DETECTION AND INTERPRETATION OF ORGANIC GUNSHOT RESIDUES

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Certificate of authorship and originality

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text. This research is supported by an Australian Government Research Training Program and by a Premier's Research and Industry Fund grant provided by the South Australian Government Department of Further Education, Employment, Science and Technology.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

Matthieu Maitre

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List of publications

The chapters presented in this thesis have been published, accepted for publication or submitted to journals as follows:

Chapter two: Forensic interpretation of GSR evidence

- Maitre M, Kirkbride KP, Horder M, Roux C, Beavis A. Current perspectives in the interpretation of gunshot residues in forensic science: A review. ***Forensic Science International***. *2017;270:1-11.*

Publication of collaborative work with the University of Lausanne:

- Hofstetter C, Maitre M, Beavis A, Roux CP, Weyermann C, Gassner A-L. A study of transfer and prevalence of organic gunshot residues. ***Forensic Science International***. *2017;277:241-51.*

Chapter three: Section III:

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Chapter four: Probabilistic approach

- Maitre M, Gittelson S, Kirkbride KP, Gassner AL, Weyermann C, Beavis A, Roux C. Application of the likelihood ratio approach to the assessment of organic gunshot residues. *Manuscript drafted.*

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Abbreviations

.357 Magnum	.357 Mag
.40 Smith & Wesson	.40 S&W
Automatic colt pistol	ACP
American Society for Testing and Material	ASTM
Bayesian network	BN
Diphenylamine	DPA
Diphenyl-d10-amine	d10-DPA
Dimethyl phthalate	DMP
Electrospray Ionisation	ESI
Ethylcentralite	EC
European Network of Forensic Science Institutes	ENFSI
Gunshot residues	GSR
Ion-Mobility Spectrometry	IMS
Inorganic gunshot residues	IGSR
Internal standard	IS
International conference on harmonisation	ICH
Likelihood ratio	LR
Limit of detection	LOD
Mass spectrometer	MS
Methylcentralite	MC
Multi reaction monitoring	MRM
New South Wales	NSW
New South Wales Police Force	NSWPF
N-nitrosodiphenyamine	*N*-nDPA
Organic gunshot residues	OGSR
Principal component analysis	PCA
Principal component	PC
Probability density function	PDF
Part per billion	ppb

Part per million	ppm
Person of interest	POI
Quality control	QC
Relative retention time	RRt
Relative Standard Deviation	RSD
Retention time	Rt
Scanning Electron Microscopy-Energy Dispersive X-ray spectroscopy	SEM-EDX
Total ion chromatogram	TIC
Triple quadrupoles tandem mass spectrometer	QqQ-MS
Ultra performance liquid chromatography	UPLC

Abstract

The traces produced when a firearm is discharged, called gunshot residues and abbreviated GSR, can provide important information in cases when questions regarding the association of a person of interest (POI) with the event are raised. In most forensic laboratories, routine GSR analyses focus on the detection and characterisation of the inorganic components (abbreviated IGSR), which are mainly particles containing mixtures of lead, barium and antimony, originating from the ammunition primer. The increasing prevalence of heavy metal-free ammunition challenges the current protocols used for IGSR analysis. To provide complementary information to IGSR particles, the current project concentrated on the organic components (abbreviated OGSR), which are arising from the combustion of the ammunition propellant powder.

The overall aim of this project was to develop additional knowledge about OGSR in order to assess the possibilities of using these organic traces to provide a complementary to the IGSR and as a complementary tool in cases where heavy-metal free ammunition might be suspected. The project aimed at evaluating the relevancy of OGSR by assessing the persistence and secondary transfer, which are two crucial parameters when approaching forensic traces. This project focused on the detection of four compounds well-known as being part of OGSR: ethylcentralite (EC), methylcentralite (MC), diphenylamine (DPA), *N*-nitrosodiphenylamine (*N*-nDPA). The specimens were liquid-extracted and analysed by UPLC-QqQ-MS, which was validated using the "International Conference on Harmonisation of technical requirements for registration of pharmaceuticals for human use guidelines" (ICH guidelines). Two studies were carried out during this project.

Throughout the project, it was observed that the research studies highlighted a successful detection of three of the four compounds of interest in specimens arising from the firearm discharges.

The first part of the project tackled the study of the persistence of OGSR traces on a shooter's hands. The overall study aim was to provide additional information regarding OGSR retention, which can be integrated into an appropriate interpretation framework as recommended by the recent guidelines for "Evaluative Reporting in Forensic Science" of the European Network of Forensic Science Institutes (ENFSI). The persistence was studied through several intervals ranging from immediately after discharge to four hours, and two ammunition calibres were chosen: .40 S&W, used by the NSW Police Force; and .357 Magnum, which is frequently encountered in Australian casework. This study successfully detected three compounds of interest up to four hours after discharge. The trends displayed a large decrease in the amount detected during the first hour. A large variability was also observed due to numerous factors involved in the production, deposition, collection and analysis of OGSR.

The second part of the project concerned the study of the secondary transfer of OGSR. Similar to the situation with IGSR, OGSR compounds originally deposited on the shooter during the firing process may further be transferred onto another individual or surface. Hence, the aim of this study was to provide additional information regarding the risk of a secondary transfer of OGSR. Two scenarios were investigated, the first one related to the arrest process and the possibilities of a secondary transfer arising between a shooter onto a non-shooter (e.g. between a police officer and a POI). The second scenario concerned the transfer of OGSR onto the non-shooter after handling a firearm for few minutes without discharging it. One calibre was investigated, the .40 S&W calibre, used by the

NSW Police Force. A secondary transfer was observed in all cases for the two scenarios investigated, for three compounds of interest (EC, DPA and *N*-nDPA). The firearm handling scenario resulted in a larger secondary transfer to that of the arrest scenario. Overall, the amounts of OGSR detected on the non-shooter were generally lower than that detected on the shooter and controls after the arrest scenario. The results of this study provide complementary knowledge about OGSR, which can be further used to improve the current practice and the interpretation of OGSR evidence. In particular, it highlights that the secondary transfer proposition must be considered during the interpretation of forensic findings, especially when small amounts of OGSR target compounds are detected.

However, with advances in technology, the forensic challenges presented by OGSR, are moving from the analytical domain to the interpretation of the analytical results. As emphasised by the recent ENFSI guidelines, an interpretative framework, based on the application of Bayesian reasoning has to be developed for the appropriate assessment of evidence in regards to activity-related questions. This approach allows an evaluation of the evidence that is more closely aligned to judicial and investigative aims.

Therefore, the last aim of the project was to encapsulate the results obtained in the persistence and secondary transfer of OGSR into an appropriate interpretation framework with a concrete application of the Bayesian theorem for the assessment of OGSR evidence. This study showed that likelihood ratios (LR) could be calculated for each compound of interest. It was found that the magnitude of the LR obtained were consistent across the different targeted OGSR compounds, with a magnitude ranging from "moderate" to "strong" support of one of the propositions under investigation.

Finally, the application of the LR approach to assess OGSR traces highlighted that normal probability density functions were the most suitable method to assess OGSR. It was found that LR could be calculated for the three compounds of interest. It was also found that all LR were not supporting the propositions at the same level, which was found to be intrinsically linked to the degree of overlap of the different population distributions. However, the LR approach was found to be applicable to the interpretation of OGSR traces by being able to provide meaningful and relevant information because of its ability to support a proposition rather its alternative.

CHAPTER ONE: GSR FUNDAMENTAL KNOWLEDGE

1. Gunshot residues: What are they?

The traces produced during the discharge of a firearm – called gunshot residues (GSR) or firearm discharge residues (FDR) [1] – can provide valuable information for assessing relationships between an individual(s) and a sequence of activities involving the use of a firearm. GSR can also assist by allowing a wound entry hole to be distinguished from an exit hole or estimating a shooting distance [2-4]. The latter are particularly important for the reconstruction of firearm-related cases. GSR may also be relevant to establish the kind of ammunition used at the time of the shooting [5-9].

The formation process of GSR is initiated when the firing pin strikes the primer cap which initiates the primer combustion. The reaction generates a large amount of energy which is transferred through the flash hole(s) to the propellant powder also known as smokeless powder and initiates its combustion, which generates enough energy to propel the bullet through the barrel (Figure 1). Therefore, GSR term encapsulates all combustion products arising during and after the discharge process of ammunition by use of a wide range of firearms.

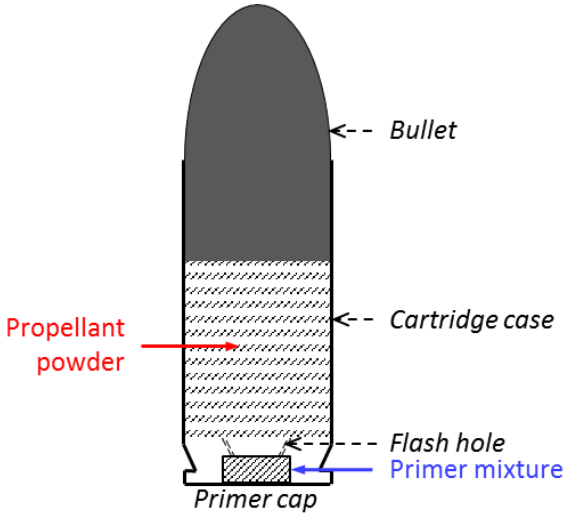


Figure 1. Cartridge diagram.

The combustion of the primer mixture generates vapours that condense as particles due to the exposure of the compounds to a large and instantaneous increase of temperature and pressure. This creates spherical shaped particles [10] with either a smooth, fuzzy or scaly surface [11, 12]. The size of typical GSR particles arising from the primer mixture is around 1 and 5 micrometres [9, 11-14], with some reaching 10 micrometres (µm), and more rarely hundreds of micrometres [8].

The combustion of the propellant components, which propels the projectile along the barrel, produces degraded organic compounds that escape, mix and deposit in a similar way with the primary components of the propellant and the inorganic particles. Finally, other sources of elements may also be present in GSR. They are formed during the forced path of the projectile through the barrel. The pressure created by the propellant powder combustion initiates frictions between the bullet and the barrel (to give a gyroscopic movement to the bullet). From those frictions is resulting the production of small metallic particles (from the barrel, grease and the bullet jacket) that mix with primer and propellant

residues [15]. In addition, Collins et al. have observed traces of glass in residues arising from the primer mixture [16].

In summary, GSR can be defined as a complex mixture of volatile, gaseous products as well as metallic particulate matter formed during and after a firearm is discharged [1, 17]. The residues are composed of burnt and unburnt particles arising from the primer (inorganic GSR - IGSR), propellant (organic GSR - OGSR) and other materials coming from the cartridge case, the projectile(s) and the firearm itself [10, 15, 18-20].

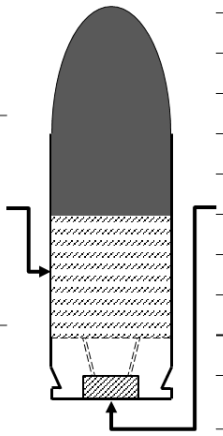
The residues escape predominantly from the muzzle of the firearm. As a result, GSR may deposit on the target as well as surfaces surrounding the discharged firearm, which could include the shooter's hand, face, hair and on objects in the close vicinity [21-24] following Locard's principle [25-27]. The distribution of GSR is influenced by different factors such as the type of firearm, the barrel length [28] ammunition used, the location (outdoor, indoor) [29] as well as environmental factors [18]. For instance, a semi-automatic pistol has the ejection port either on the right or the left side of the firearm. Due to its construction, a greater amount of GSR might be expelled on the side of the shooter where the ejection port is. On the other hand, a revolver has a drum chambers which creates small gaps between the barrel and the drum chambers. This difference fundamentally changes the dispersion and deposition process of GSR as the plume expands from the firearm more symmetrically from the gap between the drum and the barrel.

Several collection techniques have been developed and optimised to facilitate in situ recovery of GSR [30-32]. The first and the most widely used technique involves a stub coated in an adhesive surface. The stub is dabbed many times against the sampled surfaces (hands, face or hair) in order to collect GSR [33-35]. Swabbing methods have also been developed [33, 36, 37]. This technique is generally a piece of cotton moistened with an

organic solvent rubbed on the skin surface usually used to collect OGSR [36]. Finally, vacuum lifting has also been used for GSR recovering from clothing items [33, 38]. Vacuum lift allows for the recovery of GSR from the complex and large matrix of the garment where traditional stubs are contaminated by the garment fibres due to the adhesive surface.

When GSR traces are collected, the specimens[1] must be analysed. As mentioned previously, GSR can be separated in two distinguishable categories: IGSR and OGSR and illustrated in Figure 2.

PROPELLANT – Smokeless powder	
Compounds	**Role**
Nitrocellulose (NC)	Primary explosive Different powder types: Single base: NC Double base: NC+NG Triple base: NC+NG+NGU
Nitroglycerine (NG)	
Nitroguanidine (NGU)	
Diphenylamine (DPA)	Stabilisers
Ethylcentralite (EC)	
Methylcentralite (MC)	
Resorcinol	
Akardite I (AKI)	
Akardite II (AKII)	
Akardite III (AKIII)	
Dibutylphthalate (DBP)	Plasticisers Gelatinisers
Dimethylephthalate (DMP)	
Diethylphthalate (DEP)	
Glycerol triacetate	
Dinitrotoluene (DNT)	Flash inhibitor



PRIMER MIXTURE	
Sinoxid®	
Compounds	**Role**
Lead styphnate	*Primary explosive*
Barium nitrate	*Sensitisers*
Antimony sulfate	
Tetrazene	
Calcium silicate	*Pyrotechnic system*
Lead peroxide	
Lead dioxide	
Glass powder	
Sintox® (Lead free)	
Diazole (2-diazo-4, 6-dinitrophenol)	*Primary explosive*
Tetrazene	*Sensitisers*
Zinc peroxide Titanium chloride	*Pyrotechnic system*

Figure 2. Composition of firearm ammunition – propellant powder residues are known as organic GSR (OGSR) [31, 39-42] and the primer residues are categorised as inorganic GSR (IGSR) [1, 43, 44].

[1] "Specimen" refers to a small part used for testing. It is the preferred terminology in a forensic context instead of "sample" which refers to small part that is representative a whole.

Scanning Electron Microscopy coupled with Energy Dispersive X-ray spectroscopy (SEM-EDX) is currently the most used technique for IGSR particles detection and chemical characterisation. It allows the morphological shape and the elemental composition of particles to be analysed [9, 11, 14, 45, 46]. It is a non-destructive method based on the examination of conductive carbon-coated adhesive tape (stubs) traditionally used for GSR sampling [47, 48]. The analysis with SEM-EDX is automatically managed by software, where the X-ray analysis firstly identify the presence of typical elements, e.g. lead (Pb), barium (Ba) and antimony (Sb) [11, 49]. Once the elemental analysis conducted, the operator assesses the particles morphology defined as spherical or globular with a smooth surface (diameter between 0.5 and 5.0 μm) [9, 11, 13, 14]. The combination of both elemental composition – presence of the three elements Pb/Ba/Sb – and morphology is considered when discriminating IGSR from other environmental kind of aggregates [9, 50]. Nevertheless, in 1982, Hagel and Redecker patented a new primer mixture for the manufacture of ammunitions called Sintox® and produced by Dynamit Nobel AG [51]. This new primer formula – known as lead-free or heavy-metal free primer (Figure 2) – was originally designed to minimise airborne heavy metal such as lead, barium and antimony to avoid health and environmental issues, notably in firing ranges and hunting [52]. In these ammunitions, the primary explosive lead styphnate is replaced by 2-diazo-4,6-dinitrphenol (Diazole) [19, 53].

The introduction of lead-free ammunition has introduced future challenges for forensic GSR analysis given the identification of characteristic particles is based on the presence of particles of lead in combination with barium and antimony [54-58], which are not

produced by this new primer mixture. Consequently, several authors [31, 39, 59-63] approach the identification of GSR through their organic components. There exist three main types of propellant based on the composition of the main explosive mixture as summarised in Figure 2.

Single and double base powders are the most common propellant used in the manufacture of modern ammunition. The triple base mixture is difficult to obtain in the market because it is primarily used in rockets and large calibre military weapons [64]. Additives (stabilisers, plasticisers and flash inhibitors) are also part of the propellant to increase the powder workability, stability and to control the burning rate [31, 42, 65]. Methyl- and ethylcentralite (MC and EC) are two stabilisers restricted to ammunitions and consequently are the most characteristic of propellant powder, thus the probative value of the evidence is strengthened when they are detected [59, 66]. Diphenylamine (DPA) is considered characteristic of GSR when associated to its nitro-derivatives such as *N*-nitrosodiphenylamine (*N*-nDPA), 2-nitrodiphenylamine (2-nDPA) or 4-nitrodiphenylamine (4-nDPA) [39, 65].

Several analytical techniques have been successfully utilised for the detection of OGSR [33] such as gas chromatography (GC) [65, 67-70], micellar electrokinetic capillary electrophoresis (MECE) [60, 71-73], Raman spectroscopy [64, 74-76], desorption electrospray ionisation–mass spectrometry (DESI–MS) [66, 77, 78] and liquid chromatography tandem mass spectrometry (LC-MS/MS) [39, 41, 62, 79-86]. However, the analytical aim relates to only one dimension of the forensic scientist's task. The other dimension relates to the investigative and judicial aims of forensic science. In this dimension, forensic scientists work commonly with several stakeholders in the system

such as investigators, prosecutors, defence and finally the court to help reconstruct an event under investigation and ultimately help the trier of fact reach a verdict [87, 88]. From an investigative point of view, the forensic scientist can be asked to provide fast information focusing on the incident by generating hypotheses for sustaining and orientating investigators during the initial phase of the inquiry [89]. After that phase, when a person of interest (POI) has been charged, the forensic scientist has to assess their test outcomes given a set of propositions relating to the POI and the case [89, 90] in order to assist the court in the decision-making process.

To approach the management of uncertainty relating to the distinctiveness of traces and the nature of each event, a framework to enhance the interpretation of evidence[2] has been developed [27, 92-94]. This framework can aid in the clarification of the meaning of the analytical findings in reference to the allegations presented by the court and the specific contextual circumstances surrounding the criminal case under investigation.

In regards to GSR interpretation, of primary importance is the classification of residues as IGSR particles [11]. This classification concerns the uncertainty associated with attributing the origin of residues as firearm discharge rather than something else without considering the case in question (i.e., the classification is at the sub-source level). Thus, current challenges encountered during trials concern a possible environmental or occupational source of particles rather than firearm discharge [95]. At the next level of questioning, attention then needs to be concentrated on the persistence of the traces and

[2] According to the ENFSI guideline "*The term 'evidence' is generic. From a strict scientific point of view, evidence refers to outcomes of forensic examinations (findings - results of observations, measurements and classification that are made on items of interest), at a later point, may be used by legal decision-makers in a court of law to reach a reasoned belief about a proposition. Evidence should be a term kept for lawyers.* " [91] S. Willis, L. McKenna, S. McDermott, G. O'Donell, A. Barrett, B. Rasmusson, A. Nordgaard, C. Berger, M. Sjerps, J. Lucena-Molina, ENFSI guideline for evaluative reporting in forensic science, European Network of Forensic Science Institutes (2015)..

the possibility of secondary transfer, arising through contact between the POI and police officers [96, 97] or other persons who have handled or discharged a firearm, to the possibility that the POI discharged or handled a firearm prior to the crime under investigation. Therefore, to deal with chronological factors and circumstantial information of the event, a "case-by-case" approach was proposed by Romolo and Margot in 2001 [34]. In this article, they also introduced the Bayesian theorem as interpretation framework to gunshot residues events [34].

CHAPTER TWO: FORENSIC INTERPRETATION OF GSR EVIDENCE

1. The American Society for Testing and Materials Standard: A formal approach

Seminal research relating to the interpretation of IGSR was first published in 1979 [11, 43, 44] based upon a report published in 1977 [40] about the detection and specificity of GSR analyses. The particle compositions and spheroidal, non-crystalline morphology were initially classified by Wolten et al. (1979) who reported they were specific to GSR [11]. This classification generated the first interpretative framework for GSR. The American Society for Testing and Materials (ASTM) then initiated the development of standards [49] to minimise misinterpretations due to environmental sources of GSR-like particles. Romolo and Margot [34] described the previous ASTM standard [49] as a formal approach due to the lack of consideration of the case circumstances during the interpretation.

Table 1 provides the current ASTM encapsulation of the views of Wolten et al. and others subsequently [11, 34, 43, 44, 98-100]. The classification under this standard is divided in three categories: The "characteristic" particles, defined as *"particles rarely found from any other source"* by the ASTM standards [101]. These particles may be directly linked to firearms-related events such as discharging gun, or contact with or close proximity to a discharged firearm (Table 1). The second category relates to particles "consistent with GSR", which are particles *"also found from a number of relatively common non-firearm source"* [101].

The last category relates to particles "commonly associated with GSR" defined as particles *"also commonly found in environment particles from numerous sources"*. These latter particles have to be found in combination with "characteristic" or "consistent with" particles to be considered [101].

Table 1. Current classification of particles composition detected with a SEM-EDX [101].

Characteristic (exclusive to GSR)	Consistent with GSR	Commonly associated with GSR
(1) Lead-Barium-Antimony *(Pb-Ba-Sb)* (2) Lead-Barium-calcium-silicon-tin *(Pb-Ba-Ca-Si-Sn)*	(1) Lead-Barium-Calcium-Silicon *(Pb-Ba-Ca-Si)* (2) Barium-Calcium-Silicon *(Ba-Ca-Si)* (3) Antimony-Barium *(Sb-Ba)* (4) Lead-Antimony *(Pb-Sb)* (5) Barium-Aluminium *(Ba-Al)* (6) Lead-Barium *(Pb-Ba)*	(7) Lead (Pb) (8) Antimony (Sb) (9) Barium (Ba) (10) *Suflure may be present*

Through this classification, forensic scientists compare their analytical outcomes to the ASTM standard to express their conclusion [49]. The results that are therefore related to a specific event are compared to a general and theoretical idea of the particles' origin suggested by the ASTM standard [34, 49]. In practice, as a protection against false positives, forensic scientists take into consideration the composition of the entire population of particles present in the recovered traces and not only one or few particles that meet the ASTM criteria. The results of a survey conducted by DeGaetano et al. in the early 1990s [46] demonstrated that in 41% of laboratories, detecting one particle containing the characteristic configuration was considered sufficient to indicate GSR

analyses as positive, despite variations that exist among experts about the significance of identifying a single GSR particle. This suggested that a court outcome could potentially rest on the detection and classification of a single characteristic particle without consideration of whether case circumstances tend to support or refute that opinion. These observations were supported four years later by another survey conducted by Singer et al. [102]. The famous case of the murder of the television presenter Jill Dando [103] highlights this situation, where only one particle of GSR was found inside the pocket of the defendant's coat one year after the event. The significance and relevancy of finding a single particle was discussed during the trial for many days by different forensic experts, who suggested that it was likely that the single GSR particle arose from a gun fired by the defendant. The defendant was convicted of the murder of Jill Dando. The defendant was acquitted in 2008 after the third appeal. The court concluded that the jury was misled and that the GSR particle evidence was inconclusive and could not provide any weight to the case [103].

Particles having the compositions mentioned in Table 1 may also contain one or several of the following elements: *silicon (Si), calcium (Ca), aluminium (Al), copper (Cu), iron (Fe), sulfur (S), phosphorus (P), zinc (Zn) (in combination with copper), nickel (Ni) (rare and only in combination with copper and zinc), Zirconium (Zr), tin (Sn), potassium (K) and chlorine (Cl*) [101] .

According to the latest ASTM standard [101] the criteria for identifying a particle as "characteristic" using SEM-EDX is based on the elemental composition (Table 1) and morphology of particles with a diameter usually between 0.5 and 5 μm. The observed morphology should be spheroidal and non-crystalline, although it is noted that angular

GSR particles are uncommonly encountered. Any other particle compositions can only be classified as "consistent with GSR" or "commonly associated with GSR".

The introduction of lead-free ammunition has given rise to an additional class of particles. Particles considered as characteristic contain: *(1) Gadolinium (Gd) – Titanium (Ti) – Zinc (Zn), (2) Gallium (Ga) – Copper (Cu) – Tin (Sn)*. The particle compositions considered as consistent with GSR are: *(1) Titanium (Ti) – Zinc (Zn)* and *(2) Strontium (Sr)* [101]. OGSR are not covered in the ASTM standard. This could lead to further complication with regards to GSR evidence evaluation, especially if only IGSR is considered.

The main issue with the formal interpretation framework is that the forensic scientist is unable to answer the questions typically explored during a court case involving firearms related events. The forensic scientist through the ASTM guidelines is able to provide expert testimony related to the source of the evidential material (GSR or not). However, the court interrogations concern mostly the next level of interpretation, namely the activity before, during and after the event. Forensic traces represent vestiges of the actions undertaken at the time of the event [88]. Margot (2011) defined the traces as being [88]:

"*A fragmented, imperfect, uncontrolled pattern, signal or material transferred during an event (often unknowingly by the actors of the event). It is the remnant (the memory) of the source (identity – who, with what?) and of the activity (What, how, when, why?) that produced it." [88]*

This definition includes the traces in the centre of any activities, and therefore in the centre of forensic investigations. OGSR traces represent such kind of traces as it reflects the activity of discharging a firearm and is consequently of high interest in criminal firearm-related case. However, OGSR can be heavily affected by external factors such as the time elapsed between the firearm discharges and the collection and analysis of traces.

In order to increase the value of the contribution of the forensic scientist in the investigation, Romolo and Margot (2001) proposed a case-by-case approach for the interpretation of GSR evidence [34]. As the name implies, it was recommended that evidence be evaluated in light of the particular case circumstances, including comparison of residues with the specific suspected ammunition as often as possible, and the results assessed using the logical framework of Bayes' theorem. This allows for an investigation of source and activity questions by considering the context of the case [34]. The current ASTM standard [101] recommends that examiners compare the composition of suspected GSR with a putative source such as a suspected firearm or fired cartridge case. However, when only few particles are recovered the comparison may be misleading due to the stochastic nature of GSR formation and the fact that the small number of particles might more strongly support a pollution[3] or secondary transfer hypothesis.

[3] According to Schwendener et al [104] G. Schwendener, S. Moret, K. Cavanagh-Steer, C. Roux, Can "contamination" occur in body bags? - The example of background fibres in body bags used in Australia, Forensic Science International 266 (2016) 517-526., "pollution" refers to the addition of some material due to a lack of precautions, while "contamination" refers to non-pertinent traces present before any investigation and cannot be avoided. Pollution is therefore more suitable in the context of a secondary transfer study.

2. A Bayesian perspective: GSR issues through the hierarchy of the propositions

The GSR interpretation process is crucial as the expert may add value to the analytical results by using structured reasoning incorporating the examination outcomes in the global context of the case [87]. One of the most important roles of the scientist in the traditional approach of forensic science is to articulate to the court the reasoning that lead to the formulation of their expert opinion [105] rather than present it *ipse dixit*. Indeed the judicial system requires that the forensic scientist present evidence in a manner that allows the triers of fact to rationally evaluate findings and does not leave them in an information vacuum. The forensic scientist has to provide robust evidence, be transparent, and impartial by giving clarifications on scientific issues for which they are qualified [21, 87].

To achieve these requirements, an interpretation scheme based on Bayes' theorem has been developed. In 1977, through his articles titled "A problem in forensic science", Lindley [106] posed the first iteration of the modern interpretation of evidence using Bayesian reasoning. It provided a robust and logical framework through the use of laws of probabilities. Impartiality is achieved through the constraint of the theorem to assess the evidence in under at least two competitive hypotheses [21, 90, 94, 107, 108]. Transparency is the combination of opinion – mostly arising from several years of experience – with solid references to a logical reasoning and a body of scientific knowledge [109-111]. This concept introduces the notion of the opinion of the expert in the assessment of the evidence. As emphasised by Taroni et al. in 2001, the notion of subjectivism must not be understood as a synonym of "arbitrary", but refers to the notion

of "personal" or "related to a particular individual" [112-115]. In addition, interpretation of evidence depends on contextual information [27, 92] inducing subjectivity since it depends on the understanding by the receiver of the information and it cannot be fully detached from human judgment [109, 116, 117]. Indeed the first argument in the application of Bayes' theorem (Equation 1) to a forensic context is the "calculation" of prior probabilities that concern the first degree of belief of stakeholders about each proposition: Hp (hypothesis of the prosecution) and Hd (hypothesis of the defence).

$$\frac{P(Hp\,|I)}{P(Hd\,|I)} * \frac{P(E\,|Hp,I)}{P(E\,|Hd,I)} = \frac{P(Hp\,|E,I)}{P(Hd\,|E,I)}$$

Equation 1. The odds form of the Bayes' theorem: "Hp/Hd" is a set of propositions reflecting the questioning in a specific case, "E" represents the observations (Evidence) and "I" represents the canvas of circumstances surrounding the case given to the forensic scientist.

The likelihood ratio (LR) represents the extent to which the evidence supports the respective hypotheses and measures the ability of evidence to discriminate between two competing propositions of interest [21, 107]. The application of the LR enables the prior degree of belief on a specific case to be updated into a posterior state of knowledge [27, 92, 107]. In a forensic context the subjective jury opinion (prior) is integrated by the expert opinion provided by the logical assessment of the strength of the evidence (LR) to reach their final (posterior) conviction about the specific case. The following section provides an overview of the LR assessment carried out by the forensic scientist. Three principles must be considered when assessing LR [87, 94].

The first principle concerns the relevant background information ("I" in the Equation 1) that must be considered by the forensic expert during the interpretation process. This set of information is crucial for a case specific interpretation and consequently if the circumstances change, the interpretation of evidence has to be reviewed in accordance to the new information [94]. The circumstances of interest might be provided by the POI, solicitor, prosecutor, investigator, police officer or witnesses [89, 118].

The second principle involves generating a minimum of two mutually exclusive propositions [90, 115, 119, 120]. Those propositions should be determined before knowing the outcomes of the analysis in order to avoid the defence adapting their position. However, as part of their communication strategy, the defence is not obliged to explore and set out an alternative scenario. Therefore the scientist has to work on the general defence hypothesis "*wasn't involved"* in the absence of any other scenario. Different levels of proposition – known as the hierarchy of propositions and described hereafter – have been developed to address questions asked by the judicial process and are based on different levels of understanding depending of the amount of information available to the scientist [89, 119].

The third principle concerns the outcomes or observations from technical and/or analytical analysis. It is central for a correct logical LR assessment that the forensic scientist reasons on the analytical outcomes – called evidence (E) – given both propositions. If a precise number cannot be given, the LR may be expressed as an order of magnitude that reflects the strength of the evidence which can be translated into a verbal equivalent [94, 113, 121-123]. However, recent research [124, 125] emphasises the risk of misinterpretation of verbal scales by jurors. According to Martire et al. jurors encounter challenges understanding the ascending nature of the verbal scale and the

relation between the verbal and numerical value of the LR. This can ultimately lead to confusion during the presentation of evidence by the scientist, with the effect more pronounced in the case of weak evidence [124, 125].

Through numerous studies published on forensic application of the Bayesian reasoning [27, 92-94, 107-109, 112, 119, 126, 127], the LR is currently established in the forensic research community as the appropriate tool for providing a logical and robust reasoning framework for the interpretation of evidence [91, 127]. Despite a global acceptance within the academic community, for practitioners, many challenges remain in the widespread application of the framework, notably illustrated by the *R v T* case law [128] and subsequent discussion [127, 129-131] about the interpretation of evidence and the presentation in the court by the scientist. The main practical issue arises from the lack of data available in the literature that could be used by the scientist to understand the problematic and ultimately which could be used in the LR calculation. This lack of data limits the opportunity to implement the Bayesian theorem as a routinely used interpretation framework. The current situation is also epitomised in the response of Morrison et al. [132] to the draft of the Australian Standard about forensic analysis interpretation, where the response takes the position that the Standard proposed for forensic scientists to follow enshrines bad evidence evaluation practice. The progress of forensic evidence evaluation is an ongoing educative and research journey and the recently published guidelines by the European Network of Forensic Science Institute (ENFSI) and associated workshops definitely assist this development [91] . The approach introduced by Romolo & Margot in 2001 [34] involves the assessment of LR allowing the forensic scientist to approach questions of judicial importance. When GSR are detected on the POI's hands, the key relevant question is how the evidence can

discriminate between propositions such as: *the POI has discharged (Hp) or an unknown person has discharged the firearm (Hd)* [21]. As a large prerequisite of background information is needed, the set of propositions has to be framed in collaboration and coordination between the forensic scientist and their information source(s). The questions can be classified in three levels, representing the hierarchy of propositions: the source (or sub-source) level (I), the activity level (II) and the offence level (III) [119]. Considering the field of GSR investigation, a hierarchy of proposition could be developed on each level of 'judicial' questions [90] as presented in Figure 3.

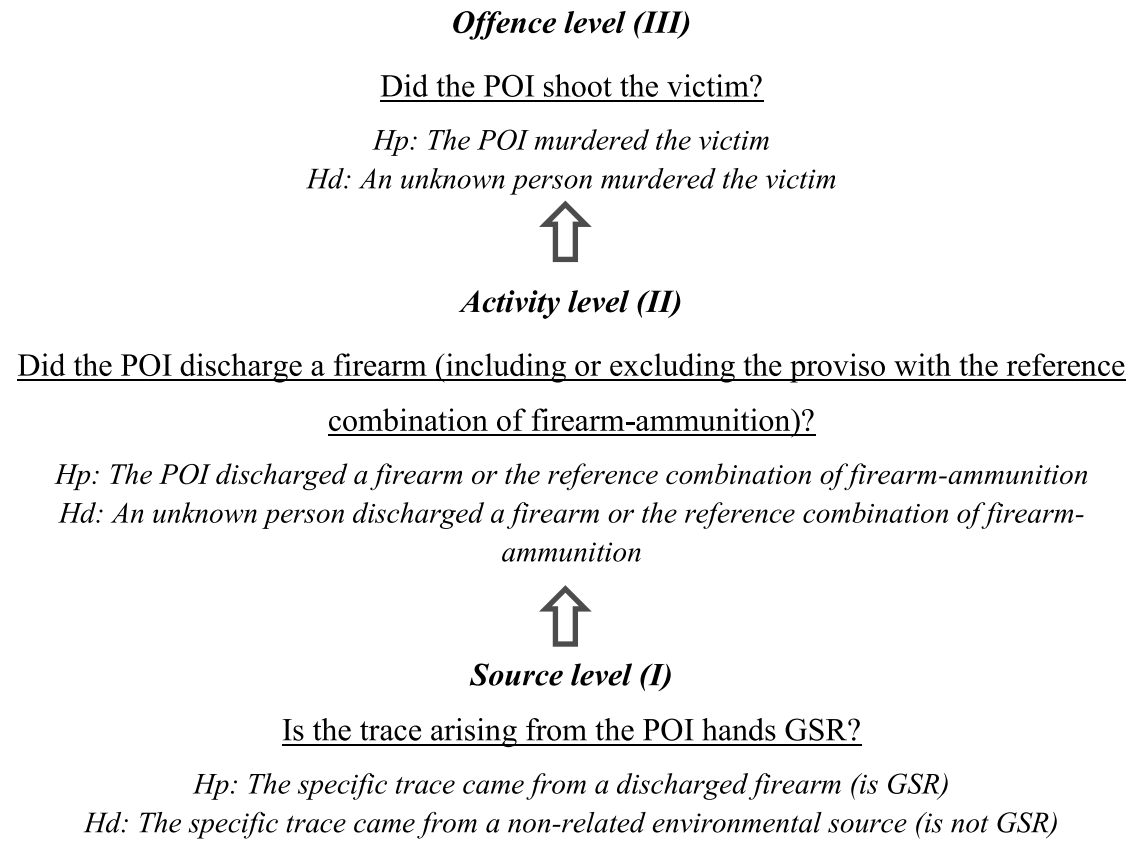


Figure 3. Example of hierarchy of proposition for GSR evidence, based on Jackson et al., 2006 [90]. It represents a question and an example of possible set of hypotheses for each level.

Level I is the lowest level, representative of more general questioning. Level III represents the highest level and, as it requires information specific to the case that is often not available to the forensic scientist for the evaluation of the evidence under offence propositions. This "ultimate question" is the domain of the triers of fact [27, 119]. In this chapter, the focus will be on the Level I and II propositions and the different parameters that are considered to assess evidence.

2.1 Source level (or Level I)

First of all, by coming back to the question presented in the Figure 3, a source level question can be stated as follow:

Is the trace recovered from the POI hands GSR?

Level I represents the first stage of the interpretation and is often the most straightforward to evaluate. It is relative to the identification of the source of the evidence, here, gunshot residues. The questioning at source level requires solely analytical information [92].

A pair of proposition can be expressed as follow:

***Hp**: The specific trace came from a discharged firearm (is GSR).*

***Hd**: The specific trace came from an non-related environmental source (is not GSR).*

The level I question represents the first stage of the interpretation process (Figure 3). At its simplest, sub-source level, this question becomes: *Are the residues GSR?* The questioning at this level requires solely analytical information and knowledge of the material under investigation [92]. Under the prosecution proposition (Hp), the interpretation process attempts to assess the degree of compatibility between the results and that expected for GSR. Under the defence proposition (Hd), the POI is regarded as a person unrelated to the case, which requires assessment of the evidence knowing that it comes from a source other than firearm discharge [119]. Another possibility could appear under the case-by-case source level assessment, for example when a firearm(s) and ammunition(s) are found during the investigation. Consequently, another question could be addressed: *Does the recovered trace have the same characteristics (or not) as the reference GSR produced by the suspected combination of firearm(s)-ammunition(s)?*

In this case, the interpretation under Hp involves a careful comparison between the recovered trace and traces produced by shooting the firearm-ammunition combination(s). Then, under Hd, the interpretation focuses on other possible sources since the combination of suspected firearm-ammunition is considered unrelated to the event [93, 119]. Indeed, this information is crucial; detection of GSR is possible in scenarios not related to a criminal event. The GSR could well arise from legitimate firearm-related sources such as hunting, during a shooting session at a firing range before or after an alleged criminal event, or due to a secondary transfer. The residues detected may also arise from an environmental pollution from sources that are external to firearm discharges. Therefore, the main aspect that has to be determined under level I propositions are the alternative populations that could be the source of GSR-like components.

An important consideration is therefore to assess the background occurrence of IGSR, OGSR and/or GSR-like components [119, 133] in the relevant population which is case-specific and relative to the circumstances [27, 134].

Other questions might arise from the analysis of GSR such as the possibility to link GSR to specific ammunition. Different experiments were carried out by Brozek-Mucha [135-137] about the possibility of using chemometrics to classify ammunitions following SEM-EDX analysis. Based on the frequency of different elements (Pb, Ba and Sb), chemometrics can be used to classify different kinds of handgun ammunition using various statistical analyses [135-137]. Collins et al. [16] used time of flight-secondary ion mass spectrometry (ToF-SIMS) and elemental profiles to compare traces of glass in residues of fired primer. They observed GSR particles with glass surfaces arising from the discharges of .22 ammunition. Such particles might originate from frictionators such as borosilicate glass and soda-lime glass [16].

Determining the composition of OGSR and IGSR is essential to identify what kind of set of compounds or particles forensic scientists are facing. However, it is even more important to know their composition in order to investigate potential alternative sources, which could lead to similar composition or shape. Such environmental or occupational sources could ultimately lead to false positive results, by associating environmental compounds or particles to OGSR or IGSR.

2.1.1 Sources of IGSR-like particles

The source level interpretation must be performed in the context of recognising potential sources of GSR-like particles. In order to identify alternative sources of such particles, several researches investigated different occupational activities. Automobile-related activities were identify by Wolten et al. in 1979 as a potential source of pollution of GSR particles [43]. Garofano (1999) also studied several potential sources of IGSR-like particles [100]. Their conclusion was that particles of Pb-Ba-Sb are to be considered as characteristic to GSR, confirming the conclusions of Wolten et al. [43, 138]. Following these studies, several authors approached the issue of IGSR pollution through automobile-related activities, with Torre et al. (2002) [138] and Cardinetti et al. in 2004 [47] exploring the possibility of GSR-like particles arising from brake pads. They concluded that it is possible to find particles from brake pads very similar in composition to GSR (Pb-Ba-Sb particles) [47, 138]. However, particles from brake pads are typically angular while the majority of GSR particles exhibit a rounded morphology, which allows simple differentiation between the two sources of particles. Furthermore, dust from brake pads is accompanied by an abundance of particles that are not commonly found in GSR and such an observation is routinely referenced to support the opinion that particles (even angular ones) are of brakes origin rather than GSR. The work of Garofano et al. (1999) resulted in particles with Ba-Sb composition being 'downgraded' from the characteristic class to the indicative (now "consistent with") class [100].

Further studies have explored the residues arising from the explosion of automotive airbags [139, 140]. Some manufacturers of hybrid airbags use percussion primer containing lead, barium and antimony as initiator of the airbag explosion. The recovery

of GSR-like particles arising from an airbag explosion is therefore possible but again the entire population of particles will contain many that are not usually associated with firearm discharge [139, 140]. With appropriate training, forensic scientists would most likely not confuse GSR with airbag residues.

Alternative potential sources of GSR-like particles such as fireworks [43, 141-143] or welding process [144] have also been explored. Mosher et al. [141] found that recovering Pb-Ba-Sb and Ba-Sb particles characteristic of GSR with a spheroid shape between 0.5-5 µm is possible from fireworks devices [141]. This challenged the findings from another study [142] were no particles were discovered containing Pb-Ba-Sb with a spheroidal shape in firework residues. In any event, other elements were detected such as chlorine and potassium [142] that are not common to GSR and therefore give indication of a possible non-GSR source. In 2015, Brozek-Mucha [144] studied the possibility of GSR-like particles arising from welding fumes. It was concluded that possible spherical particles of aluminium-titanium could be produced during the welding process that may be indistinguishable from lead-free ammunition particles.

2.1.2 Sources of OGSR-like compounds

After a firearm discharge, nitrocellulose (NC) – the main component of the propellant powder – is degraded making it indistinguishable from environmental sources of NC such as paints, varnishes and lacquers [6, 77]. Nitroglycerine (NG), used in double- and triple-base powders, is also used in pharmaceutics as a cardio-stimulant [6]. Thus the detection of NC and NG alone cannot be used unequivocally to demonstrate the presence of GSR

[39]. Stabilisers, plasticisers, deterrents and flash reducers are also present in all propellant powders to improve their properties and their detection can be utilised for identifying OGSR [39]. Diphenylamine (DPA) is a common stabiliser in propellant formulations, but it is also added to rubber and plastic products and has been reported in the 1980's and earlier as being used as an insecticide, to control attack of apples and pears, and as a stabiliser for perfumes [145]. In two early works [45,112], traces of substances identified as DPA were detected on outer garments, tyres, and on the surfaces of fruit, as well as shoes and leather [62]. DPA is also used as a precursor in the synthesis of compounds used in pharmaceuticals and dyes [145]. As a consequence, DPA could remain as a trace impurity in these materials.

As the propellant ages, the NC releases nitric oxides that catalyses its decomposition. DPA is added to eliminate the nitric oxides, with a number of by products resulting, including *N*-nDPA and various nitro- and dinitro-DPA [59, 146]. Non-propellant based sources of *N*-nDPA have not been reported. However, nitrodiphenylamines are produced in the chemical industry as precursors for certain dyes and as a consequence, similar to DPA, it is feasible that these compounds might remain as trace impurities in products that have been treated with these dyes [145]. Traces of DPA, nitro- and dinitro-diphenylamines have been detected in ground water and soil in locations where large quantities of propellant have been stored or disposed [145]. These studies suggests that detection of DPA (without any nitro-derivative) cannot be considered strongly indicative of OGSR because of its presence in the environment, which is an observation also made by Laza et al. [39]. However, a combination of DPA and its nitro-derivatives could be used as an indicator of OGSR because environmental and industrial occurrence of these is restricted [69] or non-existent in the case of *N*-nDPA. Ethylcentralite (EC) and

methylcentralite (MC) are other stabilisers also used in combination with DPA. Their usage is restricted to propellant manufacture, which has led to these compounds being described as the most characteristic residues of smokeless powder [65, 77]. However, the manufacture of propellant powder typically involves the addition of a single centralite, either EC or MC, but rarely both. Thus if one of them is detected, it reduces the possibility of an environmental source and therefore weaken the probability of the defence proposition (Equation 1, with a same numerator, leading to a higher LR) [66].

Few studies have surveyed the presence of OGSR-like components on hands of "random man". Northrop, in 2001, studied occupational occurrence of OGSR-like elements by analysing hands from 100 volunteers using micellar electrokinetic capillary electrophoresis (MECE) [71]. No compounds present in OGSR were detected or were below the limit of detection (LOD) of MECE analysis. Seven of the participants indicated that they were in contact with firearms within the previous 24 hours but all had washed their hands, which could have been sufficient to remove OGSR [71]. Bell and Seitzinger carried out a survey of OGSR in a population of West Virginia (USA) using ion mobility spectrometer (IMS) [147]. Further statistical analyses were performed using neural networks and LR-oriented analysis for differentiating a shooter from a non-shooter population [147]. It was found that successful differentiation of swabs from shooters and non-shooters using the developed neural-network, which compared mobile ion spectra and based the samples differentiation on the presence and absence of peaks [147].

In regards to the examination of matters involving discharge of a firearm, the forensic scientist must acknowledge that the POI might have been exposed to one or more of the possible sources of GSR-like components summarised in Table 2. In addition to potential environmental sources of particles, a memory effect was observed by Harris (1995)

during a study involving lead-free ammunition [54]. IGSR particles were observed even when lead-free ammunitions were shot. These particles arise from traditional ammunition shot previously that are deposited in the chamber and the barrel of the firearms, and persist even after cleaning. These observation were also supported by other studies [148, 149].

Table 2. Summary of possible environmental sources of GSR compounds.

IGSR		OGSR	
References	*Potential Sources*	*References*	*Potential Sources*
[40, 43, 141, 142]	Fireworks *Manufacture* *Handlings*	[6, 145]	Paints *Dyes* *Varnishes*
[40, 43, 47, 100, 138-140]	Automobiles-related *Brake pads* *Airbags* *Repair mechanics*	[62, 145, 150]	Rubber products *Production process* *Tyres*
[98]	Cartridge-operated tools	[6, 62, 145, 150]	Pharmaceutics/ cosmetics *Medicine* *Perfumery* *Hair dyes*
[144]	Welding fume: *particle of Al/Ti also produce by lead-free ammunition*		Chemistry/ insecticides *Synthesis* *Post-harvest*

As explained previously, the knowledge about the case is important because that will directly influence the assessment of both numerator and denominator of the LR (Equation 1). In practical terms, if an environmental source of particles is suspected it will result in a smaller than expected LR because the probability of the occurrence of the detected compounds becomes much higher and therefore the denominator of the LR increases.

2.2 Activity level (or level II)

As presented in Figure 3, an example of activity level question can be presented as follow:

Did the POI discharge a firearm (including or excluding the proviso with the reference combination of firearm-ammunition)?

This kind of question involves the action of shooting with a firearm. It also asks, in an underlying manner, if we can link the action of shooting to individual A, and for example, discriminate the shooter from a bystander.

A pair of propositions can be expressed as follows:

> ***Hp**: The POI discharged a firearm or the reference combination of firearm-ammunition.*
>
> ***Hd**: An unknown person discharged a firearm or the reference combination of firearm-ammunition.*

Although the determination of the probability of the occurrence of the particular features of IGSR and OGSR traces in the relevant population is essential at each step of the hierarchy, the question of the court frequently concerns the activity of the POI before, during and after the criminal event. To take into account the chronological factors during the interpretation, a transition to the activity level (II) is required, which implies, besides background, the assessment of additional parameters – namely transfer and persistence [89, 90]. Indeed an action leads to a transfer (Locard's principle) of traces that exhibit qualitative and quantitative characteristics and subsequent actions will impact the persistence (retention) of these traces and their characteristics [119]. Under the activity

level (Figure 1), the hypotheses involve the action of discharging a firearm. Consequently, the forensic scientist requires additional circumstantial information in order to identify appropriate propositions [119]. This contextual information is mainly about physical activities and the environmental conditions that can play a role in the deposition, retention or degradation of GSR as well as the time between the alleged event and the trace collection [8].

2.2.1 Persistence of GSR

When a trace is analysed, a question that could arise is whether the result is consistent with the case circumstances, that is, the activity of the POI and with the time spent between the event and point of the sampling. A typical defence line when GSR is detected is that the POI had legitimate contact with firearms, for example in a firing range, before or after the criminal event. Therefore the study of GSR persistence over time is critical when exploring activity level propositions. This is of particular importance when the POI is not apprehended immediately after the incident [58, 148, 151].

Concerns relating to the persistence of IGSR were first reported by Kilty in 1975 [152]. This study, using the screening method neutron activation analysis (NAA), suggested that the bulk of IGSR particles were removed within two hours, making it difficult to link residues detected after this time period to GSR. However, the high limit of detection (LOD) of NAA could potentially explain this short time period for the detection of IGSR. Afterwards, IGSR persistence was studied using SEM-EDX. In 1976, Nesbitt [14] observed a rapid decrease of the amount of GSR particles on hands depending on the

activity using SEM-EDX. However, GSR particles were still detected after approximately 2-3 hours even after vigorous activities [14]. This observation was confirmed by Andrasko with the detection of GSR 2 hours after shooting [9]. Both authors also analysed typical daily activities such as washing, rinsing hands and wiping hands. All concur that these activities decrease the amount of detectable GSR until it is removed completely [9, 152]. It was more recently confirmed by Cook (2016) who studied the potential pollution of police officers during their firearms check when they commence their shift [153].

More recently, Jalanti et al. [148] observed a poor shot to shot reproducibility of counted particles. The persistence study concluded that a rapid loss of IGSR particles was observed between 2-4 hours [148] confirming the findings of several former studies [9, 14, 151, 154] that time after discharge is a crucial factor when attempting to detect GSR. Cardinetti (2006) also studied the persistence of the IGSR particles and modelled the GSR distribution using a Poisson distribution [50]. These models were then used to interpret the evidence using the Bayesian approach [21]. In 2011, Brozek-Mucha [8] studied traces on shooter's hands with SEM-EDX and focused on specific particles having the characteristic elemental composition Pb-Ba-Sb. A significant decrease in the number of characteristic particles was observed after the first 30 minutes. A similar trend was observed for traces obtained from the shooter's clothing [8]. The high activity level involving hands was said to be responsible for the rapid loss of characteristic particles on the shooter's hands [8]. Such an observation is further supported by longer persistence patterns observed for areas such as the face and hair. Therefore, if the sampling on the POI is carried out a few hours after the alleged event, locations other than hands, such as clothes, hairs and face, should be also collected to maximise the possibility to recover IGSR particles [8, 58, 148].

Similarly to secondary transfer, few studies have focussed on the persistence of OGSR. Northrop (2001) [72] used MECE to detect and study the persistence of OGSR, with results suggesting that OGSR is not detected beyond a one hour interval [72]. However as previously emphasised, the LOD of MECE analysis may explain this short window of time.

Further studies into persistence utilising the IMS have also been conducted [58]. Using DPA as target molecule, detection has been achieved after 4 hours, but activity such as washing hands with soap or sanitiser completely removed traces of OGSR [58]. A summary of the GSR persistence studies is presented in Figure 4 illustrating a trend that an important decrease of the GSR occurs between 2 to 4 hours. These studies emphasise the fact that the first few hours of the investigation are critical for the recovery and detection of GSR on the shooter's hands. More recently, Gassner et al. investigated the detection of OGSR immediately and two hours after the discharge [155]. It was observed that a significant decrease occurred after two hours, with a factor 100 for EC and 20 for 2-nDPA. However, the six OGSR compounds of interest were still detectable after two hours on the sleeves of the jumper worn by the shooter. The right hands presented a positive detection for five of the six compounds of interest (EC, DPA, *N*-nDPA, 4-nDPA, 2-nDPA and AKII), while the left hand were positive to only two of the six compounds [155].

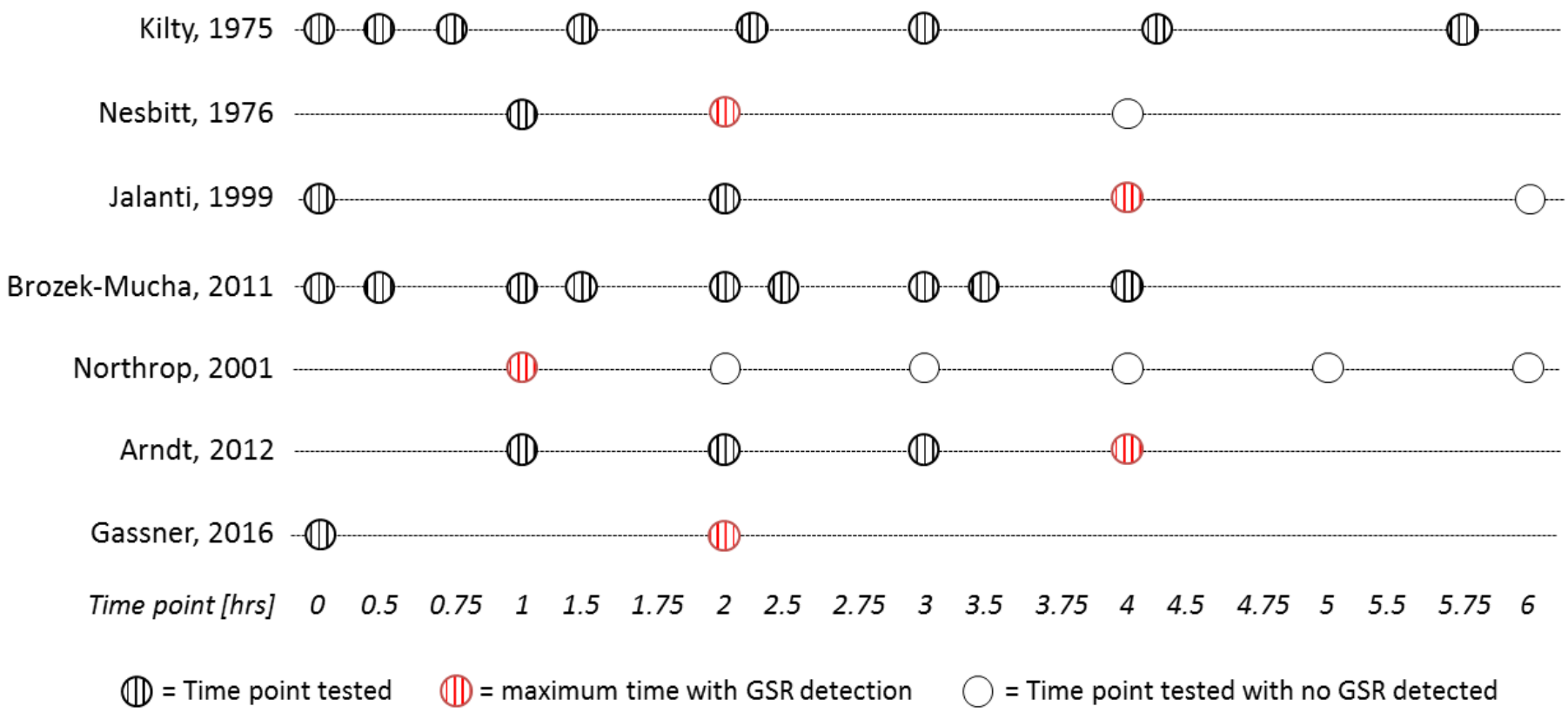


Figure 4. Persistence of GSR in the literature [8, 14, 58, 72, 73, 148, 152, 155].

Moran and Bell [156] have also explored the factors influencing the persistence of OGSR. According to this study, retention is influenced by two factors; evaporation and absorption of OGSR compounds by the skin during hours following the shot [156]. The lipophilic nature of organic compounds resulted in higher skin retention rates thus increasing their persistence rate and the time window for considering a possible detection [156]. They found that the rates of evaporation and permeation of DPA and DMP are more important than for other OGSR components such as EC, 2-nDPA, 4-nDPA [156]. Therefore, the detection of DPA or/and DMP means that the shooting event was only within few hours before the sampling. The analysis of OGSR can also be used to assess the time since last discharged. Andrasko et al. conducted an extensive study based on the persistence of volatile OGSR present inside the barrel after the shot [157-160]. The organic volatile compounds present in GSR also persist in fired cartridges and decrease with time. Therefore, they could be used in order to determine the time since discharge of a cartridge case [161, 162]. The degradation of compounds may also be used in the assessment of the propellant age [146].

The movement and retention of traces as GSR are factors influencing the evidence and therefore they have to be considered in the calculation of the LR. Their inclusion in the LR may considerably affect both the numerator and denominator and significantly influence the calculation. For managing the increasing calculation complexity by the introduction of multiple sources of uncertainty, Bayesian networks (BNs), which are tools with an underlying probabilistic framework, can be built and applied in forensic interpretation of evidence [126].

2.2.2 Secondary transfer of GSR

The interpretation of traces under the activity propositions require an understanding of the transfer parameter, as the presence of GSR on a POI does not confirm a person has handled or discharged a firearm [163]. Similarly, the potential for secondary transfer also exists (the deposition of GSR on the shooter representing the primary transfer). When GSR is identified on a POI, a typical defence proposal is the possibility of an accidental pollution, mostly since the person who arrests the POI and collects traces from them is a police officer [50] and the POI might have been in police custody. Some papers have reported upon the possibility of GSR transfer from an officer to a POI [164-166].

A study by Gialamas et al. [165] suggested that even if a firearm is carried and used regularly by officers, only a small fraction of them (7%) had characteristic GSR particles on their hands. No GSR was detected on 58% of tested officers. This low detection rate of characteristic GSR on officers suggests that the opportunity for transfer of GSR from police to a POI during arrest is relatively low [165]. Berk et al. [164] studied the possibility of contamination in Chicago police facilities. To assess the potential secondary transfer, this study focused on surfaces that the POI's hands were likely to encounter during the investigation such as police facilities (arrest cells, interview rooms) or vehicles. In 89% of the specimens (178 out 201) no particles were observed containing Pb-Ba-Sb. However, a total of 56 particles containing one or two of the three elements were observed in the remaining 11% of specimens. The majority of particles (54 of the 56) were recovered from the police facilities (table and restraining bar), with the remaining 2 particles originating from police vehicles [164]. They concluded that a minimum of 3 particles containing the Pb-Ba-Sb combination are required for reporting positive IGSR

identification [164]. Recommended guidelines for minimising potential pollution were also proposed to assist police officers in minimising the potential for secondary transfer [164]. In contrast with those conclusions, higher levels of pollution were identified during another study conducted with the Special Police Forces in Sweden [167]. It has been hypothesised that the higher pollution levels were linked to the more active use of firearms by this particular police force [166, 167]. Similar observation were made in Australia through the work of Hales [168] who observed a much higher pollution of officers who were part of the special forces. Furthermore, a study carried out by Hannigan et al. assessed the risk of detecting IGSR particles by chance. They highlighted the fact that it is more likely to detect particles by chance on clothing than on hands, emphasising that IGSR particles are persisting longer on clothing than on the skin. They also stressed that detecting particles by chance still remains a rare event as they found a probability order of 0.02 for one or two 3-components particles [169].

