Determining the Frequency of Occurrence of Certain Target Organic Gunshot Residue Components in Non-shooting Environments

By

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ABSTRACT

Understanding the prevalence of Organic Gunshot Residue (OGSR) particles in a non-shooting environment can provide useful information in forensic investigations. Previous studies have shown that certain OGSR compounds are widely used in various occupational sources. This research investigated the prevalence of OGSR in various non-shooting occupational sources. Samples were collected from the hands of 90 volunteers using polyester swabs and deionized water. A total of 119 qualified samples were analyzed for the presence of 2,6 dinitrotoluene (2, 6- DNT), trinitroglycerin (TNG), and ethyl centralite (EC) using Gas Chromatography-Mass Spectrometry (GC-MS). This research determined that of those 119 samples: 100 samples contained 2, 6- DNT, 25 samples contained EC at a threshold concentration of 0.5 ug/mL or above, and no sample contained TNG at a threshold concentration of 25 ug/mL or above. According to previous studies, the presence of TNG and EC as a pair is considered characteristic of OGSR; therefore, these findings were not characteristic of OGSR. Furthermore, these results strengthen the likelihood that certain OGSR compounds, if found in pairs, are indicative of a discharge of a firearm. The use of this knowledge in conjunction with the traditional analysis of GSR can reduce the risk of false negative results. With the aid of future studies to incorporate known shooter samples to assess the threshold levels of these compounds in real life scenarios, OGSR analysis can ultimately strengthen the probative results of forensic GSR analysis in the criminal justice system.

KEY WORDS: Gunshot residue, Organic gunshot residue, firearms, prevalence study, trace analysis

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ABBREVIATIONS

2 or 4-NDPA	2 or 4-nitrodiphenylamine
2, 4-DNDPA	2, 4–dinitrodiphenylamine
2,4- or 2,6-DNT	2,4 or 2,6 Dinitrotoluene
ASTM	American Society for Testing Material
Ba	Barium
CA	California
Cal-DOJ	California Department of Justice
CDC	Center of Disease Control and Prevention
d10- DPA	d10- diphenylamine
DI	Deionized
DNT	Dinitrotoluene
DPA	Diphenylamine
dr	Dram
EC	Ethyl centralite
GC-MS	Gas chromatography- mass spectrometry
GSR	Gunshot residue
HPLC-MS	High pressure liquid chromatography- mass spectrometry
Hr	Hour
IGSR	Inorganic gunshot residue
IGSR	Inorganic gunshot residue

IPA	Isopropyl Alcohol
IRB	International Review Board
ISTD	Internal standard
LOD	Limit of Detection
NC	Nitrocellulose
N-nDPA	N-nitrosodiphenylamine
OGSR	Organic gunshot residue
Pb	Lead
RT	Retention time
Sb	Antimony
SCCL	Sacramento County District Attorney's Crime Laboratory
SEM/EDX	Scanning Electron Microscopy/Energy-Dispersive X-ray Spectrometry
SIM	Selected Ion Monitoring
SRM	Standard Reference Material
TNG	Trinitroglycerin
TWGFEX	Technical Working Group on Fire and Explosives
UCD	University of California Davis

1. INTRODUCTION

1.1. Persistence of firearm-related cases in forensic laboratories

The involvement of firearms in crimes such as robbery, assault, and homicide remains highly prevalent in the twenty-first century. In 2018, the use of firearms contributed to approximately 68% of willful homicide in the state of California. (Figure 1). Thus, the influx of firearms-related cases is likely to persist in forensic laboratories in the coming years. Firearm-related cases submitted in forensic laboratories often encompass multiple objectives ranging from serial number restoration to firearm exposure determination.

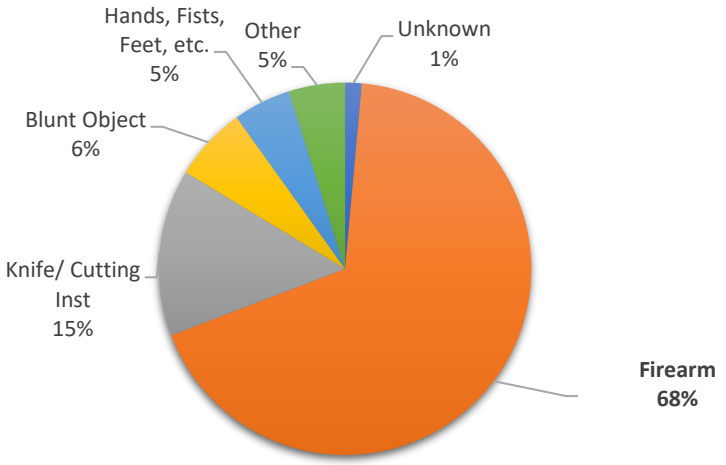


Figure 1: Willful homicide crimes reported in 2018 in the state of CA (data obtained from Cal DOJ statistics)

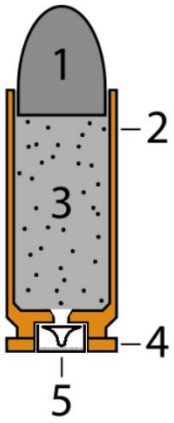
One of a forensic laboratory's main objectives in a firearm-related case is to establish or refute associations between the suspect and the use of a firearm in a crime, which can ultimately corroborate the suspect's alibi relating to firearm exposure. Firearms exposure can confirm whether the suspect has fired a gun or has been near a gun where it has been fired. This can be accomplished by screening for the residues deposited on various surface areas such as hair, hands,

face, and clothing after the discharge of a firearm; these residues are referred to as gunshot residues (GSR).

1.2. GSR composition

GSR consists of an intricate mixture of inorganic and organic components. These components come from the actual firearm, the ammunition used, and the resulting combustion products during discharge. Figure 2 exhibits a typical composition of a cartridge, which consists of five major parts: 1. bullet, 2. cartridge case (or metallic case), 3. gun powder (or propellant powder), 4. rim, and 5. primer. Organic GSR (OGSR) includes the completely or partially burned and unburned particles that originated from propellants, stabilizers, and lubricants. Inorganic GSR (IGSR) consists of the metallic particles from the primer, the projectile, the cartridge, and the firearm.

A widely accepted screening test for GSR within the forensic science community is the identification all three inorganic gunshot residue (IGSR) components through Scanning Electron Microscopy/Energy-Dispersive X-ray Spectrometry (SEM/EDX) as established by American Society of Testing Materials (ASTM) (ASTM Guide E1588095, 2001). The three IGSR components: lead (Pb), barium (Ba), and antimony (Sb) are considered characteristic of GSR. Each of these components helps the primer ignite the propellant in a cartridge. More specifically: Pb is the initiator when the firing pins hits the primer cap; Ba is the oxidizer that gives oxygen needed to burn the fuel; and Sb is the fuel that burns at a high rate, which ultimately ignites the gun powder resulting in propulsion of the bullet.



1. bullet, as the projectile; 2. metallic case, which holds all parts together; 3. propellant, for example gunpowder or cordite; 4. rim, which provides the extractor on the firearm a place to grip the case to remove it from the chamber once fired; 5. primer, which ignites the propellant.

Figure 2: Modern cartridge composition (source: https://www.warhistoryonline.com/guns/how-a-bullet-works.html)

1.3. Lead regulation

The use of lead in ammunition is regulated across the United States. There are numerous legislative bills implemented in various U.S. states that prohibits of lead and heavy metal ammunition; for example, the state of California recently passed Assembly Bill No.7, which mandates the use of lead-free ammunition in hunting effective July 1, 2019. As a result of this widespread regulation, forensic practitioners who use traditional screening tests for GSR face new challenges.

The new posed challenges with traditional GSR analysis demonstrate the ever-evolving field of Forensic Science. Numerous forensic laboratories, in response, are working diligently for a promising solution that will ultimately strengthen the credibility of GSR analysis (e.g., reduce false negative results). More specifically, suitable OGSR compounds are being considered as supporting or alternative markers for assessing firearms exposure. To this date, forensic laboratories across the nation continue to validate multiple research projects to demonstrate the value of OGSR in GSR analysis. Table 1 illustrates the type of GSR compounds, their method of detection, and whether or not they are currently being analyzed in forensic analysis.

