannot detect nitroglycerin or 2,4-DNT, samples were also run by electrochemistry, providing the necessary detection for these two OGSR compounds. From a practitioner's perspective, this study demonstrates that LC/MSMS is better suitable for OGSR

detection. but raises a warning flag that the classification of characteristic OGSR was unreliable for GC-MS detection when using alone. Combination of GC-MS with LC-MS/MS or with other screening tools like ECD shown to enhance overall performance rates. The results of GC/MS varied with ammunition type and calibers, and therefore it is recommended to evaluate its performance under other firearms, calibers, and ammunitions that more extensively represent common specimens received in casework. More sensitive instrumentation (i.e., high-resolution MS) can overcome some of the regular quadrupole GC-MS limitations. The recommendations from this study are anticipated to offer a path forward in the field.

Table 10. *Figures of Merit for GC-QqQ, GC/MS and LC/MSMS Techniques for Common OGSR Compounds, using the OGSR standards.*

	Figures of Merit					
	Compound	**LOD (ppb)**	**LOQ (ppb)**	**%RSD Intra-day**	**%RSD Inter-day**	**LDR (ppb)**	**R^2**
GC-QqQ	**NG**	130	400	13.0	18.1	400-3200	0.994
	2,4-DNT	45	140	12.0	14.1	250-1000	0.994
	DPA	25	80	2.5	4.50	100-1000	0.997
	MC	50	150	4.5	10.0	250-1000	0.998
	EC	60	180	3.7	10.0	250-1000	0.998
	2-NDPA	65	200	4.1	12.4	250-1000	0.996
	AKII	100	310	6.4	14.1	500-2000	0.998
	4-NDPA	120	370	14.5	17.2	500-2000	0.998
	Compound	**LOD (ppb)**	**LOQ (ppb)**	**%RSD Intra-day**	**%RSD Inter-day**	**LDR (ppb)**	**R^2**
GC-MS	**NG**	110	320	7.2	16.3	500-3000	0.997
	2,4-DNT	80	240	3.9	14.3	250-3000	0.996
	DPA	60	180	2.7	7.9	250-3000	0.993
	MC	40	130	4	11.3	250-3000	0.999
	EC	60	180	5	13.9	250-3000	0.999
	2-NDPA	60	180	3.3	14.3	250-3000	0.999
	AKII	80	250	10.2	18.6	250-3000	0.993
	4-NDPA	130	400	7.2	18.1	500-3000	0.998
	Compound	**LOD (ppb)**	**LOQ (ppb)**	**%RSD Intra-day**	**%RSD Inter-day**	**LDR (ppb)**	**R^2**
LC-QqQ	**DPA**	3.4	10	3.7	10	10-200	0.999
	MC	0.3	0.9	2.7	11	1-200	0.999
	EC	1.0	3.0	1.1	9.3	5-200	0.999
	2-NDPA	2.7	8.2	4.6	4.0	10-200	0.997
	AKII	0.3	0.9	1.3	4.8	1-200	0.999
	4-NDPA	3.0	9.0	7.9	6.4	10-200	0.999

Table 11. *Concentrations of Eight Major OGSR Analytes Collected from the Hands of Shooters using Four Brands of Ammunition by LC-MS/MS (AK II, MC, EC, 4NDPA, DPA) and GC-MS (NG and 2,4-DNT).*

		Summary of Analyte Concentrations Detected on hands' residues (ppb)							
		AK II	**MC**	**EC**	**4NDPA**	**DPA**	**2NDPA**	**NG**	**2,4DNT**
Federal 9mm	**Range**	0.50-55	0	10.-23	3.1-6.9	2.8-47	3.4-6.5	129-405	9.2-10
	Mean	12	0	3.78	4.04	14	4.5	200	9.6
	Median	4.5	0	1.7	3.8	7.6	4.1	133	9.6
Blazer 9mm	**Range**	0.45-150	0	0.32-150	2.7-10	2.6-138	3.3-14	139-5468	9.0-9.6
	Mean	14	0	13	5.0	18	5.6	1031	9.3
	Median	0.92	0	1.0	4.2	5.6	4.5	422	9.3
Blazer 40	**Maximum**	0.72-849	0	0.69-166	2.6-177	4.3-1343	3.8-54	128-144375	11-12
	Mean	141	0	30	35	281	18	32020	11
	Median	67	0	13	8.1	110	9.0	2102	11
Winchester 40	**Maximum**	1.0-239	0	0.78-59	6.3-42	5.0-641	3.9-46	123-1643	11
	Mean	24	0	11	17	170	15	429	11
	Median	2.0	0	6.2	13	93	12	190	11
Overall Predicted Concentration Range		0.45-849	n.a	1.0-166	2.6-177	2.6-1343	3.3-54	123-144374	9.0-12
Overall Average Predicted Concentration		323	n.a	100	59	542	30	37973	11

A critical aspect for interlaboratory testing was to demonstrate the stability and storage conditions of these standards, which we briefly described below in task 1.2.

Task 1.2: Results for the stability and collection efficiency of the developed IGSR and OGSR standard

From the solvents tested, methanol showed the best performance and was compatible with multiple techniques of interest. The main effects on storage conditions were calculated to determine the factor(s) that had the most significant impact on the tested result. In this study, the main effect was the concentration of OGSR analytes in the standard. The average response of all monitored OGSR analytes was used as the standardized response factor for further Plackett Burman calculations.

Standardized effects calculations and graphs were performed to assess the main effects of the experimental design for each OGSR solvent according to the ASTM standard for conducting ruggedness testing. **Figure 17** illustrates the results for OGSR analytes solvated in methanol.

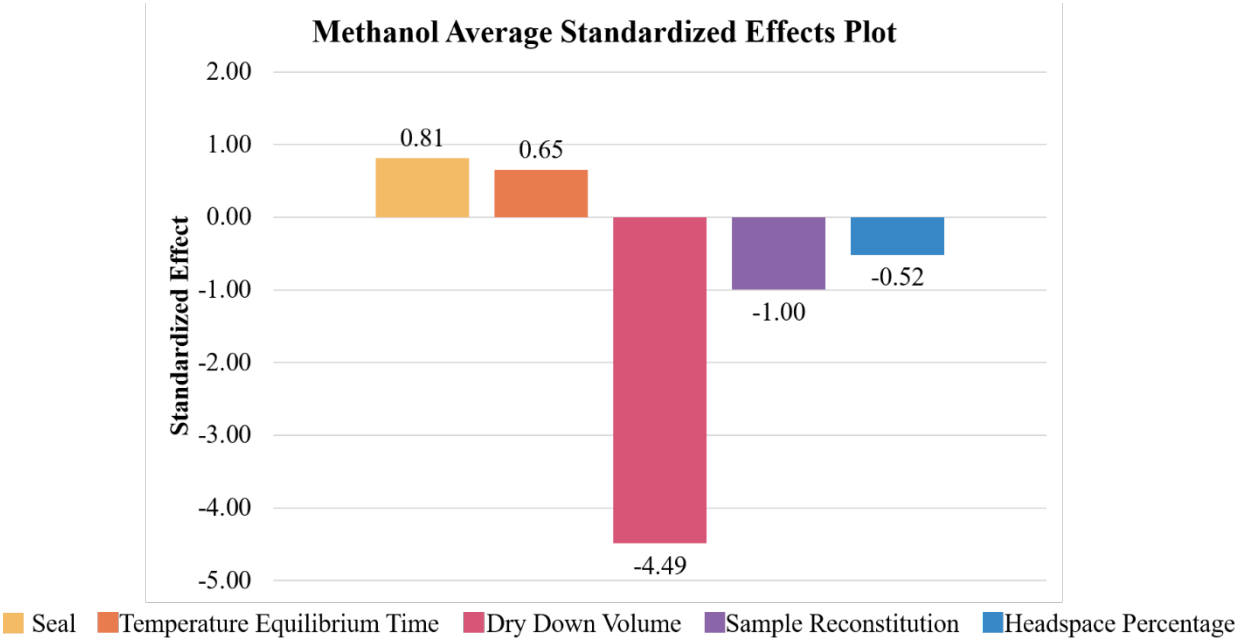


Figure 17. *Average standardized effects chart of the Plackett-Burman Ruggedness test on the methanol OGSR standard*

The factor that had the most significant impact on analyte recovery was the dry-down volume, with an absolute standardized effect of 4.49. The remaining factors equally affected the percent recovery of OGSR analytes with lower standardized effects. Therefore, the dry-down volume was the only factor ruggedly controlled. As a result, vials were dried down completely to achieve optimal percent recoveries. Lower concentrations observed in partially dried samples were due to variability in leaving 50 µl in the vial. Since the other factors had lower impacts on analyte recovery, procedures were chosen based on ease of sample preparation. Therefore, the final parameters included thawing the stock solution to room temperature for 15 minutes, reconstituting based on volumetric measurements, maintaining a maximum of 10% headspace in the standard solution, and using PolySeal® caps to prevent evaporation of solvent and analytes over time. These conditions and procedures were followed for the remainder of the studies to ensure satisfactory recovery of analytes.

LC-MSMS results of the Stability of OGSR Standard

The stability of the methanolic standards was tested at 0, 2, 4, 6, 8, 12, and 14 weeks, following the optimal storage conditions previously described. The concentration of each analyte was determined using LC/MSMS. Concentrations over the 14 weeks were assessed using ANOVA and Tukey-Kramer statistical testing. The compounds had superior stability over 14 weeks in methanol compared to ethanol or acetone storage conditions. These results confirm that organic gunshot residue standards can be stored in methanol for lengthy periods in a cooled environment.

Figure 18 shows the stability study for two OGSR analytes, Akardite II and Methyl Centralite, evaluated over 14 weeks. The central line represents the analyte's overall mean concentration, while

the main line of each diamond represents a specific group mean with a 95% confidence interval span on either side. Similar sizes of the overlapping Tukey-Kramer circles indicated comparable variances between weeks and confirmed concentration stability up to 14 weeks. This trend was observed for all six analytes capable of detection through LC-MS/MS analysis (i.e., AK II, MC, EC, 4-NDPA, DPA, 2-NDPA). Final concentrations observed for each analyte were: AK II (81.7±2.3 ppb), MC (76.7±4.3 ppb), EC (84.0±2.4 ppb), 4-NDPA (83.3±2.5 ppb), DPA (65.7±1.4 ppb), and 2-NDPA (71.0±1.3 ppb). Furthermore, intra-week variability was below 6% RSD for all analytes across the 14-week testing period. These results prove that the OGSR solution satisfied critical reference standard criteria like homogeneity, known analyte composition and concentration, and sustained stability. Ultimately, the known composition and stable concentration of the analytes offered confidence for future use of the OGSR standard in systematic deposition, recovery, transfer, and persistence studies. Continuous monitoring of these standards has shown stability for up to 52 weeks when storage conditions are controlled, and the solutions are maintained at -4C.

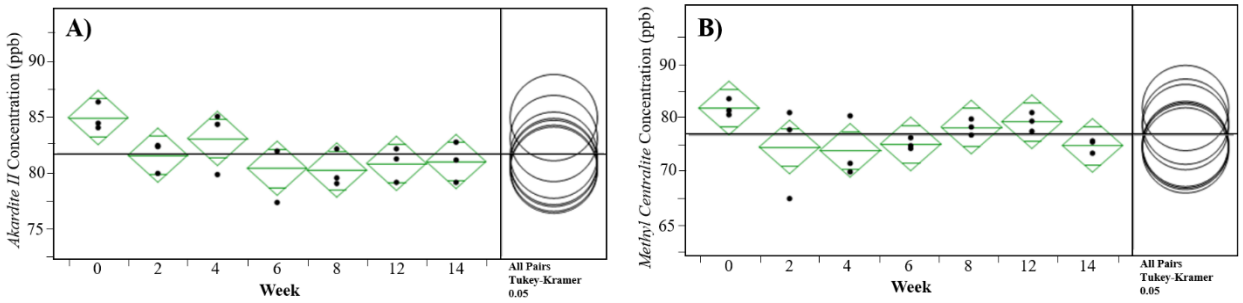


***Figure 18**. ANOVA and Tukey-Kramer results for akardite II and methyl centralite analytes contained in OGSR standard solution. Results show the stability of analytes concentration over 14 weeks.*

Electrochemical results of the Stability of OGSR Standard

Electrochemistry was also used to assess the stability of the 1-ppm OGSR standard created for the deposition onto hands and clothing for the persistence and transfer studies. OGSR compounds were chosen for both LC-MS/MS and electrochemical analysis. The OGSR standard mixture was analyzed every two weeks over a 12-week window and then again at 20 weeks. While the standard contained several common OGSR compounds, several were not detected by electrochemistry due to being below detection limits or interference with oxidation potential resolution. Nitroglycerin is a good example of this, where the oxidation peak cannot be resolved with diphenylamine when in solution at the same concentration and has been reported previously by our research group. Electrochemistry detected the stability over 20 weeks for 2,4-dinitrotoulene, ethyl centralite, diphenylamine, 4-nitrodiphenylamine, and 2-nitrodiphenylamine. (**Figure 19).** The procedure for electrochemical analysis is depicted in **Figure 20** and was repeated in triplicate at every time interval.

Following EC analysis, the peak current area signals from electrochemical detection were used to assess the stability of the standard over weeks using ANOVA analysis. The results presented in Figure **19** show good stability for all analytes with their respective Tukey Kramer output for comparing the means. Regarding the 2,4-DNT stability, the week 20 results of 2,4-DNT saw a slight drop in the oxidation signal.

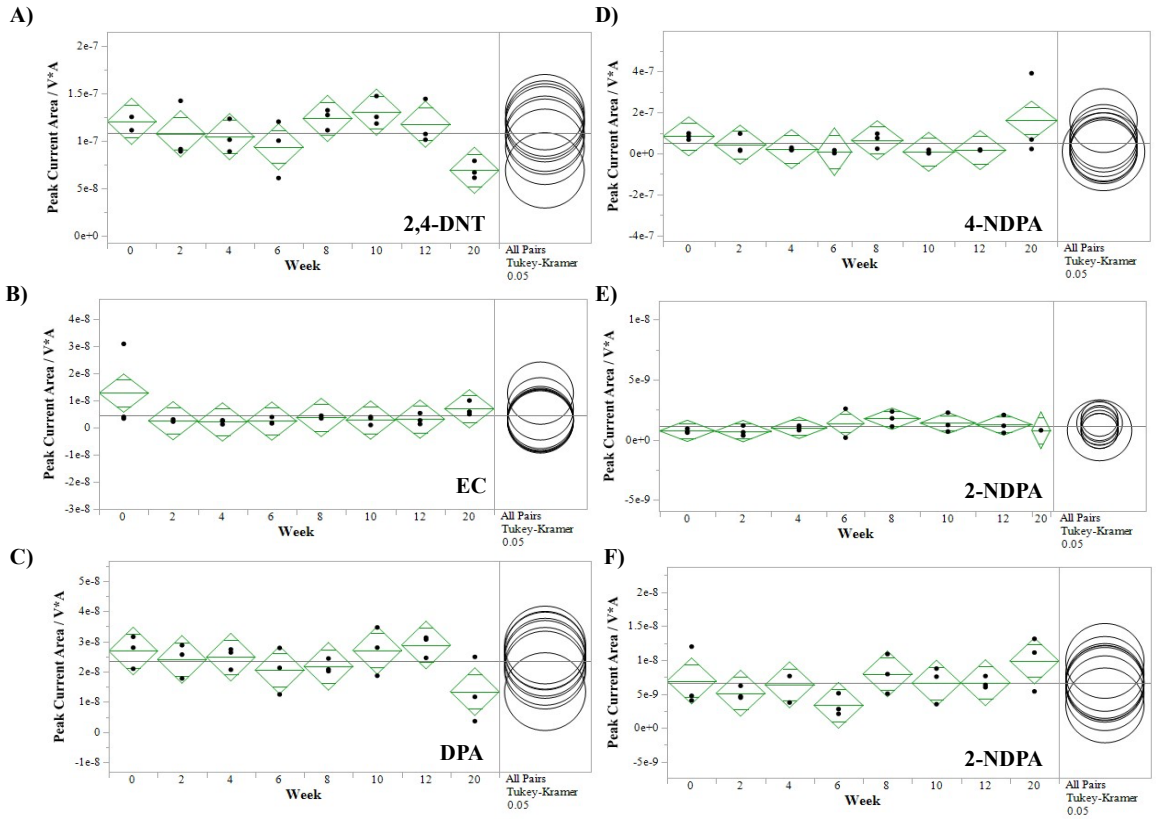


Figure 19. *ANOVA results of the 1-ppm OGSR stability via electrochemistry for the oxidation peak current areas for A) 2,4-Dinitrotoluene, B) Ethyl centralite, C) diphenylamine, D) 4-nitrodiphenylamine, E) the first oxidation of 2-nitrodiphenylamine at 0.2 V, and F) the second oxidation of 2-nitrodiphenylamine at 0.6 V.*

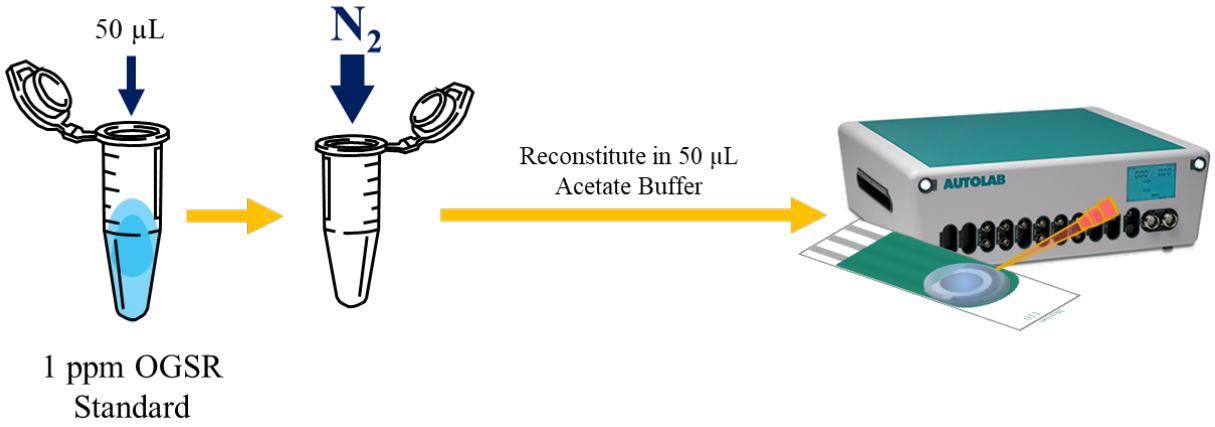


Figure 20. *Procedure for the stability study of 1-ppm OGSR standard via electrochemical detection.*

Testing for Recovery Efficiency of OGSR Standard from GSR Carbon Adhesive and Synthetic Skin Membranes

Perhaps even more impactful for the GSR field was the combination of OGSR and IGSR standards for transfer and persistence studies. A recovery testing was designed for the methanol-OGSR standard

on carbon stubs and synthetic skin membranes. **Table 12** shows the analyte percent recoveries using both extraction procedures. While both extraction procedures showed excellent recovery rates, with the lowest being 68% for MC when performing stub washes, an exhaustive extraction procedure was favorable for most analytes.

Table 12. *Percent Recoveries of OGSR Analytes from Carbon Stubs using Stub Wash and Exhaustive Extraction*

Exhaustive Carbon Extraction		*Carbon Adhesive Stub Washes*	
Analyte	**Percent Recovery**	**Analyte**	**Percent Recovery**
Akardite II	98 ± 13	**Akardite II**	92 ± 10
Methyl Centralite	73 ± 11	**Methyl Centralite**	68 ± 6
Ethyl Centralite	109 ± 14	**Ethyl Centralite**	102 ± 9
4-Nitrodiphenylamine	102 ± 13	**4-Nitrodiphenylamine**	79 ± 7
Diphenylamine	102 ± 12	**Diphenylamine**	73 ± 7
2-Nitrodiphenylamine	104 ± 12	**2-Nitrodiphenylamine**	78 ± 8

Furthermore, similar recovery studies were performed on the synthetic skin membrane of choice; Strat M. Strat M membranes were cut into thirds, and each section had 100 µL of the methanol OGSR standard deposited onto the surface. Once the solvent was dried, the residues were extracted from the membrane using two methods. The first method involved stubbing the membrane surface five times with a carbon adhesive GSR stub. This was to simulate the authentic collection method currently used in the field. Analytes collected on the carbon adhesive were then extracted using 6 x 50 µl (300 µl total) of methanol.

The second method followed an exhaustive extraction where the entire membrane section was submerged in 500 µl of methanol and sonicated for five minutes. As seen in **Table 13**, stubbing the membrane surfaces using a carbon adhesive GSR collection stub resulted in little to no recovery of analytes. This may be due to the composition and structure of the Strat M membrane.

Since synthetic skin membranes are constructed to model the movement of organic molecules (i.e., medications, skin care products) through human skin layers, the surface is more porous than the carbon adhesive of a GSR stub. Therefore, during the depositing of the OGSR standard, the solvent permeates the surface of the membrane and moves to the "inner layers," making it difficult to recover analytes from the surface. This is indicated in the high recovery percentages observed when an exhaustive extraction is performed (≥81%). Therefore, exhaustive extractions were performed in the experiments simulating the transfer and persistence of OGSR analytes. Although this is not a direct comparison to skin recoveries, performing exhaustive extractions will still provide overall information on evaporation trends and loss of OGSR over time and after performed activities. In addition, performing an exhaustive extraction of the membranes allows for IGSR particles to be collected using a carbon stub while the OGSR analytes remain in the membrane for later extraction. This allows for comprehensive IGSR and OGSR analysis on a single membrane sample.

The final recovery testing included deposition of both IGSR and OGSR standards onto the synthetic skin membrane (Strat M) and assessing the ability to recover all analytes from a single membrane. Initial investigations showed that deposition of both standards onto the Strat M had its challenges; (1) depositing acetone (IGSR) onto the membrane first caused curling of the edges, and (2) adding methanol after the acetone was dried created a solvent front that pushed IGSR particles towards the

edge of the membrane. The curling of the membrane edges resulted in low retention of the methanol (OGSR) and the solvent front resulted in low recoveries of the IGSR particulate. Furthermore, excessive dry-down times over ~90 minutes were observed when depositing both standards simultaneously.

Table 13. *LC/MSMS Recoveries of OGSR Analytes Achieved from Synthetic Skin Membranes using Two Extraction Methods. (NR: Not Recovered)*

Exhaustive Membrane Extraction		*Stub Collection and Washes*	
Analyte	**Percent Recovery (n=6)**	Analyte	**Percent Recovery (n=6)**
Akardite II	82 ± 8	Akardite II	NR
Methyl Centralite	94 ± 10	Methyl Centralite	NR
Ethyl Centralite	86 ± 8	Ethyl Centralite	NR
4-Nitrodiphenylamine	81 ± 9	4-Nitrodiphenylamine	NR
Diphenylamine	98 ± 7	Diphenylamine	NR
2-Nitrodiphenylamine	90 ± 6	2-Nitrodiphenylamine	NR

Therefore, an experiment was designed to test two different deposition procedures and IGSR ratios (**Figure 21**). Two different ratios of IGSR standards were tested to increase lead (Pb) recovery rates. Initial testing included the deposition of only a Winchester brand IGSR standard. However, low recoveries were seen for the lead when analyzed by LIBS and SEM-EDS. Therefore, TulAmmo was used in addition to Winchester. TulAmmo was chosen because it lacks barium in composition but has a high concentration of lead (~16.1 ppm) compared to Winchester (~5.0 ppm). Furthermore, a higher concentration of OGSR (5ppm) was deposited in a smaller amount (20 µl) to aid in decreasing dry-down time, while still depositing the same mass of analytes in previous studies (100 ng).

To perform the testing, the OGSR standard was deposited onto the membrane first and allowed to dry, followed by IGSR deposition. Another experiment was conducted by switching the deposition order of IGSR/OGSR. (**Figure 21**). Immediately after both sets of solvents were completely dry, each membrane was first stubbed using a carbon adhesive to collect IGSR and then exhaustively extracted to collect OGSR.

Table 14 shows the dry-down times observed during each deposition test. In general, depositing the OGSR standard first, followed by the IGSR standard, resulted in drastically lower dry-down times than the opposite deposition order. On the other hand, the ratio of IGSR standards had little effect on the overall dry-down times. The excessive dry downtime still observed in experiment D was due to curling on the membrane, so the solvent localized in one position rather than spreading into the membrane.

As a result, all membranes were secured to a flat surface using double-sided tape. It was also observed that adding the OGSR before the IGSR eliminated the creation of a solvent front and resulted in high recoveries of the IGSR particles deposited on the membrane surface. Additionally, adding acetone to the membrane after OGSR deposition did not result in substantial analyte evaporation, as

demonstrated in **Table 15**, where high recoveries were still observed for replicates A and B. While all deposition methods resulted in comparable recovery rates of OGSR analytes, IGSR recovery benefited from a 30:70 ratio of Wincehster: TulAmmo.

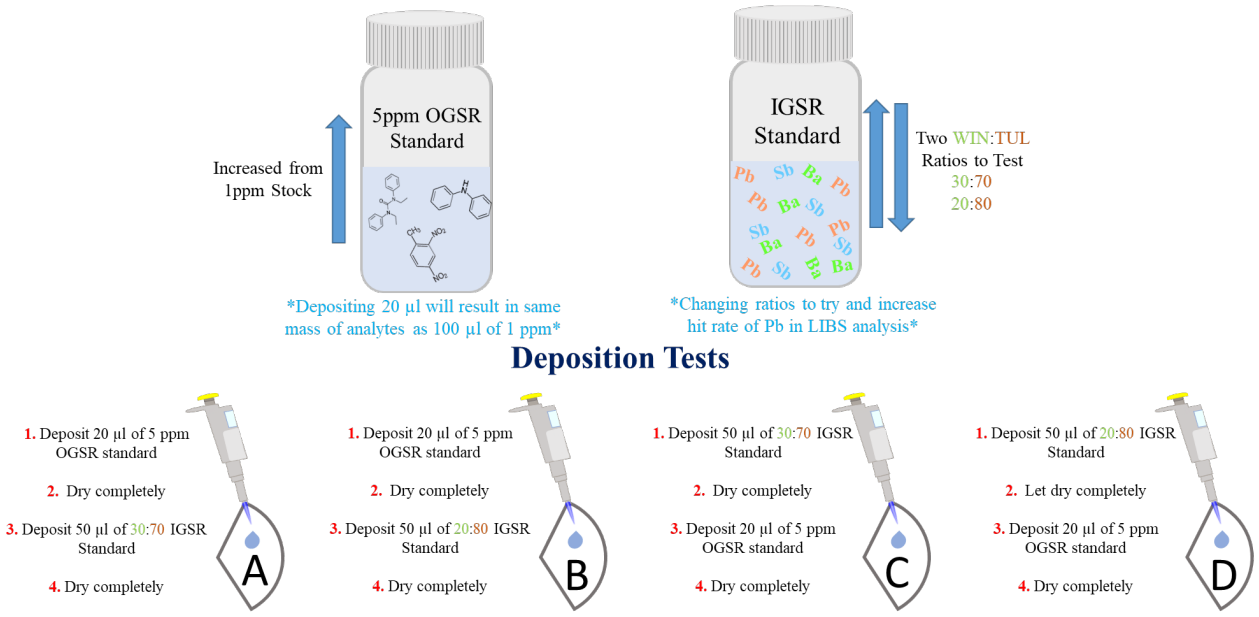


Figure 21. *OGSR and IGSR Deposition Testing Procedure*

Based on these results, the ongoing studies for transfer and persistence include the following procedure: (1) depositing 20 µl of 5ppm OGSR standard and dry, (2) depositing 50 µl of a 30:70 (WIN:TUL) IGSR standard and dry, (3) completing time interval waiting time or specified activity, (4) stubbing membrane surface to collect IGSR particles, (5) performing an exhaustive extraction on the membrane to collect OGSR analytes.

Table 14. *Observed Dry Down Times of GSR Standards During Different Deposition Procedures*

A		B		C		D	
OGSR	5 min	OGSR	4 min	IGSR	11 min	IGSR	10 min
IGSR	12 min	IGSR	10 min	OGSR	23 min	OGSR	60 min
Total	17 min	Total	14 min	Total	34 min	Total	70 min

Table 15. *Observed OGSR Analyte Recoveries during Different Deposition Procedures*

A		B		C		D	
AK II	92%	AK II	80%	AK II	75%	AK II	65%
MC	92%	MC	77%	MC	73%	MC	69%
EC	91%	EC	71%	EC	67%	EC	62%
4NDPA	79%	4NDPA	67%	4NDPA	69%	4NDPA	67%
DPA	84%	DPA	67%	DPA	75%	DPA	73%

Stability of Inorganic Gunshot Residues Micro-Particle Standard by ICP-MS

The stability of five leaded (Federal, Sellier & ANOVA Bellot, TulAmmo, Winchester) and five lead-free (CCI, Fiocchi, Heavy, Inceptor, Syntech) IGSR micro-particle standards was proven by our group over 52 weeks. For in-depth results and discussion of this study, please refer to our group's previous publication[11] and Korina Menking-Hoggatt's NIJ fellowship report (#2018-R2-CX-0009)[12]. **Figure 22** shows ANOVA and Tukey Kramer analysis examples for two tailor-made inorganic standards (*Leaded:* TulAmmo, *Lead-free*: Inceptor). Overall, the study results confirm that the elements of interest in each inorganic micro-particle standard (a.k.a. *p*GSR standard) stock solutions remain stable over a 52-week storage period.

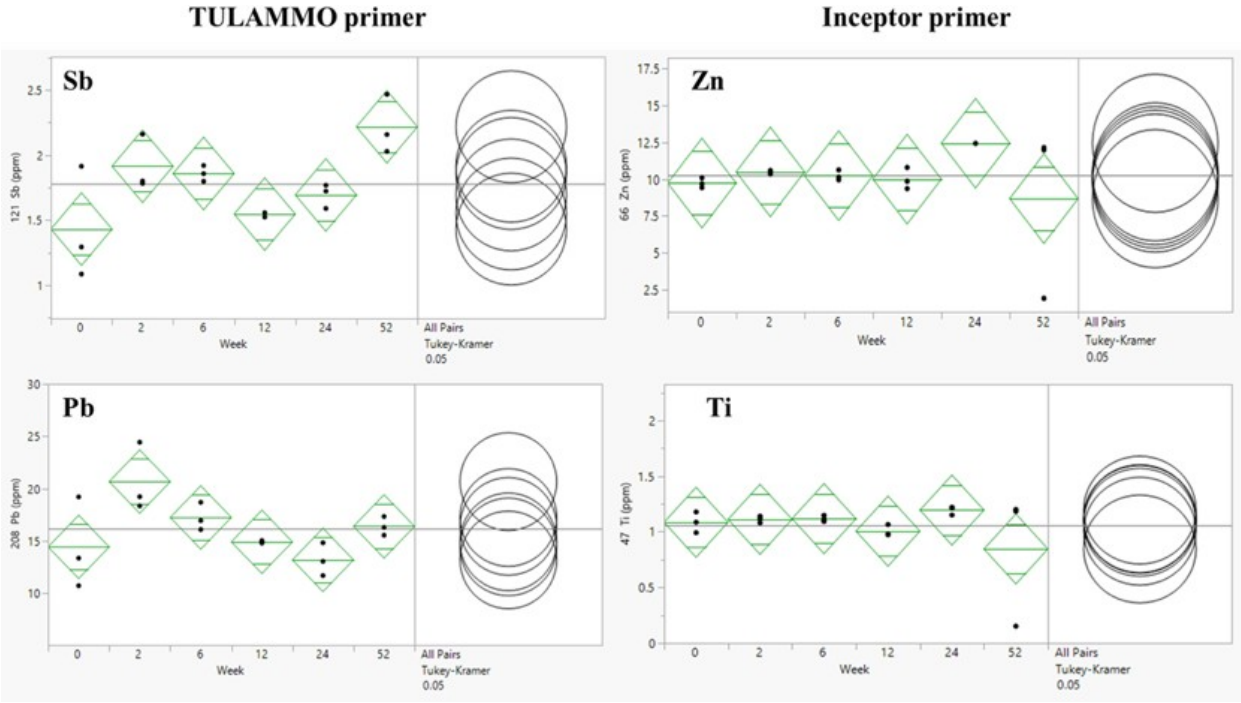


Figure 22. *ANOVA and Tukey-Kramer results for two inorganic micro-particle standards during a 52-week storage period.*

In summary, the pGSR and OGSR standards proved useful for method development, method validation, interlaboratory testing, and conducting controlled experiments where the known particle counts, and organic composition are necessary. The solvent's stability and quick evaporation allowed their use in liquid or solid form. The standards can be effectively applied in a variety of substrates (i.e., carbon stubs, adhesives, synthetic skin membranes, polymers, fabrics, glass, etc.), and are compatible with a large suite of instrumental methods. For instance, the standards have been utilized for analysis in SEM-EDS, LIBS, ECD, GC methods (GC/MS, GC/FID, GC-QqQ), DART-MS, LC-MS/MS, ICP-MS, LA-ICP-MS. [1-16] The long shelf life and the parent particle and concentrations allow thousands of uses per stock solution. After their manufacture and characterization in 2020, we have been using the stock solutions in various stages of our research with no significant changes in their performance. The in-situ combination of pGSR and OGSR standards also opens new possibilities to study the combined occurrence and identification of inorganic and organic constituents in GSR. Overall, the versatility of these standards has made possible their use in research for a multitude of

experimental designs. Indeed, after our publication in Talanta[11], various researchers and practitioners have reached out to us requesting some of our standards for testing their methods, so the standards are already having an impact on the scientific community.

Task 1.3: Results for the visualization of IGSR and OGSR after discharging a firearm

During the firing process, GSR is released in varying patterns and directions, but the mechanisms of deposition and transfer still need to be fully understood. Because of this, it can be challenging to determine if a suspect with GSR is the person who fired the firearm or simply has acquired GSR by other means, such as being a bystander to a crime. Because of the difficulty in differentiating between these two, it is important to enhance the current understanding of GSR deposition, transference, and persistence after a firing event. This study employed inexpensive custom-made atmospheric samplers that can determine the density of airborne particles and a more robust particle counting system. Additionally, high-speed videography and laser sheet scattering are proposed to investigate visual information about the flow of GSR plumes under various controlled experimental conditions. SEM-EDS and LC-MS/MS were used as complementary tools to confirm the elemental and chemical makeup of particles detected by the sensors.

Figure 23 shows the experimental display that generates multiple data to observe GSR dynamics, measure particles and detect OGSR and IGSR. We hypothesize that the counting and distribution of micron-sized particles, along with tools that can visualize the flow dynamics of their movement, can provide estimates of the number of GSR particles that are produced during a firing event under controlled conditions, estimate how long it takes to settle in surfaces located at various distances and heights from the firing spot, evaluate risks of indirect deposition and transfer, and develop enhanced protocols for sampling indoor environments. We anticipate that combining these sampling and visualization tools will provide breakthrough knowledge in forensics, which can also be expanded to other disciplines where airborne exposure is central such as environmental and public safety. Three main questions were addressed during this study's experimental design **(Table 16).**

Table 16*. Questions of interest were used during the experimental design process to direct portions of this study.*

GSR Visualization - Questions of Interest

GSR particle counts and particle size distribution	GSR transfer to bystander and passerby	Deposition and settling of GSR particles
What GSR particle sizes and distributions are observed?	Can we observe GSR particles from bystanders?	How far away can we detect GSR particles from the shooting position?
Are there differences in count/distribution when comparing a single/multiple shots?	Does the number/distribution of particles change significantly in respect to the shooter?	How long do particles persist in the enclosed area?
Are there differences when comparing a pistol vs. a revolver?	Can we observe GSR particles from a person entering the shooting room shortly after?	Does airflow in indoor environments influence the number of particles observed?
	Does the number/distribution of particles change significantly in respect to the shooter?	
	Does the GSR risk of exposure change significantly for IGSR and OGSR?	

The PMSA003 sensor outputs data in the form of particle densities (#/cm^3). However, time was also

monitored to estimate particle counts per minute. Calculating the number of particles counted in a given time was possible by measuring the sensors' flow rates with an ADM 1000 Flowmeter (Agilent Technologies, Santa Clara, CA, USA).