It is important to note that the conclusions regarding IGSR transfer possibility [164, 165] are based on background studies and not on transfer experiments. These studies draw conclusions about a parameter taking place normally during an action (i.e. contact between two persons causing traces transfer) through the assessment of the level of pollution. A small number of studies involving transfer scenarios including contact between the donor (contaminated person) and the receiver (i.e. the POI) have been explored [149, 166, 170]. Charles and Geusens [166] conducted a transfer study involving simulation of the contact that could occur between police officers and POIs during an arrest. A high degree of pollution from the police officer was identified. They belonged to the special forces unit which, similarly to the study by Pettersson [167], had higher activity levels and proximity to firearms [166]. The risk of a secondary transfer is also

heavily dependent on the method of arrest. More GSR particles are transferred when the contact is longer or more vigorous [166]. French et al. [149] conducted a study on the secondary transfer of particles via handshaking between the shooter and a second person, and a second scenario including firearm exchanges. The number of IGSR particles transferred through handshaking was found to be high (average of 82 characteristic particles transferred, maximum of 129 particles). In the first scenario, the handshake was conducted immediately after the firearm discharge when the maximum amount of particles remains on the shooter's hands. For the second scenario involving the firearm exchange, a lower number of particles were transferred (average of 39.3 particles transferred, maximum of 86 particles) [149]. It is important to note that the second transfer is lower that the primary transfer (between the firearm and the shooter) and should be considered during the interpretation process [149].

In 2015, French and Morgan [170] extended their studies by evaluating the secondary and tertiary transfer through handshaking. They observed a progressive decrease in transferred GSR particles between the shooter and the two participants. However, particles were always detected after the third transfer (second handshaking) [170]. Therefore it was concluded that it could be useful to sample different people potentially involved in a firearm-related event because of the possibility of finding GSR on the second or the third person in contact with the potential shooter [170]. Similar observations were made in the study conducted by Brozek-Mucha in 2014 [171]. The aim of the experiment was to understand persistence and transfer of GSR particles to avoid any pollution in laboratories [171]. In 2011, a study carried out by Girvan measured GSR transfer from police officers who had fired five rounds of ammunition and soon afterwards subjected a "mock" suspect to either un-resisted or more physical

apprehension activities. GSR sampling was carried out immediately after "arrest". This "worst-case" scenario for secondary transfer resulted in the detection of a few three-component particles on average, but only 10% of each stub was searched for particles [172].

Only two studies have explored the transfer of OGSR [58, 173]. Arndt et al. studied the secondary transfer using IMS analysis after handshaking between the shooter and another person [58]. No OGSR compounds were observed on traces arising from the second person. However, several limitations, including a high LOD of the instrumentation, were highlighted, which could have contributed to this results [58]. The lipophilic nature and skin absorption of the compounds perhaps accounts for such a result, making OGSR less subject to transfer [58]. This hypothesis was further supported in a study carried out by Moran and Bell on the skin permeation of OGSR compounds [156]. It is clear that significant efforts to explore the activity level interpretation have been conducted in the IGSR area, but such a focus does not exist yet in the OGSR area, aside from studies into time since discharge [157-162]. Gassner et al. investigated three different scenarios just after discharge involving handshakes, transporting a firearm and arrests [173]. The secondary transfer of OGSR was observed for all scenarios when a more sensitive analytical method, UHPLC-QqQ-MS, was utilised. The firearm transportation scenario resulted in the lowest amount transferred, followed by handshaking with the arrest scenario resulting in highest amount of OGSR transferred [173]. These studies emphasised that the risk of a secondary transfer of both IGSR and OGSR is significant. Therefore, precautions are essential to avoid a transfer of GSR when a contact between police officers and non-shooters occurs and the possibility for secondary transfer of OGSR and/or IGSR must be considered when GSR case findings are evaluated.

2.3 Offence level (or level III)

An example of offence level question, as exposed in Figure 3, can be stated as below:

Did the POI shoot the victim?

An example of propositions set under the offence level may be illustrated as follow:

Hp: *The POI murdered the victim.*

Hd: *An unknown person murdered the victim.*

This question represents the highest level of the hierarchy of proposition and is considered through the judicial proceedings. To assess evidence under the offence level, the forensic scientist requires the same parameters as those for the level II namely, knowledge about the background, persistence and transfer of OGSR. Furthermore, this level of question requires a set of circumstantial information that is more substantial than for level II interpretation and this knowledge is generally not available to the forensic scientist, explaining why, the assessment of evidence under the offence level is not considered the domain of the forensic scientist [27, 119]. The forensic identification (source level interpretation) is not the main question of interest for a juror during a trial because its final aim is to draw an inference about offence level propositions (i.e. is the suspect the offender or not?) [112].

Level III – as with level II – does not depend of observation, which means we can assess no observed evidence. Indeed the level II and III depend of two kinds of information: analytical (from the expertise: “E” in the Bayesian theorem (Equation 1) and

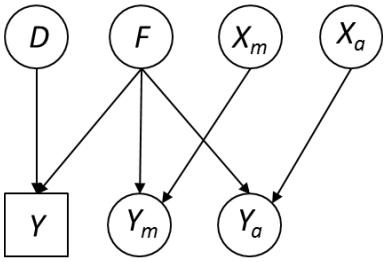
circumstantial (from the case: "I" in the Bayesian theorem (Equation 1) [119]. Therefore, we can evaluate negative observation (e.g. no GSR detected) in accordance with each proposition. Indeed, the fact of taking into account the circumstances of the case and the parameters of background, persistence and transfer enable to assess no observed evidence. On the other hand, the level I is a strict comparison, where the evidence is compared firstly to a potential source and secondly the occurrence frequency of the evidence is taken into consideration. Thereby we cannot interpret an evidence we do not observe under level I propositions.

In conclusion, as the level of proposition being addressed moves from source to offence, the potential value added by the scientific evidence increases, as does the relevance of the expertise. However, a greater amount of circumstantial information is required [119] that is not always available to the expert. Therefore, if we attempt to assess evidence under the activity, the collaboration between the forensic scientist, investigator and the defence is crucial to firstly build a set of propositions and secondly provide the suitable contextual information needed by the scientist for an appropriate interpretation framework [119]. In addition, at the offence level, the propositions are related to legal definition and the guiltiness in the actual offence (Hp/ Hd above) and forensic scientists do not participate in this debate.

2.4 Bayesian networks: graphical tools for forensic inference

BN are an alternative environment for the traditional LR calculation [174, 175]. BNs are graphical models with an underlying probabilistic framework that allows the articulation of a human reasoning through a numerical and graphical interface [176]. Complex BNs allow the propagation of the information, or new evidence, throughout the network by applying the logical framework of probabilities and making BNs truly adapted for assessing forensic evidence [126, 177, 178]. In recent years, BNs have taken an increasingly important role in evidence evaluation due to their capacity to manage the inclusion of multiple sources of uncertainty [121, 176-179] such as those discussed previously. When a firearms-related event occurs, expertise can provide different evidence relating to bullets, cartridges, firearms and GSR traces. It would be advantageous to combine those various pieces of evidence during the interpretation in order to reach a global assessment [121]. In 2006, Biedermann et al. [121] described a BN to combine the evaluation of the shooting distance analysis using GSR and bullet marks comparison. The BN is presented in Table 3.

Table 3. BN proposed by Biedermann et al. (2006) for the joint evaluation of firearm and GSR evidence [121][4].

BN	Nodes definitions
	D – *Shooting distance (D_{30cm}, D_{50cm}, D_{70cm}, D_{90cm})*
	F – *Questioned bullet fired by the POI's weapon*
	X_m – *Set of manufacturing characteristics in relevant population*
	X_a – *Set of acquired characteristics in the relevant population*
	Y – *Quantity of GSR particles observed on stubs*
	Y_m – *Manufacture characteristics of the questioned bullets*
	Y_a – *Acquired characteristics of the questioned bullets*

The BN is composed of two parts: the right part concerns the comparison between two bullets (questioned and reference) and the left part concerns the inference about the shooting distance based on the quantity of IGSR particles observed on the target. Both parts of the BN are linked through the node F: "the questioned bullet was fired by the POI's weapon" [121]. This node (F) represents the set of propositions influencing both results of bullets comparison and GSR analysis. Consequently, the assessment of manufactured and acquired characteristics of the questioned bullet (respectively Y_m and Y_a) is influenced by the set of propositions (F) and the manufactured and acquired characteristics of the reference bullets (respectively X_m and X_a). Likewise, the GSR outcome (Y) is influenced by the same set of propositions (F) and the distance shooting (D). Further work by Biedermann et al. [21] focused on the interpretation of GSR using results and data provided by Cardinetti et al [50]. The proposed BN focused on the evaluation of IGSR particle deposits [21] according to Cardinetti's approach. The BN was

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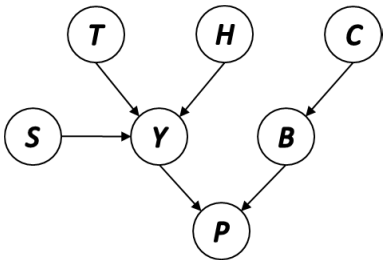
developed in order to include other parameters such as the presence of a background, contamination and efficiency of the recovery and analytical procedures [21, 180]. The BN they developed is presented in Table 4.

Table 4. proposed by Biedermann et al. [21] presenting the BN for IGSR particles interpretation[5].

BN	Nodes definitions
	T – *Time interval (in hours) between the event and the sampling* H – *Hypotheses Hp and Hd* C – *Degree of background of GSR particles on POI's hands*
	S – *Different experimental settings (specific to the case)*
	Y – *Number of particles due to the shooting of a firearm*
	B – *Number of GSR particles due to the background*
	P – *Total number of GSR particles observed*

These studies demonstrate that BNs provide a rigorous framework for evidence assessment and are ideal for scenarios involving complex reasoning [27] when the number of variables increases or multiple evidence types are involved. In addition, BNs are tools for interpreting evidence in a specific case (case-by-case) and they are thus dependent of the case information available. A paper published by Gauriot et al. [181] attempts to reach a general structure of GSR interpretation by adding a definite list of hypotheses in which the defence "picks up" one. The latter approach is however seen as part of the investigative (instead of evaluative) process by Gallidabino et al. in 2015 [182].

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In their commentary, they indeed emphasised the distinction between the investigative and evaluative opinion leading to common misunderstanding like in Gauriot et al. [181]

BNs appropriately support the scientific interpretation of evidence by offering the possibility to reach the requirements of transparency, impartiality, robustness and logic. However, dealing with multiple evidence types is not an easy task and that could easily lead to different reasoning errors during the BN building process. Furthermore a BN may be expressed at different levels of detail that is highly dependent on the available information that are empirical (general knowledge of GSR) and case-specific (circumstances surrounding the case) [21].

3. Conclusion

While the detection, recovery, and analysis of GSR, both inorganic and organic, are very well covered by the literature, peer-review data and information about the interpretation of GSR are not as common and are rather *ad hoc*. This literature review has attempted to address this gap and presents current perspectives in the interpretation of GSR in forensic science.

Most of the difficulties in the field of GSR relate to the subsequent evaluation of the analytical results in the context of the specific circumstances of the investigation and the limits they impose upon GSR transfer and persistence. Appropriate interpretation of the evidence should (1) help the investigator in orientating the investigation and (2) provide a balanced, robust and transparent expert opinion for assisting the trier of facts. Current

research continues to focus on OGSR detection through the development of a variety of methods that are increasingly more sensitive but often more complicated. However, the test outcomes must be included into an adequate interpretation structure to increase the contribution of the evidence during the decision-making process of the particular case. Indeed, stakeholders' questions often relate to POI activity, which is far beyond the simple detection of OGSR (or IGSR for that matter). This can be achieved through an appropriate method of interpretation provided by the logical framework of Bayes' theorem. In this area, BNs appear particularly promising.

Nevertheless, forensic science has to deal with the complexity of real-world casework which complicates the interpretation and make the Bayesian framework difficult to practically use – not only in GSR evidence. This mainly arises from lack of available data relevant to questions of transfer, persistence and background of evidence at the disposal of forensic scientists that would allow them to proceed to the interpretation. Other issues arise from the legal system and concern the context of circumstances that are not always clearly defined and communicated by parties to the forensic scientist or difficulties of a jury to understand the presentation of evidence by the scientist in front the court. Further, the introduction of lead-free ammunition will result in the types of particles traditionally used to identify GSR being absent; studies of non-GSR sources of particles resembling those from lead-free ammunition are fewer and novel approaches to both detection and interpretation of evidence are required.

4. Project aims

For casework involving firearm related events, a better understanding of OGSR traces detection and interpretation is needed. There is a growing push from government and health institution for a reduction in the exposure to lead and heavy-metals (Hunting season or shooting range facilities), which might increase the prevalence of lead-free and non-toxic ammunition in the general population [52]. Only through thorough experimentation can the behaviour of these traces linked to the use of these ammunition types be understood.

Consequently, the aim of this project was to provide a more comprehensive knowledge about OGSR behaviour in a context of firearm-related activities to ultimately bring a better support to police investigations and court proceedings. Such studies are becoming increasingly essential in order to understand and to meaningfully interpret OGSR traces for the different stakeholders involved in the investigation process.

Figure 5 represents a general timeline of an investigative process. It can be seen that a POI(s) is usually not apprehended at the scene, and therefore traces might have been altered by the activities undertaken after the possible firearm discharges. It is essential to assess how OGSR behave in time by investigating the persistence of such compounds, especially when investigative questions such as "*Is it worthwhile to undertake specimen collection from the POI for OGSR analysis after a certain number of hours?"* are raised. In addition, in an interpretative perspective, the persistence might be of high interest when questions such as "A*re the OGSR traces recovered from the POI's hands in accordance with the sequence of events under investigation?"* are asked by the different stakeholders.

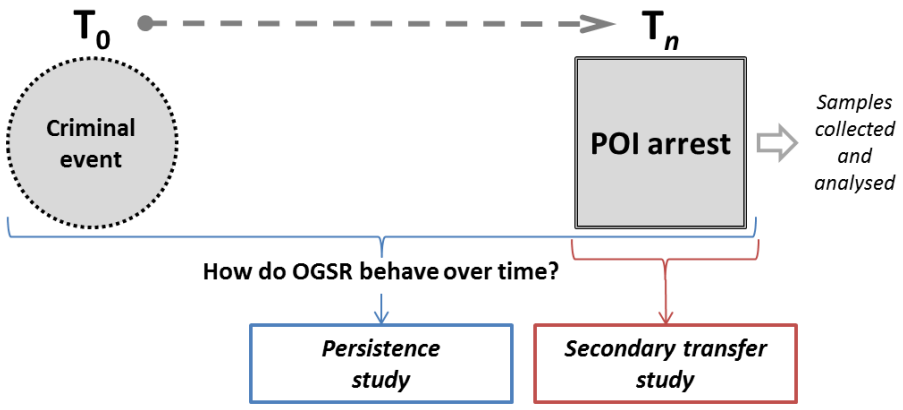


Figure 5. Questions of activities in an investigative context regarding OGSR.

Intrinsically connected to the question of OGSR retention on POI's hands is the question of a potential of transferring these traces from a POI who discharged a firearm to another individual, e.g. a non-shooter (Figure 5). Because OGSR compounds are transferred onto the hands of the shooter during and after the firearm discharge(s) process, the traces might also be susceptible to be further transferred onto other surfaces such as a non-shooter. These considerations induce a non-negligible risk of pollution of a non-shooter by someone who discharged a firearm. Hence, several questions can be raised in order to investigate the possible secondary transfer of OGSR, for instance:

(1) Is a pollution of the POI's hands through a contact with a Police Officer during an arrest possible (Figure 5)?

Another possible risk of legitimate pollution may arise from handling a firearm:

(2) Is handling a firearm (without discharging it) sufficient to transfer OGSR?

The premises behind the question raised in point *(1)* is the fact that, daily, police officers have contact with their own service weapon. Consequently, it is legitimate to raise the question about potential pollutions could occur during the circumstances of a POI arrest. As an arrest process involves a more or less vigorous contact between the police officer and the POI(s), which might lead to a secondary transfer of OGSR from the police officer onto the POI's hands, ultimately leading to significant risk of false positives results.

The last aim of the project relates to integrate the results obtained from the persistence and secondary transfer studies into an appropriate interpretative reasoning framework. Such an interpretation model is based on the Bayesian theorem, which is currently the most adequate framework for interpreting forensic evidence. This part aimed to assess likelihood ratios (LR) for different scenarios involving different circumstances.

The holistic project aim was to develop and improve the general knowledge about the OGSR evidence and, with the evaluation of LRs, to assess the utility of such traces as a relevant forensic material when casework involves firearm-related traces. Finally, the purpose of this project is to assess if OGSR could be considered as a reliable complementary source of information to the current inorganic GSR particle analysis guidelines and protocols [101].

CHAPTER THREE: EXPERIMENTAL RESEARCH

A global representation of OGSR research studies is presented in Figure 6 under the form of an iterative process. The iteration may arise as the composition of the propellant powder may evolve in the future. Therefore, the analytical studies as well as the forensic parameters will need to be re-addressed by considering the new compounds candidate. The circle highlights the main parameters to consider when studying OGSR traces. In Figure 6, the current project studies are represented by the red framed parameters.

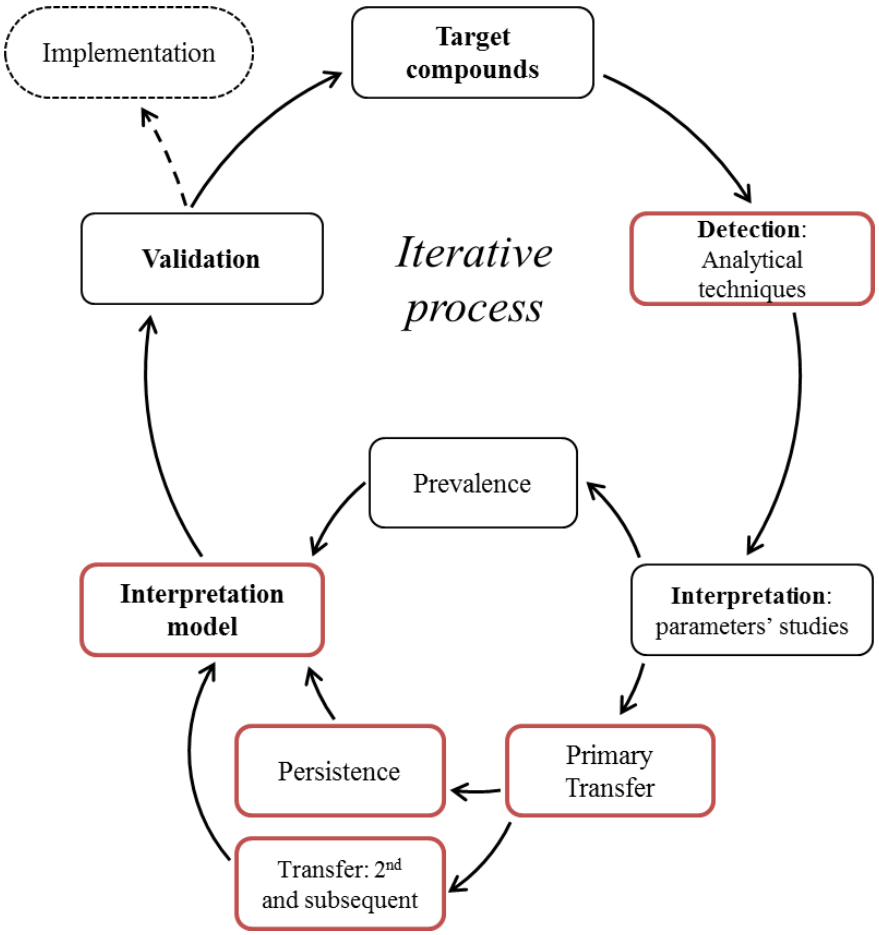


Figure 6. Organic Gunshot Residues (OGSR) iterative research circle. The current project studies are circled in red.

This chapter is articulated around the different research aims presented in Figure 6. And presented as follows:

To ensure an appropriate detection of OGSR, Section I presents the analytical method used to detect OGSR target compounds and its related validation. Section II tackled the analysis of the propellant powder before the firearm being discharged. This section aimed to determine what compounds are present within the propellant powder and therefore what compounds can be expected from firearm discharges. The second project tackled in Section II relates to the stability of the OGSR compounds after being extracted in order to assess how long the specimens can be stored without risking a degradation.

Section III approaches the persistence study of the OGSR compounds on the hands of the shooter. Several time points between T0, representing the amount detected immediately after the discharge up to 4 hours after the discharges. Finally, Section IV approached the question of the secondary transfer of OGSR in two different scenarios: an arrest process and handling a firearm without discharging it.

Throughout this project, four target compounds were chosen based on their relevance to propellant powder and supported by previous studies involving OGSR [41, 85]**.** It included three main compounds: DPA, EC and MC. The fourth compound is a nitroso-derivative of DPA, *N*-nDPA. These compounds were chosen as they are well characterised (Figure 7), through the extensive literature about OGSR detection and identification, as being part of the residues produced during the shooting process.

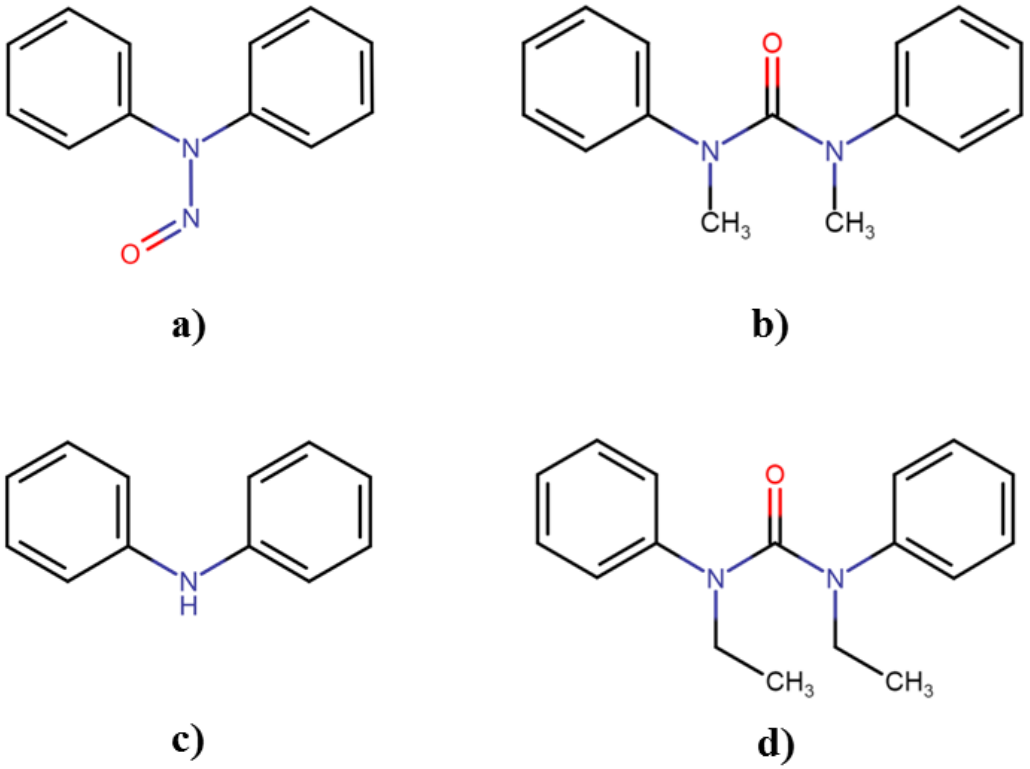


Figure 7. Target compounds identified as being part OGSR.
a) *N*-nitrosodiphenylamine – *N*-nDPA, b) Methylcentralite – MC, c) Diphenylamine – DPA, d) Ethylcentralite – EC.

Section I: Analytical method and validation protocol

1. Material and analytical method

1.1 OGSR standards and analytical reagents

The targeted compounds were chosen depending on their affiliation to OGSR based on the current literature available on the detection of OGSR (Table 5) [42]. A more comprehensive target compound list was avoided to reduce the analysis time, with the most relevant and common compounds present in propellant powder selected.

Table 5. Standard of compounds of interest.

Compounds	Supplier	Concentration	Solvent
N-nDPA	Novachem superior standard	1000 µg/mL	Methanol
MC	Novachem superior standard	100 µg/mL	Methanol-Acetonitrile (1:1)
DPA	Novachem superior standard	1000 µg/mL	Methanol
EC	Novachem superior standard	100 µg/mL	Methanol-Acetonitrile (1:1)
d10-DPA (IS)	C.D.N Isotopes	*Solid*	-

Stock solution of internal standard, d10-DPA, prepared at a concentration 1000 µg/mL (1000 part per million - ppm) in Methanol: Acetonitrile (1:1) v/v. d10-DPA was selected as the internal standard for its similar ionisation and fragmentation response to the analytes of interest. It was also used in research conducted by Ali et al. in 2016 regarding the detection of OGSR by LC-MS/MS in Pittsburgh police stations [183].

1.2 Analytical method: UPLC-QqQ-MS

The chromatographic separation was conducted using a Waters UPLC ACQUITY® system. The chromatographic method was adapted from a previously published method [85]. However, the developed method utilised UV detection. In order to increase the sensitivity of the analytical method in this research, the UPLC system was instead coupled to a Waters triple quadrupoles tandem mass spectrometer (QqQ-MS).

1.2.1 UPLC chromatographic method

The mobile phase was composed of methanol and Ultrapure Water (Table 6) to which 0.1% (v/v) formic acid was added to enhance the ionisation. The water solvent was an ultrapure Milli-Q® Water (18.2 MΩcm, Q-POD®, Merck KGaA). Before being injected into the UPLC system, the water solvent was filtered through a 0.2 µm membrane filter composed of mixed cellulose ester (47 mm, Advantec, grade A020H047A) and the methanol was similarly filtered through 0.2 µm PTFE membrane filter (47 mm, Advantec, grade J020A047A). Both solvents were degassed through sonication for two minutes in preparation for analysis.

Table 6. List of reagents used for samples preparation and UPLC-QqQ-MS analysis.

Reagents	Grade	Provider	Usage
Acetone	AR	Chem-supply Pty.	Sample preparation
Acetonitrile	LC-MS	HONEYWELL Burdick & Jackson®	Sample preparation
	Hypergrad Lichrosolv®	Merk Pty	Needle wash
Methanol	LC-MS	HONEYWELL Burdick & Jackson®	Sample preparation
	Hypergrad Lichrosolv®	Merk Pty	LC mobile phase
Water	MilliQ® 18.2 MΩcm	-	LC mobile phase
Formic acid	AR	AJAX Chemicals	LC mobile phase additive
Isopropyl alcohol	EMSURE®	Merk Pty	Needle wash

A 4.6% increase per minute of the organic solvent (methanol) gradient was used for the chromatographic separation of the sample [85]. The parameters of the UPLC system and the gradient composition are presented respectively in Table 7 and Table 8.

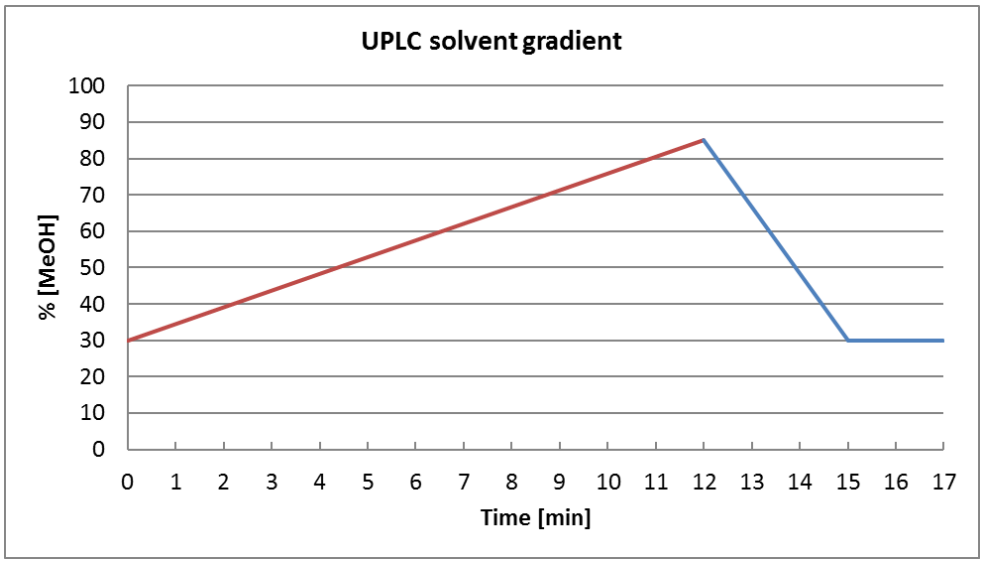
.

Table 7. UPLC system conditions.

Injection volume	2.00 µL
Flow rate	0.8 mL/min
Column	Agilent Eclipse RRHD XDB-C18, 3x10mm Particles size : 1.8µm
Guard column	Agilent UPLC guard, Eclipse XDB-C18, 3mm Particles size: 1.8µm
Column temperature	43 °C
Sample temperature	8 °C

Table 8. UPLC Gradient conditions. The color code refers to the different phase of the gradient.

Task	Time	Aqueous solvent: *Water + 0.1% v/v formic acid*	Organic solvent: *Methanol + 0.1% v/v formic acid*
Run time	0.00 min	70 %	30 %
	12.00 min	14.80 %	85.20 %
Wash period and stabilisation for subsequent runs	*15.00 min*	*70 %*	*30 %*
	17.00 min	*70 %*	*30 %*



1.2.2 QqQ-MS conditions

In order to maximise the sensitivity of the system, the detection was achieved using a QqQ-MS, a commonly used detection system for OGSR and explosives [39, 41, 85, 86, 183]. The source used was an electrospray ionisation (ESI) as it is nowadays the most popular source for QqQ analysis [184]. The detection method was carried out using Multi-Reaction-Monitoring (MRM) which enables to scan several product ion transitions for one or more precursor ions. The MRM mode is currently preferred to manual integration as it provides a faster and less biased analysis with a higher degree of automation. The conditions used in the Waters TQD® system are presented in Table 9.

Table 9. QqQ-MS conditions.

Ionisation source: ESI	
Source temperature	140 °C
Desolvation temperature	250 °C
Scanning time	12.00 min

The Waters optimisation software IntelliStart® was used in order to determine the cone and capillary voltage for two product ions for the precursor of each target molecules. The optimisation was achieved through a direct injection of each compound at a concentration of 1 ppm. The identified MRM transitions are presented in Table 10.

Table 10. QqQ-MS MRM transitions.

Time window [min]	Compounds	Precursor ion [m/z]	Product ions [m/z]	Cone Voltage [V]	Capillary Voltage [V]	ESI Polarity
0.0 to 8.1	***N*-nDPA**	199	Ion#1: 66	26	24	+
			Ion #2: 169	26	12	
	MC	241	Ion#1: 106	32	26	+
			Ion#2: 134	32	16	
7.8 to 9.2	**DPA**	170	Ion#1: 65	42	32	+
			Ion#2: 93	42	30	
	d10-DPA (IS)	180	Ion#1: 71	42	42	+
			Ion#2: 98	42	28	
9.0 to 12.0	**EC**	269	Ion#1: 120	28	24	+
			Ion#2: 148	28	14	

1.2.3 Software and data extraction

The detected peaks were integrated using the Waters QuanLynx® software. The presence of precursors to both product ion transitions was a required condition for considering the compounds as detected. The peak integration was calculated using the MRM algorithm which returns the abundance of the peak of interest based on the TIC MRM chromatogram. All peak integrations were manually checked before being exported as text file (.txt) for further processing into MS Excel®.

1.3 Method validation protocol

The optimised method underwent a validation process to ensure it was fit for purpose for the chosen target compounds. The validation protocol adopted was the internationally accepted ICH harmonised tripartite guidelines [185], as no specific forensic science guideline is currently available for OGSR analysis. According to the ICH guidelines, several parameters were assessed in the context of this validation which are the *specificity*, a*ccuracy*, *precision*, *linearity*, *LOD)*, and *robustness* (Table 11).

Table 11. Validation protocol according to the ICH guidelines [185].

Studied Parameters (ICH Guideline)		Samples
Specificity	*Selectivity*	Internal standard and compound injected independently
Linearity	*Calibration curve* *LOD*	7 points ranging from 0.01 ppm to 5 ppm
Accuracy and precision (level I)	*Repeatability – Quality Controls (QC)*	QC1: 0.05 ppm QC2: 0.5 ppm QC3: 5 ppm
Robustness	*Column temperature +/- 1 degree*	QC2: 0.5 ppm
	Solvent composition +/- 5% [MeOH]	
	Flow rate +/- 0.05ml/min	
Intermediate precision (level II)	*Procedure repeated over 2 days*	

The specificity represents the unequivocal determination of the presence of an analyte among compounds which might be expected [185]. It involves the analysis of each analyte separately (10 ppm concentration) with the aim to assess any interferences occurring between the set of compounds of interest.

The linearity depicts the ability to obtain concentration results which are directly proportional to the amount of analytes present over a specific range of concentration [185]. It was assessed by the injection of a 7 point calibration curve with a concentration ranging between 0.01 ppm to 5 ppm (Table 12).

Table 12. Calibration curve concentration range and QC.

Concentrations [ppm]						
1	2 (also QC)	3	4	5	6	7
0.01	0.05	0.1	0.5	1	2.5	5

The LOD is described by the ICH guideline as the minimum amount of an analyte which can be detected but not necessarily quantified [185]. It is calculated as shown in Equation 2:

$$Limit\ of\ Detection\ (LoD) = \frac{3.3 * \sigma}{S}$$

Equation 2. LOD equation, where σ represents the standard deviation of the analytes at the lowest concentration (0.01 ppm) and S represents the slope of its calibration curve [185, 186].

Along with the calibration standards, a set of 3 QCs were also analysed. Their concentrations cover the range of the calibration domain with a respective concentration of 0.05 ppm (QC1), 0.5 ppm (QC2) and 5 ppm (QC3). The accuracy of the method is assessed by calculating the concentration of each of them based on the respective calibration curves. The repeatability is evaluated by injecting the QCs in triplicate and measured by calculating the %RSD.

The robustness is defined as the determination of the ability of an analytical procedure to remain unaffected by minor changes in the method [185]. The robustness was studied by making small, intentional changes to various method parameters. For this study, the selected parameters were the column temperature, the solvent composition and the flow rate were modified to evaluate if a minor change affected the analysis. The robustness was studied by injecting a 0.5 ppm mix of the target compounds. The relative retention times to the internal standard (RRt) were calculated to assess the reliability of the method.

Finally, the validation protocol was repeated over 2 days in order to assess the intermediate precision of the method and the consistency of the results over 2 close days of analysis.

1.3.1 Data normalisation and pre-processing

The data resulting from the validation protocol were normalised to the internal standard (d10-DPA). In order to prepare the data for further analysis, the square root of the ratios to IS were also calculated. The premise behind the square root pre-processing is to reduce the effect of large peaks over smaller ones and therefore balancing the weight and the influence between data arising from large and smaller peaks [187, 188].

In order to be able to reliably use the data transformation on the results of this project, it was necessary to validate the normalisation by applying it to the validation data. The normalisation process is defined in Equation 3.

$$Normalised\ Peak\ area = \sqrt{\frac{Peak\ Area_{Analyte}}{Peak\ Area_{Internal\ Standard}}}$$

Equation 3. Normalisation of the peak area for each analyte targeted.

2. Results and discussion – analytical validation

Throughout the project, the analysis is carried out semi- quantitatively. Determining the concentration of OGSR is not considered as relevant. Indeed the amount present in the propellant powder is unknown and highly variable from ammunition to ammunition, batch to batch and even cartridge to cartridge [1, 64]. Furthermore, the number of factors involved in the production, deposition and retention of GSR are numerous. It includes the type of firearm, the type of ammunition, environmental conditions as well as the properties of the shooter (skin, hairiness and clothing). All these factors are typically unknown and have a significant impact on OGSR traces and therefore the quantitation of such traces does not convey additional details regarding the interpretation of the traces in the context of cases involving GSR.

The validation was successfully achieved by following the ICH guidelines as developed in subsection 1.3 (p. 56). A representative total ion chromatogram (TIC – MRM) acquired by UPLC-QqQ-MS is displayed in Figure 8.

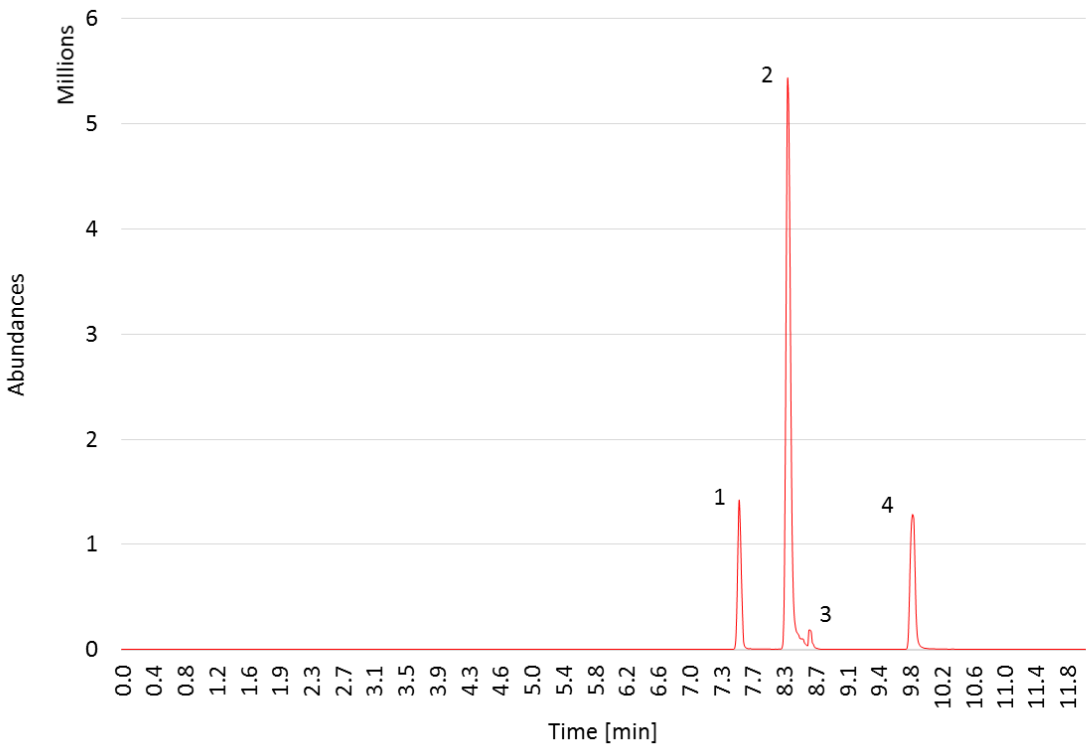


Figure 8. A representative Total Ion Chromatogram (TIC MRM) at a concentration of 0.5 ppm; 1= MC and *N*-nDPA, 2= D10-DPA (IS), 3= DPA, 4= EC.

The RRt are presented in Table 13. It was found that MC and *N*-nDPA as well as d10-DPA and DPA were co-eluting. The RRt were found to be stable over time with a %RSD below 0.0625 (Table 13).

Table 13. Relative retention time (RRt) of each compound of interest (n= 82).

RRt	*N*-nDPA	MC	DPA	EC
Average [min]	0.937	0.936	1.018	1.187
Std deviation [min]	0.006	0.004	0.001	0.002
% RSD	0.625	0.396	0.119	0.163

The co-elution on the chromatogram between MC and *N*-nDPA as well as d10-DPA and DPA did not present an issue from an analytical perspective as the two MRM transitions facilitated the calculation of accurate peak areas [184]. All the MRM transitions for each compounds of interest can be found in the Appendix II.1. All the targeted compounds were considered detected when the abundance was found above the LOD (Table 14).

The data were pre-processed with the square root (Equation 3). The validation data were also pre-processed with the square root in order to ensure that the pre-processing did not skew the results of the analysis. Several advantages of using the square root were identified in the literature [187]. Firstly, the square root allows reducing the effect of large values (IS) over smaller ones (targeted compounds) by improving the balance between small peak and larger ones. Secondly, the uncertainties in small values does not have a major effect on data analysis unlike other pre-processing, such as the logarithm [187].

The complete validation results are presented in Table 14, with the method found to be fit for purpose.

Table 14. Validation results: Summary table.

Parameters		Samples	Results
Specificity	*Selectivity*	Separated standards	Interference < 19 %
Linearity	*Calibration curve*	7pts: 0.01 to 5 ppm	$R^2 > 0.995$
		LOD	*N*-nDPA: 5.64×10^{-03} ppm
			MC: 1.75×10^{-04} ppm
			DPA: 2.09×10^{-03} ppm
			EC: 3.82×10^{-04} ppm
Accuracy/ precision (level I)	*Repeatability – Calibration curve and QCs*	QC1: 0.05 ppm	> 92 % accuracy
		QC2: 0.5 ppm	> 91 % accuracy
		QC3: 5 ppm	> 90 % accuracy
Robustness	*Column temperature +/- 1 ºC*	QC2: 0.5 ppm	97 % < RRt < 101 %
	Solvent composition +/- 5 % methanol		
	Flow rate +/- 0.05 ml/min		
Precision (level II)	*Repeated over 2 days*		% RSD < 15 %

2.1 Specificity

The specificity of the method was assessed by injecting each compound separately to ensure there was no interference between them. The data generated are presented in Figure 9. The data processing involved normalising the compounds abundance to the targeted compounds. Accordingly, each compound was detected with abundance lower than 10%, except the internal standard d10-DPA which was once detected at a level of 18.7% (sample of *N*-nDPA). It was considered as a carry over as it was not detected in any of further samples.

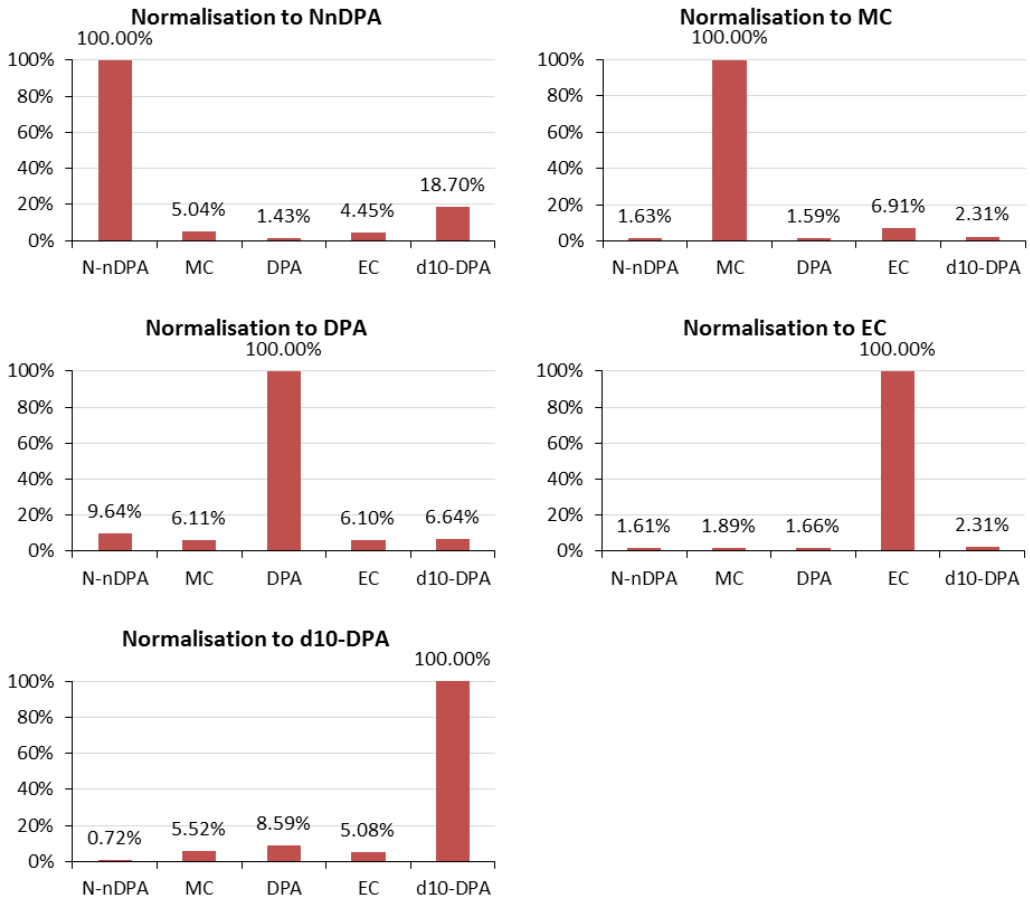
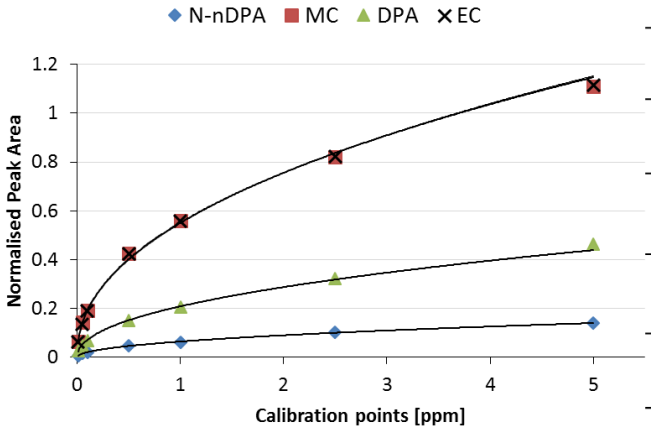


Figure 9. Assessment of the specificity of the analytical method. Each compounds investigated is normalised to itself.

2.2 Linearity and limit of detection

The linearity was assessed by generating a 7 point calibration curve ranging between 0.01 ppm and 5 ppm. The linearity criterion is fulfilled as the coefficient of determination, R^2 (RSQ), is greater than 0.995 for each of the targeted compounds (Figure 10).



Compounds	Regression Equation	RSQ
N-nDPA	$y= 0.0645x^{0.4821}$	0.9972
MC	$y= 0.5515x^{0.4549}$	0.9989
DPA	$y= 0.2088x^{0.4612}$	0.9951
EC	$y= 0.5484x^{0.4614}$	0.9990

Figure 10. Summary of the linearity of the calibration curves. The normalisation of the peak area is performed as detail in 1.3.1 (p. 59). Separated calibration curves and QCs are available in the Appendix II.2.

The LOD were calculated according to the ICH guidelines. The average LOD values ranged between 1.75×10^{-4} and 5.64×10^{-3} ppm (MC and *N*-nDPA respectively). The LOD can be found in Table 14 (p. 63). These values are acceptable for analysing samples of compounds of interest at trace level.

2.3 Accuracy and precision

The accuracy and repeatability sustain the results of linearity, three QCs were analysed along with the calibration curve (0.05 ppm, 0.5 ppm and 5 ppm). The concentration calculated for each compound present in each QC report accuracy greater than 90 % (Table 15).

Table 15. Accuracy and precision for the targeted OGSR compounds analysed by UPLC-QqQ-MS.

		Average Normalised Peak area	Standard Deviation Normalised Peak area	Repeatability [%RSD]	Concentration [ppm]	Accuracy [% Difference]
N-nDPA	QC1 [*0.05 ppm*]	0.02	1.50E-03	**9.75**	0.054	**8.37**
	QC2 [*0.5 ppm*]	0.04	4.20E-03	**9.00**	0.51	**2.68**
	QC3 [*5 ppm*]	0.14	6.00E-04	**0.45**	4.88	**2.28**
MC	QC1 [*0.05 ppm*]	0.14	1.80E-03	**1.31**	0.047	**5.12**
	QC2 [*0.5 ppm*]	0.42	1.80E-03	**0.43**	0.54	**8.61**
	QC3 [*5 ppm*]	1.09	8.20E-03	**0.74**	4.50	**9.94**
DPA	QC1 [*0.05 ppm*]	0.05	1.10E-03	**2.23**	0.046	**7.99**
	QC2 [*0.5 ppm*]	0.15	4.00E-03	**2.68**	0.49	**1.75**
	QC3 [*5 ppm*]	0.46	7.30E-03	**1.58**	5.53	**10.69**
EC	QC1 [*0.05 ppm*]	0.13	1.60E-03	**1.17**	0.047	**7.05**
	QC2 [*0.5 ppm*]	0.41	5.00E-03	**1.21**	0.53	**6.99**
	QC3 [*5 ppm*]	1.10	4.70E-03	**0.43**	4.54	**9.16**

The intermediate precision of the method was assessed as adequate with an inter-day %RSD below 10%, with the exception of DPA – QC3 (10.7%), for each compound present in each sample representing each point of the calibration curve (Figure 11 and Table 16).

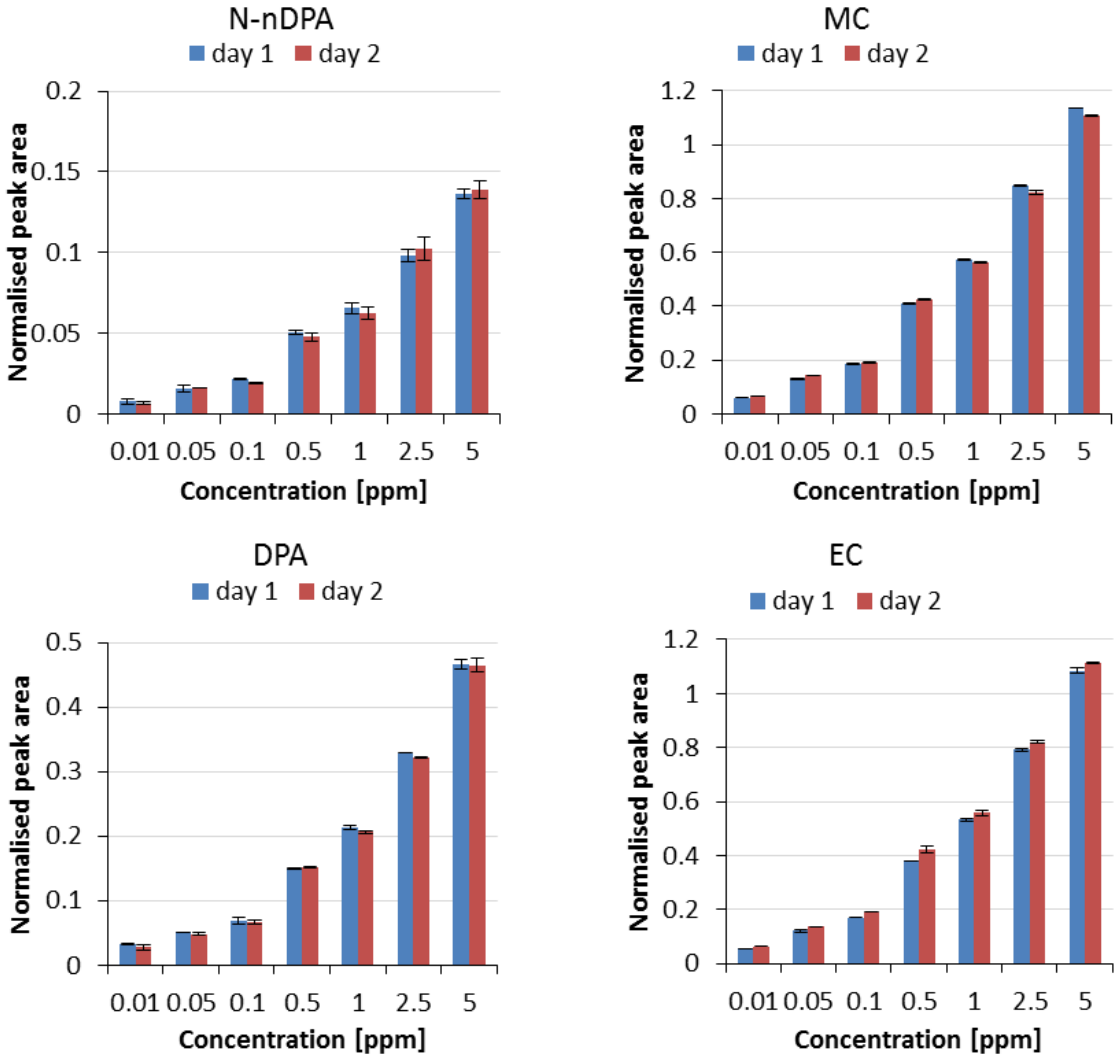


Figure 11. Inter-day of the analysis of calibration curves. The error bars represent the standard deviation between the two days of analysis.

Table 16. Inter-day %RSD of the normalised peak area of each compounds of interest.

	%RSD between two days of analysis			
Calibration curve points	*N*-nDPA	MC	DPA	EC
0.01 ppm	14.47%	4.48%	12.18%	6.34%
0.05 ppm	7.60%	4.36%	3.26%	5.70%
0.1 ppm	6.70%	2.47%	5.96%	5.03%
0.5 ppm	4.34%	2.29%	0.75%	4.85%
1 ppm	4.50%	1.26%	1.86%	2.60%
2.5 ppm	5.30%	1.88%	1.42%	2.08%
5 ppm	4.24%	1.39%	2.06%	1.64%

2.4 Robustness

The robustness involved changes in the chromatographic method. The results are shown in Figure 12. It displays the retention time (Rt) for each compound after each of the changes to the analytical conditions.

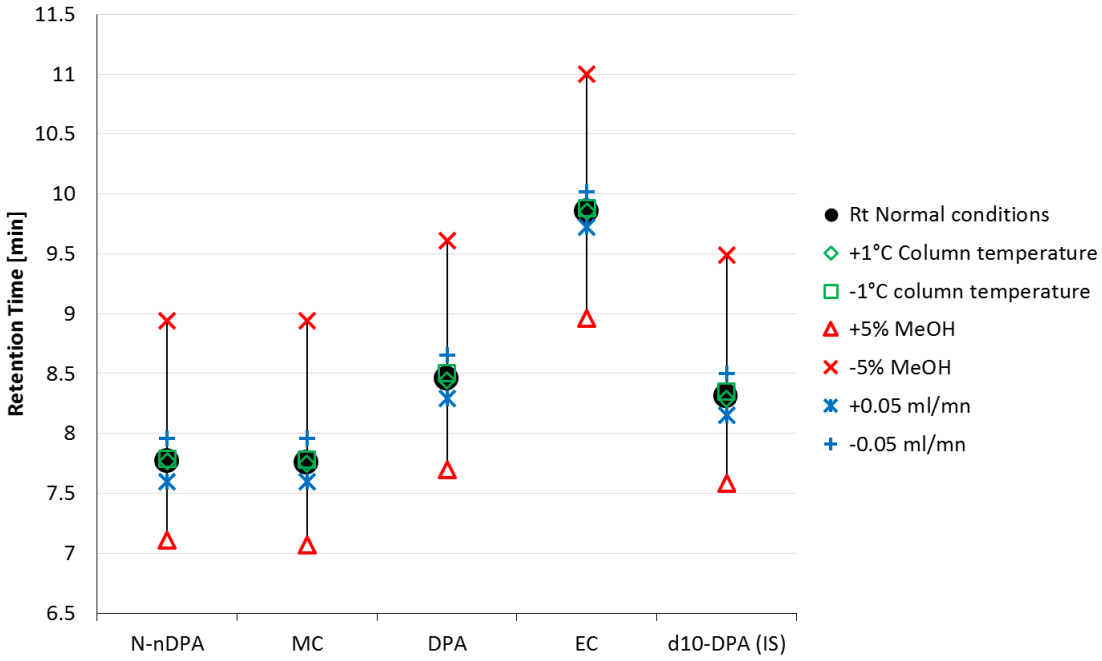


Figure 12. Robustness results. The details of each parameters are available in Appendix II.2.

It can be observed that for every change operated on the analytical method, all compounds showed similar shifts in their respective Rts. This observation highlights that the modifications applied to the method had a similar effect on each targeted compound.

When the Rt of each compounds of interest was normalised to the internal standard (d10-DPA), the RRt were found to be stable (Figure 13). A variation between 97% and 101% was observed when compared to the corresponding RRt observed under normal conditions. Such results highlights the reliability of the method to keep consistent results, even with small alterations of the analytical method.

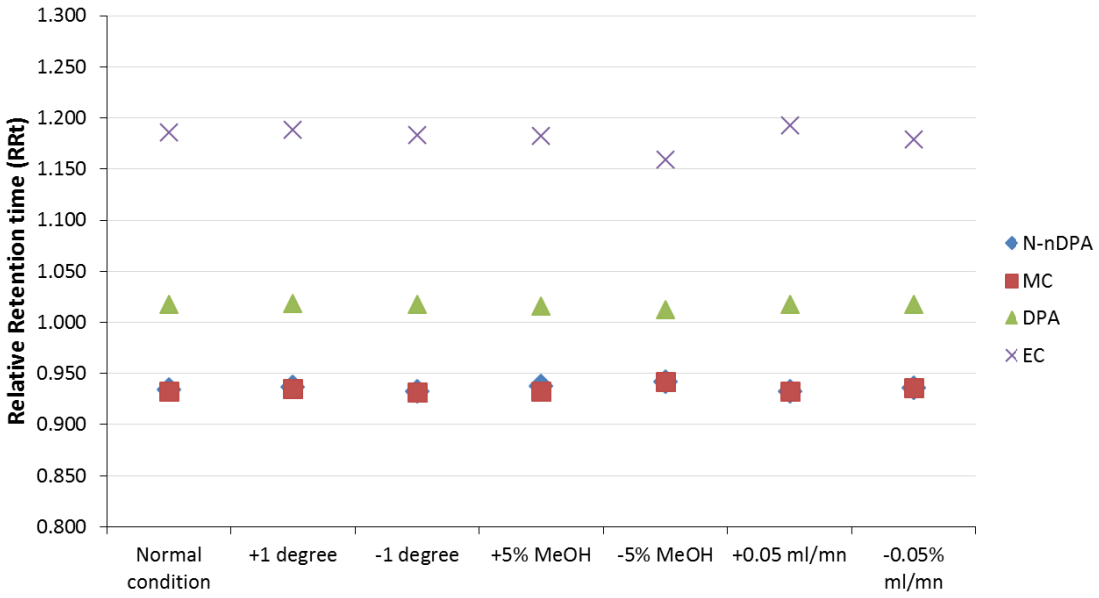


Figure 13. Relative retention time (RRt) for each parameters assessed for the analytical method robustness. The normal conditions represent the optimised conditions of the UPLC-QqQ-MS method. Detailed results are available in Appendix II.2.

3. Conclusion: Impact on subsequent samples analysis

The detection of OGSR is performed qualitatively. Indeed, the determination of the concentration of each targeted compound is not considered relevant as the original amount present in the ammunition and deposited on the shooter is unknown. Therefore, such information does not provide meaningful information regarding the discharging process. The analytical validation, per ICH guidelines [185], enabled to determine the method as fit for OGSR analysis purposes. The criterion of specificity, linearity, accuracy and robustness were fulfilled for the analysis of the four compounds of interest: *N*-nDPA, MC, DPA and EC. The analytical method was therefore used in order to detect OGSR originating from different conditions inherent to the persistence and secondary transfer studies.

The instrument used was part of a governmental laboratory and therefore was required to fulfil other analysis beside this project on OGSR. Consequently, it was essential to control the quality and performance of the system before every analytical run. To do so, each worklist of specimens analysed by UPLC-QqQ-MS started with the injection, in duplicate, of a five-point calibration curve (concentration between 0.01 ppm & 1 ppm) and one quality control (QC, concentration 0.05 ppm). A fresh calibration curve was run every time to ensure that the sensitivity and the linearity criterion of the instrument was fulfilled for the analysis of the specimens of interest.

Section II: Propellant powder analysis and stability study

The first part of this section concerns the analysis of the propellant powder. The aim was to determine what compounds of interest are present in the propellant powder before proceeding to the ammunition discharges in order to ensure that the compounds detected in OGSR specimens are arising from the ammunition propellant.

The second part of this section relates to the stability study. The aim of this study was to provide additional information regarding the stability of OGSR compounds after extraction. The aim is to assess the period of time for which specimens can be stored.