Table 1: Summary of comparison between the two types of GSR

Type	Origin	Characteristic compounds	Method of detection	Used in forensic analysis
Inorganic	metallic particles from primer, projectile, cartridge, and firearm	Pb, Ba, SB	SEM/EDX	Yes
Organic	propellants, stabilizers, and lubricants	unknown	unknown	No

1.4. OGSR analytes

As mentioned previously, OGSR compounds originate from combination of propellants, stabilizers, and lubricants; however, it is important to note that various OGSR compounds also exist in various environmental and occupational materials. Hence, as part of the global-wide efforts to provide a more comprehensive interpretation of the value of OGSR in GSR analysis, this research focuses on analyzing the prevalence of certain OGSR on subjects from non-shooting environments in various occupations.

The OGSR compounds targeted in this research varied throughout the four phases of the project: phase 1 targeted ethyl centralite (EC), dinitrotoluene (DNT), trinitroglycerin (TNG), phase two targeted EC, N-nitrosodiphenylamine (N-nDPA), diphenylamine (DPA), phase three targeted EC, DPA, and 2,6- dinitrotolouene (2,6- DNT), and phase four targeted EC, TNG, and 2,6 DNT. These four phases were a part of the method development. The final phase was deemed to be the most appropriate method to be used on the samples; and therefore, only the analytes targeted in phase four were further assessed. It is important to note, however, that the compounds mentioned in phase one and two are the same compounds analyzed by the method the researcher tried to replicate. The method used in each phase will be discussed thoroughly in the discussion part of the paper.

The OGSR compounds analyzed in the samples collected were ethyl centralite (figure 3), 2, 6- DNT (figure 4), and trinitroglycerin (figure 5). Table 2 illustrates the physical characteristics of each of these compounds. These compounds were selected because of their prominence in the smokeless powder database for reloading ammunition from the Technical Working Group of Fire and Explosives (TWGFEX), availability of standard reference materials (SRM), and their function in gunpowder.

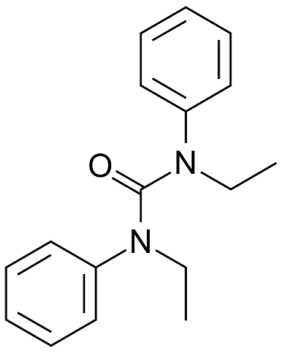


Figure 3: Chemical structure of ethyl centralite

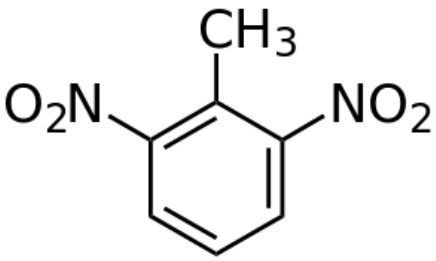


Figure 4: Chemical structure of 2,6- DNT

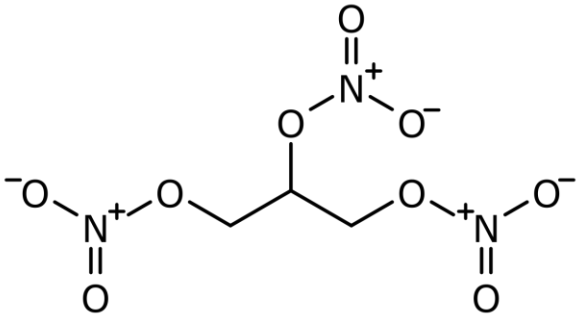


Figure 5: Chemical structure of trinitroglycerin

Table 2: Physical characteristics of target analytes

Compound	CAS number	Molecular weight (g/mol)	Boiling point (ºC)
Ethyl centralite	85-98-3	268.35	218
Trinitroglycerin	55-63-0	227.11	218
2,6- dinitrotoulene	606-20-2	182.14	291

All target analytes in this research serve a vital role in the ammunition materials. EC serves as a burning rate moderator and a stabilizer, TNG serves as an energizer and propellant, and 2, 6-DNT serves as a plasticizer. Mach et. al 1978 considered EC as the most characteristic OGSR compound and although 2, 6- DNT was considered an impurity in the manufacturing process of GSR, it can still add more credence to the characterization of OGSR if it originated from TNG. This research investigated this premise by looking into the extent that EC, TNG, and 2, 6- DNT are present in other sources than ammunition materials.

1.5. Environmental sources of target analytes

This research investigated the extent that EC, TNG, and 2, 6- DNT are present in other sources than ammunition materials. According to Wu et. al 1999, most of the OGSR compounds including the target analytes in this research are widely used in other fields other than GSR, which can diminish their evidential value in GSR analysis. For example: TNG is commonly used as an explosive ingredient and in pharmaceutical preparation (i.e. medication to relieve chest pain), and 2, 6-DNT compound is used in production of explosives, surface coatings, and dyes. Lastly, a study by Lleget and Lott 1989 inferred that fruits such as grapefruit, oranges and pears have shown to produce peaks at the same retention time as EC.

1.6.Targeted population

The targeted population of this research are subjects from different environments that have not been exposed to a shooting event in the past 8 hours. These subjects include but are not limited to Law Enforcement officers, Firefighters, Electricians, Pharmacists, Emergency Medical Technicians, Farmers, Construction Workers, Mechanics, Chemists, Biologists, and Textile, Apparel, and Furnishing Workers. This research aimed to recruit approximately 100 professionals

that primarily work with their hands and handle various substances. The variation in the population is imperative in order to assess any possible association of the target analytes in different occupations.

2. GOALS AND OBJECTIVES

The overall goal of this project is to test the hypothesis that OGSR components will be present on the subjects from non-shooting environment.

The specific hypothesis and null hypothesis for this project are as follows:

H_1 OGSR components will be present on the subjects from non-shooting environment

H_0 OGSR components will not be present on the subjects from non-shooting environment

This project expanded on the research findings in Tobin 2012. Tobin 2012 detected various OGSR compounds from known shooter samples; and therefore, the specific objectives of this study are to:

1. Assess the presence of EC, TNG, or 2,6- DNT as preliminary study on the prevalence of each analyte in non-shooting environments.
2. Assess the presence TNG and EC together as characteristic of OGSR, as proposed by Tobin (2012).

3. MATERIALS AND METHODS

3.1.Study participant recruitment process

A comprehensive application was submitted to the International Review Board (IRB) under the University of California: Office of the President prior to recruiting potential volunteers. In essence, IRB provides an administrative oversight on research projects involving human subjects. IRB evaluated the potential risks in safety and privacy of this project and deemed that the safety and privacy of the volunteers for this project are secured. Both the researcher and principal investigator (PI) underwent formalized online training through the IRB, which included lectures and quizzes. Once all requirements set by the IRB were satisfied, the researcher started the volunteer recruitment process. The recruitment process involved direct solicitation, and the posting and distributing of an approved advertisement (figure 6). This advertisement highlighted the summary of study as well as volunteer eligibility and requirements. Interested volunteers and the recruiter both signed a comprehensive consent form, which answered the following questions:

1. Why is this study being done?
2. What does the study involve?
3. Who should participate in this study?
4. How will the study help the volunteer or others?
5. What are the risks of this study?
6. Who is working on this study?
7. Is the volunteer's participation in this study voluntary?
8. How will the privacy of the volunteer be protected?
9. Who can the volunteer reach for questions or problems?

Figure 6: Recruitment posting explaining what is expected for volunteers

The consent form was followed by a five-question screening survey. The subjects were asked about their occupation, handedness (right or left), if they have handled a gun in the last 8 hours (yes or no), if they have fired a gun or been near a gun when it was fired in the last 8 hours (yes or no), and the last time their hands were washed (0-2 hours, 2-4 hours, 4-6 hours, 6-8 hours, or greater than 8 hours) The samples from subjects who answered yes to the questions regarding firearms were excluded in this study.

3.2. Sample collection and materials

Once the survey and consent forms were completed, both hands of the volunteers were swabbed using deionized (DI) water as the collection solvent and polyester swab as the swabbing medium (figure 7) These collection materials were deemed most appropriate for the study because they pose the least amount of risk to volunteers. The swabs used in the collection

process were labeled to distinguish the samples from their source (left or right), no other identifying information is attached to the swabs (figure 8).