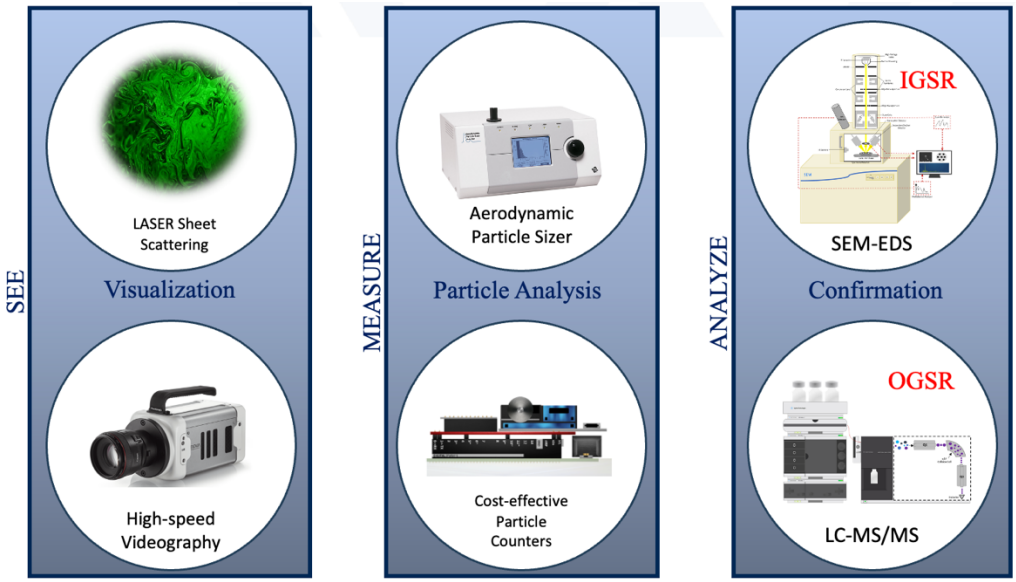


Figure 23 *Overview of the experimental display that generates multiple data to observe GSR dynamics, measure particles, and detect OGSR and IGSR*

This was helpful, as the device was adapted with a carbon stub on the flow output to retain particles on the adhesive and monitor the particle composition and morphology with SEM-EDS. This set-up also aims to evaluate its potential use as an airborne particulate pre-concentration device in indoor settings. However, it is important to note that the correlation between particles seen by these sensors in atmospheric sampling and the particles deposited on surfaces surrounding firing (i.e., particles collected from an individual's skin or surface using carbon tape) is still unknown and will be evaluated in future experiments.

The first piece of information obtained in this preliminary study was the average particle size distribution. The sensors bin particles into six sizes: 0.3 µm, 0.5 µm, 1.0 µm, 2.5 µm, 5.0 µm, and 10.0 µm. The particle size distribution for a single shot from the Springfield XD-9 can be seen in **Figure 24.** This was taken across sensors 1 to 4, which were determined to be the most comparable due to their placement near the shooter and directly beside each other. As can be seen in the figure, the vast majority (93%) of particles are smaller than 1.0µm. This is an important finding, as particles of this size are not easily characterized in SEM-EDS analysis, with 1µm usually being the lower limit that can be accurately detected by most SEM-EDS methods used at forensic laboratories. This critical finding is worth sharing with the forensic and chemistry communities; however, we recognize that the particle size bins must be cross-calibrated on the particle sizers before drawing any generalizations.

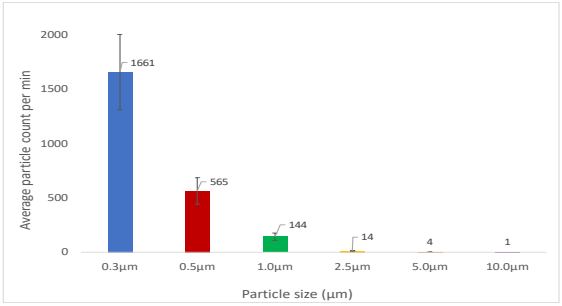


Figure 24. A graph detailing the number of particles

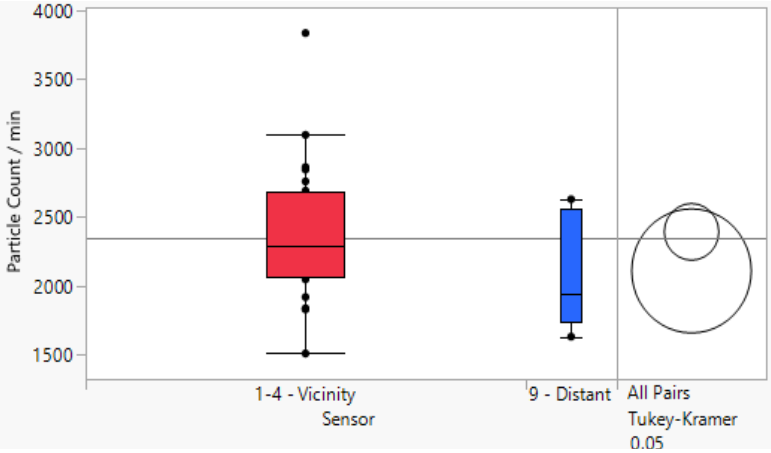


Figure 25. Comparison of average particle counts per minute between vicinity particle counters and the distant particle counter with the Springfield XD-9

Figure 25 shows an example of the time-elapsed particle counts per size monitored on one of the sensors at the vicinity of the shooter. Some interesting similarities can be seen by comparing sensors 1 to 4 near the shooter, with distant sensor 9, located 14 feet behind the shooter. There is some lower level of particles in the distant sensor, but the mean particle counts are not significantly different (t-test, 95% confidence, p=0.05, **Figure 26**). However, it took the particles' cloud, on average, 80 seconds to reach the distant sensor. At a distance of 14 feet from the firearm, this experiment shows the speed at which a GSR particle cloud can move in an indoor environment with no airflow disturbances (i.e., no open windows or doors, no AC). These findings show that the number of particles released in a shooting event is large and can quickly populate indoor rooms. Therefore, those particles can settle and deposit in areas or individuals near the firing.

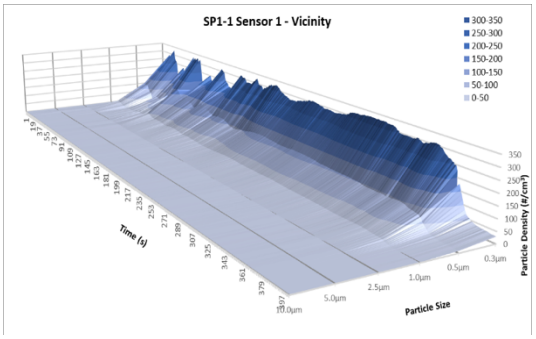


Figure 26. A graph showing the particle density recorded each second for a total of 400 seconds

During the extended sampling study, the particles reached a maximum concentration of approximately 325 particles per cubic centimeter within seconds of the shot being fired. However, this continually fell linearly over the next hour. By finding the slope of this line and extrapolating it, it is possible to predict how long it will take for the particle concentration to return to pre-shooting background levels. For the smallest of particles at 0.3µm, it was estimated that it would take approximately 3 hours for most particles to settle and airborne particulates to return to baseline levels. These findings were further confirmed by repeating the experiment for longer collection times (Figure 31)

While the revolver and pistol showed statistically similar particle counts, the size distribution of particles differed greatly between single and multiple shots. As shown in **Figure 27**, a significantly higher number of 0.5 µm particles were detected after 5 shots, while 0.3 µm dominated the one-shot firings. However, it was determined that there was no significant difference in particle counts when comparing the pistol and revolver, as shown in **Figure 28.** On the other hand, the number of particles generally increased with the number of shots although there is not a linear relationship.

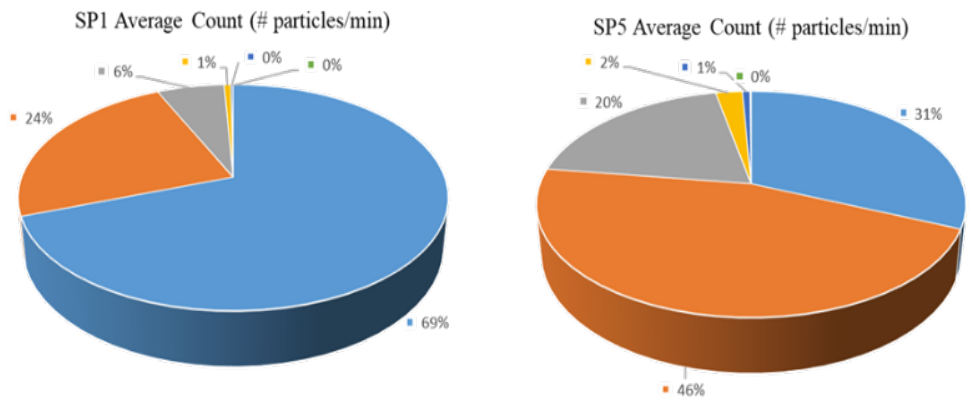


Figure 27. Particle distributions displayed in a pie chart to compare the relative ratios of particle sizes observed in a single vs multiple shots

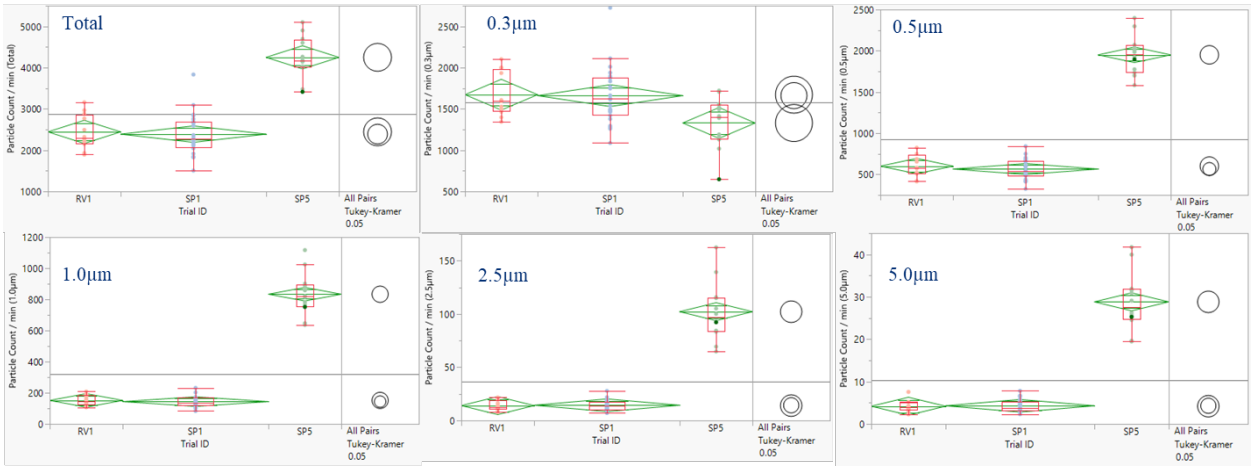
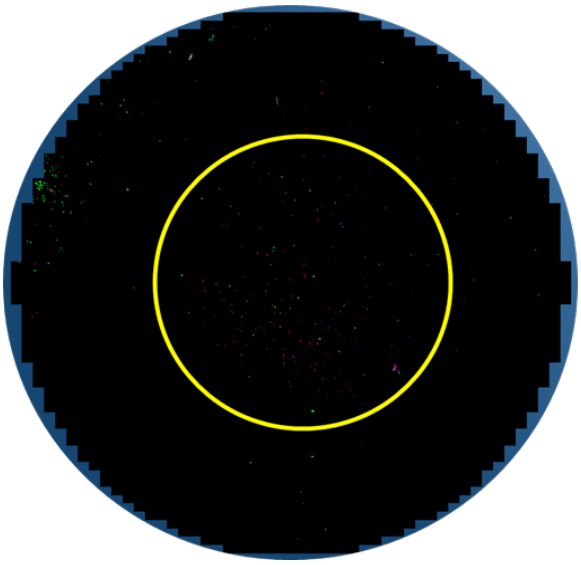


Figure 28. ANOVA of the six particle sizes binned by the custom-made atmospheric samplers comparing the average number of particles counted per minute separated by firearm type and number of shots fired.

The results of preliminary preconcentration testing are shown in **Figure 29**. This carbon adhesive was fitted to sensor 1. The sensor was turned on for five minutes following a single shot by the Springfield XD-9. The area of the carbon adhesive that was directly exposed to the sampler outlet shows the highest concentration of particles. In total, 377 GSR particles were detected. While this is fewer than the number of particles that pass through the sampler in a given trial, it is important to remember that our SEM-EDS system is set to detect particles above 1 µm, and atmospheric samplers are non-discriminatory in their counting and, therefore, can count particles that are not considered GSR (i.e., dust and debris).



Class	Rank	Features
Pb Sb Ba	Characteristic - Pb bearing	123
Pb Ba Ca Si	Consistent - Pb bearing	1
Ba Ca Si	Consistent - Pb bearing	22
Sb Ba	Consistent - Pb bearing	15
Pb Sb	Consistent - Pb bearing	56
Ba Al	Consistent - Pb bearing	2
Pb Ba	Consistent - Pb bearing	8
Gd Ti Zn	Characteristic Pb-Free/Non-Toxic	0
Ga Cu Sn	Characteristic Pb-Free/Non-Toxic	0
Ti Zn	Consistent Lead-Free/Non-Toxic	2
Sr	Consistent Lead-Free/Non-Toxic	0
Pb	Commonly Associated with GSR	60
Sb	Commonly Associated with GSR	22
Ba	Commonly Associated with GSR	52
	No Classification	377

Figure 29. Results from SEM-EDS analysis of carbon adhesive tape fitted to the air outlet of a custom-d t heri le Are i led i llo d t the that di tl d t the tlet

The information captured via particle sensors is complemented by high-resolution videos that provide a wealth of information about the creation, movement, and deposition of GSR during and after a firing event. **Figure 30** illustrates an image of the GSR cloud expansion 5 seconds after firing. This study provides a proof of principle on the utility of the proposed visualization tools and particle counters to provide insights into the factors that can influence the deposition and transfer of GSR at a level never discussed before.

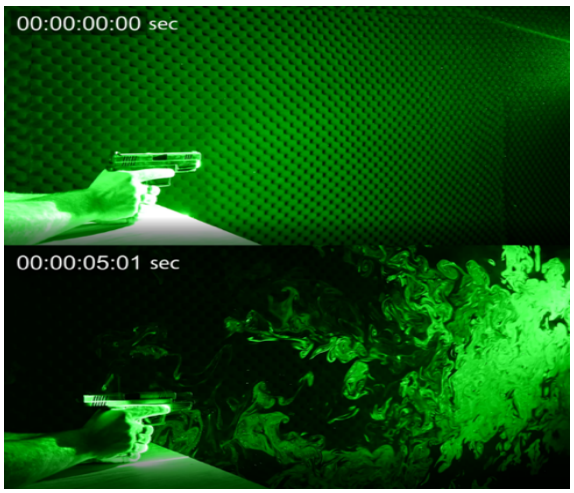


Figure 30 An example of what is observed visually with the laser sheet. Before (top) five shots were fired, and immediately after (bottom).

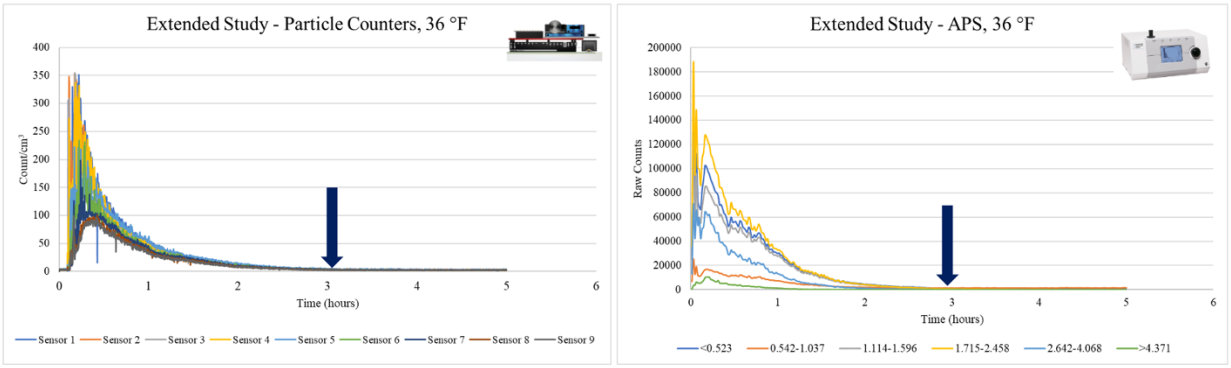


Figure 31. *Curves produced by particle counters (top) and APS (bottom) over a five-hour sampling period.*

The LC-MS/MS analysis collected from the sensors showed interesting results in **Table 17**. Of the 45 samples analyzed by LC-MS/MS from passive sampling, only two compounds were observed above LODs. AKII was observed in only one sample. However, in 14 of 15 synthetic skin samples, DPA was observed close to the detection limit. Due to the high prevalence of DPA unaccompanied by other OGSR compounds, it was hypothesized that a naturally small amount of DPA was found due to the formulation of the synthetic skin. Another study later confirmed this. These results suggest that OGSR is not deposited in high enough quantities to be observed by our LC-MS/MS method. The primary hypothesis for this is that the surface area of the carbon adhesive and synthetic skin is not great enough to allow for the deposition of a large enough amount of OGSR and that passive deposition on other surfaces is not as common for OGSR as it is for pGSR, where SEM-EDS analysis of the same trials yielded positive results.

Table 17. *Results of LC-MS/MS analysis of experiment 2 (PREC = preconcentrated stub, PASS = passive stub, VCN = vicinity, BYS = bystander, PBY = passerby, ND = not detected)*

Sample	AKII	MC	EC	4-NDPA	DPA	2-NDPA
01LC_PREC_VCN	ND	ND	ND	ND	ND	ND
01LC_PASS_VCN	ND	ND	ND	ND	ND	ND
01LC_PREC_BYS	ND	ND	ND	ND	ND	ND
01LC_PASS_BYS	ND	ND	ND	ND	ND	ND
01LC_PREC_PBY	ND	ND	ND	ND	ND	ND
01LC_PASS_PBY	ND	ND	ND	ND	ND	ND
02LC_PREC_VCN	ND	ND	ND	ND	ND	ND
02LC_PASS_VCN	ND	ND	ND	ND	ND	ND
02LC_PREC_BYS	ND	ND	ND	ND	ND	ND
02LC_PASS_BYS	ND	ND	ND	ND	ND	ND
02LC_PREC_PBY	ND	ND	ND	ND	ND	ND
02LC_PASS_PBY	ND	ND	ND	ND	ND	ND
03LC_PREC_VCN	ND	ND	ND	ND	ND	ND
03LC_PASS_VCN	ND	ND	ND	ND	ND	ND
03LC_PREC_BYS	ND	ND	ND	ND	ND	ND
03LC_PASS_BYS	ND	ND	ND	ND	ND	ND
03LC_PREC_PBY	ND	ND	ND	ND	ND	ND

Sample	AKII	MC	EC	4-NDPA	DPA	2-NDPA
03LC_PASS_PBY	ND	ND	ND	ND	ND	ND
04LC_PREC_VCN	ND	ND	ND	ND	ND	ND
04LC_PASS_VCN	ND	ND	ND	ND	ND	ND
04LC_PREC_BYS	ND	ND	ND	ND	ND	ND
04LC_PASS_BYS	ND	ND	ND	ND	ND	ND
04LC_PREC_PBY	ND	ND	ND	ND	ND	ND
04LC_PASS_PBY	ND	ND	ND	ND	ND	ND
05LC_PREC_VCN	ND	ND	ND	ND	ND	ND
05LC_PASS_VCN	ND	ND	ND	ND	ND	ND
05LC_PREC_BYS	ND	ND	ND	ND	ND	ND
05LC_PASS_BYS	ND	ND	ND	ND	ND	ND
05LC_PREC_PBY	ND	ND	ND	ND	ND	ND
05LC_PASS_PBY	ND	ND	ND	ND	ND	ND
01LC_SKIN_VCN	ND	ND	ND	ND	4.79	ND
01LC_SKIN_BYS	ND	ND	ND	ND	4.61	ND
01LC_SKIN_PBY	ND	ND	ND	ND	5.71	ND
02LC_SKIN_VCN	ND	ND	ND	ND	4.90	ND
02LC_SKIN_BYS	ND	ND	ND	ND	5.72	ND
02LC_SKIN_PBY	ND	ND	ND	ND	3.89	ND
03LC_SKIN_VCN	ND	ND	ND	ND	4.56	ND
03LC_SKIN_BYS	ND	ND	ND	ND	4.77	ND
03LC_SKIN_PBY	ND	ND	ND	ND	4.63	ND
04LC_SKIN_VCN	2.12	ND	ND	ND	5.10	ND
04LC_SKIN_BYS	ND	ND	ND	ND	ND	ND
04LC_SKIN_PBY	ND	ND	ND	ND	4.26	ND
05LC_SKIN_VCN	ND	ND	ND	ND	4.99	ND
05LC_SKIN_BYS	ND	ND	ND	ND	4.98	ND
05LC_SKIN_PBY	ND	ND	ND	ND	4.58	ND

SEM-EDS analysis was completed for four out of five trials. Unlike the results from the OGSR portion of this experiment, some IGSR was deposited on every stub by passive means. However, the number of particles depended greatly on the position of the stub to the firearm. Regardless of classification (characteristic, consistent with, or commonly associated with), the number of particles deposited onto the passive carbon stubs decreased as expected. In all trials, the stub located directly near the shooter had greater counts of particles than those located farther from the firearm (bystander, passerby). These comparisons can be seen in **Figure 32**. The trends in the results for passive collection stubs suggest that both distance & time have an inverse effect on the amount of IGSR deposited onto a stub.

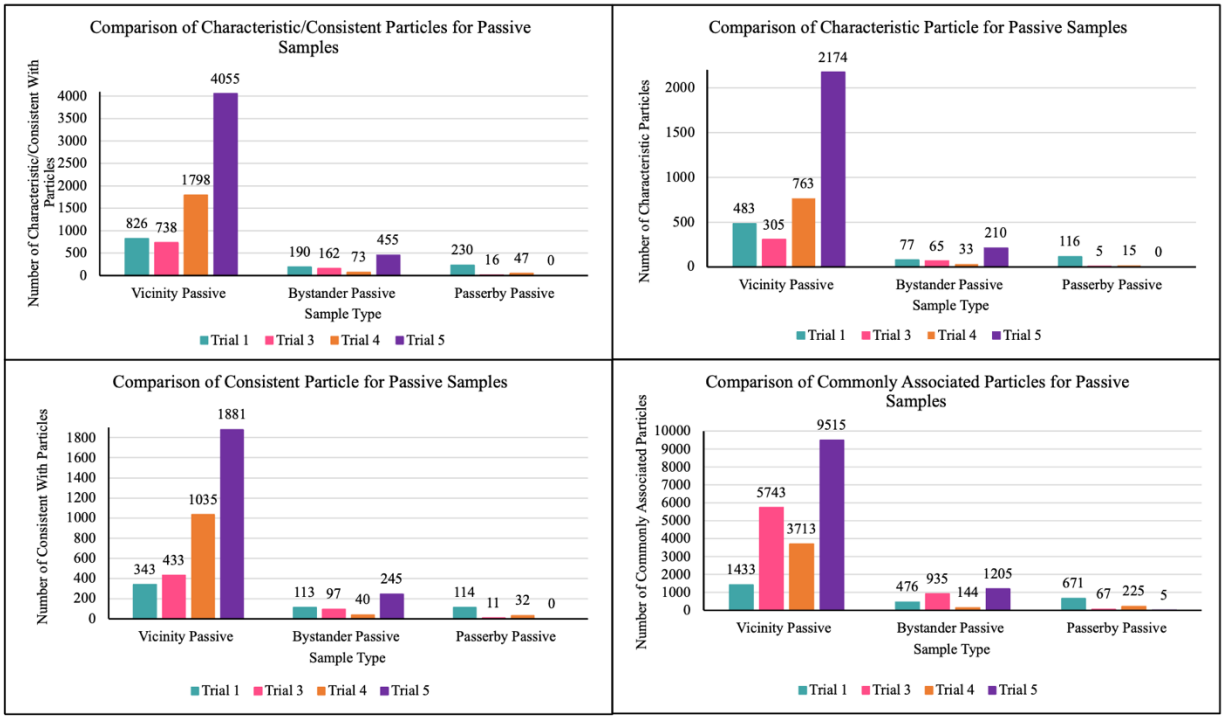


Figure 32. *Results of SEM-EDS analysis of passive collection carbon stubs separated by classification criteria.*

Because IGSR was present on every stub, it was also possible to evaluate the preconcentrating ability of the sensors. **Table 18** shows an interesting trend in this regard. For the vicinity sensors, the preconcentrated stubs had fewer particles deposited onto them in comparison to the passive stubs. However, the opposite trend was seen for the bystander and passerby preconcentrated stubs. This difference is under investigation and will be evaluated in future studies.

Table 18. *Averages and standard deviations from trials 1-4 are separated by location and collection type. Red triangles denote that preconcentrated stubs had fewer particles than passive stubs. Green triangles denote that preconcentrated stubs had more particles than passive stubs. A yellow square denotes similarity.*

Averages and Standard Deviations (Trials 1-4)					
Sample ID	Characteristic (Pb, Ba, Sb)	Consistent	Characteristic/ Consistent	Commonly Associated with	Total
Vicinity Passive	400 ± 300	466 ± 413	866 ± 701	2769 ± 2463	3634 ± 2871
Vicinity Preconcentration	132 ± 25 ▼	164 ± 46 ▼	296 ± 64 ▼	986 ± 490 ▼	1282 ± 550 ▼
Bystander Passive	48 ± 29	66 ± 46	114 ± 75	400 ± 402	514 ± 464
Bystander Preconcentration	169 ± 61 ▲	163 ± 36 ▲	331 ± 91 ▲	650 ± 77 ▲	981 ± 103 ▲
Passerby Passive	35 ± 54	42 ± 49	77 ± 103	248 ± 295	325 ± 397
Passerby Preconcentration	56 ± 53 ■	96 ± 75 ▲	152 ± 118 ▲	1287 ± 1885 ▲	1439 ± 1920 ▲
Hand	1259	1428	2687	31155	33842

In experiment regarding the mechanisms of deposition and exposure risks, the hands of the shooter, bystander, and passerby were sampled in addition to collecting OGSR with passive stubs. This was done to allow for deposition onto a larger surface area in a commonly sampled location, such as a hand. As hypothesized, a few samples (8 of 60) had OGSR compounds detected in LC-MS/MS analysis. However, the samples taken from hands provided more information regarding the risk of

indirect OGSR deposition onto a bystander or passerby. These results are shown in **Table 19.** In these samples, AKII, EC, and DPA were detected above LODs in all six shooter's hand samples. AKII and DPA were not detected in the bystander and passerby samples. AKII was not detected in five of six samples. OGSR transfer from the shooting event to the hands of a bystander or passerby is unlikely. Moreover, the distinction between the shooter and non-shooters is clear. This suggests that the mechanism of deposition and transfer for particles of organic nature is highly dependent on the distance of the deposited substrate and the source of the particles.

The presence of OGSR compounds in high concentrations on a shooter's hand relative to concentrations on a bystander or passerby's hand is a very important finding. When comparing this finding to the results obtained for IGSR analysis in experiment 2, it becomes evident that the analysis of OGSR as a complimentary tool provides excellent confirmatory information. With the combined analysis techniques, it is possible to enhance the confidence of results when attempting to determine if an individual of interest fired a gun or was merely present in the room during a shooting event.

Table 19. *Results of LC-MS/MS analysis of experiment 3 for hand samples taken from a shooter, bystander, and passerby.*

Shooter's Hand Sample

Sample	AKII	MC	EC	4-NDPA	DPA	2-NDPA
01_HAND_SHO	71.77	ND	10.30	4.14	36.09	3.15
02_HAND_SHO	23.85	ND	3.85	ND	6.34	ND
03_HAND_SHO	27.31	ND	5.17	ND	26.00	ND
04_HAND_SHO	7.16	ND	15.57	ND	7.89	ND
05_HAND_SHO	105.57	ND	65.50	11.19	166.44	7.54
06_HAND_SHO	29.65	ND	6.74	ND	25.81	ND

Bystander's Hand Sample

Sample	AKII	MC	EC	4-NDPA	DPA	2-NDPA
01_HAND_BYS	ND	ND	ND	ND	ND	ND
02_HAND_BYS	ND	ND	ND	ND	ND	ND
03_HAND_BYS	ND	ND	ND	ND	ND	ND
04_HAND_BYS	ND	ND	1.31	ND	ND	ND
05_HAND_BYS	ND	ND	ND	ND	ND	ND
06_HAND_BYS	ND	ND	ND	ND	ND	ND

Passerby's Hand Sample

Sample	AKII	MC	EC	4-NDPA	DPA	2-NDPA
01_HAND_PBY	ND	ND	ND	ND	ND	ND
02_HAND_PBY	ND	ND	ND	ND	ND	ND
03_HAND_PBY	ND	ND	ND	ND	ND	ND
04_HAND_PBY	ND	ND	ND	ND	ND	ND
05_HAND_PBY	ND	ND	ND	ND	ND	ND
06_HAND_PBY	ND	ND	ND	ND	ND	ND

Task 1.4. Interlaboratory exercises using the newly developed OGSR-IGSR standard mix

Interlaboratory studies for the in-house IGSR and OGSR standards were conducted by SEM-EDS, LAICPMS, ECD, and LC-MS/MS to account for multiple instrumental techniques, analysts, and laboratories. A new variable introduced in the interlaboratory testing was their stability through transportation via email to other states, an aspect of critical interest in these materials.

Interlaboratory study for an in-house IGSR and OGSR standard by SEM-EDS

Intraday variability was evaluated when the triplicate deposits on the same stub were run in one day, and interday variability assessed the reproducibility of the same deposited spot (either A, B, or C) on different days. We compared the similarities and differences in instrument configuration by comparing

the results from two laboratories (SCDACL and WVU). **Table 20** compares the results of the same three-day sample run from different instruments, operators, and laboratories.

The leaded pGSR standard WIN is composed of heavy atomic weight elements that are not as sensitive to changes in contrast and brightness over time. This can be seen by the lower relative standard deviation percentages (%RSD) for intraday experiments in **Table 20**. We observed comparable particle counts at the two laboratory sites, with overall particle count means, across various days and depositions, of 266 ± 40 and 274 ± 46 for SCDACL and WVU labs, respectively.

On the other hand, for the lead-free pGSR standard INC, we observed more variability in the interday and intraday data for both laboratories and instruments. The most likely reason for this is the difference in the elemental composition of the particles. Titanium and Zinc are light atomic weight elements; therefore, they are more sensitive to small changes in contrast and brightness on SEM imaging. This setting is adjusted daily before beginning automated analysis with additional modifications during the automatic sample mapping, which takes about 12 hours per stub. Therefore, small increases and decreases in contrast and brightness over time can affect the particles the software detects when using low atomic particle composition.

Table 20. *Comparison of SCDACL and WVU ILS results for the particle counts of a leaded Winchester (WIN) and a lead-free Inceptor (INC) pGSR standard.*

Winchester results from SCDACL (leaded pGSR)

Intraday Variability (PbBaSb)			
	Day 1	**Day 2**	**Day 3**
Mean	234	239	328
St.Dev.	55	30	4
%RSD	23	12	1

Interday Variability (PbBaSb)			
	Spot A	**Spot B**	**Spot C**
Mean	305	237	258
St.Dev.	44	57	64
%RSD	14	24	25

Winchester results from WVU (leaded pGSR)

Intraday Variability (PbBaSb)			
	Day 1	**Day 2**	**Day 3**
Mean	252	222	348
St.Dev.	50	10	44
%RSD	20	5	13

Interday Variability (PbBaSb)			
	Spot A	**Spot B**	**Spot C**
Mean	306	243	274
St.Dev.	72	48	84
%RSD	24	20	31

Inceptor results from SCDACL (lead-free pGSR)

Intraday Variability (TiZn)			
	Day 1	**Day 2**	**Day 3**
Mean	108	167	197
St.Dev.	40	41	188
%RSD	37	25	96

Interday Variability (TiZn)			
	Spot A	**Spot B**	**Spot C**
Mean	261	103	109
St.Dev.	136	26	41
%RSD	52	26	37

Inceptor results from WVU (lead-free pGSR)

Intraday Variability (TiZn)			
	Day 1	**Day 2**	**Day 3**
Mean	13	36	71
St.Dev.	2	12	16
%RSD	13	35	22

Interday Variability (TiZn)			
	Spot A	**Spot B**	**Spot C**
Mean	51	35	34
St.Dev.	37	28	24
%RSD	72	81	69

Overall, both laboratories' SEM-EDS particle count data shows that the counting of particles of leaded pGSR standard is more reproducible than lead-free formulations. Compared to other instrumental analyses performed on the same standards, SEM-EDS particle counts were more variable than bulk and micro-bulk methods (i.e., ICP-MS and LIBS inter and intraday reproducibility better than 10%RSD). For this reason, we recommend using only leaded *p*GSR standards when SEM-EDS particle count is required. Lead-free ammunition is more prone to SEM-EDS inter-day variation, and these results further prove the challenges that examiners may face in the future with changes in modern green ammunition components. Also, we developed an interlaboratory exercise with our collaborators at Iowa State University, Chemistry Department. The standards were characterized by single particle- ICP-TOF. A publication describing the results was recently published.[5]

Interlaboratory study for an in-house IGSR standard by LC/MSMS

Another interlaboratory study was conducted between West Virginia University (WVU) and the National Institute of Standards and Technology (NIST). Reproducibility was evaluated by comparable composition and concentration of organic analytes present in a 1 ppm standard mix suspended in methanol.

After analysis, analyte concentrations in the OGSR standard were calculated using equations produced by nine-level calibration curves ranging from 0 – 200 ppb. As seen in **Table 21**, each laboratory produced good repeatability results, with standard deviation values lower than 5.3 for both instruments across all analytes. The results also showed reproducibility between the two labs in this interlaboratory study. However, some analytes performed better than others. For example, Ethyl Centralite was predicted to have a concentration of 72.2 ± 2.1 ppb by the NIST LC-MS/MS and a concentration of 71.6 ± 0.3 ppb by the WVU instrument, which shows minimal variability in concentrations.

However, analytes like DPA and 4NDPA had higher, but reasonable, differences observed for their predicted concentration. This may be due to come loss or preconcentration of analytes and solvent during transport of the samples. While samples were kept in a cooler during transport to NIST, some minimal loss could have still occurred during the 3-hour car transport. For the DPA difference, Akardite II can fragment into a DPA-like ion during the ionization process, which may contribute to the higher concentration seen at NIST, as they co-eluted during analysis. Overall, the differences in concentration were both minimal and explainable and therefore not of concern for first pilot testing. This interlaboratory study demonstrates how the OGSR standard solvated in methanol created at WVU can be used by other laboratories as a reference standard for method development and experimentation.

Table 21. *Summary of Analyte Concentrations in OGSR Stock Solution Predicted by NIST and WVU LC-MS/MS Instruments*

Compound	Average expected concentration (ppb)	NIST Predicted Concentration (ppb)	NIST %bias	WVU Predicted Concentration (ppb)	WVU % bias
Diphenylamine	67.0 ± 3.2	84.0 ± 1.1	25%	74.3 ± 1.5	11%
Akardite II	81.9 ± 2.3	83.4 ± 1.4	1.8%	83.6 ± 1.3	2.1%
Methyl Centralite	84.2 ± 2.3	79.7 ± 2.1	5.3%	85.4 ± 1.2	1.4%
Ethyl Centralite	76.1 ± 4.4	72.2 ± 2.1	5.1%	71.6 ± 0.3	5.9%
2-Nitrodiphenylamine	71.7 ± 2.5	74.5 ± 5.3	3.9%	77.2 ± 2.1	7.6%
4-Nitrodiphenylamine	83.4 ± 2.4	77.5 ± 3.6	7.0%	84.1 ± 0.6	0.8%

Electrochemistry Results for the Interlaboratory Study

Finally, another interlaboratory study was conducted between West Virginia University and the New Jersey State Police Office of Forensic Sciences (NJSP OFS). WVU Graduate Research Assistant Kourtney Dalzell visited the NJSP OFS for two weeks to perform on-site training and validation on the portable electrochemical unit, including training on electrochemistry theory, sample preparation, software, and data analysis. Validation was completed by analyzing calibration curves, quality control mixtures, pGSR/OGSR standards, and authentic shooter and background population collected at the NJSP OFS. Calibration curves were completed using individual stock solutions to create 5-point curves in triplicate on screen-printed carbon electrodes. Each IGSR and OGSR analyte had figures of merit determined from the calibration curves, including potential window, linear dynamic range, R^2, repeatability, detection limits, and quantitation (**Table 22**). All calibration curves were performed with R^2 values greater than 0.983 and most analytes' repeatability under 20%, except for ethyl centralite. Limits of detection were comparable to WVU LODs for antimony, copper, diphenylamine, and ethyl centralite. NJSP calibration curves were more sensitive with the lowest LODs for lead and nitroglycerin. Our recommendation to NJSP OFS is to use the limit of quantitation for reporting purposes. These results can be compared with the performance characteristics table for the portable instrument in the comparison between benchtop and portable electrochemical instruments section.