Nowadays, forensic laboratories have to deal with an increasing number of specimens that may ultimately lead to backlogs. Moreover, it is not unusual to have analytical instrumentation break down, such as UPLC-QqQ-MS, which eventually leads to delays in the specimen analysis. In addition, decisions have to be made regarding the prioritisation of specimens in order to reduce the cost while optimising the chance of results. These different factors which impact routine analysis require specimens to be stored, typically at 4 °C. It is consequently essential to study the stability of OGSR in order to assess the timeframe available after the extraction to analyse specimens (by UPLC-QqQ-MS) in order to minimise the risk of degradation. Additionally, the prioritisation of specimens can be improved by acquiring such knowledge about the stability of OGSR.

The stability study is split in two sections. The first section focused on the stability of OGSR compounds standards while the second section focus on the stability of the same compounds but collected from the discharge of a revolver.

1. Methodology

1.1 Propellant analysis

The analysis of the propellant powder was the first step in order to identify and confirm the presence of the molecule of interest. For that purpose, three cartridges of each ammunition (Table 17) studied during this project were opened, with the propellant powder removed and stored at 4 °C. The powder was extracted and analysed within 24 hours of the cartridges being opened.

Table 17. Ammunition and propellant powder investigated during this project. .40 S&W= .40 Smith and Wesson.

Nominal calibre - imperial *(metric)*	Ammunition
.40 S&W *(10 x 22 mm)*	Winchester WinClean® 180 Gr. Brass Enclosed Base
.357 Magnum *(9 x 33 mm R)*	PPU Ammunition® 158 Gr. Semi-Jacketed Hollow point (SJHP)

Three samples were prepared from each propellant powder (nine samples per calibre, Table 18). 10 mg of powder were weighed and placed in 10 mL of MeOH: ACN (1:1, v/v). The solution was sonicated for five minutes at room temperature. The solution was then filtered through a 0.2 µm PTFE filter to remove remaining particles. Then the solution was diluted 1000 times in order to avoid overloading the analytical system. The samples were analysed by UPLC-QqQ-MS per described in Section I.

Table 18. Propellant powder analysis samples list.

Calibre	Propellant powder	Samples
.357 Magnum	A	A1
		A2
		A3
	B	B1
		B2
		B3
	C	C1
		C2
		C3
.40 S&W	D	D1
		D2
		D3
	E	E1
		E2
		E3
	F	F1
		F2
		F3

1.2 Specimens arising from shooting experiments:

1.2.1 Specimens nomenclature

A significant number of specimens were collected throughout the project, with specific names assigned. Blanks and specimens were collected from both hands of the shooter, and it was important to differentiate the hands by their role in the firing process, the dominant hand is typically the one with a firm grip of the firearm and pulls the trigger while the non-dominant is the hand that supports the firearm from the side or the bottom depending on the position adopted (Figure 14).

In addition, the construction of the firearm is another criterion to consider. A semi-automatic pistol will have an ejection port with a position that may change from firearm to firearm according to the brand and model. The ejection port position is another important factor in the deposition of GSR as the residues predominantly escape the firearm during the breech opening and the cartridge ejection (Figure 14).

This distinction allows the use of the same specimens label and nomenclature whether the shooter is right-handed or left-handed. Subsequently, both hands are distinguished based on their degree of implication in the shooting process rather than their physiological position. The dominant hand represents the favourite hand of the shooter (or POI) which is the most likely to have pulled the trigger in an ordinary shooting event configuration (Figure 14).

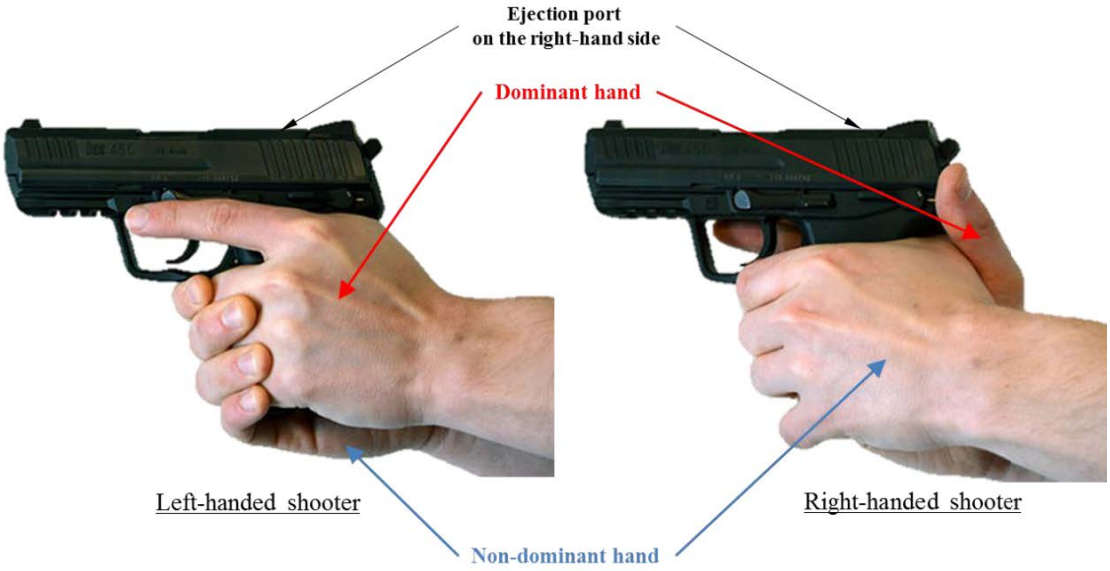


Figure 14. Illustration of the position of the hands for a left handed and right handed shooter configuration[6].

[6] Images imported from Google Image

1.2.2 Collection and extraction protocol

The collection of OGSR traces was conducted using SEM-EDX stubs. They were selected based on their widespread use in police services for GSR sampling, with recent research suggests that GSR stubs are more efficient than alcoholic swabs for recovering organic residues (recovery rate of about 80%) [41, 189]. The collection method using the stubs was approved by UTS ethic committee (reference number 2015000480). It involved an information sheet to be read by the participant and a consent form to be filled (Appendix I). GSR stubs kits were purchased from Ted Pella, Inc. (USA). The forensic kits acquired contains a box of 5 carbon-coated stubs (12.5 mm diameter) along with labels and a pair of gloves making the kit ready for collection in the field.

When a firearm is discharged, the hands and forearms of the shooter are the areas most likely to be in contact with the GSR plume. Therefore, the area of interest when sampling GSR is known as the web area (thumb-index area at the back of the hand) as well as the thumb area and the wrist. Both hands of the POI are sampled (Figure 15) [17]. Throughout the research, the shooter (n=1) performed the firearm discharges in the NSW Police Force premises, he was right-handed (dominant hand), both hands of the shooter were sampled for OGSR. The stubs were dabbed on the surface of the hands until it has lost all stickiness (30-50 dabs) [41, 164, 173, 189].

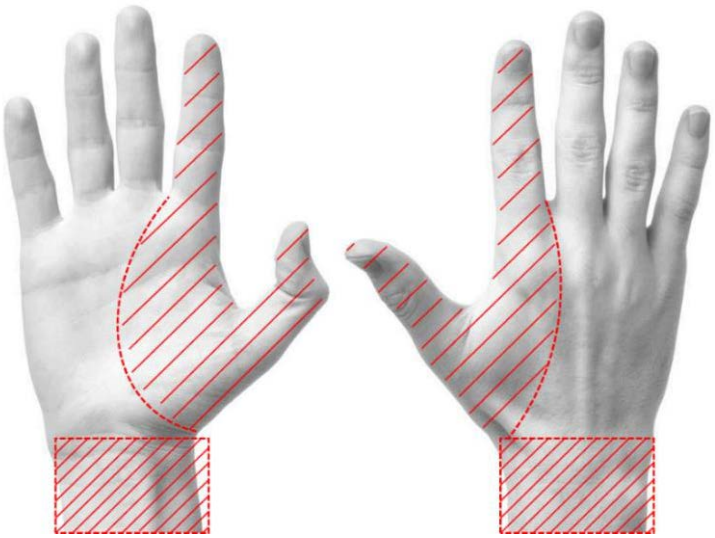


Figure 15. Area of interest on POI hands for GSR collection.

One stub was used collect both hand and wrist area (Figure 15). Therefore, one stub was used for the sampling of each hand. Once specimens are collected and packaged appropriately (covered and placed in their boxes), they are stored until extraction at 4 ºC [155]. The extractions were carried out as soon as possible after the collection as research indicates significant degradation of the OGSR compounds occurs during the first 4 days after collection [190].

The extraction protocol was optimised and recently published by Taudte et al. [189]. The protocol is as follow (Figure 16): The stubs were removed from the plastic base and placed into a 20 mL scintillation vial. Approximately 5 mL of acetone was added in order to completely immerse the stubs which were sonicated at room temperature for 5 minutes. Once the sonication was finished, the stub was removed and the eluent filtered through a 0.2µm PTFE filter to remove any particulate arising from the stubs. Once filtered, the

eluent was evaporated under a stream of nitrogen to about 1 mL and transferred into a LC vial before finishing the evaporation to complete dryness. When the specimen was dry, it was reconstituted with 196 µL methanol and acetonitrile (1:1 v/v), and finally 4 µL of internal standard (1000 ppm of d10-DPA) was added. The internal standard final concentration was 20 ppm. After the extraction, the specimens were stored at 4 °C before being analysed by UPLC-QqQ-MS.

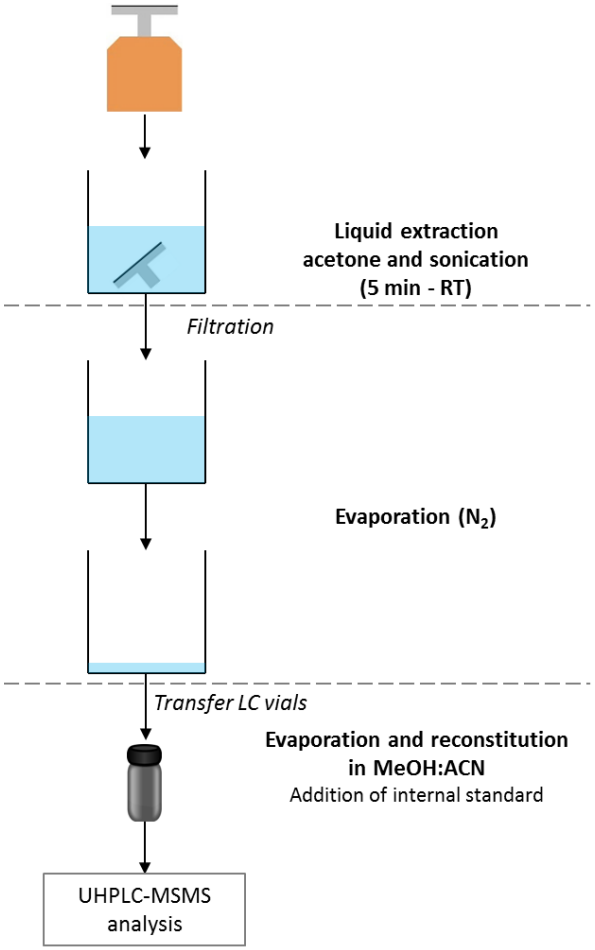


Figure 16. Extraction protocol utilised during this project and taken from Taudte et al [189]. RT = Room temperature.

1.3 Stability of OGSR compounds in solution

Following the validation process, it was essential to assess the stability of specimens in the perspective of the laboratory strategy. After the collection process, specimens are usually sent to the forensic laboratory for GSR analysis. This stability study aimed to determine the optimal storage duration of extracted specimens to minimise degradation of the target compounds.

A previous study carried out by Taudte et al. (2016) [190] assessed the stability of OGSR compounds onto the collection devices, in this case carbon-coated aluminium stubs. It was found that when stored at 4 °C, the largest degree of degradation was observed during the first 4 days of storage [190]. This research suggests that specimens, once received, have to be extracted as soon as possible in order to limit the degradation of OGSR specimens. Consequently, the present stability research is pursuing the assessment of the stability of OGSR compounds by studying their stability in solution after having been extracted following the protocol described in Figure 16.

Two different sets of samples were analysed (Table 19): using a 5-point calibration curve and specimens arising from both hands of a shooter who discharged three times a revolver 686 calibre .357 Magnum (.357 Mag) presented in Table 17. The specimens were collected immediately after the discharge in order to maximise the amount of OGSR detected. The GSR specimens were extracted the same day as collection using the protocol presented in Figure 16. All the specimens were stored at 4 °C.

Table 19. Stability study samples list. Each of the sample were analysed the following day (fresh), after one week and after two weeks of storage.

	specimens
Standards *Calibration curve*	0.01 ppm
	0.05 ppm
	0.1 ppm
	0.5 ppm
	1 ppm
Firearm discharges *Revolver S&W model 686, .357 Mag, discharged 3 times, ammunition in Table 17*	Specimen 1
	Specimen 2
	Specimen 3

Availability of the UPLC-QqQ instrument allowed for the weekly analysis of specimens. Therefore, the specimens and calibration curve were analysed the day following their preparation (fresh), then after one and two weeks of storage. The results of the analysis were normalised and pre-processed as developed in the Section I, part 1.3.1 (p. 59), with the results of each week then compared to assess the stability of standards samples as well as GSR specimens from firearm discharge conditions.

2. Results and discussion

2.1 Propellant powder analysis

The propellant was taken out of three cartridges of each calibre studied throughout this project. The propellant powder was photographed (Figure 17) and weighed (Table 20). It was observed that both propellant powders were in the shape of flakes with a significant difference of size between both powders.

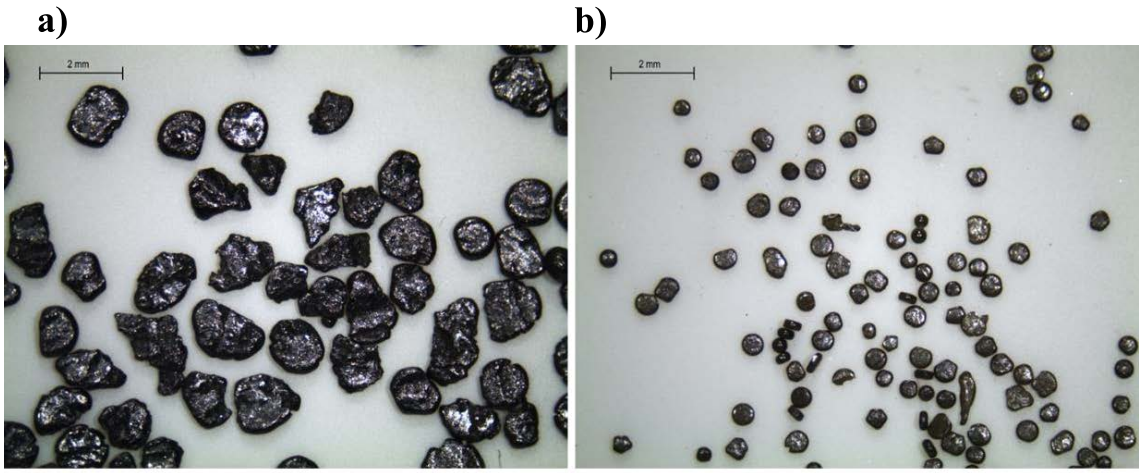


Figure 17. Photographs of the propellant powder under macroscope, magnification 12x. a) .40 S&W, Winchester WinClean® and b) .357 Mag, PPU ammunition.

Table 20. Propellant powder weight.

		.40 S&W	.357 Mag
Propellant	Average [mg]	336	853
	Standard deviation [mg]	16	21

The analysis by UPLC-QqQ-MS, presented in Table 21, showed that three out of the four compounds of interest were detected in both propellant powders. Indeed, MC was not detected in any of the propellant powders analysed, therefore the compounds successfully detected were DPA, *N*-nDPA and EC (Table 21). This is not surprising given the manufacture of smokeless powder typically includes MC or EC, rarely both in combination. A representative chromatogram (TIC MRM) of the analysis of the propellant powder (.357 Mag) diluted 1000 times is presented in Figure 18 (in blue). It is compared to the chromatogram of a standard mix (0.5 ppm) of the compounds of interest (in red).

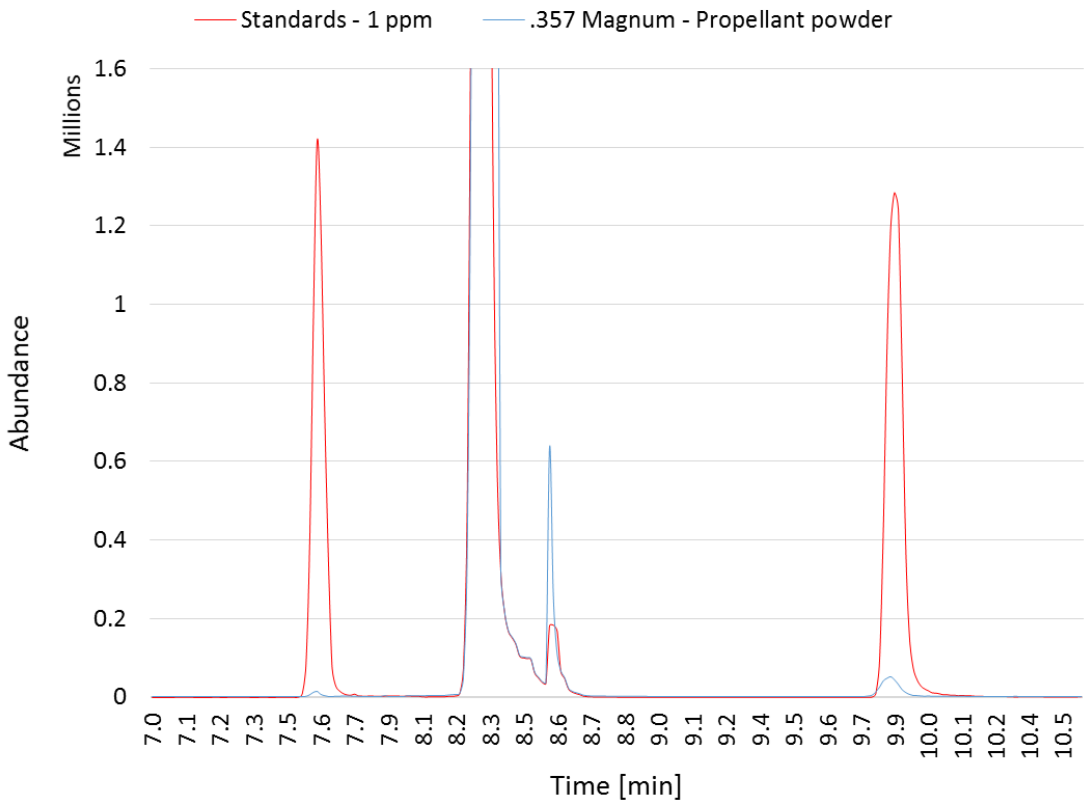


Figure 18. Comparison between the chromatogram (TIC MRM) obtained from a standard mix of the compounds of interest (in red) and the chromatogram of the propellant powder analysis diluted 1000 times (.357 Mag – in blue).

A large % RSD was also observed for some compounds (.40 S&W, EC and .357 Mag, *N*-nDPA) as shown in Table 21. This result may be attributable to the heterogeneity between the three propellant powders analysed. Three different cartridges were open for each calibre investigated, therefore, the large % RSD observed may be due to the heterogeneity in the manufacture of the propellant powder.

Table 21. Average response (normalised peak area) between the three unfired propellant powders analysed for each compounds. N/D= Not detected.

Calibre	Compounds	Average	Std. deviation	% RSD
.40 S&W	*N*-nDPA	0.01	1.86×10^{-3}	15.26%
	MC	N/D	N/D	--
	DPA	0.02	2.71×10^{-3}	15.11%
	EC	0.01	3.92×10^{-3}	36.93%
.357 Mag	*N*-nDPA	0.01	2.98×10^{-3}	26.08%
	MC	N/D	N/D	--
	DPA	0.04	6.17×10^{-3}	16.81%
	EC	0.19	3.33×10^{-2}	17.89%

2.2 Stability of OGSR compounds in solution

In order to optimise the results generated by forensic laboratories and to avoid any impact of the subsequent investigation, it was important to assess the stability of OGSR compounds in different conditions (e.g. on the collection device or in solution). A previous study approached the study of the organic compounds of GSR on the collection device [190]. It was found that the larger degradation occurred during the first four days, concluding that the extraction and analysis process should be done as soon as the specimens were collected. No recent study has explored the stability of OGSR compounds in solution, once having been extracted.

2.2.1 Stability after storage I: Standards and calibration curve

The five-point calibration curves were analysed by UPLC-QqQ-MS three times: within 24 hours (Fresh), one week after preparation and two weeks after preparation. The samples were stored at 4 °C until analysis.

The first observation shown in Figure 19 (a and b), presents the successful detection of the four compounds of interest at each concentration, ranging from 0.01 ppm to 1 ppm. The impact of the storage is observed in form of a lower amount detected after one and/or two weeks. In Figure 19, *N*-nDPA presented a lower amount detected after one week, with a significant decrease observed after two weeks of storage. MC and EC levels were stable after one week of storage, with a significant decrease visible after two weeks of storage. DPA is an exception as the detection after one week showed a higher detection

than the fresh calibration curve (Figure 19a). To perform a T-test to assess the significance of this result, the criterion of normality must be satisfied. Razali et al. studied several normality tests [191]. It was found that, among four tests investigated, Shapiro-Wilk was the most powerful test to assess the normality of small and large datasets [191].

Therefore, Shapiro-Wilk tests were performed on the stability study datasets at 95% confidence interval (CI, $\alpha = 0.05$). It was found that the dataset seemed to be normally distributed (p-value > 0.05), the results of these tests are presented in appendix III.1.1. Then, a T-test (two tails, $\alpha = 0.05$) was performed between the fresh and 1-week old samples and resulted in a non-significant difference between the two weeks of analysis (p-value = 0.880). This observation may be attributable to the degradation of *N*-nDPA to DPA after a week of storage. *N*-nDPA is a derivative product of DPA produced in smokeless powder when DPA scavenged nitroso-radicals to produce nitro-derivatives such as *N*-nDPA, 2-nDPA or 4-nDPA. After two weeks of storage it seemed that the amount of DPA stabilised with an amount close to the fresh analysis (Figure 19a).

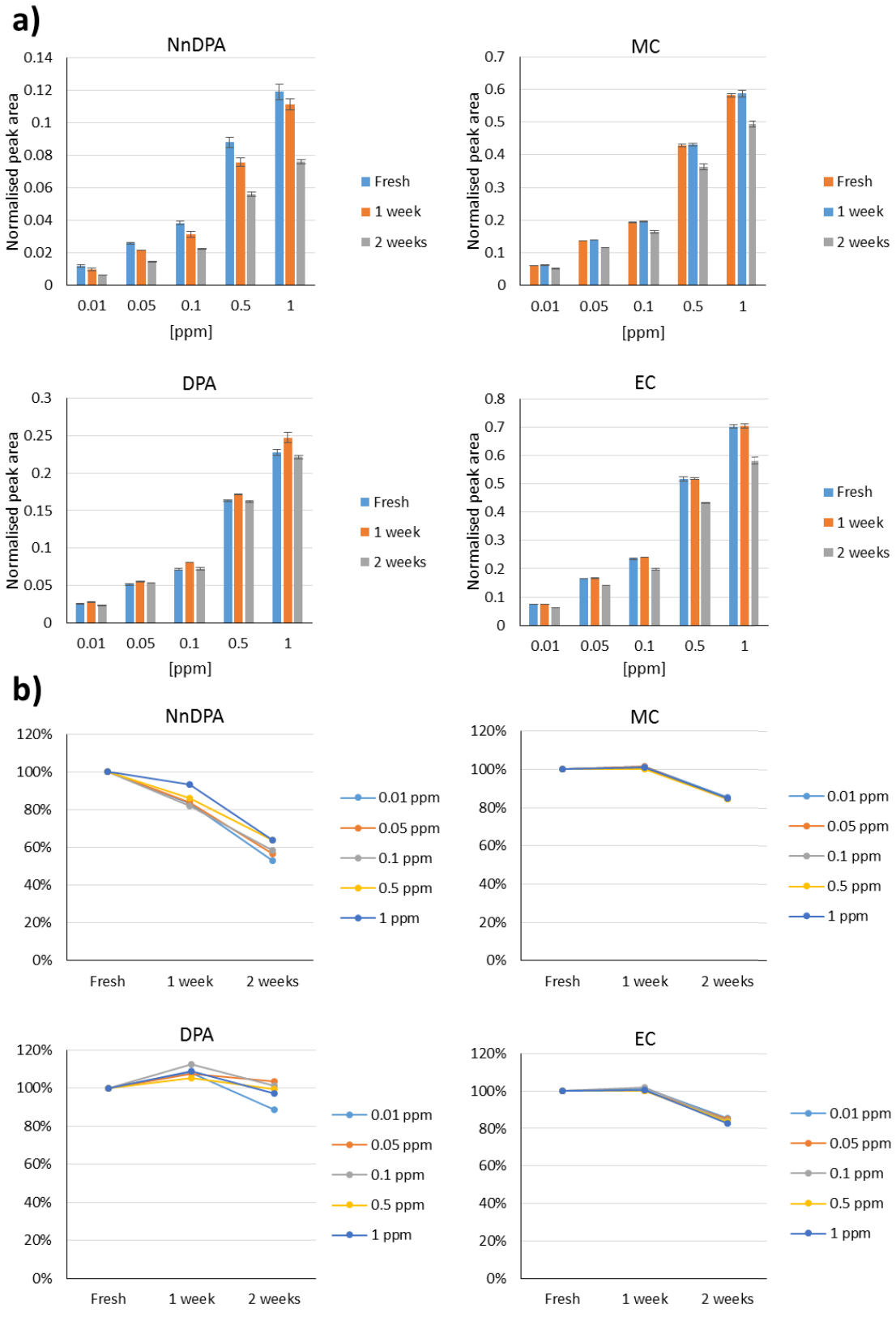


Figure 19. Storage effect on standards: a) Amount detected and b) Each calibration point is normalised to their respective "Fresh" response and presented as percentages.

Figure 19b represents the amount detected for each calibration points when normalised to the fresh response. After two weeks of storage, *N*-nDPA was observed to have degraded most significantly, to approximately 60% of the original level. The amount detected after one week being higher by about 10%, the degradation occurring during the second week of storage lead to results being close to the amount originally detected (~100%). MC and EC were found to be stable during the first week of storage with a variation in the amount below 2%. A decrease was observed after two weeks of storage where both compounds presented a decrease of approximately 20% (Figure 19b).

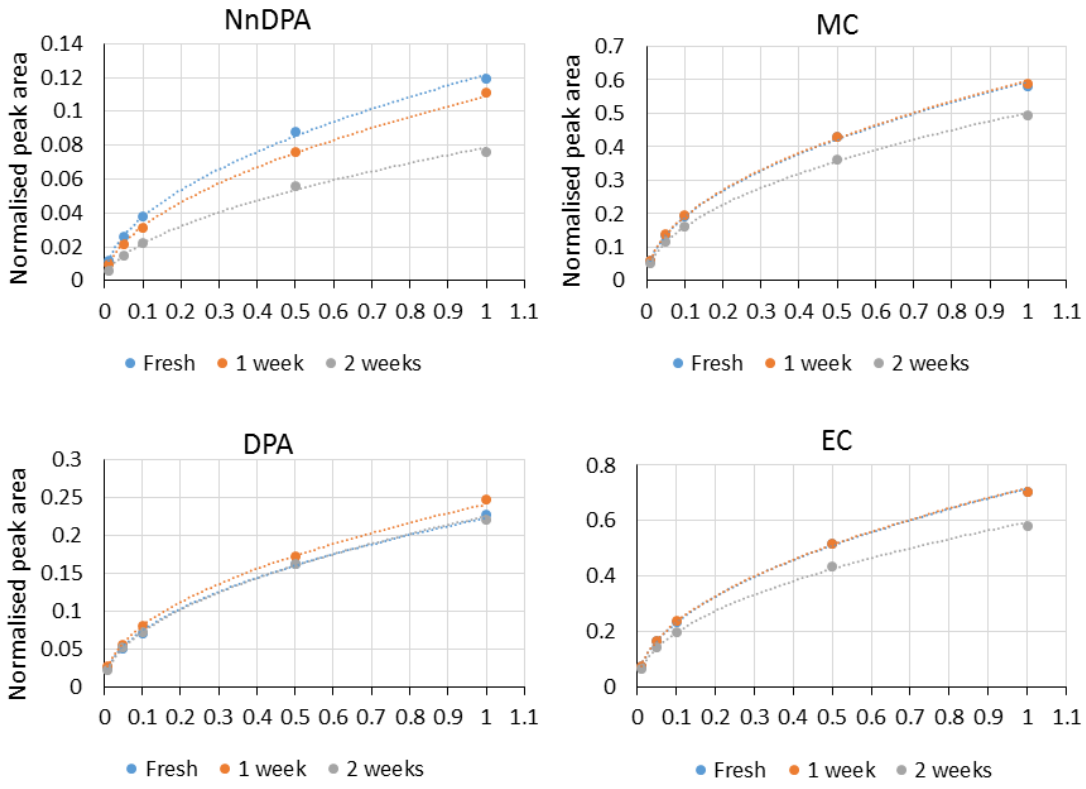


Figure 20. Storage effect on the standards: Calibration curve.

Even though the amount observed was decreasing during two weeks of storage, it did not impact on the linearity of the calibration curve (Figure 20 and Table 22). The RSQ displayed in Table 22 are found stable after different storage times, with %RSDs ranging from 0.0048 % to 0.0624 %. The stability of the curve's linearity is also visible within the slopes of each calibration curve which showed a RSD ranging from 0.22% to 4.12% (Table 22).

Table 22. Slopes and RSQ of each calibration curve.

	Slope				**RSQ**			
	N-nDPA	MC	DPA	EC	*N*-nDPA	MC	DPA	EC
Fresh	*0.51*	*0.49*	*0.48*	*0.49*	*0.9993*	*0.9997*	*0.9982*	*0.9997*
1 week	*0.53*	*0.49*	*0.48*	*0.49*	*0.9992*	*0.9998*	*0.9986*	*0.9996*
2 weeks	*0.55*	*0.49*	*0.49*	*0.48*	*0.9990*	*0.9998*	*0.9994*	*0.9996*
Average	**0.53**	**0.49**	**0.48**	**0.49**	**0.9992**	**0.9997**	**0.9987**	**0.9996**
Std Dev	**2.19E-02**	**1.08E-03**	**9.29E-03**	**3.46E-03**	**1.59E-04**	**4.84E-05**	**6.23E-04**	**6.78E-05**
% RSD	**4.12**	**0.22**	**1.93**	**0.71**	**0.0159**	**0.0048**	**0.0624**	**0.0068**
	Percent - Slope				**Percent - RSQ**			
	N-nDPA	MC	DPA	EC	*N*-nDPA	MC	DPA	EC
Fresh	*100%*	*100%*	*100%*	*100%*	*100%*	*100%*	*100%*	*100%*
1 week	104.29%	99.61%	99.58%	99.58%	99.99%	100.01%	100.03%	99.99%
2 weeks	108.60%	99.63%	103.14%	98.63%	99.97%	100.01%	100.12%	99.99%

Figure 21 represents the variation of the slope of the calibration curves in percent. For *N*-nDPA the slope resulted in an increase from the first analysis (Fresh) to the analyses performed after one and two weeks of storage. This observation might be due to the different rate of degradation between the different concentrations. Lowest concentrations are expected to degrade faster than the highest concentrations, which leads to an increase of the slope from the fresh analysis to the analysis after one and two weeks of storage. DPA also demonstrated an increase of the slope but only between one week and two weeks of storage. On the other hand, the two centralites presented a stable slope from the first analysis up to the analysis carried out after two weeks from preparation with a variation below 2% (Figure 21) which emphasised a better stability of these compounds that DPA and its derivative.

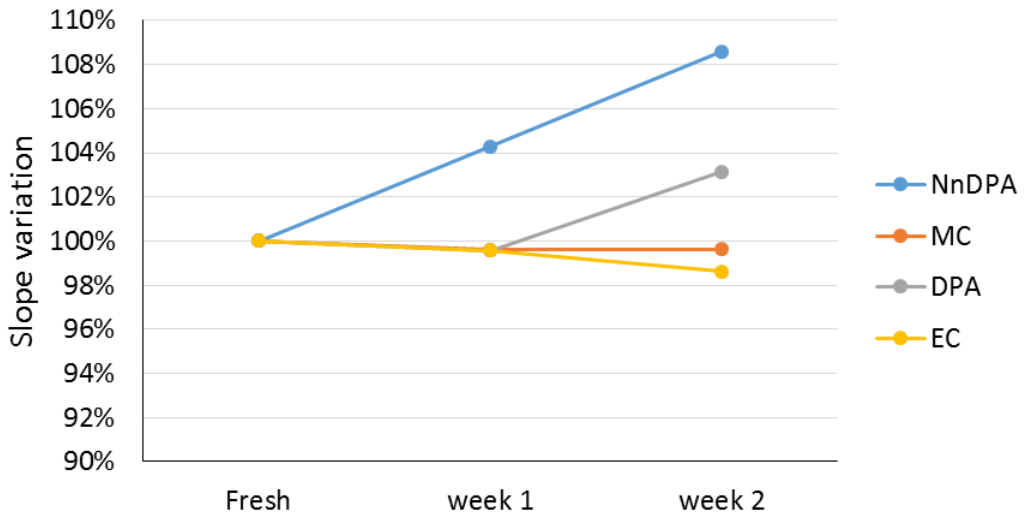


Figure 21. Percent variation of the slope from fresh analysis to the analysis after one and two weeks.

In conclusion, the stability of the targeted compounds in standards solution was assessed by analysing a five points calibration curve at three time points following preparation. The standards were stored in the fridge (4 °C) between the different analyses. The stability was found to be variable across the different compounds. *N*-nDPA presented the largest decrease across the time period with a decrease of approximately 40% after 2 weeks. The results for DPA presented a different trend with an increase of about 10% after one week. This increase was found to be not signficant (T-test, two tails, α=0.05, P-value=0.880). After two weeks of storage, the amount detected was close to the amount observed during the fresh analysis (~100%). MC and EC presented a similar trend, both compounds were found stable after one week of storage (<2% variation), with a 20% decrease occuring after two weeks of storage.

The next section approached the second aim of this stability project by exploring the stability of specimens arising from cartridge discharges. The premise of this additional set of analysis relates to the different origin of the compounds of interest. The standards of the targeted molecules previously analysed provided the compounds in solution (MeOH:ACN, 1:1 v/v). However, the same compounds present in OGSR are collected after the combustion of smokeless powder and need to be extracted before being analysed. As the specimen's source is completely different between the standards and the GSR specimens, it was essential to also assess the stability of the compounds collected after firearm discharges.

2.2.2 Stability after storage II: Specimens from firearm discharges

Three specimens were collected from three consecutive discharges of a revolver (S&W Model 686), calibre .357 Mag. Both hands of the shooter were sampled separately. The specimens were collected immediately after the discharge (T0) in order to maximise the amount of OGSR. A representative chromatogram (TIC MRM) of the analysis of a specimen arising from the firearm discharges .357 Mag ammunition is presented in Figure 22 (in blue). It is compared to the chromatogram of the propellant powder analysis of the same ammunition (in red). The three compounds of interest detected in the propellant powder prior to discharge (Section II, 2.1) were successfully detected in the specimens arising from the hands of the shooter after discharges.

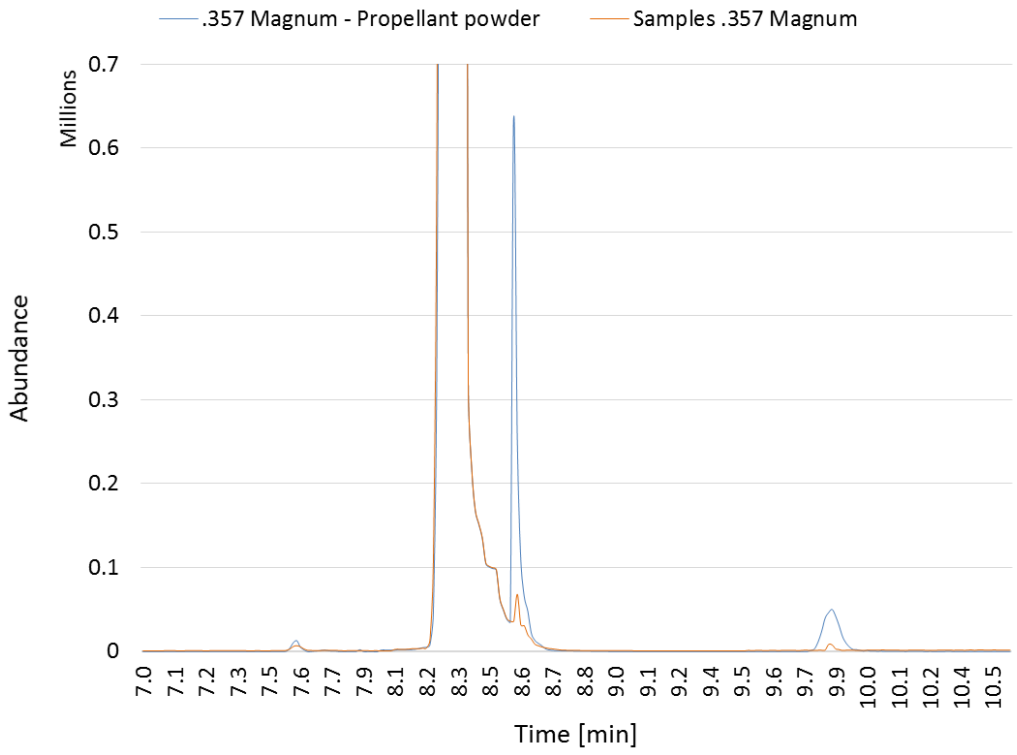


Figure 22. Comparison between the chromatogram (TIC MRM) obtained from a specimens arising from a three discharges of a .357 Mag ammunition (in red) and the chromatogram of the propellant powder diluted 1000 times (.357 Mag – in blue).

Figure 23 presents the average amount detected by UPLC-QqQ-MS for each compounds of interest on both dominant and non-dominant hand. *N*-nDPA presented a regular decrease between the fresh analysis and the analysis carried out one and two weeks after storage. This observation is consistent with observation made during the evaluation of the stability of the calibration standards (Figure 19). In Table 23, approximately 50% of *N*-nDPA was observed after two weeks of storage. This observation was consistent with the observation made with the analysis of standards with approximately 60% of the original response detected after two weeks.

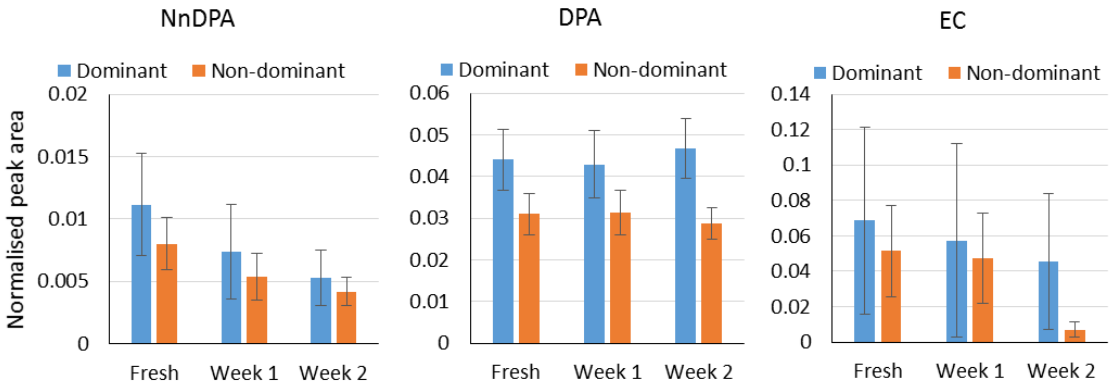


Figure 23. Storage effect on shooting specimens: Average normalised peak area, the error bars represent the standard deviation.

Table 23 represents the amount detected in percentage once normalised to their respective fresh response. *N*-nDPA, DPA and EC were successfully detected in the three specimens. For DPA, the amount detected remained stable after one week of storage (~98% and ~100%, Table 23). However, after two weeks, an increase was observed on the dominant hand (~105% in Table 23). For the non-dominant hand, a slight decrease is observed after two weeks (93% in Table 23).

To perform an ANOVA in order to assess the significance of the impact of two weeks of storage on DPA, the criterion of normality must be fulfilled. Shapiro-Wilk tests were assessed at 95% CI (α= 0.05) on each DPA population and the data were found to be normally distributed (p-values > 0.05), the results of these tests are presented in appendix III.1.2. The normality criterion being satisfied, an ANOVA (single factor, α= 0.05) was performed and it was found that the variations observed for DPA after storing specimens for two weeks were found to be not significant (p-value= 0.82).

EC presented a similar trend to that observed with the standards solutions. The amount detected was stable after one week of storage (~90% in Table 23) and a decrease was observed after two weeks (66% in Table 23). However, the decrease was more important for specimens collected from the non-dominant hands (14% in Table 23).

Table 23. Each specimen is normalised to their respective "Fresh" response. The results are displayed as percentages.

	N-nDPA		DPA		EC	
	D	ND	D	ND	D	ND
Fresh	*100%*	*100%*	*100%*	*100%*	*100%*	*100%*
1 Week	66.3%	67.3%	97.5%	101.1%	83.7%	91.9%
2 Weeks	47.3%	52.3%	106.1%	92.8%	66.3%	13.7%

2.2.3 Impact on subsequent analysis

The study of Taudte et al. highlighted that a large degradation occurred within the first four days after OGSR specimens were collected [190]. They emphasised that the extraction of the specimens has to be performed rapidly. In addition to the Taudte et al study, the present study also highlighted that, after the extraction, the specimen analyses should be performed as soon as possible, as a degradation was observed for some compounds (*N*-nDPA, EC) after one week of storage.

Nevertheless, limitation to this study can be identified, the instrumentation was always available the same days of the week. Therefore, studying the stability of OGSR compounds within a shorter period was not possible. Studying the stability of specimens during shorter period of storage might be considered in future research in order to improve the time window available for the specimen analyses.

3. Conclusion

The analysis of the propellant powder highlighted the presence of *N*-nDPA, DPA and EC in both ammunitions studied (.40 S&W and .357 Mag). MC is typically used as an EC substituent in some propellant powder manufacture, therefore as EC was detected, it was not surprising to not detect any traces of MC.

The stability study revealed that specimens arising from firearm discharges should be extracted and analysed as soon as possible as degradation was observed for two of the three target compounds (*N*-nDPA and EC), while DPA showed a better stability after one week, showing first signs of degradation after two weeks of storage. Moreover a previous study focusing on the stability of OGSR compounds on the collection device [190] suggested that specimens must be treated quickly as degradation occurred during the first four days. In summary, these two studies emphasise the importance of extracting and analysing OGSR specimens within a few days of collection in order to avoid an accumulation of specimen degradation, either on the collection devices or in solution once the specimens are extracted.

The study of the persistence and secondary transfer of OGSR presented in the following sections considered the conclusions reached in the stability study. Every specimen collected, which arose from shooting discharges, were extracted within 24 hours after collection and the UPLC-QqQ-MS analyses were carried out within three days after collection/ extraction.

Section III: Persistence study

The study emphasised on the ability to detect OGSR compounds from the hands of a shooter after a certain amount of time has elapsed. The persistence is essential to assess the usability of OGSR as a reliable source of information for investigation involving firearm-related event and GSR analysis. The procedure for the study required access to a shooting range where we could undertake the experiments as well as an authorised individual to discharge the firearm.

Consequently, the project was carried out in collaboration with the New South Wales Police Force (NSWPF). The firearm discharges were achieved by a police officer in an indoor shooting range.

1. Methodology: Shooting session procedure

The study focused on the recovery and detection of the four compounds of interest: DPA, EC, MC, *N*-nDPA [41, 85]. The collection was performed immediately after firearm discharge (T0), 30 minutes after discharge (T0.5h), an hour (T1h), two hours (T2h) and four hours (T4h) after discharge. The specimens were extracted within 24 hours and analysed by UPLC-QqQ-MS (Section I and II) within three days.

Two calibres and firearms were chosen based on the occurrence in NSW casework (Table 24). The .40 S&W was chosen as it is the calibre of the service ammunition of the NSWPF. Its characteristics are also close to the calibre .45 ACP (Automatic Colt Pistol –

11.43 x 23mm) which is also often encountered by the NSWPF. The firearm used to discharge .40 S&W ammunition is the Glock 22®, a service weapon of NSW police officers. A revolver S&W (model 686) was used for discharging the second calibre which is the .357 Mag, the most powerful 9 mm revolver ammunition often encountered in NSW casework.

Table 24. Calibre – firearm combination used in this project.

Nominal calibre - imperial *(metric)*	Ammunition	Firearm used
.40 S&W *(10 x 22 mm)*	Winchester WinClean® 180 Gr. Brass Enclosed Base	Semi-automatic pistol Glock 22®
.357 Mag *(9 x 33 mm R)*	PPU Ammunition® 158 Gr. Semi-Jacketed Hollow point (SJHP)	Revolver S&W model 686 Barrel length: 4 inches

The shooting process is presented in Figure 3. This required the shooter (n=1) to decontaminate their hands with D-Lead® hand soap, before blanks were collected using the GSR stubs. Following three discharges of the firearm holding the gun with both hands, the shooter continued with their daily activities (with the only restriction to not wash their hands) for the studied time intervals (immediately after discharge (T0), 30 minutes (T0.5h), 1 hours (T1h), 2 hours (T2h) and 4 hours (T4h) after discharge. Three discharges were performed in order to ensure a sufficient amount of OGSR transferred on the shooter. The same number of discharges were performed throughout the project. The specimens were collected with GSR stubs after the respective time had elapsed. The stubs

were dabbed on the shooter's hands until it has lost all stickiness. The experiment was repeated in quintuplicate for each time point (in triplicate for T4h) and both firearm-ammunition combinations.

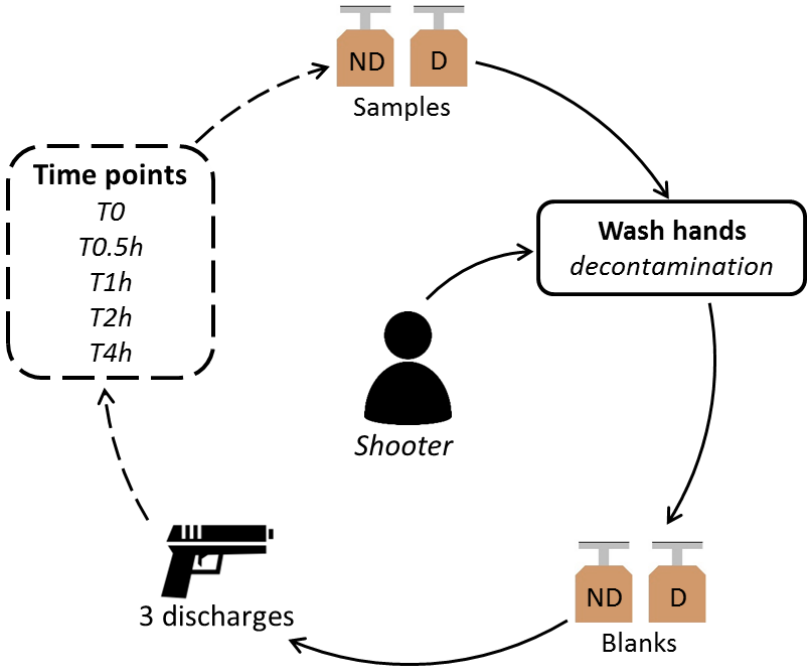


Figure 24. Persistence experimental methodology. The shooting sessions are carried out in collaboration with the NSWPF (Sydney, Australia).

Once the specimens are collected, the extraction was performed in the laboratory the same day per the protocol detailed in Figure 16 (p. 78). The specimens were stored at 4ºC until the analysis which was performed by UPLC-QqQ-MS the following day (Section I, 1.2, p. 51). A five points calibration and a QC were also run at the same time as the specimen and instrument QCs.

1.1 Data normalisation and pre-processing

The data processing involved the normalisation to the internal standard and for each specimen; the associated blank is subtracted in order to keep the amount of residues resulting from the discharge and removing any contamination (Equation 4).

$$Normalised\ Peak\ Area_{\ analyte} = \sqrt{\frac{(Peak\ Area\ analyte_{sample}) - (Peak\ Area\ analyte_{\ blank})}{Peak\ Area_{\ Internal\ Standard}}}$$

Equation 4. Normalisation of the peak area for each analyte targeted detected on specimens arising from shooting experiments.

2. Results and discussion

Throughout the project, calibration curves were analysed in order to assess the conditions of the analytical run. The peak areas were found to be stable across various days of analysis with the calibration curves found to be linear during each analytical run ($R^2 > 0.99$).

All the targeted compounds were considered as detected when the abundance was found above the LOD presented in Table 14 (Section I). Every specimen underwent a blank subtraction (Equation 4) in order to take into account possible contaminations. Although the shooter is a police officer, it was surprising that even after hand washing some blanks were found to contain OGSR, as washing has previously been indicated [58] to completely remove traces of it. The difference in results might arise from the use of different soap and sanitisers. Arndt et al have studied different soap containing alcohol reagents [58], which might be more efficient to remove OGSR. In this study the soap used was a D-lead® hand soap used to decontaminate the hands of heavy metals, such soap might not be sufficient enough to remove OGSR. In addition, different instrumentation were used, which differ in sensitivity and the associated LODs [58]. Arndt et al. have analysed samples by IMS which typically produces high LODs [58]. Conversely, highly sensitive UPLC-QqQ-MS instrumentation was used in this study, and it was found that hand washing was not sufficient to completely remove OGSR traces.

2.1 OGSR: An investigative perspective

In an investigative perspective, the ability to estimate the likelihood of detecting traces on a suspect at a particular time point after an offence is valuable. The pre-assessment of specimens is central for a laboratory in order to be able to efficiently prioritise analyses according to the probability of obtaining positive (or negative) outcomes. To acquire knowledge about traces and what to expect from specimens taken at various time points after an incident is, therefore, a main factor to consider.

The results presented in Figure 25 demonstrate that OGSR compounds were detected up to four hours after discharge. Figure 25 represents the proportion of specimens for which the three compounds of interest were detected simultaneously on both the dominant and non-dominant hands for the two calibres studied (.40 S&W and .357 Mag). For the calibre .40 S&W, It was observed that when combining the results for both hands in the overall graph (far right) that the number of specimens considered detected decreases over time, reducing from 93% at T0 to 72% at T4h. The trends are similar for the calibre .357 Mag, it was observed in the overall graph that the number of specimens considered detected decreases over time, reducing from 93% at T0 to 77% at T2h. However, the number of specimens in which all three compounds were detected increases to 89%. It is essential to emphasise that the number of positive specimens is highly sensitive to the number of specimens analysed. The last time point (T4h) was analysed in triplicate, not quintuplicate. This result confirmed that it is necessary to extend the research by collecting and analysing additional specimens after four hours.

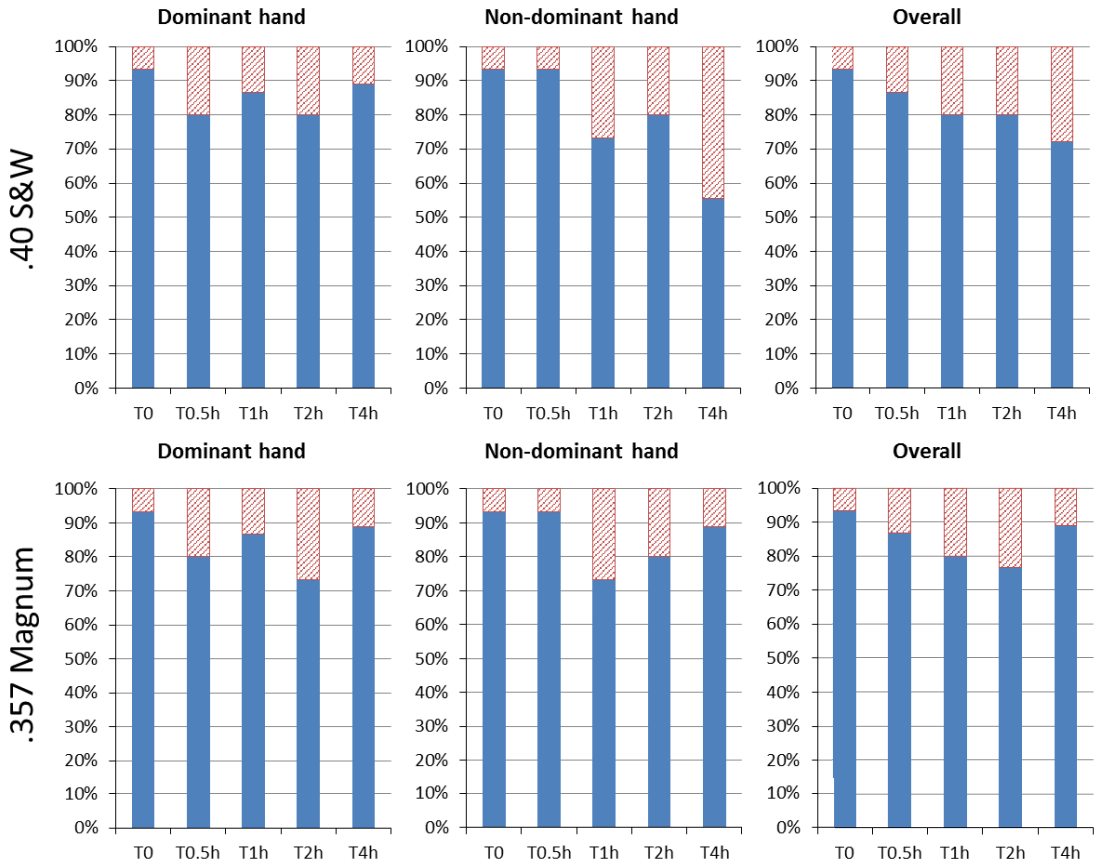


Figure 25. Percentage of specimens considered positive. The number of positive specimens is defined as the number of specimens in which the three compounds of interest were detected simultaneously out of the total number of specimens analysed. The overall graph characterises the combination of both dominant and non-dominant hands.

When looking closer at the contribution from each hand for the .40 S&W at T4h, it was observed that all targeted compounds were detected in 89% of the specimens from the dominant hand, with all three OGSR compounds detected in only 56% of the specimens from the non-dominant hand (Figure 25). This observation is not surprising as the non-dominant hand is typically less exposed to the GSR plume than the dominant hand due to the grip used to hold of the firearm. Indeed the dominant hand usually holds the firearm and is involved in pulling the trigger, hence is more exposed to the GSR plume, while the other hand acts as a support at the bottom of the firearm and is, therefore, less exposed to the residues.

However, when looking at the contribution from each hand at T2h for the second calibre (.357 Mag), it is observed that 73% of the specimens from the dominant hand were detected, while 80% of the specimens from the non-dominant hand were detected. This result suggests that the level of activity of each hand in daily activities is a key factor in the persistence of OGSR. Indeed the dominant hand is the more likely to be in contact with GSR due to the primary transfer occurring from the firearm to the hands of the shooter during the firing process. Similarly, the dominant hand is typically more predisposed to be used for other activities post-discharge, increasing the likelihood of residues loss, influencing the persistence parameter. Consequently, when compared to the non-dominant hand, there might be a higher proportion of OGSR lost on the dominant hand over time. Conversely, due to more limited involvement in regular activities, the non-dominant hand might be more likely to preserve OSGR on the surface of the skin. According to these observations, when a long time elapsed, it might be more valuable to target compounds on the non-dominant hand in order to increase the likelihood of detecting OGSR.

This section emphasises the utility of OGSR from an investigative and prioritisation perspective. Indeed it was found that OGSR can be successfully detected up to four hours using two different ammunition and firearm combinations (semi-automatic and revolver). For the two calibres of investigated, Figure 26 illustrates the amount of each targeted compound detected for the five different time points of interest. Similar trends were observed for both calibres and firearms studied. A decrease in the amount detected was observed across the four hours; however, the three compounds were still simultaneously identifiable up to four hours in the majority of specimens as discussed above. It was also observed that there was a large variation in the amount detected of each compound on

each hand at the various time points (Figure 26). This observation can be attributed to the numerous variables involved in the production, dispersion and deposition of GSR traces. This includes external factors, such as position of the hands, condition of the skin, or the heterogeneity in the propellant composition which can differ from ammunition to ammunition as well as from batch to batch of the same ammunition [192, 193]. In addition, the type of firearm used was drastically different and played a large role in the amount of OGSR deposited on the shooter. Due to the larger gaps in a revolver, a larger quantity of GSR might escape and deposit on the shooter, while a semi-automatic pistol will have a more enclosed body preventing the GSR plume to escape.

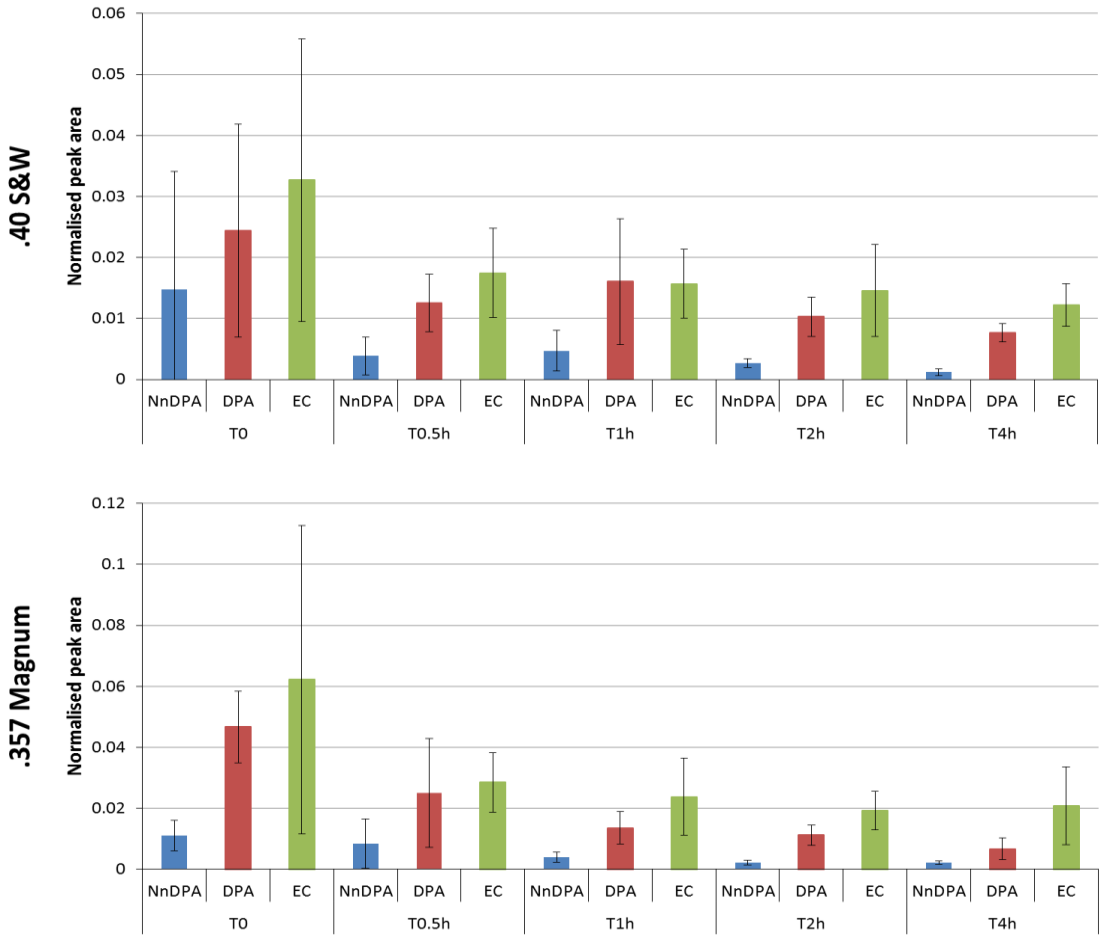


Figure 26. Normalised peak area of both hands combined for each compound at T0, T0.5h, T1h, T2h and T4h. The error bars represent the standard deviation.