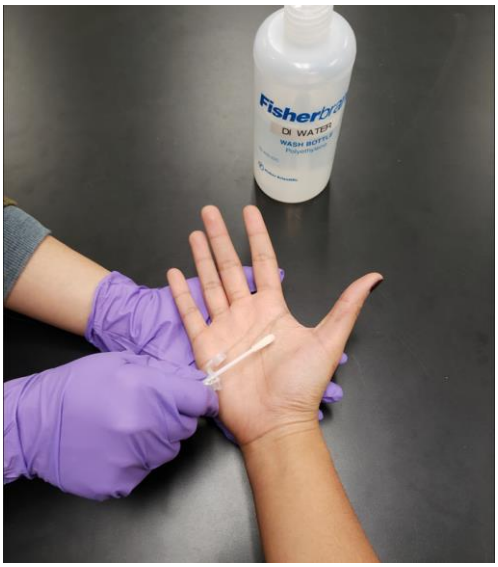


Figure 7: Sample collection using polyester swab and DI water

Figure 8: Samples are labeled to distinguish the sample from their source (left or right). No identifying information is attached to the samples.

The polyester swabs initially used for this study were Puritan 6" sterile standard polyester plastic handle swab & tube (figure 9). The manufacturer classified these swabs as regular polyester tipped applicator with polystyrene handle in dry transport tube (SKU#: 25-806 1PD BT). The transport tubes were non-aerated, which caused mold growth in the some of the samples collected. The samples that were affected with mold were discarded.

To prevent mold growth and ultimately preserve the integrity of samples during storage, a new set of polyester swabs with aerated tips were purchased and used (figure 10). The new swabs were Cap-Shure 6" sterile elongated flock swab & protective cap w/ polystyrene handle (SKU#: 25-3206-U EC). The DI water used in the study were sourced from both Sacramento County District Attorney's Crime Laboratory (SCCL) and University of California Davis (UCD) Forensic Science Graduate Program's Criminalistics Laboratory.

According to the Center of Disease Control and Prevention (CDC), mold can thrive on any substance if moisture is present; thus, great efforts and care were given to ensure that the swabs were dry after collection and were stored away from moisture. By implementing these safeguards, the researcher was able to prevent mold from developing in the samples.

Figure 9: Polyester swab with non-aerated transport tube

Figure 10: Packaging of polyester swab with aerated tip (top) and polyester swab with aerated tip (bottom).

3.3. Solvents and analysis materials

The solvent used in this study is 40:60::acetone:IPA as proposed in Tobin 2012. The acetone and IPA were purchased from Sigma-Aldrich (Item #: 179124) and Fischer Scientific (Catalog #: A461-500), respectively.

The internal standard (ISTD) selected for this study is 2-naphthol. The standard reference material of 2-naphthol was used in another GSR study; and therefore, readily available for use. After determining its solubility and resolution amongst the other standard, the researcher decided to use 2-napththol as the ISTD for this research as well. This ISTD was purchased in solid form from Sigma-Aldrich (PCode: 101773704; Lot #BCBP0986V).

The standard reference materials for the targeted analytes were purchased from different vendors and came in different forms. EC was purchased in solid form from Sigma-Aldrich (PCode: 100617942 372889-100G; Lot #: 05107LFV). TNG was purchased in liquid form from Cerilliant (Product #: T-002; Lot # FN07171701). Lastly, 2, 6-DNT was purchased in solid form Sigma-Aldrich (PCode: 31565-250MG; Lot #: SZBD123XV).

The glass vials used in study were 2 dram (dr) Fisherbrand Class A Clear Glass Threaded Vials and 2 mL snap top vials with caps purchased from Fischer Scientific (Catalog #: 03-339-25C) and Agilent Technologies (Part #: 5190-2240), respectively. The conical inserts used inside the 2 mL vial was an 8mm autosample inserts purchased from Thermo Fisher Scientific (Catalog #: C4012-529).

3.4. Extraction

The researcher followed the sample extraction developed by Tobin 2012 with some adjustments to account for availability of materials and equipment. The steps are shown in Figure 11. The first step is cut the swab and only include the polyester end into a 2 dr glass vial (vial A) followed by the addition of 995 uL of solvent and 2 uL of ISTD. This mixture is then vortexed for 15-20 seconds and set aside for 15 minutes. After the 15-minute waiting period, vial A is vortexed once again for 15-20 seconds and its contents is transferred to a 2 mL GC-MS glass vial (vial B). Vial B is then placed on a hot plate set at 100 ℃ and allowed to evaporate to dryness. Once dried, vial B is reconstituted with 40 uL solvent and shaken by hand. Ultimately, this mixture is transferred to a conical vial insert where 1 uL is drawn for a GC-MS analysis.

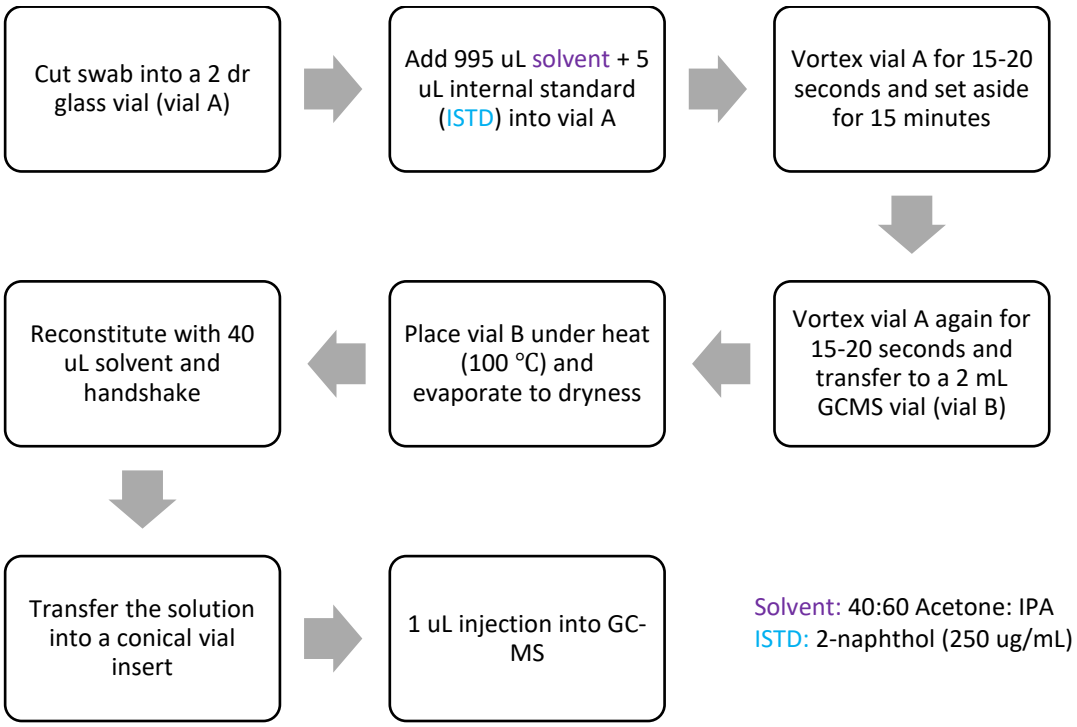


Figure 11: Extraction procedure of the collection swab

3.5. GC-MS specifications and parameters

The Gas Chromatography- Mass Spectrometer used in this study is located at the UCD Forensic Graduate Program's Criminalistics Lab. The GC component was an Agilent 6890N Network GC system paired with an Agilent 7883 injector. The column was 30 m x 0.25 mm i.d. x 0.25µm film thickness non-polar 5% phenyl – 95% dimethyl polysiloxane (Model number: Rxi-5MS) and the carrier gas used was Helium. The MS component was an Agilent 5973 Network Mass Selective Detector. Selective ion monitoring (SIM) was run at values published in Tobin 2012 for each target analyte and ISTD. The values for the target analytes are (most abundant listed first): 120, 148, and 268 (EC), 165, 63, and 89 (2, 6- DNT), and 46 and 76

(TNG). The values for the ISTD are (most abundant listed first): 115 and 144. See appendix for the MS spectrum of each target analyte.

The GC-MS parameters including the temperature program parameters are shown in Table 3.

Table 3: GC-MS and temperature program parameters used in GC-MS analysis.

GC	MS	Temperature Program
Injection Temp: 180 ˚C	Solvent Cutoff Time: 5 min	Initial Oven Temp: 45 ˚C;
Interface Temp: 230 ˚C	Acquisition Mode: SIM	hold for 3 min
Control Mode:	Scan Speed: 1000	Program Rate: 15 ˚C/min to
Pulsed Splitless	Scan Range: 40-480 amu	150 ˚C hold for 0 minutes
Pulse pressure: 50 psi	Sampling Rate: 0.50 sec	40 ˚C/min to 265 ˚C hold for
Purge flow: 50 mL/min		8 minutes
Purge time: 0.90 min		Total run time: 20.87 min
Total flow: 53.6 mL/min		
Column Flow: 0.90 ml/min		

4. RESULTS AND DISCUSSION

4.1. Four phases of method development

As briefly mentioned in the introduction, this study included four phases as part of method development. Table 4 shows the summary of each phase, which include the instrument type, method used, targeted analytes, and conclusion.