Table 22. *Performance characteristics calculated based on the PalmSens4 portable instrument at the NJSP OFS.*

IGSR	Potential (V)	Linear Range (µg/mL)	R^2	Repeatability (%RSD, n=3)	LOD (µg/mL)	LLOQ (µg/mL)
Lead	-0.80 +/- 0.02	0.1-2	0.999	4.0	0.07 +/- 0.003	0.1
Antimony*	-0.42 +/- 0.01	0.1-4	0.986	18	0.21 +/- 0.04	0.1
Copper	-0.31 +/- 0.03	0.05-1	0.999	0.81	0.005 +/- 0.00004	0.05
OGSR	**Potential (V)**	**Linear Range (µg/mL)**	**R^2**	**Repeatability (%RSD, n=3)**	**LOD (µg/mL)**	**LLOQ (µg/mL)**
2,4-Dinitrotoluene*	-0.17 +/- 0.01	1-10	0.983	5.1	0.14 +/- 0.01	1
Diphenylamine	0.41 +/- 0.01	1-6	0.998	12	0.11 +/- 0.01	1
Nitroglycerin	0.52 +/- 0.01	0.5-8	0.988	14	0.08 +/- 0.01	0.5
Ethyl centralite	0.92 +/- 0.01	3-6	0.997	32	0.63 +/- 0.21	3

** Antimony and 2,4-DNT was assessed as peak current height whereas all other analytes are assessed as peak current area*

Quality Control mixtures were analyzed over two weeks and performed before the analysis of authentic shooter and background samples. **Figure 33** demonstrates lead and nitroglycerin peak current area from the 2.5 ppm mixture where lead concentration is 0.05 ppm and nitroglycerin concentration is 2.5 ppm, in addition to the Tul pGSR standard control chart.

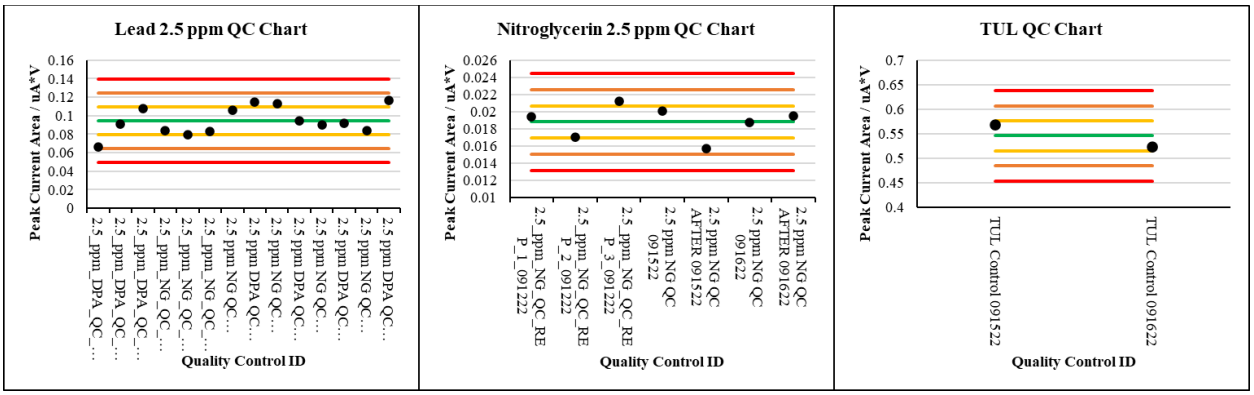


Figure 33. *Quality control plots of peak current areas for 0.05 ppm lead (left) and 2.5 ppm nitroglycerin (center) in the 2.5 ppm mixtures and the pGSR TUL control (right).*

Validation concluded with the analysis of a small population of authentic shooters and background samples. To this end, a total of 25 background samples were collected from volunteers from various forensic disciplines at the laboratory. The volunteers were asked a series of questions to ensure they did not handle or fire a firearm in the past 24 hours or participate in activities considered to be high-risk for GSR-type residues. The authentic shooter population consisted of 25 samples collected at the NJSP Ballistics range using a Springfield XD 9mm firearm with 3 different types of ammunition. Ammunition utilized in this collection was Blazer Brass (8 samples), Remington UMC (8 samples), and Federal American Eagle (9 samples).

An analyte was considered positive if the peak current area was above the critical threshold. A sample was positive if there were two or more IGSR or IGSR and OGSR analytes positive in the sample. All NJSP backgrounds had less than 4% for lead, copper, and nitroglycerin in the background population. Lead and copper were most prevalent in the NJSP shooter samples at 100% and 84%, respectively. Overall, **Table 23** demonstrated that the method's performance found 100% true negatives and 84% true positives. The accuracy of the method determined was 92%. Additionally, authentic shooter samples were also analyzed by SEM-EDS after electrochemical analysis to demonstrate that that sample could undergo confirmatory analysis by the ASTM 1588 standard. A cutoff limit of 20 characteristics (Pb, Ba, and Sb) particles was set, and all shooter samples reached the cutoff threshold for SEM analysis.

Table 23. *Performance measures of the shooter and background populations by ECD, using 2 or more IGSR, IGSR, and OGSR positive sample criteria.*

Performance Measures	
True Positives (Sensitivity)	21 (84%)
False Negatives	4 (16%)
True Negatives (Specificity)	25 (100%)
False Positives	0 (0%)
Accuracy	**92%**

Results of Task 2 (Objective 2)—To develop systematic methods for the transfer and persistence of gunshot residues, using in-house OGSR & IGSR microparticle standards

Summary of IGSR/OGSR transfer and persistence study.
This study employed an innovative approach to evaluating the transfer and persistence of samples containing both pGSR and OGSR, monitoring in-house characterized pGSR/OGSR standards in synthetic skin membranes and comparing the findings to authentic shooter specimens. The pGSR/OGSR standards provided a feasible model to monitor the deposition, persistence, and transfer of inorganic particles and organic compounds with known initial compositions for the first time. The results show that the OGSR standard methanol solution can be sequentially deposited with a pGSR acetone solution without interferences or loss of organic analytes or inorganic particles. Another advantage of the standard solutions is that they can be made or deposited at varying concentrations that represent typical concentrations on real specimens and are compatible with various substrates.

The in-depth transfer and persistence studies performed here added to the knowledge of the fate and behavior of gunshot residues post-deposition. Unlike previous studies, experiments benefitted from initial concentration and particle count knowledge of OGSR and IGSR, respectively—furthermore, a synthetic skin membrane allowed for controlled factors that complemented the interpretation of the results. The study included the collection and analysis of over 650 samples, including 247 collections from human skin after firing a gun (107 hands and 240 face and hair areas) and 405 synthetic skin and fabric substrates after depositing a characterized IGSR/OGSR standard. The transfer and persistence of IGSR and OGSR were evaluated on different substrates (hands, ears, nostrils, forehead, hair, fabrics, and synthetic skin), simulating collection at different times after firing (from time zero to up to 6 hours), and common post-shooting activities (rubbing hands, handshaking, running, washing hands, and washing fabrics).

The research revealed that the StratM® membranes could efficiently simulate the behavior of both inorganic and organic gunshot residues after deposition on authentic human skin. The novelty of this development in gunshot residue research is that it provides safe alternative matrices to perform in-depth behavior studies using the proposed standard solutions. It also provides, for the first time, simultaneous information about pGSR and OGSR transfer and persistence on the same specimens. Moreover, the synthetic skin membranes allowed an understanding of volatile OGSR evaporation and how compounds permeate through the different layers of human skin, adding another interpretation

of organic behavior that currently has limited knowledge. Incorporating synthetic skin membranes into fate and behavior studies is anticipated to provide an alternative method for performing more advanced and rigorous activity studies that are typically limited by safety concerns and other human factors. Combining ground truth pGSR particle counts, OGSR analyte concentrations, and accurate skin modeling substrates allows advanced interpretation of how gunshot residues' inorganic and organic constituents transfer and persist on human skin.

Overall, it was found that the behavior of organics is highly dependent on compounds' physicochemical properties, while that of inorganics is more dependent on physical properties. The results show that inorganic particles persist longer than organic compounds. Unless acted upon by an outside force, pGSR will remain on a surface or individual until physically removed by action or movement. However, they are easily lost and transferred by common activities. On the other hand, organic gunshot residues experienced a more significant loss 6 hours after deposition, indicating that residues are lost over time even without outside forces. Organic compounds can also be lost by permeation through the stratum corneum layer of human skin. While this remains true for hand samples, authentic shooter studies indicated that other collection locations, such as noses, ears, foreheads, and hair, do not provide substantial additional information on the deposition or persistence of gunshot residues at the experimental conditions used in this study. However, it should be noted that only one firearm and ammunition type were used during authentic shootings, and results may vary with different firing configurations.

This study showed that inorganic GSR particles transfer more easily than organic compounds during different activities. These findings also agreed with laser scattering visualization and onsite sensor monitoring experiments conducted in our group. The implications of these results can be crucial in future steps the community can take in the overarching interpretation of GSR evidence, and thus, replication and extended studies are recommended to corroborate these findings by additional researchers and laboratories. The observations can be explained by looking at the residues' properties. In the case of pGSR, the microscopic particles tend to remain on the surface of an individual's hands, which allows easy secondary transfers. This experiment showed that even minimal contact during shaking hands could secondarily transfer over 100 characteristic particles to another individual. As the length and intensity of contact increase, particle transfer also increases. For example, almost an equal distribution of 300 particles occurred between the donor and recipient membranes when rubbing hands together. In contrast, organic compounds tend to evaporate, permeate, and adsorb at some level in the skin and their affinity to the skin composition makes it more difficult to be transferred by contact. Thus, for organic compounds, no transfer was detected to the recipient membrane, no matter the length of contact or intensity, due to potential evaporation and absorption through the StratM® or hands. However, both residues experienced the most loss when other factors, like soap and water, were included. Additionally, although inorganic information may be gone after washing hands, organic residues may still be present and are equally crucial for interpreting events. These results indicate that incorporating knowledge of both inorganic and organic gunshot residues will increase confidence in interpreting the results under alternative hypothesis, as various transfer and persistence situations affect differently the inorganic and organic species.

On the other hand, the interaction of GSR on fabrics is more challenging due to the variety of chemical compositions of the fibers and their construction patterns in the end product. Although pGSR results proved difficult for clothing samples, some information about the organic transfer was still gained. First, clothing construction does not heavily influence the behavior of organic residues. In other words, the fiber type and construction did not result in significantly different baseline percent recoveries. However, the chemical properties of the OGSR analytes heavily influenced their recovery

rates after activities. Overall, after intense interactions such as struggling events, the friction that occurs leads to the evaporation of analytes with more significant evaporation of analytes with higher vapor pressures like DPA and 4-NDPA. On the other hand, those analytes, along with 2-NDPA, showed increased recovery after washing fabrics in water over other analytes with high water solubility properties like MC, EC, and AK II. Therefore, it is possible, to some extent, to recover OGSR from a suspect or victim's clothing even after fabric washing.

These findings are anticipated to help crime scene personnel and forensic examiners in their collection strategies, analytical workflows, and interpretation of GSR evidence. At the same time, investigators can construct more advanced investigative leads that include hypotheses for activity and timelines of events. It is, however, essential to note that the mechanisms of transfer and persistence are complex, and more experimentation with additional variables and combined factors can provide additional insights for the interpretation of IGSR/OGSR evidence. Furthermore, the expansion of novel synthetic skin membrane studies to the firearm-related field opens opportunities for several other OGSR and pGSR-controlled studies. Future endeavors of this study will include data from screening methods of analysis developed in our group, such as LIBS and EC, to overcome SEM-EDS limitations encountered for clothing samples and the time-consuming factor of SEM-EDS analyses.

Part of these studies have been reported in Vander Pyl et al.[3,14] and are described in the dissertation of one of our doctoral studies. The following sections summarize some of the major findings.

Task 2.1. To assess the effect of activity on the transfer and persistence of IGSR and OGSR on hands

Baseline Collection Efficiency of OGSR Standard Solution on Carbon Stubs and Synthetic Skin Membranes

Baseline recovery procedures were performed to determine the ability to retrieve analytes after deposition onto a substrate of choice (carbon adhesive and StratM®). Initial evaluations were performed on the current SEM-EDS collection substrate for gunshot residue. Two extraction methods were compared: washing the carbon stub with sequential aliquots of methanol (surface method) or submerging the carbon adhesive in methanol and sonicating to extricate analytes (exhaustive approach). **Table 24** shows analyte percent recoveries obtained from both extraction protocols. While both extraction procedures showed reasonable recovery rates, with the lowest being 69% for MC stub washes, an exhaustive extraction procedure was favorable for all analytes. Submerging the carbon adhesive resulted in recovery rates above 89% with low standard deviations and indicated reproducible recovery capabilities.

Similar recovery studies were performed on the synthetic skin membrane of choice; StratM®. The first extraction method involved stubbing the membrane surface five times with a carbon adhesive stub to coincide with authentic GSR collection methods from hands currently used in the field, followed by washing the stub surface with methanol. The second method followed an exhaustive extraction where the entire membrane section was submerged in methanol and sonicated for five minutes. As seen in **Table 24,** stubbing the membrane surfaces with carbon adhesive resulted in no recovery of analytes. This may be due to the surface and structure of the StratM® membrane. The smooth, porous nature of the StratM® caused a weaker tack interaction between the carbon adhesive and membrane surface than human skin. Furthermore, the OGSR compounds have shown to permeate the membrane shortly after deposition. This combination of factors caused inefficient recovery of analytes from the membrane surface. However, high recovery percentages were observed when an exhaustive extraction

was performed (≥81%). Therefore, the exhaustive method was selected for the experiments simulating the transfer and persistence of OGSR analytes in the carbon and StratM substrates. Although this approach was not directly comparable to human skin specimen collections, it still provided general information on the trends of the OGSR movement. In addition, exhaustive extraction of the membranes permitted the initial recovery of pGSR particles by carbon stub. At the same time, the OGSR analytes remained in the membrane for extraction, which allowed for comprehensive IGSR and OGSR analysis on a single membrane sample. Overall, these studies showed that the OGSR standard solution was effectively recovered from multiple substrates with calculated percent loss from ground-truth knowledge of controlled pGSR/OGSR deposits, making it helpful for assessing the transfer and persistence of gunshot residues.

***Table 24**. Percent Recoveries of OGSR Analytes from Carbon Adhesive and StratM® using Surface and Exhaustive Extraction Methods (NR: Not Recovered)*

Carbon Adhesive				StratM®			
Exhaustive		Stub Washes		Exhaustive		Stub Washes	
Analyte	Recovery (%)	Analyte	Recovery (%)	Analyte	Recovery (%)	Analyte	Recovery (%)
AK II	89 ± 6	AK II	87 ± 7	AK II	82 ± 8	AK II	NR
MC	96 ± 1	MC	69 ± 6	MC	94 ± 10	MC	NR
EC	96 ± 1	EC	93 ± 2	EC	86 ± 8	EC	NR
4-NDPA	98 ± 6	4-NDPA	79 ± 7	4-NDPA	81 ± 9	4-NDPA	NR
DPA	96 ± 2	DPA	78 ± 7	DPA	98 ± 7	DPA	NR
2-NDPA	95 ± 1	2-NDPA	78 ± 8	2-NDPA	90 ± 6	2-NDPA	NR

Transfer and Persistence of OGSR using Synthetic Skin Membranes

The first set of experiments used StratM® membranes and activities individuals commonly perform with their hands. No-activity membranes were collected daily as baselines to assess the percent loss of OGSR analytes after rubbing, shaking, and washing the membranes. Two activities were performed in this part of the study to evaluate the secondary transfer of organic residues: rubbing two membranes together to simulate rubbing hands and attaching membranes on two individuals before shaking hands. After analysis, little concentration of OGSR analytes was observed from the extraction of "secondary transfer" membranes. Results suggested that OGSR is not readily transferred from one StratM® membrane to another (i.e., simulating secondary transfer from one individual to another individual). Therefore, this study determined that, unlike pGSR, OGSR residues are not as easily transferred between materials and surfaces. Although limited studies have been performed on OGSR secondary transfer, this corroboration of trends between the StratM® and authentic shooters further established that the synthetic skin membrane can be accurately used to interpret GSR transference. Since the secondary transfer of OGSR was not substantial, the following discussion focuses on residues extracted from the spiked StratM® portions. All analytes generally behaved similarly in simulated hand activities using the StratM® (**see table 25**). Results from these simulated tests can be extrapolated to authentic shooters based on knowledge gained in this study.

***Table 25**. Relative Percent Recoveries and Losses of OGSR Analytes Observed during StratM® Transfer Studies*

Hands Activity	AK II		MC		EC	
	% Recovery	% Loss	% Recovery	% Loss	% Recovery	%% Loss
None	78±6%	--	79±10%	--	78±3%	--
Rubbing	75±5%	4%	69±5%	10%	74±6%	5%
Shaking	78±5%	0%	77±6%	2%	78±6%	0%
Washing	68±7%	13%	66±7%	16%	68±7%	13%
Hands Activity	**4NDPA**		**DPA**		**2NDPA**	
	% Recovery	% Loss	% Recovery	% Loss	% Recovery	% Loss
None	69±5%	--	69±4%	--	75±5%	--
Rubbing	63±5%	7%	65±4%	6%	67±5%	9%
Shaking	66±5%	3%	67±4%	3%	72±5%	4%
Washing	56±5%	19%	58±6%	16%	59±6%	21%

The following conclusions can be made about the transfer and persistence of organic gunshot residues after activity:

(1) Organic gunshot residues are generally not readily transferred from a primary surface to a secondary surface after deposition.
(2) Activities having limited contact and force do not result in significant OGSR loss from a primary surface after deposition.
(3) Analyte loss is related to the extent and rigor of the activity performed. More analyte loss is observed for activities that require more extended contact (i.e., rubbing hands) or more intense interactions (i.e., washing hands with soap and water).
(4) Specific analyte loss also depends on the physiochemical properties of each OGSR compound and its interaction with the activity environment.

Transfer and Persistence of pGSR on Skin Membranes

For the membranes spiked with pGSR, the resulting particle counts from each activity were compared to those obtained for baseline substrates with no activity performed on them. These are referred to as "none" samples in the tables. Similar to OGSR, the StratM® no activity samples presented high recovery rates of particles from the synthetic skin membranes, with ~700 characteristic particles and ~3000 consistent particles, further indicating accurate deposition and recovery of particles from the StratM® (**Table 26**).

***Table 26**. Average Particle Recoveries and Percent Losses during Membrane Transfer Studies*

Hands Activity	Characteristic		Consistent	
	Particles Recovered	% Loss/ % Transfer	Particles Recovered	% Loss/ % Transfer
None	690±104	--	2973±593	--
Rubbing A-donor	308±114	55% Loss	1466±498	51% Loss
Rubbing B-recipient	219±52	32% Transfer	1028±308	35% Transfer
Shaking A-donor	790±129	14% Loss	3633±786	22% Loss
Shaking B-recipient	147±137	21% Transfer	702±693	24% Transfer
Washing	2±1	99% Loss	16±7	99% Loss

The pressure and extent of contact applied during the "rubbing" hands activity led to a significant transfer of particles from the membrane deposited (A, donor) on and the clean membrane (B,

recipient). For example, after rubbing hands, a 55% loss of characteristic particles was seen between the "no activity" samples and the spiked sample (A). Furthermore, most particles lost from membrane A were transferred to membrane B, as indicated by the average of 219 characteristic particles detected by SEM-EDS (32% transfer). This was also corroborated with particles with the classification of "consistent with GSR."

Furthermore, with known deposited particle counts, the percentage of particle transfer between hands could be calculated, unlike in authentic shootings, where particle deposition is random and often results in highly variable numbers of primarily transferred residues to the shooter's hands. Therefore, the StratM® was determined to be a feasible and safe alternative for demonstrating ground truth transfer studies for both inorganic and organic gunshot residues. Thus, the results can be extrapolated to shooter individuals: particles could transfer between a shooter's left and right hand when a rubbing motion is performed after discharging a firearm. These findings corroborated previous authentic activity studies performed in our group in the hands of shooters where significant particle transfer was observed between a shooter's left and right hand after discharging a firearm and rubbing hands together for 60 seconds. While many particles were transferred while rubbing hands together, not as many were transferred while shaking hands (**Figure 34).** This was most likely due to longer and more vigorous contact times for rubbing hands. In contrast, shaking hands was a brief contact, and the membranes needed to be perfectly lined up between the two volunteers. The membranes were carried vertically during the handshake, which may have caused particles to fall to the surface below once participants detached their hands. Due to this, the number of particles was more variable, and relatively lower transfer occurred to the second surface, as shown in **Table 26.** Interestingly, characteristic and consistent particles continue to follow the same trend. However, results did show that it is possible to secondarily transfer a large number of particles to an individual who did not discharge a firearm, unlike OGSR compounds that were determined to experience little to no secondary transfer.

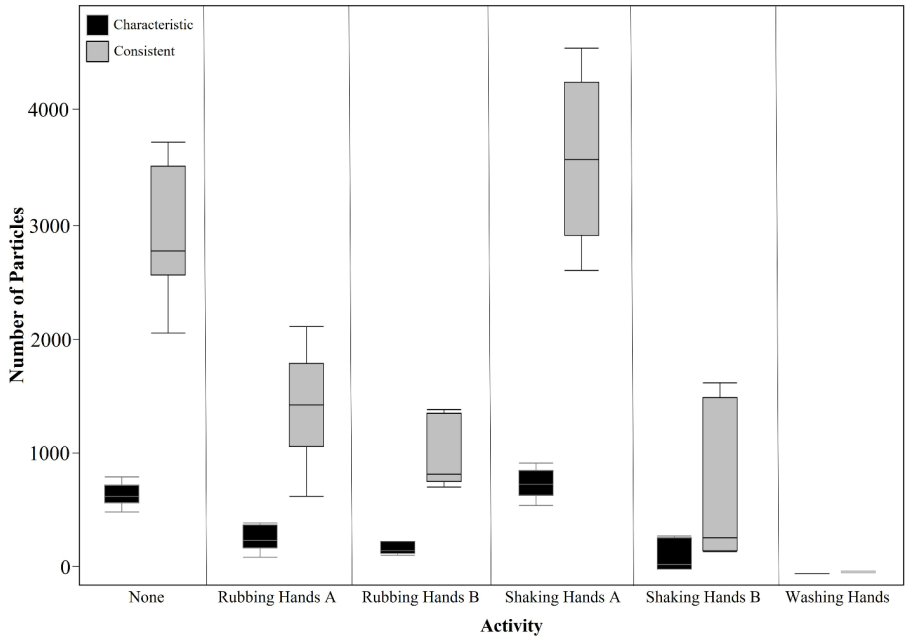


Figure 34*. Box plot showing characteristic and consistent particle trends collected from synthetic membrane samples after performing hands activities.*

As expected, washing with soap and water significantly affected pGSR retention, with a 99% loss of particles observed for both characteristic and consistent particles. However, some particles were still collected by the carbon adhesive and detected by SEM-EDS analysis. An average of two characteristic particles and 16 consistent particles were still recovered from the StratM® surface, revealing that particles can remain on a surface, such as an individual's hands, after hand washing but appear in much less proportions than other activities performed in this study.

Several conclusions can be made about pGSR transfer based on the observed trends:

(1) In general, the transfer of pGSR particles is heavily influenced by the rigor, duration, and intensity of contact with another surface.
(2) Some particles can be transferred during a brief handshake. However, the number of particles is much less than an activity requiring longer or stronger forced contact.
(3) Washing hands with soap and water followed by drying with a paper towel led to an almost complete loss of GSR particles, characteristic and consistent composition.
(4) Secondary transfer of pGSR particles is more likely than OGSR under identical experimental conditions conducted on the same sample.

Electrochemistry results for the membrane activity transfer study

Electrochemical analysis totaled 180 samples processed, separated into two subsets regarding the analysis sequence. Thirty-six samples were analyzed by SEM-EDS, followed by the LIBS and EC analysis scheme, while only the LIBS and EC scheme analyzed the other one-hundred and forty-four samples. The subset of samples ran by SEM before the screening methods allowed for ground truth to be known beforehand, adding reliability to electrochemical results. Due to similar trends being observed in both sample subsets, only the samples processed by SEM, LIBS, and EC will be shown in this discussion.

The GSR loss was visualized using box plots to compare the activity groups. First, the dry rubbing of membrane A (donor) resulted in a significant loss of pGSR and transfer of pGSR for lead to the recipient membrane (B) compared to the no-activity controls. The box plots in **Figure 35** summarizes the findings. These observations are also seen in the ANOVA and Dunnett's control statistical analysis, where the rubbing grouping is significantly different from the no-activity group (**Figure 36**). Like the rubbing hands, the shaking hands activity resulted in loss and transfer. Still, a smaller relative loss was observed compared to the no activity sample, wherein the ANOVA and Dunnett's control test, membrane A of shaking hand, is considered not significantly different from the no activity control group. The transfer to the second membrane for shaking hands was significantly different from the control group. A 99% relative lead loss was observed for the washing hands activity compared to the no activity, consistent with the SEM-EDS observations on the same samples.

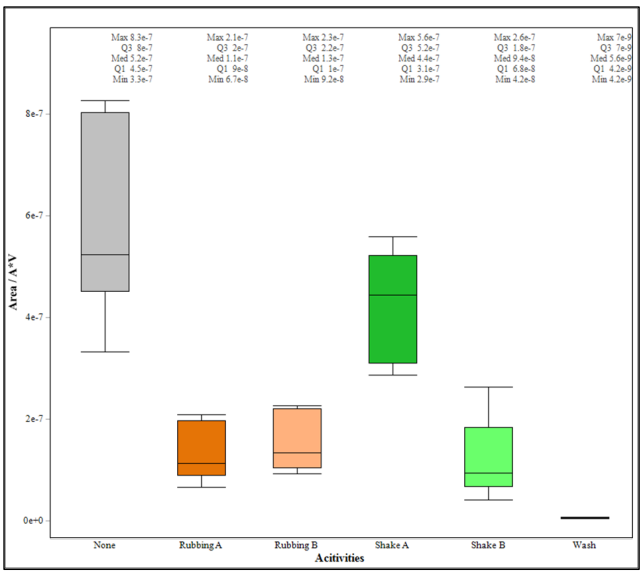


Figure 35. *Box plot of time versus the peak current area of lead for the different activities No Activity (gray), Dry Rubbing (orange), Shaking Hands (green), and washing hands (blue) on the synthetic skin membrane of 36 samples analyzed by SEM, LIBS, and EC.*

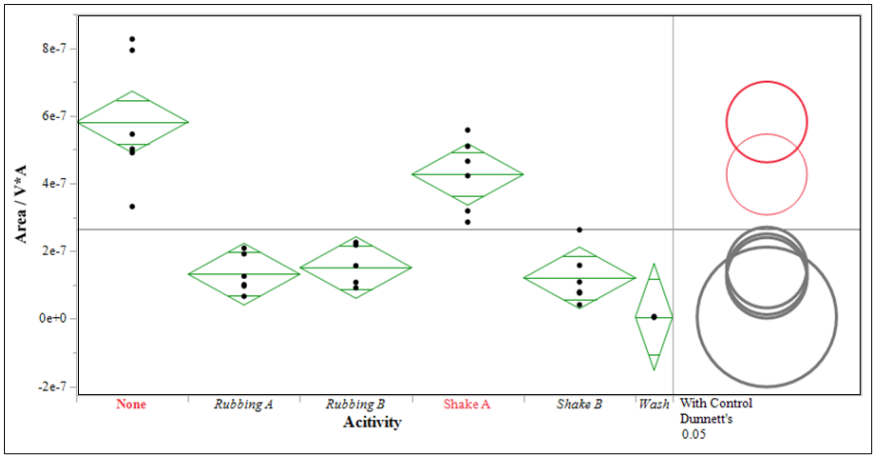


Figure 36. *ANOVA and Dunnett's control of time versus the peak current area of lead for the different activities: No Activity, Dry Rubbing, Shaking Hands, and washing hands on the synthetic skin membrane of 36 samples analyzed by SEM, LIBS, and EC.*

Task 2.2: To evaluate deposition, transfer, and persistence of GSR on hands over time

A total of 120 sample sets were analyzed to assess the persistence of GSR over six-time intervals (0, 1, 3, and 6 hours) using actual shooting compared to the IGSR/OSGR standard depositions. Collection at time zero estimates the initial amount of GSR deposited. A waiting time of 3 minutes is used for the shooting experiments to allow particle deposition. After application, the substrate is left in a dedicated sampling room for synthetic skin experiments to prevent cross-contamination in laboratory areas.

Since our in-house standards are produced from ammunition that has been characterized before shooting (e.g., propellant composition) and after shooting (e.g., primer and GSR characterized standards), we conducted a comparative analysis of the transfer and persistence of OGSR and IGSR under these deposition approaches. All conditions between the experiments remained consistent, with the only varying factor being the GSR deposition process.

Authentic Shooter Persistence Studies (hands, nose, ears, forehead, and hair)

Authentic shooter persistence samples were collected using a 9 mm Springfield XD9 semi-automatic pistol loaded with CCI magnum standard leaded primer ammunition. Each shooter fired five consecutive shots within the range before moving to the designated collection area. After waiting the allotted persistence time (0 hrs, 1 hr, or 3 hrs), a separate individual collected from each of the shooters using one GSR stub (12 mm) for both hands. Each shooter washed their hands, and then returned to their station to collect residues from all other designated locations (nose, ears, forehead, hair, in that order.) For collection from nose and ears, one stub (9mm) was used for both nostrils and the crevices surrounding the nostril, and one stub (9mm) was used for both ears. To be consistent between all individuals, collection was set to 5 dabs per nostril and 10 dabs per ear. For forehead and hair one stub (12mm) was used for each location, with 20 dabs used per area. Final collection resulted in 5 different stubs per individual.

All sets of collected stubs were then randomly distributed between each instrumental technique, to achieve OGSR and IGSR analysis throughout the whole sample set. Sample sets were distributed so LC/MSMS and LIBS/EC analyzed at least 12 sample sets, while SEM-EDS analyzed 6 sample sets due to the lengthy analysis required. It should be noted that facial areas showed little retention of both IGSR and OGSR analytes for 0 hr and 1 hr collections. Therefore, only hands were collected from the 3-hour persistence studies. **Table 27** below summarizes the number of samples collected from each area for the three different persistence times.

Table 27. *Number of Samples Collected per Location at Different Persistence Time Intervals*

	Hands	Nose	Ears	Forehead	Hair
0hr persistence	50	30	30	30	30
1hr persistence	34	30	30	30	30
3hr persistence	30	--	--	--	--
Total	114	60	60	60	60

Under our experimental conditions, only hand samples provided sufficient IGSR/OGSR recovery; other collection areas of the face supplied no significant residues, with few samples positive for OGSR analytes and pGSR particles. Immediately after firing, only four samples from these alternative collection regions contained AK II, and four others contained EC. Furthermore, the analytes (i.e., AK II and EC) were present on different samples, not on the same individual. Furthermore, no characteristic GSR particles were detected by SEM-EDS on any face samples collected immediately after or one hour after firing. Insufficient evidence of persistence trends on any face samples suggests those locations may not be effective for collection during real-case firearm investigations, at least under the circumstances and conditions investigated in this study. Therefore, the remainder of this study focused on information gained from the hands of authentic shooters.

OGSR Persistence over Time in Human Skin after Discharging a Firearm and Under Controlled Conditions on Synthetic Skin Specimens

Two different persistence experiments were conducted for comparison. One set of samples was collected from both hands of authentic shooters after discharging a firearm and waiting for a designated time. The second set of samples was collected under controlled conditions in a laboratory using StratM® synthetic skin membranes and an OGSR standard solution. Using StratM® membranes allowed additional experiments with longer wait times and knowledge of known OGSR concentrations. Therefore, authentic information was only provided up to three hours, while synthetic studies provided information about OGSR compound behavior up to six hours.

The first observation about authentic shooters is that the propellant consisted of analytes in various low ppb concentrations. (Immediately after discharge, ethyl centralite and diphenylamine were detected at high concentrations (an average of 22 ppb for both, with considerable variation ranging from 3 ppb to 50 ppb). In contrast, other analytes like 4-NDPA, 2-NDPA, and AK II were detected at concentrations lower than 5 ppb. This observation was unsurprising since EC and DPA are primary stabilizers, while other compounds like 2-or-4-NDPA are often combustion or degradation byproducts. Methyl centralite was not detected in authentic shooter samples, indicating that it was not used in the propellant formulation or produced in concentrations below the detection limits of the LC-MS/MS instrument used in this study.

Authentic shooter data showed that GSR recovery was highly variable due to the random and uncontrolled GSR formation and deposition mechanisms during a firing event. This adds an advantage to studies that used synthetic skin membranes. The OGSR standard solution was explicitly made to observe all analytes at relatively similar concentrations (**Figure 37**). Therefore, unlike authentic shooters, persistence behaviors could be uniformly observed between analytes without misinterpreting results due to concentration variations. Furthermore, MC information was only gained through synthetic membrane studies since no information was gained from authentic shooter samples with the ammunition used. Regardless of these concentration differences at "time zero," authentic shooters and synthetic skin experiments show similar relative persistence trends for the compounds of interest, demonstrating the synthetic skin membranes are a feasible model for studying OGSR.

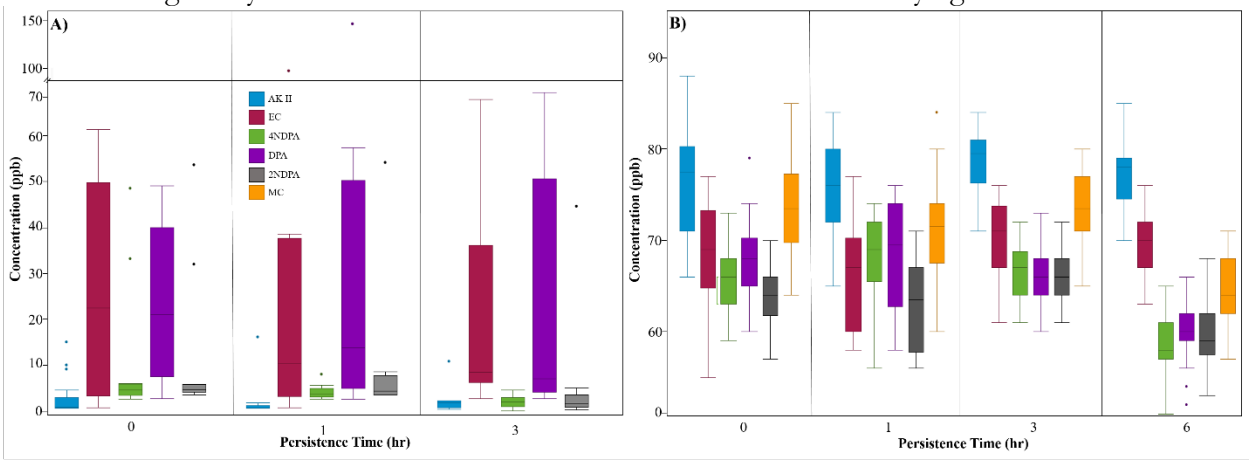


Figure 37 Observed concentrations of OGSR analytes AK II, EC, 4NDPA, DPA, 2NDPA, and MC in authentic shooter samples (left) and synthetic membrane samples (right) at 0 hr, 1 hr, 3 hrs, and 6 hrs.

For authentic shooters, EC, DPA, and AK II were present in 100% of samples collected across the three persistence times (0 hr, 1 hr, 3 hr). Despite the low concentrations observed for AK II immediately after firing, the concentrations were not significantly different after a three-hour wait period, as determined by ANOVA and Dunnett's control test (**Figure 38**). The control groups in this study were "0 hr" persistence samples and "No activity" transfer samples, as the question at large is how treatments are compared to baselines rather than how different treatments are compared to each other. Therefore, for authentic shooters, analyte concentrations up to three hours for EC, DPA, AK II, and 2-NDPA were not significantly different from concentrations determined immediately after discharge. In contrast, 4-NDPA was determined to decrease over time to significantly different concentrations from time zero (p-value: 0.0012) and fewer positive samples 3 hours post-discharge.

Similar trends were observed with the synthetic skin membrane. Like authentic shooters, excellent persistence was observed for important OGSR compounds of AK II, EC, 2-NDPA, and DPA. However, the significant difference at the 3-hour interval for 4-NDPA on authentic skin specimens was not observed during synthetic studies. The decrease in 4-NDPA was insignificant in synthetic skin until it reached the 6-hour mark. This minor discrepancy in the retention of 4-NDPA can be explained in a few ways: chemical properties of analytes, manner of deposition, and interaction of analytes with the different substrates (authentic skin versus synthetic skin). Chemical properties like vapor pressure and surface permeability heavily influence the persistence of organic compounds on an individual's hands. Analytes with higher vapor pressures have lower boiling points, evaporating more easily. Analytes of interest in this study have determined vapor pressures (mmHg) of $6.39x^{-4}$ (DPA), $4.53x^{-5}$ (MC), $1.66x^{-5}$ (4-NDPA), $1.00x^{-5}$ (2-NDPA), $6.75x^{-6}$ (EC), and $5.00x^{-7}$ (AK II). Additionally, the heat of combustion during discharge (160-180 °C) and the typical temperature from the hands of a shooter (36 °C) can cause vapors to evaporate quicker than compounds in controlled laboratory experiments at room temperature (~65 °C). This is one possible explanation for why 4-NDPA is lost more rapidly in authentic shootings after 3 hours than in synthetic samples. However, vapor pressure alone does not explain why DPA was not lost after three hours in authentic shooters, but 4-NDPA was, even though it has a lower vapor pressure. This different behavior can be explained using partition coefficients and chemical-skin interactions.