It must be emphasised that in purpose of Figure 26 each specimens from each time point were of a different discharge trials and therefore the amount deposited on the hand of the shooter immediately after a discharge for T0.5h and T1h specimens is unknown.

The comparison of each ammunition type indicates that the amount detected from the .40 S&W and the .357 Mag were different. It was observed that the amount detected from the .357 Mag is greater than for the .40 S&W at T0 and T0.5h. This was an expected observation as each calibre contains different amount of propellant powder. Both .40 S&W and .357 Mag bullets weigh approximately 10 grams, however the velocity and energy reached by the .357 Mag is approximately 430 m/s for 950J while the .40 S&W only reach 350 m/s for 620J [20]. In order to reach higher pressure and energy, .357 Mag ammunitions require a larger load of propellant powder.

The difference in the amount of propellant powder originally present in the ammunition may have also led to the recovery of higher amounts of OGSR from the hands of the shooter. Figure 27 presents the trend lines from the amount detected at each time point for both calibres studied. It was found that the power trend lines function produced the best fit with coefficients of determination (RSQ) between 0.811 (Figure 27,.40 S&W, DPA) and 0.97 (Figure 27,.357 Mag, DPA).

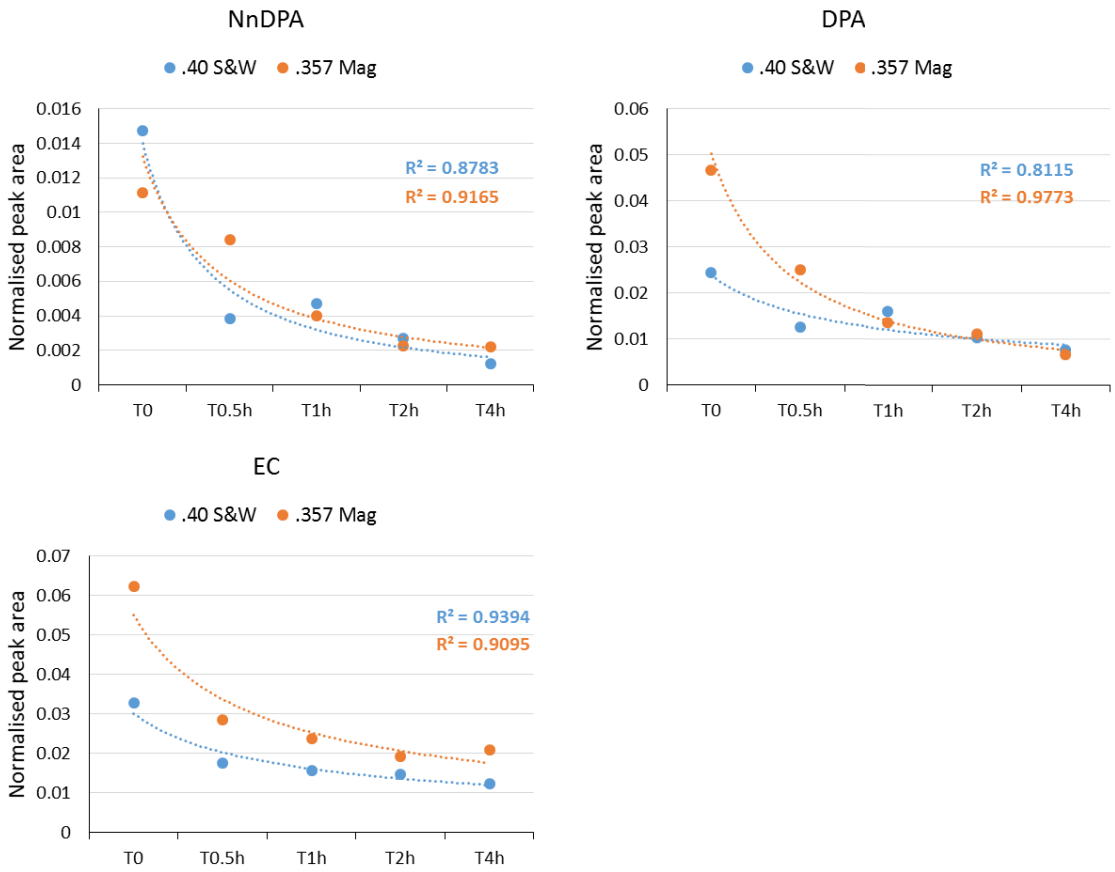


Figure 27. Normalisation of the peak area for both calibres studied. The trend lines are generated from the power function.

However, from the one hour time points (T1h), the difference in the amount detected is reduced between the two calibres. The difference between both calibres is presented in Figure 28 and it suggests that after half an hour the amount detected becomes similar for both calibres as the difference is largely decreasing.

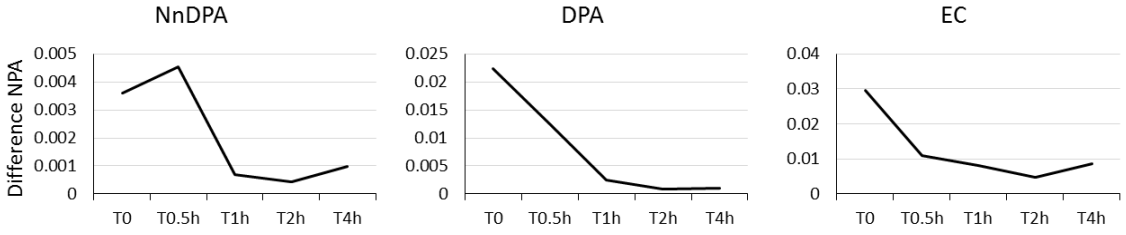


Figure 28. Difference in the normalised peak area between the calibre .40 S&W and .357 Mag.

This observation could be attributable to the level of activities undertaken by the shooter after the discharge. The more vigorous is the activity the larger might be the loss of OGSR, leading to the reduction of the difference between the two calibres. In addition, the lower is the amount recovered, the smaller becomes the difference between the two calibres.

Furthermore, the collection devices may be a significant contributor to this observation. The stubs were dabbed onto the shooter skin until the complete loss of stickiness. Therefore, their abilities to collect a small amount might become limited. After a certain amount of time (T2-4h), the quantities left on the shooter's skin is substantially smaller and the efficiency of the stubs might become limited. Also the different step undertaken in order to extract the specimen (Figure 16, p. 78), may greatly influence the amount detected.

Additionally, the results presented illustrate a large variability which can be attributed to a substantial number of factors affecting the production and deposition of OGSR leading to significant variation in the amount detected. Furthermore, the research suggests that the dominant hand collects more OGSR than the non-dominant hand while also being more predisposed to losing OGSR traces due to higher degree of activities undertaken.

Knowledge of the persistence of OGSR traces is critical when prioritising the analysis of specimens during the investigative phase of casework. Indeed, as resources of forensic laboratories are limited, it is essential to triage specimens in order to maximise the potential results while optimising the use of resources.

Given the observation that OGSR traces were detected in over 2/3 of specimens collected after two and four hours post discharge, the analysis of OGSR has been found to have a clear potential value. Using this additional information about traces presented in this study may improve the central role of the forensic scientist in the decision-making process and, more particularly, in a pre-assessment perspective. In combination with the circumstances of the case under investigation and the available resources, the understanding of OGSR traces increases the ability of the forensic scientist to prioritise the analysis of specimens to maximise outcomes while improving the efficiency of the forensic investigation.

2.2 Persistence of OGSR over time

The three compounds of interest were successfully detected up to four hours after the firearm discharges, where OGSR traces were detected at a significant amount. In comparison to the LODs determined in Chapter three (Section I, p. 50), it was found that, after four hours, the OGSR traces were detected at levels ranging from an average of 1.6 times (.40 S&W, EC, ND) to 73 times (.357 Mag, EC, D) the LOD (Table 25). These results suggest that OGSR might still be detectable after longer time points. However, several limitations can affect the positive recovery of such traces after such long times with regards to casework, such as longer and more intense activities, washing and rubbing the hands which can wash the residues off the skin.

Table 25. Ratio between the response at T4h and the LODs for both studied calibres. It represents the ratio T4h/LOD. T4h-1 to 3 represent the three replicates performed. The N/A values represent missing values.

.40 S&W						
	N-nDPA		DPA		EC	
	D	ND	D	ND	D	ND
T4h-1	9.50	*N/A*	39.83	*N/A*	15.49	*N/A*
T4h-2	11.91	13.26	35.41	32.78	26.57	1.48
T4h-3	*N/A*	17.73	39.28	*N/A*	4.20	1.73
Average	**10.70**	**15.50**	**38.18**	**32.78**	**15.42**	**1.60**

.357 Mag						
	N-nDPA		DPA		EC	
	D	ND	D	ND	D	ND
T4h-1	51.56	44.49	45.83	5.31	41.45	19.89
T4h-2	33.03	37.48	3.95	32.56	*N/A*	37.07
T4h-3	35.49	24.19	28.74	*N/A*	106.30	5.56
Average	**40.03**	**35.39**	**26.17**	**18.94**	**73.88**	**20.84**

Figure 29 presents the same dataset as Figure 27, however the results are presented in order to highlight the inherent intra-variability between each experiment replicate. Figure 29 presents the normalised peak area of the targeted compounds for each calibre investigated at each time point (T0 - T4h). The shooter held the firearm with both hands as is the usual practice in the police force. The results presented include the combined amount (average) detected on both dominant and non-dominant hand of the shooter. It was observed that a large decrease occurs during the first hour (Figure 29).

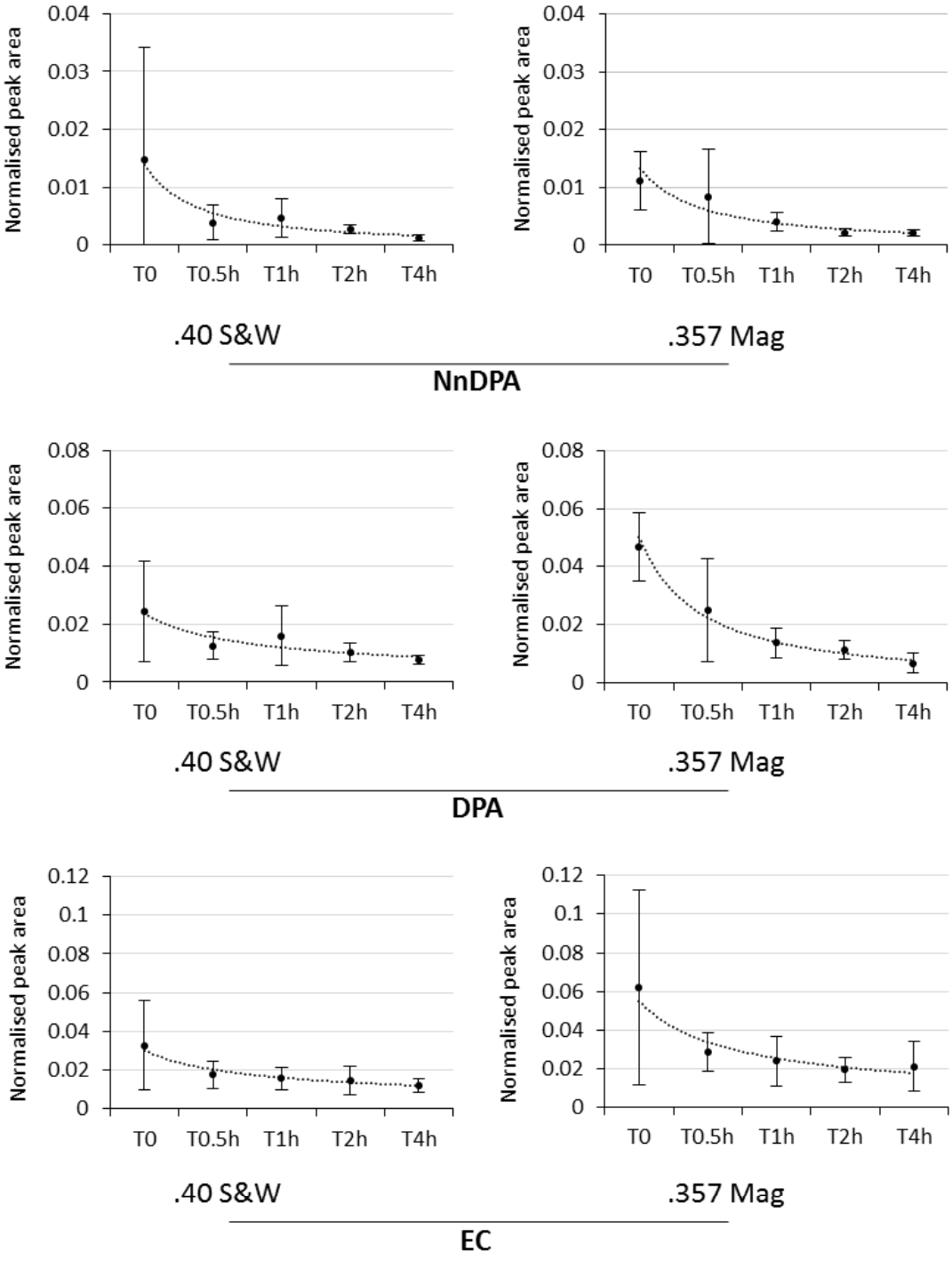


Figure 29. Average normalised peak area (both hands combined) of each targeted compound, the error bars represent the Standard deviation. The trend lines are generated from the power function.

Table 26 represents the relative percentage of the amount detected once normalised to T0. Consistent trends were observed with a large decrease in the amount of each compound detected after the first hour following discharge: a mean of 34% for *N*-nDPA, 40% for DPA and 43% for EC were detected, referenced to T0. After four hours, the mean amount remaining relative to the T0 point was 14% for *N*-nDPA, 23% for DPA and 35% for EC were still detectable (Table 26).

Table 26. Percentage of the average normalised peak area (both hands combined) of each targeted compound when each time point is normalised to T0.

	N-nDPA		DPA		EC	
	.40 S&W	.357 Mag	.40 S&W	.357 Mag	.40 S&W	.357 Mag
T0	*100%*	*100%*	*100%*	*100%*	*100%*	*100%*
T0.5h	26.3%	75.6%	51.3%	53.6%	53.4%	45.8%
T1h	31.9%	36.1%	51.7%	29.1%	47.9%	38.1%
T2h	18.2%	20.1%	42.1%	23.9%	44.6%	30.9%
T4h	8.2%	19.6%	31.5%	14.3%	37.3%	33.5%

Figure 30 shows each replicate collected for each shooting experiment at the studied time points. For the .40 S&W ammunition discharged with the Glock 22, a larger amount was detected on the dominant hand of the shooter when compared to the non-dominant hand. This observation can be explained by construction of the firearm, as the ejection port was located on the right side of the firearm, a greater amount of OGSR expelling on the right-hand side of the shooter leading to such results (Figure 30). The trends for the revolver S&W model 686 were different. Indeed, the construction of the firearm is completely different, with a drum instead of an ejection port. This difference fundamentally changes the dispersion and deposition process of OGSR as the plume expands from the firearm more symmetrically from the gap between the drum and the barrel, which may lead to a more homogeneous deposition between both hands of the shooter (Figure 30) [28].

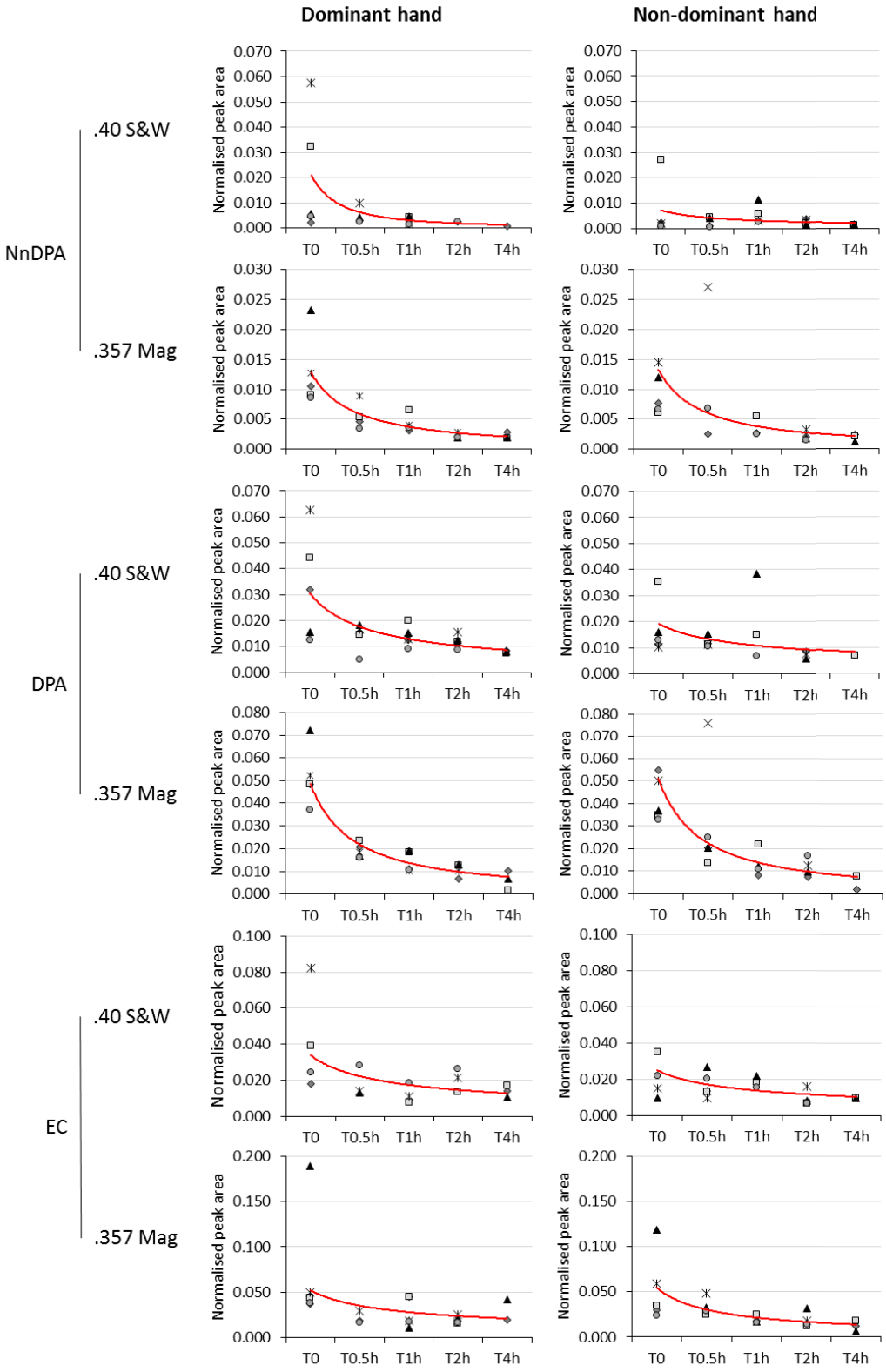


Figure 30. Normalised peak area of each targeted compound on both hand of the shooter. Each data point represents a replicate of the experiment (n=5 for T0, 0.5h, 1h, 2h and n=3 for 4h). The trend lines are generated from the power function.

The results presented in Figure 29 and Figure 30 are consistent with two previous research efforts regarding the persistence of OGSR [41, 58]. Arndt et al. also successfully detected DPA at four hours after the discharge [58]. Similarly, the results obtained in this current study, a large decrease in the amount of OGSR after one hour was also reported by Gassner et al. [41].

The aim of the study was to assess the effect of activities on the retention of OGSR traces on the hands of the shooter. It is essential to emphasise the limitations, as this was a controlled experiment, where the shooter did not wash their hands across the studied time period and thus represents a 'best-case' scenario. Previous research indicates that hand washing and wiping has a drastic effect on the amount of GSR remaining on the surface of the hands [9, 152]. Arndt et al. observed that the activity of washing hands completely removed all traces of DPA on the hands of the shooter, although as previously detailed, it must be pointed out that those researchers used a different detection technique and used swabs for collection of GSR [58]. According to the observed results, it seems that hands washing has an effect ranging from partial to complete removal of OGSR.

In this particular case, the activities undertaken after firearm discharges involved routine police and office duties (without hand washing and without any additional contact with firearms). The observed decrease in the amount detected was likely to be highly dependent on the kind of activities undertaken by the person on whom the deposits are present. It would be reasonable to expect that the more vigorous the activity the greater potential for the loss of residues. In addition to the activity effect, a previous study conducted by Moran et al. (2014) hypothesised that evaporation and absorption of the organic compounds by the skin are also significant parameters in the retention and recovery of OGSR [156]. Additionally, it has been previously emphasised that the

lipophilic aspect of the organic residues may increase their retention on the surface of the skin when compared to the inorganic particles that may be more easily lost or removed [58].

The general trends shown in Figure 29 were consistent with previous studies involving IGSR, where the most significant decrease occurs within the first two hours post discharge [8, 148]. Brozek-Mucha recorded a 96% decrease in the amount of particles during the first 30 minutes after discharge [8]. However, it is important to note that most studies on the persistence of IGSR do not provide enough information about the trends over time to proceed to a more reliable comparison with persistence of OGSR. Nevertheless, the results of this study show that the decrease of OGSR over time seems less significant than the loss of IGSR mentioned in the current literature with an average amount detected, across the targeted compounds, of 43.83% for .40 S&W and 34.43% for the .357 Mag after one hour (Figure 29). Such results may arose from the different physical and chemical nature between IGSR and OGSR, which may support the suggestion that the lipophilicity of OGSR is an important factor in the persistence of OGSR on the skin. OGSR traces persist for longer suggesting that they could be a more suitable target traces for understanding the activity level scenario for investigations involving firearms.

When comparing different types of firearms, it is commonly reported that the amount of GSR detected is greater with a revolver than a semi-automatic pistol [28]. Revolvers have a more rudimentary construction that presents larger gaps for the GSR plume to exit the firearm (e.g. cylinder, firing pin, and trigger). The results from this study report that the amount of residues detected from the .357 Mag (a revolver) are in the same range as that of the .40 S&W calibre fired with a semi-automatic Glock 22® (Figure 30).

2.2.1 Variability of OGSR deposition and analysis

Significant variation was observed between the replicates at each time point studied. This was particularly pronounced at T0 suggesting that shot to shot variation was a significant source of large %RSD. Each data point was arising from a separate and totally different shooting experiment as shown in Figure 24, this suggests that the variability observed at T0 is the consequence of the OGSR production, dispersion and primary transfer. The factors influencing the variability of the primary transfer are numerous during the firing process.

A secondary transfer onto the hands of the shooter can occur before the firearm discharge through a contamination of the grip, which is due to previous discharges of the weapon. During the firing process, the combustion of the propellant powder may vary from shot to shot as the composition and/or loading of the propellant may vary slightly from cartridge to cartridge (e.g. due to different storage conditions or a heterogeneous manufacturing process) which causes the production of variable amounts of OGSR.

Lastly, after the firing process, different environmental conditions (e.g. airflow) also greatly affect the dispersion of GSR plume, and the conditions of the shooter, such as their skin, hairiness and the clothing, greatly influence deposition of OGSR traces. These factors collectively result in the highly variable primary transfer as observed in Figure 29 and illustrated in Figure 30 with the large scattering of the replicates at T0.

Consequently, for every time point longer than T0, the original amount deposited on the shooter's hands is unknown and cannot be extrapolated to other time points. For instance, a high amount of DPA is detected on the non-dominant hand at T0.5h for the .357 Mag

ammunition. This reflects the primary transfer variability with a potentially high amount deposited on the hands of the shooter at the time of discharge for this particular specimen. A similar observation was seen in the .40 S&W calibre on the non-dominant hand at T1h. When comparing with the existing literature, several studies observed large variations in the detection of OGSR. Gassner et al. observed a high variability in the amount recovered and detected, which was associated to the high variability of the OGSR production, deposition and recovery rate [41].

Other human factors must be considered when observing persistence data that includes unrestrained activities such as daily work. One key consideration is the role of the dominant hand, which is the preferred hand when undertaking activities such as handling objects, opening doors and many others. The different involvement of the two hands in such activities may highly influence the degree of retention of OGSR traces with a rapid decrease observed on the dominant hand compared to the non-dominant one. Conversely, the non-dominant hand, may preserve the OGSR traces on the surface of the skin for longer due to reduced role in activities. In addition, another factor concerns the possible redistribution of OGSR on the hands during the time of the experiment as the two hands may enter in contact with each other spreading the traces over their surface [155]. When the inorganic component of GSR is considered it appears that high variability is also observed. Jalanti et al. reported a poor reproducibility in the counts in particles and suggested that the particle retention was not dependent of their chemical composition [148].

2.3 Exploratory Data Analysis (EDA)

Further analysis was conducted to assess the possible differentiation of the results obtained between the time points studied. The boxplots presented in Figure 31 illustrate the variations of the data obtained for each time points studied. It confirms the high variability, with the largest variation observed immediately after the discharge (T0). The results confirm that a large part of the variations arises from the discharge process and the primary transfer occurring between the firearm and the shooter. In addition, it is seen that the largest decrease occurred during the first 30 minutes.

It is important to note that the shape of the trends observed in Figure 29, Figure 30 and Figure 31 followed an exponential decrease. Such trends are commonly encountered in the persistence of other traces, notably with fibres and glass fragments [194-196].

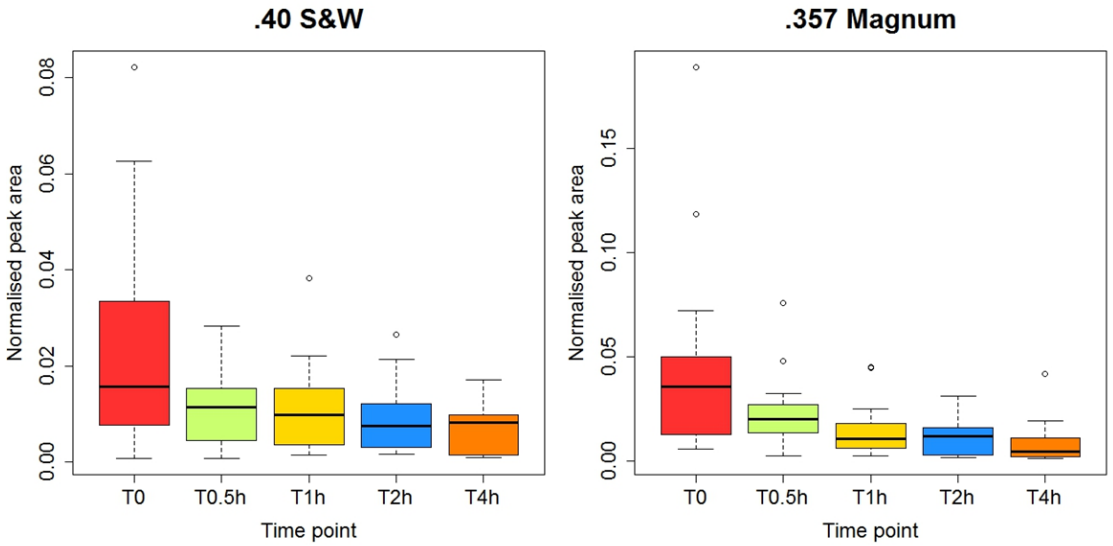


Figure 31. Boxplot of each time point for both .40 S&W and .357 Mag. Both dominant and non-dominant hands were averaged.

To perform an ANOVA (or a T-test), the criterion of normality of the dataset must be fulfilled. It was assessed at 95% CI (α= 0.05). A Shapiro-Wilk test was conducted on each time point, hand and for each calibre. A total of 60 tests were conducted, the detailed results are presented in Appendix III.2. It was found that 82% of the tests (45/55) returned a p-value above the threshold of 0.05 indicating a normally distributed dataset. However, such results have to be interpreted carefully as only 5 data points were available to assess the normality. This resulted in 18% (10/55) of the tests returning non-normal dataset (Appendix III.2).

Consequently, the criterion of normality was considered fulfilled and an ANOVA (one factor) was performed on both .40 S&W and .357 Mag dataset. ANOVA assesses the significant difference between the averages of each group, i.e. the different time points. It was found that for the .40 S&W and .357 Mag the *P*-values were significantly lower than the threshold of 0.05 (α) with values of 1.15×10^{-4} and 1.89×10^{-6} respectively. Therefore, the results of the ANOVA found that, for both calibres, at least one of the group was different. The ANOVA test was, then, followed by a post-hoc analysis in order to identify which group(s) presented a difference. The Tukey-Kramer test (HSD) was conducted, paring each group. The results are presented in Table 27.

Table 27. Persistence study: Tukey-Kramer (HSD) test results.
Df = degree of freedom.

pairing	.40 S&W			.357 Mag		
	q stat	**q critic** α = 0.05 n = 5 Df = 110	Tukey's test conclusion	**q stat**	**q critic** α = 0.05 n = 5 Df = 114	Tukey's test conclusion
T0 vs T0.5h	4.874	3.917	Difference	4.559	3.917	Difference
T0 vs T1h	5.007		Difference	6.260		Difference
T0 vs T2h	5.962		Difference	6.911		Difference
T0 vs T4h	5.402		Difference	6.748		Difference
T0.5h vs T1h	0.231		No difference	1.628		No difference
T0.5h vs T2h	1.169		No difference	2.356		No difference
T0.5h vs T4h	1.429		No difference	2.669		No difference
T1h vs T2h	0.920		No difference	0.762		No difference
T1h vs T4h	1.220		No difference	1.230		No difference
T2h vs T4h	0.449		No difference	0.533		No difference

The results of the Tukey-Kramer test showed that differences were found significant between T0 and the other time points (Table 27). However, the results showed that no differences were found between the different time points, emphasising on the rapid decrease observed during the 30 minutes after the discharges (Figure 31). As observed from the boxplots illustrated in Figure 31, the no difference observed in the results of the Tukey-Kramer test is due to the closeness of the mean between the different time points, which is caused by the inherent variability of the OGSR traces and previously detailed.

2.3.1 Principal Components Analysis (PCA)

Principal components analysis (PCA) is a multivariate, non-parametric technique which transforms correlated variables into variables uncorrelated from each other, called principal component (PC). The PCA algorithm calculates linear combinations of a set of variables in order to maximise the variance, reducing the dimensionality of a large dataset while minimising the loss of information, resulting in a less redundant information [187, 188, 197]. PCA is widely used as an exploratory analysis in order to visualise the properties of a dataset [187]. It allows one to graphically observe a dataset by combining PCs which explain the maximum of the variance.

A drawback of PCA is that difficulties are encountered when the variables present missing data, such as no detection of OGSR compounds for some specimens [187]. In order to overcome this obstacle, it was essential to accommodate the missing data. The techniques chosen was to replace the missing values by values extrapolated from the dataset obtained from the experiments. This was done in order to avoid skewing the dataset by including zero values.

Two techniques were investigated to replace missing values; the median, which is less sensitive to extreme data than the average, and the half-minimum value, which is often a default choice [187]. The two techniques were tested on both .40 S&W and .357 Mag persistence datasets. It has to be noted that the missing values were replaced only for the PCA. The rest of the analyses were performed on the original dataset. The results of the replacement of the missing values are shown in Figure 32 and Figure 33 and the trends were found to be similar between both calibres investigated. It was found that replacing the missing values by the median of the respective dataset was the most suitable

technique, as the boxplots of the median (Figure 32 and Figure 33, Median) are highly similar to the original dataset (Figure 32 and Figure 33, RAW) indicating that replacing the missing values by the median did not skew the properties of the dataset.

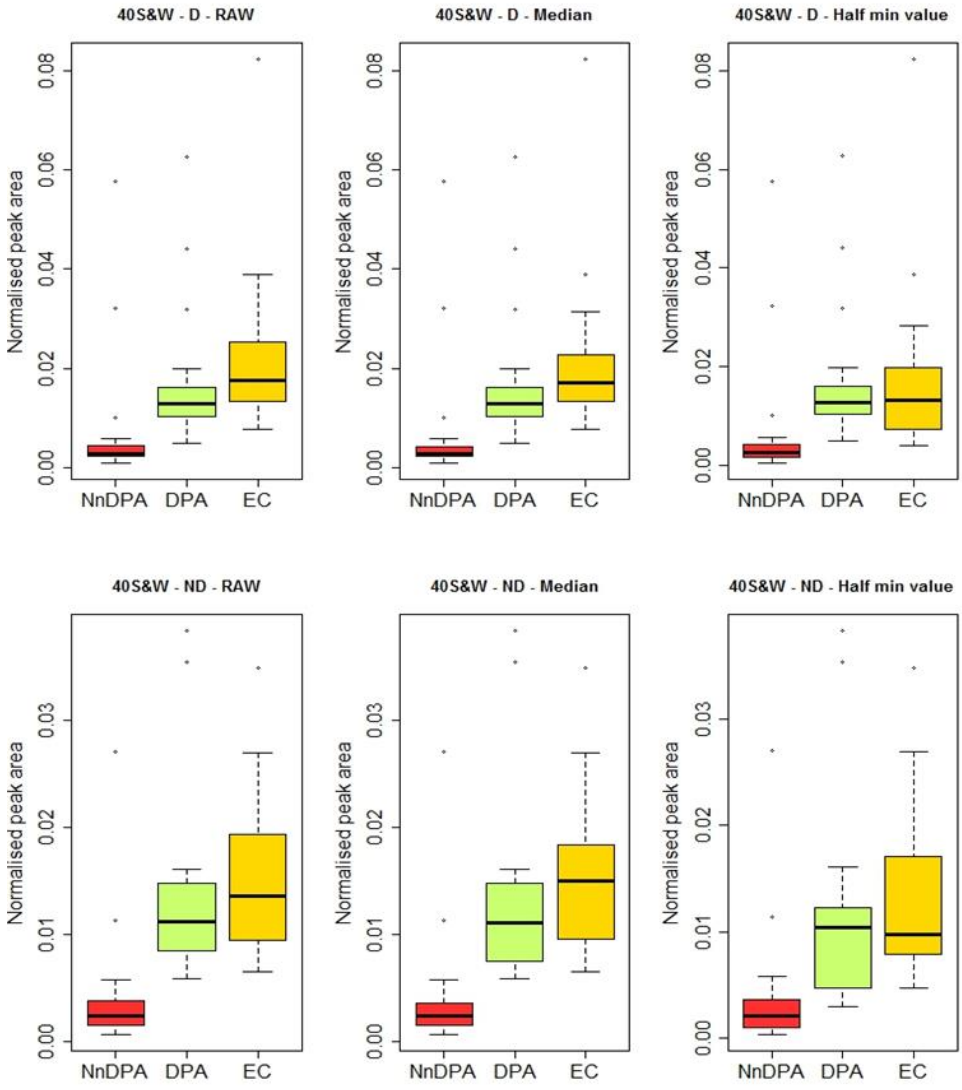


Figure 32. Results of the missing values replacement for the .40 S&W dataset. D= dominant hand, ND= Non-dominant hand. Raw = original dataset with missing values, Median = missing values replaced by the median if the dataset and Half min values = missing values replaced by the half-minimum values.

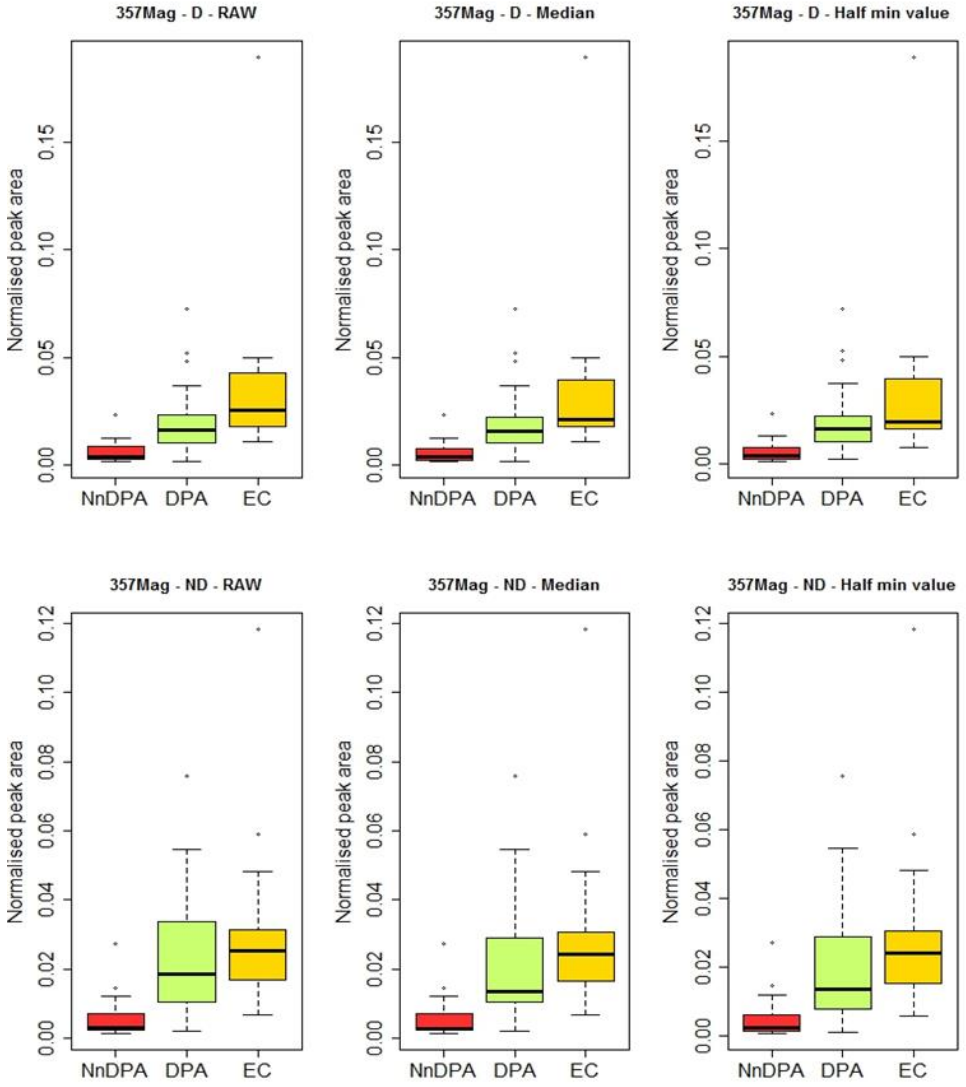


Figure 33. Results of the missing values replacement for the .357 Mag dataset. D= dominant hand, ND= Non-dominant hand. Raw = original dataset with missing values, Median = missing values replaced by the median if the dataset and Half min value = missing values replaced by the half-minimum values.

The PCA were performed with the statistical computing software R®. Two separate analyses were conducted. The first PCA was conducted to visualise the possible distinction between the different time points investigated in this study. In addition, PCA evaluating both dominant and non-dominant hand for visualising separately the results from the two hands of the shooter.

The results of the PCA for the .40 S&W calibre is presented in Figure 34. The results showed that the PCA plots of the first and second PC maximised the percentage of variance explained with a total percentage of 98.5% and 94.8% for the dominant and non-dominant hand respectively.

Regarding the second calibre, .357 Mag, the results of the PCA for the .357 Mag is highly similar to the results observed for the .40 S&W, with a large total percentage of variance explained by the PCA, respectively 99.9% and 99.3% for the dominant and non-dominant hand (Figure 35).

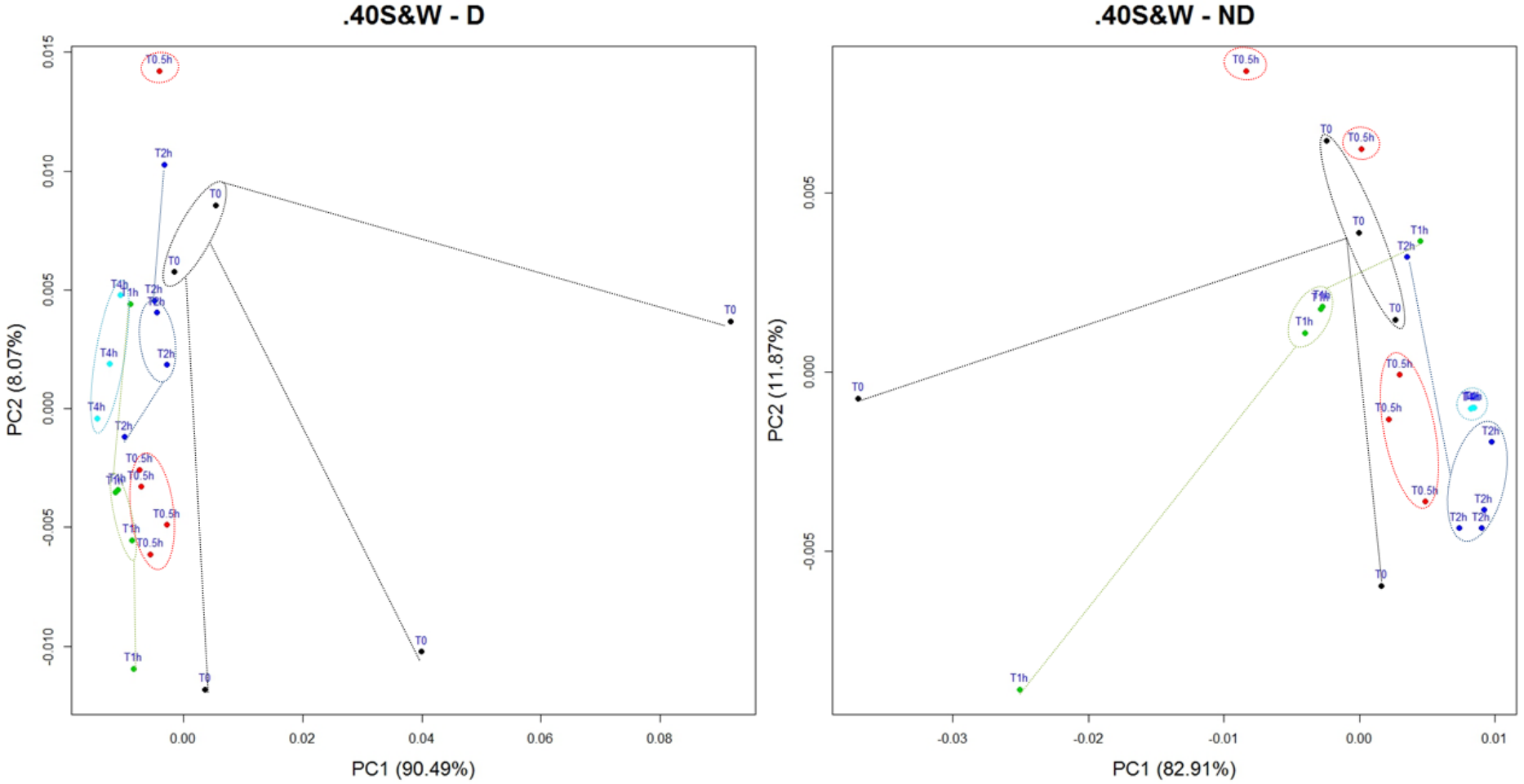


Figure 34. PCA plots for the calibre .40 S&W. D= dominant hand, ND= non-dominant hand. Each time points is represented as a cluster. The large variability can be observed within T0 which highlights the statistically difference with the other time points. The proximity between T0.5h, T1h, T2h and T4h clusters illustrates the results of the statistical test resulting in no significant difference between the time points past T0.5h (Table 27).

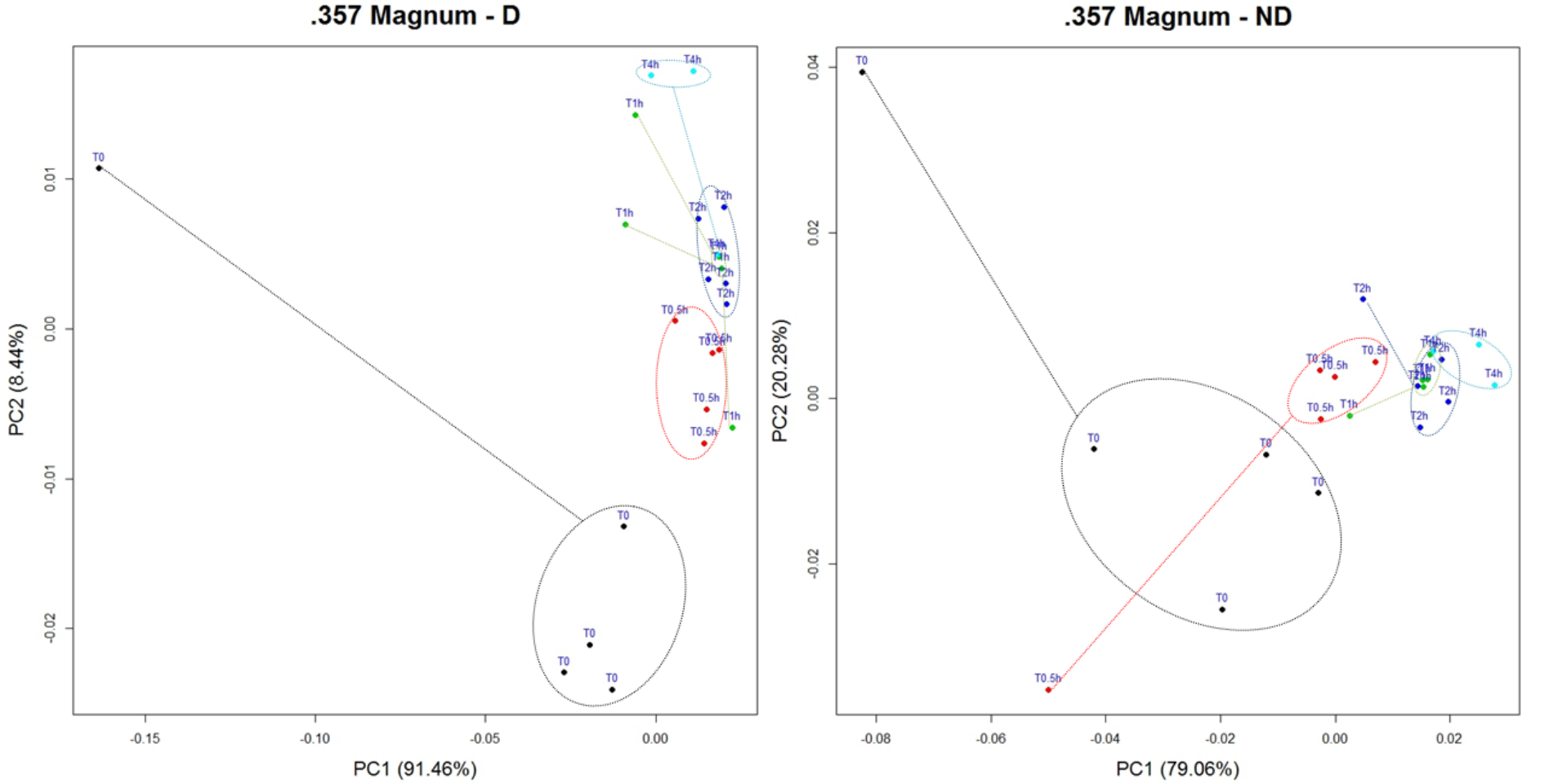


Figure 35. PCA plots for the calibre .357 Mag. D= dominant hand, ND= non-dominant hand. Each time points is represented as a cluster. Similar observation to the .40 S&W can be made for the .357 Mag calibre, which highlights the same statistical tests outcomes (Table 27).

The large variability observed in the persistence data and detailed previously has been reinforced by the PCA plots (Figure 34 and Figure 35), with a large scatter of the data points (e.g. T0). For the .40 S&W, it can be observed that data points arising from the same time point have the tendency to cluster together (illustrated by the ovals on Figure 34). Conversely, for the .357 Mag (Figure 35), it can be observed that no difference can be made between the time point T1h, T2h and T4h as they all cluster together (illustrated by the circle on Figure 35). This result supported the observation made from the boxplots presented in Figure 31 that the median and spread of the three time points was similar.

In addition, the statistical test (ANOVA and Tukey-Kramer test) performed previously and displayed in Table 27 concluded that there were no significant differences between the time points that exceeded T0. Such results are illustrated by the PCAs plots performed on both calibres. Even though a clustering was performed, it can be observed that most of the clusters were stacked close to each other, with a large spread of some of the data points in the form of outliers (illustrated by the lines on Figure 34 and Figure 35).

The scatter of these data points suggests that it is not possible to differentiate between the five time points studied. This also reflects the inherent complexity associated with the analysis of OGSR. As mentioned earlier, there is a countless number of parameters involved in the production and deposition of OGSR leading to a large variability in the recovery and analyses of such traces.

Some investigations may require the forensic scientist to attempt to determine the type of ammunition used based on the detection of GSR.

Figure 36 presents the PCA of the combination of T0 data points for both .40 S&W and .357 Mag. The aim of this analysis was to investigate the possible differentiation of the calibre based on their respective OGSR responses to the three compounds detected, *N*-nDPA, DPA and EC. The total percentage of variance explained by the two first PCs are 97.03% and 97.1% for the dominant and non-dominant hand respectively (Figure 36).

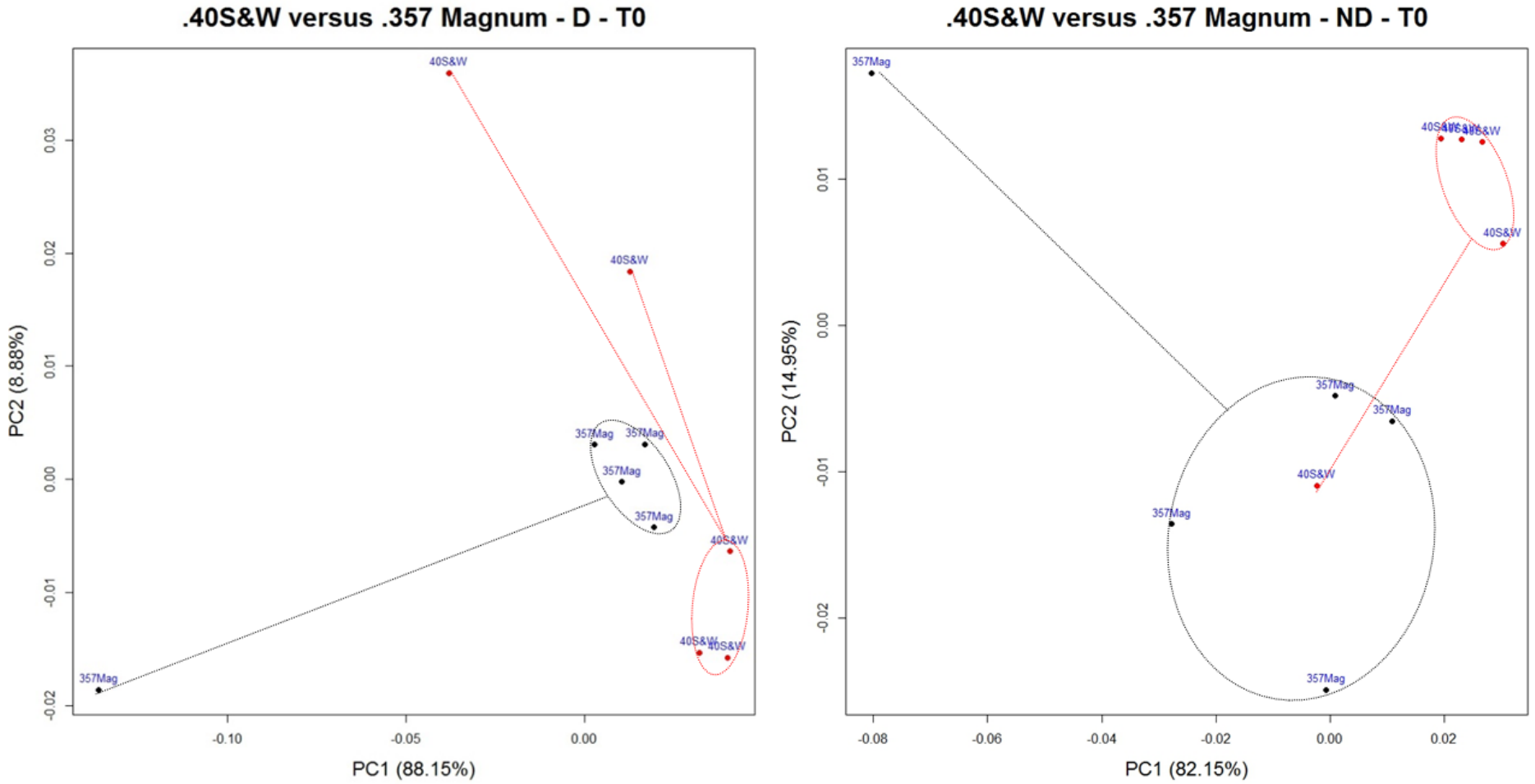


Figure 36. PCA plots for the comparison between the calibre .40 S&W and .357 Mag at T0. D= dominant hand, ND= non-dominant hand. Each calibre is represented as a cluster. Both calibre at T0 are represented. The large variation within the dataset can be observed through a large scatter of the data points for both calibres.

The results illustrate a distinct clustering of both calibres (circle on Figure 36). However, as previously outlined, there are some data points scattered from the main cluster, illustrated by the lines on Figure 36. A T-test (two tails, α=0.05) was performed in order to assess the significance of the clustering between the .40 S&W and .357 Mag at T0. The T-test resulted in P-value of 0.0318, suggesting a significant difference between the two populations. However, as Figure 36 indicates, a number of the replicates remain separated from the bulk of the population, limiting the potential differentiation of either the time points after the discharges or the calibres used. The PCA presented represent the response detected at T0, e.g. immediately after the discharges. However, the PCA between the two calibres were performed, in the same manner, for each time point studied (T0.5h, T1h, T2h and T4h). They are presented in the appendices as the results reached similar conclusions (Appendix III.3).

A T-test (two tails, α= 0.05) was subsequently performed on each time points in order to assess the difference between the .40 S&W and the .357 Mag. The results of each T-test is presented in Table 28. It was observed that after T0.5h there is no differentiation between the two calibres. This observation was also observed previously in both Figure 27 and Figure 28 (p. 106), in which it was found that for T0.5h, the differences between the responses of the two calibres becomes similar.

Table 28. T-test results between the .40 S&W and .357 Mag for each time point investigated.

T-test (two tails): .40 S&W vs .357 Mag		
Time point	*P*-values (α=0.05)	Conclusion
T0	0.0319	Difference
T0.5h	0.0047	Difference
T1h	0.2098	No difference
T2h	0.2354	No difference
T4h	0.6990	No difference

Principal component analysis was carried out on the persistence dataset of both .40 S&W and .357 Mag in order to, firstly differentiate the different time points investigated during the persistence experiment and, secondly, differentiate the different calibres studied. It was found that the three compounds targeted throughout this study did not allow significant differentiation of the five time points studied, due to the high intra-variability of the OGSR traces (Figure 28). However, it was possible to differentiate between the two calibres, but the large variability inherent to OGSR traces creates outliers which were observed on each of the PCA plots presented. A larger dataset might provide additional information regarding the frequency of the OGSR and might improve the performance of such chemometric analysis.

Hierarchical Cluster Analysis (HCA) was also performed on the persistence dataset for both ammunitions. In summary, similar conclusions to the PCA were identified, and they are presented in appendices (Appendix III.4 and III.5) as complementary information.

Several studies in the literature have approached the question of differentiating ammunition [74, 135, 136]. Brozek-Mucha et al. also investigated the differentiation of GSR [135]. They studied the classification of the inorganic particles based on their elemental composition. Their results emphasised that only one calibre could be differentiated from the other calibres investigated [135]. They further investigated a larger set of ammunition and calibres and classifying the inorganic particles based on the frequency of lead-barium-antimony particles [136]. They found that the frequency of some elements was a discriminative feature to classify IGSR particles as originating from a particular calibre [136].

Bueno et al. have also studied the ammunition discrimination by analysing GSR by mean of Raman spectroscopy [74]. They have emphasised that the results presented were preliminary and found their method suitable to differentiate GSR based on a set of chemical bonds. A limitation to this study concerns the nature of these bonds used to classify GSR, such as nitro (NO_2), carbonate (CO_3) and other aromatic groups. These bonds did not relate to GSR themselves as they are common chemical structure of many environmental compounds.

2.4 Future considerations

As a final point, this study provides valuable information for both forensic science and legal practitioners. As mentioned by the ENFSI guidelines [91], to achieve a proper and meaningful interpretation of traces such as OGSR, it is essential to integrate the results into the context and the chronology of the case under investigation. The interpretation of forensic traces at the activity level of the hierarchy of propositions must take into account factors such as persistence and secondary transfer [91], which relate to activities affecting the properties of OGSR traces. The focus of this research was to evaluate the effect of activities on the retention of OGSR on the hands of a shooter as persons of interest are rarely apprehended immediately at the scene of the suspected crime. During this study, the activities undertaken by the shooter involved daily office work, including computer and paper work. Indeed, being part of the police force, the shooter was asked to avoid contaminations (e.g. contact with firearms). Therefore, as previously emphasised, the simulations reflect idealistic situations.

Further studies are required in order to extend the simulations with scenarios involving a larger degree of activities, which may see alternative trends identified. Hofstetter et al. studied the transfer of OGSR on different surfaces, including hands, wrists, forearms, face and clothes of the shooter [17]. The results suggested that the amount detected was larger on clothing. It was also suggested that the clothes, wrist and forearm might be good alternatives to hands for collection of OGSR as traces may persist longer due less activity taking place at these particular areas [17]. Therefore, extending persistence studies to also include sampling of such areas is recommended in order to provide a more extensive knowledge about the retention of OGSR traces.

3. Conclusion

The aim of this study was to investigate the persistence of OGSR after discharge. The UPLC-QqQ-MS method was validated and found to be fit for purpose for the detection of four compounds associated to OGSR: DPA; *N*-nDPA; MC and EC.

The three compounds of interest were successfully detected in more than 70% of the specimens up to four hours after the discharge, with the most significant decrease in the OGSR amount occurring during the first hour post discharge. The observed trends were comparable to IGSR, but OGSR was retained for a longer period on the hands of the shooter. It is recommended that further research is conducted with a larger degree of activities, different firearms and different targeted surfaces such as forearms and clothing in order to extend OGSR persistence knowledge.

It can be worthwhile to include persistence information into the interpretation process to consider the chronology between the event under investigation, the kind of activities undertaken and the time between deposition and sampling. Finally, persistence information can be used in prioritisation of cases under investigation according to the time delay between suspected discharge of a firearm and collection of residues.

Additionally, as observed in previous studies involving the retention of inorganic particles, a high variability in the OGSR amount detected from shot to shot was observed. These observations can be attributable to numerous factors involved in the formation, dispersion and deposition of the residues. Nevertheless, the trends observed suggest that OGSR is a useful and meaningful source of information as a complement to the inorganic particles analysed by SEM-EDX. Finally, this study provides better knowledge regarding

the behaviour of OGSR traces, which can be used to improve the interpretation of organic gunshot residues. The PCA analysis allowed to observe clusters of the different time points studied. However, because of the large variability of the OGSR compounds, the results of the ANOVA and Tukey-Kramer test determined that the difference between the time points were found not to be significant. Such outcomes may have an important impact while interpreting the evidence. It highlights that a clear distinction between the time points might not be completely achievable because of the large intra-variability observed.