Table 4: Summary of method development phases explored in this research.

Phase	Instrument	Method	Target analytes	Conclusion
One	HPLC-MS	In house method from SCCL in collaboration with Agilent technologies	EC, DNT, TNG	Not viable: Could not pass QC check
Two		Maitre et al. 2018	EC, n-NDPA, DPA	Not viable: Known standards not detected
Three	GC-MS	Tobin 2012 (scan method)	EC, DPA, 2,6-DNT	Not viable: Scan method not ideal for trace analysis
Four		Tobin 2012	EC, TNG, 2,6-DNT	2,6 DNT deemed as contaminant (Mach et al 1978); therefore, analyte on its own has limited value in characterization of OGSR

The High Pressure Liquid Chromatography- Mass Spectrometer (HPLC-MS) located at the SCCL was utilized in phase one and two of this project. Phase one of this project involved an in-house method from SCCL in collaboration with Agilent technologies was validation study

performed by Agilent Technologies titled *Analysis of Gunshot Residue*, which involved a multisource detector. The target analytes were EC, DNT, and TNG. The known standards for these analytes were successfully detected during the validation study performed by Agilent. During the phase one of the project, however, the instrument could not pass the quality check.

After phase one, the project moved to phase two, which involved a new method that still utilized HPLC-MS with a switch from multisource detector to an electrospray ionization detector. A recent study in 2018, by Maitre et al, titled *a forensic investigation on the persistence of organic gunshot residue* investigated identifying three compounds from smokeless powder: diphenylamine (DPA), N- nitrosodiphenylamine (N-nDPA) and ethyl centralite (EC). Unfortunately, this method was not able to detect the known standards using the HPLC-MS in the SCCL.

Due to the multiple issues with the HPLC-MS, this project considered using the GC-MS instrument for phases three and four of this project. Phases three and four explored the method proposed by Tobin in 2012 titled *The Development of a Gas Chromatography/Mass Spectrometry (GC/MS) Method for the Separation and Identification of Components of Organic Gunshot Residue and Its Use as a Forensic Tool for Association of Firearms Related Evidence*. Phase three had a slight variation with the acquisition mode programmed in the instrument. This project looked into obtaining the MS data by scan method instead of Selected Ion Monitoring (SIM), which decreases the sensitivity of the instrument but provides a full MS profile of each analyte detected. The target analytes in phase three were EC, DPA, and 2, 6- DNT. Through multiple trials, this variation in method was deemed inappropriate for trace analysis. In addition, the DPA reference standard purchased was the incorrect standard needed for this research. Therefore, the final phase of the project reverted to the SIM acquisition mode as originally proposed by Tobin 2012 and eliminated DPA as a target analyte. The target analytes in phase four were EC, TNG, and 2, 6-

DNT. Although 2, 6- DNT was deemed as a contaminant by Mach et Al 1978, this project perceived that it is still valuable in characterizing OGSR if detected with other known OGSR compound/s.

4.2.Limit of detection

Prior to analyzing the samples, the lowest concentration levels of the target analytes and ISTD that can be detected during analysis were determined. This establishes what is known as the limit of detection (LOD). A series of dilutions were run for each analyte. Table 5 and 6 show the concentrations in relation to the peak area detected for each analyte. These values were then plotted and evaluated for a linear decrease (figures 12-15). The lowest concentration of the ISTD that can be detected in the instrument used in this study is 12.5 ug/mL; this information is then used to determine the appropriate volume of ISTD used during extraction procedure. The lowest concentration of each analyte that the instrument can detected are as follows:

- 2, 6- DNT: 0.5 ug/mL
- TNG: 25 ug/mL
- EC: 0.5 ug/mL

By determining the LOD of each analyte, this study can determine if any of the target analytes can be detected within their respective LOD.

Table 5: Peak areas of 2, 6- DNT, TNG, and 2-naphthol in relation to varying concentrations.

Concentration (ug/mL)	Peak area 2, 6- DNT	Peak area TNG	Peak area 2-naphthol (ISTD)
100	10367300	4352145	19020251
75	7601754	3644109	12756589
50	4602681	2468614	7275422
25	2080898	275761	2786399
12.5	920767	-	91910
2.5	30925	-	-
0.5	4367	-	-

Table 6: Peak areas of EC in relation to varying concentrations.

Concentration (ug/mL)	Peak area EC
62.5	24719422
50	17911819
37.5	13098989
25	8148093
12.5	3776963
6.25	1713106
1.25	275413
0.25	43259
0.5	6219