A partition coefficient (LogP) is a compound's ability to move from an organic phase to an aqueous phase and is inversely related to the water solubility of a compound. A compound with a large partition coefficient has a higher affinity for an organic phase and, therefore, has a lower water solubility. Thus, compounds with larger LogP values may be able to permeate through the selectively permeable stratum corneum of the skin. The stratum corneum allows more hydrophobic substances (less water soluble) to pass through the barrier. Diphenylamine has a LogP value of 3.50, while 4-nitrodiphenylamine has a LogP value of 3.75, meaning 4-NDPA will permeate through the skin faster. Additionally, 4-NDPA appears in authentic shooter skin specimens at a lower concentration than DPA, which can make minor losses more substantial if some of the analyte is absorbed through the stratum corneum and can no longer be collected from the surface of the hand by the carbon stub. This is not observed during synthetic studies since the StratM® was exhaustively extracted, which allowed analytes to be recovered from any layer, unlike the surface extraction of human hands. Therefore, the difference observed for 4-NDPA between authentic shooters and synthetic skin membranes was considered explainable and did not diminish the validity of the StratM® to accurately model the persistence of OGSR compounds through human skin, especially when all other analyte trends were comparable.

Using synthetic membrane studies allowed information to be gained about OGSR for an additional persistence time of 6 hrs. After this time interval, some loss of analyte concentration was observed for DPA, 2-NDPA, 4-NDPA, and MC. However, AK II and EC still presented good concentration recovery. This was expected as AK II and EC have vapor pressures two and three orders of magnitude smaller than the rest of the analytes, evaporating slower. One great advantage of performing studies with synthetic skin membranes is gaining enhanced interpretation of trends. In authentic shooters, it cannot be entirely determined whether 4-NDPA was lost to evaporation or permeation through the epidermis. However, using synthetic skin membranes and an exhaustive extraction technique, we could establish that the loss of analytes was due to evaporation and not permeation. Therefore, not only did the StratM® accurately model authentic shooters, but it also provided additional important information on the specific movement and loss of organic gunshot residue compounds. Thus, it was concluded that although slight differences were observed between the authentic and synthetic collections, general trend information is corroborated between the two sample types.

The most significant findings regarding OGSR persistence are that:

(1) Most OGSR compounds are generally detectable with minimal loss in human skin and synthetic skin for up to 3 hours when no rigorous activity is performed after deposition.
(2) Although some OGSR loss was observed on the synthetic skin after 6 hours, all compounds were still detectable.
(3) The synthetic skin model corroborated general persistence trends observed in authentic specimens, offering more control of factors of interest and the ability to know true concentrations at time zero.

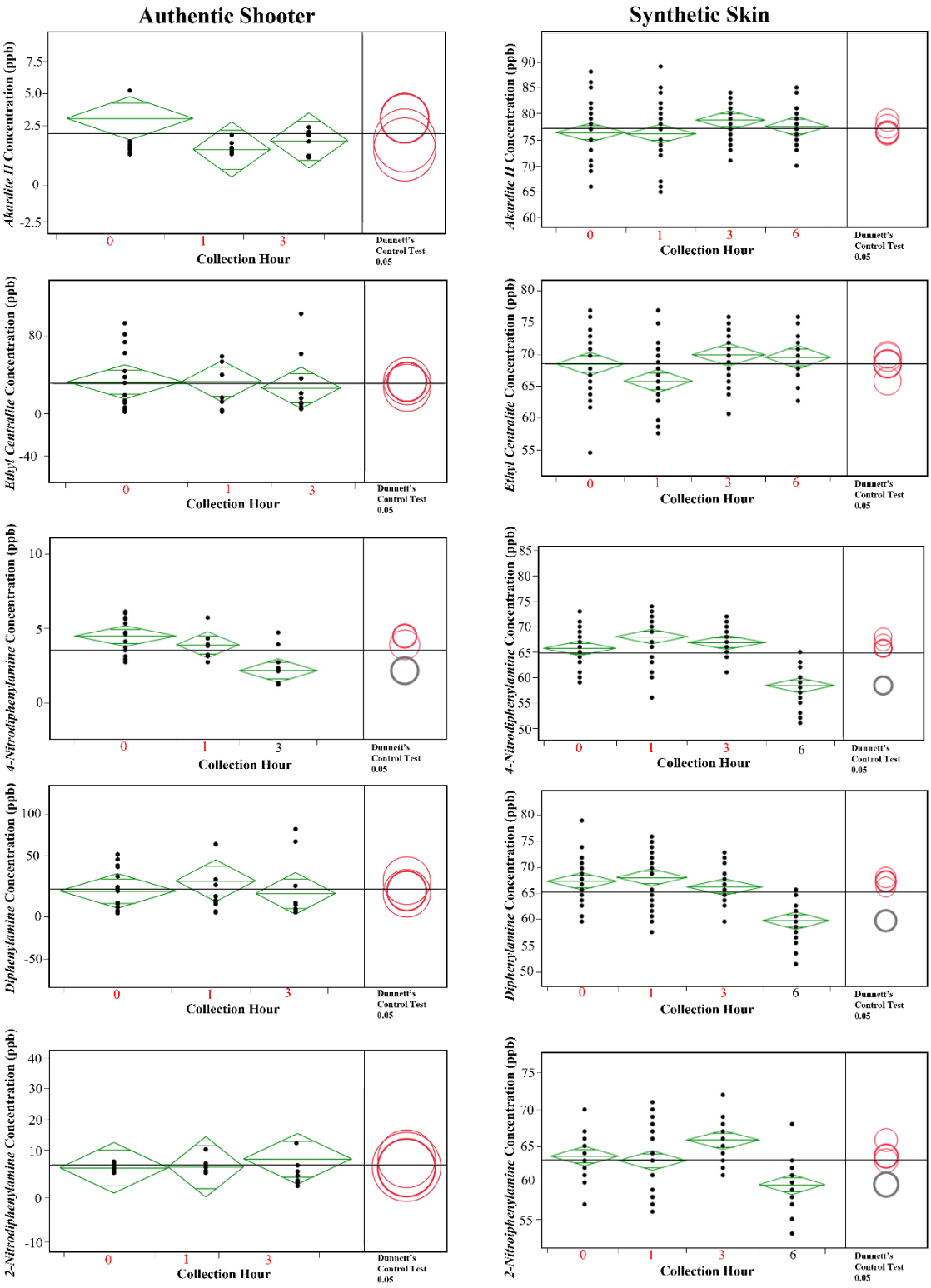


Figure 38. Persistence Dunnett's control test for OGSR analytes collected from authentic shooters (left) and synthetic skin membranes (right). Time intervals in red indicate belonging to the same group and time intervals in grey indicate means determined significantly different by Dunnett's Test.

pGSR Persistence Over Time in Human Skin after Discharging a Firearm and Under Controlled Conditions on Synthetic Skin Specimens

Inorganic gunshot residue trends were also compared using authentic shooter samples and StratM® membrane specimens spiked with a pGSR standard. Authentic shooters utilized ammunition loaded with CCI magnum primers containing unknown levels of primer constituents. For synthetic models, controlled particle counts were spiked onto samples using characterized pGSR standards mixed at known Pb, Ba, and Sb ratios. A Winchester (WIN) primer (~ 5.0 ppm of Pb, 6.1 ppm of Ba, and 3.1 ppm of Sb) and a TulAmmo (TUL) primer (~ 16.1 ppm of Pb, no Ba, and 1.7 ppm of Sb) were combined in 30:70 ratio. The number of characteristic and consistent particles detected throughout the study was controlled by introducing a primer with higher lead content. This is apparent in the number of particles observed for authentic shooter samples compared to synthetic membrane samples at time zero. Authentic shooters generally produced about 1250 particles between characteristic and consistent classifications. In contrast, simulated samples produced about 5000 particles between the two classifications for the TUL and WIN mixture (**Table 28**). Therefore, relative overall trends were evaluated compared to the initial particles at time zero instead of direct comparisons using particle counts.

Table 28*. Mean Particle Counts for Authentic and Synthetic Persistence Samples (% loss refers to the percentage of particles recovered compared to counts achieved in 0 hour samples)*

	Authentic Shooter				Synthetic Skin			
	Characteristic		Consistent		Characteristic		Consistent	
Hour	**Particles Recovered**	**% Loss**	**Particles Recovered**	**% Loss**	**Particles Recovered**	**% Loss**	**Particles Recovered**	**% Loss**
0	479±186	--	757±535	--	963±218	--	3990±625	--
1	315±243	34%	598±572	21%	783±78	16%	3611±630	9%
3	158±150	67%	521±360	31%	851±177	12%	3345±181	16%
6	**--**	**--**	**--**		881±214	9%	3349±734	16%

For authentic shooter samples, a decrease in characteristic particle counts was observed over time, with an overall 67% loss three hours post-firing. Although less substantial (31%), this same trend was observed for consistent particles collected from the hands of authentic shooters. A Dunnett's control test revealed loss of characteristic particles after three hours was significant compared to baseline particle counts collected immediately after firing. However, the number of consistent particles remained relatively constant for one to three hours, as demonstrated by boxplots in **Figure 39**. This outcome is similar to previous studies where GSR particles were still recovered from office workers hours after discharging a firearm. However, our research demonstrated the difference in loss of characteristic versus consistent particles, adding additional information to the interpretation. The most important takeaway from these results is that many particles, and positive GSR samples, can still be obtained three hours post-discharge when minimal activities have been performed.

Similar trends in the loss of pGSR particles were also observed on the synthetic skin samples. However, the loss was less drastic than that observed in authentic skin samples, and a significant difference in particle numbers was not observed even after 6 hours (**Figure 39**). The reasoning was equated to synthetic skin samples sitting in a laboratory undisturbed for different time intervals. Once spiked, the membranes were not handled until extraction, limiting the possibility of particles being removed or lost from the membranes. This can be related to post-shooting circumstances in suicide

case studies, where large particle counts are achieved days after the incident took place. In other words, when particles were on a stagnant surface, little to no loss of particles occurred. However, a significant loss of characteristic particles occurred at 3 hours when human factors were involved, even with limited activity.

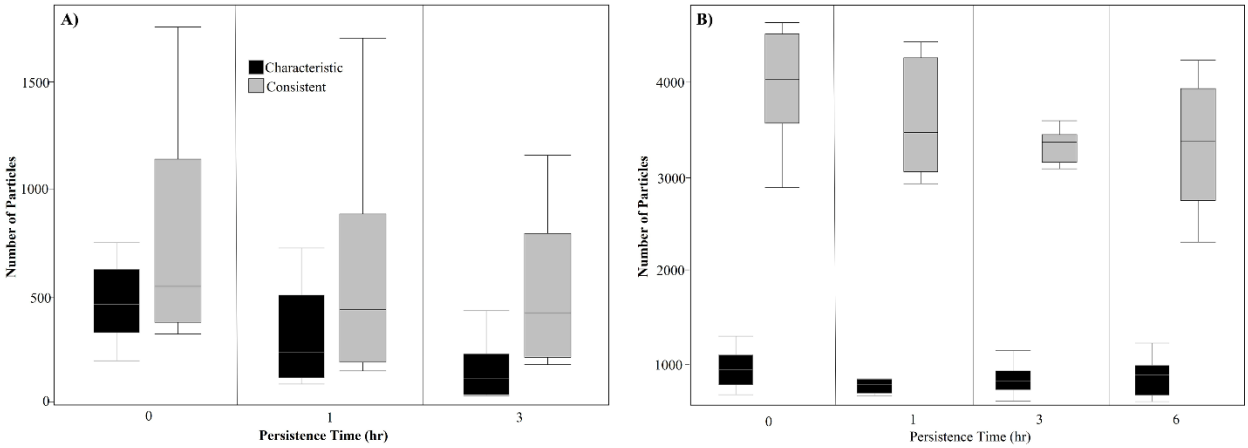


Figure 39. *Box plots showing characteristic and consistent particle distribution and loss after different time intervals for authentic shooters (left) and simulated studies using StratM® membranes and pGSR standards (right)*

Furthermore, we are controlling the number of particles deposited on the surface of the membrane by introducing the pGSR standard with known particle counts. During authentic shootings, the random nature of the event leads to higher uncertainty of deposited particles. Therefore, we provided higher confidence in results obtained during simulated studies, while complementing those observations on authentic shooter studies. The composition of ammunition sources explained the differences in characteristic and consistent particles. These findings show that the StratM® membrane is feasible to model static pGSR behavior and persistence on authentic shooters.

Although there were observed differences in the authentic and synthetic skin samples mostly due to differences in ammunition sources, overall trends and significant findings are congruent:

(1) Detecting characteristic and consistent pGSR particles is feasible for up to 3 hours after deposition. Detection at longer intervals is observed when relatively minor post-shooting activities are performed.

(2) Physical pGSR particles persist over time and are not significantly lost unless outside forces are imposed on the particles; a critical aspect to consider when investigative questions arise about potential suicide and homicide victims.

LIBS results for the authentic shooter persistence study.

LIBS analysis was first performed on the authentic 0-, 1-, and 3-hour authentic persistence samples using the micro-spatial method developed in phase 1 of the grant. Data analysis focused on the three major elements of interest for GSR analysis (Pb, Ba, and Sb).

LIBS Hand Samples Time Persistence Results

Box plots were created to represent the trends between the different sample sets visually and focused on Pb, Ba, and Sb trends. **Figure 40** shows the box plots that display the signal-to-noise ratio (SNR) on the y-axis and the hour of collection on the x-axis. The dotted line represents the critical threshold (CT) and is also written in the bottom right corner for each element of interest. The data spread in the box plots varies from hour to hour, with no substantial analyte lost over the 3 hours, corroborating observations by SEM-EDS. Following the box plots, an Analysis of Variance (ANOVA) was performed, using a Dunnett's test with time zero as control. No significant differences in Ba, Sb, or Pb content were found after 3 hours.

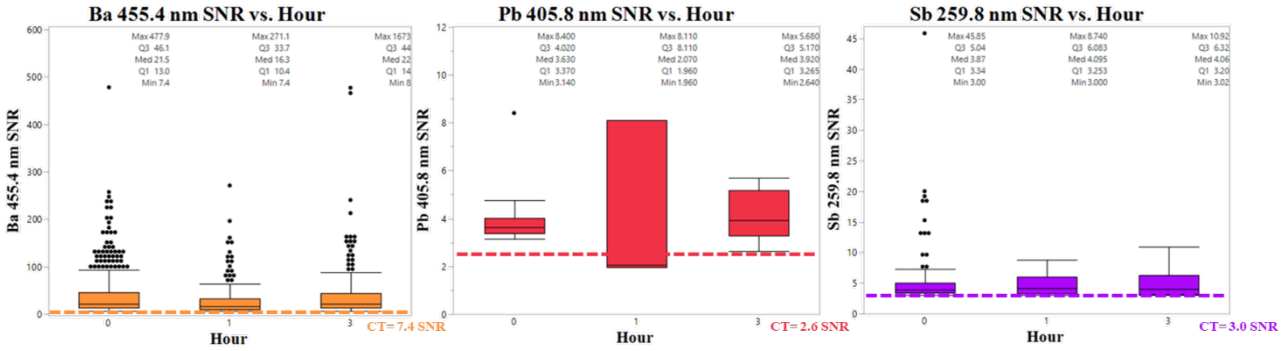


Figure 40. *Box plot comparison of hand persistence sample for 0-, 1-, and 3-hour periods for barium (orange), lead (red), and antimony (purple) with five number summaries and their respective critical thresholds represented by the dotted lines and in the bottom right corner.*

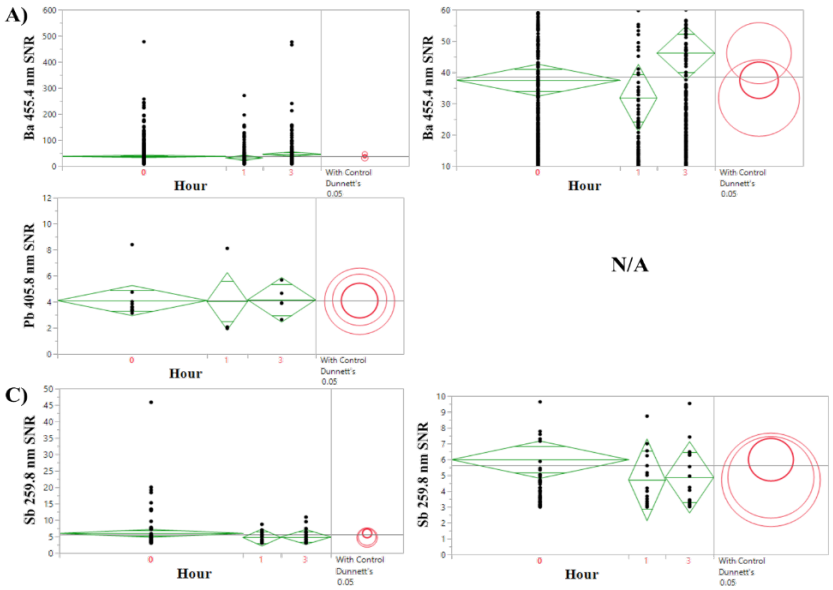


Figure 41. *Analysis of Variance report for Ba (A), Pb (B) and Sb (C) for the 0-, 1-, and 3-hour time periods with respective Dunnett's report where the 0-hour acted as the control group.*

LIBS Results for Persistence of Facial Areas

Exploratory analysis using boxplots was also performed on the facial samples. Due to low detection observed at time zero, the only times performed for this set were 0- and 1-hour. In **Figure 42,** we can observe a slight decrease in the SNRs for the 1-hour samples. The graphs have been zoomed in to visualize the smaller values better. These values represent only a few samples above the respective critical thresholds.

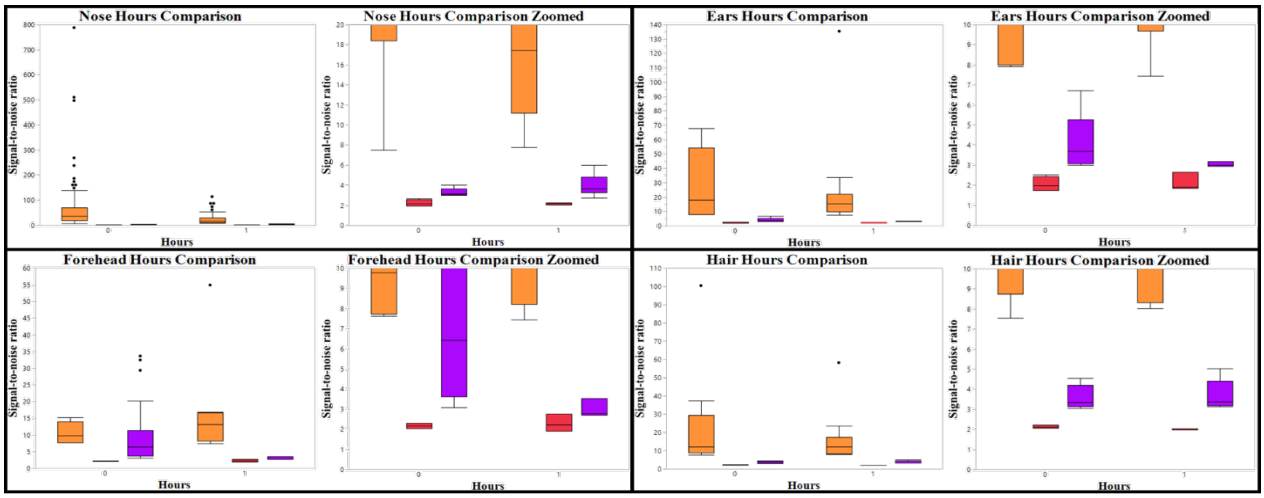


Figure 42. *Box plot graphs of the Ba (orange), Pb (red), and Sb (purple) SNR found in their respective facial collection areas.*

Table 29. *Positive analyte and sample calls using critical thresholds and a SNR of 3 for facial areas comparing the 0-hour and 1-hour time periods.*

Positive Analyte and Sample Calls								
	Above Critical Threshold				Above SNR of 3			
Collection time after shooting	Sb	Pb	Ba	Positive	Sb	Pb	Ba	Positive
Nose								
0 Hours (N=18)	5 (27.8%)	1 (5.6%)	8 (44.4%)	1 (5.6%)	6 (33.3%)	0 (0%)	16 (88.9%)	2 (11.1%)
1 Hour (N=11)	4 (36.4%)	0 (0%)	3 (27.3%)	2 (18.2%)	4 (36.4%)	0 (0%)	9 (81.2%)	2 (18.2%)
Ear								
0 Hours (N=18)	5 (27.8%)	0 (0%)	4 (22.2%)	0 (0%)	5 (27.8%)	0 (0%)	16 (88.9%)	0 (0%)
1 Hour (N=11)	1 (9.1%)	1 (9.1%)	6 (54.4%)	2 (18.2%)	1 (9.1%)	0 (0%)	9 (81.2%)	1 (9.1%)
Forehead								
0 Hours (N=18)	10 (55.6%)	0 (0%)	3 (16.7%)	1 (5.6%)	10 (55.6%)	0 (0%)	14 (77.8%)	2 (11.1%)
1 Hour (N=11)	1 (9.1%)	1 (9.1%)	4 (36.4%)	0 (0%)	1 (9.1%)	0 (0%)	9 (81.2%)	0 (0%)
Hair								
0 Hours (N=18)	5 (27.8%)	0 (0%)	5 (27.8%)	0 (0%)	5 (27.8%)	0 (0%)	13 (83.3%)	1 (5.6%)
1 Hour (N=11)	5 (45.5%)	0 (0%)	4 (36.4%)	1 (9.1%)	5 (45.5%)	0 (0%)	9 (81.2%)	1 (9.1%)

These results indicate that very little GSR accumulates in these areas. Also, there is some additional loss over time despite the participants not conducting any activity during the waiting times. Positive calls were also determined for each facial area and are summarized in **Table 29**. As the box plots displayed, very little Pb and a small amount of Sb were detected. This is also reflected in the low number of overall positive samples (0-<18%). Despite Sb and Ba being detected, they were rarely in the same spot on the stub. Thus, the results indicate that collecting from these alternative locations does not add much to the hands sampling for GSR analysis by LIBS.

Electrochemical results from the authentic shooter and membrane persistence study

Following the LIBS analysis of the persistence samples, electrochemical analysis was performed on the 0-, 1, and 3-hour samples using the previous electrochemical methods reported in phase I of the grant.

Electrochemistry Hand Sample Results

Graphical, exploratory data analysis and critical thresholds were used in the initial assessment of the persistence study samples by electrochemistry. Box plots were utilized to visualize the trends in peak current areas for lead, copper, and nitroglycerin (**Figure 43**), where the peak current area is shown on the y-axis for comparison to the periods on the x-axis. The dotted lines represent the respective critical thresholds for each analyte as calculated from the non-shooter population of 350 samples previously reported in phase I of the grant. A slightly decreasing trend for lead is shown, where the median current area decreases as the time between shooting and collection becomes longer. Less noticeable trends are viewed in copper and nitroglycerin, wherein the 1 hour, approximately three samples had noticeably higher copper signals and two samples had higher nitroglycerin signals.

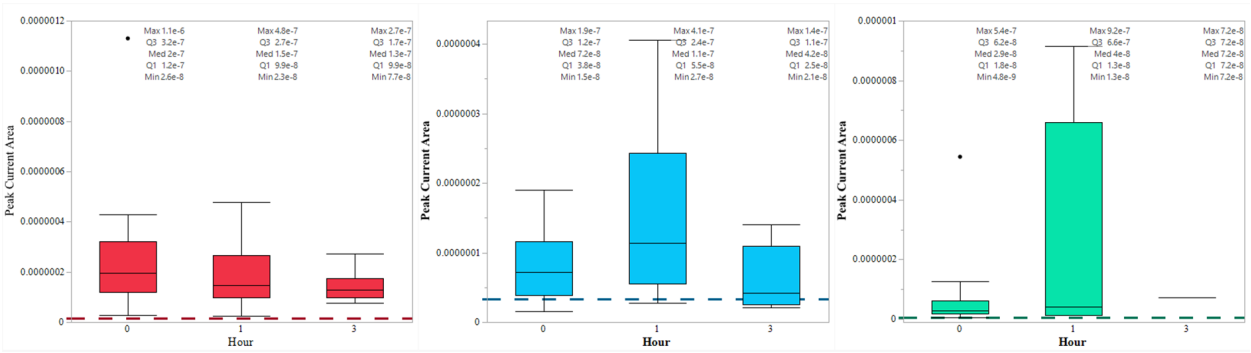


Figure 43. *Box plot comparison of hand persistence sample for 0-, 1-, and 3-hour time periods for lead (red), copper (blue), and nitroglycerin (green) with five number summaries and their respective critical thresholds represented by the dotted lines.*

Analysis of Variance (ANOVA) with Dunnett's control was used for lead, copper, and nitroglycerin (**Figure 44**). Results from the test found no difference in mean for both IGSR and OGSR analytes. For nitroglycerin, results were skewed as only one signal for nitroglycerin was found to be positive above the critical threshold in time 3h. Student's t was also used for the assessment of means and variance, which also found no difference between the periods.

A sample is deemed positive if 2 or more GSR analytes are present above the critical threshold values, which can be a combination of two IGSRs or an IGSR and OGSR analyte. **Table 30** demonstrates the results for all individual samples analyzed by electrochemistry for the persistence study and the final percent identification of the critical threshold. Lead persisted the most, with 100% of samples at

each time called positive. A small difference in copper was found at the 0- and 1-hour times (48% and 58%, respectively), with a sharp decrease to 25% in the 3-hour samples. The nitroglycerin signal diminished from 78% at 0 hours to 8% in the 3-hour samples. GSR positive samples (2 or more analytes) followed a steady progression of loss, where approximately a 30% drop was seen between periods (83% (0-hour) to 58% (1 hour) to 33% (3 hours). These results agreed with the LIBS data.

Table 30. *Positive calls and percentages for lead, copper, and nitroglycerin for hand samples and overall positive calls for two or more analytes for 0-hour (A), 1-hour (B), and 3-hour (C) periods.*

Positive Analyte and Sample Calls above Critical Threshold				
Collection time after shooting	**Pb**	**Cu**	**NG**	**Positive**
0 Hours (N=23)	23 (100%)	11 (48%)	17 (74%)	19 (83%)
1 Hour (N=12)	12 (100%)	7 (58%)	5 (42%)	7 (58%)
3 Hour (N=12)	12 (100%)	3 (25%)	1 (8%)	4 (33%)

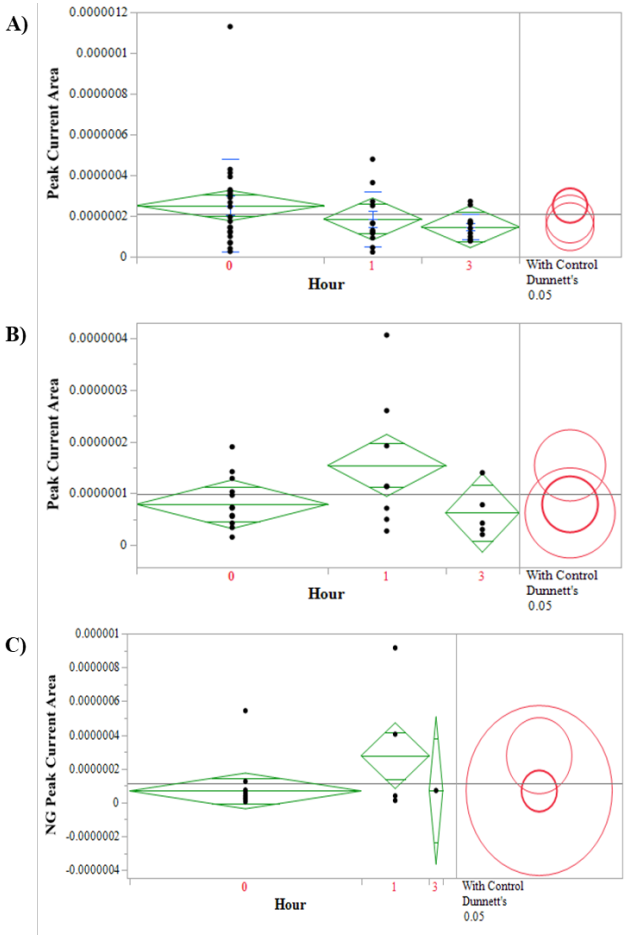


Figure 44. *Analysis of Variance report for lead (A), copper (B) and nitroglycerin (C) for the 0-, 1-, and 3-hour time periods with respective Dunnett's report where the 0-hour acted as the control group.*

Electrochemistry Persistence of Facial Area Results

The box plot in **Figure 45** demonstrates a generally decreasing trend from 0 to 1 hour; however, the GSR analyte signals were very limited, with only 1 to 7 positive calls made in a given period out of the possible 17 samples (0-hour) and 12 samples (1-hour). Due to the lower prevalence of the individual analytes in these samples, statistical analysis of this sample set would be extremely limited and would not provide generalized information. However, based on the results, several observations can be discussed. First, as expected, the levels of the individual analytes were generally higher in the 0-hour than in the 1-hour collections. It is also important to note in box plots the absence of some analytes, where these were not observed in the facial samples and were much lower compared to hand specimens.

Table 31 provides the breakdown for each sample set and the facial area time. The 0-hour had a total number of analyte identification above the critical threshold of nine samples for lead, one for copper, and six for nitroglycerin. This was compared to the 1-hour with seven samples for lead, zero for copper, and six for nitroglycerin. Interestingly, the nose resulted in most lead above the critical threshold with 7 samples in the 0 hours, which decreased to 4 for the 1 hour. These comparisons were also provided based on using the lowest calibrator as the classifier of positive or negative since the previous population study only addressed background levels on hands, although these comparisons were similar, with only minor differences. Finally, a low number of positives was observed. Indeed, only one sample was classified as positive for GSR out of both periods, and this was a nose sample collected at time zero. As a result, it was decided to forego testing of the facial areas by electrochemistry at longer time intervals.

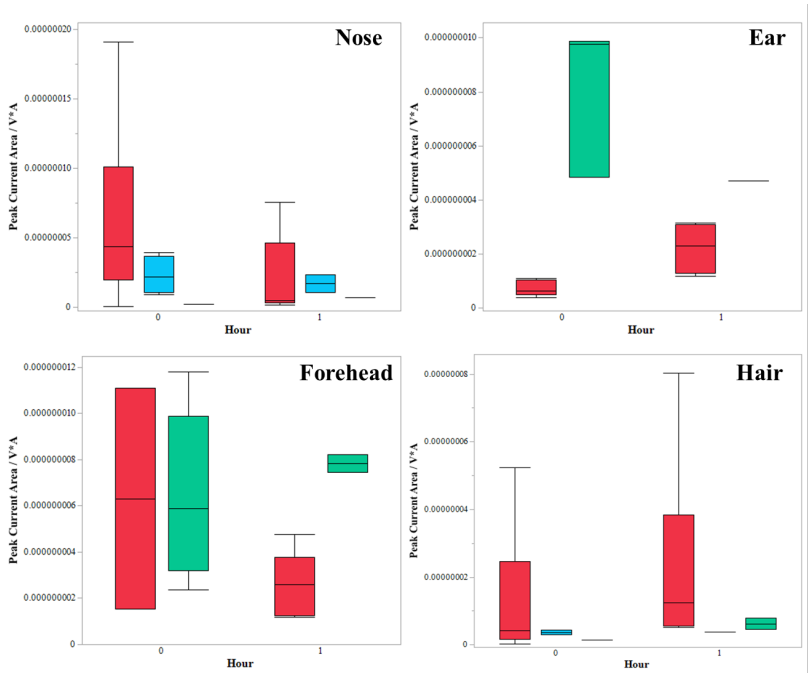


Figure 45. *Box plot graphs of the lead (red), copper (blue), and nitroglycerin (green) peak current areas found in their respective facial collection areas.*

Table 31. *Positive analyte and sample calls using critical thresholds and the average lowest calibrator for facial areas comparing the 0-hour and 1-hour time periods.*

Positive Analyte and Sample Calls in Facial Areas								
	Above Critical Threshold				Above Average Lowest Calibrator			
Collection time after shooting	Pb	Cu	NG	Positive	Pb	Cu	NG	Positive
Nose								
0 Hours (N=17)	7 (41%)	1 (6%)	0 (0%)	1 (6%)	4 (24%)	1 (6%)	1 (6%)	1 (6%)
1 Hour (N=12)	4 (33%)	0 (0%)	1 (8%)	0 (0%)	2 (17%)	0 (0%)	1 (8%)	0 (0%)
Ear								
0 Hours (N=17)	0 (0%)	0 (0%)	3 (18%)	0 (0%)	0 (0%)	0 (0%)	3 (18%)	0 (0%)
1 Hour (N=17)	0 (0%)	0 (0%)	1 (8%)	0 (0%)	0 (0%)	0 (0%)	1 (8%)	0 (0%)
Forehead								
0 Hours (N=17)	0 (0%)	0 (0%)	3 (18%)	0 (0%)	0 (0%)	0 (0%)	5 (29%)	0 (0%)
1 Hour (N=12)	0 (0%)	0 (0%)	2 (17%)	0 (0%)	0 (0%)	0 (0%)	2 (17%)	0 (0%)
Hair								
0 Hours (N=17)	2 (12%)	0 (0%)	0 (0%)	0 (0%)	1 (6%)	0 (0%)	1 (6%)	0 (0%)
1 Hour (N=12)	3 (25%)	0 (0%)	2 (17%)	0 (0%)	1 (8%)	0 (0%)	2 (17%)	0 (0%)

Task 2.3: To determine the influence of fiber type on the persistence of IGSR and OGSR on clothing substrates

Figure 46 illustrates the design for the clothing experiments. Samples were processed by LC/MSMS and SEM-EDS.

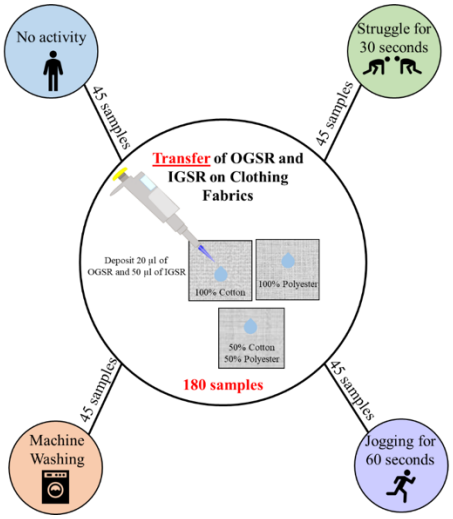


Figure 46. *Project and experiment outline for transfer studies completed on three different fabric types.*

Transfer and Persistence of OGSR on Fabrics

For transfer and persistence studies involving fabrics, it was essential to understand how the combined fabric composition (fiber type and construction) influenced the base recovery of analytes. For example, the 100% cotton-woven fabric had a tight weave pattern that dried slowly, the 35%Cotton/65%Polyester-woven fabric had a loose weave pattern that dried at a medium rate, and the 100% polyester-knit fabric had an open knit pattern that dried at a faster pace. Baseline recoveries achieved for each analyte when no activity was performed can be seen in **Figure 47**. In general, full recovery of OGSR compounds in baseline sets was not feasible due to the retention and OGSR-affinity properties to the substrates. The cotton fabric tended to have higher concentration recoveries for all analytes (except 4-NDPA), compared to the other two fabrics, with recoveries ranging from 61% to 84% and an average recovery rate of 73% across analytes. The fabric with the lowest analyte recovery was polyester, with an average of 68% across analytes, and the cotton/polyester mix fell between with an average recovery rate of 71%. Therefore, differences between each were considered in the experimental design, and all percent losses observed for activity samples were calculated accordingly based on the average "base recoveries" of each fabric type. Furthermore, these baselines show that fabric type did not significantly affect OGSR analyte evaporation or retention.