Section IV: Secondary transfer study

The aim of the current study was to extend the body of knowledge regarding the secondary transfer of OGSR as only two studies tackled such questions, and only one analysed specimen with a highly sensitive instrument. The current ammunition used by Australian police forces is the calibre .40 S&W, which has never been studied in the context of secondary transfer. Generally, police officer(s) carry their service firearms while on duty and may potentially come in contact with non-shooter(s) during police investigations and operations. It is, therefore, essential to assess the degree of transfer that could potentially take place if physical altercations such as an arrest procedure occurs between police officer(s) and non-shooter(s). This study considered two scenarios: the first one related to an arrest scenario between the shooter (i.e. Police officer) and a non-shooter (i.e. a 'clean' POI). The aim of this first scenario was to study the potential pollution of POI by police officers during the arrest process. The second scenario involved a non-shooter handling a firearm without discharging it. The aim of the second scenario was to determine the amount of OGSR transferred by handling the firearm when compared to a person who discharged it.

1. Methodology

For this study, one authorised officer in an Australian State police force facility performed the firearm discharges in an indoor shooting range. The different transfer scenarios were performed outside of the firing range to minimise the risk of polluting the hands of the non-shooter from the environment. One calibre and firearm were selected: A Glock 22® calibre .40 S&W. It is the service weapon and calibre of a number of Australian State police forces. The lead-free Winchester WinClean® (180Gr. Brass Enclosed Base) ammunition was chosen.

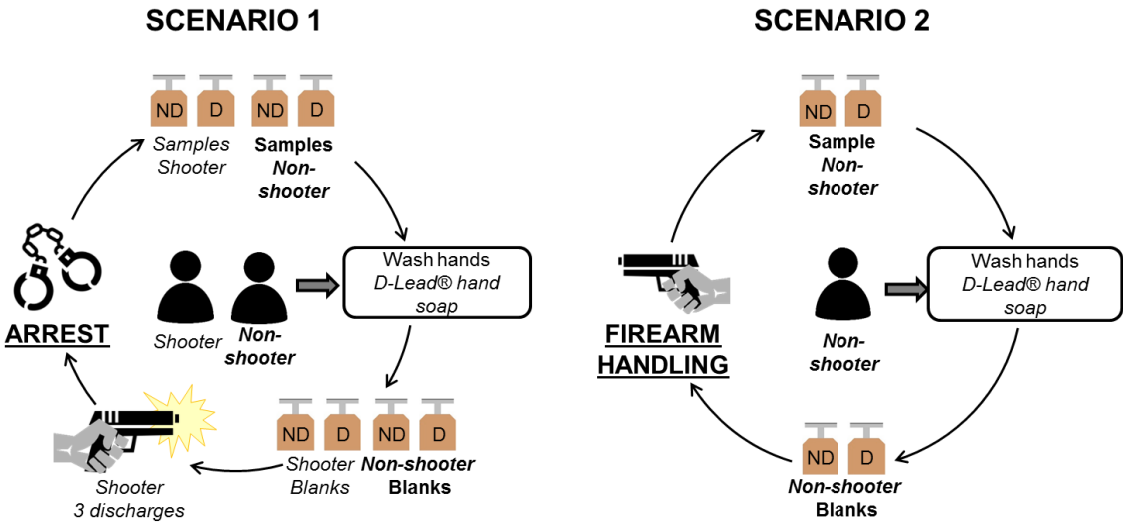


Figure 37. The sampling procedure of the secondary transfer experiments for both scenario 1 and 2 (n=5). D= dominant hand, ND= Non-dominant hand.

The method for both scenarios is presented in Figure 37. This involved both participants (one shooter and one non-shooter) thoroughly washing their hands prior to the blanks being collected. The next step required the shooter to discharge three rounds of

ammunition with the Glock® 22 held with two hands. To avoid pollution, the non-shooter did not enter the range at any point during the scenario. Following the firearm discharge, the shooter left the firing range and conducted the arrest scenario: the non-shooter put their hands on the top of their head before the shooter gripped the hands of the non-shooter and put them behind their back before they were handcuffed (Figure 37, scenario 1). The handcuffing procedure simulated a typical arrest procedure. The non-shooter was also asked to resist the arrest during the handcuffing process in order to simulate a realistic scenario.

After 1-2 minutes, the shooter removed the handcuffs and the specimens were collected from both hands of the non-shooter, as well as the shooter. The collection of OGSR was performed with GSR stubs (Ted Pella Inc, USA). The thumb-forefinger region of the palm and back of the hand, as well as the wrist, were thoroughly sampled until the stub surface was no longer sticky [17]. Each hand was sampled using an individual stub with the collected specimens packaged separately by sealing with the cover and placed in their respective boxes. The specimens were stored at 4 ºC until extraction. The extraction was performed within 24 hours of collection to avoid degradation of the specimens [190].

For the second scenario, the non-shooter washed their hands before blanks were taken outside the firing range while the shooter discharged the firearm three times. The non-shooter then picked up, held and handled the firearm with both hands for 5-10 minutes, without discharging it (Figure 37, scenario 2). Specimens from both hands were collected after the time elapsed as described previously. Both scenarios presented in Figure 37 were repeated in quintuplicate. Controls specimens were also collected. These specimens were collected from the same shooter immediately after having discharged three rounds of the same ammunition with the same firearm. The shooter did not make contact with any other

surfaces. These control specimens can be reasonably expected to contain a greater amount of OGSR because of the absence of potential losses due to further activities. Consequently, control specimens represent, on average, the maximum amount of OGSR detected from the hands of the shooter.

The specimens were stored at 4ºC until the analysis which was performed by UPLC-QqQ-MS the following day (Section I). A five points calibration and a QC were also run at the same time as the sample as instrument QCs. The results from the UPLC-QqQ-MS analysis are reported as a normalised response (Section III, 1.3.1, p. 99).

2. Results and discussion

During this study, calibration curves were analysed along with the specimens and showed consistent and stable responses across each analytical analysis with the calibration curves found to be linear during each analytical run (average $R^2 > 0.99$).

The targeted compounds were considered detected when the abundance was found above the LOD (Table 14, p. 63). Blank subtractions were performed on every specimen from each of the shooter and the non-shooter to account for possible contaminations. The blank specimens arising from the non-shooter where found to be clean, i.e. with none of the OGSR compounds detected. Similarly to the persistence study presented in Section III, it was surprising that even after hand washing, some blanks from the shooter were found to contain OGSR. The D-lead® hand soap, already used in the persistence study, was also used in this study. Such soap might not be sufficient enough to remove OGSR. If the hand blank was found to contain a larger amount of OGSR than that of its corresponding

specimen from the experimentation, that particular specimen was considered negative for OGSR traces to avoid skewing the results.

2.1 Scenario I: Arrest simulation

During their duties, police officers may come into contact with a potential POI for a particular investigation. When it occurs, there is a question of potential pollution of the non-shooter by the police officer. In order to assess this, an arrest scenario was performed and investigated. Three of the four target compounds were detected (EC, DPA and *N*-nDPA). The manufacture of propellant powder typically involves the addition of a single centralite, either EC or MC but rarely both. Figure 38 represents the amount detected (normalised response) for the three compounds of interest collected from both hands (dominant and non-dominant) of the shooter and non-shooter in the context of the arrest scenario. Figure 38a represents the details of the replicates, each bar-plot represents the amount detected on each hand. Figure 38b represents the averaged amount detected from each set of replicate specimens.

It was observed that the three compounds of interest were identified in each of the five replicates of the arrest scenario. The amount detected of these compounds followed the same trend across each of the dominant and non-dominant hands, with a lower amount detected on the latter when compared to the dominant hand. As expected, the largest amount was detected in the control (Figure 38a and b), which relates to the detection of OGSR on the hands of the shooter immediately after the firearm discharges. The second largest amount detected were from specimens arising from the hands of the shooter after

they arrested the non-shooter. Finally, a secondary transfer was observed for the three compounds of interest on the non-shooter after being handcuffed by the shooter (Figure 38). The non-shooter did not have any prior contact with any potential source of OGSR, and their blanks were found to be free of OGSR. This emphasises the fact that the OGSR found on the non-shooter arose primarily from the transfer of OGSR from the shooter during the arrest. It must be noted that the wrists were also sampled. Hence, the handcuffs might also have contributed to the secondary transfer of OGSR onto the hands of the non-shooter in addition to the transfer from the shooter during the duration of the arrest process.

The results observed in Figure 38b on the hands of the shooter also suggest the presence of a secondary transfer as the amount detected on the hands of the shooter is lower than the amount detected in the controls. The only difference between the control specimens and the shooter specimens was the arrest scenario. Hence, the responses detected, for each of the compounds on both hands of the shooter, were found to be lower than the controls. Such a result is likely due to the transfer of OGSR off the hands of the shooter onto the hands of the non-shooter while performing the arrest simulations. However, losses to the environment or redistribution of the OGSR onto surfaces that were not collected may also have contributed to the results. An exception is regarding DPA on the non-dominant hand (Figure 38 and Table 29), where it can be observed that adding together the amounts of DPA on the shooter and non-shooter resulted in a larger amount than the control. Such a result may be the consequence of the high variability observed between specimens as illustrated by the error bars in Figure 38b.

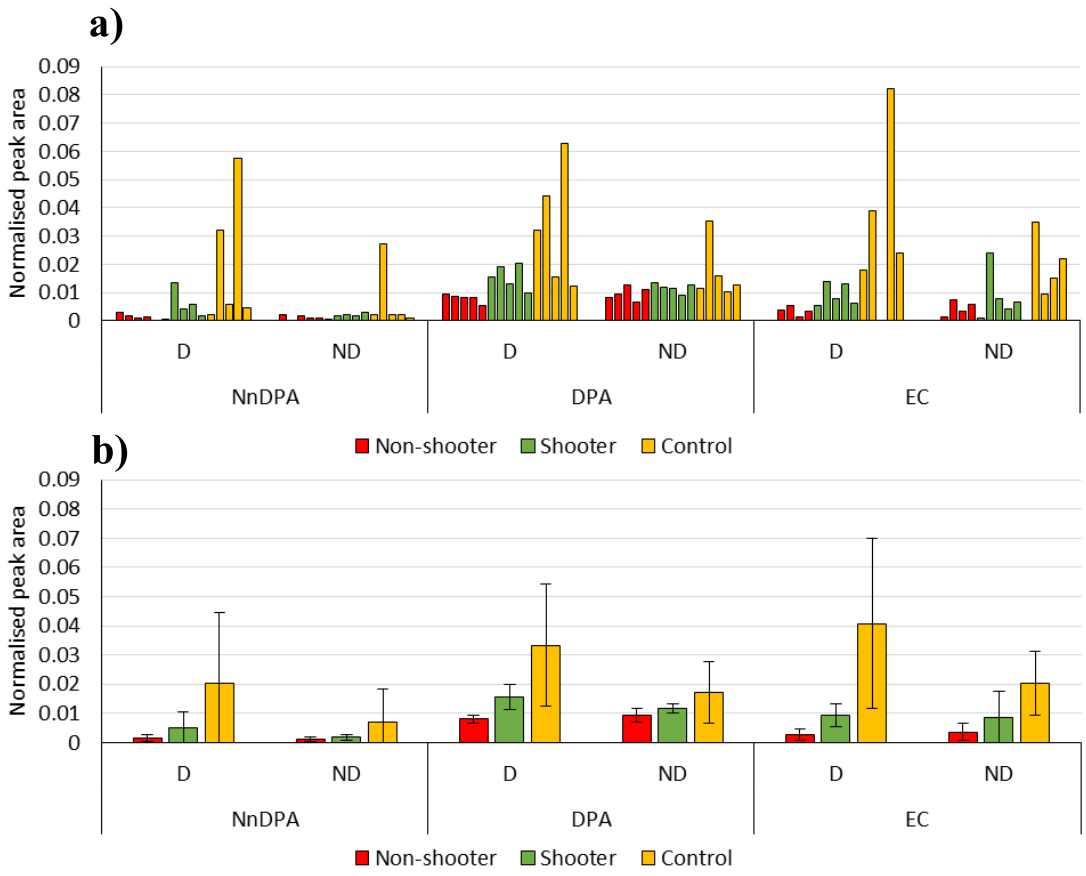


Figure 38. Scenario 1: Arrest process. Level of OGSR detected (normalised response) from both hands of each participant. D= dominant hand, ND= Non-dominant hand. a) Each replicates separately (n=5); b) Averaged amount; the error bars represent the standard deviation. The "non-shooter" represents the individual being arrested who did not have any contact with the firearm; the "Shooter" is the police officer who arrested the non-shooter after discharging the firearm. "Controls" represent the amount detected from the shooter immediately after three discharges, without having entered in contact with any other surfaces.

Different trends were observed between the non-shooter when compared to the shooter and the controls when comparing the dominant and non-dominant hand (Figure 38b). It was found that the detection of compounds was more consistent between both hands on the non-shooter while larger differences between hands was seen in the shooter and control specimens. Greater amounts of OGSR were detected on the dominant hand of the shooter and control when compared to the amount detected on the non-dominant hand. When semi-automatic pistols are considered, the deposition of GSR is highly dependent on the make of firearm as that dictates relative positioning of the hands to the ejection port. In this case the ejection port of the firearm was on the right. As a result, a greater amount of GSR is typically deposited on the right hand if that is the dominant hand. The difference in the amount is consequently highly due to the activity of holding and discharging a firearm. Whereas on the non-shooter, the result related to the handcuffing scenario for which both hands of the non-shooter were usually clutched in the same manner in order to put them behind the back. This led to a lower and more consistent level of OGSR being transferred across both hands of the non-shooter than that seen in the shooter and control specimens. The large variability was also observed in Figure 38b (as demonstrated by the error bars) for both shooter and control. This variability reflects the numerous factors influencing the primary transfer of OGSR compounds such as the firearm, the ammunition, the plume dispersion at the muzzle and the ejection port, the shooter position, skin conditions and environmental conditions. Such variations were also observed in previous studies [41, 173].

Table 29 represents the percentage when the detected responses were normalised to the control. The controls represent the largest amount of residues available as they were collected immediately after discharging the firearm (100%). A large difference was observed between the specimens (Table 29), with the amount detected on the shooter post arrest ranging between 23% (EC, D) and 68% (DPA, ND). Conversely, that of the non-shooter ranged between 9% (*N*-nDPA, EC, D) up to 55% (DPA, ND).

Table 29. Scenario 1: Average percent of the level of OGSR detected when normalised to Control. D= dominant hand, ND= non-dominant hand.

	N-nDPA		DPA		EC		AVERAGES
	D	ND	D	ND	D	ND	
Control	*100%*	*100%*	*100%*	*100%*	*100%*	*100%*	*100%*
Shooter	25.7%	27.3%	46.9%	68.1%	22.8%	42.5%	38.9%
Non-shooter	8.6%	21.0%	24.1%	55.4%	8.4%	22.4%	23.3%

When comparing the overall averages (Table 29), it was observed that after the arrest scenario an amount corresponding to 39 % of the control were still observable from the shooter, while on the arrested non-shooter, the amount detected after secondary transfer was about 23 %, suggesting that a pollution of a non-shooter from shooter who proceeded to the arrest was significant.

Similar trends were observed by Gassner et al. [173] with detection of OGSR on a non-shooter after an arrest scenario. The study involved the use of a different calibre (9 mm Luger) with the arrest scenario conducted differently as the non-shooter was handcuffed on the ground and then helped to get back up. They identified that a significant amount of OGSR (41.9% for *N*-nDPA) was transferred during an arrest simulation. As they sampled both hands on the same GSR stub [173], the results from the present study (e.g. D and ND) were summed together for comparison purposes, and a secondary transfer of 29.6% for *N*-nDPA was found. Considering the large variability observed between discharges, the difference in the observed secondary transfer is likely to arise from the simulations and the way the arrests were conducted. In the study by Gassner et al. [173], the fact that the non-shooter was lying down and helped to get back up may result in a longer and more vigorous contact, which may ultimately lead to a larger amount of OGSR being transferred.

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2.2 Scenario II: Firearm handling

In Scenario 2, a firearm was discharged three times and then handled by a non-shooter for approximately 10 minutes without being further discharged. The holding activity was not restricted and the non-shooter also manipulated the firearm by opening and closing the breech. In Figure 39a and b, it was observed that *N*-nDPA, DPA and EC were successfully detected on the hands of the non-shooter, confirming that secondary transfer was possible through handling of the firearm, with OGSR detected in all 5 replicates. The amount detected was consistently lower than that of the control (Figure 39a). Figure 39b represents the average amount (normalised response) for each participant (non-shooter and shooter) and the error bars reflect the standard deviations.

After discharge, OGSR traces might deposit onto the body of the firearm, generating an accumulation of residues on its surface. Furthermore, if the firearm is typically not cleaned frequently there may be accumulation of GSR on its outer surfaces. The source of the OGSR traces detected on the non-shooter hands is, therefore, likely to arise from the OGSR background present on the firearm during the holding contact and manipulations. Consequently, the secondary transfer of OGSR when handling a firearm might be a significant or even the main contributor to the deposition of OGSR on the non-dominant hand of a shooter.

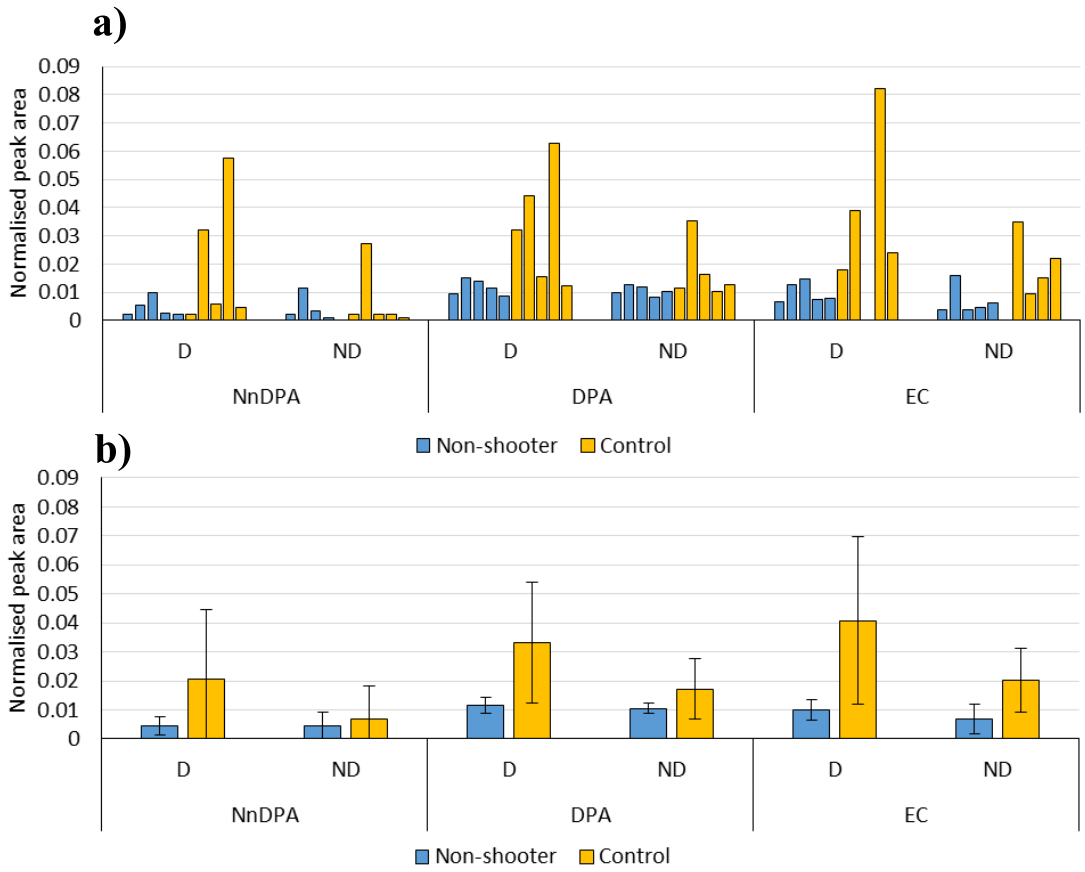


Figure 39. Scenario 2: Firearm handling. Level of OGSR detected (normalised response) from both hands of each participant. D= dominant hand, ND= Non-dominant hand. a) Each replicates separately (n=5); b) Averaged amount; the error bars represent the standard deviation. The "non-shooter" represents the individual who handled the firearm; "Controls" represent the amount detected from the shooter immediately after three discharges, without having entered in contact with any other surfaces.

It is interesting to observe that for *N*-nDPA on the non-dominant hand of the non-shooter (Figure 39), handling the firearm resulted in a similar amount of OGSR being transferred to the amount detected in control specimens. As indicated above, due to the construction of the firearm, the position of the ejection port (on the right) and the position of the hands while discharging the firearm, the non-dominant hand is often less exposed to the GSR plume when firing a gun than the dominant hand. Therefore, a significant portion of the total amount of OGSR transferred onto the non-dominant hand might arise from the contact with the grip of the firearm as it usually acts as a support when holding the firearm, or by more generally handling the firearm immediately prior to discharging it.

However, as the non-shooter held the firearm without any restriction, on occasions, the firearm was passed between the two hands exposing both the dominant and non-dominant hand to OGSR. This manipulation might have likely contributed to the secondary transfer on the non-dominant hand. Furthermore, the duration of the contact was longer during the scenario (approximately 10 minutes) to that of the controls, where the specimens were collected immediately after discharge. A prolonged contact would also impact the level of OGSR transfer onto the surface of the hands.

When comparing the trends, Table 30 illustrates the percentage of OGSR detected on both hands of the non-shooter normalised to the control. On the dominant hand, a range of 22 % - 35 % was detected. While on the non-dominant hand, a wider range of 33% - 65 % for the three compounds was detected (Table 30). On average, an amount of 40 %, when compared to the control specimens, was still detected after handling the firearm. The results emphasise that handling a firearm for several minutes, without discharging it, is sufficient to successfully transfer OGSR traces in a substantial amount. The discrepancies between the dominant and non-dominant hand observed in Table 30 arose

from the normalisation to the control (Figure 39). In the control (Figure 39), a lower amount was detected on the non-dominant hand, which resulted, after the normalisation, in larger percentages.

Table 30. Scenario 2: average Percent of the level of OGSR detected when normalised to Control. D= dominant hand, ND= non-dominant hand.

	N-nDPA		DPA		EC		AVERAGE
	D	ND	D	ND	D	ND	
Control	*100%*	*100%*	*100%*	*100%*	*100%*	*100%*	*100%*
Non-shooter	21.7%	65.2%	35.1%	61.3%	24.1%	33.4%	40.2%

Gassner et al. also observed a secondary transfer of OGSR by only touching the surface of a firearm when transporting it [173]. They observed that the amount detected after touching the firearm was lower than after the arrest, while in this study the opposite trend was observed. The different conditions of the firearm manipulation and the timeframe of the experiment are likely the source of the different trends observed between the two studies. Indeed, their experiment involved transporting a firearm without any further handling, whereas in the present study, the non-shooter was allowed to manipulate the firearm, including opening and closing the breech. Opening and closing the breech provides access to additional sources of OGSR inside the weapon, leading to a potentially higher secondary transfer. The second substantial factor is the contact duration. In their study, contact was about 10 seconds [173], while here the non-shooter held the gun approximately 10 minutes. Such difference in the duration of the contact with the firearm may greatly influence the amount of OGSR transferred as a longer contact may result in a larger amount of OGSR transferred.

2.3 Scenarios comparison

Figure 40 illustrates the amount detected across the two scenarios performed in this study. It was found that a secondary transfer of OGSR occurred following both the arrest and firearm handling experiments, with similar trends observed across the three compounds detected. A substantial reduction of the response detected between the controls and the non-shooter after the arrest was observed (Figure 40).

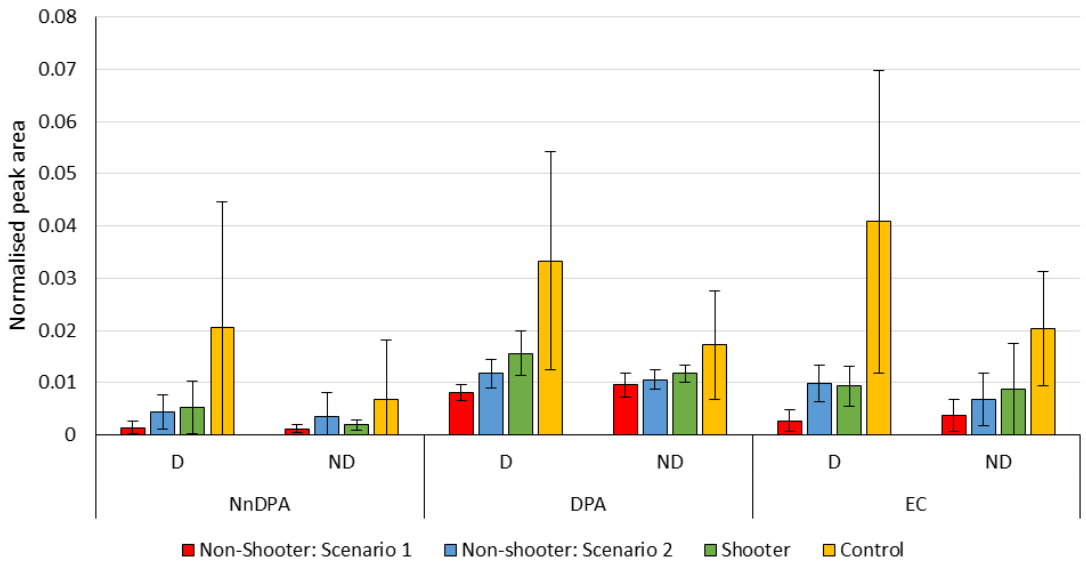


Figure 40. Comparison between scenario 1 and 2. D= dominant hand, ND= non-dominant hand.

It was also observed that handling a firearm resulted in a higher degree of secondary transfer (Table 30, average of 40% observed) than being arrested by the shooter (Table 29, average of 23% observed). The amount detected on the shooter after the arrest scenario is similar to the non-shooter after having handled a firearm (Figure 40, Table 29 and Table 30, 38.9% and 40.2% respectively). Minor exceptions were seen for DPA (D)

for which a larger response was observed on the shooter when compared to the non-shooter (firearm handling). The opposite result was observed with *N*-nDPA on the non-dominant hand, of which a difference of almost 38% was observed between the two scenarios (27% in Table 29 and 65% in Table 30). This is due to a high amount detected on the hand of the non-shooter after handling the gun and a low amount detected on the shooter after the arrest. These results emphasise the influence of the shooting process and the associated variability in the amount of OGSR recovered and detected. Finally, on average, the largest responses detected arose from specimens taken from the controls, which were taken from the shooter immediately after the firearm discharges and without any contact with any other surfaces.

In summary, the results of this study demonstrate that the risk of secondary transfer is significant. From an investigation and interpretation perspective, it is recommended that precautions should be taken to minimise instances of secondary transfer to avoid false positive results. Because of the numerous factors involved in the formation, deposition and analysis of OGSR, as well as the unique sequence of activities that may lead to different extent of secondary transfer, it is essential to consider assessing every investigation using case-specific information in order to ensure to interpret OGSR results in an appropriate manner in view of the circumstances surrounding the investigation [34].

The results suggest that standard practice should include documentation of any contact between officers, firearms and the POI(s). Further, the results suggest that officers' firearm, handcuffs, clothes and other equipment should be cleaned and decontaminated regularly. It would be valuable for contact records to be provided to the forensic scientist in order to assist in the evaluation of the OGSR evidence. This will allow forensic scientists to approach the interpretation of the results with a better understanding of the

context in which the specimens were obtained. For instance, a police officer who discharged a firearm shortly before or during an intervention should not come into contact with POI(s) in order to restrict possible pollution and ultimately reduce the risk of false positive. If such a situation is unavoidable and occurs, sampling the police officer who arrested the POI might be valuable. Such specimens could be used as controls to assess the degree of OGSR pollution of the police officer as soon as possible.

In the global context of GSR, no direct comparison of transfer and persistence data can be performed between IGSR and OGSR because of the different formation process, physical and chemical composition as well as transfer and persistence mechanism. Several studies have examined the secondary transfer of IGSR [149, 166, 170, 172]. In the context of an arrest scenario, Charles and Geusens [166] have studied two separate simulations involving a low and high level of contamination of the police officers. For the low contamination simulation, in average, 2 characteristic particles (Pb-Ba-Sb) were detected on the shooter while only 1 was detected on the non-shooter, resulting in 33% of transfer [166]. For the high simulation, however, an average of 66 particles were found on the shooter, while only 3 on the non-shooter (4% of transfer) [166]. Girvan et al [172] have also studied the secondary transfer of IGSR through the arrest process. They found a secondary transfer of 40% for the characteristic Pb-Ba-Sb particles [172]. Such studies highlight the inherent variability of IGSR deposition and analysis, which was also observed for OGSR in this study.

These results on the secondary transfer of IGSR [166, 172] have shown similar trends when compared to the results observed for OGSR presented in this study, emphasising that a secondary transfer of both IGSR and OGSR is possible in the context of an arrest. The differences are likely to arise from the different retention and transfer properties of

IGSR when compared to OGSR. Consequently, such results stress the caution that should be exercised when interpreting GSR evidence, especially when a low number of particles of IGSR or a low amount of OGSR are detected. This study has emphasised the necessity and the importance of assessing the secondary transfer of OGSR.

Such results might be used in order to improve the interpretation of such traces in the context of forensic investigations. An interpretative model can be developed through the use of a probabilistic framework such as Bayesian theorem, which would enable forensic scientists to assess the LR for OGSR outcomes in the light of the propositions of interest as well as the case circumstances. The advantages of the Bayesian approach is that it allows forensic scientists to take into account the possibility of a secondary transfer as well as the persistence when calculating the LR.

2.4 Exploratory Data Analysis (EDA)

Similarly to the persistence study (Section III), further exploratory data analysis were performed on the secondary transfer dataset. The aim was to assess a possible distinction between the different activities involved in the scenarios, embodied by the different individuals such as the non-shooter, the shooter and the control.

The boxplots presented in Figure 41 represents the variability of the data points for each set of specimens. Both hands are combined in order to have a general overview of the distribution of the results.

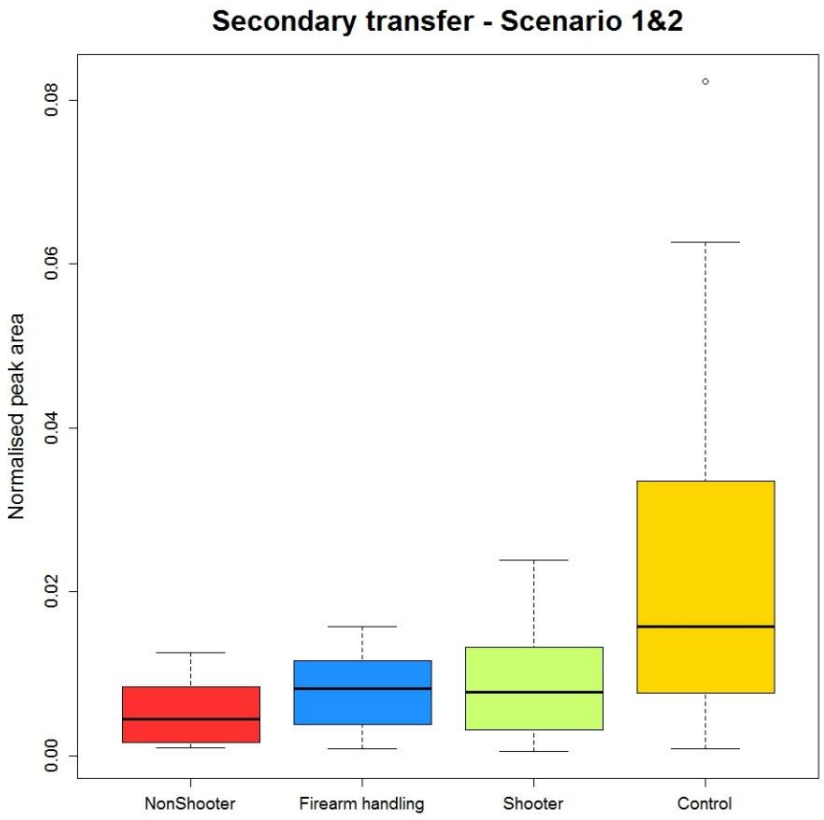


Figure 41. Boxplot secondary transfer. Both hands combined.
Non-shooter= after arrest, Firearm handling= non-shooter after handling the firearm, Shooter= after the arrest. The black lines represent the median of the dataset.

It can be seen that the lowest response was obtained by the non-shooter after the arrest scenario and highest by the controls. However, the non-shooter after the firearm handling is highly similar to the shooter after the arrest, indicating that a distinction between both activities is highly unlikely.

To assess the normality criterion, Shapiro-Wilk tests were conducted, on each population at a 95% CI (α= 0.05). This was performed in order to determine if an ANOVA can be conducted. A total of 18 tests were conducted, the detailed results are presented in appendix III.2. It was found that 94.4% (17/18) of the tests returned a p-value above the 0.05 threshold indicating that the normality of the datasets cannot be rejected. 5.6% of the tests (1/18) returned a result indicating a non-normal population (appendix III.2). However, such results have to be interpreted carefully as only 5 data points were available to assess the normality.

Consequently, the criterion of normality was considered fulfilled and, in order to assess if there are any significant differences between the different populations (individuals involved), an ANOVA (one factor) was performed on the dataset for each scenario, similarly to the persistence study. It was found that the P-value was significantly lower than the threshold of 0.05 (α) with values of 8.91×10^{-8}, suggesting that at least one group is different. In order to assess which group present a difference, the Tukey-Kramer post-hoc test (HSD) was conducted on each pair of groups. The results are presented in Table 31.

Table 31. Secondary transfer study: Tukey-Kramer (HSD) test results.
Df = degree of freedom, SC1 & 2 = Scenario 1 and 2.

pairing	Secondary transfer		
	q stat	**q critic** α = 0.05 n = 4 Df = 109	Tukey's test conclusion
Control vs Shooter	6.788	3.680	Difference
Control vs Non-shooter (SC2)	7.046		Difference
Control vs Non-shooter (SC1)	8.266		Difference
Shooter vs Non-shooter (SC2)	0.319		No difference
Shooter vs Non-shooter (SC1)	1.745		No difference
Non-shooter (SC2) vs Non-shooter (SC1)	1.424		No difference

The results of the Tukey-Kramer test highlighted than there were significant differences between the controls and the other populations (Table 31). Nevertheless, no differences were observed between the three other individuals. This was also observed in the boxplots in Figure 41 with the median and the scatter of the non-shooters and the shooter are close from each other, while the control presented a larger response.

The boxplots presented in Figure 42 illustrate the variability and the scatter of the response observed in both scenarios investigated. Both hands are observed separately and it can be seen that the trends are generally highly similar, with the lowest response obtained from the non-shooter after the arrest scenario and the highest response observed from the control specimens. Both non-shooter after the firearm handling scenario and shooter after the arrest scenario were found to be very similar, with the exception of DPA on the dominant hand, which present a larger response observed on the shooter after arrest when compared to the non-shooter after the firearm handling. A second exception can be seen on the non-dominant from the *N*-nDPA, where the firearm handling presented an abnormally high response when compared to the other specimens. This is be due to a larger secondary transfer occurring on the hands of the non-shooter during the second scenario.

This observation emphasised the previous discussion in which it was noted that there was no restriction in the way of handling the firearm. Thus, it is possible than the individual held the grip with the non-dominant hand causing a secondary transfer. This may have led to a larger amount being transfer than the primary transfer during a firearm discharge, as the non-dominant hand was less prone to enter in contact with the grip and the OGSR plume (Section III, 2.1, p. 101).

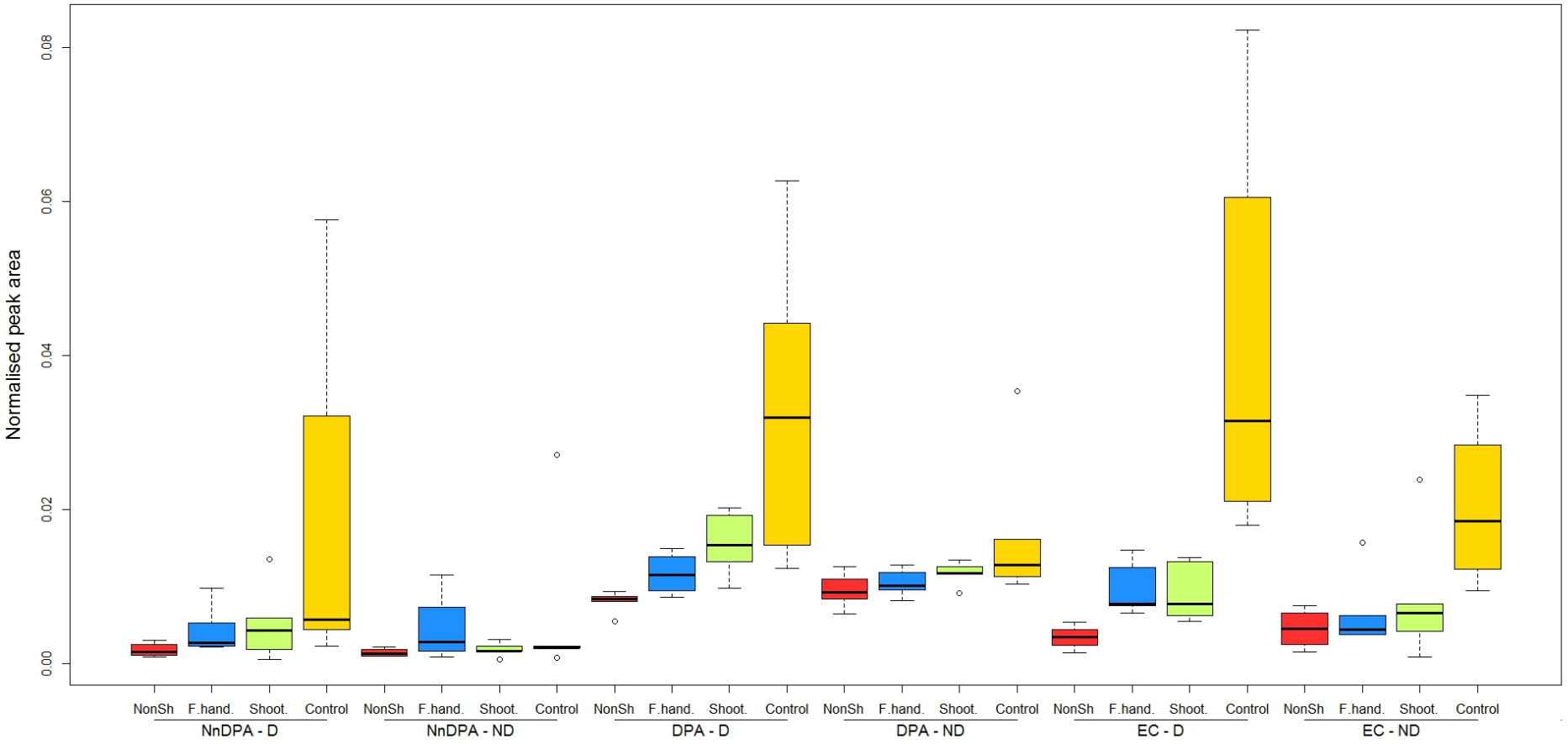


Figure 42. Boxplot secondary transfer. The two scenarios studied are represented.
D= dominant hand, ND= non-dominant hand. NonSh= Non-shooter of the arrest scenario, F.hand.= Non-shooter of the firearm handling scenario, Shoot.= Shooter after the arrest scenario.

2.4.1 Principal Components Analysis (PCA)

Because of the inherent variability of the primary transfer and the nature of OGSR, some experiments were found to not contain OGSR (<LODs). Similarly, to the persistence study, it was essential to assess how to deal with missing values.

Two methods to replace the missing values were investigated, replacing the missing values by the median values of the respective dataset as well as the half of the minimum values of the respective dataset [187]. Replacing the missing values was performed in order to avoid introducing zeros in the dataset, which would skew the results of the PCA.

The results are presented in Figure 43 and Figure 44, which present the original dataset (Raw), the dataset with the missing values replaced by the median (Median) and the dataset with the missing values replaced with the half minimum values (Half min value). It was observed that replacing the missing value by either the median or half minimum value lead to good results when compared to the original dataset (Figure 43 and Figure 44) as either method did not affect the general scatter and median of the dataset.

The results of the test for replacing the missing values performed on the secondary transfer datasets are highly similar to the tests performed on the persistence dataset presented in Section III (part 2.3). Therefore, the median, which was used in the persistence dataset, was also used to replace the missing values in the secondary transfer dataset. It is important to note that the replacement of the missing values was only achieved in order to perform the PCA, and not for any further analysis.

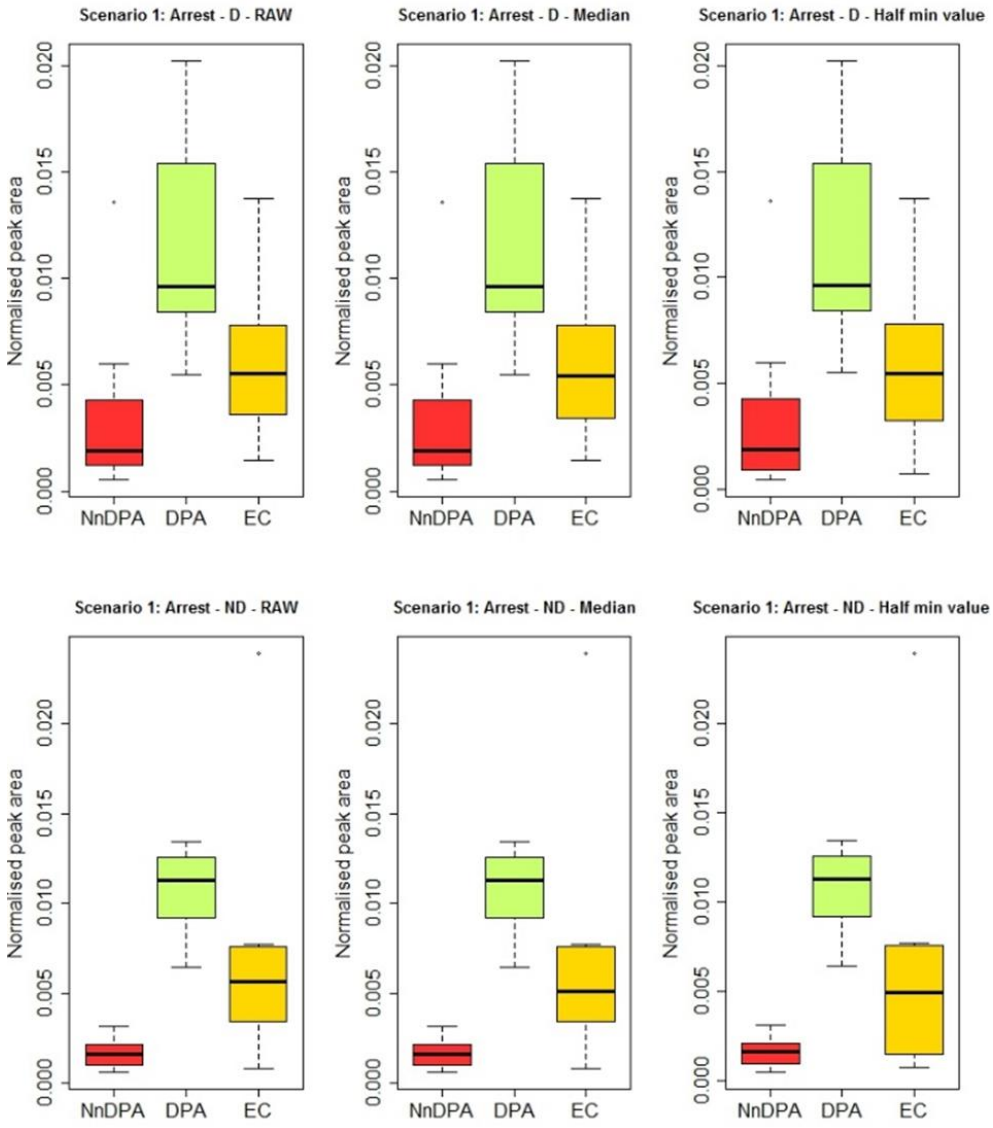


Figure 43. Results of the missing values replacement for the scenario 1 of the secondary transfer study. D= dominant hand, ND= Non-dominant hand. Raw = original dataset with missing values, Median = missing values replaced by the median if the dataset and Half min values = missing values replaced by the half-minimum values. F.Hand= Firearm handling scenario.

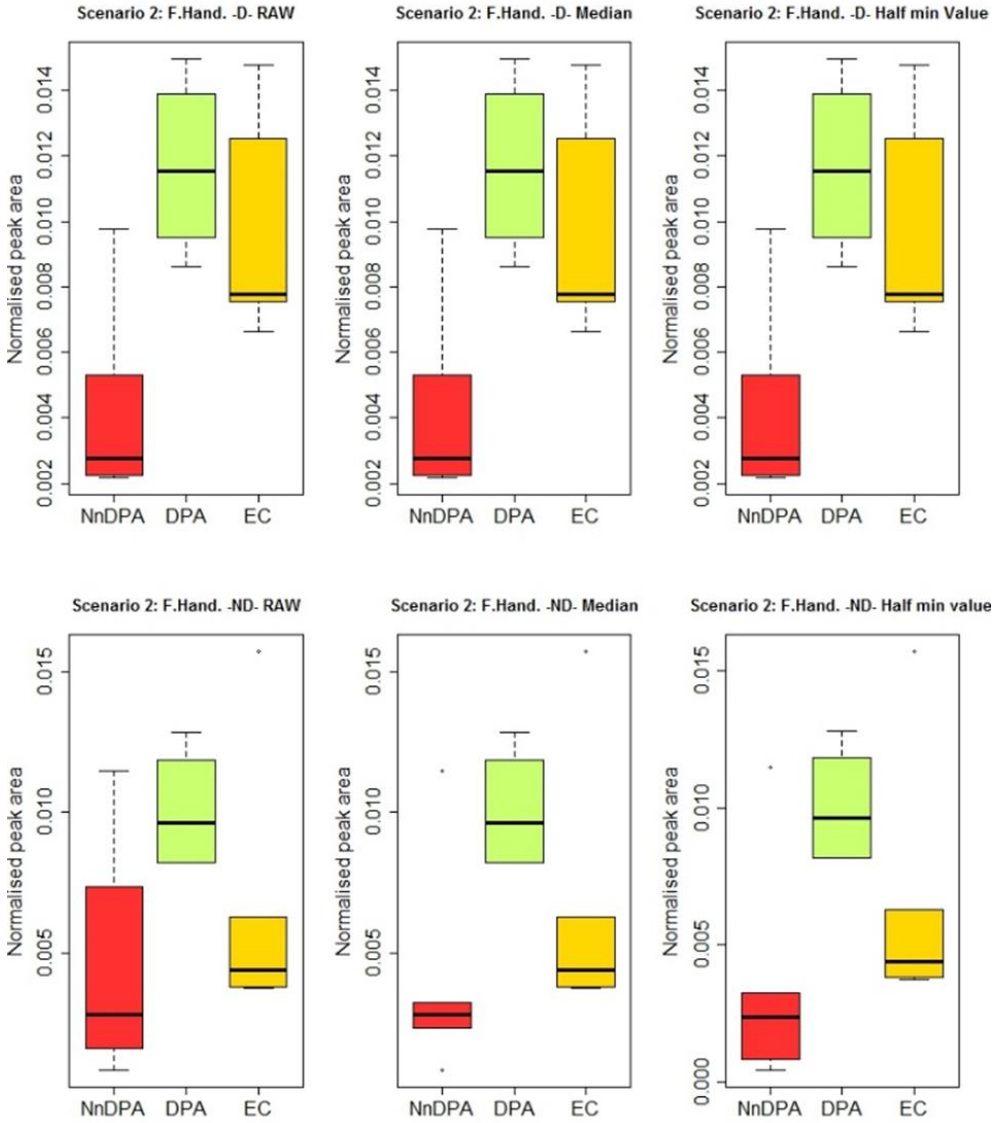


Figure 44. Results of the missing values replacement for the scenario2 of the secondary transfer study. D= dominant hand, ND= Non-dominant hand. Raw= original dataset with missing values, Median= missing values replaced by the median if the dataset and Half min values = missing values replaced by the half-minimum values. F.Hand= Firearm handling scenario.

The PCA were performed in the same manner as persistence study. The PCA were conducted by mean of the statistical software R®. The first PCA was conducted for the scenario 1, the arrest simulation. The aim was to assess if any differentiation could be made between the different protagonists. The results are presented in Figure 45, it was observed that that the PCA plots of the first and second PC maximised the percentage of variance explained with a total percentage of 98.0% and 98.5% for the dominant and non-dominant hand respectively.

Regarding the second scenario, the firearm handling simulation, the PCA results are presented in Figure 46. The results are highly similar to the results observed for the scenario 1, with a large total percentage of variance explained by the PCA, respectively 98.2% and 97.6% for the dominant and non-dominant hand (Figure 46).

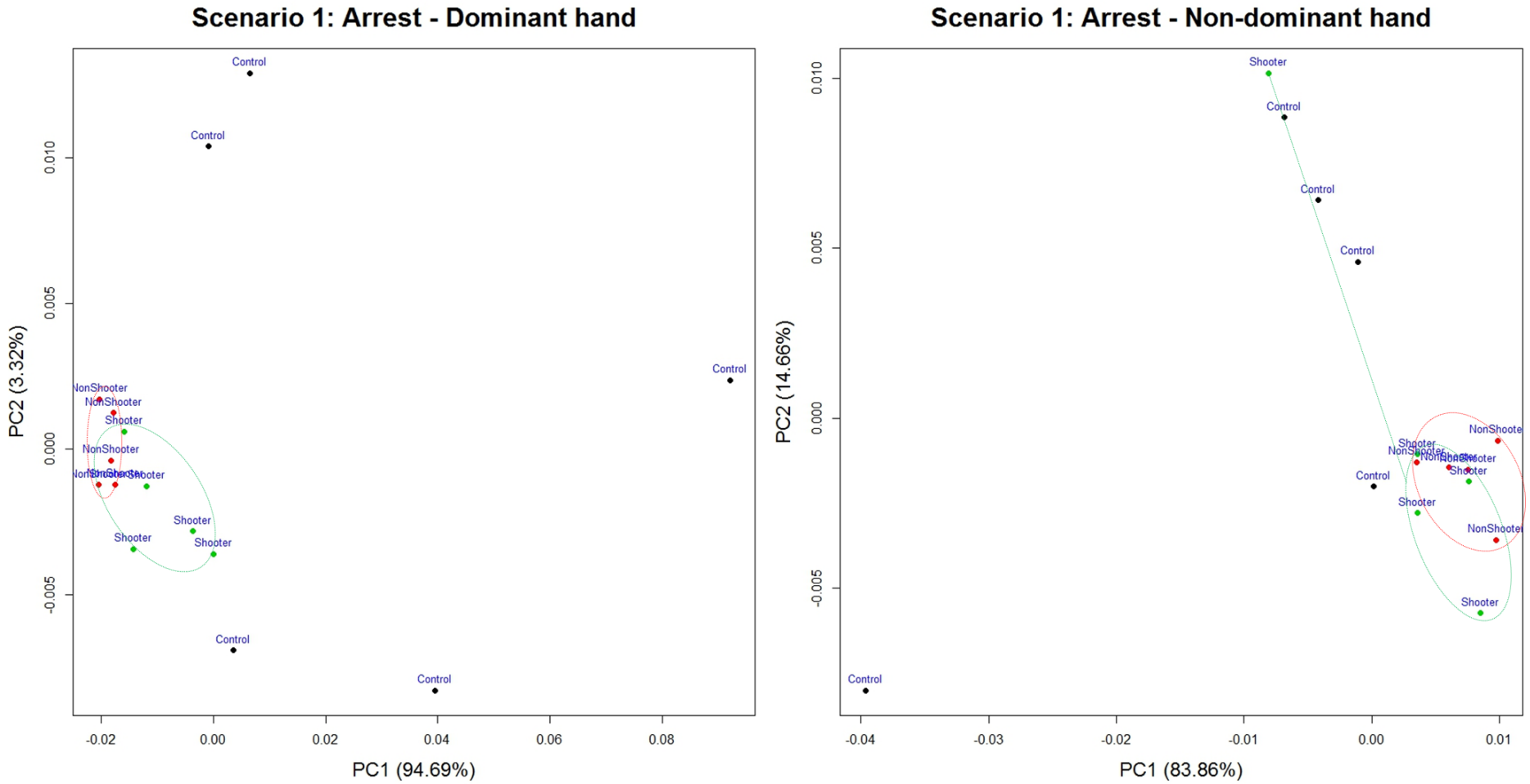


Figure 45. PCA plots –Scenario 1: arrest scenario. The Control highlights the large variability observed when analysis OGSR from the hands of the shooter. Both non-shooter and shooter are represented as clusters. However, a large overlap can be seen, highlighting the non-statistical differences between the two populations (Table 31).

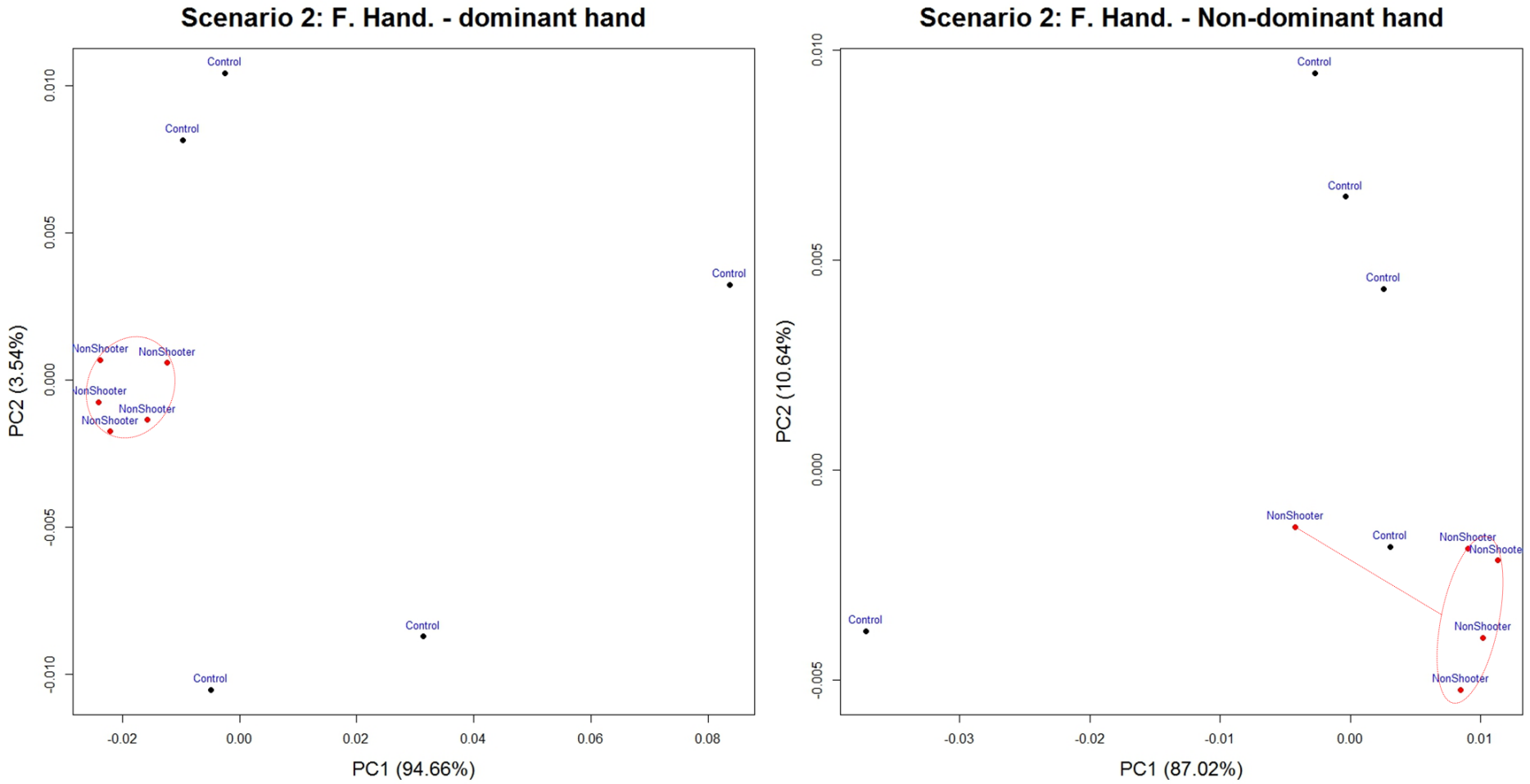


Figure 46. PCA plots – Scenario 2: Firearm handling scenario. The Control highlights the large variability observed when analysis OGSR from the hands of the shooter, while the non-shooter clusters closely. This result highlights the statistical difference observed between the control and non-shooter (Table 31).

A large variability (scatter of the data points in Figure 45 and Figure 46) was observed for the control specimens. This result was previously observed in the persistence study. It was due to the intrinsic variation of OGSR formation, deposition on the shooter during the firing process. This was also observed with the scatter of the "shooter" data points, which are the specimens taken from the shooter after the arrest scenario (green circle on Figure 45).

Nevertheless the data points from the non-shooter (Figure 45 and Figure 46) illustrates a good clustering of the specimens arising from the individual who was arrested by the shooter (red circle in Figure 45) and the individual who handled the firearm for five to ten minutes (red circle in Figure 46). Such results might relate to a better reproducibility of the secondary transfer when compared to the primary transfer occurring during the firearm discharges.

It is also observed that for scenario 1, the clusters of the data points arising from the non-shooter and the shooter are close to each other. Such results suggest that the differentiation between the two clustered populations is not significant. This is also supported by the ANOVA and Tukey-Kramer test previously performed on the dataset, which concluded that there was no difference between the non-shooter and the shooter (Table 31).

Whereas, for scenario 2, the ANOVA and Tukey-Kramer test resulted in a significant difference between the non-shooter and the control (Table 31), which is illustrated in Figure 46 by the close clustering of the non-shooter data points (red circle) to that of the controls.

The following PCA was performed by combining the two scenarios in order to have a general overview of the possible discrimination between the different individuals. The results are presented in Figure 47. The overall percentage explained by the two first PCs are of 97.09% and 97.51% for the dominant and non-dominant hand respectively.

For the dominant hand, it was observed that, with the exception of the controls, the three populations (Non-shooter-arrest, non-shooter-firearm handling and shooter) are clustering together (Figure 47). Even though clusters were observed, they were found to be close to each other, The ANOVA and Tukey-Kramer test performed earlier concluded that there were no significant differences between the three populations, which is emphasised in Figure 47 by the closeness of the three clusters.

The results of the statistical tests is also emphasised by the PCA observed on the non-dominant hand, which presents different trends. Indeed, it was observed that all the data points are largely overlapping with each other. This result emphasised that no differences could be seen in order to differentiate the non-shooters and shooter (Figure 47).

An additional observation concerned the variability of the different populations. It can be seen in Figure 47 that both non-shooters (NS and FH) present a small cluster, while the controls present a larger scatter of the data points. This scattering is caused by the inherent variability of the primary transfer (firearm onto the shooter), as well as the many factors involved in the formation, deposition and collection of OGSR traces.