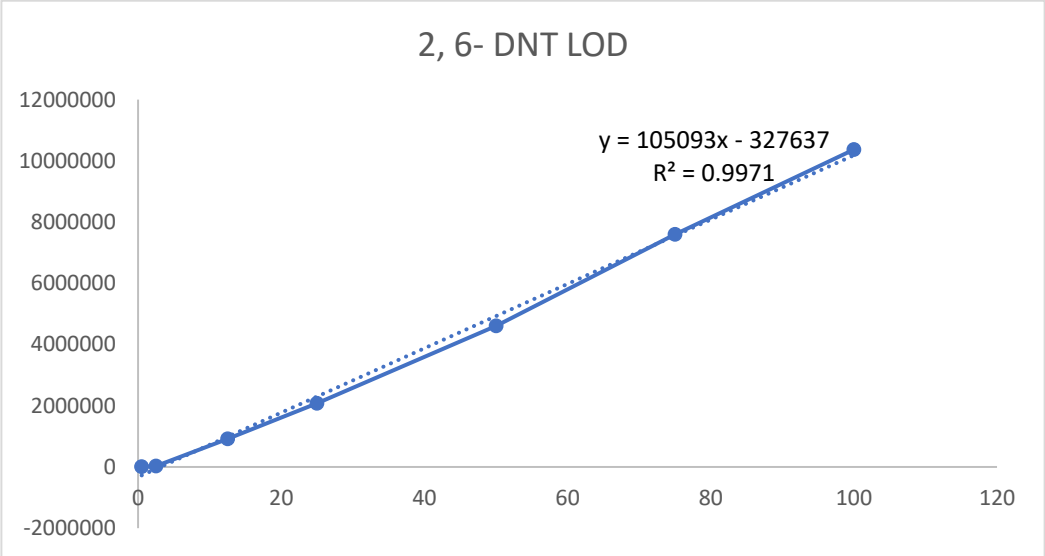


Figure 12: Concentration of 2,6- DNT versus the corresponding peak area

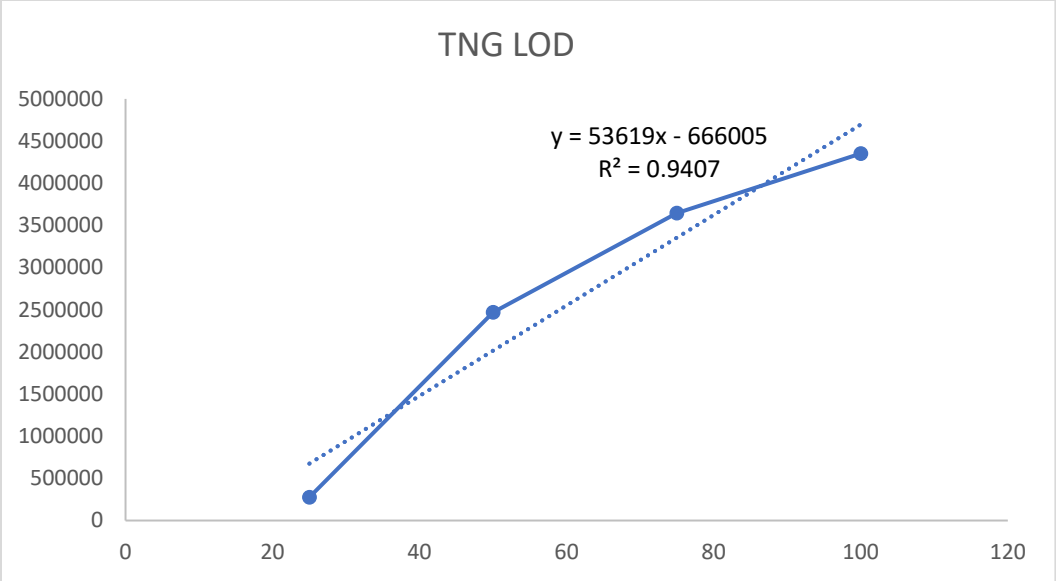


Figure 13: Concentration of TNG versus the corresponding peak area

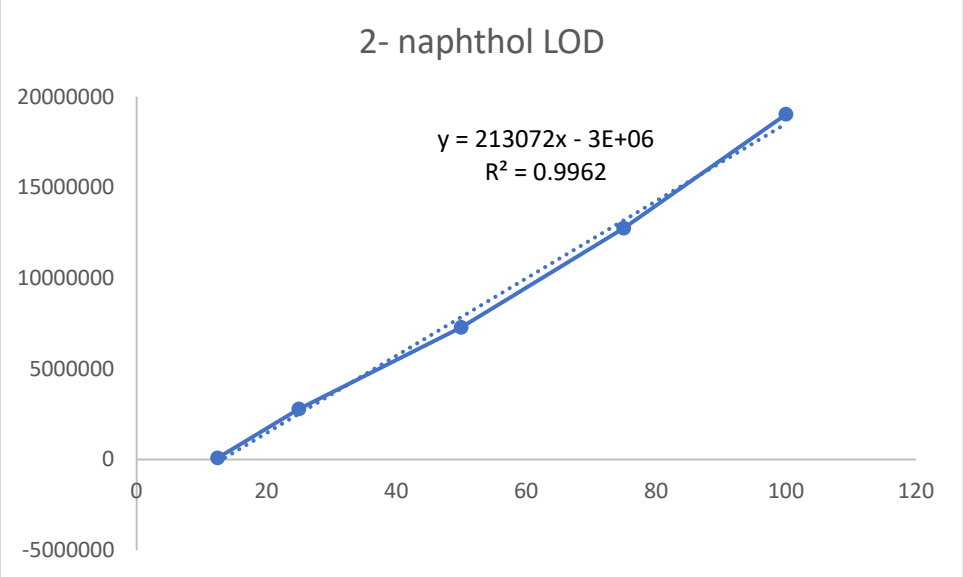


Figure 14: Concentration of 2-naphthol versus the corresponding peak area

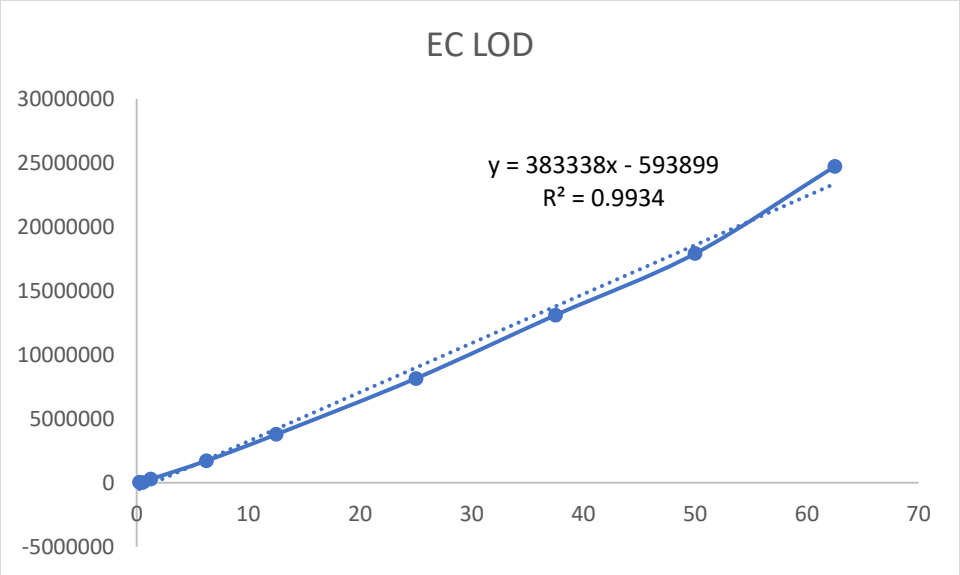


Figure 15: Concentration of EC versus the corresponding peak area

4.3.Data analysis: volunteer demographics

There was a total of 163 samples run during the course of this research. However, for the purpose of this research, data analysis was only performed on the samples ran on the final method. Although all subjects except for one provided a set of samples (one from each hand), each sample was analyzed individually. In addition, the majority of the subjects are right-handed; and therefore, the handedness of the subjects were not considered in the final analysis of the results.

A total of 119 qualified samples were analyzed using the settings established in phase four of the project. These samples were screened for the presence of 2, 6- DNT, EC, and TNG. It is important to note that this research is a qualitative study, so no quantification was performed for analytes detected in any samples. The qualified samples were categorized using the source of the samples (right or left hand), occupations, and time-lapsed on handwashing prior to sample collection (0-2 hours, 2-4 hours, 4-6 hours, 6-8 hours, or greater than 8 hours). The qualified samples comprised of 61 and 58 samples from right and left hand, respectively (Table 7). Most

subjects had washed their hands 0-2 hours prior to sample collection followed by 2-4 hours (Table 8).

Table 7: Number of qualified samples in relation to their hand source (left or right)

Sample source	
Right	**Left**
61	58

Table 8: Number qualified samples in relation the time-lapsed on handwashing prior to collection

Time-lapsed on handwashing prior to collection (hr)				
0-2	**2-4**	**4-6**	**6-8**	**8+**
54	38	10	10	7

The occupations of the subjects included (most common listed first): police, laborer, mechanic, healthcare personnel, miscellaneous, industrial drivers, laboratory personnel, and restaurant personnel. Figure 16 shows each count and percentage of each occupation as it comprised the total number of samples ran in the final method of the study. Due to variations in the job titles with similar duties, some jobs and occupations of the volunteers in the study were grouped together or classified into a more appropriate category (i.e., miscellaneous). For example, jobs that are relatively in the same field including pharmacist, pharmacist technician, and caregiver are grouped together as healthcare personnel. Another example are jobs that are primarily based in an office-setting were classified as miscellaneous; these jobs include realtor and administrative assistant. Table 9 enumerates the jobs and occupation within each category.

Table 9: Jobs and occupation comprising each category.

Category	Occupation
Healthcare Personnel	Pharmacist, Pharmacist Technician
Industrial Drivers	Truck Driver, Bus Driver, Transit Driver, Agricultural Chemical Delivery Driver
Laboratory Personnel	Laboratory Technician, Research Assistant, Biochemist, Undergraduate Biochemistry Researcher
Laborer	Farm Chemical Worker, Alarm Technician, Field Man Worker Pest Control Advisor, Construction Worker, Custodian, Maintenance Worker, Laborer, Painter
Mechanic	Mechanic, Agricultural Mechanic
Miscellaneous	Realtor, Administrative Assistant
Police	Police Cadet
Restaurant Personnel	Cook, Food Preparation Worker

The majority of the samples analyzed in this study were from police, followed by laborers. Figure 16 illustrates the proportion of each occupation in the total number of samples. Each proportion exhibits the count and percentage of a respective occupation and is illustrated with a distinct color scheme.

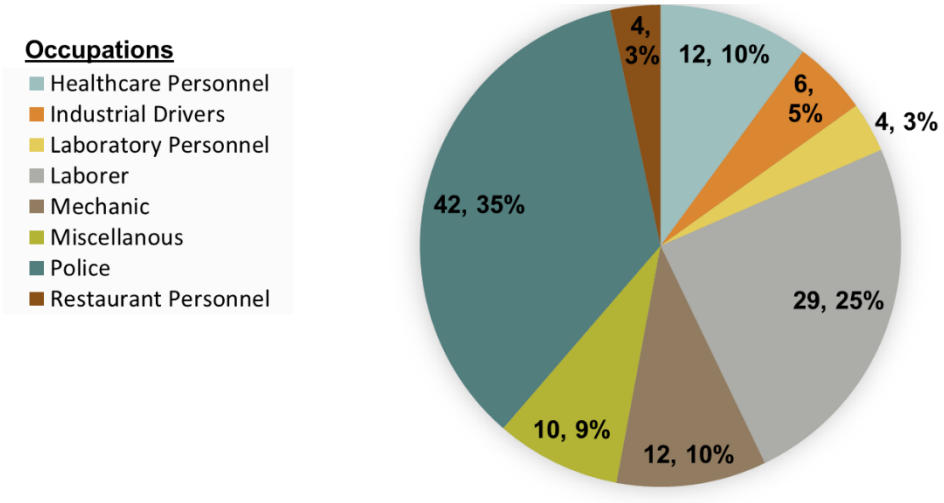


Figure 16: Occupations of each sample ran on the final method of the research. Each category shows the count and percentage of the respective occupation.