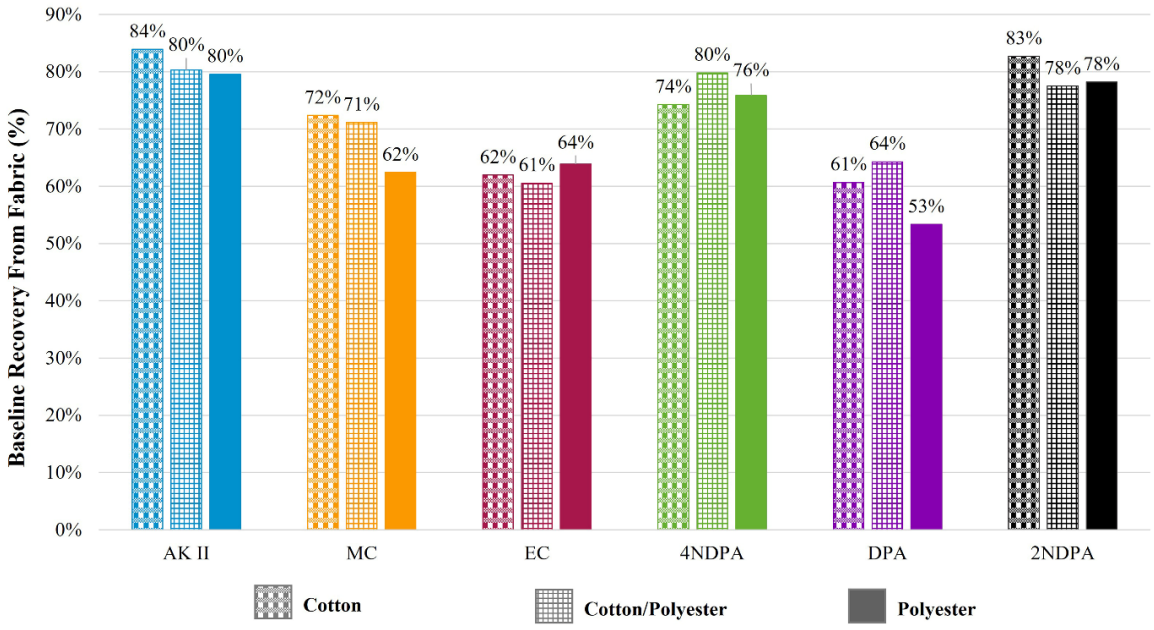


***Figure 47**. Baseline recovery results of analytes from three fabric types: cotton, cotton/polyester, and polyester.*

Activity studies performed on fabric samples showed similar OGSR trends to StratM® transfer studies. In other words, as the level of interaction and robustness of activity increased, the more significant observed loss of OGSR analytes (**Table 32**). For example, running resulted in high retention of OGSR analytes, with the most considerable percent loss being 4% for EC on cotton fabric. The polyester and blended fabrics had similar results. These results were unsurprising as running did not require extensive interaction with the fabric samples leaving the analytes primarily undisturbed.

Two fabric pieces were placed on top of each out when simulating a struggle and moved back and forth while applying pressure. Interestingly, the blended fabric exhibited a higher loss during the experiments than the single-composition fabric samples (100% cotton, 100% polyester), which were comparable. As seen in **Table 32,** cotton yielded percent losses between 11% and 20%, polyester produced losses between 10% and 20%, and the blended fabric resulted in losses ranging from 26% to 43%. It is important to note that composition and construction were compound factors that are not separated in this experimental design. Therefore, the higher loss may be a combination of knit interlacing and the blended cotton/polyester fiber types. Regardless of the fabric type, DPA was the most lost analyte. This result was expected since DPA has the highest vapor pressure out of all analytes, and the struggle procedure was comparable to that performed for rubbing hands, yielding similar results.

Washing fabric samples presented the most interesting results. As expected, it resulted in the most significant reduction of the analyte concentration of the activities performed. However, as seen in **Figure 48**, not all analytes followed the same trend. Akardite II lost the most on average, with a 2% average recovery across fabrics (%loss ~98%). This was followed by methyl and ethyl centralite with average recoveries of 5% and 7% (%loss ~92% and 89%, respectively). However, DPA and its derivatives showed higher recovery of analytes for cotton, polyester, and blended fabrics. The water solubility properties of the compounds can explain this. Unlike the StratM® samples, clothing samples were fully submerged in a detergent solution and clean DI water for 60 seconds.

Furthermore, the samples were agitated during submersion using a whisk spun back and forth. This allowed the water to interact with the fabric and extract the OGSR analytes. Akardite II is soluble in water up to a concentration of 10 ppm, corresponding to 98% of the concentration moving from the fabric into the detergent solution and going undetected. Similarly, MC and EC are soluble up to a concentration of 5 ppm. This was also observed in concentration results where MC and EC were lost but retained in the fabric more than AK II. However, DPA, 2-NDPA, and 4-NDPA all have less than 1 ppm water solubilities. Therefore, a higher concentration was expected to remain in fabric samples.

The increase in analyte retention can be seen in **Table 32**, where 4-NDPA had average percent recoveries between 26%-37%, 2-NDPA had average recoveries between 32%-46%, and DPA had average percent recoveries between 16%-22%, depending on the fabric type. These results show that even after fabric washing, it was possible to extract and detect the stabilizer DPA and its combustion by-products, 4-NDPA and 2-NDPA, from three different fabric types and is a good sampling source for casework. The only activity that did not result in a significant loss of OGSR concentration was running, as little disruption of OGSR from the substrate is explainable.

Table 32. *Summary of OGSR Analyte Recovery and Losses from Three Different Fabric Constructions After Activity*

	Cotton											
	AK II		MC		EC		4NDPA		DPA		2NDPA	
Clothing Activity	% Recovery	% Loss	% Recovery	% Loss	% Recovery	% Loss	% Recovery	% Loss	% Recovery	% Loss	% Recovery	% Loss
None	87±8%	--	74±10%	--	64±5%	--	76±5%	--	61±6%	--	84±5%	--
Running	84±6%	3%	77±7%	4%	63±5%	2%	73±5%	4%	60±5%	2%	84±7%	0%
Struggle	76±6%	13%	59±5%	20%	57±5%	11%	65±5%	14%	50±6%	18%	72±5%	14%
Washing	1±0%	99%	4±0%	95%	10±1%	84%	35±7%	54%	18±1%	70%	32±3%	61%

	Polyester											
	AK II		MC		EC		4NDPA		DPA		2NDPA	
Clothing Activity	% Recovery	% Loss	% Recovery	% Loss	% Recovery	% Loss	% Recovery	% Loss	% Recovery	% Loss	% Recovery	% Loss
None	80±6%	--	63±11%	--	64±9%	--	77±7%	--	54±12%	--	79±5%	--
Running	84±4%	5%	63±5%	0%	62±4%	3%	75±3%	3%	54±3%	0%	83±3%	5%
Struggle	72±4%	10%	54±4%	14%	56±4%	13%	68±5%	12%	43±3%	20%	70±6%	11%
Washing	2±1%	98%	5±1%	92%	6±0%	91%	26±1%	66%	16±1%	70%	35±2%	56%

	Cotton (35%) /Polyester (65%)											
	AK II		MC		EC		4NDPA		DPA		2NDPA	
Clothing Activity	% Recovery	% Loss	% Recovery	% Loss	% Recovery	% Loss	% Recovery	% Loss	% Recovery	% Loss	% Recovery	% Loss
None	81±5%	--	71±7%	--	61±5%	--	81±7%	--	65±5%	--	78±3%	--
Running	81±2%	0%	75±2%	6%	64±3%	5%	79±2%	3%	66±2%	2%	80±3%	3%
Struggle	60±3%	26%	50±4%	30%	44±4%	28%	55±4%	32%	37±5%	43%	57±6%	27%
Washing	2±0%	98%	5±0%	92%	7±1%	89%	37±2%	54%	22±1%	66%	46±2%	41%

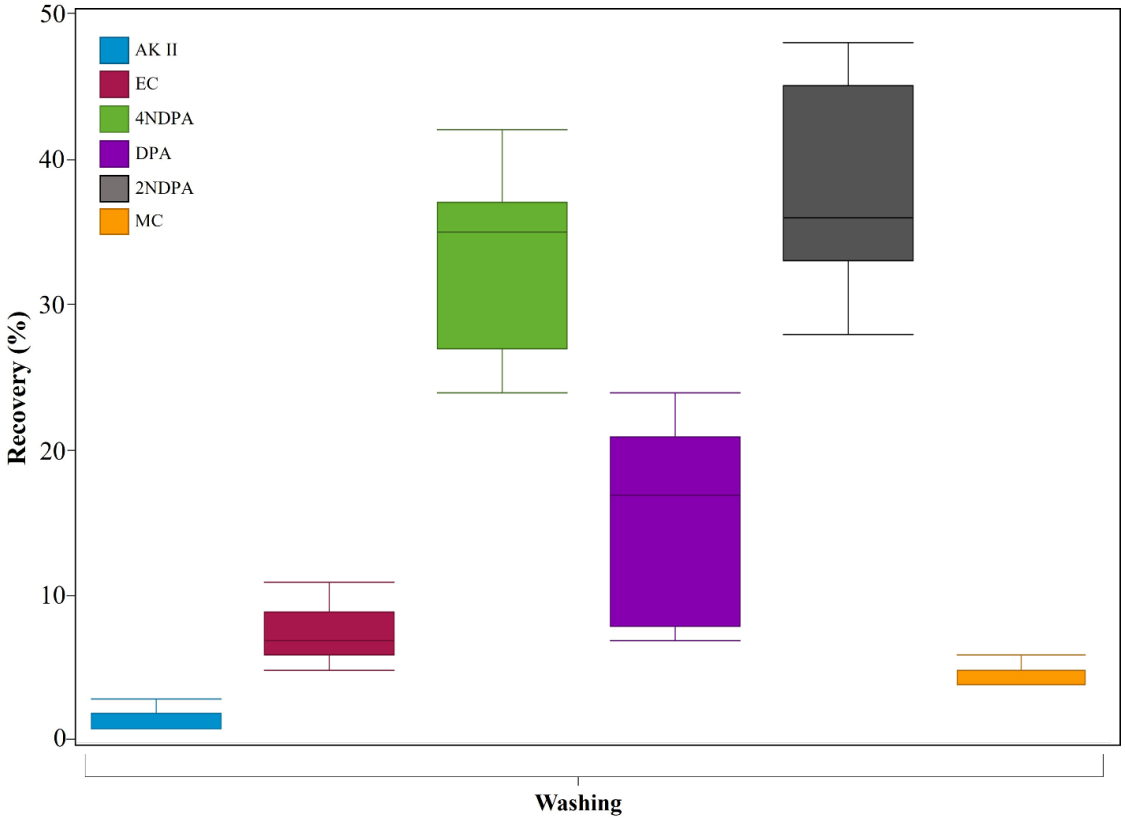


Figure 48. *Box plots with average OGSR analyte recovery across all fabric compositions after washing.*

The most significant findings about OGSR clothing transfer and persistence are as follows:

(1) Overall, OGSR compounds present at trace levels are not easily recovered from fabrics; thus, more exhaustive solvent extractions may be preferred over typical carbon adhesive stubbing. The fabric composition and fiber type evaluated in this study do not significantly impact the retention or loss of OGSR compounds.

(2) If a struggle (i.e., arrest) ensues, some OGSR compounds can be lost from the suspect's clothing but are unlikely to transfer to a second individual or arresting officer.

(3) Recovery of OGSR from clothing is feasible, even after washing. After washing a piece of clothing, analysts are more likely to recover DPA and its derivatives than other stabilizing materials due to solubility and partition properties.

Transfer and persistence of IGSR in fabrics

Clothing interpretation was more challenging for pGSR, as less than 100 total characteristic and consistent particles were recovered from samples for all fabric types when no activity was performed. There are multiple reasons for this poor recovery. First, fibers appeared bright against the carbon background using the backscatter detector during SEM-EDS automated particle mapping. The GSR particles also appeared bright and could have been masked by the fibers, resulting in pGSR particles going undetected. Second, is the nature of the fabric design. The stitching of the fabrics created different weave and knit patterns that varied fiber construction. For example, cotton and mixed fabrics both had weave patterns. However, the mixture had a plain weave with substantially larger gaps than the cotton fabric, which was constructed in a twill weave pattern and had deep grooves (**Figure 49**).

The polyester was created using an interlocking knit pattern, resulting in large empty spaces between each fiber. These grooves and gaps may have caused particles to embed or fall through threads and settle on the substrate below. Therefore, some of these particles were not recovered when stubbing the fabric's surface. For these reasons, insufficient particle data was collected during this part of the study to make informed conclusions. Another analytical technique may be used to overcome some limitations, such as LIBS, which can ablate through the interfering surface fibers on the carbon stub and provide information on any pGSR concealed beneath or trapped in the woven or knit constructions.

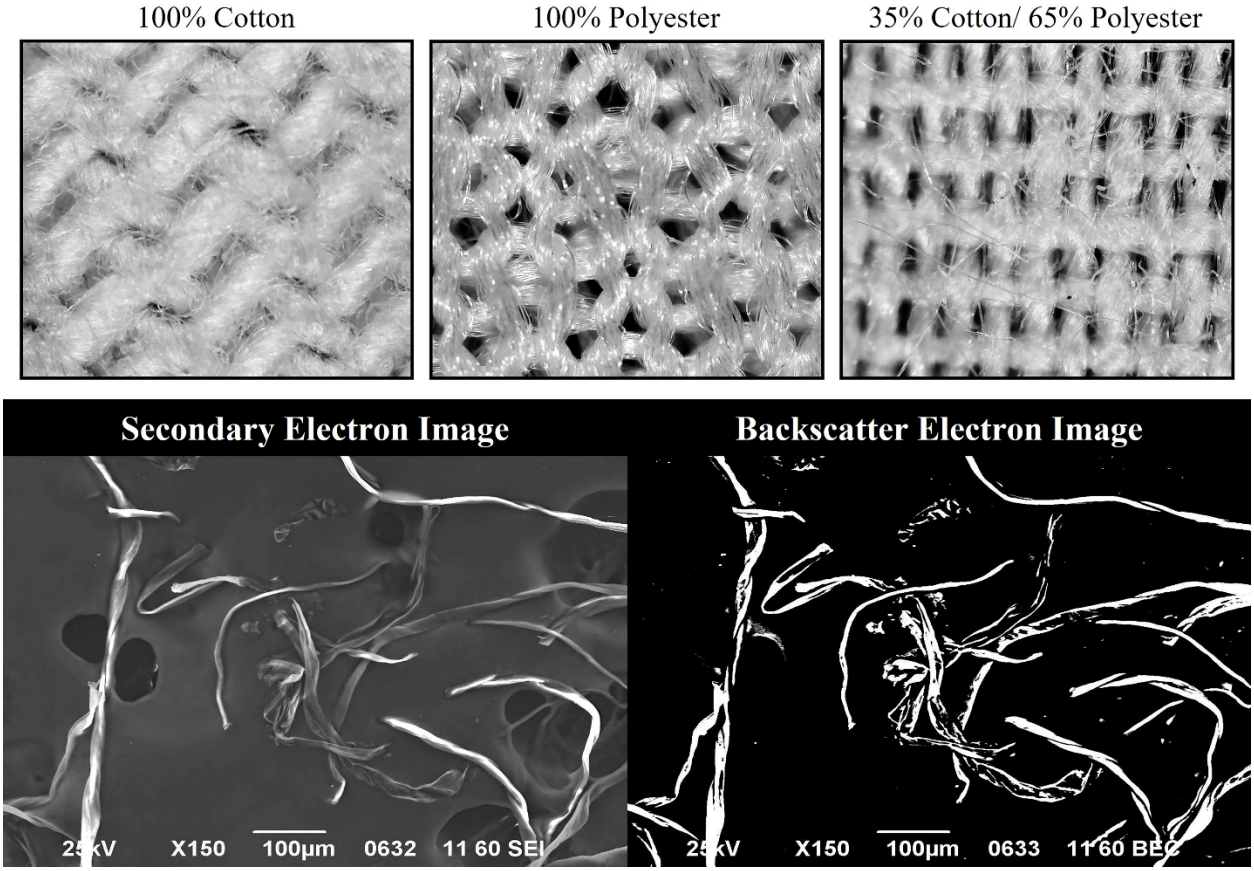


***Figure 49**. Top: Magnified images of fiber construction for three types of fabrics used in this study. Bottom: Secondary and backscatter electron images of interfering fibers on SEM-EDS stub surface.*

Results from Task 3 (Objective 3)— Testing of portable LIBS and EC units for the detection of GSR at crime scenes and laboratories, and comparative studies of cost-efficiency and reliability of bench-top and portable units

In this task, we focused on working with industry partners in developing LIBS and EC portable configurations and comparing their performance to benchtop instruments already validated in our group for GSR examinations. The performance was evaluated using the IGSR/OGSR standard materials, sets of authentic specimens collected from the hands of individuals of interest, and through mock crime scenes of various levels of complexity that simulate real situations one may encounter in

firearm-related investigations. A summary of the main findings is provided below, while more information can be found in Vander Pyl et al. [2,3] and Dalzell et al. [6]

Task 3.1. Comparison of the performance of bench-top LIBS and ECD instruments to the portable version of the instrumentation using the OGSR-IGSR standard mix and authentic samples

Feasibility study for LIBS portable instrumentation

This study evaluated a mobile LIBS instrument to detect inorganic GSR and compared its performance to a previously validated laboratory instrument. The mobile LIBS is designed with advanced configurations specifically for on-site GSR analysis, including a CMOS detector and a sampling chamber that holds up to six typical GSR collection devices with separate gas flow ports to prevent cross-contamination. A significant novelty incorporated to the portable instrument is a camera with high resolution and image magnification. The imaging system allows the user to target micron-size particulates typically encountered in GSR, which allows quick searching and visualization of GSR particle morphology for direct single-particle analysis (see **Figure 50)**. The single-particle elemental composition is one of a kind and offers superior confirmatory features for GSR.

The mobile LIBS performance was evaluated for residues collected from the hands of shooters (100 samples) and non-shooters (200 background samples), analyzed sequentially by the mobile instrument and then the laboratory instrument. Interestingly, the portable instrument was on-par with the benchtop one, achieving accuracy better than 98.8% (See **Table 33**).

The validation studies revealed that the mobile instrument reached detection levels needed for trace levels of IGSR specimens that were equal to or better than the laboratory method. The mobile instrument showed LODs of 0.2 ng (Ba), 2.0 ng (Pb), and 2.0 ng (Sb) compared to 0.2 ng (Ba), 50 ng (Pb), and 220 ng (Sb) for the laboratory instrument, demonstrating how the specialized configurations (i.e., laser wavelength, detector type, sampling chamber, and magnification) adapted for mobile GSR analysis allows portability of the unit without sacrificing performance of the method.

Noteworthy, we are not suggesting replacing SEM-EDS analysis with LIBS since SEM-EDS still provides much superior resolution and magnification for morphological analysis and is non-destructive. Instead, we envision the LIBS technology as a potential breakthrough in the field of GSR by providing fast and highly accurate screening alternatives that are otherwise not available. LIBS can therefore improve decision-making efficiency at the crime scene and assist forensic laboratories with triage and case management in the laboratory for increased confidence in the results, reduced backlogs, and a prompter service to end-users.

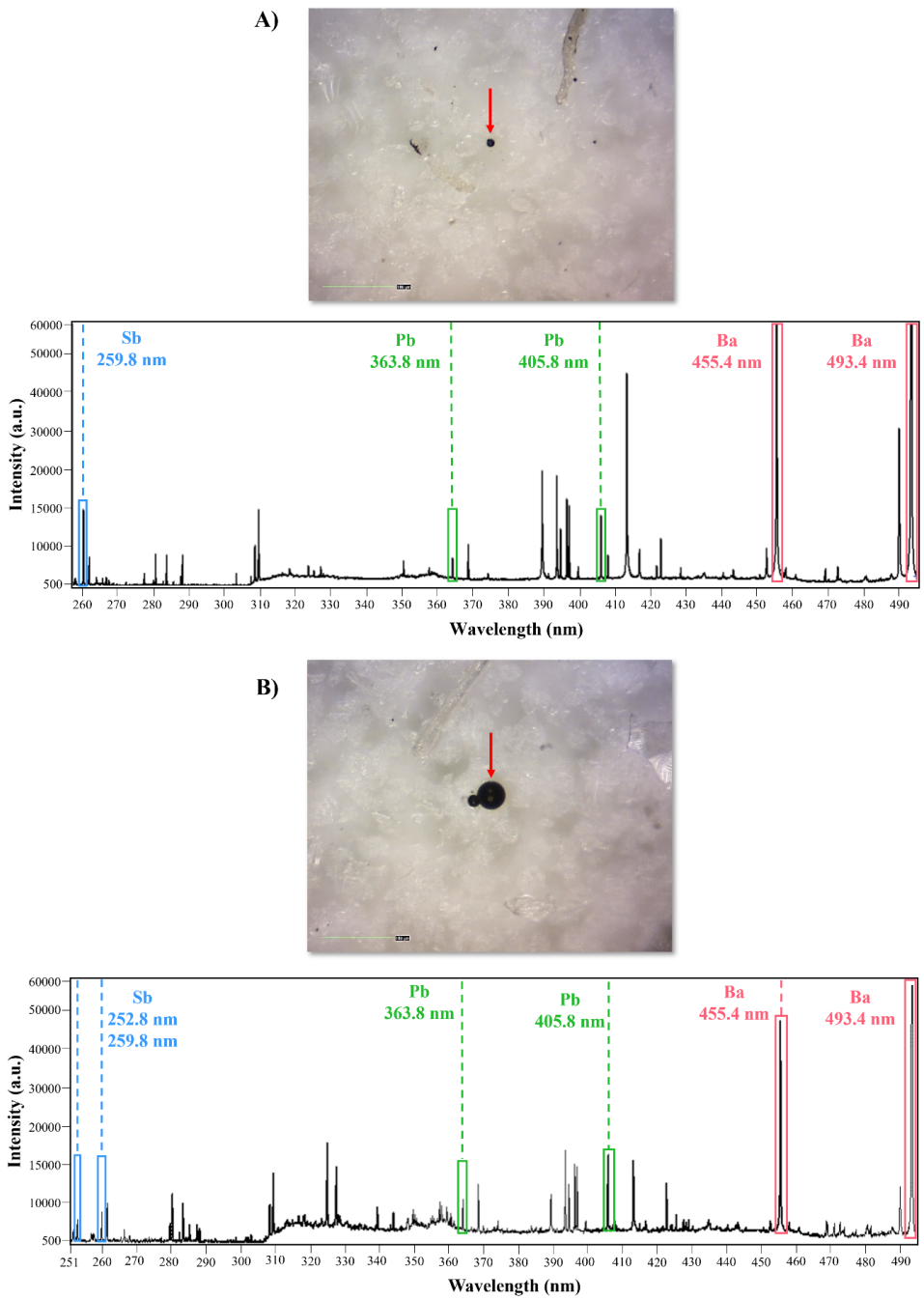


Figure 50. *Mobile LIBS images of gunshot residue particles collected from shooters' hands at sizes A) ~8 um and B) ~20 um, with corresponding LIBS spectra for each particle.*

The results demonstrate the scientific validity and reliability of the LIBS laboratory and mobile methods for authentic casework-like samples at trace levels (e.g., skin residues). Overall, this study furthers the necessary scientific foundations to implement this LIBS technology as a mobile detection technique for gunshot residue evidence.

Table 33. *Performance Rates for Authentic Shooter and Non-Shooter Hand Samples by Laboratory and Mobile LIBS Instruments*

	Laboratory J200 LIBS		Mobile LIBS	
	Number of Samples	**Percentage**	**Number of Samples**	**Percentage**
True Positive (n=100)	100	100%	100	100%
True Negative (n=200)	197	98.5%	195	97.5%
False Positive (n=200)	3	1.5%	5	2.5%
False Negative (n=100)	0	0%	0	0%
Accuracy	99.0%		98.8%	

Feasibility study for ECD portable instrumentation

The results demonstrate equivalent identification of GSR between the two ECD instruments, which provides a foundation for further implementation for preliminary testing of suspected GSR at forensic laboratories and the crime scene. The first step in this study was the optimization of instrumental parameters and evaluation of their figures of merit. **Tables 34** and **35** summarize these results.

Table 34. *Square-wave voltammetry parameters comparison used with bare screen-printed carbon electrodes for the Metrohm Autolab and PalmSens4 potentiostats.*

Parameter	**Benchtop Instrument**	**Portable Instrument**
Deposition Time	120 s	120 s
Deposition Potential	-0.95 V	-0.95 V
Start Potential	-1.0 V	-1.0 V
End Potential	1.2 V	1.2 V
Potential Step	0.004 V	0.005 V
Amplitude	0.025 V	0.025 V
Frequency	8 Hz	11 Hz

The sample preparation method provided the ability to analyze the same specimens on both instruments with ease of analysis taking under 5-10 min per sample and resulting in data directly comparable between instruments for the authentic samples. Electrochemical performance characteristics demonstrated the comparable specificity and sensitivity between the benchtop and portable potentiostats for simultaneous IGSR and OGSR detection with limits of detection below 0.6 µg/ml for both instruments. The most significant difference was that the benchtop potentiostat demonstrated better repeatability (**Tables 35 and 36**)

Table 35. *Performance characteristics calculated based on the Metrohm Autolab benchtop instrument.*

IGSR	Potential (V)	Linear Range (µg/mL)	R^2	Repeatability (%RSD, n=3)	LOD (µg/mL)
Lead	-0.784 ± 0.035	0.10 to 2.0	0.999	4.4	0.055 ± 0.01
Antimony	-0.401 ± 0.027	0.75 to 7.5	0.986	10	0.183 ± 0.07
Copper	-0.292 ± 0.053	0.05 to 1.0	0.990	2.3	0.012 ± 0.001
OGSR	**Potential (V)**	**Linear Range (µg/mL)**	**R^2**	**Repeatability (%RSD, n=3)**	**LOD (µg/mL)**
2,4-Dinitrotoluene *	−0.132 ± 0.032	1.0 to 20	0.982	5.6	0.200 ± 0.03
Diphenylamine	0.406 ± 0.018	1.0 to 8.0	0.987	6.2	0.462 ± 0.06
Nitroglycerin	0.509 ± 0.010	0.50 to 8.0	0.998	10	0.147 ± 0.08

Table 36. *Performance characteristics calculated based on the PalmSens4 portable instrument.*

IGSR	Potential (V)	Linear Range (µg/mL)	R^2	Repeatability (%RSD, n=3)	LOD (µg/mL)
Lead	-0.790 ± 0.017	0.10 to 2.0	0.995	4.6	0.278 ± 0.13
Antimony*	-0.391 ± 0.017	0.1 to 2	0.992	16	0.235 ± 0.39
Copper	-0.317 ± 0.021	0.05 to 1.0	0.999	4.2	0.009 ± 0.004
OGSR	**Potential (V)**	**Linear Range (µg/mL)**	**R^2**	**Repeatability (%RSD, n=3)**	**LOD (µg/mL)**
2,4-Dinitrotoluene*	−0.148 ± 0.025	1.0 to 10	0.998	14	0.061 ± 0.09
Diphenylamine	0.417 ± 0.008	1.0 to 8.0	0.999	29	0.152 ± 0.44
Nitroglycerin	0.523 ± 0.007	0.50 to 8.0	0.995	33	0.438 ± 1.46
Ethyl centralite	0.945 ± 0.004	2.0 to 10	0.926	30	0.566 ± 1.67

** Antimony and 2,4-DNT were assessed as peak current height whereas all other analytes were assessed as peak current area.*

Following the assessment of performance characteristics, the electrochemical quality controls were analyzed on the portable PalmSens. These quality controls use two mixtures of the IGSR and OGSR analytes. The first is a solution of IGSR (2 ppm Pb, 0.2 ppm Cu, and 8 ppm Sb) and 10 ppm (2,4-DNT, DPA or NG, and EC). The second solution is the same; however, DPA is replaced with NG to evaluate their peak potential since peak resolution is difficult to achieve when DPA and NG are in solution together. These two solutions are the 10 ppm NG QC and 10 ppm DPA QC. Then 1:4 dilutions are made to generate a mixture of 2.5 ppm of OGSR analytes and 0.5 ppm Pb, 0.05 ppm Cu, and 2 ppm Sb for the IGSR analytes. **Figure 51** demonstrates the voltammograms of the quality control mixtures. The mixture voltammograms were comparable to those obtained using the Metrohm benchtop potentiostat, with a possible slight loss in sensitivity at the low concentration for the portable instrument.

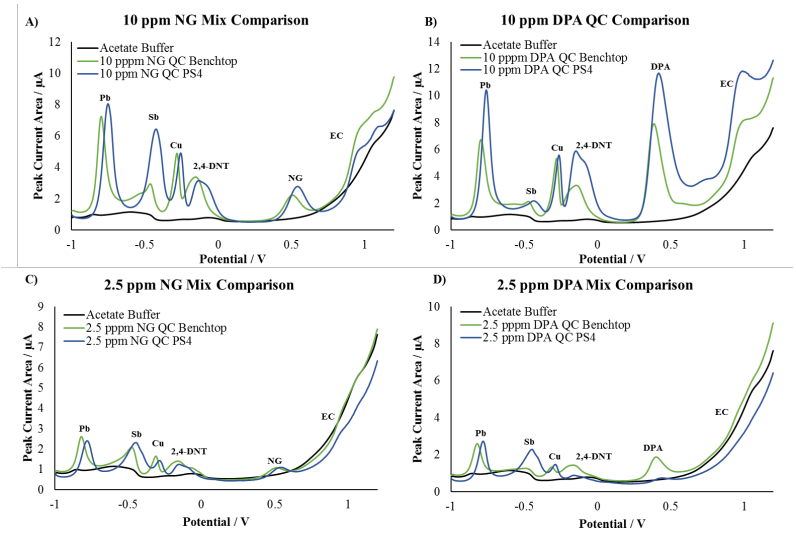


Figure 51*. Comparison voltammograms of the quality control mixtures for the 10 ppm NG QC (A), 10 ppm DPA QC (B), 2.5 ppm NG QC (C), and 2.5 ppm DPA QC (D) for the portable and benchtop instruments.*

Most importantly, both instruments provided GSR identification for lead, copper, and nitroglycerin with accuracies over 95% for the classification of samples as shooter or non-shooter based on combined IGSR/OGSR profiles. This demonstrated the scientific reliability of the portable electrochemical method for casework-like samples. Assessing the application of the portable potentiostat laid the groundwork for this screening approach as a future tool for forensic laboratories.

Following the extraction procedure reported by Ott et al., half the sample was run on the portable instrument, and the other half was run on the benchtop. This procedure can be seen in **Figure 52**. In this way, the results from the analysis could be compared directly from the same specimens.

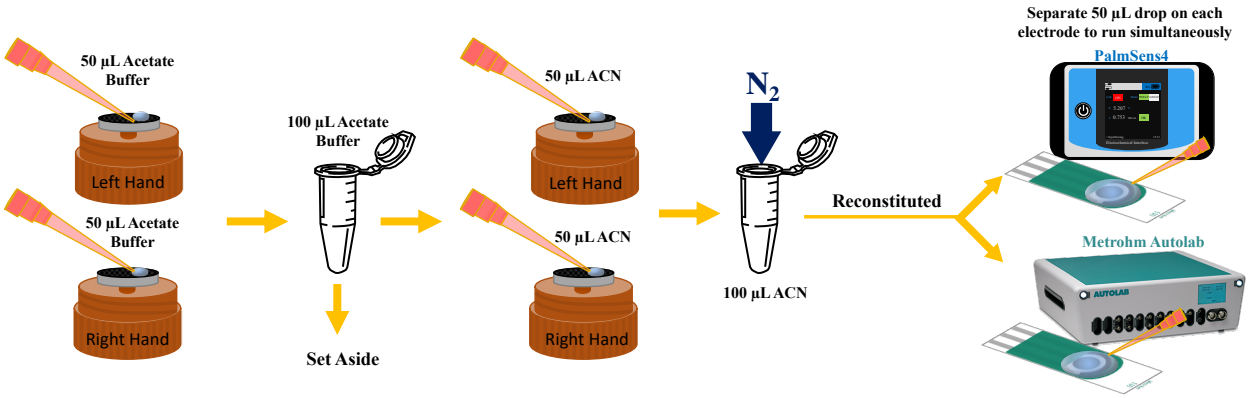


Figure 52*. The analytical procedure of the extraction process from both the left and right hand of the sample set for electrochemical analysis.*

Table 37 below demonstrates the performance measures for the background and leaded shooter samples compared between the benchtop and portable instrument. Both instruments accurately determined all 100 background samples as not having the presence of GSR compounds, resulting in a 100% true negative rate. In the case of the leaded shooter samples, a minimal difference was seen

between the benchtop and portable instrument, with over 97% of the shooter samples correctly reporting positive results for GSR. This demonstrated electrochemistry's strength to screen for IGSR/OGSR and the ability of the portable instrument to analyze and produce results almost identical to the benchtop model.

Table 37. *Comparison of low-risk background performance measures and leaded shooter populations between the benchtop (green) and portable (blue) instrumentation.*

Background and Shooter Samples Performance Rates by Critical Threshold				
	Metrohm Benchtop Instrument		**PalmSens4 Portable Instrument**	
	Background	**Shooter**	**Background**	**Shooter**
Number of Sets	200	100	200	100
True Positive	N/A	97* (97%)	N/A	99 (99%)
False Negative	N/A	3 (3%)	N/A	1 (1%)
True Negative	200 (100%)	N/A	200 (100%)	N/A
False Positive	0 (0%)	N/A	0 (0%)	N/A
Accuracy	99.0%		99.7%	

**Electrical issue caused the loss of two samples.*

Additionally, the prevalence of each of the three analytes, lead (Pb), copper (Cu), and Nitroglycerin (NG), above the critical threshold values, was assessed for GSR derived from firing lead-free and leaded ammunition (**Figure 53**). Similar signals were observed with both systems for the respective datasets.

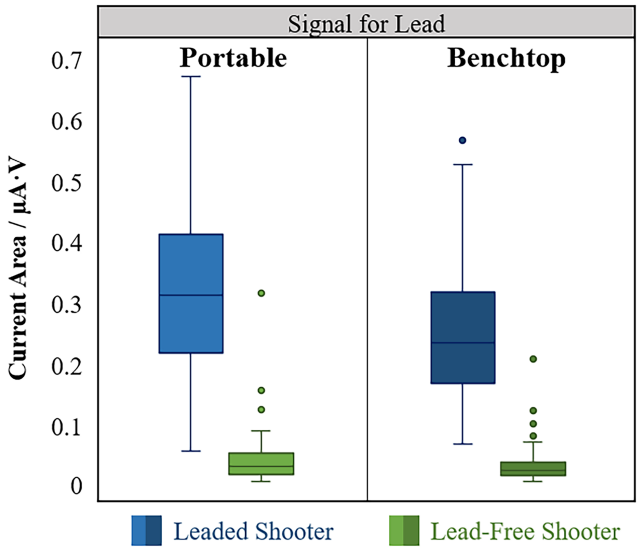


Figure 53. *Box plot comparison of the peak current area signal for lead (Pb) between the leaded and lead-free populations for the benchtop (right) and portable (left) potentiostats.*

The advantage of this portable system is that it provides a rapid and sensitive GSR field-screening method to minimize the disconnect of decisions between the crime scene and laboratory analysis within the discipline. Additionally, portable devices can help triage at crime scenes and laboratories to provide a cost-efficient screening method that can decrease backlogs and allow for further confirmatory testing when needed. Most importantly, fast decision-making at crime scenes can significantly aid the collection of relevant information.

Continued research in portable electrochemical potentiostats by our research group will include collaborating with forensic laboratories to showcase the importance and efficiency of on-site GSR screening.

Task 3.2. Testing of the portable and bench-top methods at mock crime scenes

The motivation for this study was to demonstrate the ruggedness of the mobile instrumentation in case-like scenarios and to provide recommendations on the workflow scheme when analyzing both IGSR and OGSR. The GSR workflow study investigated the difference between typical carbon adhesive and the in-house stub adhesive efficiency for collecting GSR and analysis by portable methods. The purpose of evaluating mock crime scenes was to assess the capabilities of mobile instrumentation for detecting GSR when there are potential transfer and persistence factors during the arrest and at the scene.

GSR Workflow Study and Adhesive Comparison Results

In this study, we evaluated the difference in the adhesive composition of the carbon tabs and scotch permanent double-sided tape to use clear adhesive to image the GSR particles by LIBS easily. The adhesive properties were evaluated by microscopic examination and FTIR, summarized in **Table 38**. Physical attributes of the double-sided adhesive found a much smoother surface with a thin adhesive layer compared to the carbon tabs, which have a rough surface and are thicker when looking at cross sections of the adhesives.

Figure 54 provides microscopic images and average measurements taken on the cross sections of the carbon and double-sided adhesives. From the measurements, a single layer of the carbon adhesive is approximately two times thicker than the double-sided adhesive when comparing single layers to each other (46μm for double side tape versus 118μm for carbon). The rougher carbon surface also allows for more surface area interaction when performing the stubbing on the hands.