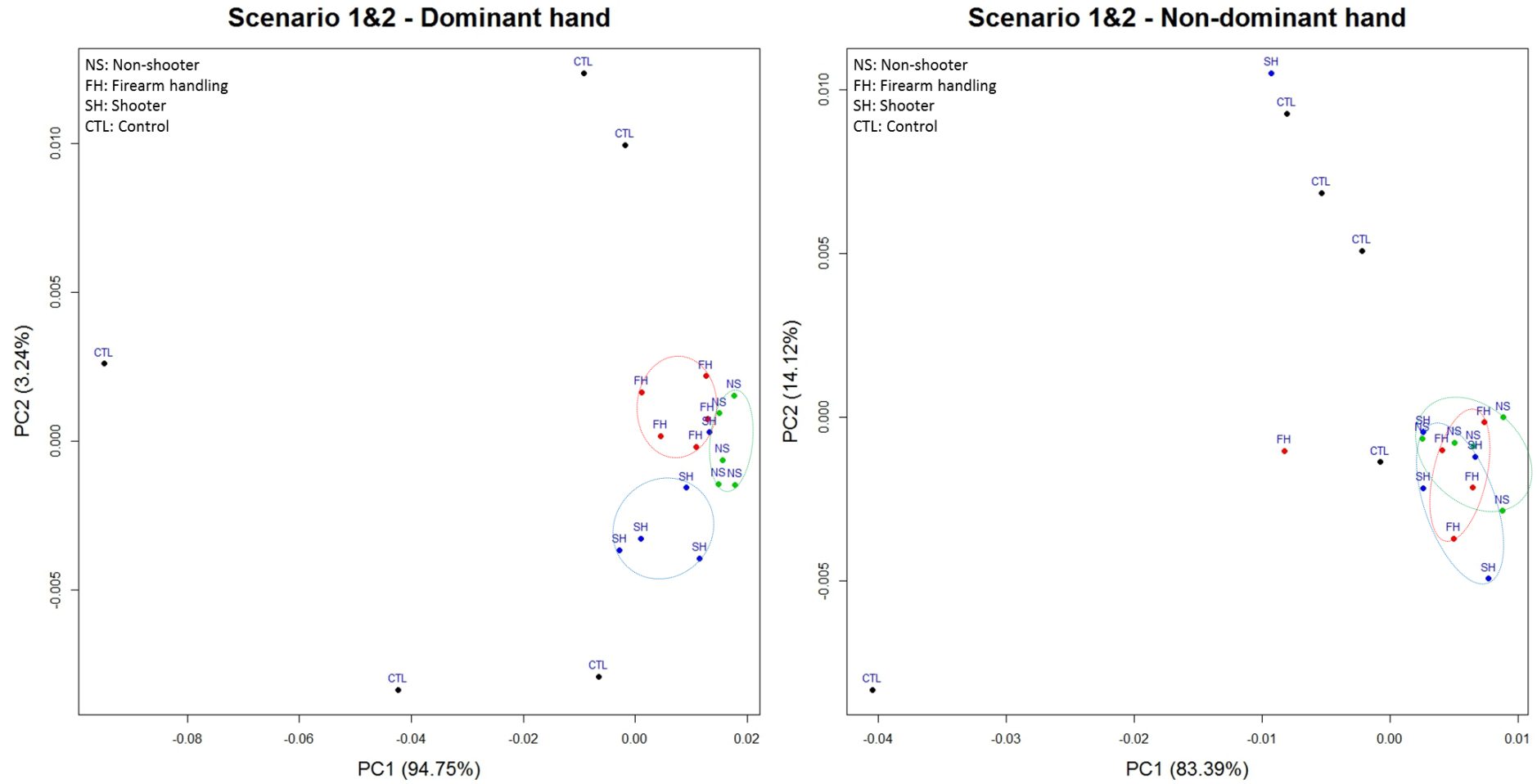


Figure 47. PCA plots – Scenario 1 and 2 combined. NS = non-shooter after the arrest, FH= non-shooter after the firearm handling, SH= Shooter after the arrest and CTL= controls, e.g. shooter immediately after the discharge. The three populations (SH, NS and FH) are found to form separate clusters, however, the proximity between these clusters highlights that there is non-statistically significant differences between the populations (Table 31).

On the other hand, the shooter, which related to the shooter after having arrested the non-shooter, presented a cluster similar to the two non-shooter datasets (Figure 47). This observation is surprising as the formation and deposition process of the traces onto the shooter is the same as the controls, involving the discharge of a firearm three times. Therefore, a similar variability could have been expected.

Such results might arise from the secondary transfer. The fact that undertaking an arrest scenario after having discharged the firearm may have led to a spread of the OGSR traces across the surfaces of the hands. This may have resulted in a more homogenous distribution of the residues after the secondary transfer scenario to that observed immediately after the discharge without having undertaken any additional activities.

2.5 Future considerations

It has to be noted that the scenarios performed were designed to maximise the secondary transfer, representing the worst case scenario for forensic practice. As POIs are typically in contact with police facilities, several opportunities are available for secondary transfer such as during arrest, transportation in police vehicles and detainment in police facilities.

In order to acquire additional information regarding OGSR pollution and secondary transfer mechanisms, it would be beneficial to increase the number of scenarios by changing parameters such as the time between the discharge and the arrest or the time between the arrest and the specimens collection time. It would be also interesting to add steps into the arrest process such as exposing the suspect to police vehicles and facilities prior to sampling. This would generate a range of more authentic scenarios reflective of

standard police practices. In addition, it would be valuable to acquire information in regards to other combinations of firearms and ammunitions as not all police forces use the same ammunitions and/or firearms. Experiments that include several calibres and firearms would be essential in order to assess the impact of the weapon systems on the deposition and secondary transfer of OGSR.

3. Conclusion

This study evaluated and explored critical questions regarding the secondary transfer of OGSR between a shooter, who previously discharged a firearm, and a non-shooter, with no immediate prior contact with a firearm. Two scenarios were investigated, one relating to the arrest of a non-shooter by a shooter, the second one approached the handling of a firearm by a non-shooter without discharging it. A secondary transfer occurred during both scenarios, with OGSR detected in each specimen collected from the non-shooter following an arrest process. OGSR was also detected when a non-shooter handled a firearm with similar levels of OGSR detected when compared to a shooter who discharged their firearm followed by conducting an arrest scenario. On average, the amount of OGSR detected did not exceed the amount identified on the shooter who was sampled immediately after discharge.

Interestingly, the amount of OGSR detected on the hands of a shooter did not greatly exceed that detected on the hands (especially the non-dominant hands) of persons who simply handled a gun. It therefore appears logical that the amount of OGSR detected on a shooter is actually the combination of OGSR compounds deposited during firing and the compounds transferred to the hands by handling the gun. Direct transfer of GSR from

the gun is usually not controlled in GSR detection experiments and the experimental results presented here indicate that it may be a significant factor.

It is essential to assess such scenarios in order to develop a better understanding of OGSR behaviour. In addition, such research provides complementary information to forensic scientists in order to improve the interpretation process. When approaching the assessment of traces such as OGSR, questions such as secondary transfer and persistence become essential. In practice, standardised protocols restricting or mitigating contact between police officers who discharged a firearm and the POI are advisable as well as recommending that firearms are cleaned on a regular basis to limit the accumulation of OGSR. Additionally, this information and the context of the arrest should be documented and provided to the forensic scientist for the evaluation of OGSR results. Such information allows the results of the analysis to be included into the global context of the case, to be combined with other findings such as IGSR particles. It also allows for consideration of the chronology of the event, the time of sampling and potential sources of pollution such as an arrest process undertaken by contaminated officers. In addition, the results presented in this study can inform an evaluative framework. The secondary transfer can be included in the interpretation process of OGSR in order to provide a more meaningful assessment of such traces. Doing so, this would allow to have a better understanding of such findings by including them in the global context of the case under investigation.

CHAPTER FOUR: PROBABILISTIC APPROACH

1. Introduction

Forensic investigations are typically conducted in the context of case specific circumstances. Forensic scientists must, therefore, be able to adjust the interpretation of the analytical results in accordance with the case circumstances, such as the time and the conditions where the specimens were collected (Chapter two) [93].

In addition, a question often arising in firearm-related event relates to the activity level, when referring to the hierarchy of the propositions. For instance, *did the POI(s) discharge a firearm?*

Such questions require consideration of factors such as the retention properties of the OGSR traces and the possibilities of a pollution through a secondary transfer in order to provide a meaningful interpretation of the results [89, 90]. It is essential to include this information into the interpretative framework as they can significantly influence the outcomes of the assessment process. For instance, if a relatively low amount of OGSR is detected, it might be interpreted as pollution response, however, if the forensic scientist knows that the specimens were collected two hours after the alleged discharge(s), then the interpretation can be completely reframed and the outcomes might be fundamentally different.

As detailed in Chapter two, the Bayesian approach is largely accepted by the forensic community as the most appropriate method to interpret forensic trace results [127, 132]. It is also emphasised by the recent publication of the ENFSI guidelines on the evaluative reporting in forensic science [91], which details the use of the Bayesian approach as the method of choice. The Bayes theorem enables the assessment of results in light of at least two propositions, mutually exclusive, often denoted as the prosecutor hypothesis and the defence hypothesis, abbreviated as Hp and Hd respectively [90, 119] providing a balanced and impartial evaluation during the interpretation process. The results of the interpretation is provided by a LR representing the strength of the evidence, which aimed to support more or less greatly a proposition rather the alternative one.

To use the information acquired in the persistence and secondary transfer studies, the first step was to combine the obtained data in order to have a global visualisation of the available datasets. Figure 48 represents the comparison of the data from the persistence and the secondary transfer studies. It can be observed that the non-shooter after the arrest is predominantly similar, in terms of amount detected, to the latest time points studied during the persistence either T2h or T4h. Such results highlight that a distinction between a shooter from whom the specimens were collected after two to four hours and a non-shooter who was contaminated during the arrest process might be difficult to achieve (Figure 48).

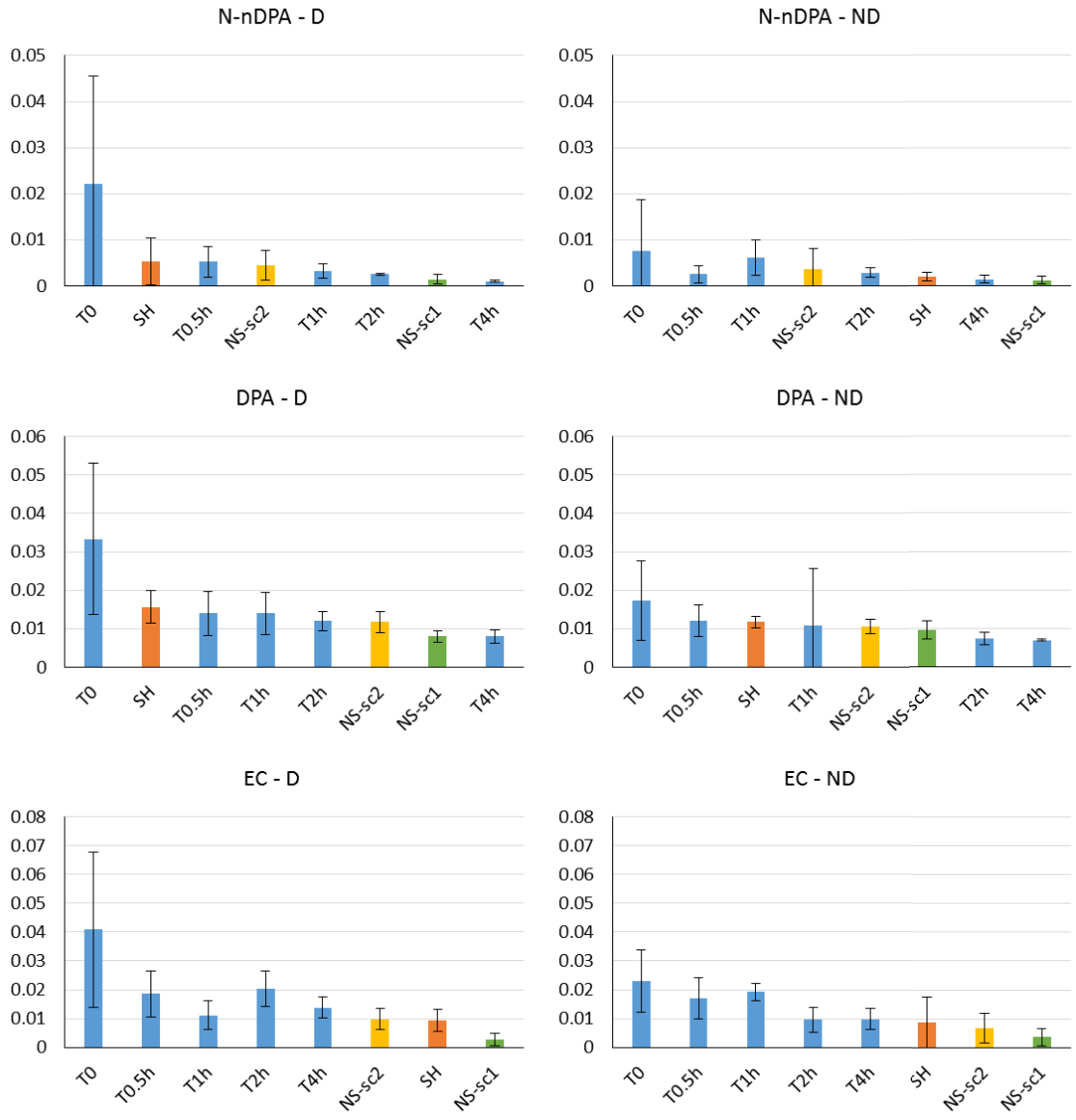


Figure 48. Comparison of the persistence and secondary transfer study. SH= Shooter after the arrest scenario, NS-sc1= Non-shooter after the arrest scenario (scenario 1), NS-sc2= Non-shooter after handling the firearm (scenario 2).

This observation is also presented in Table 32 with the data normalised to the T0 control specimen. The amount of OGSR detected on the non-shooter after the arrest is found to be lower than the response from specimens collected after two hours but higher than specimens collected after four hours.

Table 32. Comparison between the persistence and the secondary transfer when normalised to T0. D= dominant hand, ND= Non-dominant hand. Shooter= Shooter after the arrest, NS SC1= Non-shooter after the arrest, NS SC2= Non-shooter after handling the firearm.

	N-nDPA		DPA		EC		AVERAGES
	D	ND	D	ND	D	ND	
T0	*100%*	*100%*	*100%*	*100%*	*100%*	*100%*	*100%*
Shooter	23.80%	32.71%	46.87%	70.50%	45.51%	73.81%	48.81%
T0.5h	23.40%	81.73%	41.89%	68.07%	27.30%	83.74%	54.35%
NS SC2	20.12%	47.53%	41.84%	63.19%	49.90%	41.72%	44.05%
T1h	14.38%	38.02%	36.03%	61.30%	33.89%	42.24%	37.65%
T2h	11.33%	24.89%	35.13%	55.40%	24.15%	37.53%	31.40%
NS SC1	6.37%	18.70%	24.09%	42.83%	22.81%	29.45%	24.04%
T4h	4.49%	15.30%	23.87%	40.01%	6.70%	15.78%	17.69%

The following part of this chapter presents the interpretation process applied to OGSR traces by taking into account the persistence and secondary transfer studies. The aims is to provide examples of the application of the Bayesian assessment process when analytical results have to be interpreted in a particular context. The first stages aimed at determining the most appropriate distribution for the OGSR interpretation model.

2. Probability distribution identification

Both persistence and secondary transfer studies related to the detection of OGSR compounds under different conditions. The distribution of the OGSR data for both the persistence and transfer scenarios is continuous as the detected amounts can take any value exceeding zero. Consequently, the first step to evaluate the data in a probabilistic framework involved identifying the most appropriate probability density function (PDF) for the data. The process involved a goodness-of-fit test of the datasets with a series of the most recurrent probabilities density functions. The tests of goodness-of-fit were carried out with the Minitab® 17 Statistical software (Minitab Pty Ltd, version: 17.1.0) with the *"individual distribution function"*. 14 different probability density functions were tested as well as the Box-Cox transformation, which transform the data to improve the goodness-of-fit of the dataset with the normal distribution (Table 33).

Table 33. The list of the probability distribution functions (PDF) assessed during the goodness-of-fit tests.

Normal
Normal after Box-Cox transformation
Lognormal
3-Parameter Lognormal
Exponential
2-Parameter Exponential
Weibull
3-Parameter Weibull
Smallest Extreme Value
Largest Extreme Value
Gamma
3-Parameter Gamma
Logistic
Loglogistic
3-Parameter Loglogistic

Table 34. Total number of tests conducted in order to assess the fittest probability density function.
D= Dominant hand, ND= Non-dominant hand. N= number of data points.

PERSISTENCE					SECONDARY TRANSFER			
.40 S&W					.40 S&W			
T0	NnDPA	D	N=5		NonShooter	NnDPA	D	N=5
		ND	N=5				ND	N=5
	DPA	D	N=5			DPA	D	N=5
		ND	N=5				ND	N=5
	EC	D	N=5			EC	D	N=5
		ND	N=5				ND	N=5
T0.5h	NnDPA	D	N=5		Shooter	NnDPA	D	N=5
		ND	N=5				ND	N=5
	DPA	D	N=5			DPA	D	N=5
		ND	N=5				ND	N=5
	EC	D	N=5			EC	D	N=5
		ND	N=5				ND	N=5
T1h	NnDPA	D	N=5		Firearm handling	NnDPA	D	N=5
		ND	N=5				ND	N=5
	DPA	D	N=5			DPA	D	N=5
		ND	N=5				ND	N=5
	EC	D	N=5			EC	D	N=5
		ND	N=5				ND	N=5
T2h	NnDPA	D	N=5					
		ND	N=5					
	DPA	D	N=5					
		ND	N=5					
	EC	D	N=5					
		ND	N=5					
T4h	NnDPA	D	N=3					
		ND	N=3					
	DPA	D	N=3					
		ND	N=3					
	EC	D	N=3					
		ND	N=3					
.357 Magnum								
T0	NnDPA	D	N=5					
		ND	N=5					
	DPA	D	N=5					
		ND	N=5					
	EC	D	N=5					
		ND	N=5					
T0.5h	NnDPA	D	N=5					
		ND	N=5					
	DPA	D	N=5					
		ND	N=5					
	EC	D	N=5					
		ND	N=5					
T1h	NnDPA	D	N=5					
		ND	N=5					
	DPA	D	N=5					
		ND	N=5					
	EC	D	N=5					
		ND	N=5					
T2h	NnDPA	D	N=5					
		ND	N=5					
	DPA	D	N=5					
		ND	N=5					
	EC	D	N=5					
		ND	N=5					
T4h	NnDPA	D	N=3					
		ND	N=3					
	DPA	D	N=3					
		ND	N=3					
	EC	D	N=3					
		ND	N=3					

The individual distribution function was applied to each data set involved in both persistence and secondary transfer as presented in Table 34. 78 tests were conducted to identify the best probability distribution function for each set of data. Each of the tests assessed 15 probability density functions presented in Table 34, generating 1170 individual tests and graphs.

To diminish the number of potential PDF candidates, based on the entire set of tests generated, the four fittest probability density functions were kept and considered relevant for this study. The selection of the best PDF was based on two different statistics provided for each test (n=77): the Anderson-Darling statistic (AD), which is a statistical test assessing whether a given population of data is drawn from a particular probability distribution [198]. A lower AD statistic suggests a superior fit between the dataset and the tested probability density function.

The second statistic returned was the p-value, which represents the calculated probability of finding the outcomes when the hypothesis H0 (the dataset fits the probability density function) is true [187]. When the p-value exceeds the threshold (α= 0.05), H0 is verified at a 95% confidence level. Therefore, the higher the p-value, the better is the fit between the tested dataset and the tested distribution.

The results of the tests conducted are presented in Table 35, which represents the number of tests for which the probability distribution was returned in the four fittest distributions with the particular statistic (AD and p-value).

Table 35. Results of the goodness-of-fit test. It represents the number of test for which the particular PDF is returned as one of the 4 fittest PDF for each descriptive statistic.

Probability distribution tested	Frequency			
	Anderson-Darling statistic (AD) n= 77	Percentage	p-value n= 77	Percentage
Normal	**34**	**44.2%**	**32**	**41.6%**
Box-Cox Transformation	**74**	**96.1%**	**68**	**88.3%**
Lognormal	**58**	**75.3%**	**47**	**61.0%**
3-Parameter Lognormal	6	7.8%	2	2.6%
Exponential	5	6.5%	18	23.4%
2-Parameter Exponential	**34**	**44.2%**	**27**	**35.1%**
Weibull	5	6.5%	6	7.8%
3-Parameter Weibull	12	15.6%	**63**	**81.8%**
Smallest Extreme Value	3	3.9%	7	9.1%
Largest Extreme Value	2	2.6%	5	6.5%
Gamma	3	3.9%	14	18.2%
3-Parameter Gamma	1	1.3%	0	0.0%
Logistic	12	15.6%	6	7.8%
Loglogistic	24	31.2%	13	16.9%
3-Parameter Loglogistic	**35**	**45.5%**	0	0.0%

It was observed for the two measured statistics (Table 35, Figure 49), that the four most suitable probability density functions are: the normal distribution, the normal distribution after the Box-Cox transformation, the lognormal and the 2-parameters exponential (Figure 49). The graphs illustrating the goodness-of-fit of these four probabilities distribution functions are presented in Appendix IV.

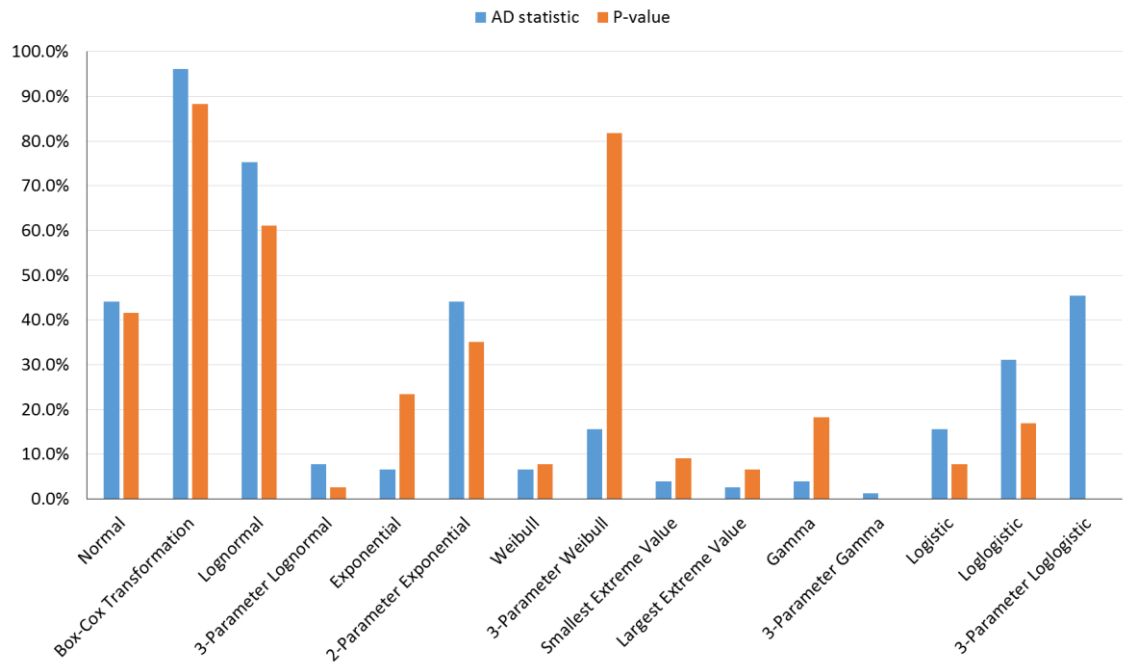


Figure 49. Probability density functions identification results (n=77). AD= Anderson-Darling statistic.

According to the tests conducted, the outcome of the lognormal PDF is close to the outcome provided by the normal PDF, while the 2-parameters exponential PDF was not found be fit with the datasets (Appendix IV). Therefore, it was concluded that the normal distribution and normal distribution after the Box-Cox transformation are suitable distributions for the persistence and secondary transfer data sets. However, the Box-Cox transformation has a significant limitation. Such a transformation can drastically changes the scale of the dataset, which may skew the differences between the data distributions (e.g. time points) and greatly impact the further analysis carried out with the different persistence and secondary transfer populations.

The data set is constituted of five data points for each population (T0, T0.5h, T1h, T2h and secondary transfer study), with the exception of T4h which is based on three replicates. Because of the limited amount of data, the identification of the most adapted

probability density function cannot be performed with a high degree of confidence. In addition, to maintain the inherent variability of the different populations, such as the time points of the persistence study as well as the different activities involved in the secondary transfer study, the normal distribution was selected as the most suitable probability distribution function.

The selection of the normal distribution is strengthened by the Shapiro-Wilk tests conducted in Chapter three. Both persistence (Section III – 2.3, p. 118) and secondary transfer datasets (Section IV – 2.4, p. 154) were tested and presented in Appendix III.2. It was found that 81.9 % of the tests performed on the persistence dataset and 94.4 % of the tests conducted on the secondary transfer dataset resulted in normally distributed data. The normal distribution was also used in a previous publication on the interpretation of OGSR compounds, naphthalene, to assess the time after discharge of cartridge cases [162].

3. Probability density functions

A normal distribution is defined by two parameters: the mean (μ) and the variance (σ^2) of each population as presented in Equation 5.

$$X \sim N(\mu,\ \sigma^2)$$

Equation 5. Normal distribution parameters.

The associated normal probability density function (normal PDF) is presented in Equation 6. In the context of OGSR analysis, "*x*" represents any given normalised peak area, while μ and σ^2 represent the respective mean and variance for the relevant population.

$$f(x\ |\mu,\ \sigma^2) = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{(x-\mu)^2}{2\sigma^2}}$$

Equation 6. Normal probability density function (Normal PDF).

3.1 Persistence dataset

In the context of the persistence study, the normal probability distribution can be calculated for both calibres, each time point and compounds. In this case, both dominant and non-dominant hand of the shooter were averaged for presentation purposes. The normal PDF for the entire set of data, of both dominant and non-dominant hands, for each compound at each time points can be found in the Appendix V.

The mean (μ) and variance (σ^2) were calculated for the two calibres studied (.40 S&W and .357 Mag) for both hands combined in order to generate an overall representation of the different time points investigated (Table 36).

Table 36. Mean (μ) and variance (σ^2) parameters for each time points and compound investigated during the persistence study.

		.40 S&W		.357 Mag	
		μ	σ^2	μ	σ^2
T0	*N*-nDPA	0.014	3.67E-04	0.011	2.54E-05
	DPA	0.025	3.13E-04	0.047	1.43E-04
	EC	0.030	5.31E-04	0.062	2.70E-03
T0.5h	*N*-nDPA	0.004	8.47E-06	0.008	7.23E-05
	DPA	0.013	1.52E-05	0.025	3.30E-04
	EC	0.017	4.90E-05	0.028	9.59E-05
T1h	*N*-nDPA	0.004	8.30E-06	0.004	2.14E-06
	DPA	0.016	9.59E-05	0.013	2.42E-05
	EC	0.015	2.79E-05	0.024	1.58E-04
T2h	*N*-nDPA	0.002	5.25E-07	0.002	3.76E-07
	DPA	0.010	9.64E-06	0.011	1.07E-05
	EC	0.013	5.61E-05	0.019	4.13E-05
T4h	*N*-nDPA	0.001	7.88E-08	0.002	3.08E-07
	DPA	0.008	4.39E-07	0.006	1.37E-05
	EC	0.012	1.06E-05	0.019	1.80E-04

Based on the mean (μ) and variance (σ^2), the normal PDF can be calculated using Equation 6 for every population (Table 36) with their respective parameters. Figure 50 and Figure 51 illustrates the normal density functions for both calibres and for each compound at the different time points investigated in the persistence study. It was observed in Chapter three that the general trends included a decreasing amount of OGSR being detected as time elapsed since discharged, while the spread of the replicates at the longer time periods was significantly lower than T0.

As observed in Figure 50 and Figure 51 an overlap is observed between the different normal PDF, indicating that the complete differentiation between the time points is not achievable.

These results are consistent with previous observations from Chapter three (Section III), in which some compounds were found to be detected in similar amount across the different time points studied (Figure 29, p. 110, .40 S&W - EC). They also confirm the results obtained from the ANOVA and Tukey-Kramer test perfomed during the exploratory data analysis presented in Chapter three (p. 118), in which no differentiation was found between the different time points after T0.

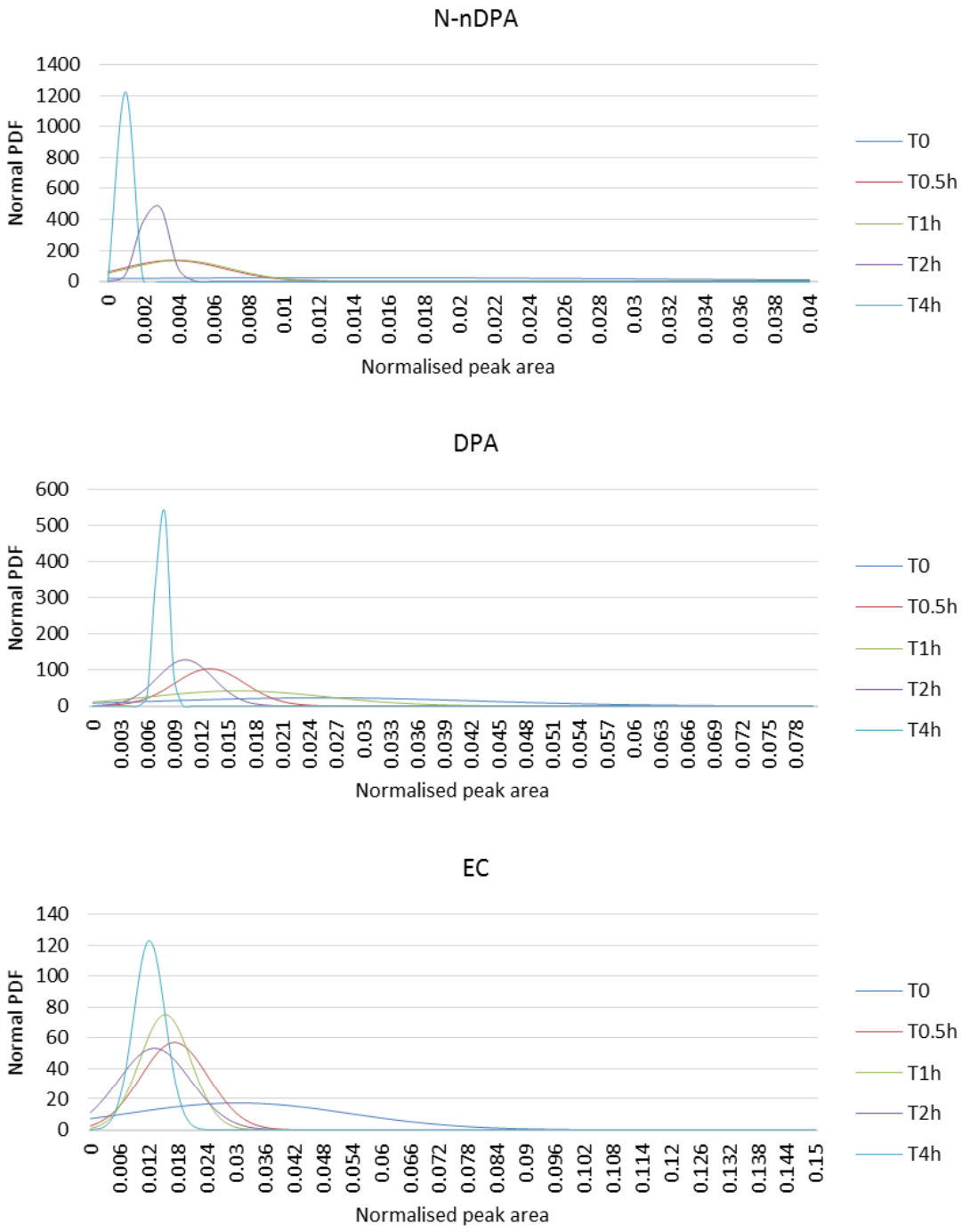


Figure 50. .40 S&W: Normal probability density function of the combined hand for each time point investigated during the persistence study.

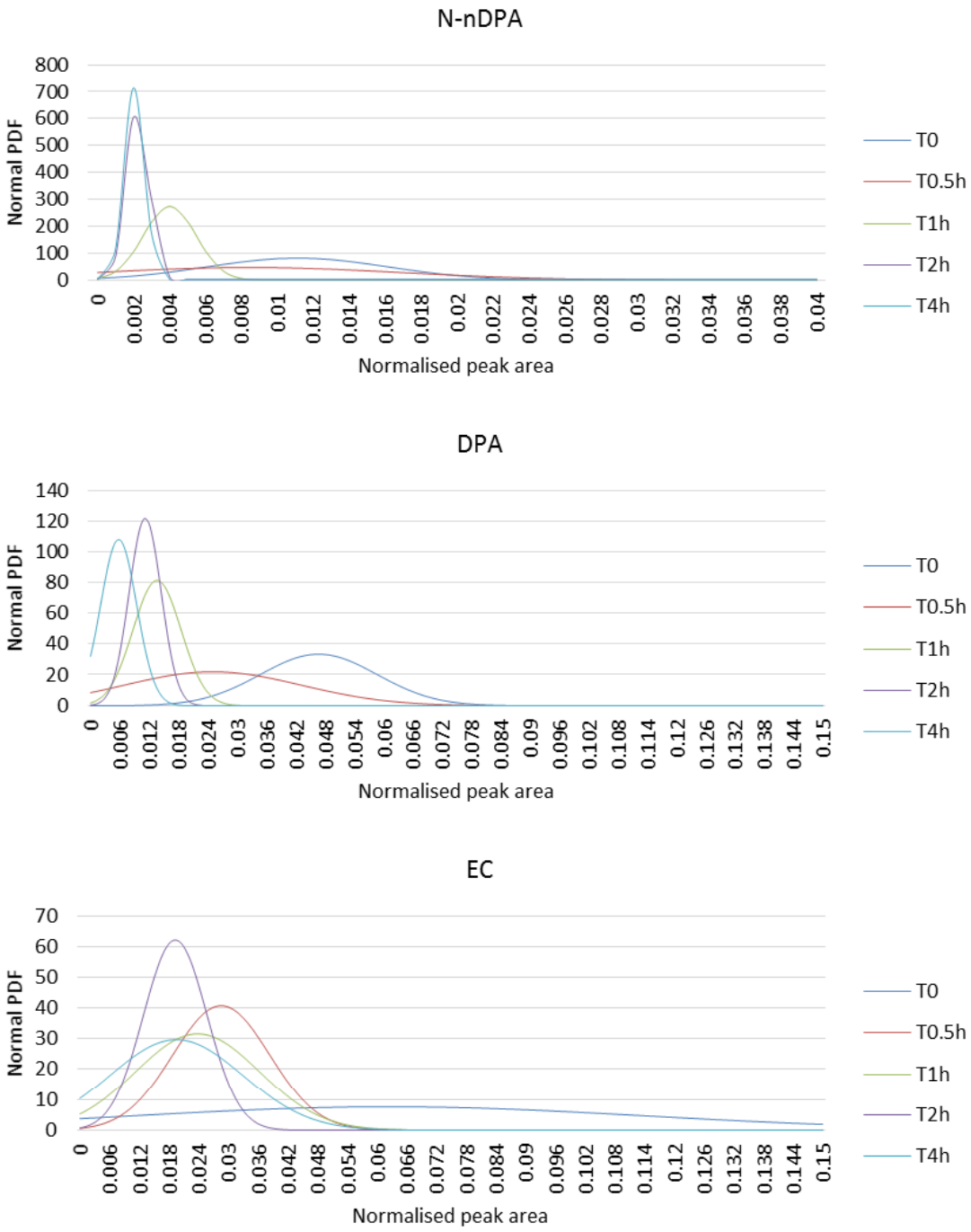


Figure 51. .357 Mag: Normal probability density function of the combined hand for each time point investigated during the persistence study.

3.2 Secondary transfer dataset

A similar process was carried out with the secondary transfer data set. The mean and variance were calculated for both hands combined in order to generate an overall representation of the different activities involved in the secondary transfer scenario (Table 37).

Table 37. Mean (μ) and variance (σ2) parameters for compound and each participant investigated during the secondary transfer study. SC1= scenario 1: arrest, SC2= scenario 2: Firearm handling, Shooter= Shooter after the arrest scenario.

		μ	σ^2
Non-shooter - SC1	*N*-nDPA	0.001	5.24E-07
	DPA	0.009	4.11E-06
	EC	0.004	4.43E-06
Shooter	*N*-nDPA	0.003	1.52E-05
	DPA	0.014	1.35E-05
	EC	0.009	4.22E-05
Non-shooter - SC2	*N*-nDPA	0.004	1.38E-05
	DPA	0.011	5.18E-06
	EC	0.008	1.98E-05
Control	*N*-nDPA	0.014	5.24E-07
	DPA	0.025	4.11E-06
	EC	0.030	4.43E-06

Based on the mean (μ) and variance (σ2), the normal PDF can be calculated using Equation 6 for every population (Table 37) with their respective parameters. Every normal PDF for the entire set of data, separating both dominant and non-dominant hands can be found in Appendix V.

It was observed in Chapter three, that a lower mean amount of OGSR was detected on the non-shooter after the arrest process (Figure 38, p. 142). This observation is reinforced by Figure 52, with a normal PDF close to zero and a narrower normal PDF, while the largest amount detected arose from the controls, illustrated by the higher mean and a larger variance.

The amount detected from the non-shooter after the firearm handling scenario (Scenario 2) was similar to that detected on the shooter after the arrest (Figure 39, p. 147). This observation had a large impact on the normal PDF. As presented in Figure 52, for *N*-nDPA and EC, the two normal PDF for non-shooter after scenario 2 and the shooter after the arrest are almost indistinguishable, making the differentiation between the two populations impossible. However, for DPA, it is possible to attempt a differentiation of the relevant populations.

These results are consistent with the previous outcomes obtained from the ANOVA, Tukey-Kramer test (Table 31, p. 156) and the PCA (p. 159), which resulted in no significant difference between the populations with the exception of the controls.

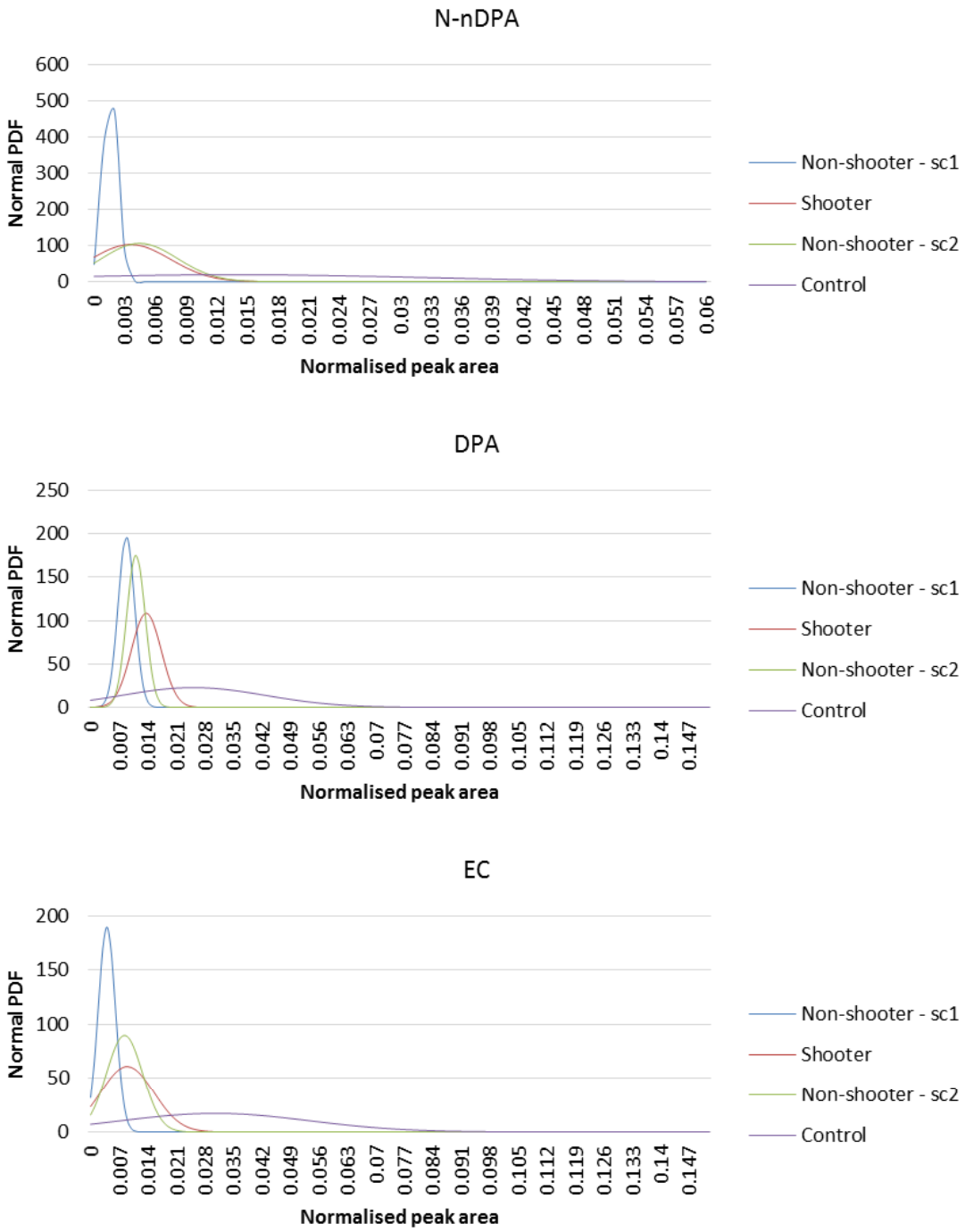


Figure 52. Normal probability density function of the combined hand for each activities investigated during the secondary transfer study. Shooter= shooter after the arrest process, sc1= scenario1: arrest process scenario, sc2= firearm handling scenario.

4. Interpretation simulations

This section will detail how the proposed interpretation framework could be applied to two different simulations, which are based on the persistence and secondary transfer studies. The purposes of these studies were to provide additional information to forensic scientists about OGSR behaviour. This knowledge can support the interpretation of such traces by considering variations over time and possible pollutions. Therefore, the aim of this section is to use the persistence and secondary transfer study to illustrate the interpretation process and the calculation of LRs, and how such studies help to improve the integration of OGSR traces in the overall context of the case during the interpretation process.

4.1 Results and discussion

4.1.1 Simulation 1: Chronology of the event

The first simulation involves a mock case where specimens were taken from both hands of a POI. The POI was suspected to have discharged a firearm (calibre .40 S&W) few minutes prior to specimen collection. The POI does not contest they discharged a firearm, however, it attested that the last discharges were carried out an hour ago.

The first step of the interpretation process involved to identify and define the most relevant set of propositions for the case under investigation. This simulation relates to the chronology of the event, taking place at the activity level (II). The interpretation of the

specimens at the source level, implying the assessment of the results as originating from a firearm discharges or another unknown environment source, is not conducted. In this particular case, the POI did not question the fact of a firearm having been discharged, only question the *time* at which the discharges occurred.

In this case, the specimens were alleged to have been collected from the POI a few minutes after the firearm discharges, while the alternative proposition indicates that the specimens arose from discharges that occurred one hour ago. Therefore, the two alternative propositions can be stated as follow:

Hp: The POI discharged a firearm minutes before the specimens collection.

Hd: The POI discharged a firearm one hour prior the specimens collection.

The specimens were further analysed by UPLC-QqQ-MS for OGSR and the normalised peak area (N_{PA}) were calculated. The laboratory results of the specimens are presented in Table 38. The three compounds of interest were detected on both dominant and non-dominant hand specimens.

Table 38. Simulation 1: Results of the OGSR samples collected from both hands of the POI. N_{PA}= Normalised peak area.

Trace - N_{PA}	Dominant hand	Non-dominant hand
N-nDPA	$E_{NnDPA, d} = 0.009$	$E_{NnDPA, nd} = 0.018$
DPA	$E_{DPA, d} = 0.032$	$E_{DPA, nd} = 0.020$
EC	$E_{EC, d} = 0.035$	$E_{EC, nd} = 0.027$

Based on the two propositions, two different populations of interest were investigated based on the data resulting from the persistence studies (for the calibre .40 S&W; Chapter three, Section III, 2.2, p. 113) and the normal PDF was generated for both propositions of interest. The population T0 is used in order to determine the normal PDF for Hp as it is the closest distribution to the time frame hypothesised in the proposition Hp. For the proposition Hd, the normal PDF was generated from the population T1h (Chapter three, Section III, 2.2, p. 113).

Both hands of the POI were sampled and the three compounds of interest were detected, resulting in six normal distribution being calculated with their respective mean (μ) and variance (σ^2) parameters, presented in Table 39.

Table 39. Simulation 1: normal PDF parameters for the two propositions of interest.

	Dominant hand				Non-dominant hand			
	Hp: *T0*		Hd: *T1h*		Hp: *T0*		Hd: *T1h*	
	$\mu_{d, t0}$	$\sigma^2_{d, t0}$	$\mu_{d, t1h}$	$\sigma^2_{d, t1h}$	$\mu_{nd, t0}$	$\sigma^2_{nd, t0}$	$\mu_{nd, t1h}$	$\sigma^2_{nd, t1h}$
N-nDPA	0.020	$5.82x10^{-4}$	0.003	$1.82x10^{-6}$	0.007	$1.28x10^{-4}$	0.005	$1.37x10^{-5}$
DPA	0.033	$4.34x10^{-4}$	0.014	$1.65x10^{-5}$	0.017	$1.08x10^{-4}$	0.020	$2.67x10^{-4}$
EC	0.041	$8.40x10^{-4}$	0.012	$2.87x10^{-5}$	0.020	$1.20x10^{-4}$	0.019	$1.00x10^{-5}$

The normal PDF were calculated as detailed previously (Equation 6). The equation can be adapted to the particular simulation as shown in Equation 7. For each compound, hand and both Hp and Hd (Table 39), where *"x"* represents the normalised peak areas.

$$f\left(x \mid \mu_{Hp},\, \sigma_{Hp}^2\right) = \frac{1}{\sqrt{2\pi\sigma_{Hp}^2}} e^{-\frac{(x-\mu_{Hp})^2}{2\sigma_{Hp}^2}}$$

$$f(x \mid \mu_{Hd},\, \sigma_{Hd}^2) = \frac{1}{\sqrt{2\pi\sigma_{Hd}^2}} e^{-\frac{(x-\mu_{Hd})^2}{2\sigma_{Hd}^2}}$$

Equation 7. Simulation 1: general normal PDF calculation for both Hp and Hd.

Figure 53 presents the normal PDF of each compound for both dominant and non-dominant hands, for each proposition of interest (Hp/ Hd). As presented, Hd, representing the distribution at T1h, has a narrower distribution and a lower mean (i.e. lower normalised peak area), while the Hp distribution has a larger mean (i.e. a larger normalised peak area) and a wider peak, representing a larger variance.

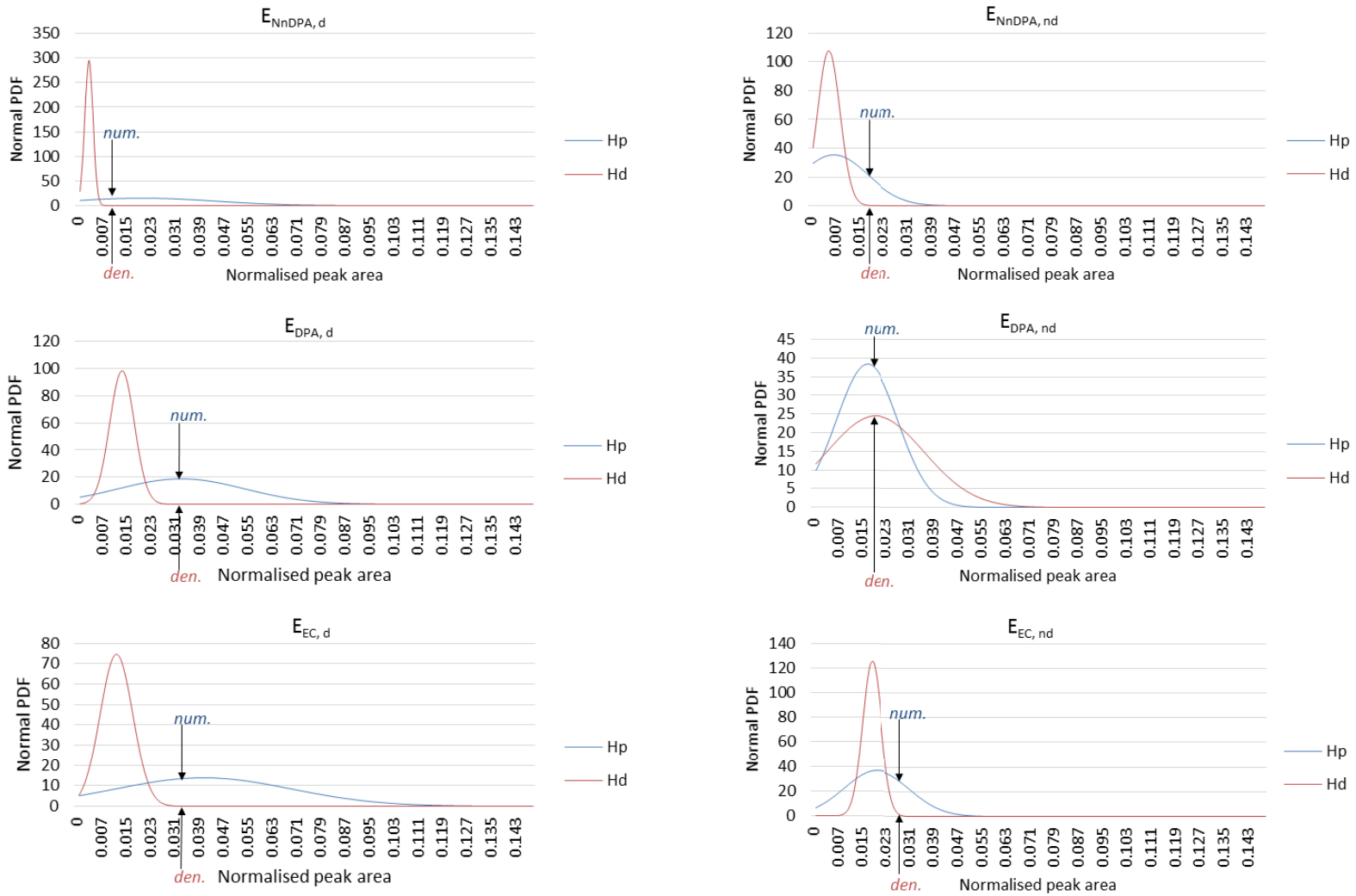


Figure 53. Simulation 1: Normal probability density function (PDF) for each proposition of interest; num= LR numerator, den= LR denominator. The black arrows represent the N_{PA} for the OGSR traces under investigation.

From the probability density functions, the LR, can be calculated for each N_{PA} evidence obtained (Table 38). The LR is resulted from the Bayesian theorem presented in Equation 1 (p. 15), and assesses the evidence according to each of the proposition of interest (Hp and Hd):

$$LR = \frac{p(E\ |Hp)}{p(E\ |Hd)} = \frac{p(E\ |N{\sim}(\mu_{Hp}, \sigma_{Hp}^2))}{p(E\ |N{\sim}(\mu_{Hd}, \sigma_{Hd}^2))}$$

The LR can therefore be detailed with the calculation of the probability for the evidence given the normal PDF for both propositions of interest, with the parameters presented in Table 39. It represents the ratio of both equations presented in Equation 7, and displayed below:

$$LR = \frac{p\left(E\ \middle|N{\sim}(\mu_{Hp},\ \sigma_{Hp}^2)\right)}{p\left(E\ \middle|N{\sim}(\mu_{Hd},\ \sigma_{Hd}^2)\right)} = \frac{\frac{1}{\sqrt{2\pi\sigma_{Hp}^2}} e^{-\frac{(E-\mu_{Hp})^2}{2\sigma_{Hp}^2}}}{\frac{1}{\sqrt{2\pi\sigma_{Hd}^2}} e^{-\frac{(E-\mu_{Hd})^2}{2\sigma_{Hd}^2}}}$$

Equation 8. Simulation 1: LR detailed formula.

A graphical representation of the LR numerator and denominator can be seen in Figure 53, where the normalised peak area of each OGSR compound detected in the questioned specimens (Table 38) are illustrated by the black arrows. Both numerator and denominator of the LR can be defined by the Hp and Hd probability density function

curve at that particular value. The results of the LR calculation for each OGSR evidence is presented hereafter:

$$LR_{NnDPA,d} = \frac{p(E_{NnDPA,d} = 0.009 \,|N\sim(\mu_{NnDPA,d,t0} = 0.020,\ \sigma^2_{NnDPA,d,t0} = 5.82e-4))}{p(E_{NnDPA,d} = 0.009 \,|N\sim(\mu_{NnDPA,d,\,t1h} = 0.003,\ \sigma^2_{NnDPA,d,t1h} = 1.82e-6))} = \frac{14.770}{0.0115} = 1282.9 \cong 1283$$

$$LR_{NnDPA,nd} = \frac{p(E_{NnDPA,nd} = 0.018 \,|N\sim(\mu_{NnDPA,nd,t0} = 0.007,\ \sigma^2_{NnDPA,nd,t0} = 1.28e-4))}{p(E_{NnDPA,nd} = 0.018 \,|N\sim(\mu_{NnDPA,nd,\,t1h} = 0.005,\ \sigma^2_{NnDPA,nd,t1h} = 1.37e-5))} = \frac{21.714}{0.2626} = 82.7 \cong 82.5$$

$$LR_{DPA,d} = \frac{p(E_{DPA,d} = 0.032 \,|N\sim(\mu_{DPA,d,t0} = 0.033, \sigma^2_{DPA,d,t0} = 4.34e-4))}{p(E_{DPA,d} = 0.032 \,|N\sim(\mu_{DPA,d,t1h} = 0.014, \sigma^2_{DPA,d,t1h} = 1.65e-5))} = \frac{19.103}{0.004} = 4468.4 \cong 4468$$

$$LR_{DPA,nd} = \frac{p(E_{DPA,nd} = 0.020 \,|N\sim(\mu_{DPA,nd,t0} = 0.017,\ \sigma^2_{DPA,nd,t0} = 1.08e-4))}{p(E_{DPA,nd} = 0.020 \,|N\sim(\mu_{DPA,nd,t1h} = 0.020,\ \sigma^2_{DPA,nd,t1h} = 2.67e-4))} = \frac{36.962}{24.428} = 1.51 \cong 1.5$$

$$LR_{EC,d} = \frac{p(E_{EC,d} = 0.035|N\sim(\mu_{EC,d,t0} = 0.041,\ \sigma^2_{EC,d,t0} = 8.40e-4))}{p(E_{EC,d} = 0.035|N\sim(\mu_{EC,d,t1h} = 0.012,\ \sigma^2_{EC,d,t1h} = 2.87e-5))} = \frac{12.839}{0.309} = 41.4 \cong 41.5$$

$$LR_{EC,nd} = \frac{p(E_{EC,nd} = 0.027 \,|N\sim(\mu_{EC,nd,t0} = 0.020,\ \sigma^2_{EC,nd,t0} = 1.20e-4))}{p(E_{EC,nd} = 0.027 \,|N\sim(\mu_{EC,nd,t1h} = 0.019,\ \sigma^2_{EC,nd,t1h} = 1.00e-5))} = \frac{30.289}{4.030} = 7.51 \cong 7.5$$

The summary of the LR obtained is presented in Table 40. Firstly, it can be observed that each LR exceeds 1. Therefore, it is more likely to observe the questioned specimens if they were collected minutes before discharges rather than they were collected one hour after the discharges.

Table 40. Simulation 1: LRs obtained for each OGSR evidence.

Evidence	LR	Verbal equivalent according to Evett et al. [94]
$E_{NnDPA, d}$	**1283**	***Strong*** *support to Hp rather than Hd*
$E_{NnDPA, nd}$	**82.5**	***Moderate*** *support to Hp rather than Hd*
$E_{DPA, d}$	**4468**	***Strong*** *support to Hp rather than Hd*
$E_{DPA, nd}$	**1.5**	***Weak*** *support to Hp rather than Hd*
$E_{EC, d}$	**41.5**	***Moderate*** *support to Hp rather than Hd*
$E_{EC, nd}$	**7.5**	***Weak*** *support to Hp rather than Hd*

However, all the LRs are not supporting the Hp to a similar degree. Indeed, it can be seen in Table 40 that the specimens collected from the dominant hand of the POI produced a larger LR presenting moderate (EC) to strong (*N*-nDPA and DPA) support of proposition Hp rather than proposition Hd. This results can be observed in Figure 53, with a better separation of both Hp and Hd distributions ($E_{NnDPA,d}$ and $E_{DPA,d}$), leading to larger LRs.

Conversely, the results of the specimens collected from the non-dominant hand of the POI, the LRs present a moderate (*N*-nDPA) to weak (EC) support to proposition Hp rather than proposition Hd, while DPA provides a weak support to any of the propositions of interest. This can be observed in Figure 53 with a large overlapping of both Hp and Hd

distributions ($E_{DPA,nd}$ and $E_{EC,nd}$). Because of this large overlap, the reasonable separation between Hp and Hd distributions is not achievable, resulting in LR close to one.

Figure 54 and Figure 55 present the evolution of the LRs at any given normalised peak areas for that particular set of propositions. The axis at 0 represents a LR=1 (Log(1)= 0). The Log(LRs) below 0 support Hd rather than Hp, while log(LRs) above 0 support Hp rather than Hd. The results showed to be coherent with the expected trends, where detecting a larger amount in the specimen will result in greater LR supporting Hp (T0) rather than Hd (T1h). It can be observed that for *N*-nDPA the LRs are increasing more quickly than EC and DPA. This observation is the consequence of the increased separation of the Hp and Hd distribution (Figure 53, $E_{NnDPA,d}$) to that of DPA and EC (Figure 53, $E_{DPA,d}$ and $E_{EC,d}$). For DPA and EC, it is observed that the LRs support Hd rather than Hp between a normalised peak area of 0 and 0.022 and 0.023, respectively. At these particular values of normalised peak area, the LRs are approximately one, which represent the intersection between the Hp and Hd distributions (Figure 53, $E_{DPA,d}$ and $E_{EC,d}$). Past these values of normalised peak area, the LRs support the proposition Hp rather than Hd.

Similar observations are seen for the LRs for the non-dominant hand (Figure 55), with a support of Hd between a normalised peak area of 0 to 0.011 for *N*-nDPA and between 0.013 to 0.024 for EC. However, DPA is an exception, with a different trend, where the LRs do not change significantly from a value of one (Figure 55), a result of the large overlap of the two distributions (Figure 53, $E_{DPA,nd}$).