4.4.Data analysis: analytes detection

This research detected the presence of 2, 6-DNT and EC compounds in the span of the qualified samples; no TNG compound was detected. More specifically, 83% (99 count) and 21% (25 count) of the samples contained 2, 6-DNT and EC, respectively.

In relation to occupations, a majority of the samples that contained 2, 6- DNT were from police while majority of the samples that contained EC were from laborers. Figure 17 illustrates the proportion of each occupation in the overall number of samples containing either 2, 6- DNT or EC. Table 10 shows the percentage of each analyte detected in relation to the samples ran for specific occupation. At least one sample from each occupation contained 2, 6-DNT. More specifically, 100% of the samples belonging to industrial drivers, restaurant personnel, laboratory personnel, and miscellaneous contained 2, 6-DNT. EC was detected in all of the samples from each occupation except healthcare personnel. The occupation group that contained the most EC are industrial drivers: 50% of the samples.

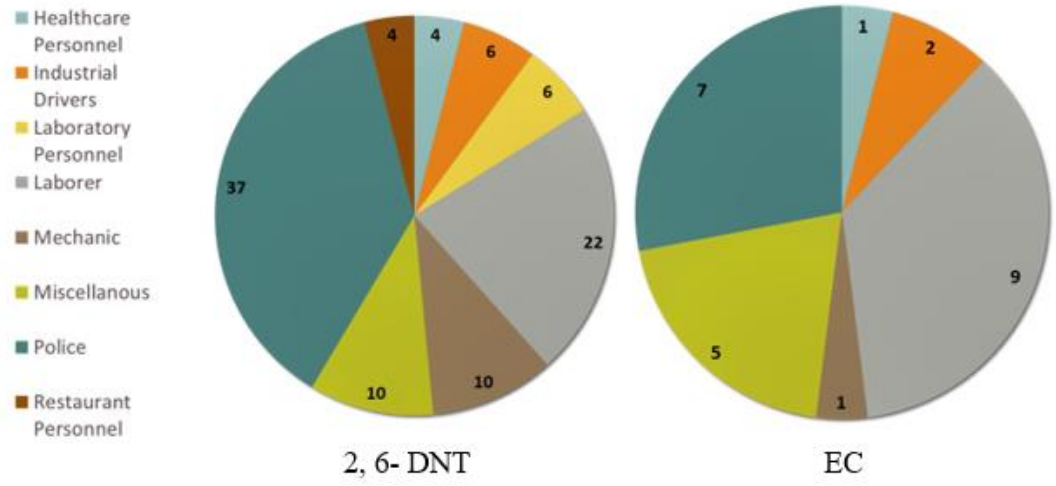


Figure 17: Analytes detected in each occupation.

Table 10: Percentage of detected analyte in relation the samples collected from each respective job category

Occupation	2,6 DNT	EC
Healthcare Personnel	33%	0%
Industrial Drivers	100%	50%
Laboratory Personnel	100%	25%
Laborer	79%	10%
Mechanic	83%	8%
Miscellanous	100%	20%
Police	86%	33%
Restaurant Personnel	100%	25%

In relation to the source of the samples (right or left hand), 77% and 90% of 2, 6- DNT was detected from samples collected from right and left hands, respectively. In contrast, EC was detected 18% and 24% in the samples from right and left hands, respectively. Figure 18 compares each analyte in relation to their respective source. The trend shows that majority of both compounds were detected from the left hand.

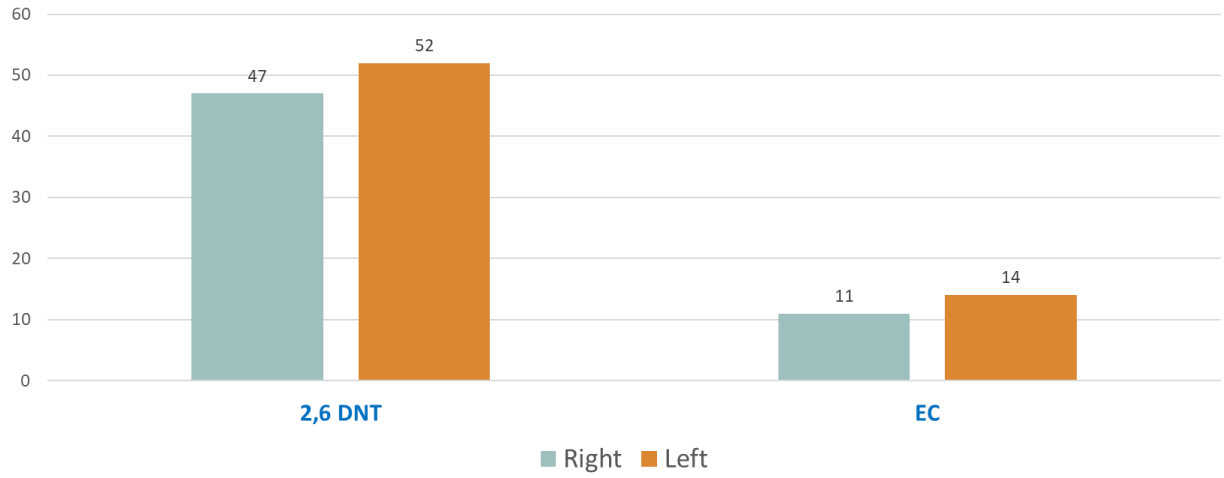


Figure 18: Counts of each detected analyte from right and left hands source

In relation to the time-lapsed (hr) since the volunteers have washed their hands, this research determined that both analytes were detected across different times. Table 11 shows the

percentage of each analyte detected in relation to the samples ran for specific time-lapsed. All of samples collected form 6-8 hour time-lapsed contained 2,6- DNT. The time-lapsed group of 2-4 hour showed the most EC detected at 32%. However, each time-lapsed is not comprised of the same number of samples. Figure 19 illustrates the count of samples containing the target analyte. The most of count of 2,6- DNT was detected from samples collected from volunteers who have washed their hands 0-2 hour prior to collection while most samples that contained EC was from 2-4 hour prior to collection.

Table 11: Percentage of detected analyte in relation the samples collected from each respective time-lapsed category

Time lapsed (hr) prior to collection	2,6 DNT	EC
0-2	89%	11%
2-4	87%	32%
4-6	60%	20%
6-8	100%	30%
8+	29%	29%