Table 38. *Summary of physical and chemical characteristics of the carbon and double-sided tape adhesive.*

Properties	PELCO Carbon Tabs		Scotch Permanent Adhesive
	Single Layer	Double Layer	
Texture	Rough	Rough	Smooth
Thickness	118 ± 31 μm	266 ± 18 μm	46 ± 1 μm
FTIR Analysis	poly(butyl methacrylate)		poly(butyl acrylate: acrylic acid)
Library Match Score	0.8823		0.9289

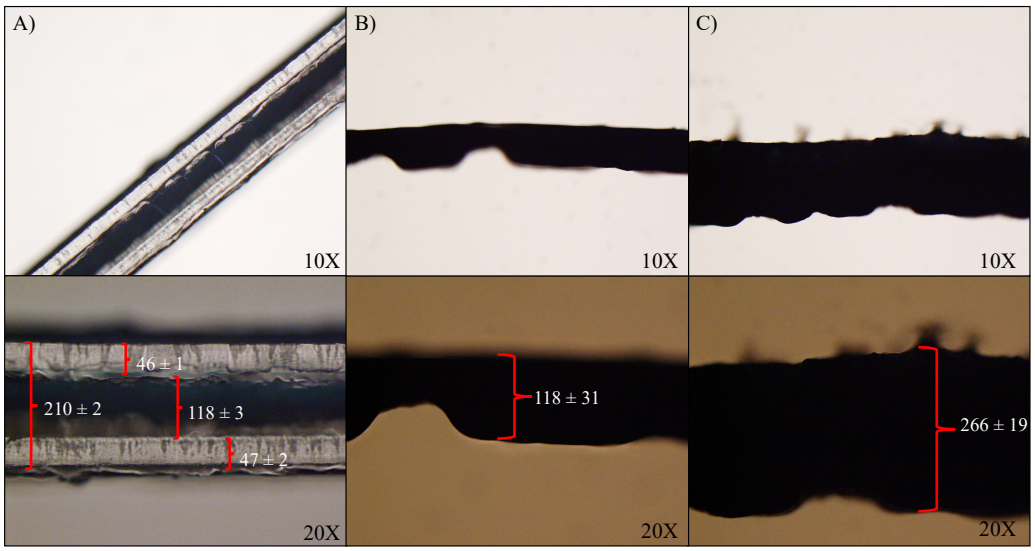


Figure 54. *Microscopic images of the A) grid stub layering and double-sided adhesive, B) single-layer carbon adhesive, and C) double-layer carbon adhesive at 10X (top) and 20X (bottom), where measurements (in micrometers) of the layer composition of the handmade stub were taken using 5 replicates.*

FTIR analysis was performed to determine the chemical structure of the adhesives using ATR attachment (**Figure 55**). Both adhesives demonstrated bands relating to the isoprene backbones of the molecules at approximately 1453, 1376m, and 840 cm^{-1}. The double-sided adhesive spectra contain an additional band, which may correlate to a C_5-tackifying resin at 967 cm^{-1}. It should also be noted the increased background in the carbon adhesive is due to the absorption of the carbon black. FTIR analysis showed that the two adhesives have different compositions, which leads us to hypothesize that adhesives may have different efficiencies for collecting, recovering, and preserving gunshot residues.

OGSR Recovery Testing Results

The microscopic and FTIR analysis determined that the adhesives differ in physical and chemical composition. To determine how this played a role in the collection, recovery, and preservation of OGSR, an experiment was designed to determine if there were significant differences between the two adhesives in the recovery of OGSR using the workflow following LIBS, EC, and LC methods. The OGSR standard developed by our group was used to determine the adhesive's performance in recovering OGSR compounds after LIBS analysis. Spiked samples were evaluated (referred to as "spikes" in figures and tables) to obtain ground truth concentrations of the OGSR standard used on the stub surface. Carbon and clear adhesive (white stubs) were evaluated in two ways: 1) immediate extraction and drying after deposition and 2) extraction after being under argon flow (1.3 L/min.) for one and a half hours to simulate extreme situations of several samples waiting on the ablation chamber.

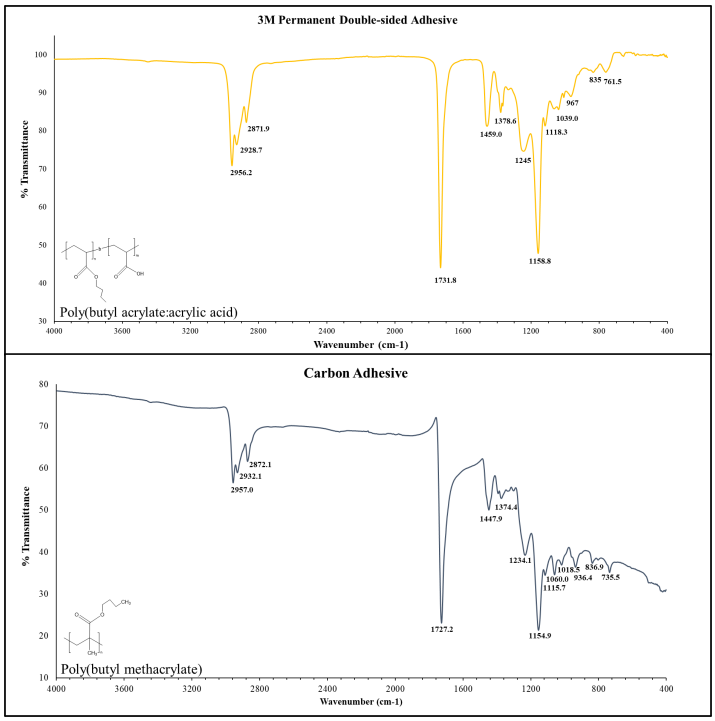


Figure 55. *FTIR spectra of the double-sided adhesive (top) and carbon adhesive (bottom) where the chemical structure of the top library hit is provided for each type of adhesive.*

Table 39 presents the average concentration and percent recoveries found from the two treatment groups. Observations show that carbon stubs recover higher concentrations for OGSR compounds except for DPA and its derivative compounds, where the recovery from the white handmade stubs is not statistically different from the carbon. Carbon stub recovery ranges from 69-104% with and without argon exposure for all compounds. The handmade clear stubs have a much wider range of percent recoveries, 59-116% without argon exposure and 40-77% with argon exposure. Comparison of carbon stubs demonstrates recovery differences between 1-33%, where carbon stubs experience a greater loss for DPA and derivates under argon flow. However, the handmade stubs demonstrate percent recovery differences between 19-39%, a wider window than the carbon stubs. These differences can be visualized using boxplots shown in **Figure 56**.

These differences in group means were statistically tested using Analysis of Variance (ANOVA), and a Dunnet's Control test was performed. The data met the assumptions for normality and variance. Results are depicted in **Figure 57,** where the white stubs exposed to Argon show a significant loss of the OGSR compared to the carbon stubs. The advantage of the white stubs is the capability to visualize GSR using the LIBS instrument, and the white background does not interfere with the LIBS analysis. However, this workflow study demonstrates the limitation of these stubs is the inability to preserve OGSR on the stub under the LIBS conditions for the analysis periods.

To overcome these limitations, a new stub collection device was developed to create a hybrid of the white handmade and carbon stubs to provide the necessary OGSR recovery but still allowing IGSR visualization using the mobile LIBS instrument. These half-carbon, half-white stubs were tested on authentic samples to determine if the decreased surface area of carbon could detect OGSR within the method of interest limits of detection and if the argon flow would affect the recovery of authentic OGSR. Sample extraction, analysis, and interpretation by LIBS, ECD, and LC-MS/MS was

conducted. The ten hybrid stubs yielded 80% positive by LIBS (n= 5), 90% positive by ECD, and 100% positive by LC. In comparison, the ten carbon stubs were 100% positive by ECD and 90% positive by LC. Mobile LIBS could not analyze carbon stubs because the black surface caused interference in identifying the particles. The results from the study highlight the hybrid stub's ability to perform sequential LIBS, ECD, and LC analysis and detection of OGSR.

Table 39. *Average concentrations (in parts per billion) and percent recoveries of OGSR compounds for the spikes, extracted immediately (carbon and white stubs) and those which were exposed to argon flow for 1.5 hours (Argon Carbon and Argon White stubs). Percent Recovery was calculated using the average concentration of the spike samples.*

Average Concentrations and Percent Recovery of OGSR						
Sample ID	**AKII**	**MC**	**EC**	**4NDPA**	**DPA**	**2NDPA**
Spikes (n=8)	126.8 ± 16	114.5 ± 21	115.5 ± 21	120.2 ± 18	87.5 ± 34	113.6 ± 23
Carbon Stubs (n=13)	95.7 ± 18	96.1 ± 21	96.4 ± 19	73.3 ± 12	70.7 ± 13	76.7 ± 17
	76%	104%	104%	103%	102%	102%
Carbon Stubs exposed to Argon (n=8)	95.3 ± 20	90.7 ± 21	92.9 ± 20	62.7 ± 12	48.0 ± 9	58.6 ± 14
	75%	98%	100%	88%	69%	78%
White Stubs (n=13)	74.8 ± 11	60.7 ± 12	76.8 ± 13	68.5 ± 12	80.3 ± 17	84.0 ± 20
	59%	66%	83%	96%	116%	112%
White Stubs exposed to Argon (n=8)	51.0 ± 13	40.9 ± 10	49.7 ± 15	44.7 ± 12	53.0 ± 20	56.4 ± 14
	40%	44%	54%	63%	77%	75%

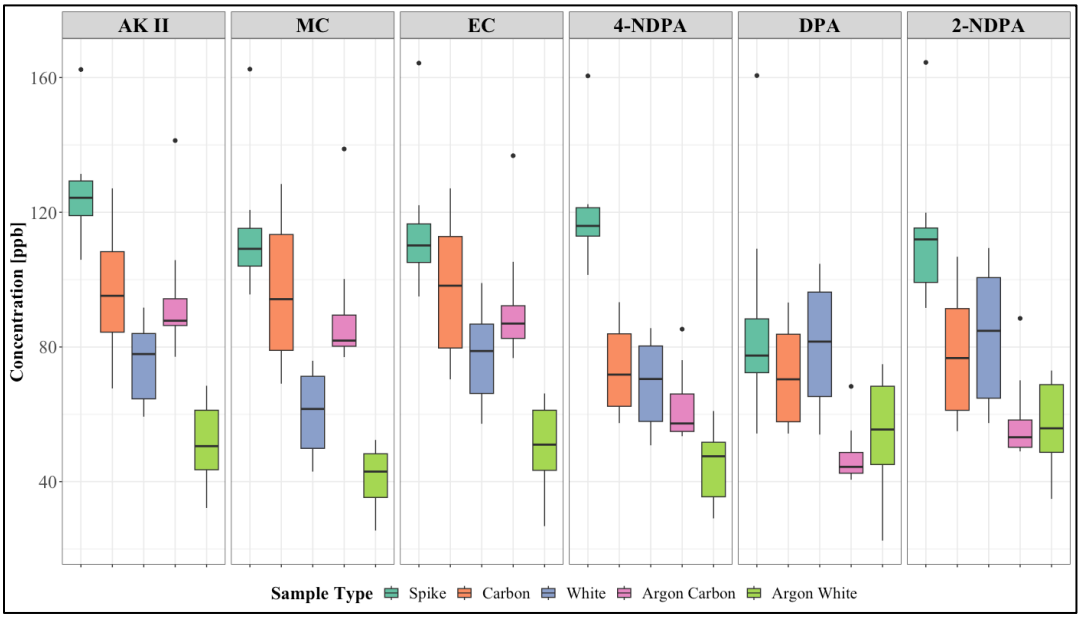


Figure 56. *Boxplot comparison of the calculated concentrations of the spike, carbon, and handmade samples with and without exposure to argon.*

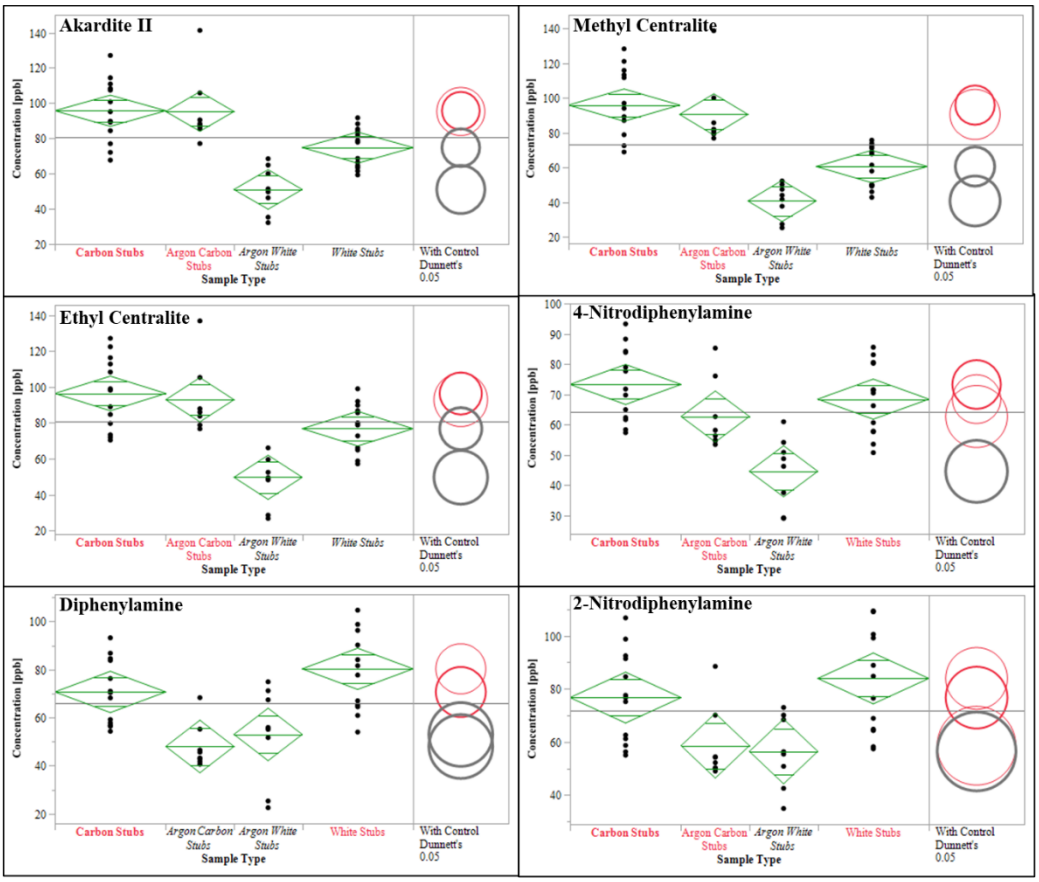


Figure 57. *Dunnet's control test for carbon and white stub samples. White stubs exposed to Argon caused a significant loss in OGSR, shown in gray. Groups with similar means to carbon stubs are shown in red.*

Mock Crime Scene Results

The four mock crime scene scenarios evaluated different transfer and persistence factors that might be experienced in case-like situations. To assess these factors, samples were assessed by LIBS, ECD, and LC analysis, where the results of the methods are summarized in **Tables 40-44**. Several conclusions were made from each scenario regarding the transfer and persistence of IGSR and OGSR.

Arrest Scenario 1

In the first arrest scenario, the first objective was to evaluate the transfer of GSR to a person of interest (POI) from manipulating a firearm without firing. The action of the POI handling the firearm for one minute resulted in observing some IGSR transfer to the individual. The sample was positive for IGSR by LIBS (Pb and Ba) and ECD (Pb and Cu), while both ECD and LC analysis found no OGSR above the method detection limits. This suggests handling of the firearm can lead to the transfer and persistence of IGSR particulates, but OGSR may not be readily transferred in this scenario. This is unexpected as we have observed low transference of OGSR on skin and synthetic membranes. Since the metal and polymer surfaces of the firearm are non-porous, it is unlikely that the compounds absorb or adsorb and remain on the surface over time.

The second objective of this scenario was to assess the potential secondary transfer and persistence from a POI who handles (but does not fire) a gun to the arresting officer who has washed hands before the arrest. The prior and post-arrest samples of the officer were found to be negative by LIBS, ECD, and LC analysis, shown in **Table 40**. These results indicate that washing hands after handling a firearm removes any transfer GSR from handling the gun. The officer's negative sample collected after performing the arrest suggests that no secondary transfer of GSR occurred during the arrest or it was not above the method limits of detection.

Table 40. *Summary table of positive samples by mobile screening methods (LIBS and electrochemistry), confirmatory OGSR testing LC-MS/MS, and overall positive samples for arrest scenario 1.*

Arrest Scenario 1 (transfer and persistence during handling a firearm)				
Sample ID	**LIBS Positive?**	**EC Positive?**	**LC-MS/MS >LOD?**	**Overall?**
Officer Control (before)	ND	ND	ND	Negative
POI (Handled Firearm)	Pb, Ba	Pb, Cu	ND	Pb, Ba, Cu (IGSR)
Officer After Arrest	ND	ND	ND	Negative

Arrest Scenario 2

The second arrest scenario assessed two different situations for the transfer and persistence to and from a POI to an officer, where **Table 41** summarizes all results. The first transfer and persistence situation evaluated the hands of an officer who handled their firearm (without firing). Then, she arrested someone who had not been near or fired a gun recently (POI 1 clean). The prior and post-arrest samples of the officer 1 were found to be negative by all analytical methods. This data contradicts the results in the first arrest scenario where the firearm handling without firing did result in IGSR transfer to the individual. Nonetheless, the difference in this scenario is the increased activity of the officer who performed more vigorous activity (i.e., simulating driving and the arrest) after handling a firearm, which we hypothesize resulted in losing any GSR transferred to the officer. Additionally, secondary transfer of GSR was not observed during the arrest from the POI, which was confirmed to have negative results by all detection methods.

Table 41. *Summary table of positive samples by mobile screening methods (LIBS and electrochemistry), confirmatory OGSR testing LC-MS/MS, and overall positive samples for arrest scenario 2. *non-detect (ND)*

Arrest Scenario 2 (transfer to POI and officer during arrest)				
Sample ID	**LIBS Positive?**	**EC Positive?**	**LC-MS/MS >LOD?**	**Overall?**
POI 1 (Clean)	ND	ND	ND	Negative
Officer 1 (Handled firearm)	ND	ND	ND	Negative
Officer 1 (after Arrest)	ND	ND	ND	Negative

The second transfer and persistence situation evaluated an officer (officer 2) who had washed his hands and then arrests a POI within 30 minutes of discharging the weapon (**Table 42**). Both IGSR and OGSR were detected on the POI by LIBS (Sb, Ba, and Pb), ECD (Pb and Cu), and LC (AKII, EC, and DPA). These results strengthen the capabilities of our analytical workflow for detecting IGSR and OGSR. It also demonstrates how finding IGSR and OGSR increases confidence in determining if an individual is a potential shooter when questions of handling versus firing are being investigated. LIBS, ECD, and LC analysis found the officer's prior and post-arrest samples were negative. Again, these results support that washing hands after handling a firearm removes any transfer of GSR from the firearm.

Additionally, the negative finding of the post-arrest sample of the officer indicates that no secondary transfer of GSR occurred during the arrest of the person of interest who had discharged the firearm. It is noteworthy to stress that the arrest followed low-mid contact from a compliant suspect, and the officer did not touch the hands of the POI during the arrest. Thus, the results should not be generalized under different arrest protocols.

Table 42. *Summary table of positive samples by mobile screening methods (LIBS and electrochemistry), confirmatory OGSR testing LC-MS/MS, and overall positive samples for arrest scenario 2.*

Arrest Scenario 2 (transfer from POI and officer during arrest)				
Sample ID	**LIBS Positive?**	**EC Positive?**	**LC-MS/MS >LOD?**	**Overall?**
Suspect 2 (Fired)	Pb, Ba, Sb	Pb, Cu	AKII, EC, DPA	IGSR and OGSR
Officer 2 (Washed Hands)	ND	ND	ND	Negative
Officer 2 (after Arrest)	ND	ND	ND	Negative

Suicide Scenario

The suicide scenario considered the transfer and persistence mechanism where a victim was discovered after 30 minutes of the shooting, and a second individual (i.e., a family member) interacted with the victim's body by checking her pulse. One of the transfer mechanisms of interest in this case scenario is the potential for finding GSR on one or both hands of the victim. Many factors can affect these results, such as the individual's dominant hand, position of the hands and firearm during firing, time of collection post-shooting, and firearm type. While we did find GSR on both hands, some limitations to this scenario were imposed for safety purposes. For instance, the individual acting as the victim used both hands while firing down the range with the firearm. The down-range firing is likely to affect how the plum of GSR develops. For this scenario, the victim's left and right hands were sampled on separate stubs to evaluate GSR transfer behavior. While both hands of the victim were positive for IGSR and OGSR, some differences were found between the right and left hands of the victim. The left hand was positive by all three analytical techniques, while the right was only positive by LIBS and ECD. This agrees with the position of the hands used for shooting the gun.

This study demonstrates that IGSR and OGSR persist on the victim who had no activity for 30 minutes after discharging the firearm (**Table 43)**. Furthermore, the family member specimen collected after the interaction was negative, suggesting no secondary transfer of GSR from shaking or checking the victim's pulse. Again, the family-member contact was minimal, and therefore the results should not be taken out of the context of the experimental design.

Table 43. *Summary table of positive samples by mobile screening methods (LIBS and electrochemistry), confirmatory OGSR testing LC-MS/MS, and overall positive samples for suicide scenario.*

Suicide Scenario 3 (transfer and persistence during suicide scenario)				
Sample ID	**LIBS Positive?**	**EC Positive?**	**LC-MS/MS Positive? >LOD**	**Overall?**
Victim (Left Hand)	Pb, Ba, Sb	Pb, Cu, NG	AKII, EC, DPA, 2-NDPA, 4-NDPA	IGSR & OGSR
Victim (Right Hand)	Pb, Ba	Pb, Cu, NG	ND	IGSR & OGSR
Family Member (Interaction)	ND	ND	ND	Negative

Homicide Scenario

In the homicide scenario, the situation was to evaluate GSR detection from the hands of a POI and cloth substrates that were targeted to simulate the shirt of a victim at the crime scene. (**Table 44**). The POI sample detected the presence of IGSR by LIBS (Sb, Ba, and Pb) and ECD (Pb and Cu), but no OGSR was detected. The unusual result of not detecting OGSR implies the deposited OGSR was not above our detection limits.

The cloth substrate was sampled using the half-carbon, half-white stub around the bullet wipe and a two-centimeter radius around the entrance hole. Both IGSR and OGSR by LIBS (Pb and Ba), ECD (Pb, Cu, and NG), and LC (AKII, EC, 4-NDPA, DPA, and 2-NDPA) were detected on both bullet holes (**Figure 58**). These results highlight that stubbing of the fabric is a quick technique for sampling GSR from alternative substrates that can be performed at the crime scene.

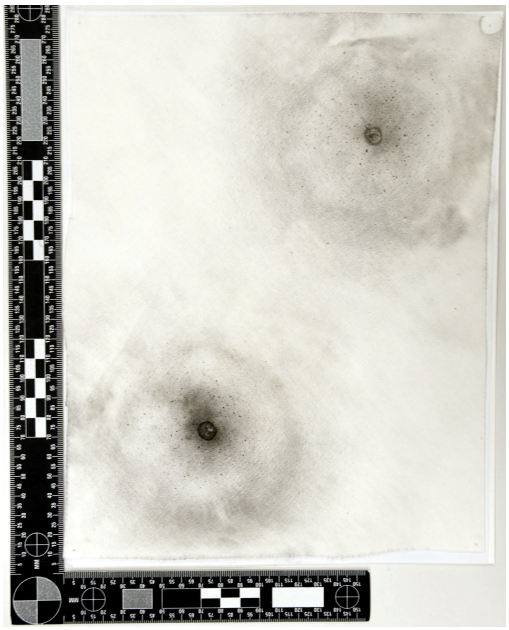


Figure 58. *Photograph documentation of the substrate fired by two rounds of ammunition where bullet hole 1 is the top right and bullet hole 2 is the bottom left.*

Table 44. *Summary table of positive samples by mobile screening methods (LIBS and electrochemistry), confirmatory OGSR testing LC-MS/MS, and overall positive samples for homicide scenario.*

Homicide Scenario (detection of GSR on various substrates)				
Sample ID	**LIBS Positive?**	**EC Positive?**	**LC-MS/MS >LOD?**	**Overall?**
Suspect	Pb, Ba	Pb, Cu	ND	IGSR
Bullet Hole 1	Pb, Ba	Pb, Cu & NG	AKII, EC, 4NDPA, DPA, 2NDPA	IGSR & OGSR
Bullet Hole 2	Pb, Sb	Pb, Cu & NG	AKII, EC, 4NDPA, DPA, 2NDPA	IGSR & OGSR

Mock Crime Scenes Workflow: Conclusions and Future Directions

The workflow and mock crime scene presented in this study enhanced the capabilities of the screening techniques by optimizing the collection and preservation of OGSR using our analysis approach. More importantly, the proposed analytical scheme incorporated OGSR detection into the current GSR confirmatory methods practice. The use of authentic GSR samples allowed us to observe the potential for secondary GSR transfer mechanisms like handling of a firearm, arrests, and other contact interactions, as well as the persistence of GSR in these arrests, suicide, and homicide scenarios. The data obtained from these authentic samples, in open and indoor environments, provided insight into how these residues were affected by activities and contacts established during such scenarios and the implications for the investigation outcomes.

The workflow study explored important variables about screening methods and the OGSR workflow for the collection and preservation of OGSR. First, the carbon and double-sided adhesives provided different recoveries of OGSR when exposed to the Argon flow needed for mobile LIBS analysis. Recovery from carbon after mobile LIBS analysis ranges between 69-100% with argon exposure, while the performance of the white stubs was between 40-75%, making this a limitation of using the white stub for OGSR collection. The benefits of carbon adhesive are the greater recovery of OGSR compounds and their use in SEM analysis; however, the advantage of the white stub is the ability to visualize and perform single-particle analysis by the mobile LIBS method. To overcome this obstacle, the half-carbon and half-white stubs were created to gain the advantage of both sample collection mediums. The half-carbon and half-white stubs are easy to assemble and allow for performing the LIBS, ECD, and LC workflow on the samples with detection similar to the typical carbon stubs by EC and LC. The performance of the in-house stubs demonstrates that they are as efficient as the traditional carbon stub collection method.

Moreover, the mock crime scenes highlight the modified collection devices, the screening techniques, and the IGSR and OGSR behavior assessment in simulated crime scene scenarios. The modified collection devices for our screening technology are simple to use and implement into the GSR workflow scheme. The screening methods showed the feasibility of GSR detection and the ability to provide useful information on the presence of IGSR and OGSR in various scenarios, with data available at the scene in less than 10 minutes. The arrest scenario demonstrates that handling a firearm can lead to the transfer of pGSR to the handler. Still, the persistence of the pGSR is dependent on factors post-handling such as time, driving, or vigorous activity (i.e., performing an arrest). The study also implies that washing your hands after handling a firearm removes traces of transferred GSR.

Additionally, the POI who fired in the second arrest scenario found both IGSR and OGSR. In this case, the handling demonstrated the transfer of only IGSR, which can benefit investigators in providing better confidence when determining a shooter from other alternative hypotheses. However, it should be noted that finding both IGSR and OGSR may not be the case for every situation, and in this study, the mock crime scene scenarios presented were only replicated once. Additional studies are required before drawing generalizations.

The suicide scenario highlighted how GSR can be distributed to both hands under the conditions of this experiment, and residues are not lost after 30 minutes or interaction from the individual who discovered the victim. The interaction did not result in any secondary GSR transfer from the victim to the "family member." The homicide scenario highlights the collection device and screening methods to sample from surfaces other than trace hand samples.

Overall, the collection, preservation, and analysis of GSR is a difficult task. This study has provided recommendations for these tasks. For instance, carbon adhesive is optimal when considering OGSR and confirmatory SEM analysis. The white stubs are optimal for mobile LIBS analysis for quick visualization and detection of IGSR. If an analyst wants to perform a multi-step approach for IGSR and OGSR, the half-carbon, half-white stubs can complete screening and confirmatory techniques. Transfer and persistence of authentic GSR agreed with findings in previous larger studies performed by our research group. The variables to consider in interpreting GSR evidence include post-shooting activities performed and detection of IGSR, OGSR, or both.

This study aimed to evaluate collection strategies, analytical workflows, and interpretation of GSR evidence to guide forensic examiners and crime scene personnel in decision-making processes at the scene and laboratory. Furthermore, these simulations of case-like evidence helped construct hypotheses about activities and events during investigations and to preliminary testing onsite. Future research will continue with mock crime scene simulations to provide replicates and corroboration of the results presented here. This will include studies on transfer and persistence in different arresting procedures and evaluating the performance of different measures for preventing contamination during the collection and preservation of GSR evidence.

Results of Task 4 (Objective 4)— To apply data from population datasets and transfer and persistent studies in the assessment of a probabilistic approach for the interpretation of GSR evidence.

Bayesian networks (BN) is a graphical decision network method that uses underlying Bayesian approaches to statistically and mathematical compute probabilities that can handle uncertainty. The Bayesian networks use a succession of nodes to represent linked variables to describe causal relationships. In forensic science, an increased interest in Bayesian networks has surged due to the advantages of assisting with evidence evaluation and the ability to handle complex relationships between variables. Although a few studies reported BN as an attractive model for gunshot residue (GSR) evidence, [17-24] the interpretation of gunshot residue evidence is particularly challenging due to the random nature of the deposition of particles to the person or object of interest and its vicinity. In addition, much still needs to be understood about GSR's transfer and persistence mechanisms. This study aims to expand the knowledge base in this field. Here, we construct a BN using data acquired from two previous studies to interpret GSR evidence using activity propositions.[7] As a first proof of concept, we focused on using scanning electron microscopy-energy dispersive x-ray spectrometry (SEM-EDS) data to construct the network. Before BN creation, the data were mined to obtain

important information about the background occurrence, transfer, and persistence behaviors of gunshot residue and investigate the expectations of how often these events happen.

This study used background data, and transfer and persistence SEM-EDS data into the Bayesian network to provide additional understanding of the transfer and persistence of GSR, a reliable decision-making tool, and probabilistic outcomes as a model for reporting the weight to the evidence. To do this, we performed data mining on two datasets our research group collected, including screening and confirmatory analysis. For the proof of concept, the data analyzed by SEM-EDS acted as the basis for the ground truth, and to reflect data that correlates to the instrumentation that most laboratories use for pGSR analysis. This study investigated the number of characteristic particles containing lead, barium, and antimony due to the higher weight those particles hold in identifying GSR as per the ASTM 1588-20 standard for leaded ammunition.[23] Lead-free ammunition characteristic particle criteria utilized the ASTM 1588-20 suggestions to included additional elements such as copper, zinc, titanium, gallium, tin, gadolinium, and aluminum, depending on the lead-free ammunition composition. To develop the Bayesian network, the first task was exploratory data analysis on the information and expected outcomes each dataset provides and how it can help in providing support toward the two competing hypotheses. An outline of the most crucial steps in the transfer and persistence of GSR was developed to provide the theoretical foundation of the Bayesian network before implementing and designing the network in the software using the data.

Exploratory data analysis

Population Dataset: Hands of Persons of Interest

Regarding the first dataset, data exploration began with gathering samples into various classifications by shooter or non-shooter, and firearm type. These samples were collected using a 10-particle cutoff where the number of samples and frequency of the subpopulations of data are grouped by the intervals of the number of characteristic particles found on the sample as shown in **Tables 45 and 46**. The leaded and lead-free shooter population has a higher frequency of observing ten or more particles (above 90.3%) compared to the low and high risk background group, where only one high-risk sample observed ten or more characteristic GSR particles. In the low-risk population, we see a 92.4% frequency of observing no characteristic particles on the samples, with only one sample that detected 3 – 7 characteristic particles. The high-risk background samples varied more, where 48% of samples had no particles; however, the false positive samples were from individuals whose occupations revolve around firearms, mechanics, and agriculture, which have been studied that can lead to higher risk of mimicking GSR.[11-14]

The histograms in **Figure 59** depict the frequencies graphically and demonstrate a good separation between shooter and non-shooter populations. To ensure the variables follow the assumption of independence for Bayesian networks in future analysis, a chi-square test for independence was performed for the column and row variables, (i.e., number of characteristic particles, and shooter and non-shooter population). The resulting chi-square test statistic and p-value (191.33, df = 12, p-value $\leq$ 0.0001) Since the p-value < 0.05, the null hypothesis can be rejected and thus the variables are independent of each other **(Figure 60**).

Table 45. *Conditional probability table of dataset I counts and frequency of shooter and non-shooter populations for the number of characteristic particles observed by SEM-EDS. The data shows the number of occurrences and the probability in parentheses.*

Number of Characteristic Particles	Shooter (n=104)		Non-shooter (n=78)	
	Leaded shooter (n=52)	Lead-free Shooter (n=52)	Low risk Background (n=53)	High risk Background (n=25)
None (0 particles)	0	0	49 (0.924)	12 (0.48)
Little (1-2 Particles)	2 (0.038)	1 (0.019)	3 (0.056)	4 (0.16)
Few (3-7 Particles)	2 (0.038)	2 (0.038)	1 (0.018)	4 (0.16)
Some (8-10 Particles)	1 (0.019)	0	0	4 (0.16)
Many (10+ Particles)	47 (0.903)	49 (0.942)	0	1 (0.04)

Additionally, the shooter data contained samples collected with two different firearms, a revolver and a pistol. The conditional probability table (**Table 47**) allows for easy comparison between the subpopulations of data where the type of firearm results in a high frequency of 0.808 or higher for detecting ten or more GSR particles. These data are important to consider as the results from detecting characteristic particles reflect the expectation of the number of particles found immediately after discharging a weapon and expected counts on the general shooters' population.