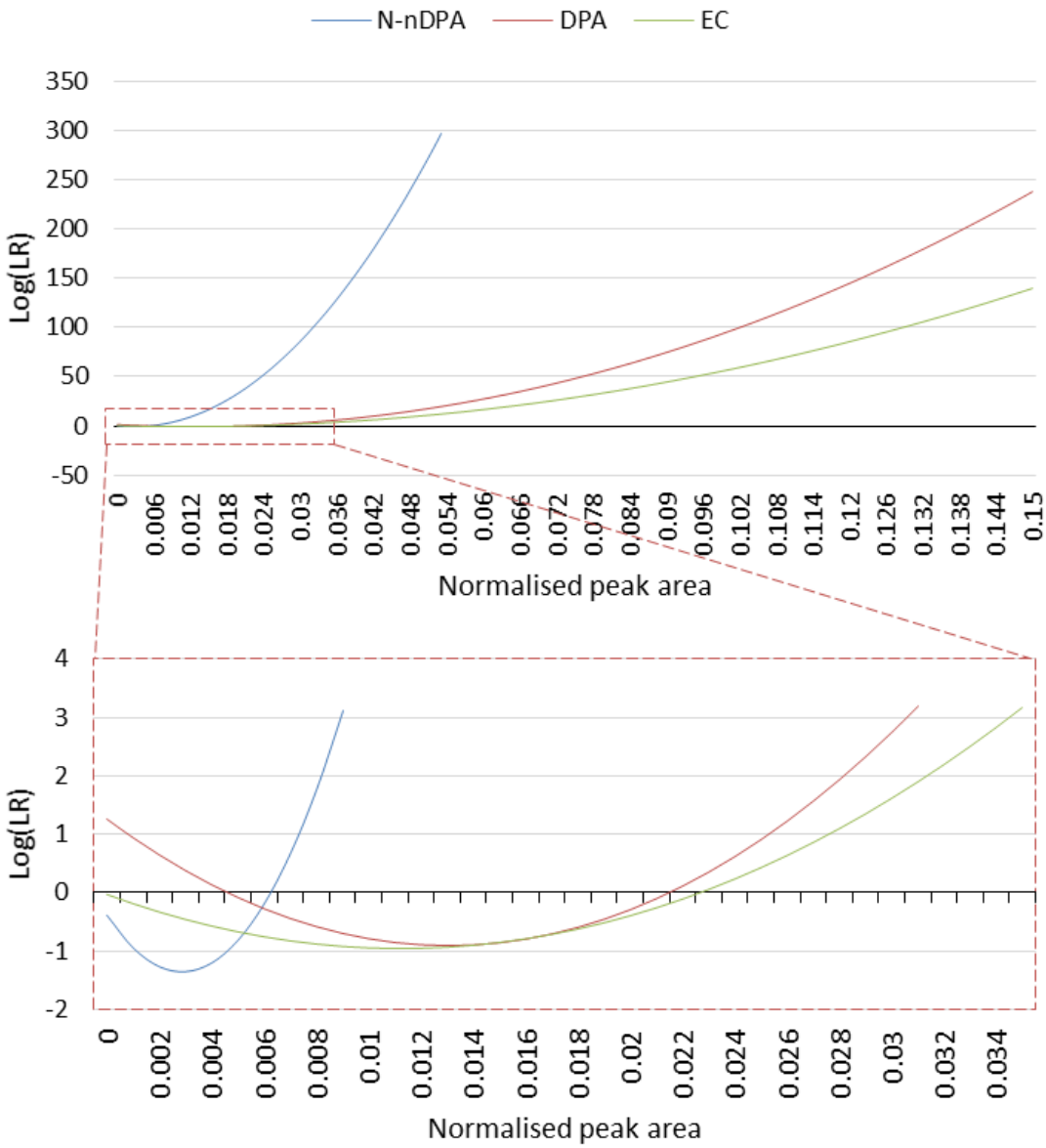


Figure 54. Simulation 1: Evolution of the LR for the dominant hand for any normalised peak area and for the particular set of propositions of simulation 1. The bottom graph presents a zoom-in of the red framed area.

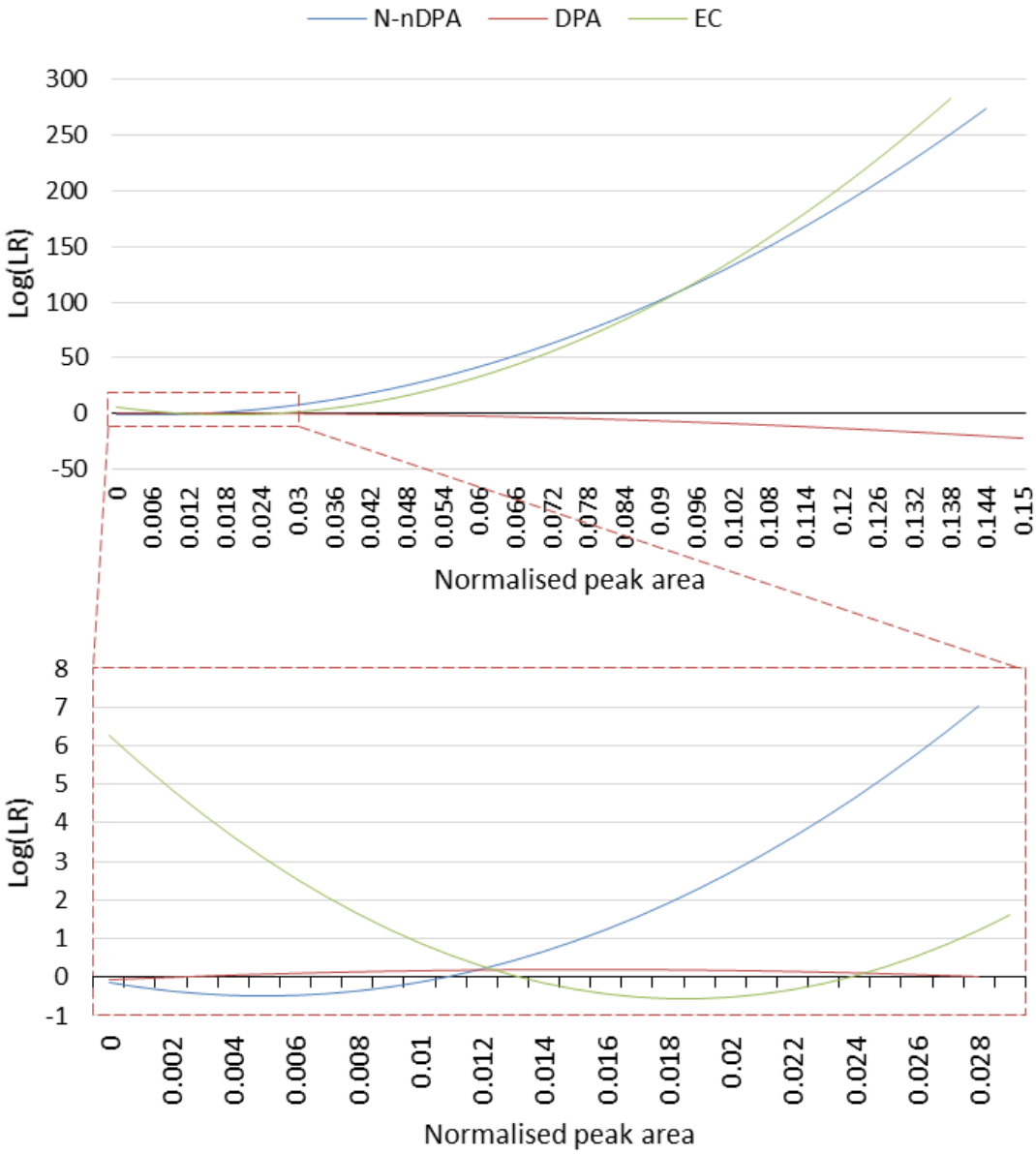


Figure 55. Simulation 1: Evolution of the LR for the non-dominant hand for any normalised peak area and for the particular set of propositions of simulation 1. The bottom graph presents a zoom-in of the red framed area.

4.1.2 Simulation 2: POI pollution

A second simulation involved a mock case where specimens were collected from both hands of an arrested POI. The POI is suspected to have discharged a firearm immediately prior to the arrest, however the POI claims that they were not involved in such an event but got contaminated by the police officer who arrested him, as a service firearm (calibre .40 S&W) was held at the time of the arrest.

The first step of the interpretation process involved to identify and define the most relevant set of propositions for the case under investigation. In this simulation, the specimens were suspected to have been collected from the POI a few minutes after the discharge, while the alternative proposition suggests that the POI has been contaminated by the police officer who performed the arrest, with the two alternative propositions stated as follows:

Hp: The OGSR traces detected on the hands of the POI originate from the discharges of a firearm minutes before the arrest.

Hd: The OGSR traces detected on the hands of the POI originate from the pollution by the police officer during the arrest process.

In this particular case, the police officer was not sampled for OGSR and therefore no results were available regarding the possible contamination of the officer's hands at the time of the arrest.

The specimens were analysed for OGSR and the normalised peak area (N_{PA}) were calculated. The laboratory results of the specimens for this simulation are presented in Table 41. The three compounds of interest were detected in both dominant and non-dominant hand specimens.

Table 41. Simulation 2: Results of the OGSR specimens collected from both hands of the POI. N_{PA}= Normalised peak area.

Evidence - N_{PA}	Dominant hand	Non-dominant hand
N-nDPA	$E_{NnDPA, d} = 0.002$	$E_{NnDPA, nd} = 0.003$
DPA	$E_{DPA, d} = 0.008$	$E_{DPA, nd} = 0.006$
EC	$E_{EC, d} = 0.004$	$E_{EC, nd} = 0.005$

Based on the two propositions of interest, two different populations of interest can be investigated. For Hp, the "control" population (specimens arising from the discharges of a firearm calibre .40 S&W at T0) was used in order to generate the normal PDF as it is the closest distribution to the time frame hypothesised in the proposition Hp. For the proposition Hd, the normal PDF was generated from the "non-shooter" population (scenario 1: after the arrest process) investigated in the secondary transfer study (Chapter three, Section IV).

As both hands of the POI were sampled and the three compounds of interest were detected, the six normal PDFs with their respective mean (μ) and variance (σ^2) parameters, are presented in Table 42.

Table 42. Simulation 2: normal PDF parameters for the two propositions of interest.

	Dominant hand				Non-dominant hand			
	Hp: *Control*		Hd: *Non-shooter*		Hp: *Control*		Hd: *Non-shooter*	
	$\mu_{d, ctrl}$	$\sigma^2_{d, ctrl}$	$\mu_{d, NS}$	$\sigma^2_{d, NS}$	$\mu_{nd, ctrl}$	$\sigma^2_{nd, ctrl}$	$\mu_{nd, NS}$	$\sigma^2_{nd, NS}$
N-nDPA	0.020	$5.82x10^{-4}$	0.002	$8.53x10^{-7}$	0.007	$1.28x10^{-4}$	0.001	$3.02x10^{-7}$
DPA	0.033	$4.34x10^{-4}$	0.008	$2.23x10^{-6}$	0.017	$1.08x10^{-4}$	0.009	$5.61x10^{-6}$
EC	0.041	$8.40x10^{-4}$	0.003	$2.57x10^{-6}$	0.020	$1.20x10^{-4}$	0.004	$6.91x10^{-6}$

The normal PDF were calculated as detailed previously (equation 7). The equation can be adapted to the particular simulation. For each compound, hand and both Hp and Hd (Table 42), where *"x"* represents any possible normalised peak area (equation 7). The normal PDF generated for Hp and Hd for each compound and each dominant and non-dominant hand are presented in Figure 56. It can be seen that the controls present a larger mean and broader peak, a consequence of the large variance observed during the OGSR analysis. However, the Hd distribution presents a lower mean, resulting from the lower amount detected on the non-shooter after the arrest process to that on the hand of a shooter (control) but also a narrower peak emphasising a lower variance (Figure 56), supporting the observation made in Chapter three, Section III.

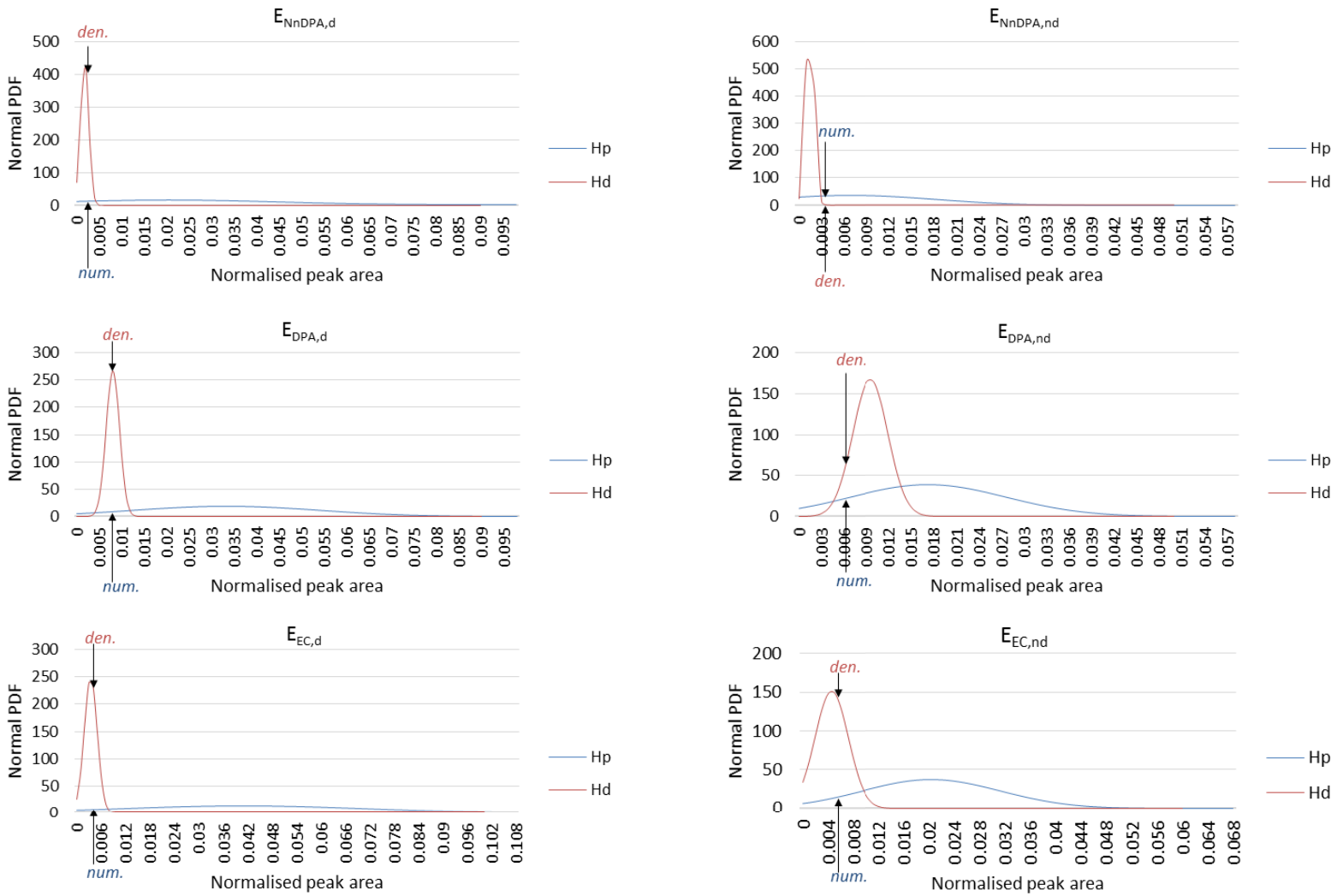


Figure 56. Simulation 2: Normal probability density function (PDF) for each proposition of interest; num= LR numerator, den= LR denominator. The black arrows represent the N_{PA} for the OGSR traces under investigation.

The LR can be calculated in the same way as in the simulation 1, with the LR formula presented in Equation 9. The detailed formula of the LR calculation is the same as the one used in simulation 1 (Equation 8).

$$LR = \frac{p(E\ |Hp)}{p(E\ |Hd)} = \frac{p(E\ |N{\sim}(\mu_{Hp}, \sigma_{Hp}^2))}{p(E\ |N{\sim}(\mu_{Hd}, \sigma_{Hd}^2))}$$

Equation 9. Simulation 2: LR equation.

This formula was used to perform the calculation of the LR for each evidence collected in this present simulation (Table 41) according to the parameters of the respective proposition of interest presented in Table 42. In addition, a graphical representation of the probability densities for the assessment of the LR can be seen in Figure 56, where the normalised peak area of each OGSR compound detected in the questioned specimens (Table 41) are illustrated by the black arrows and both numerator and denominator of the LR can be defined by the Hp and Hd probability density function curve.

The results of the LR calculation for each OGSR evidence is presented hereafter.

$$LR_{NnDPA,d} = \frac{p(E_{NnPDA,d} = 0.002 \,|N\sim(\mu_{NnDPA,d,\,ctrl} = 0.020, \sigma^2_{NnDPA,d,ctrl} = 5.82e-4))}{p(E_{NnDPA,d} = 0.002 \,|N\sim(\mu_{NnDPA,d,\,NS} = 0.0018, \sigma^2_{NnDPA,d,NS} = 8.53e-7))} = \frac{12.34}{417.54} = 0.0295 \cong 0.03$$

$$LR_{NnDPA,nd} = \frac{p(E_{NnDPA,nd} = 0.003 \,|N\sim(\mu_{NnDPA,nd,ctrl} = 0.007,\ \sigma^2_{NnDPA,nd,ctrl} = 1.28e-4))}{p(E_{NnDPA,nd} = 0.003 \,|N\sim(\mu_{NnDPA,nd,\,NS} = 0.0014, \sigma^2_{NnDPA,nd,NS} = 3.02e-7))} = \frac{33.24}{12.94} = 2.6585 \cong 2.65$$

$$LR_{DPA,d} = \frac{p(E_{DPA,d} = 0.008 \,|N\sim(\mu_{DPA,d,ctrl} = 0.033, \sigma^2_{DPA,d,ctrl} = 4.34e-4))}{p(E_{DPA,d} = 0.008 \,|N\sim(\mu_{DPA,d,NS} = 0.008, \sigma^2_{DPA,d,NS} = 2.23e-6))} = \frac{9.16}{267.00} = 0.0343 \cong 0.035$$

$$LR_{DPA,nd} = \frac{p(E_{DPA,nd} = 0.006|N\sim(\mu_{DPA,nd,ctrl} = 0.017, \sigma^2_{DPA,nd,ctrl} = 1.08e-4))}{p(E_{DPA,nd} = 0.006|N\sim(\mu_{DPA,nd,NS} = 0.0095, \sigma^2_{DPA,nd,NS} = 5.61e-6))} = \frac{21.50}{55.77} = 0.3856 \cong 0.4$$

$$LR_{EC,d} = \frac{p(E_{EC,d} = 0.004|N\sim(\mu_{EC,d,ctrl} = 0.041,\ \sigma^2_{EC,d,ctrl} = 8.40e-4))}{p(E_{EC,d} = 0.004|N\sim(\mu_{EC,d,NS} = 0.0034,\ \sigma^2_{EC,d,NS} = 2.57e-6))} = \frac{6.14}{232.78} = 0.0264 \cong 0.025$$

$$LR_{EC,nd} = \frac{p(E_{EC,nd} = 0.005|N\sim(\mu_{EC,nd,ctrl} = 0.020,\ \sigma^2_{EC,nd,ctrl} = 1.20e-4))}{p(E_{EC,nd} = 0.005|N\sim(\mu_{EC,nd,NS} = 0.0045,\ \sigma^2_{EC,nd,NS} = 6.91e-6))} = \frac{13.65}{149.49} = 0.0913 \cong 0.09$$

The summary table of the calculated LR can be seen in Table 43. For 5 out of the 6 OGSR evidence (Table 43), the interpretation resulted in LR below one, supporting the alternative proposition, that it is more likely to observe the evidence if the POI was contaminated by the police officer during the arrest process (Hd) rather than the POI discharged a firearm (Hp).

However, all the LR do not support Hd at a same degree (Table 43). For *N*-nDPA and DPA, the LR obtained from the evidence collected on the dominant hand ($E_{NnDPA,\ d}$ and $E_{DPA,\ d}$) are lower than these obtained from the non-dominant hand, which provide a larger support to Hd. For EC, both dominant and non-dominant hands LR support moderately Hd rather than Hp (Table 43).

Table 43. Simulation 2: LR obtained for each OGSR evidence.

Evidence	LR	*Verbal equivalent [94]*
$E_{NnDPA,\ d}$	**0.03**	***Moderate** support to Hd rather than Hp*
$E_{NnDPA,\ nd}$	**2.65**	***Weak** support to Hp rather than Hd*
$E_{DPA,\ d}$	**0.035**	***Moderate** support to Hd rather than Hp*
$E_{DPA,\ nd}$	**0.4**	***Weak** support to Hd rather than Hp*
$E_{EC,\ d}$	**0.025**	***Moderate** support to Hd rather than Hp*
$E_{EC,\ nd}$	**0.09**	***Moderate** support to Hd rather than Hp*

An exception can be seen with *N*-nDPA on the non-dominant hand (Table 43), which resulted in a LR above 1 (2.65), providing a weak support to Hp rather than Hd. As the LR is very close to 1, it does not provide meaningful support of either proposition of interest and contradicts the results of the other calculated LRs (Table 43). This results arose from the evidence being close to the intersection of both Hp and Hd normal PDF (Figure 56, $E_{NnDPA,nd}$). The probability densities for that particular evidence does not provide enough separation to differentiate both populations.

When balancing the interpretation of OGSR findings as a whole, it might be concluded that the OGSR evidence provides a weak-moderate support to Hd. This simulation highlighted the fact that interpreting each OGSR compounds individually can lead towards variable LRs. In this case the conflicting LR was found to be very close to 1 (2.65, Table 43) providing only a weak support towards Hp, which can be balanced by the 5 other LRs supporting Hd. However, if larger discrepancies arise between LR, the interpretation process must be balanced in order to provide a global assessment of the findings.

The LR found for each evidence is interesting as it is often assumed that detecting traces on a POI, such as OGSR is leading to a conclusion that the person discharged a firearm. However, this case resulted in counter-intuitive conclusions, where detecting OGSR on the POI supports the proposition that the traces are the consequence of a pollution during the arrest rather than a firearm discharges.

Figure 57 and Figure 58 represent the evolution of the LRs for any given normalised peak area and for the particular set of propositions of simulation 2, for both dominant and non-dominant hands respectively. The axis at 0 equals a LR=1, with log(LRs) < 0 supporting Hd rather than Hp, while log(LRs) > 0 support Hp rather than Hd.

It can be observed for *N*-nDPA and DPA (Figure 57) that detecting a small normalised peak area, between 0 and 0.04 and 0.075, respectively, result in LRs supporting Hd: a pollution, rather than Hp: firearm discharges. However, the detection of larger normalised peak areas, which relates to larger amounts of OGSR lead to LRs supporting Hp: firearm discharges rather than Hd. Similar observations can be made for the LRs on non-dominant hand (Figure 58), with a support to Hd rather than Hp at smaller normalised peak area (equalling lower amount), but when the amount detected increases, the LRs support Hp: firearm discharges rather than Hd: pollution.

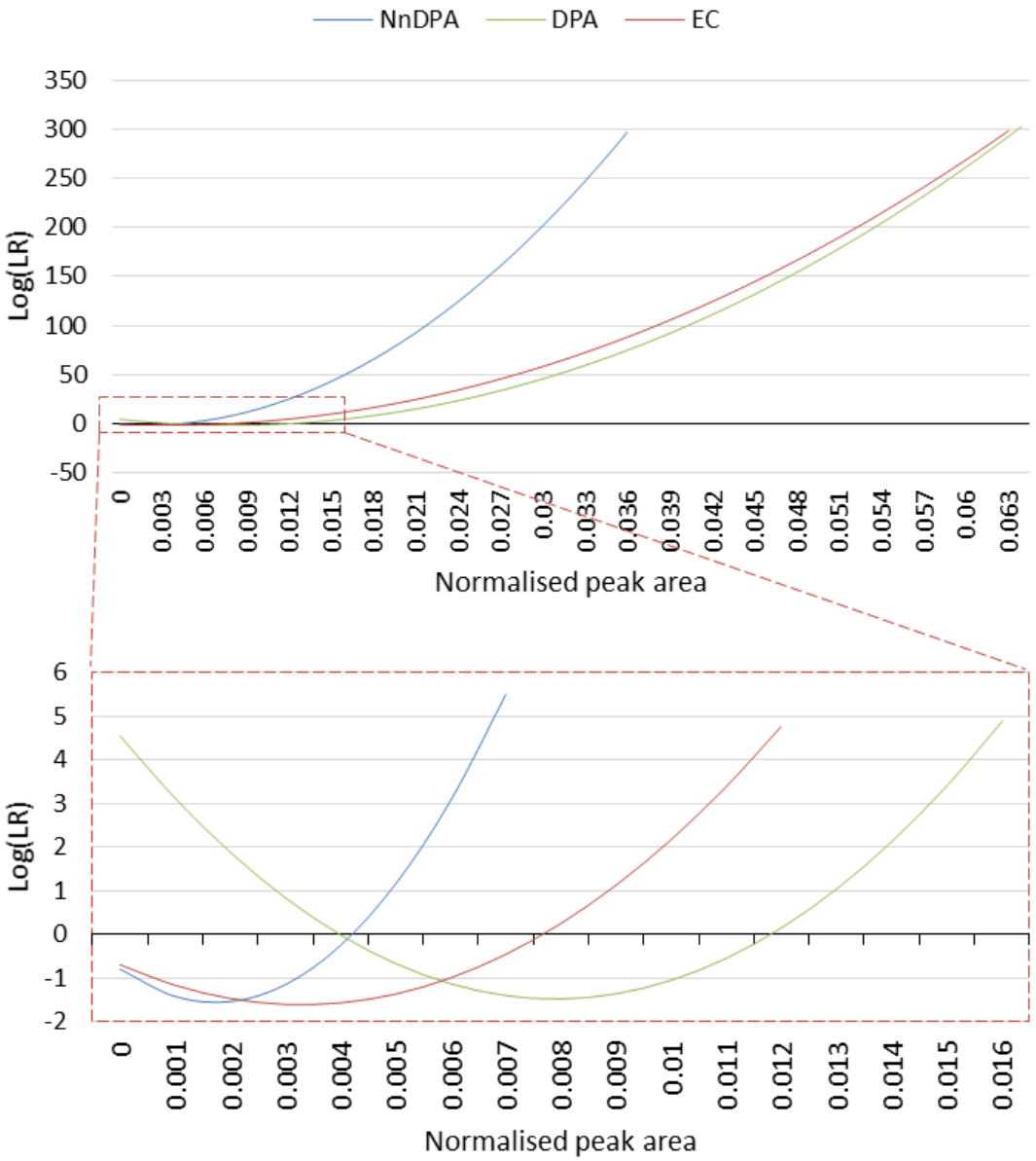


Figure 57. Simulation 2: Evolution of the LR for the dominant hand for any normalised peak area and for the particular set of propositions of simulation 2. The bottom graph presents a zoom-in of the red framed area.

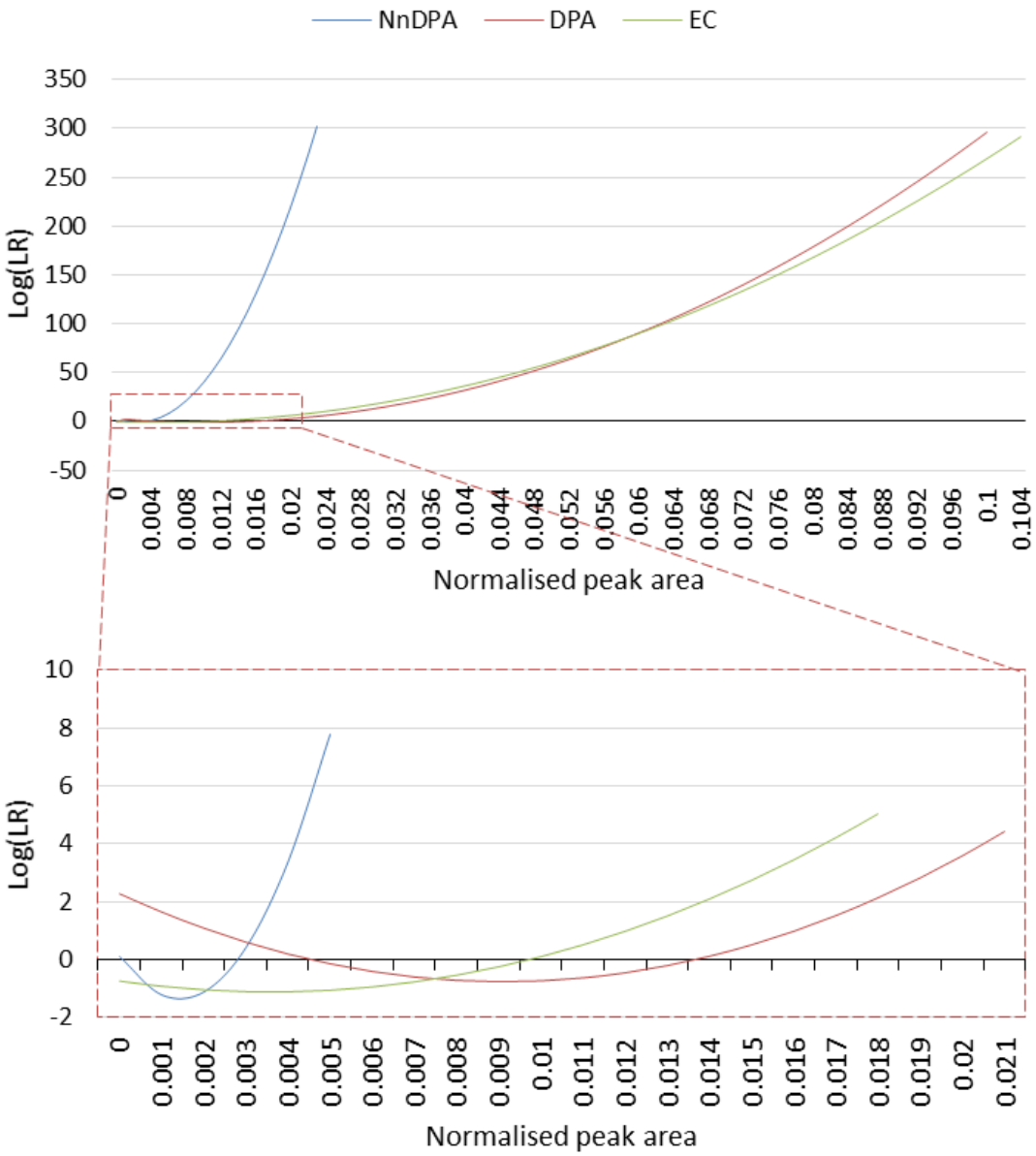


Figure 58. Simulation 2: Evolution of the LR for the non-dominant hand for any normalised peak area and for the particular set of propositions of simulation 2. The bottom graph presents a zoom-in of the red framed area.

Simulation 2, as well as Figure 57 and Figure 58, highlights the importance of secondary studies as detecting low amount of OGSR (applicable to any forensic traces) from a POI does not always support the prosecution hypothesis (Hp). It is therefore essential to develop a better understanding of such traces to ensure an appropriate interpretative framework, to limit the risk of false positives, which could have a dramatic impact on the outcomes of forensic investigations.

4.2 Future considerations

A limitation of the interpretation process presented in this chapter relates to the possibilities of contradictory LRs in relation to OGSR traces. In Chapter four, each compound was assessed independently to each other resulting in 6 different LRs (three compounds on two hands). Consequently, it is conceivable to obtain LRs which could hypothetically challenge each other with different strength of the evidence. For instance, a compound which supports Hp, while another one supports Hd, as observed with *N*-nDPA from the non-dominant hand in the simulation 2 (4.1.2). It is therefore worthwhile to consider how to deal with such situations.

Several steps can be undertaken in order to improve the interpretative process of OGSR such as considering the assessment of the OGSR traces in a multivariate perspective. In this project, the interpretation was performed on each compound taken separately, however, the three compounds of interest were detected from the same specimen and hence are part of the same OGSR trace. The multivariate approach to the interpretation of OGSR traces is essential as there is a dependency factor between the different

compounds, which prevent the possibility to multiply each compounds LR to form a global LR for the entire OGSR trace. Such an approach has been developed previously for the elemental composition of glass, in which three elemental ratios were used to develop a multivariate LR [199]. Consequently, *N*-nDPA, DPA and EC, in a multivariate approach, have to be considered simultaneously, in the form of a vector, to calculate the LR. Therefore, the evidence could be written as shown in Equation 10:

$$E_d = \begin{Bmatrix} NPA_{NnDPA,d} \\ NPA_{DPA,d} \\ NPA_{EC,d} \end{Bmatrix} \quad and \quad E_{nd} = \begin{Bmatrix} NPA_{NnDPA,nd} \\ NPA_{DPA,nd} \\ NPA_{EC,nd} \end{Bmatrix}$$

Equation 10. Multivariate consideration for traces collected from both hands of a POI. E_d= Evidence on the dominant hand, E_{nd}= Evidence on the non-dominant hand, NPA= Normalised peak area.

In addition to the multivariate approach, the time point used to assess the LR were accurate and represented a time point investigated in the persistence study. However, in a real case scenario, forensic scientists may only have at their disposal a time window more or less precise. T0, T0.5h, T1h, t2h and T4h were investigated, quid of specimens collected 15 min after discharge? Or after 1.5 h? It would be beneficial to improve the LR model by implementing an integration model when calculating the LR which would account for variations in the timeframe under investigation as shown in Equation 11.

$$\frac{\int_t^{dt} P\left(E\ |Hp,\ N{\sim}\left(\mu_{Hp}, \sigma_{Hp}^2\right)\right)\ dt}{\int_t^{dt} P\left(E\ |Hd,\ N{\sim}\left(\mu_{Hd}, \sigma_{Hd}^2\right)\right) dt}$$

Equation 11. Integration model for the assessment of LRs.

As emphasised in Chapter two, BNs are graphical interfaces, which include an underlying probabilistic framework that allows the propagation of the information throughout the network by respecting the logical framework of probabilities. The BN development was beyond the scope of this project. However, such networks facilitate the combination of different parameters such as the persistence, the secondary transfer and the prevalence of OGSR in a single interface for the calculation of LRs. BN could also be highly relevant when approaching the interpretation of OGSR in a multivariate perspective, by respecting the logical probabilistic reasoning. BN would also be beneficial to combine OGSR and IGSR outcomes by integrating the different parameters within a single BN and therefore considering the outcomes of both IGSR and OGSR, therefore assessing the strength of GSR traces as a whole.

5. Conclusions

This chapter highlights the importance of OGSR interpretation. The assessment of the results is a crucial part of any forensic investigation as it represents the opinion of the forensic scientists based on the assessment of the strength of the evidence, which is evaluated with the calculation of the LR. The interpretation of OGSR evidence imply to consider the different circumstances surrounding the particular case under investigation. This allows the forensic scientists to integrate the OGSR evidence into the global context of the case, taking into account the chronology of the case and the different possibilities of pollutions, which may occur.

This chapter emphasised on two different scenarios that illustrates the use of data generated through persistence and secondary transfer studies. In the first scenario, different discharge times were questioned. The first proposition stated that the traced arose from a firearm discharge, which occurred minutes before the collection (Hp), while the alternative proposition stated that the trace arose from discharges which occurred an hour before the specimen's collection (Hd). The assessment of the evidence given the propositions of interest led to LR supporting Hp rather than Hd (from a weak to a strong support).

In the second scenario, the question of interest related to the possible pollution of the POI by the police officer who performed the arrest. The first proposition stated that the traces collected arose from discharges, which occurred minutes before the collection (Hp), while the alternative proposition stated that the evidence arose from the pollution of the hands of the POI by the police officer who carried out the arrest (Hd). The assessment of the

evidence given the propositions of interest led to LR supporting a larger likelihood if the evidence arose from Hd rather than Hp (from weak to moderate support). This scenario highlighted the importance of such factors during the LR assessment in order to avoid misleading results, which could lead to false positive and largely impacting the outcome of the forensic investigation.

Nonetheless, some future considerations can be important to increase the utility of OGSR evidence. In this project, each compound was interpreted individually from each other, generating LR for each compound. However, interpreting OGSR evidence through a multivariate approach, by combining the compounds together, could strengthen the LR and the information provided by OGSR traces. In addition, combining the different parameter of persistence, secondary transfer and prevalence (if any data is collected) into BNs would enable the forensic scientist to interpret OGSR evidence by following the logical framework of conditional probabilities. The BN can also permit to provide a case-by-case interpretation of OGSR evidence by considering the different circumstances of the case, such as possible pollution and the chronology of the event. Ultimately, an integration of OGSR with IGSR within the same BN could potentially lead toward a more holistic approach to assess GSR traces.

CHAPTER FIVE: GENERAL CONCLUSION

1. A better understanding of OGSR evidence

OGSR have the potential to provide crucial information in firearm-related event. OGSR compounds arise from the combustion of the propellant powder during the firearm discharges and can be a potential complementary information to IGSR particles, conventionally analysed by SEM-EDX.

In this project, four compounds of interest were chosen based on their frequency in the composition of propellant powder, respectively, *N*-nDPA, DPA, MC and EC. OGSR specimens were analysed by UPLC-QqQ-MS. The analytical method was fully validated for four compounds of interest (*N*-nDPA, DPA, MC and EC) using ICH guidelines [185] and found to be fit for purpose (Chapter three, Section I). The propellant powder from the ammunition used was analysed pre-discharge to confirm the presence of the compounds of interest. Three out of the four compounds of interest (*N*-nDPA, DPA and EC) were identified in the two ammunitions types used (.40 S&W and .357 Mag, Chapter three, Section II).

Further analysis was performed to assess the stability of the specimens after the OGSR extraction process (Chapter three, Section II). Indeed, forensic laboratories will routinely face analysis backlogs and need to be able to store specimens, at 4 °C, without risking degradation. This study followed a study performed by Taudte et al., which had studied

the stability of OGSR compounds on the collection device before being subjected to the extraction process [190].

Two sets of specimens were studied: OGSR compounds standards (calibration curves) and specimens collected from the discharges of a firearm (.357 Mag). The Results showed that a degradation was observed after one week of storage for two of the three compounds of interest (*N*-nDPA and EC), while DPA was found to be more stable after one week, with degradation initiating after two weeks of storage. This study emphasised on the importance of processing the specimens as early as possible in the investigation. By doing so, it would improve the chance of detecting OGSR compounds and maximising the outcomes of the analytical analysis.

The subsequent studies involved specimens being collected from both hands of the shooter after the firearms were discharged. It was found that the three compounds of interest,
N-nDPA, DPA and EC, previously detected in the propellant powder, could be successfully identified in specimens arising from the discharges of a firearm. DPA and EC are compounds well-known as being stabilisers in the composition of the propellant powder, while *N*-nDPA is known to be a nitroso-derivative of DPA present in OGSR. Therefore, detecting such compounds altogether from hands specimens improve the degree of identification of residues as originating from firearm discharges rather than environmental sources.

The investigation of forensic material has the particularity to relate to past events which occurred with a precise chronology. The time elapsed between the investigated event (i.e. firearm discharges) has an undeniable impact on the OGSR traces. The traces collected

from the hands of a POI were subjected to different activities between their deposition and the time of the specimens were collected. Hence, it is essential to assess how OGSR traces behave over time to evaluate the potential information given by such traces under different activities conditions.

The first project focussed on the assessment of OGSR persistence. The purpose was to assess for how long OGSR traces can still be collected and the compounds being successfully detected from the hands of a shooter. Five time points were selected ranging from immediate collection to a period of 4 hours post discharge (T0, T0.5h, T1h, T2h and T4h). The results showed that the three compounds of interest could be detected up to four hours after discharge. Similar trends were observed for each compound of interest displaying a larger decrease in the amount detected occurring during the first hour. Nevertheless, after four hours, the three compounds of interest could be detected often up to 70 times higher than the LOD.

The information provided by the persistence study is found to be forensically relevant as OGSR can be a potential source of complementary information to the inorganic particles (IGSR) traditionally targeted. This is especially important when larger time periods have elapsed between the firearm discharges and the specimens collection as studies showed that IGSR persistence largely decreases within four hours [148]. Consequently, combining the IGSR information with the detection of OGSR compounds would potentially strengthen GSR evidence, particularly when only few particles are detected.

The second project concerned the likelihoods of a secondary transfer of OGSR. A primary transfer of OGSR occurs when the firearm is discharged with a transfer of OGSR on the hands of shooter and their close vicinity. From then, it was essential to investigate the possibilities of further transfer of OGSR traces.

Two scenarios were investigated: The first scenario regards the fact that police officers carry and handle daily their service weapon, there is a risk of pollution during the arrest process. The results showed that it was possible to detect OGSR from the hands of a non-shooter after an arrest by a shooter (chapter three, Section III). This information is crucial to provide stronger protocols to limit contacts between a POI and officers who had a previous contact with their firearm.

The second scenario investigated concerned a secondary transfer, which could occur while holding a firearm, without discharging it. The results of this scenario showed the amount transferred was significant (chapter three, Section IV). Consequently, forensic scientists must be aware of the context of the case when analysing and more importantly when interpreting OGSR evidence. In addition, a standard operating procedure (SOP) can be recommended to minimise the risks of pollution of POI. Police officers who discharged their firearm prior to the arrest should not enter in contact with the POI to avoid transferring OGSR. Moreover, it is recommended that the firearms as well as the handcuffs are regularly cleaned in order to minimise the accumulation of OGSR and therefore reducing risk of further pollutions of POI.

Additionally, a similar result was observed in both the study of persistence and secondary transfer. This related to the high degree of variability observed in the amount of OGSR detected shot to shot. These observations were due to numerous factors involved in the

formation, dispersion and deposition of the residues. Such variations are consistent with previous studies on the retention of inorganic particles, which also concluded that the numerous parameters involved in the discharge process, such as the type of ammunition, calibre, number of discharges, firearm construction, has a massive impact on the production and deposition of GSR (both inorganic and organic).

The studies of persistence and secondary transfer of OGSR also emphasised the importance of transmitting contextual information to the forensic scientist. Despite a strong and ongoing debate in the literature about the potential contextual bias in forensic science [200-203], having this complementary information enables the forensic scientist to consider the circumstances when interpreting the strength of the forensic findings. Providing contextual background of the specimens would allow forensic scientist to tackle the interpretation of OGSR evidence in a more conservative manner in order to minimise the risks of false classification of the results as arising from a firearm discharge.

To appropriately interpret OGSR evidence, the final chapter of this research tackled the probabilistic interpretation of OGSR evidence. The aim was to highlight the use of the results obtained in the persistence and secondary transfer studies to appropriately and meaningfully interpret OGSR results. In order to apply the probabilistic approach to the interpretation of the OGSR, two simulations were explored using Bayes theorem. The first one relates to different scenarios questioning the chronology of the event. The first proposition stated that the specimens arose from a firearm discharges few minutes before the specimen collections (Hp), while the alternative proposition stated that the POI was in a shooting range an hour ago (Hd – Chapter four, 4.1.1). For this simulation, the

interpretation results found that the LR supported Hp rather than Hp with LR ranging between 10 and 1000. These results indicate that OGSR evidence can potentially provide meaningful and relevant information to forensic investigations, supporting that OGSR can be complementary to IGSR information.

A second simulation was investigated, with the questioned specimens suspected to originate from a firearm being discharged by the POI (Hp), while the alternative proposition stated that the POI was polluted by the police officer who carried out the arrest (Hd – Chapter four, 4.1.2). The results found that results supported the pollution of the POI by the arresting officer (Hd) rather than the firearm discharge scenario with LR ranging between 0.1 and 0.001. Intuitively, detecting small amounts would suggest that the POI discharged a firearm, however, it was observed in this case that even if a small amount is detected, such a result does not always support the fact that the POI discharged a firearm but that a significant risk of a pollution exists.

As a final conclusion, OGSR traces were found to be a relevant source of information in firearm-related forensic investigations. Nevertheless, several steps can be performed in order to improve the holistic approach of OGSR analysis. Firstly, as both IGSR and OGSR are collected on the same devices, it is crucial to assess if the organic analysis process, which is destructive for the collection device, can be performed in sequence with the SEM-EDX inorganic particles analysis. At the time of this writing, there is only one published work about the influence of the SEM-EDX conditions, such as the vacuum and the electron beam, on the recovery and analysis of OGSR [189]. They have found that OGSR could be detected after being exposed to SEM-EDX conditions required for the

detection of IGSR particles [189]. A more extensive research would be an important step to undertake in order to truly assess the feasibility of routine OGSR analysis in complement to the inorganic particle detection.

Secondly, further research is needed to refine the approach used for the interpretation of OGSR. This research considered each compound separately in order to compute a LR per compounds. To generate a holistic approach to the interpretation of OGSR, it would be recommended to encapsulate all detected compounds in a single interpretation framework. Such model can be achieved through a multivariate approach by considering the OGSR traces evidence as a vector of "X" values referring to "X" detected compounds, as previously approached for the multivariate approach to interpret glass findings [199]. In addition, with a better integration within IGSR analysis, the interpretation of both IGSR and OGSR could be combined within the LR framework in order to achieve a more global assessment of GSR traces by considering it as whole rather than two separated components.

Appendices

Appendix I: Ethics and consent forms

Persistence consent form

UNIVERSITY OF TECHNOLOGY SYDNEY

PRINTED ON UTS (and/or joint) LETTERHEAD

CONSENT FORM: Persistence study

I _____ *(participant's name)* agree to participate in the research project "*Forensic gunshot residues analysis: detection and interpretation of organic compounds*" being conducted by Matthieu Maitre, PO Box 123, Broadway NSW 2007, mobile number 0478668600, of the University of Technology Sydney for his doctoral degree in Forensic Science.

I understand that the purpose of this study is the analysis of the *persistence of organic gunshot residues on the shooter's hands*.

I understand that I have been asked to participate in this research because I am holding a valid firearms licence, have access to a firing range and am located close to Sydney central. My participation in this research will involve shooting different weapons with different ammunitions several times. The duration of the research will involve approximately 11 hours of your time spread over 10 days (1 hour/day). After shooting, my hands will be sampled[1]. I will not be provided with any kind of recompense as this is a fully voluntary participation.

I am aware that I can contact Matthieu Maitre or his supervisor Dr. Alison Beavis if I have any concerns about the research. I also understand that I am free to withdraw my participation from this research project at any time I wish, without consequences, and without giving a reason.

I agree that Matthieu Maitre has answered all my questions fully and clearly.

I agree that the research data gathered from this project will be taken in handwritten and non-identifiable form. Matthieu Maitre and Dr. Alison Beavis will have access to the raw data. The data may be published in a form that does not identify me in any way in conference papers, a thesis or journal articles and possible additional form in the future.

_____ ___/___/___
Signature (participant)

_____ ___/___/___
Signature (researcher or delegate)

NOTE:
This study has been approved by the University of Technology, Sydney Human Research Ethics Committee. If you have any complaints or reservations about any aspect of your participation in this research which you cannot resolve with the researcher, you may contact the Ethics Committee through the Research Ethics Officer (ph: +61 2 9514 9772 Research.Ethics@uts.edu.au) and quote the UTS HREC reference number. Any complaint you make will be treated in confidence and investigated fully and you will be informed of the outcome.

[1]For further information please refers to the infosheet.

Persistence information sheet

UNIVERSITY OF TECHNOLOGY SYDNEY

PRINTED ON UTS (and/or joint) LETTERHEAD

INFORMATION SHEET

Forensic gunshot residues analysis: detection and interpretation of organic compounds

WHO IS DOING THE RESEARCH?

My name is Matthieu Maitre and I am a PhD student at the University of Technology Sydney (UTS). (My supervisor is Dr. Alison Beavis)

WHAT IS THIS RESEARCH ABOUT?

This research is focusing on the study of the **persistence** of organic gunshot residues on shooter's hands.

IF I SAY YES, WHAT WILL IT INVOLVE?

You will be invited to volunteer 10 days according to your availability, with approximately 1 hour of process per day (overall time between 10-11 hours). You will ask to shoot a particular weapon several times. Your hands will then be sampled over 8 time periods following the shooting: 30 minutes, 1 hour, 2 hours, 4 hours, 6 hours and eventually 8 hour and 24 hours. The sampling will involve tape-lifting which consists of gently dabbing a double-sided adhesive tape over the area of interest that is around the thumb and the forefinger. You will ask to return to your normal activities between the 8 time periods. This research will not include any expenses for you. The data collected will be used in any non-identifiable form in the future.

ARE THERE ANY RISKS/INCONVENIENCE?

There are very few if any risks because the research has been carefully designed. No chemicals are used during the sampling process. The research will require circa 11 hours spread over 10 days and will involve firing range facilities close to Sydney Central.

WHY HAVE I BEEN ASKED?

You hold a firearms licence, have access to a firing range and are located close to Sydney central.

DO I HAVE TO SAY YES?

You don't have to say yes.

WHAT WILL HAPPEN IF I SAY NO?

Nothing. I will thank you for your time so far and won't contact you about this research again.

IF I SAY YES, CAN I CHANGE MY MIND LATER?

You can change your mind at any time and you don't have to say why. I will thank you for your time so far and won't contact you about this research again.

WHAT IF I HAVE CONCERNS OR A COMPLAINT?

If you have concerns about the research that you think I or my supervisor can help you with, please feel free to contact me on my mobile phone +61 478668600 or Dr. Alison Beavis on +61 2 9514 1761.

If you would like to talk to someone who is not connected with the research, you may contact the Research Ethics Officer on 02 9514 9772, and quote this number 2012000558

Secondary transfer consent form

UNIVERSITY OF TECHNOLOGY SYDNEY

PRINTED ON UTS (and/or joint) LETTERHEAD

CONSENT FORM: Secondary transfer study

I _____ *(participant's name)* agree to participate in the research project "*Forensic gunshot residues analysis: detection and interpretation of organic compounds*" being conducted by Matthieu Maitre, PO Box 123, Broadway NSW 2007, mobile number 0478668600, of the University of Technology Sydney for his doctoral degree in Forensic Science. **My participation is required from the 01/01/2018 to the 20/12/2018.**

I understand that the purpose of this study is the analysis of the *secondary transfer of organic gunshot residues on the shooter's hands*.

I understand that I have been asked to participate in this research because I have previously been involved in OGSR project and are available along the project or because I am holding a valid firearms licence, have access to a firing range and am located close to Sydney central.
The participation in this research will involve shooting different weapons with different ammunitions several times and undertaking scenarios involving a secondary transfer of OGSR. The duration of the research will involve approximately 8 hours of your time spread over 5 days (approx. 1.5 hour/day). After shooting, my hands will be sampled[1]. I will not be provided with any kind of recompense as this is a fully voluntary participation.

I am aware that I can contact Matthieu Maitre or his supervisor Dr. Alison Beavis if I have any concerns about the research. I also understand that I am free to withdraw my participation from this research project at any time I wish, without consequences, and without giving a reason.

I agree that Matthieu Maitre has answered all my questions fully and clearly.

I agree that the research data gathered from this project will be taken in handwritten and non-identifiable form. Matthieu Maitre and Dr. Alison Beavis will have access to the raw data. The data may be published in a form that does not identify me in any way in conference papers, a thesis or journal articles and possible additional form in the future.

_____ from the 01/01/2018 to the 20/12/2018
Signature (participant)

_____
Signature (researcher or delegate)

NOTE:
This study has been approved by the University of Technology, Sydney Human Research Ethics Committee. If you have any complaints or reservations about any aspect of your participation in this research which you cannot resolve with the researcher, you may contact the Ethics Committee through the Research Ethics Officer (ph: +61 2 9514 9772 Research.Ethics@uts.edu.au) and quote the UTS HREC reference number. Any complaint you make will be treated in confidence and investigated fully and you will be informed of the outcome.

[1]For further information please refers to the infosheet

Secondary transfer information sheet

UNIVERSITY OF TECHNOLOGY SYDNEY

PRINTED ON UTS (and/or joint) LETTERHEAD

INFORMATION SHEET

Forensic gunshot residues analysis: detection and interpretation of organic compounds

WHO IS DOING THE RESEARCH?

My name is Matthieu Maitre and I am a PhD student at the University of Technology Sydney (UTS). (My supervisor is Dr. Alison Beavis)

WHAT IS THIS RESEARCH ABOUT?

This research is focusing on the study of the **secondary transfer** of organic gunshot residues on shooter's hands.

IF I SAY YES, WHAT WILL IT INVOLVE?

You will be invited to volunteer 5 days according to your availability, with approximately 1.5 hour of process per day (overall time between 7-8 hours).

- The first individual will be asked to discharge a particular weapon several times. The shooting session will be followed by undertaking different scenarios, which involves a contact with a POI e.g. an arrest process.
- The second individual agreed to be involved in the scenario as a POI and therefore agreed to undertake an arrest scenario in order to experiment the secondary transfer of GSR from the shooter unto you.

Your hands will then be sampled twice over five replicate of two scenarios. The sampling will involve tape-lifting which consists of gently dabbing a double-sided adhesive tape over the area of interest that is around the thumb and the forefinger. This research will not include any expenses for you. The data collected will be used in any non-identifiable form in the future.

ARE THERE ANY RISKS/INCONVENIENCE?

There are very few if any risks because the research has been carefully designed. No chemicals are used during the sampling process. The research will require circa 8 hours spread over 5 days and will involve firing range facilities close to Sydney Central.

WHY HAVE I BEEN ASKED?

You have previously worked in project related to OGSR and are available along the project or you hold a firearms licence, have access to a firing range and are located close to Sydney central.

DO I HAVE TO SAY YES?

You don't have to say yes.

WHAT WILL HAPPEN IF I SAY NO?

Nothing. I will thank you for your time so far and won't contact you about this research again.

IF I SAY YES, CAN I CHANGE MY MIND LATER?

You can change your mind at any time and you don't have to say why. I will thank you for your time so far and won't contact you about this research again.

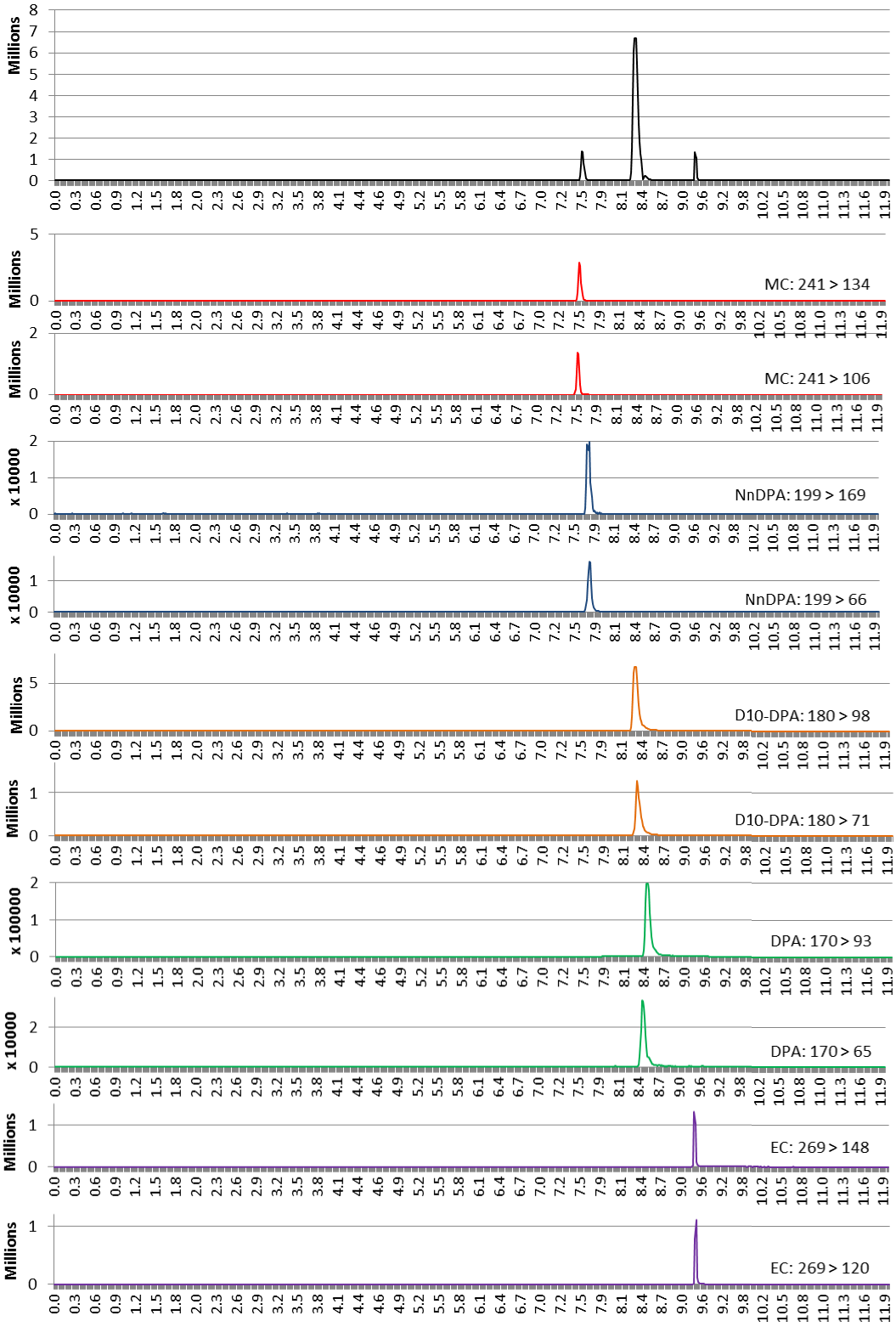
WHAT IF I HAVE CONCERNS OR A COMPLAINT?

If you have concerns about the research that you think I or my supervisor can help you with, please feel free to contact me on my mobile phone [redacted] or Dr. Alison Beavis on +61 2 9514 1761.