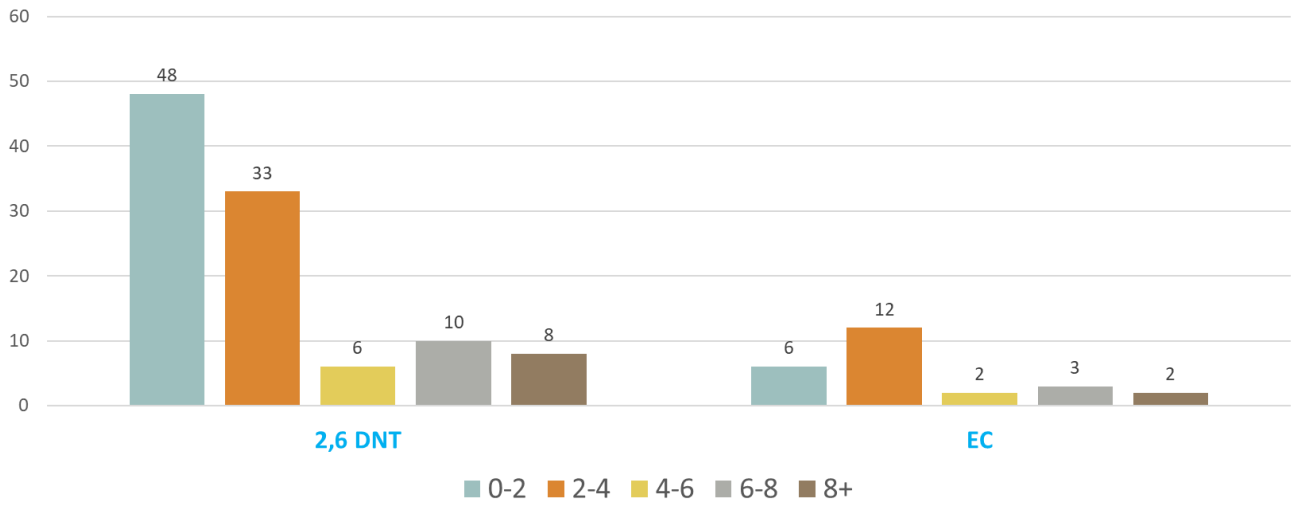


Figure 19: Counts of each detected analyte from each time-lapsed category

5. CONCLUSION

The results of our research allowed us to accept the hypothesis that OGSR component(s) will be present on subjects from non-shooting environments. However, this finding is limited by the current concentration sensitivity threshold as established for each compound. Two of the three OGSR compounds targeted in this research, namely 2, 6- DNT and EC, were detected across the samples collected from qualified volunteers. This supports the previous studies by Leggett and Loft 1989 and Wu et. al 1999 regarding the prevalence of some OGSR compounds from other fields than GSR. However, this is the first scientific research study to date to support that the detection of EC and 2, 6- DNT either on their own, or, as a pair, on non-shooting environments is not characteristic for OGSR. Hence, this finding is promising for crime laboratories, particularly in that it arose from samples that were collected from non-shooting environments. It is important to note, however, that the identification of EC and 2, 6-DNT is based on their respective expected retention time and MS spectrum, which only contains prominent ions as established in the Tobin 2012 paper. Based on previous forensic lab experience, analysis such as that performed here has not yet been considered probative for the identification or characterization of any OGSR compound at crime scenes. Thus, this research supports the development of a new investigative tool that can be used for preliminary crime lab assessment of the need for additional testing.

This research also determined that the absence of TNG and EC together as a pair in the samples from various non-shooting environments supports the likelihood that these compounds are characteristic of OGSR as proposed by Tobin (2012). Overall, the findings of this research provide a presumptive conclusion on OGSR evidence that can help crime labs streamline the

related analysis process. As forensic labs can reduce their overall workload volume by narrowing in on specific compounds to test for, these data may prove valuable to crime labs in the future.

While the results of our study demonstrated the presence of specific OGSR compounds in non-shooting environments, they also indicated that certain combinations of OGSR components are imperative in order to deem them of probative value in crime scene investigation. Furthermore, additional research needs to be done before this method can be directly implemented in a forensic laboratory setting. Among the missing elements yet to be established are OGSR component thresholds in comparison to a practicable known shooter sample used as a positive control. Crime labs are cautioned that if any of the OGSR compounds identified in this study are not detectable in comparison to a corresponding known shooter sample control, then they will not yet be appropriate for forensic application. As such, future studies are also needed to address instrument sensitivity and accuracy in detecting specific OGSR compounds compared to known shooter sample controls.

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APPENDIX

Chromatogram

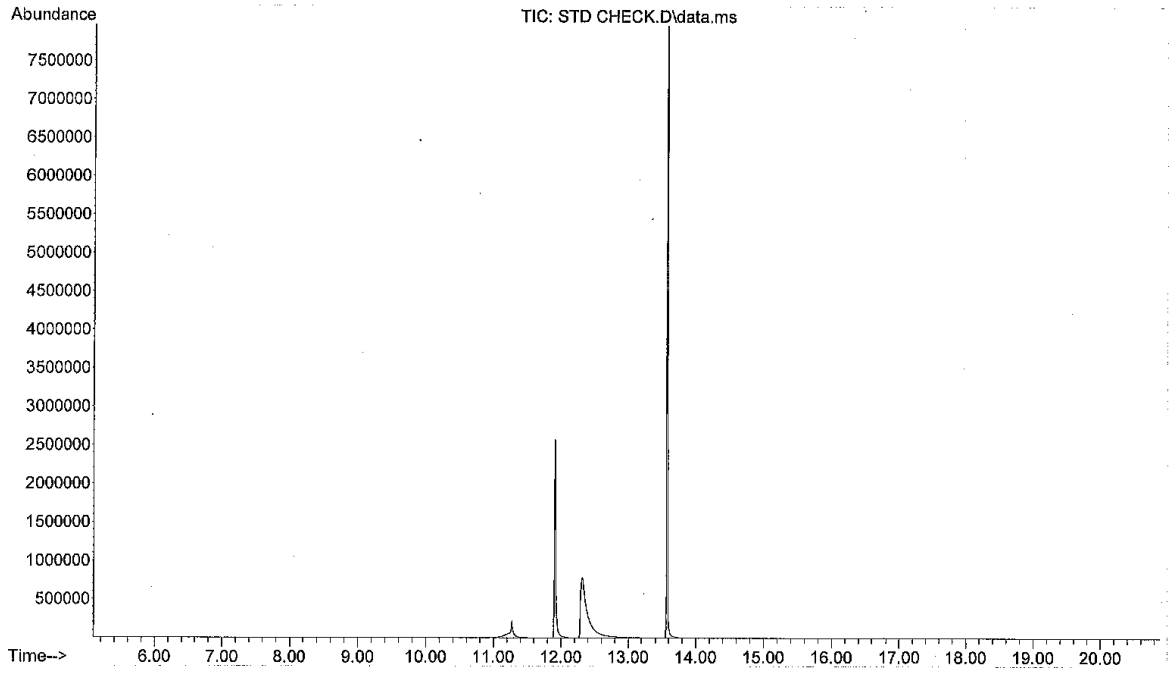

Figure A1: Chromatogram of known standards in this order: TNG, 2,6-DNT, 2-naphthol, and EC

Mass Spectrum

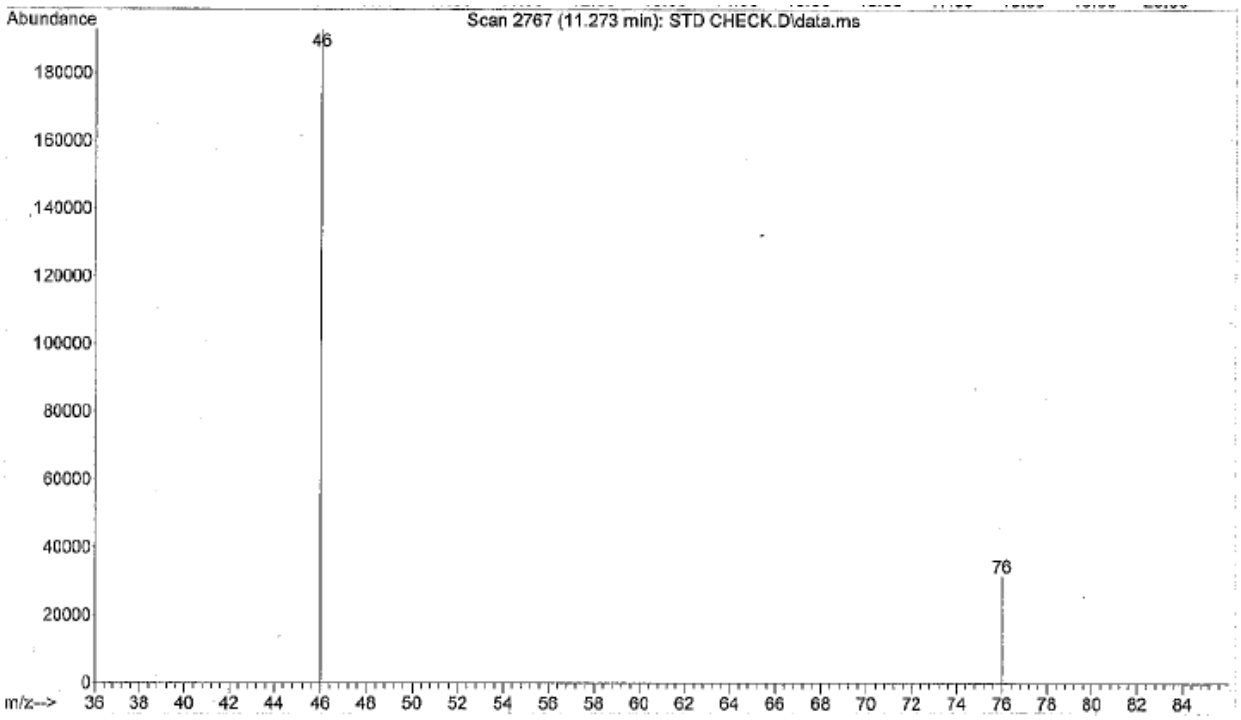

Figure A2: Reference mass spectrum for TNG (SIM)