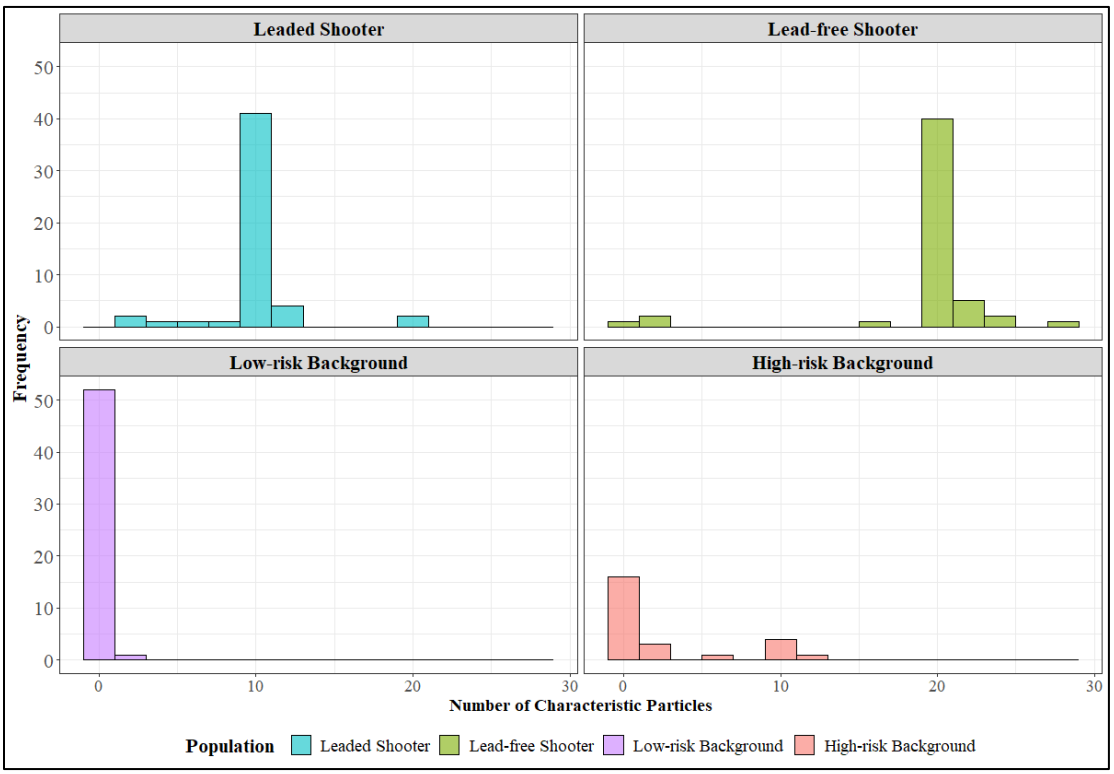


Figure 59. *Histogram of frequency of characteristic particles observed in the dataset I leaded shooter, lead-free shooter, low-risk, and high-risk background population.*

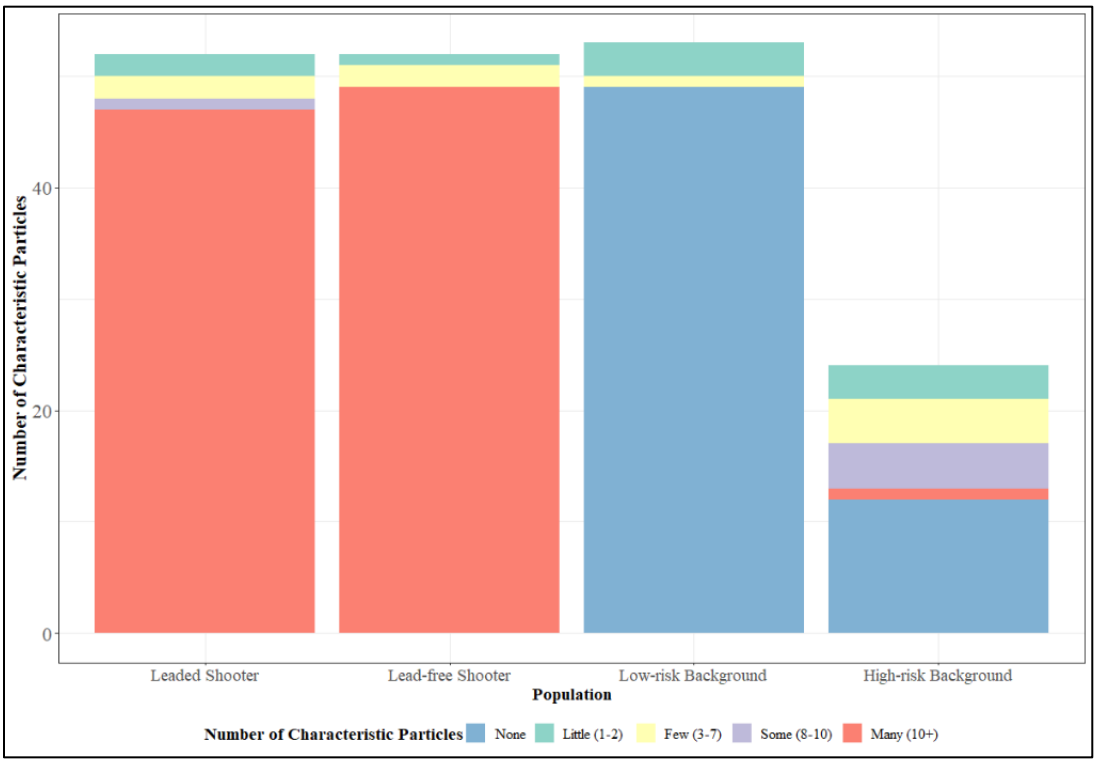


Figure 60. *A mosaic plot of the frequency of characteristic particles observed in the leaded shooter, lead-free shooter, low-risk, and high-risk background population of dataset I.*

Table 46. *Conditional probability table of dataset 1 counts and frequency of the type of firearm for the number of characteristic particles observed on the samples observed by SEM-EDS. The data shows the number of occurrences and the probability in parentheses.*

Number of Characteristic Particles	**Firearm (n=52)**	
	Pistol (n=26)	**Revolver (n=26)**
None (0 particles)	0	0
Little (1-2 Particles)	2 (0.077)	0
Few (3-7 Particles)	3 (0.115)	0
Some (8-10 Particles)	0	0
Many (10+ Particles)	21 (0.808)	26 (1.00)

The last subset from this data was the 54 activity samples, which observed various post-shooting activities. **Table 47** provides the number of samples, observed frequencies, and average number of characteristic particles detected for each observation group. The post-shooting activities included a control group for no activity, and rubbing, running, and hand sanitizing. From Table 4, a frequency of 1.0 was observed for finding ten or more characteristic particles for no activity and rubbing hands. Running resulted in some loss for two of the samples where only 88.9% frequency was observed for having ten or more characteristic particles. The application of hand sanitizer after firing shows a 94.4% frequency of detecting ten or more particles, where one sample found less than seven characteristic particles. The data collected from the post-shooting activities demonstrate the expected distributions when considering the persistence of GSR on the hands after discharging a weapon.

Table 47. *Conditional probability table activity samples from dataset I, frequency, and average number of particles for each type of activity for the number of characteristic particles observed on the samples by SEM-EDS.*

Authentic Activity Frequencies								
# of Characteristic Particles / Activity Type	**No Activity (n= 18)**		**Rubbing Hands (n=18)**		**Running (n=18)**		**Hand Sanitizer (n=18)**	
	Count (Frequency)	**Average Number of Characteristic Particles**	**Count (Frequency)**	**Average Number of Characteristic Particles**	**Count (Frequency)**	**Average Number of Characteristic Particles**	**Count (Frequency)**	**Average Number of Characteristic Particles**
None	0	**10 ± 1**	0	**10 ± 1**	1 (0.056)	**9 ± 3**	0	**10 ± 1**
Little (1-2 Particles)	0		0		0		0	
Few (3-7 Particles)	0		0		1 (0.056)		1 (0.009)	
Some (8-10 Particles)	0		0		0		0	
Many (10+Particles)	18 (1.00)		18 (1.00)		16 (0.889)		17 (0.944)	

Transfer and Persistence Dataset II

The second dataset used for creating Bayesian networks studied the transfer and persistence of GSR using authentic and synthetic skin to model these behaviors. The first part of the study compared authentic and synthetic skin models to the behavior of GSR at various post-shooting collection times. For the authentic samples, the post-shooting times study included collections immediately after shooting (time 0) or 1 and 3 hours after discharging five rounds of ammunition. Highlighted in gray in **Table 48**, the authentic samples found over ten particles on all samples; however, there was a noticeable decreasing trend where the average number of samples 479, 315, and 158 characteristic particles for 0, 1, and 3 hours, respectively. The study was repeated using a pGSR standard to deposit a known concentration of characteristic particles onto the synthetic skin membrane and collect after 0, 1, 3, and 6 hours (**Table 48** non-shaded cells). This synthetic skin model also found over 10 characteristic particles on all samples but a less significant decrease in the number of particles found over the time intervals shown in **Figure 61**. The difference between authentic and synthetic samples can be attributed to the human factors during the collection of authentic samples, as the membrane samples were left undistributed in a laboratory setting. Both persistence studies observing the detection of GSR over time concluded that IGSR can be detected up to 3 hours after shooting, and the number of particles is not significantly lost unless outside forces act upon the particles. The results from the study demonstrate the expected particle counts when GSR evidence is collected up to 6 hours after the primary transfer of GSR particles under optimal conditions without having any compounding effects from post-shooting activities.

Table 48. *Conditional probability table persistence samples from Vander Pyl dataset II. For each activity the left column represents the count of occurrences (frequency), and the right column represent average number of characteristic particles observed on the samples.*

Synthetic Skin Samples (25% Mapping)								
# of Characteristic Particles/ Post-shooting Collection Times	**Time 0 (n= 6)**		**Time 1 (n=6)**		**Time 3 (n=6)**		**Time 6 (n=6)**	
	Count (Frequency)	**Average Number of Characteristic Particles**	**Count (Frequency)**	**Average Number of Characteristic Particles**	**Count (Frequency)**	**Average Number of Characteristic Particles**	**Count (Frequency)**	**Average Number of Characteristic Particles**
None	0	**963 ± 218**	0	**783 ± 78**	0	**851 ± 177**	0	**881 ± 214**
Little (1-2 Particles)	0		0		0		0	
Few (3-7 Particles)	0		0		0		0	
Some (8-10 Particles)	0		0		0		0	
Many (10+Particles)	6 (1.00)		6 (1.00)		6 (1.00)		6 (1.00)	
Authentic Skin Samples (25% Mapping)								
# of Characteristic Particles/ Post-shooting Collection Times	**Time 0 (n= 6)**		**Time 1 (n=6)**		**Time 3 (n=6)**			
	Count (Frequency)	**Average Number of Characteristic Particles**	**Count (Frequency)**	**Average Number of Characteristic Particles**	**Count (Frequency)**	**Average Number of Characteristic Particles**		
None	0	**479 ± 186**	0	**315 ± 243**	0	**158 ± 150**		
Little (1-2 Particles)	0		0		0			
Few (3-7 Particles)	0		0		0			
Some (8-10 Particles)	0		0		0			
Many (10+Particles)	6 (1.00)		6 (1.00)		6 (1.00)			

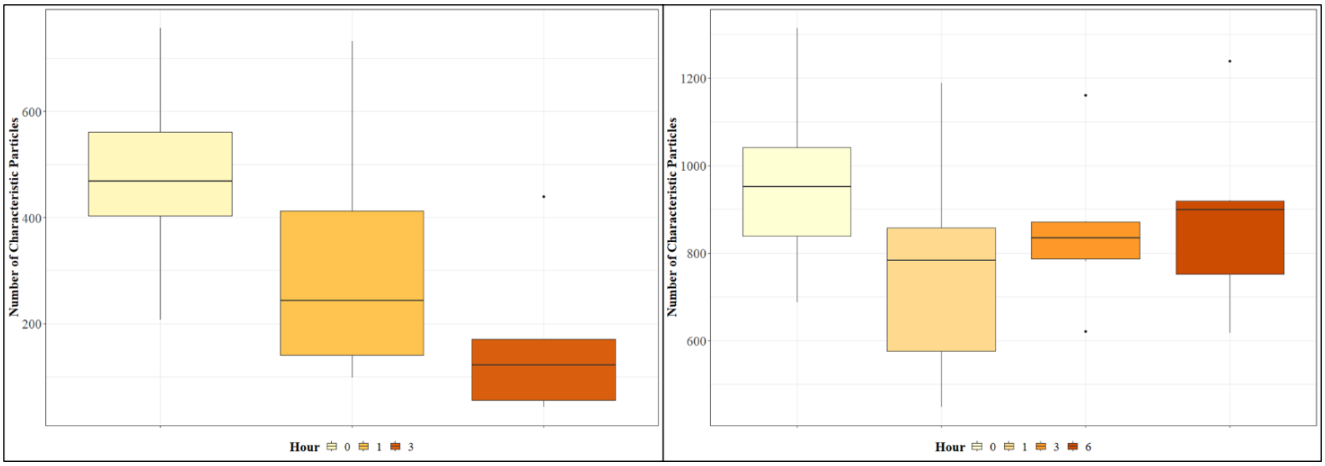


Figure 61. *Boxplot of the number of characteristic particles observed in the persistence sample collected from authentic samples (left) and synthetic skin study (right) analyzed by SEM-EDS.*

The second part of the synthetic skin study evaluated four post-shooting activities that may be commonly performed in a gun-related event. The activities included no activity as a control, and shaking hands, rubbing hands, and washing hands (**Table 49**). The data shows over ten characteristic particles for all samples from the no activity, shaking, and rubbing hands sets. On the other hand, the washing activity removed a large number of particles, with most of the samples retaining less than two particles.

Handshaking and rubbing samples also observed a secondary transfer from the first membrane, where the standard was deposited onto (denoted A), to a clean second membrane (denoted B). Trends within it show about a 14% loss from the first membrane (A) and a 21% transfer to the second membrane (B) caused by shaking hands, where the mean number of particles was similar to the no activity control samples for deposited samples. A 55% loss was found due to rubbing hands together, where approximately 32% was transferred to the second membrane. In **Figure 62**, the bar graphs demonstrate that fewer particles transfer to the clean membrane during handshaking. For hand rubbing, the bar graph highlights the higher percentage of transfer of particles to the clean membrane. Washing hands resulted in a frequency of 83% of observing 1 – 2 particles on the samples. This demonstrates most particles (99%) are lost when an individual washes their hands after the deposition of GSR. These trends are highlighted in **Figure 63,** which shows the activities with only the membrane on which it was deposited. The results demonstrate how the vigor of the activity can lead to lower persistence of IGSR, which is important to consider in assessing GSR evidence. Transfer and persistence are challenging to study because they can occur multiple times and have compounded effects on each other. It is essential to have an idea of how many times these activities are performed in an average person at a given timeframe to provide an understanding of what is likely during a firearm-involved crime.

Table 49. *Conditional probability table activity samples from Vander Pyl dataset II. For each activity the left column represents the count of occurrences (frequency), and the right column represent average number of characteristic particles observed on the samples.*

Synthetic Membrane Activities Samples								
# of Characteristic Particles/ Activity Type	**No Activity (n= 6)**		**Shaking Hands (n=6)**		**Rubbing Hands (n=6)**		**Washing Hands (n=6)**	
	Count (Frequency)	**Average Number of Characteristic Particles**	**Count (Frequency)**	**Average Number of Characteristic Particles**	**Count (Frequency)**	**Average Number of Characteristic Particles**	**Count (Frequency)**	**Average Number of Characteristic Particles**
None	0	**690 ± 104**	0	**790 ± 129**	0	**308 ± 114**	0	**2 ± 1**
Little (1-2 Particles)	0		0		0		5 (0.833)	
Few (3-7 Particles)	0		0		0		1 (0.166)	
Some (8-9 Particles)	0		0		0		0	
Many (10+Particles)	6 (1.00)		6 (1.00)		6 (1.00)		0	

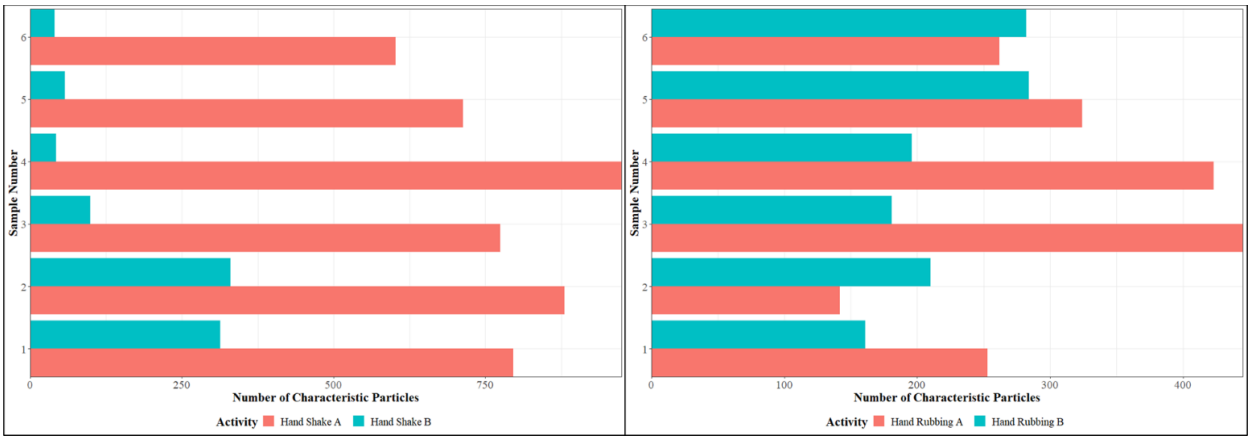


Figure 62. *Bar graph of the number of characteristic particles observed in the handshaking (left) and hand rubbing (right) samples collected from synthetic skin study analyzed by SEM-EDS where the red is the persisted particles after the activity and blue is the number of particles transferred.*

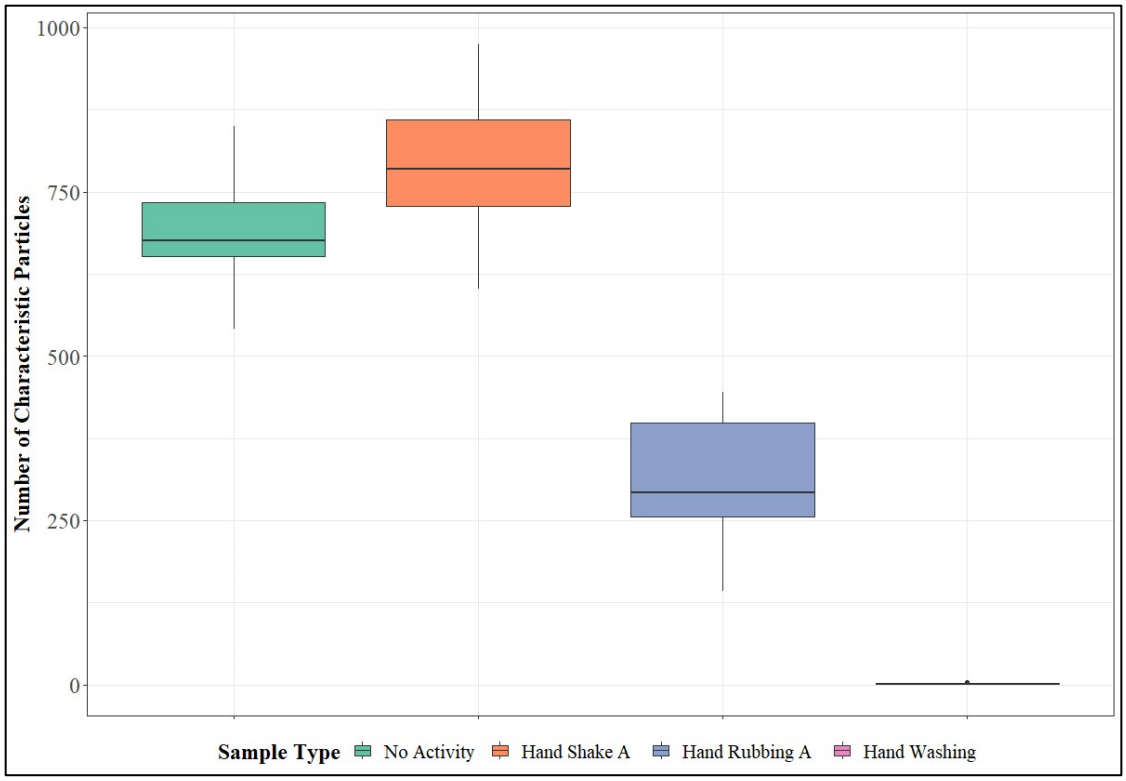


Figure 63. *Boxplot of the number of characteristic particles observed in the activity sample collected from synthetic skin study analyzed by SEM-EDS.*

Survey Results

A survey was conducted to determine everyday activities and how often they are performed by individuals on average. Thirteen questions were developed to understand the daily environment, activity levels, and hygiene activities that can affect the transfer and persistence of GSR (**Table 50**). The survey collected 98 responses. The results of daily environment questions are shown in **Figure 64**. The typical work environment was an office setting with 42 responses, followed by laboratory with 33 responses, and working from home with 28 responses. The other responses included working in a

car, outdoors, school, hospital, restaurant, *etc.* The most common modes of transportation to get to work are a personal vehicle or carpool, walking, or taking a bus. For individuals who use a car, the typical usage was 1-2 times a day, with few responses about using a vehicle 3-4 or 5 or more times a day. Finally, computer usage in the modern era plays a factor in an object commonly being touched for long periods of time. Approximately half of individuals responded they use a computer for one to two hours a day; meanwhile, 27 and 11 individuals stated they use it for at least 3 – 4 hours or five or more hours daily. Ten individuals responded that they do not use a computer daily. Daily activities help understand people's common environments they make contact with, which can provide context in firearm-related investigations containing GSR evidence

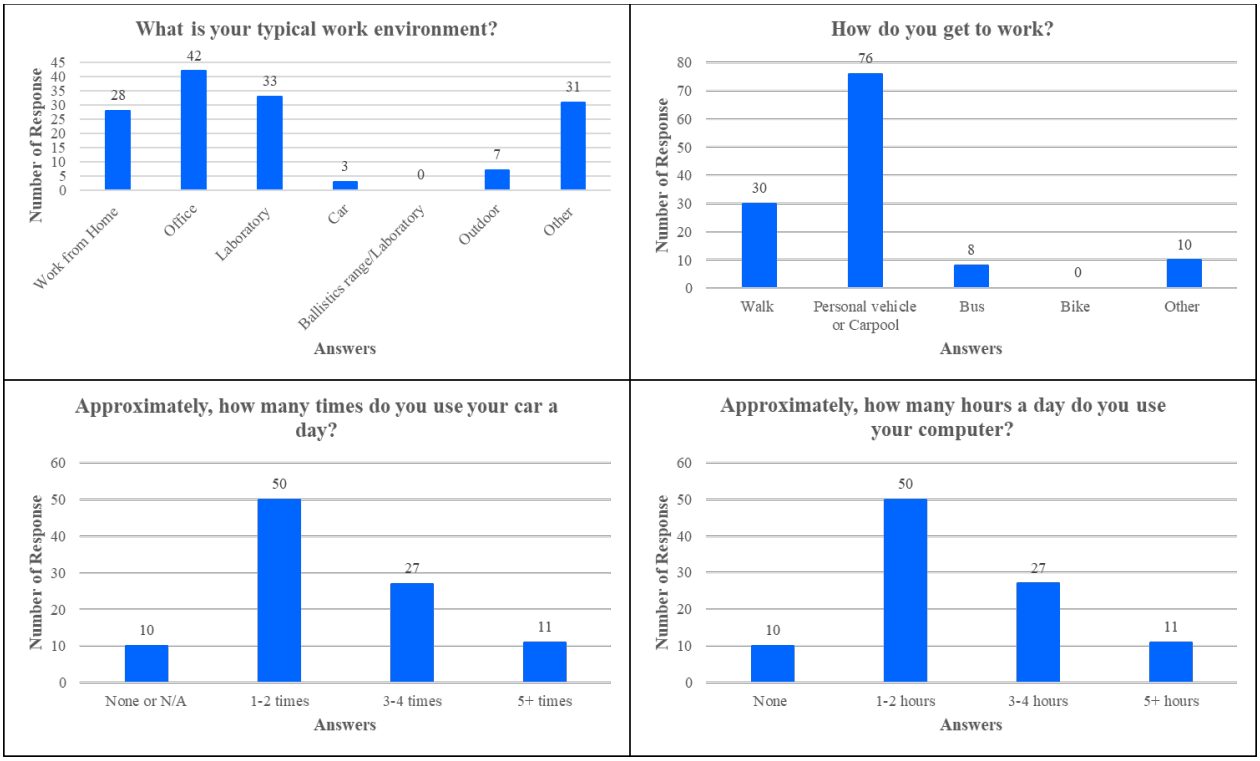


Figure 64. *Bar graph of the number of responses to the four daily environment questions from the background survey.*

The daily activities also evaluated some variables with rubbing hands, shaking hands, and exercise habits. For example, for rubbing hands, 65% of individuals estimate they perform this activity five or more times a day. At least 42% of the responders say they shake hands once or twice a day, and 40 % responded that they shake hands with someone at least three to four times a day. Regarding exercise habits, 80% of respondents take ≥ 5000 steps a day. Most participants live an active lifestyle, where the majority exercise at least one to two times a week, in comparison to the 17% that stated they do not exercise. During the exercise session, 47% of the participants ran or walked at least 1-2 miles. These habits tell us about the occurrence of GSR's potential transfer and persistence mechanisms. The results demonstrate that rubbing or shaking frequency can help forensic analysts interpret results in combination with studies like those by Vander Pyl *et al.* [3]

Table 50. *Summary table for daily activities with the answers and number of responses.*

Question Number	Question	Answers and Number of Responses				
5	Approximately, how many times a day do you rub your hands together?	None/NA	1-2 times	3-4 times	5+ times	
		7	8	19	64	
6	Approximately, how many times a week do you shake hands with someone else?	None/NA	1-2 times	3-4 times	5+ times	
		17	41	22	18	
7	Approximately, how many steps do you take daily?	0-2500 steps	2500-5000 steps	5000-7500 steps	7500-10000 steps	
		1	18	44	35	
8	Approximately, how many days do you run or exercise a week?	None/NA	1-2 times	3-4 times	5+ times	
		17	28	37	16	
9	When you run or exercise, approximately how far is your average distance?	None/NA	1-2 miles	3-4 miles	5-7 miles	10 + miles
		24	46	18	7	3

Finally, as shown in the transfer and persistence data, washing hands removes almost all traces of GSR from the hands. Additionally, Vander Pyl *et al.* concluded in their transfer study on clothing that washing also removes a significant amount of OGSR, whereas IGSR analysis was more challenging to study due to poor recovery from the fabrics, as explained by the authors.[3] Due to these results, the occurrence of these hygiene habits that can significantly diminish the persistence of GSR. Regarding daily hand washing, surveyed individuals found no individuals reported never washing their hands. Most individuals wash their hands at least three or more times a day. The two other questions of interest for washing your face and hair were also crucial as these are instances where hands are exposed to soap and water.

Lastly, the question on clothing cleaning habits, specifically upper garments, was interesting, as when a firearm is discharged, these are the surfaces closest to the plume of GSR. This question found that upper clothing garments are typically washed after one or two wears; however, 18 survey participants indicated the type of upper garment determines how many times they will wear it before cleaning. Shirts were commonly reported to be washed after wearing them 1-2 times, sweatshirts every four times, sweaters every five times, and jackets take upwards of 11 times of use before washing. This can be useful when determining what evidence to test if clothing evidence is submitted for GSR. If a shirt is submitted for GSR analysis, it will likely be washed more frequently than a jacket and still hold valuable evidence. However, this information should be taken cautiously and introduces other challenges because less washing may lead to more background contamination, especially jackets/coats may have been worn during a firing which took place quite some time prior when considering frequency of use. Hand and clothes washing reports can be important background information for a forensic analyst for the expected outcomes of IGSR analysis.

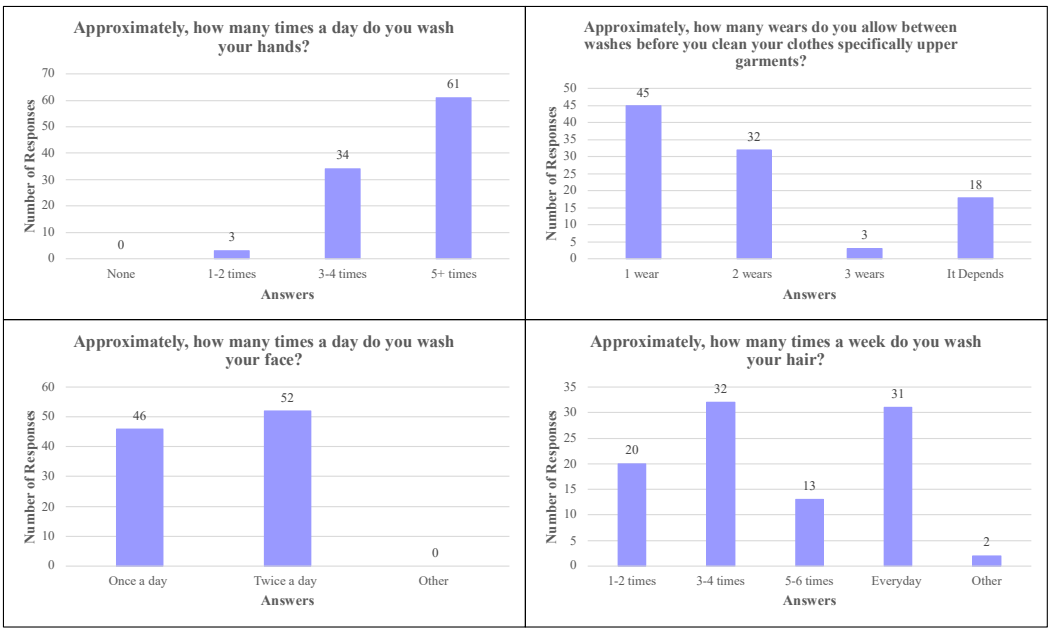


Figure 65. *Bar graph of the number of responses to the four hygiene habits questions from the background survey.*

Bayesian Network for IGSR

In the gunshot residue discipline, the interpretation of GSR evidence can be challenging due to the different levels of propositions that a scientist needs to address to respond to the information required by the trier of fact. Source-level propositions are relatively easy to determine if a trace residue is GSR or not. The Bayesian networks developed in this study focused on the second proposition level, *viz.* activity. This level can be more challenging to assess due to requiring evidentiary information on the background occurrence, transfer, and persistence behaviors. Under this level, the question requiring resolution is whether the person of interest (POI) discharged the firearm. The hypothesis for testing this will state the prosecutorial (or null) hypothesis (H_p) as the POI discharged the firearm. For the defense (or alternative) hypothesis (H_d), all of the hypotheses must be mutually exclusive and exhaustive, this includes the null hypothesis. The first defense hypothesis can be stated as someone other than the POI discharged the firearm; however, other more specific alternative hypothesis may be tested, such as the POI was standing close to the firearm when it was discharged, the POI was standing far away when the firearm was discharged, the POI handled but did not fire the/a gun, the POI acquired GSR particles by secondary transfer by shaking the hand of the person who fired the gun, *etc.* While the authors acknowledge the other hypothesis, the conditions for this Bayesian network will focus on the prosecutorial hypothesis as the POI discharged the firearm and the defense hypothesis that someone other than the POI discharged the firearm.

Bayesian networks provide a model for the cause-and-effect linkages between variables when considering the hypotheses node which can include more than two states. A framework was developed for exploring the relationships in for interpreting GSR evidence. The BN will provide data on how beliefs are affected using the information contained in the datasets by utilizing the background occurrence, transfer, and persistence of GSR. **Figure 66** depicts a simple graphical model for the transfer and persistence of GSR. **Table 51** summarizes the nodes, their abbreviation, descriptions, and states. The hypothesis node is the parent node to the primary transfer, which contains the two competing hypotheses. The primary transfer node describes the number of initial particles transferred

to the shooter's hand during the discharge event. The primary transfer of particles is affected by the type of firearm, ammunition used, distance of the shooter to target, and number of rounds fired.

The variables influence the number of characteristic particles likely to be observed from the primary transfer. It is also affected by the background occurrence of GSR on the hands of the POI for low-risk and high-risk background populations. The nodes linked to the primary transfer node influence the beliefs under the prosecutorial hypothesis of expecting a large number of particles and the defense hypothesis of observing none or a small number of particles on the hand of the POI. It is also influenced by the occurrence of having GSR on the hands before the discharge of a firearm using the background node. The box around the primary transfer node represents the possibility of the event happening multiple times if someone discharges more than one round or fires at different times during the event of a crime. The primary transfer is linked as the parent node to the persistence node. The persistence node describes the number of characteristic particles that persist on the hand of a POI. The persistence of GSR is affected by the surface from which the sample is collected, post-shooting activities, and the post-shooting collection time. The box represents that the variables influencing persistence can occur multiple times, *e.g.* someone washing their hands twice a day. Finally, the persistence node is parent to the observed data node, representing the laboratory results obtained from the sample. This node links the cause-and-effect relationships from the primary transfer of GSR to their persistence on the hands and the experimental data obtained from the evidence. The data are read into the BN (nodes, states, and edges) and the software the calculates the conditional probability tables which are then used during the instantiation of the network. Additionally, the network can be used to generate likelihood ratios when a certain number of particles is observed from the actual evidence.

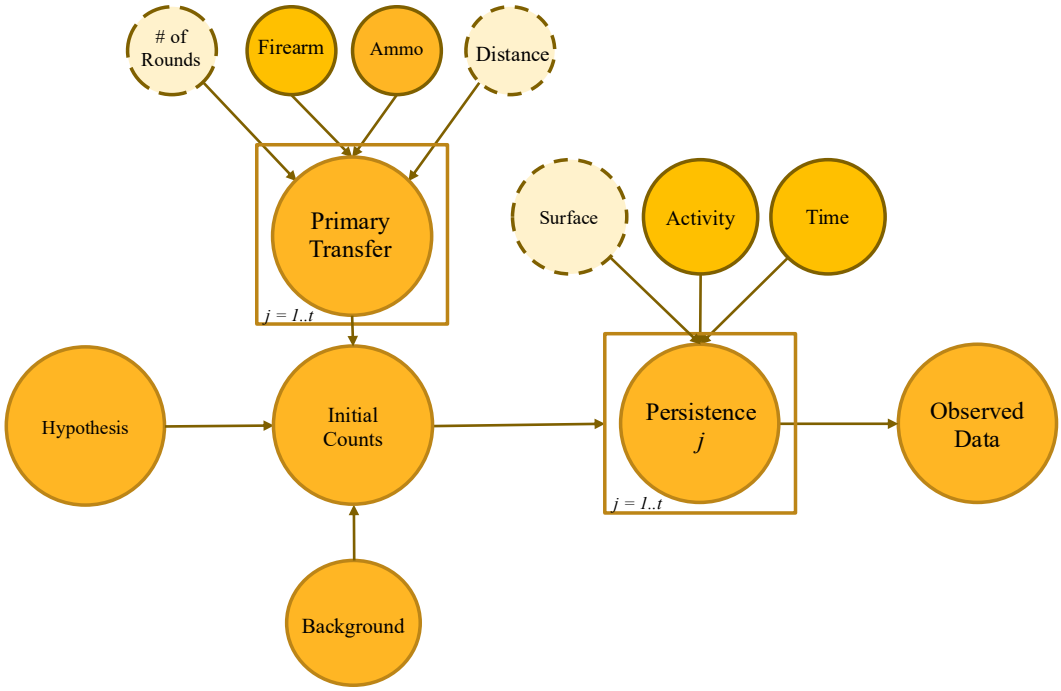


Figure 66. *A simple graphical model for the transfer and persistence of gunshot residue where solid circles are nodes for which data is available. The dashed circles can influence the transfer and persistence, but no data has been obtained yet to feed the BN.*

Table 51. *Summary of the nodes, their abbreviation, description, and states.*

Node	Abbreviation	Description	States
Hypothesis	H	Operating the network under the prosecutorial or defense hypothesis	H= H_p, H_d
Primary Transfer	PT	Number of initial GSR particles transfer to the shooter's hands from the shooting event	PT = 0-3,4-10, 10-200, 200-400, 400-600, 800-1000, 1000-2000
Background	B	Number of characteristic particle distribution in background populations	B = 0-3,4-10, 10-200, 200-400, 400-600, 800-1000, 1000-2000
Low-risk Background	LR		LR = 0-1,1-3, 3-4, 4-10
High-risk Background	HR		HR= 0-3, 3-4, 4-10, 10-20
Pistol	p	Number of characteristic particle distributions using a pistol or revolver used during the shooting event	p = 0-3,4-10, 10-200, 200-400, 400-600, 800-1000, 1000-2000
Revolver	R		R = 0-3,4-10, 10-200, 200-400, 400-600, 800-1000, 1000-2000
Leaded Ammunition	L	Number of characteristic particle distribution-based types of primer ammunition fired during shooting event	L = 0-3,4-10, 10-200, 200-400, 400-600, 800-1000, 1000-2000
Lead-free Ammunition	LF		LF = 0-3,4-10, 10-200, 200-400, 400-600, 800-1000, 1000-2000
Persistence	P	Number of GSR particles persisting on the suspect's hands from the shooting event after post-shooting activities	P = 0-3,4-10, 10-200, 200-400, 400-600, 800-1000, 1000-2000
Time	T	Number of characteristic particle distribution-based time interval between firing event and collection of GSR particles	0, 1, 3, 6 hours
Activity	A	Number of characteristic particle distribution based post-shooting activities performed between the firing event and collection of GSR particles	A= None, Running, Shaking Hands, Rubbing Hands, Washing Hands, Sanitizer
Observed Data	O	Number of observed GSR particles on the suspect's hands (due to shooting a firearm and background content of GSR)	O= 0-3,4-10, 10-200, 200-400, 400-600, 800-1000, 1000-2000

The Netica software was used to create the Bayesian Network (**Figure 67**). Data was inputted into the network after being normalized to 1000, as described in the methodology section to ensure that the data between the two sets were comparable. A Bayesian network was also created using the normalized data to the 10-particle cutoff that is not shown here. The nodes modeled containing data from the particle counts were set to be continuous nodes discretized into particle counts in intervals shown in **Table 51**. Additional nodes were created to set up the conditional probability tables in the software, but the cause-and-effect relations are the same as described above. The probability of the hypothesis node was set as H_d equal to 0.5 and H_p equal to 0.5. When the hypothesis node is instantiated to H_p, the initial counts' node uses the conditional probability tables of the primary transfer and background nodes to calculate the probability of observing each number of particles. Under the defense hypothesis, the initial counts will only consider the conditional probabilities from the background node to calculate probabilities. The node dealing with time and activity persistence of GSR where separate to create conditional probabilities for time and activity observations. The persistence time and persistence activity nodes were modeled as a function of loss using the data from the transfer and persistence studies. By instantiating a time or activity, the persistence time node updates the probabilities by subtracting the expected number of lost particles from the initial count node. The observed data node is simply the probability of the persistence activity node. Additionally, the Bayesian network feedback loop can be used by inputting an observed number of particles into the observed data node, and the probability distribution will update for all nodes as well as the hypothesis node, which can be used to obtain the likelihood ratio.

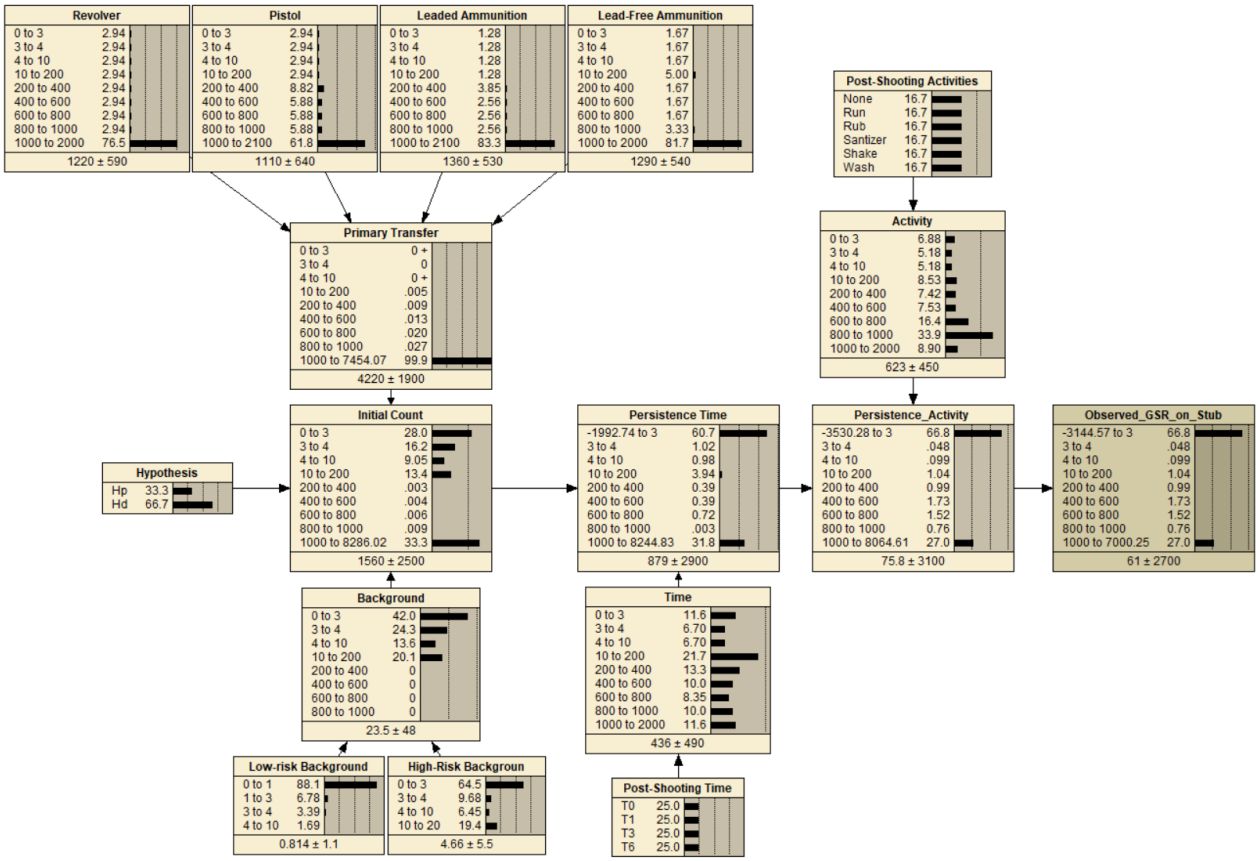


Figure 67. *Diagram of the Bayesian network created in the Netica software.*

Bayesian Networks: Conclusions and Future Work

This study has provided a proof-of-concept for using Bayesian networks as a tool for GSR interpretation. GSR interpretation challenges lay with the difficulty of modeling and studying the transfer and persistence mechanisms. The dataset used here follows experimental conditions that are a basis for creating a Bayesian network; however, the conditions of the study are also a limitation. The one provided here leans toward the best-case scenario using the authentic shooter and synthetic skin model data under controlled conditions. Additionally, there are other variables to include, like incorporating OGSR evidence to provide greater confidence in detecting GSR and the potential for differentiating between the shooter, bystander, or passerby as the competing hypotheses using the combination of IGSR and OGSR data. The present results demonstrate the promise of using Bayesian networks as an interpretation tool to evaluate evidence and aid in reporting GSR.

Future work will include generating likelihood ratios for data collected for different mock scenarios using the current network and validation with a new set of samples analyzed by SEM-EDS. Additionally, further analysis will expand the network to include confirmatory OGSR. This work encourages continued efforts to study the transfer and persistence of GSR and tools like the Bayesian network to assist with interpretation and reporting.

2.3. Limitations

The main limitation encountered in this project has been the creation of large datasets and enough replication of studies in the transfer and persistence of GSR to draw generalizations. To the best of our knowledge, our group has created the largest available repository of IGSR and OGSR data through this award and previous awards. With over 100,000 data files of GSR data from multiple subpopulations, analytical tools, and sensors, the scientific foundation of the findings is substantial.

However, the more we learn about IGSR and OGSR transfer and persistence mechanisms, the more factors become evident that require additional experimental designs to fully understand these complex processes. To that end, we have been fortunate to receive new NIJ funding to continue these studies and support from a strong partnership of practitioners, research, and industry. Additional replication and, most importantly, testing across multiple users will be essential to fully incorporate the new knowledge on IGSR and OGSR transfer and persistence into practical and comprehensive models of evidence interpretation. Our overarching goal is to ultimately answer the most relevant questions to the trier of fact (i.e., did the person of interest fired the gun?). The work produced here has laid the foundations to move in the right direction to modernize and strengthen the GSR discipline.

III ARTIFACTS

3.1 List of products

3.1.1 Publications at scientific peer-reviewed journals and dissertations

Published products in scientific peer-reviewed journals

1. Ledergerber TD, Feeney W, Arroyo L, Trejos T. A feasibility study of direct analysis in real time-mass spectrometry for screening organic gunshot residues from various substrates. *Analytical Methods.* 2023;15(36):4744-57. DOI: 10.1039/D3AY01258A
2. Vander Pyl C, Menking-Hoggatt K, Arroyo L, Gonzalez J, Liu C, Yoo J, Russo RE, Trejos T. Evolution of LIBS technology to mobile instrumentation for expediting firearm-related investigations at the laboratory and the crime scene. *Spectrochimica Acta Part B: Atomic Spectroscopy.* 2023 Jul 5:106741. https://doi.org/10.1016/j.sab.2023.106741
3. C Vander Pyl, K Dalzell, K Menking-Hoggatt, T Ledergerber, L Arroyo, T Trejos. Transfer and persistence studies of inorganic and organic gunshot residues using synthetic skin membranes. *Forensic Chemistry*, 34, 2023, 100498, https://doi.org/10.1016/j.forc.2023.100498
4. C Vander Pyl, W Feeney, L Arroyo, T Trejos, Assessment of the Limitations and Capabilities of GC-MS and LC-MS/MS for Trace Detection of Organic Gunshot Residues from Skin Specimens. *Forensic Chemistry*, 33, 2023, https://doi.org/10.1016/j.forc.2023.100471
5. Sarah Szakas, Korina Menking-Hoggatt, Tatiana Trejos, Alexander Gundlach-Graham. Elemental Characterization of Leaded and Lead-Free Inorganic Primer Gunshot Residue Standards by spICP-TOFMS. *Applied Spectroscopy.* 2022. Doi10.1177/00037028221142624
6. K Dalzell, C Ott, T Trejos, L Arroyo. Comparison of portable and benchtop electrochemical instruments for detection of inorganic and organic gunshot residues in authentic shooter samples, *J Forensic Sci*, 2022, https://doi.org/10.1111/1556-4029.15049
7. K Menking-Hoggatt, C Ott, C Vander Pyl, K Dalzell, J Curran, L Arroyo, T Trejos. Prevalence and Probabilistic Assessment of Organic and Inorganic Gunshot Residue and Background Profiles using LIBS, Electrochemistry, and SEM-EDS. *Forensic Chemistry*, 2022, https://doi.org/10.1016/j.forc.2022.100429
8. W Feeney, K Menking-Hoggatt, L Arroyo, J Curran, S Bell, T Trejos. Evaluation of organic and inorganic gunshot residues in various populations using LC-MS/MS, *Forensic Chemistry*, 2022, 27, https://doi.org/10.1016/j.forc.2021.100389.
9. W Feeney, K Menking-Hoggatt, C Vander Pyl, C Ott, S Bell, L Arroyo, T Trejos. Detection of organic and inorganic gunshot residues from hands using complexing agents and LC-MS/MS. *Analytical Methods.* 2021, 13, 3024-3039, https://doi.org/10.1039/D1AY00778E (Journal COVER PAGE and hot article)
10. C Vander Pyl, C Martinez-Lopez, K Menking-Hoggatt, T Trejos. Analysis of primer gunshot residue particles by Laser Induced Breakdown Spectroscopy and Laser Ablation Inductively Coupled Plasma Mass Spectrometry. *Analyst.* 2021. https://doi.org/10.1039/D1AN00689D
11. K Menking-Hoggatt, C Martinez, C Vander Pyl, E Heller, E Pollock, L Arroyo, and T. Trejos. Development of Tailor-Made Inorganic Gunshot Residue (IGSR) Microparticle Standards and Characterization with a Multi-technique Approach. *Talanta.* April 2021, 225, https://doi.org/10.1016/j.talanta.2020.121984

Published thesis and dissertations.

1. Korina Menking Hoggatt, Ph.D, Spring 2021. WVU Department of Forensic and Investigative Science, Characterization of modern ammunition and background profiles: A novel approach and probabilistic interpretation of inorganic gunshot residue. Graduate Theses, Dissertations, and Problem Reports. 8336. https://researchrepository.wvu.edu/etd/8336
2. William Feeney, Ph.D., Fall 2021.WVU Department of Chemistry, Modified firearm discharge residue analysis utilizing advanced analytical techniques, complexing agents, and molecular dynamics. "Modified Firearm Discharge Residue Analysis utilizing Advanced Analytical Techniques, Complexing Agents, and Quantum Chemical Calculations"

(2021). *Graduate Theses, Dissertations, and Problem Reports*. 10302. https://researchrepository.wvu.edu/etd/10302

3. Courtney Vander Pyl, Ph.D., Fall 2022. WVU Department of Forensic and Investigative Science, Expanding the capabilities of firearm investigations: Novel sampling and analytical methods for gunshot residue detection. Graduate Theses, Dissertations, and Problem Reports. 11509. https://researchrepository.wvu.edu/etd/11509
4. Dalzell KA. Electrochemical and mass spectrometry methods for identification of gunshot residues (GSR) in forensic investigations. Graduate Theses, Dissertations, and Problem Reports. 11354. https://researchrepository.wvu.edu/etd/11354/

3.1.2. Presentations at Scientific Meetings

1) November 2023. Thomas Ledergerber, Monica Joshi, Luis Arroyo, and Tatiana Trejos. Evaluation of Gas Chromatography-Triple Quadrupole Mass Spectrometry for the Identification of Organic Gunshot Residues from Known Shooters and Non-Shooters. 2023 Annual Eastern Analytical Symposium, New Jersey (invited speaker)
2) November 2023. Tatiana Trejos, Luis Arroyo, Kourtney Dalzell, Thomas Ledergerber, Leah Thomas, and Madison Lindung. Development of Strategic Analytical Methods to Support the Modernization of Gunshot Residue Practice in Forensic Science.2023 Annual Eastern Analytical Symposium, New Jersey (invited speaker)
3) October 2023. Tatiana Trejos, Luis Arroyo, Kourtney Dalzell, Leah Thomas, Madison Lindung, and Thomas Ledergerber. Streamlining Decision-making Processes at the Crime Scene and the Laboratory by Incorporating Fast Screening Tools into Current Gunshot Residue Workflows. FACSS SCIX Annual Meeting, Reno, NV (invited speaker)
4) October 2023. Kourtney Dalzell, Leah Thomas, Thomas Ledergerber, Courtney Vander Pyl, Tatiana Trejos, Luis Arroyo, Jhanis Gonzalez, Chunyi Liu, Jong Yoo, and Richard E. Russo. Advancement of LIBS Mobile Technology for the Detection of Firearm Discharge Residue from Various Substrates and Assessment at Mock Crime Scenes. FACSS SCIX Annual Meeting, Reno, NV
5) October 2023. Luis Arroyo, Tatiana Trejos, Kourtney Dalzell, Thomas Ledergerber, Leah Thomas. Strategies to streamline firearm-related investigations at the crime scene and the laboratory using modern screening tools for GSR detection. 2023 CFS Physical Sciences Symposium, Center of Forensic Science, Toronto, Canada. (invited speaker)
6) October 2023. Tatiana Trejos, Luis Arroyo, Mat Staymates, Courtney Vander Pyl, Thomas Ledergerber, Kourtney Dalzell, and Madison Lindung. Lessons learned about the transfer and persistence of inorganic and organic gunshot residue. 2023 CFS Physical Sciences Symposium, Center of Forensic Science, Toronto, Canada. (invited speaker)
7) August 2023. Thomas Ledergerber, Kourtney Dalzell, Matt Staymates, Luis Arroyo, Tatiana Trejos. Gunshot residue visualization using laser sheet scattering, high speed videography, atmospheric particle samplers, and analytical techniques. Midwestern Association of Forensic Scientists (MAFS) 52nd Annual Meeting, Detroit, MI (oral presentation)
8) May 2023. Kourtney A. Dalzell, Courtney Vander Pyl, Leah Thomas, Colby E. Ott, Korina Menking-Hoggatt, Tatiana Trejos, and Luis E. Arroyo. Advancements in por technology for gunshot residue detection using electrochemical and Laser-Induced Breakdown Spectroscopy (LIBS) devices. NIJ National Research Conference, Arlington, Virginia
9) April 2023, Tatiana Trejos, Kourtney Dalzell, Thomas Ledergerber, Zach Andrews, Lacey Leatherland, Sharon Kalb. CSI Behind the Scenes: Discovering Clues through Science. WVU ERUREKA STEM Camp Forensic Workshop. Weston, WV
10) April 2023. Madison Lindung, Tatiana Trejos, Luis Arroyo. West Virginia University

Congressionally Directed Spending Programs visit to the US Capitol and meetings with members of US Congress. Research at WVU-FIS on Firearm-Related Investigations. (invited contribution).

11) April 2023. Shippensburg University. Seminar Presentation. "Emerging Analytical Tools for the Forensic Analysis of Drugs and Gunshot Residues". Sharon Kalb, Kourtney Dalzell and Luis Arroyo. Friday, April 14, 2023. (Guest speaker, Seminar Series 2023).
12) March 2023, Kourtney A. Dalzell, Courtney Vander Pyl, Tatiana Trejos, Luis E. Arroyo. Innovative sampling and screening methods for IGSR and OGSR analysis in firearm reconstruction investigations using electrochemistry and statistical analysis. Pittcon 2023. (Poster presentation).
13) March 2023, Luis Arroyo, Tatiana Trejos, Matt Staymates, Korina Menking-Hoggatt, Colby Ott, Courtney Vander Pyl, Kourtney A. Dalzell, Thomas Ledergerber, and Bill Feeney. Pittcon 2023. NIJ Session. Advancements in the Analysis of Forensic Trace Evidence (G. Dutton session coordinator). Novel Tools for the Analysis of Organic and Inorganic Gunshot Residues: moving technology to the forefront. (Oral presentation)
14) February 2023, Thomas Ledergerber, William Feeney, Edward Scisco, Luis Arroyo, Tatiana Trejos. Investigation of Authentic Organic Gunshot Residues by Direct Analysis in Real Time-Mass Spectrometry. AAFS meeting, Orlando, FL (poster presentation)
15) January 2023, Tatiana Trejos. Expanding the Capabilities of Gunshot Residues: Novel Sampling and Analytical Methods, virtual presentation to the NIST-OSAC GSR Subcommittee (invited oral presentation).
16) January 2023, Madison Lindung, Kourtney Dalzell, Tatiana Trejos. Transfer and Persistence of Gunshot Residue on Clothing and Synthetic Skin Substrates by SEM-EDS, 20th Annual Undergraduate Research Day at the Capitol (poster presentation)
17) January 2023, Leah Thomas, Kourtney Dalzell, Tatiana Trejos. Portable Screening Solution to Firearm-related Crimes using Laser Induced Breakdown Spectroscopy (LIBS), 20th Annual Undergraduate Research Day at the Capitol. (poster presentation)
18) December 3rd, 2022. Madison Lindung, Korina Menking-Hoggatt, Courtney Vander Pyl, Kourtney Dalzell, and Tatiana Trejos. Transfer and Persistence of Gunshot Residue on Synthetic Skin by SEM-EDS. Fall Undergraduate Research Symposium, Morgantown, WV (Oral presentation)
19) October 28th, 2022. Tatiana Trejos, Luis Arroyo, Korina Menking-Hoggatt, Courtney Vander Pyl, Kourtney Dalzell. Métodos novedosos para la identificación de particulas orgánicas e inorgánicas de residuos de disparo XIII Congreso Internacional de Medicina Legal y Ciencias Forenses, Panama (Oral presentation, invited speaker)
20) September 2022, Korina Menking-Hoggatt, Courtney Vander Pyl, Kourtney Dalzell, Colby E. Ott, Luis Arroyo, Tatiana Trejos. FTCoE National Forensic Week. Persistence, Prevalence, and Probabilistic Study of Inorganic and Organic Gunshot Residue in Shooter and Non-Shooter Populations (Poster-virtual, invited speaker)
21) September 16th, 2022. Tatiana Trejos, Luis Arroyo, Korina Menking-Hoggatt, Courtney Vander Pyl, Kourtney Dalzell, Colby Ott, and Thomas Ledergerber. Development of innovative, reliable, and fast screening methods for the detection of organic and inorganic gunshot residues. MAFS 51st Annual Fall Meeting A Joint Meeting with ASTEE Des Moines, Iowa. (Oral presentation)
22) July 28th, 2022. Madison Lindung, Leah Thomas, Korina Menking-Hoggatt, Courtney Vander Pyl, and Tatiana Trejos. Portable versus Benchtop LIBS Comparison for Screening of Gunshot Residue. WVU Summer Undergraduate Symposium, Morgantown, WV (Poster, 2nd place award)
23) May 31st, 2022. Tatiana Trejos and Luis Arroyo. Analysis and Interpretation of Organic and

Inorganic Gunshot Residues: Lessons Learned from a Large Population Study. European Academy of Forensic Sciences (EAFS) conference, Stockholm, Sweden (Oral presentation, invited keynote speaker).

24) February 2022. Kourtney Dalzell, Courtney Vander Pyl, MS, Tatiana Trejos. and Luis Arroyo, Combining novel sampling techniques and electrochemical detection in GSR analysis for bullet hole identification and distance determination, Seattle, AAFS Conferences, WA (Poster Presentation).
25) February 2022. Courtney Vander Pyl, MS, Korina Menking-Hoggatt, Tatiana Trejos. Rapid Spectrochemical Mapping Techniques for Enhanced Detection and Visualization of Gunshot Residue Patterns. AAFS Conference, Seattle, WA Poster Presentation).
26) February 2022. William Feeney, Korina Menking-Hoggatt, Luis Arroyo, James Curran, Suzanne Bell, Tatiana Trejos. An Evaluation of Organic and Inorganic Gunshot Residues in Various Populations Using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). AAFS Conference, Seattle, WA. (Oral presentation)
27) February 2022 Korina Menking-Hoggatt, Colby E. Ott, Courtney Vander Pyl, Kourtney Dalzell, James Curran, Luis Arroyo, Tatiana Trejos. Probabilistic Interpretation of a Large Population Study of Gunshot Residue and Background Profiles using LIBS, Electrochemistry, and SEM-EDS, Seattle, WA. (Oral presentation)
28) December 2021, Jessica Friedel, Korina Menking-Hoggatt, Tatiana Trejos. A Study of Gunshot Residue Prevalence in the General Population of Morgantown West Virginia, WVU Fall Undergraduate Symposium (Oral Presentation)
29) October 2021. Courtney Vander Pyl, Kourtney Dalzell, Tatiana Trejos, and Luis Arroyo. Emerging Analytical Techniques for the Reconstruction of Firearm-Related Incidents. Brazil 3rd School of Forensic Science (Virtual, Oral Presentation).
30) October 2021. Korina Menking-Hoggatt; William Feeney, Tatiana Trejos, Luis Arroyo. Study of various types of Gunshot Residue and Background Populations using LIBS, Electrochemistry, SEM-EDS, LC/MS/MS and probabilistic Interpretation. Brazilian Winter School Program (Virtual, Oral Presentation).
31) September 23, 2021, Korina Menking-Hoggatt, Edward "Chip" Pollock, Emily Heller, Courtney Vander Pyl, Claudia Martinez, Tatiana Trejos. Inorganic Gunshot Residue (IGSR) Micro-particle Standard with Application to Method Development and Understanding Modern Ammunition. Mid-Atlantic Association of Forensic Scientist and ASTEE joint meeting (oral, MAAFS Annual Scholarship Winner)
32) September 23, 2021. Courtney Vander Pyl, Korina Menking-Hoggatt, Tatiana Trejos. Rapid Analytical Screening Methods for the Investigation of Firearm Related Crimes. Mid-Atlantic Association of Forensic Scientist and ASTEE joint meeting (oral)
33) July 29th, 2021. Declan Revenew, Courtney Vander Pyl, Bill Feeney, Tatiana Trejos. Evaluating GC-MS and LC-MS/MS Efficacy for Characterization of a Developed Organic Gunshot Residue Standard**.** 13th Annual summer undergraduate research symposium, Morgantown, WV https://www.youtube.com/watch?v=1yszuB1nH60
34) July 28th, 2021. Jessica Friedel, Korina Menking-Hoggatt, and Tatiana Trejos. Prevalence of Particles Characteristic and Consistent with GSR Found in A Background Population Study. Current Trends in Forensic Trace Analysis 2021 Online Forensic Symposium. (poster)
35) July 28th, 2021. Courtney Vander Pyl, Claudia Martinez-Lopez, Korina Menking-Hoggatt, Tatiana Trejos. Rapid Laser-Based Methods for the Detection of Modern Gunshot Residues. Current Trends in Forensic Trace Analysis 2021 Online Forensic Symposium. (Poster, Best poster Elsevier Forensic Chemistry award)
36) July 28th, 2021. Bill Feeney, Suzanne Bell, Tatiana Trejos. Exploring the probabilistic interpretation of gunshot residue in various populations using LC-MS/MS. Current Trends in

Forensic Trace Analysis 2021 Online Forensic Symposium. (poster)

37) April 2021, Tatiana Trejos. Perfiles químicos como pistas en escenas del crimen. 30th Anniversary of Costa Rican Scientific Highschool Systems (invited oral)
38) March 2021, Tatiana Trejos. Experiences from a Forensic Scientist. A.O.E. Junior Academy for Young Women in STEM (invited oral)
39) March 2021, Luis Arroyo, Tatiana Trejos, Korina Menking Hoggatt, Colby Ott, Courtney Vander Pyl, Kourtney Dalzell, Bill Feeney. Detection of gunshot residues from leaded and non-leaded ammunition by electrochemical sensors and LIBS, PITTCON 2021 (invited speaker)
40) March 2021, Tatiana Trejos, Luis Arroyo, Korina Menking Hoggatt and Courtney Vander Pyl. LIBS as an emerging method for the detection of firearm discharge residues, NIJ (National Institute of Justice) -Emerging Analytical Methods for Chemical and Biological Forensic Evidence Session, PITTCON 2021 (invited speaker)
41) January 2021, Bill Feeney, Tatiana Trejos. Detection of OGSR and IGSR from the same collection stub using complexing agents and LC/MS/MS, 4th event Global Lecture Series, Crossing Forensic Borders (invited speaker)
42) January 2021, Korina Menking-Hoggatt, C. Ott, Tatiana Trejos, Luis Arroyo. Novel rapid detection of inorganic and organic gunshot residues using LIBS and electrochemistry: a population study, 4th event Global Lecture Series, Crossing Forensic Borders. (Invited speaker)
43) February 2021, Courtney Vander Pyl, Korina Menking-Hoggatt, Claudia Martinez, Tatiana Trejos. Application of Laser-Based Methods for the Analysis of Gunshot Residue Originating from Modern Ammunition. AAFS 2021 (poster presentation)
44) February 2021, Kourtney A. Dalzell, Korina Menking-Hoggatt, Colby E. Ott, Tatiana Trejos, and Luis Arroyo. Detection of Lead-Free Inorganic and Organic Gunshot Residue Using LIBS, Electrochemistry, and Machine Learning. AAFS 2021 (oral presentation)
45) February 2021, Kourtney A. Dalzell, Korina Menking-Hoggatt, Colby E. Ott, Tatiana Trejos, and Luis Arroyo. Detection of Lead-Free Inorganic and Organic Gunshot Residue Using LIBS, Electrochemistry, and Machine Learning. AAFS 2021 (oral presentation)

3.1.3. Website(s) or other Internet site(s)

Our research has been highlighted in the following media:

1. Chemical and Engineering News:

Green ammunition's organic residues - C&EN

https://cen.acs.org/analytical-chemistry/forensic-science/Green-ammunitions-organic-residues/101/i23

2. WVU today

WVU forensics lab cracks case on newer, 'greener' gunshot residue

https://wvutoday.wvu.edu/stories/2023/07/06/wvu-forensics-lab-cracks-case-on-newer-greener-gunshot-residue

3.Open access government

US forensic scientists make gunshot residue breakthrough

https://www.openaccessgovernment.org/us-forensic-scientists-make-gunshot-residue-breakthrough/163233/

4.Homeland security News Wire

https://www.homelandsecuritynewswire.com/dr20230709-forensics-lab-cracks-case-on-newer-greener-gunshot-residue

5.Bollyinside

Analytical Goalposts Shift as Crime Scenes Embrace Environmentally Friendly Ammunition

https://www.bollyinside.com/news/latest-science-news/analytical-goalposts-shift-as-crime-scenes-embrace-environmentally-friendly-ammunition/

6.This Week in Forensic Science ISHI

https://www.ishinews.com/fs-news-week-of-july-03-2023/

7.Technology networks applied sciences

"Greener" Ammunition Is Moving the Analytical Goalposts at Crime Scenes

https://www.technologynetworks.com/applied-sciences/news/greener-ammunition-is-moving-the-analytical-goalposts-at-crime-scenes-375853

Moreover, our research was selected to be featured in the AAFS TV2024 Thought Leadership Film Series. They are interested in profiling NIJ-funded WVU's pioneering work in optimizing the reliability and efficiency of methods enhancing trace evidence. They are especially looking to highlight thought leaders whose work pushes the boundaries in the integrated value of trace evidence and its potential to revolutionize crime scene investigations and forensic laboratories' workload. The film will be released at the AAFS 2024 Annual Meeting and disseminated through AAFS TV, YouTube, and other media.

3.2. Data sets generated.

According to our data management plan, the data resulting from this research was curated and compiled into a centralized dataset repository. The dataset generated in this study consists of a digital collection of about 35,530 files containing documents and data pertaining to this grant, including raw and processed data from various populations and analytical tools (LIBS, EC, SEM-EDS, LC-MS/MS, particle counters). Each subfolder of data contains the respective READ ME files explaining folder contents. The datasets are accompanied by a codebook, a master inventory excel file and Standard Operating Procedures.

Data Storage and File Descriptions

The stored data folder is named after the grant number "2020-DQ-BX-0010". This folder contains two main folders, documents and data. The document folder contained the pdf files required for grant data submission. The data folder contains three subfolders split by the objectives of the grant (see **Figure 68**). Each folder is further broken down into the tasks associated with each objective, and an example of the data organization is shown in **Figure 69.**

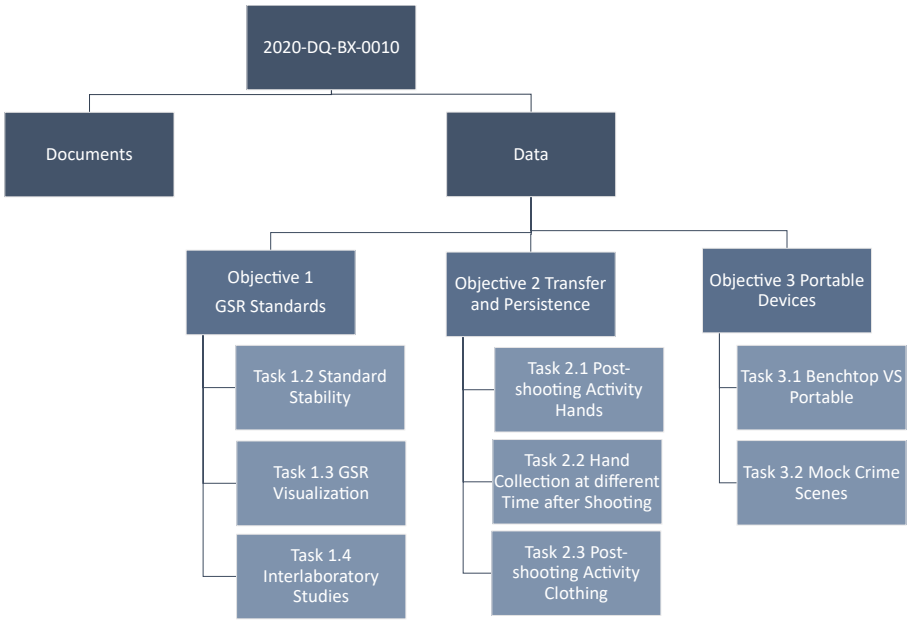


Figure 68. *2020-DQ-BX-0010 folder structure diagram for the GSR data storage.*

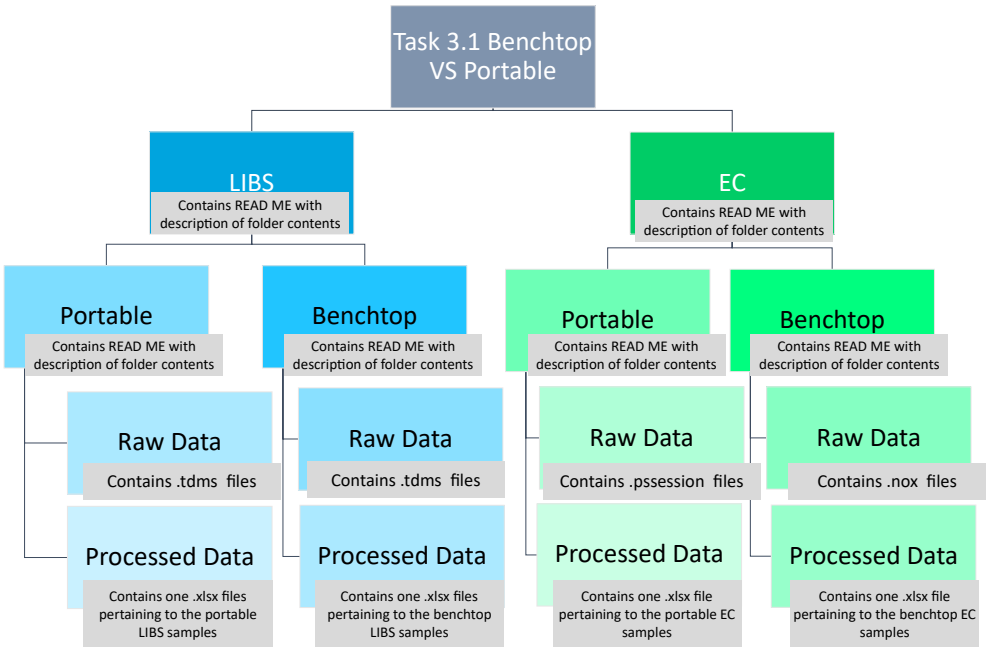


Figure 69. *Example of flow diagram of the folder structure of Task 3.1 Benchtop VS Portable folder.*

3.3. Dissemination activities

The main dissemination routes have been publishing manuscripts and presenting research results at scientific meetings. Moreover, we collaborated with the Sacramento Crime Laboratory to evaluate the utility of the proposed approaches. The laboratory has a LIBS system and is interested in adopting the methodologies. They have shown great interest in utilizing electrochemical detection to attend crime scenes, particularly in those cases where acoustic systems are used by law enforcement to identify gunshot sounds to participate in the scene in a matter of minutes after a shooting has occurred. Additionally, we created a memorandum of understanding (MOU) with the New Jersey State Police (NJSP) Division of Forensic Science to deliver a workshop on the portable electrochemical unit.

Also, we have established networking with the NIST-OSAC GSR Subcommittee to collaborate and participate in designing an interlaboratory study to examine OGSR. The in-house standards being developed in our group would serve these purposes. Additionally, we presented at 45 scientific meetings like the AAFS, EAFS, PITCON, SCIX, and the Undergraduate Research Symposium at the West Virginia State Capitol and the 14th Annual Undergraduate Research Symposium at WVU.

IV PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS

This research has provided a robust platform for training the next generations of forensic scientists in trace evidence, gunshot residue, and experimental design in forensics. This research has provided research opportunities for undergraduate students and graduate students (Master and Doctoral). **Table 52** lists the main participants and collaborators.

Moreover, this project's resources and research settings have provided all undergraduate and graduate students the unique opportunity to present their results at scientific venues. The opportunities provided to undergraduate researchers, some of the first-generation university students or minority students, have served as an essential foundation to their professional development. Four of our PhD students and one undergraduate student joined the workforce, two undergraduates are applying to graduate school, one master's students completed her degree and started in the doctoral program. All remaining students continue conducting research in our group. These student's achievements and STEM professional preparation are, in our opinion, the most valuable product of NIJ-funded efforts like this one.

This project also allowed a valuable collaboration across disciplines and between academia, practitioners, researchers, and industry, exposing the students, faculty, and practitioners to an enriching multi- and inter-disciplinary environment to develop solutions for our criminal justice system.

Table 52. *List of main participants and collaborating organizations*

Participant Name	Affiliation	Role	Contributions
Tatiana Trejos	West Virginia University	Principal investigator, Associate Professor	Managed the project and directly supervised students on experimental designs, sample collection, method development, and statistical interpretation of the data. Supervised dissemination plans, data curation and management plans.
Tatiana Trejos	West Virginia University	Principal investigator, Associate Professor	Managed the project and directly supervised students on experimental designs, sample collection, method development, and statistical interpretation of the data. Supervised dissemination plans, data curation and management plans.
Keith Morris and Roger Jefferys	West Virginia University	Collaboration with Ballistics Shooting Range	Assisted as officials on charge for the coordination of the use of the shooting range, and storage and management of firearms and ammunition. Dr. Morris also contributed with the Bayesian Network.
James Curran	University of Auckland	Statistician Collaborator (subaward), Head of the Statistics	Collaborated as expert in statistical analysis and interpretation of the data and as co-author of manuscripts.
Rick Russo and Jhanis Gonzalez	Applied Spectra	Industry partner	Main collaborators at Applied Spectra, who helped with the manufacturing of a custom-made prototype for LIBS mobile analyses
Matt Staymates	NIST	Scientist	Scientist engineer at NIST expert in flow dynamics. He has provided key support in the visualization of GSR using laser scattering and sensors.
Korina Menking-Hoggatt	West Virginia University	Graduate student (PhD) and post-doc	PhD graduate student working at the Trejos's group, who later was hired as post-doc. Korina was the lead student researcher, contributed with collections, physical and digital database, the data acquisition, analysis and interpretation. She has been a primary contributor to the manuscripts and dissemination of results.
Courtney Vander Pyl	West Virginia University	Graduate Student (PhD)	Graduate student working at the Trejos's group. Primary contributor to the project, the manuscripts and dissemination of results.

Participant Name	Affiliation	Role	Contributions
William Feeney	West Virginia University	Graduate Student (PhD)	Graduate student working at the Trejos's group. Primary contributor to the project, the manuscripts and dissemination of results.
Colby Ott	West Virginia University	Graduate Student (PhD)	Graduate student working at the Arroyo's group. Primary contributor to the project, the manuscripts and dissemination of results.
Kourtney Dalzell	West Virginia University	Graduate Student (MSFS and PhD)	Graduate student working at the Arroyo's group. Primary contributor to the project, the manuscripts and dissemination of results.
Thomas Ledergerber	West Virginia University	Graduate Student (PhD)	Graduate student working at the Trejos's group. Primary contributor to the project, particularly in the area of OGSR.
Leah Thomas	West Virginia University	Undergraduate and Graduate Student (MSFS)	Student working at the Trejos's group. Primary contributor to the project (LIBS and SEM-EDS).
Jessica Friedel	West Virginia University	Undegraduate Student	Undergraduate student working at the Trejos's group (sampling and SEM-EDS)
Declan Revenew	West Virginia University	Undegraduate Student	Undergraduate student working at the Trejos's group (sampling and transfer and persistence)
Madison Lindung	West Virginia University	Undegraduate Student	Undergraduate student working at the Trejos's group (sampling, sample preparation, LIBS, SEM-EDS)
Addio Fiordigigli	West Virginia University	Undegraduate Student	Undergraduate student working at the Trejos's group (sample preparation, LCMSMS and GCMS)
Liliana Barbosa	West Virginia University	Undegraduate Student	Undergraduate student working at the Trejos's group (sample preparation, LCMSMS and GCMS
Ian Balestieri	West Virginia University	Undegraduate Student	Undergraduate student working at the Trejos's group (sample preparation, LCMSMS and GCMS)

V CHANGES IN APPROACH

Nothing to report.

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