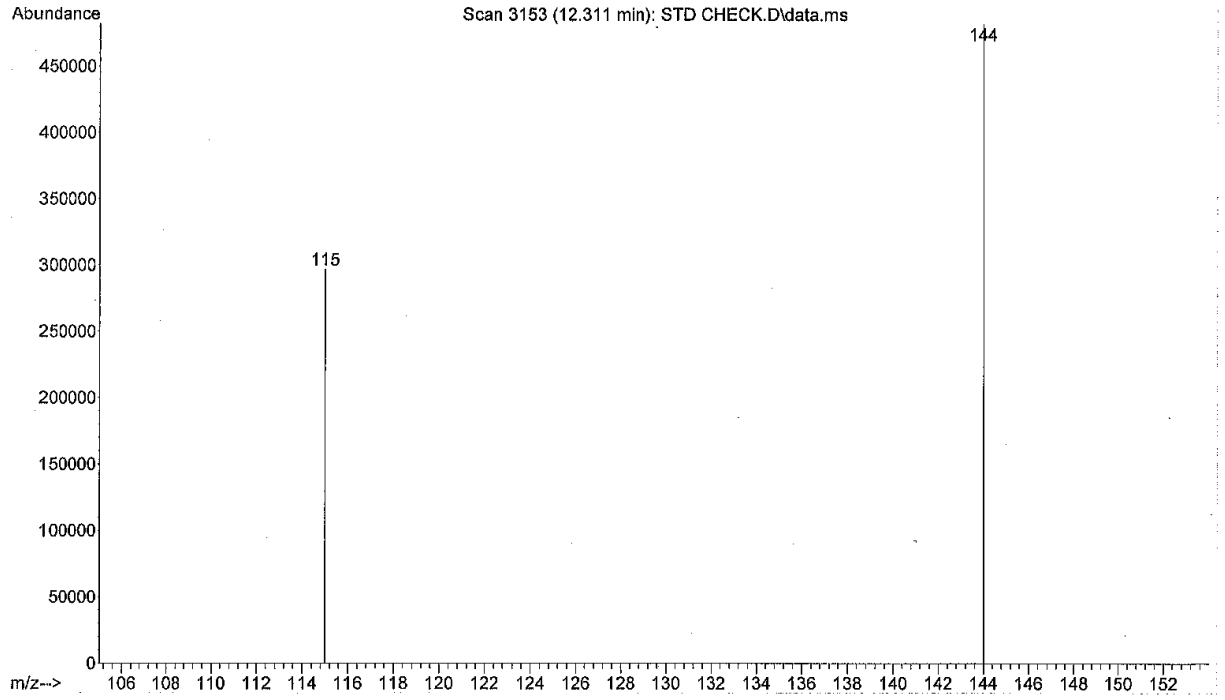

Figure A3: Reference mass spectrum for 2-naphthol (SIM)

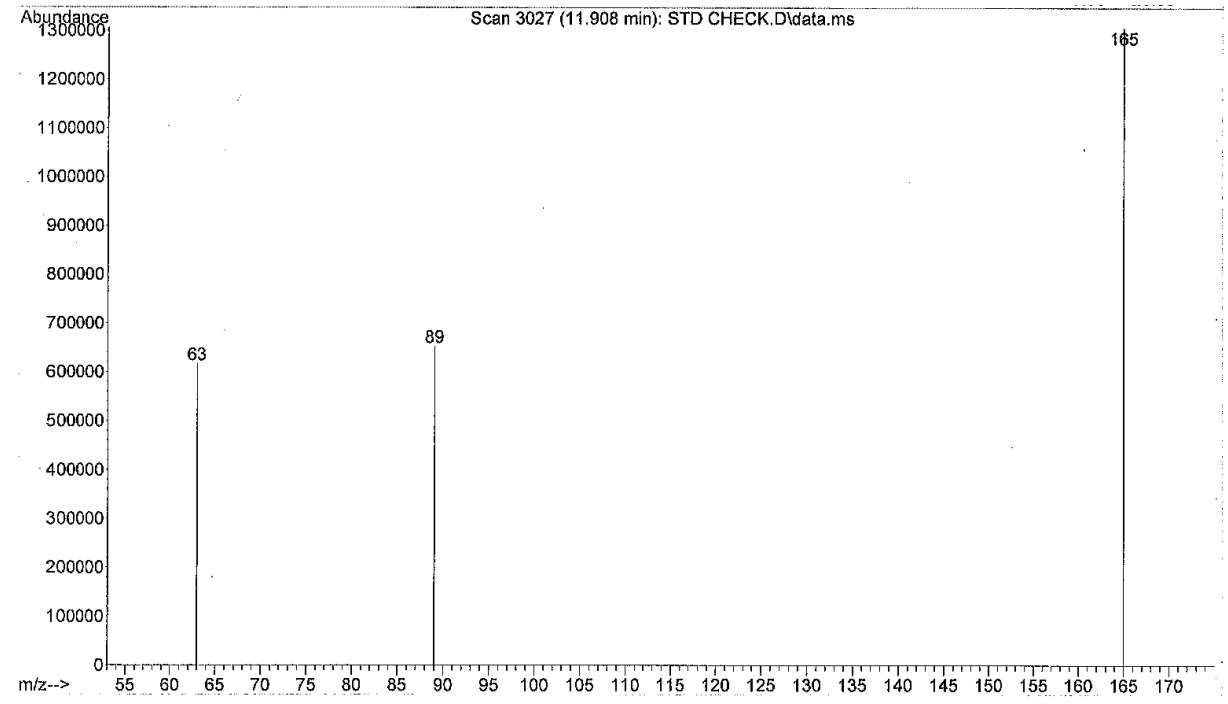

Figure A4: Reference mass spectrum for 2,6- DNT (SIM)

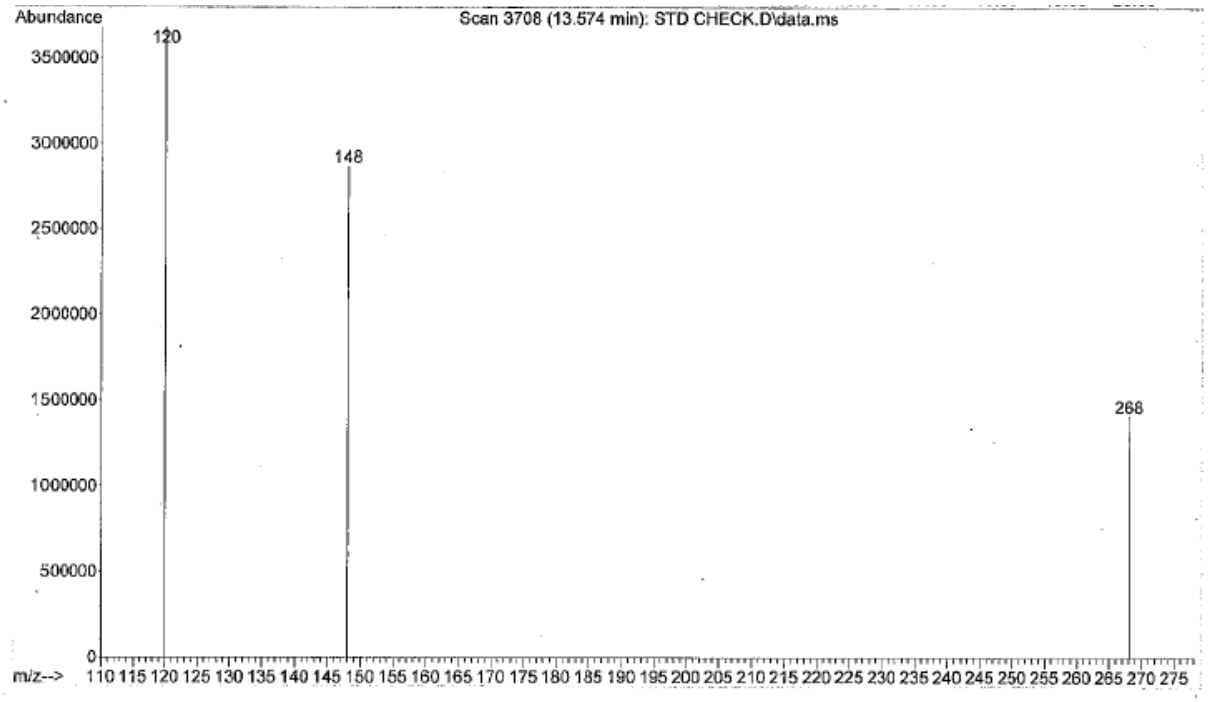

Figure A5: Reference mass spectrum for EC (SIM)