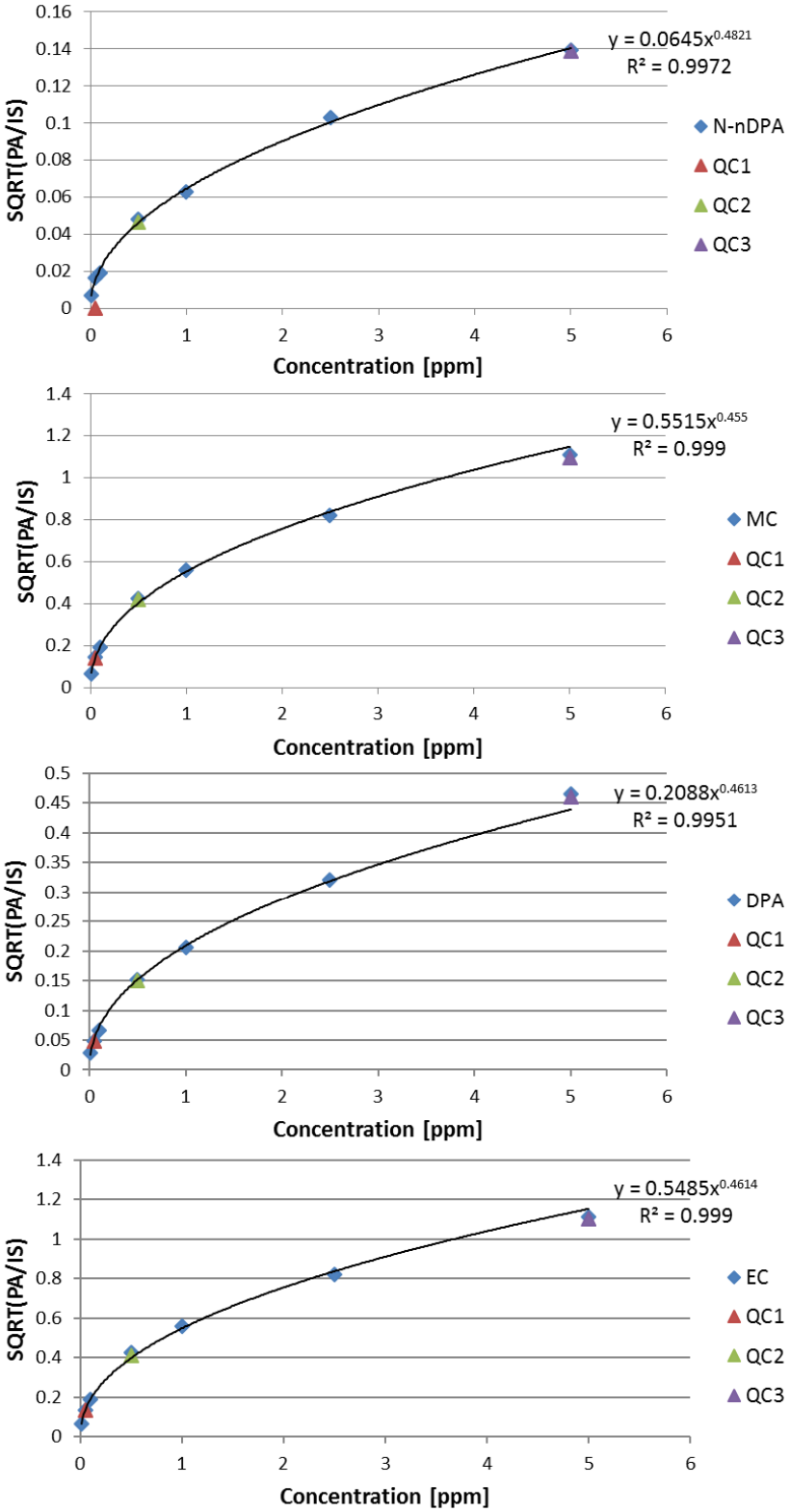
If you would like to talk to someone who is not connected with the research, you may contact the Research Ethics Officer on 02 9514 9772, and quote this number 2012000558

Appendix II: Analytical method and validation

Appendix II.1: TIC – MRM



Appendix II.2: Calibration curve for each compounds with each of the associated QCs



Calibration curves: complementary information

	Av. slope	Av. intercept	Av. RSQ	%RSD slope	%RSD RSQ
N-nDPA	0.004	0.000	**0.999**	**7.356%**	**0.110%**
MC	0.240	0.037	**0.993**	**9.456%**	**0.364%**
DPA	0.043	0.001	**0.999**	**0.663%**	**0.084%**
EC	0.232	0.031	**0.996**	**7.084%**	**0.250%**

Robustness: complementary data

***N*-nDPA**	*Rt*	*Rt (IS)*	*RRt*	*average RRt*	*sd RRt*	***%RSD***	**% difference**
+1 degree	7.77	*8.29*	0.94	0.94	3.53E-03	**0.38**	**100.31**
-1 degree	7.79	*8.35*	0.93				**99.84**
+5% MeOH	7.11	*7.58*	0.94				**100.39**
-5% MeOH	8.94	*9.49*	0.94				**100.82**
+0.05 ml/mn	7.60	*8.15*	0.93				**99.80**
-0.05% ml/mn	7.96	*8.5*	0.93				**100.22**
Normal condition	7.76	*8.25*	0.93				**100.00**
MC	*Rt*	*Rt (IS)*	*RRt*	*average RRt*	*sd RRt*	***%RSD***	**% difference**
+1 degree	7.75	*8.29*	0.93	0.93	3.84E-03	**0.41**	**100.20**
-1 degree	7.78	*8.35*	0.93				**99.87**
+5% MeOH	7.07	*7.58*	0.93				**99.97**
-5% MeOH	8.94	*9.49*	0.94				**100.97**
+0.05 ml/mn	7.60	*8.15*	0.93				**99.95**
-0.05% ml/mn	7.96	*8.5*	0.93				**100.37**
Normal condition	7.75	*8.25*	0.93				**100.00**
DPA	*Rt*	*Rt (IS)*	*RRt*	*average RRt*	*sd RRt*	***%RSD***	**% difference**
+1 degree	8.44	*8.29*	1.02	1.02	2.09E-03	**0.20**	**100.06**
-1 degree	8.50	*8.35*	1.02				**100.05**
+5% MeOH	7.70	*7.58*	1.01				**99.84**
-5% MeOH	9.61	*9.49*	1.01				**99.52**
+0.05 ml/mn	8.29	*8.15*	1.02				**99.97**
-0.05% ml/mn	8.65	*8.5*	1.02				**100.02**
Normal condition	8.45	*8.25*	1.02				**100.00**
EC	*Rt*	*Rt (IS)*	*RRt*	*average RRt*	*sd RRt*	***%RSD***	**% difference**
+1 degree	9.85	*8.29*	1.19	1.18	1.16E-02	**0.98**	**100.23**
-1 degree	9.88	*8.35*	1.18				**99.81**
+5% MeOH	8.96	*7.58*	1.18				**99.71**
-5% MeOH	11.0	*9.49*	1.16				**97.78**
+0.05 ml/mn	9.72	*8.15*	1.19				**100.60**
-0.05% ml/mn	10.02	*8.5*	1.18				**99.44**
Normal condition	9.85	*8.25*	1.18				**100.00**

Appendix III: Exploratory Data Analysis: Complementary results

III.1 - Shapiro-Wilk tests conducted on the dataset of DPA of the stability study

III.1.1 - Standards samples

The Shapiro-Wilk tests were performed at a 95% Confidence interval (CI), with an alpha threshold of 0.05.

		p-values α= 0.05
DPA	Fresh	0.4455
	1 week	0.5003

III.1.2 – Shooting sessions specimens

The Shapiro-Wilk tests were performed at a 95% Confidence interval (CI), with an alpha threshold of 0.05.

			p-values α= 0.05
DPA	Fresh	D	0.365
		ND	0.124
	1 week	D	0.723
		ND	0.394
	2 weeks	D	0.605
		ND	0.353

III.2 – Shapiro-Wilk test conducted on the persistence and secondary transfer dataset

The values in red represents p-values above the threshold α=0.05 or the data for which the test could not be conducted.

PERSISTENCE

				p-values α= 0.05
40SW	T0	NnDPA	D	0.106
			ND	0.001
		DPA	D	0.641
			ND	0.020
		EC	D	0.266
			ND	0.792
	T0.5h	NnDPA	D	0.076
			ND	0.144
		DPA	D	0.087
			ND	0.033
		EC	D	0.115
			ND	0.553
	T1h	NnDPA	D	0.476
			ND	0.050
		DPA	D	0.817
			ND	0.471
		EC	D	0.550
			ND	0.817
	T2h	NnDPA	D	0.799
			ND	0.175
		DPA	D	0.646
			ND	0.718
		EC	D	0.761
			ND	0.009
	T4h	NnDPA	D	*no test conducted*
			ND	*no test conducted*
		DPA	D	0.218
			ND	*no test conducted*
		EC	D	0.990
			ND	*no test conducted*

				p-values α= 0.05
357MAG	T0	NnDPA	D	0.053
			ND	0.316
		DPA	D	0.396
			ND	0.202
		EC	D	0.001
			ND	0.088
	T0.5h	NnDPA	D	0.481
			ND	0.315
		DPA	D	0.577
			ND	0.011
		EC	D	0.232
			ND	0.119
	T1h	NnDPA	D	0.197
			ND	0.153
		DPA	D	0.043
			ND	0.266
		EC	D	0.097
			ND	0.034
	T2h	NnDPA	D	0.028
			ND	0.317
		DPA	D	0.448
			ND	0.884
		EC	D	0.508
			ND	0.223
	T4h	NnDPA	D	0.234
			ND	0.662
		DPA	D	0.798
			ND	*no test conducted*
		EC	D	0.990
			ND	0.901

SECONDARY TRANSFER

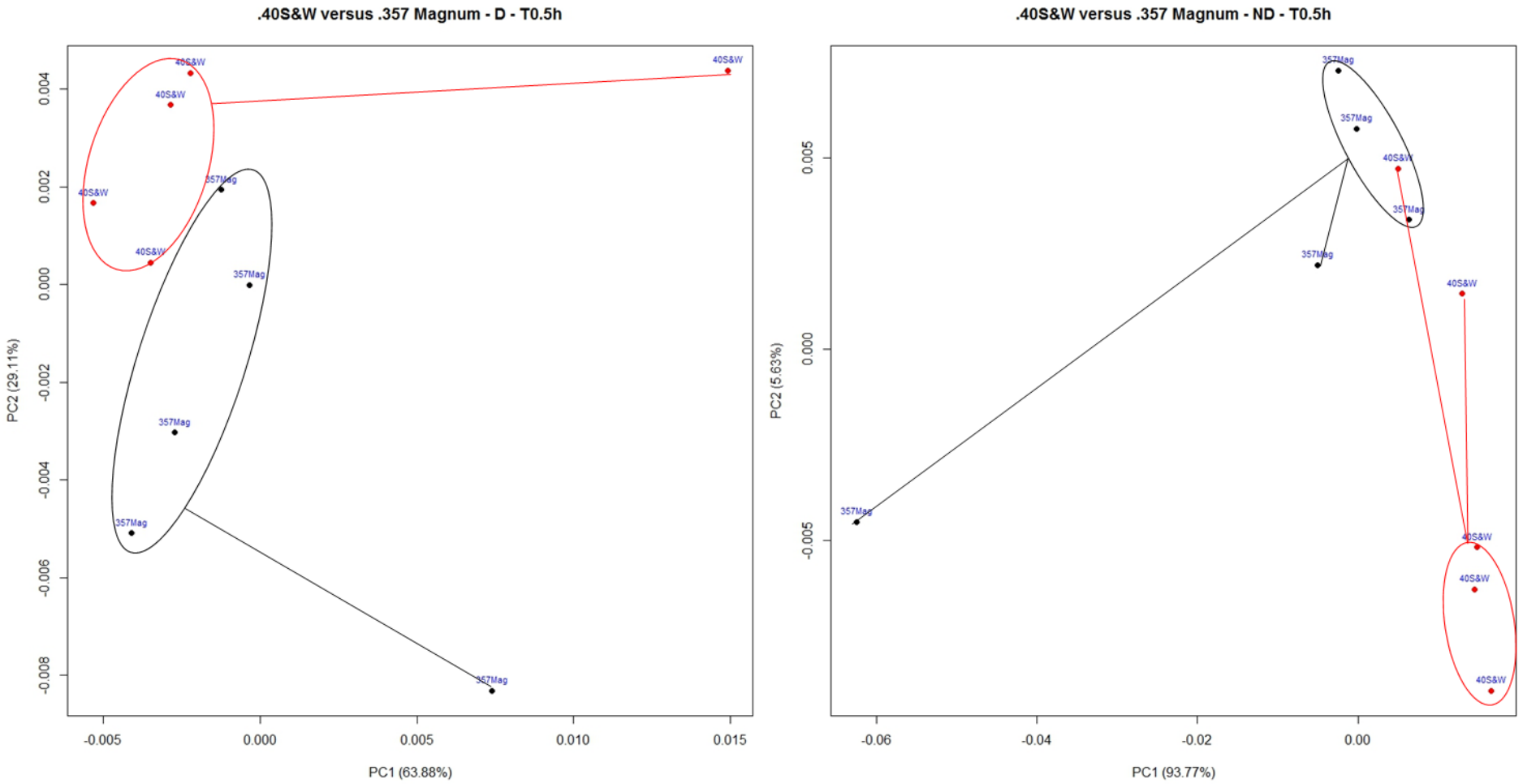
				p-values α= 0.05
40SW	NonShooter	NnDPA	D	0.619
			ND	0.333
		DPA	D	0.193
			ND	0.991
		EC	D	0.887
			ND	0.947
	Shooter	NnDPA	D	0.077
			ND	0.133
		DPA	D	0.625
			ND	0.902
		EC	D	0.205
			ND	0.011
	Firearm handling	NnDPA	D	0.371
			ND	0.886
		DPA	D	0.712
			ND	0.582
		EC	D	0.156
			ND	0.121

Summary tables for the Shapiro-Wilk tests

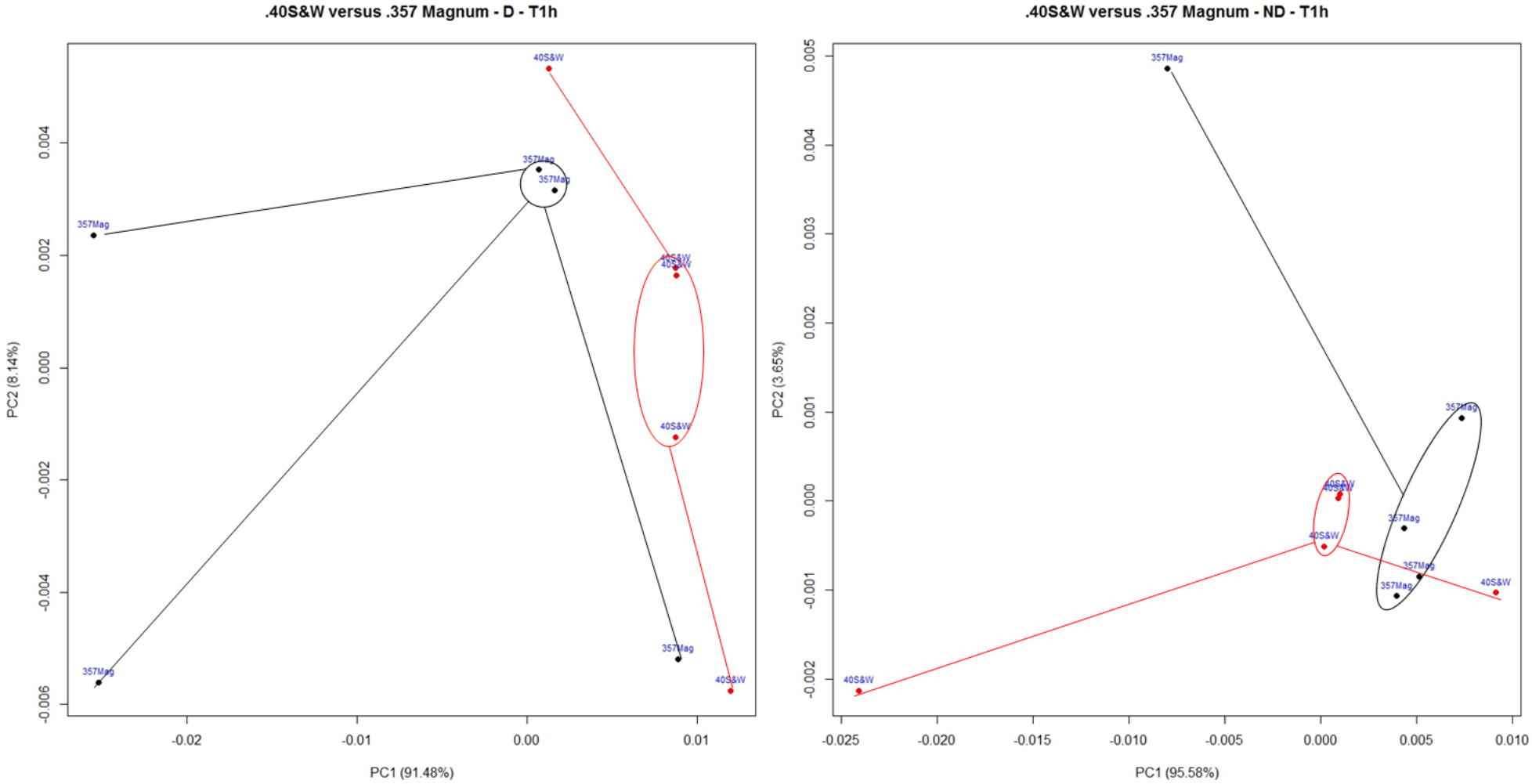
Persistence Dataset (n=60)		
p-values	above alpha 0.05	45
	below alpha 0.05	10
	no test conducted	*5*
	Total number of tests conducted	55
Percentage	above alpha 0.05	81.82%
	below alpha 0.05	18.18%
	Total	100.00%

Secondary transfer dataset (n=18)		
p-values	above alpha 0.05	17
	below alpha 0.05	1
	no test conducted	*0*
	Total number of test conducted	18
Percentage	ABOVE	94.44%
	BELOW	5.56%
	Total	100.00%

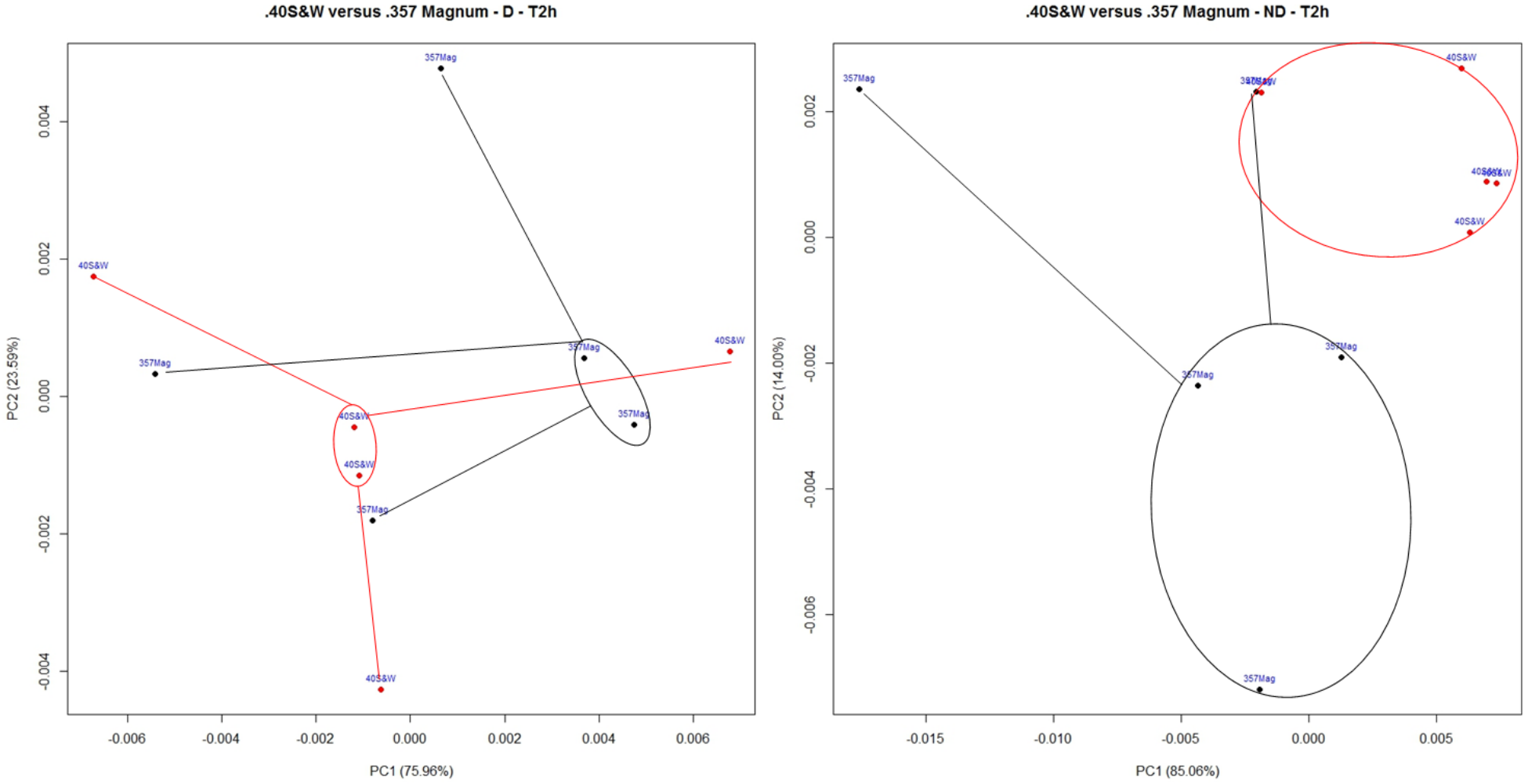
III.3 – PCA conducted between the 2 calibres investigated at each time points



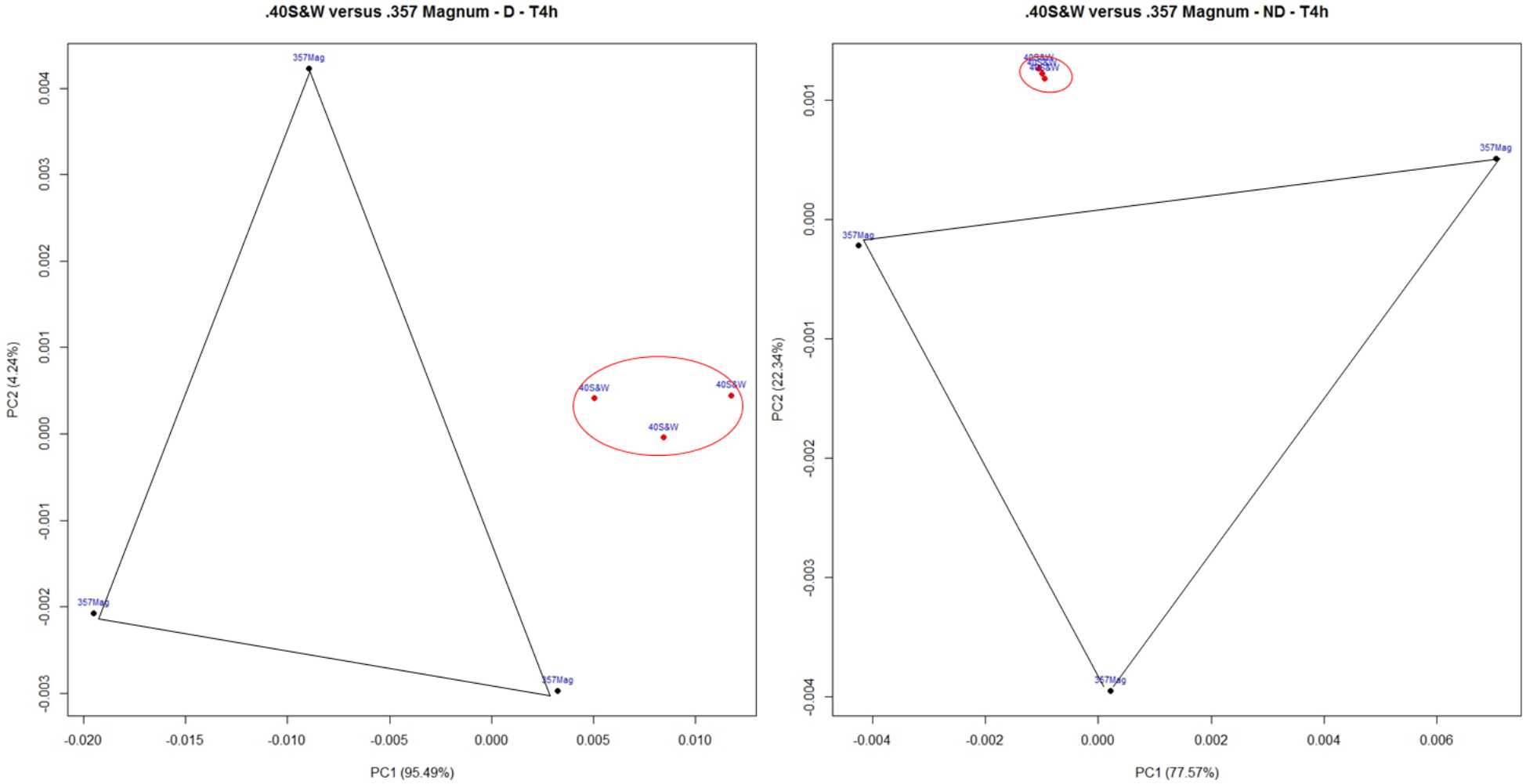
PCA plots for the comparison between the calibre .40 S&W and .357 Mag at T0.5h. D= dominant hand, ND= non-dominant hand. Clusters of both calibres can be observed. A large scatter of the data point is also visible.



PCA plots for the comparison between the calibres .40 S&W and .357 Mag at T1h. D= dominant hand, ND= non-dominant hand. Clusters of both calibres can be observed. A large scatter of the data point is also visible.

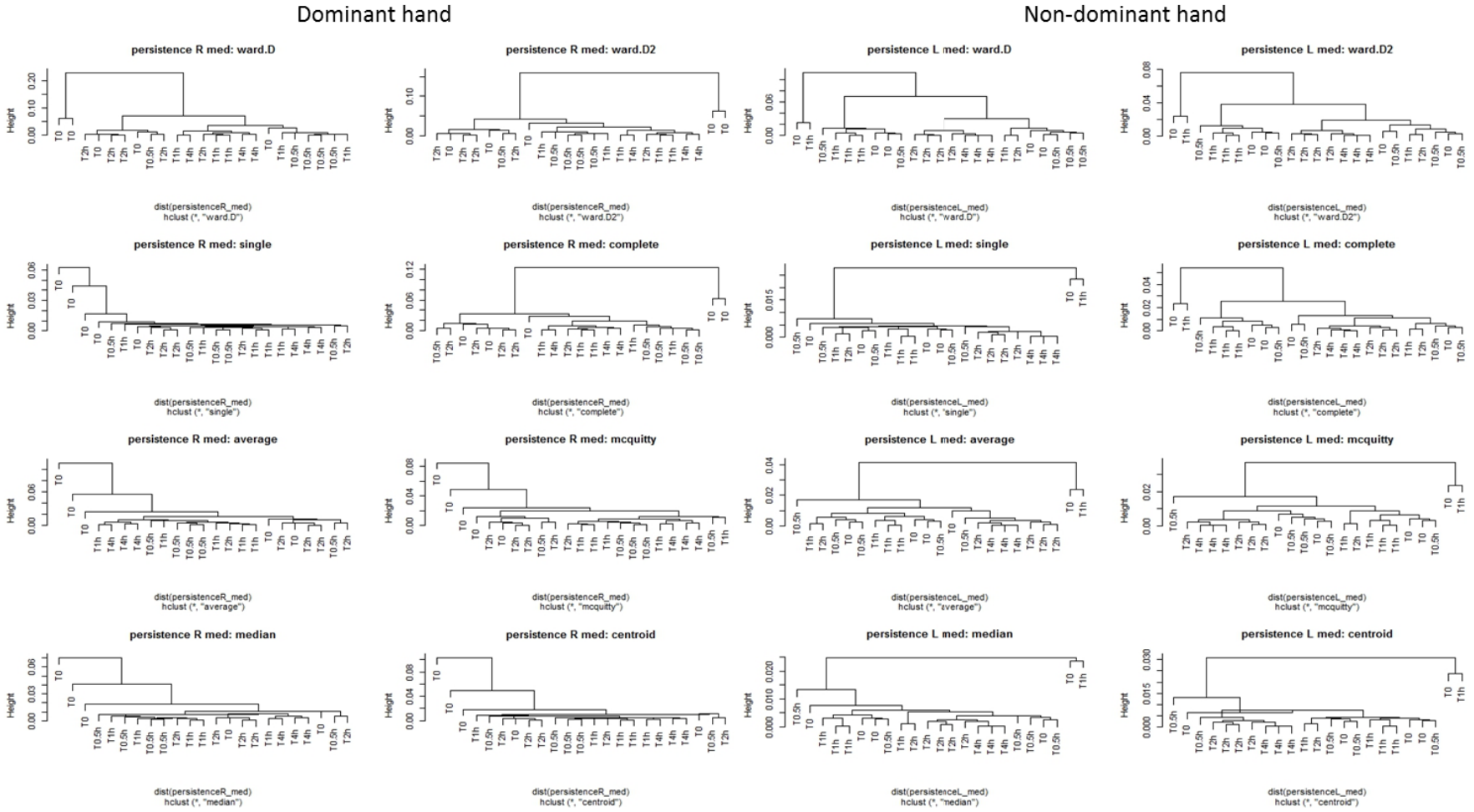


PCA plots for the comparison between the calibre .40 S&W and .357 Mag at T2h. D= dominant hand, ND= non-dominant hand. Clusters of both calibres can be observed. A large scatter and overlap of the data point is also visible.



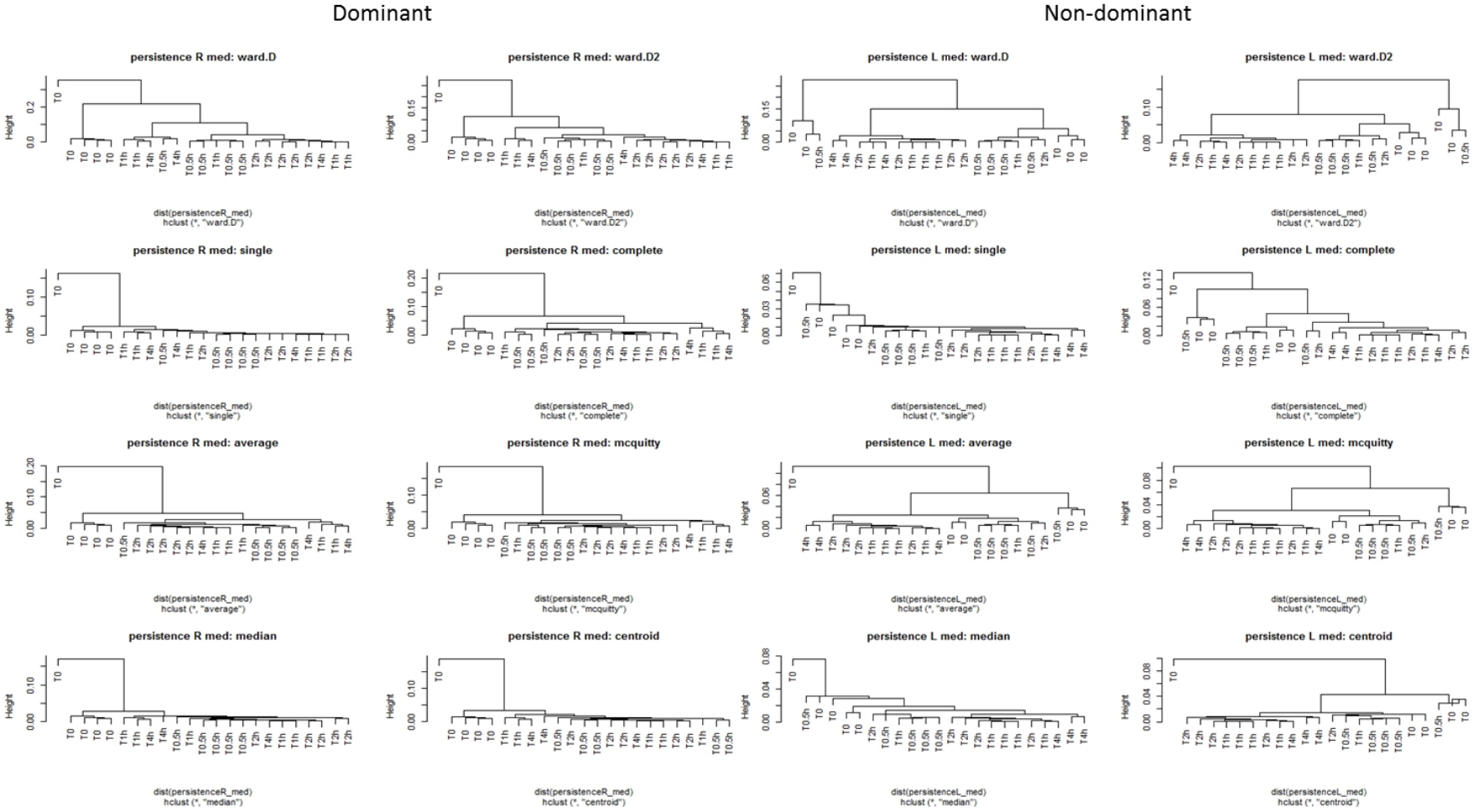
PCA plots for the comparison between the calibre .40 S&W and .357 Mag at T4h. D= dominant hand, ND= non-dominant hand. Clusters of both calibres can be observed. A large scatter of the data point is also visible.

III.4 - Hierarchical Cluster Analysis (HCA) performed for the calibre .40 S&W: 8 different measures tested.



R= Dominant hand, L= Non-dominant hand. The HCA were performed in R studio with the function hclust(). Each HCA cluster represents a different measure of correlation (n= 8): Ward.D, Ward.D2, Single, Complete, Average, McQuitty, Median and Centroid.

III.5 - Hierarchical Cluster Analysis (HCA) performed for the calibre ..357 Mag: 8 different measures tested.

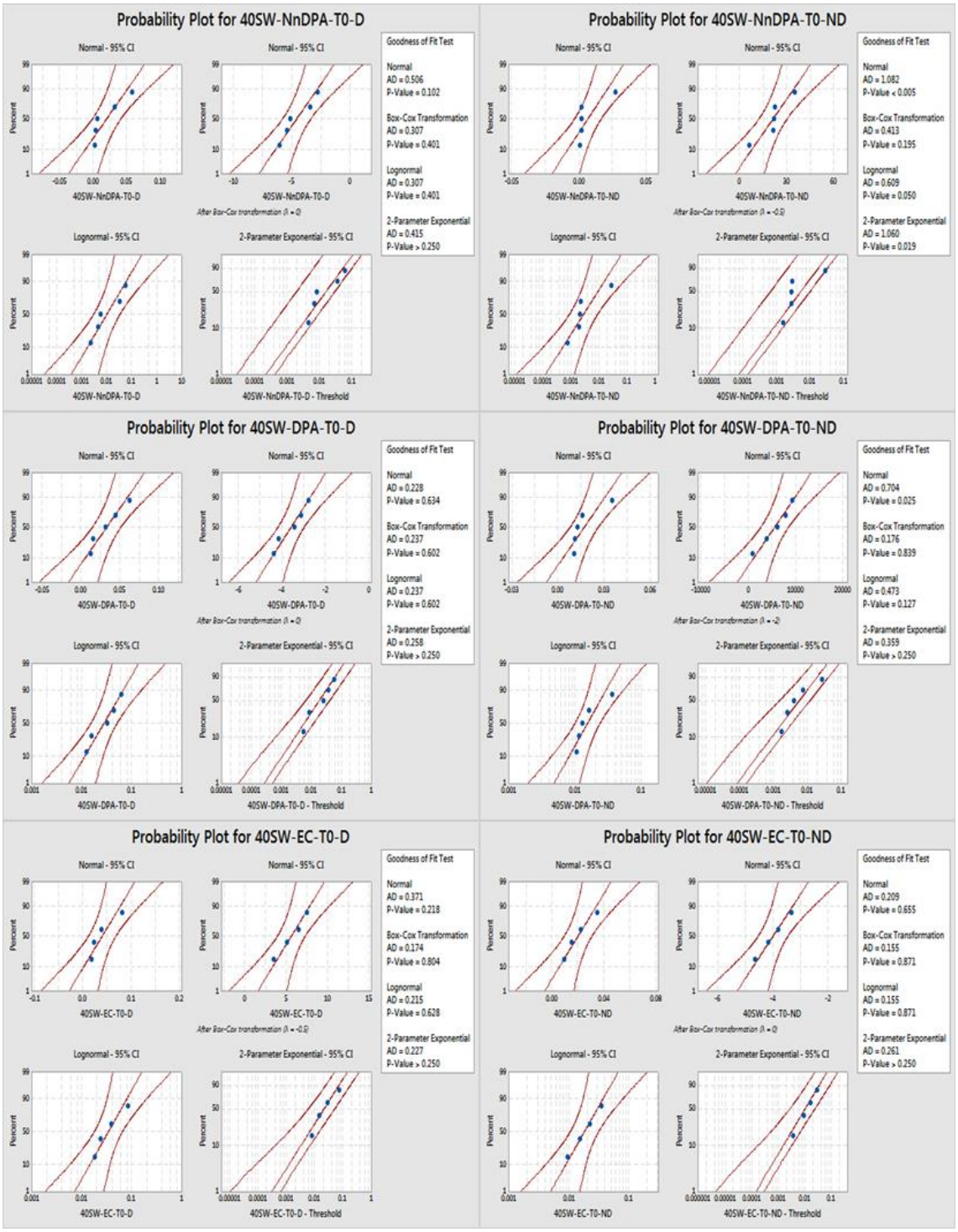


R= Dominant hand, L= Non-dominant hand. The HCA were performed in R studio with the function hclust(). Each HCA cluster represents a different measure of correlation (n= 8): Ward.D, Ward.D2, Single, Complete, Average, McQuitty, Median and Centroid.

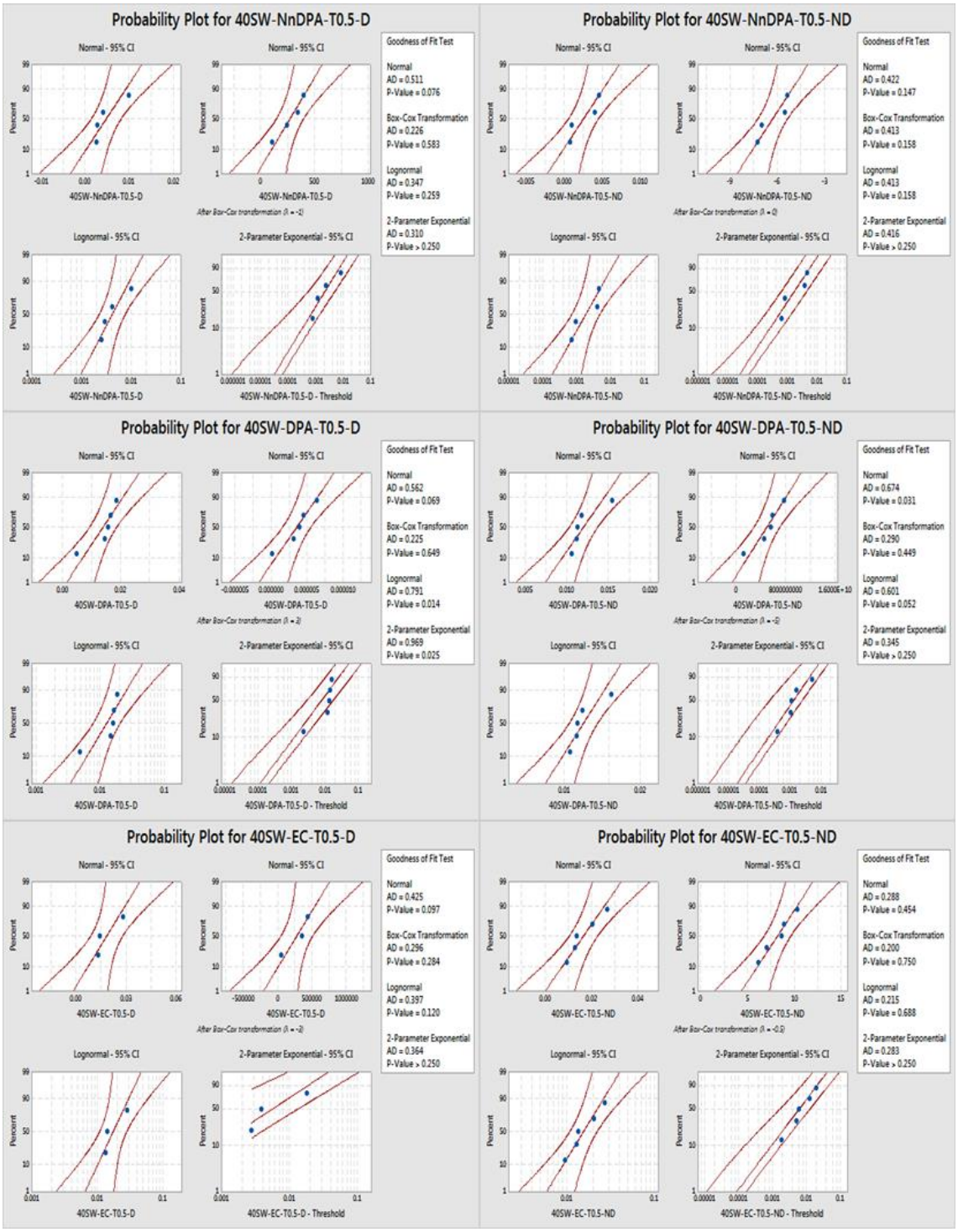
Appendix IV: Probability distribution: Goodness-of-fit

.40 S&W

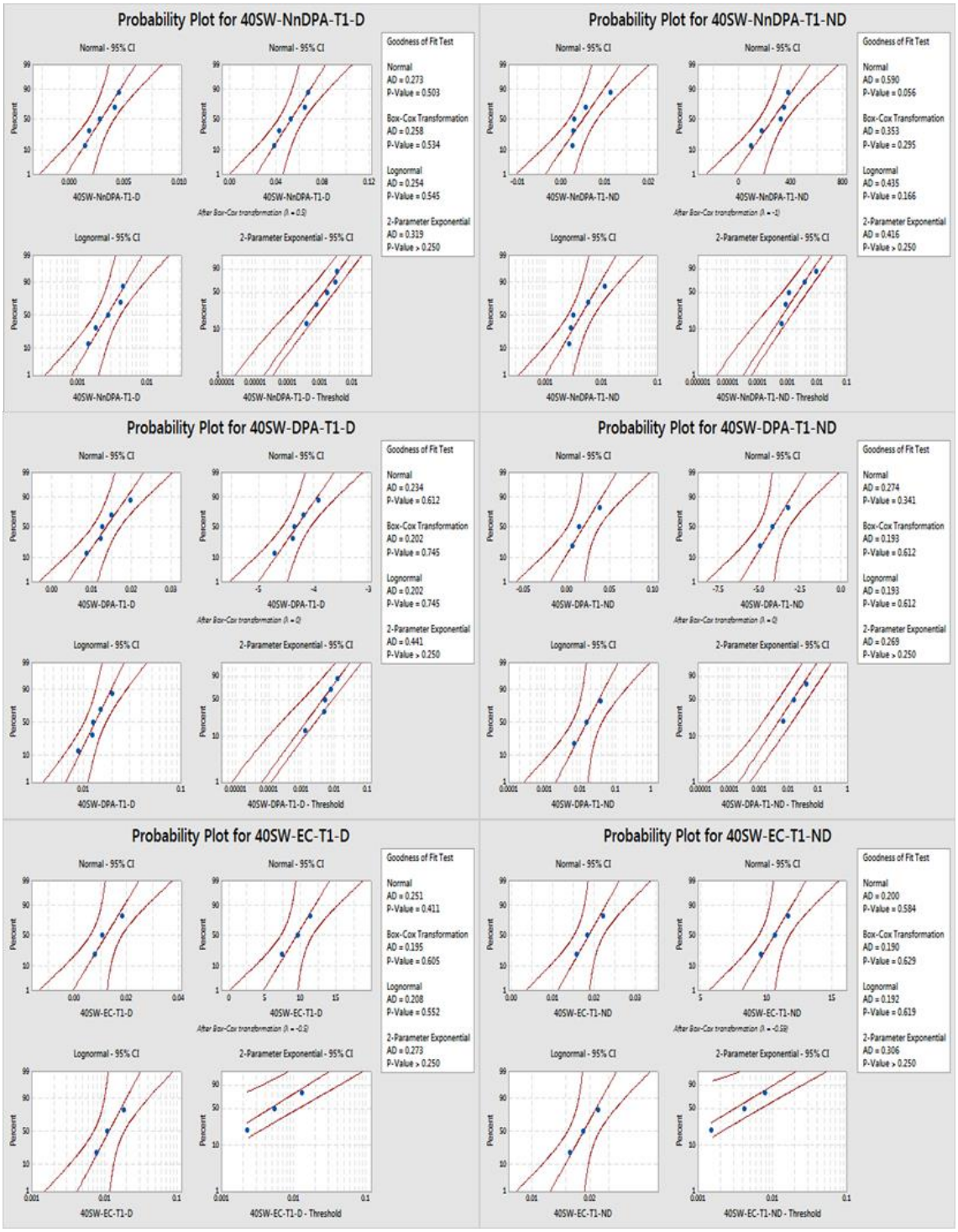
Each graph displays the four fittest distributions **at T0** for each compound, each hand for the calibre **.40 S&W**. The AD and P-value statistics can be found in the table on the right-hand side.



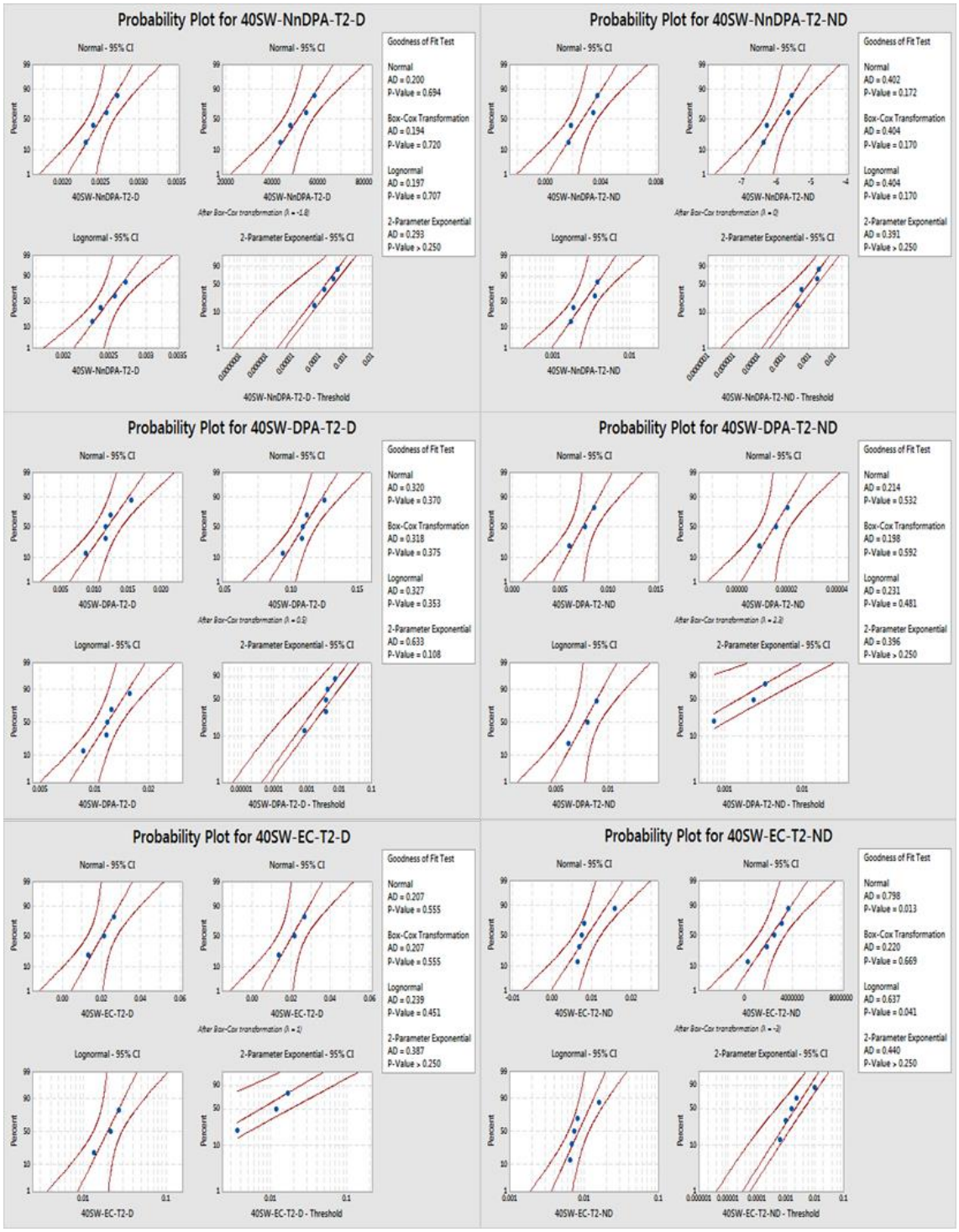
Each graph displays the four fittest distributions **at T0.5h** for each compound, each hand for the calibre **.40 S&W**. The AD and P-value statistics can be found in the table on the right-hand side.



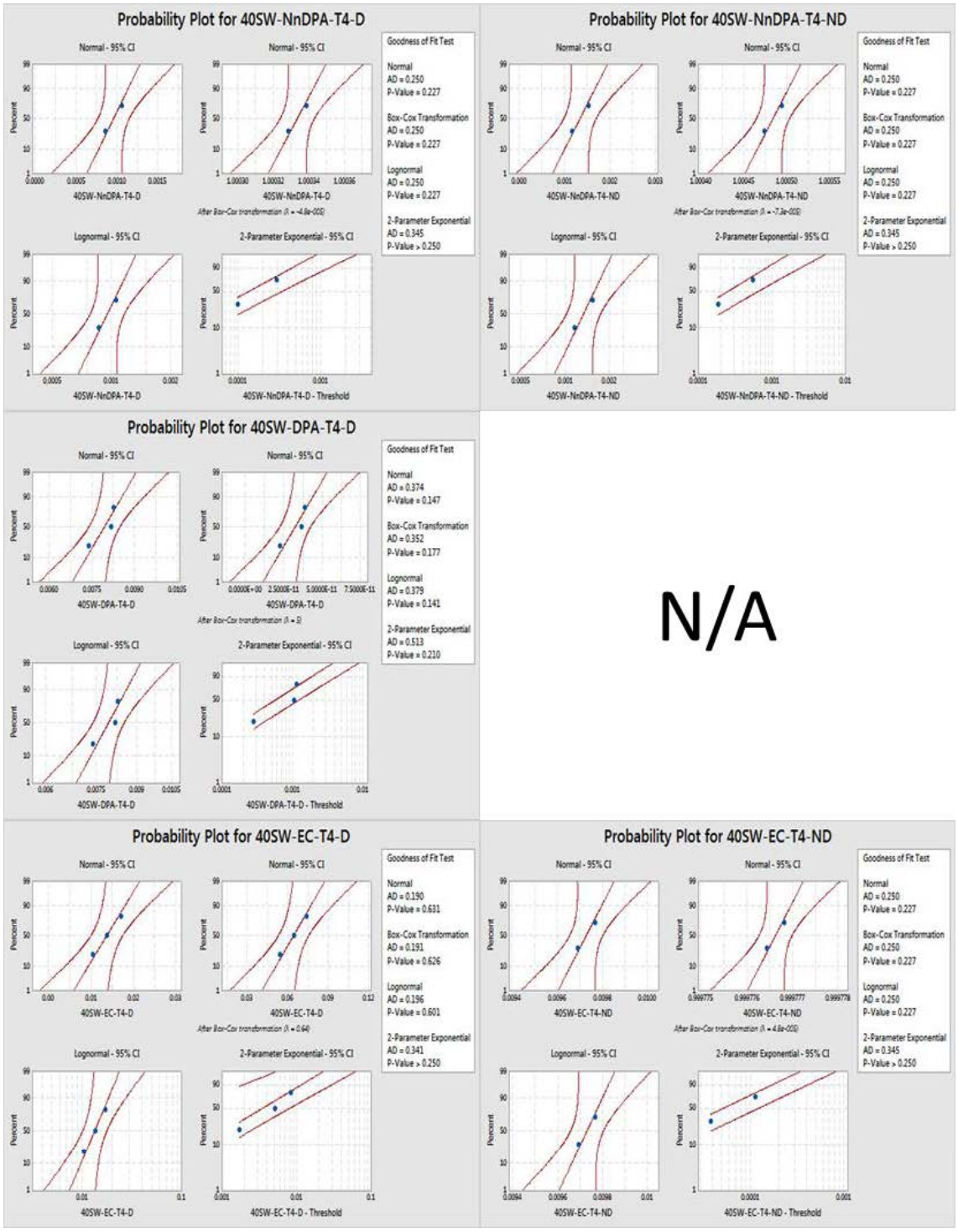
Each graph displays the four fittest distributions **at T1h** for each compound, each hand for the calibre **.40 S&W**. The AD and P-value statistics can be found in the table on the right-hand side.



Each graph displays the four fittest distributions **at T2h** for each compound, each hand for the calibre **.40 S&W**. The AD and P-value statistics can be found in the table on the right-hand side.

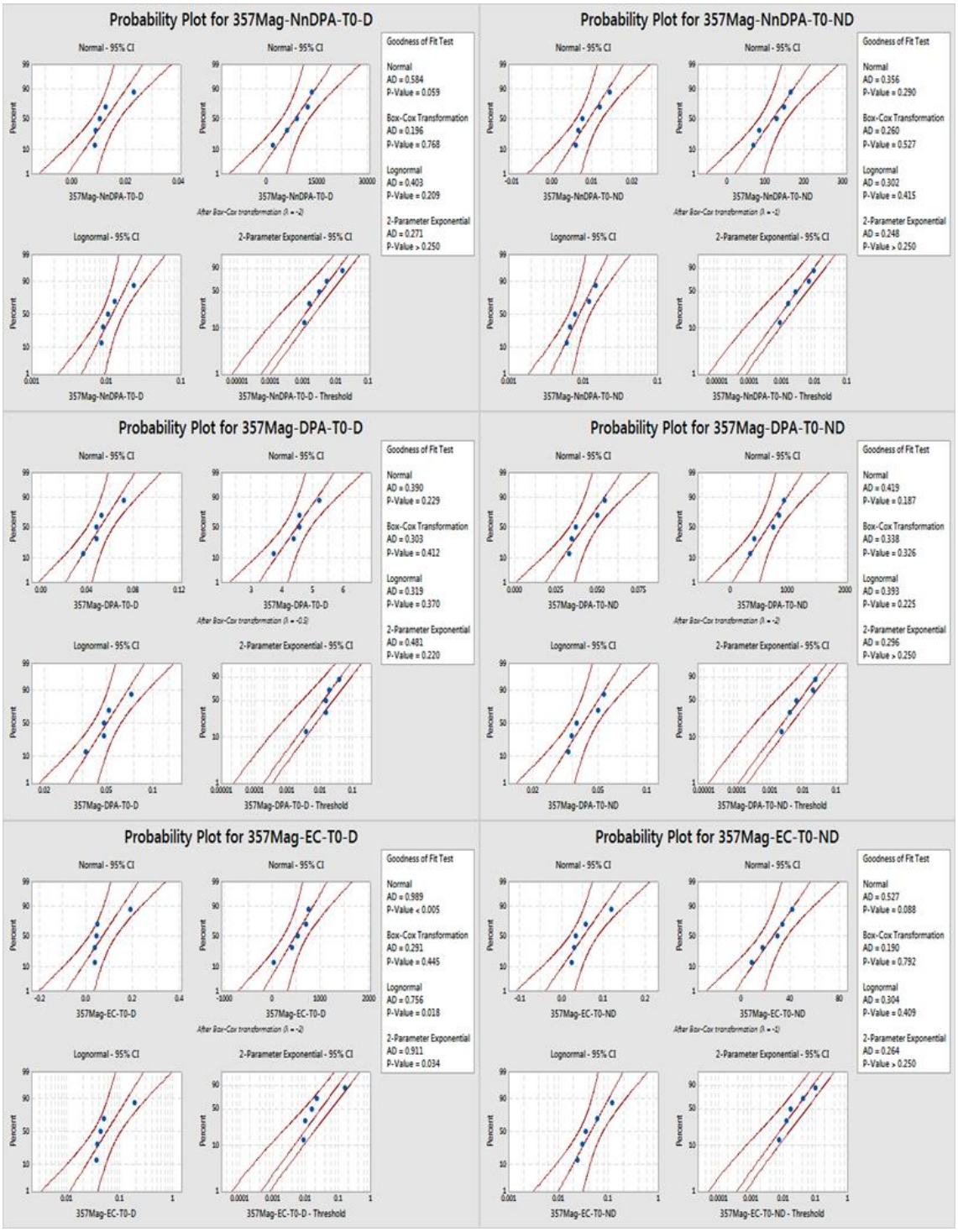


Each graph displays the four fittest distributions **at T4h** for each compound, each hand for the calibre **.40 S&W**. The AD and P-value statistics can be found in the table on the right-hand side.

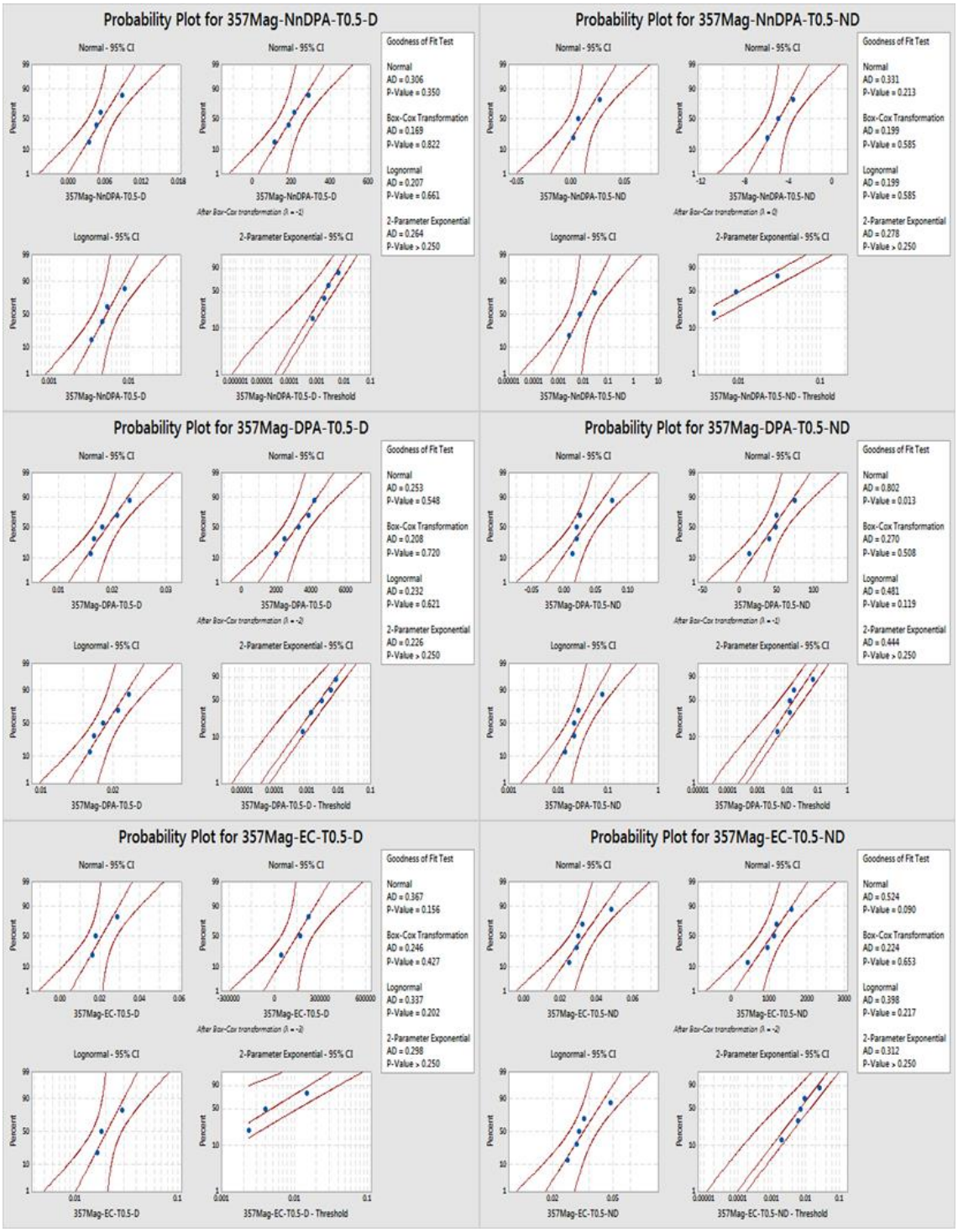


.357 Mag

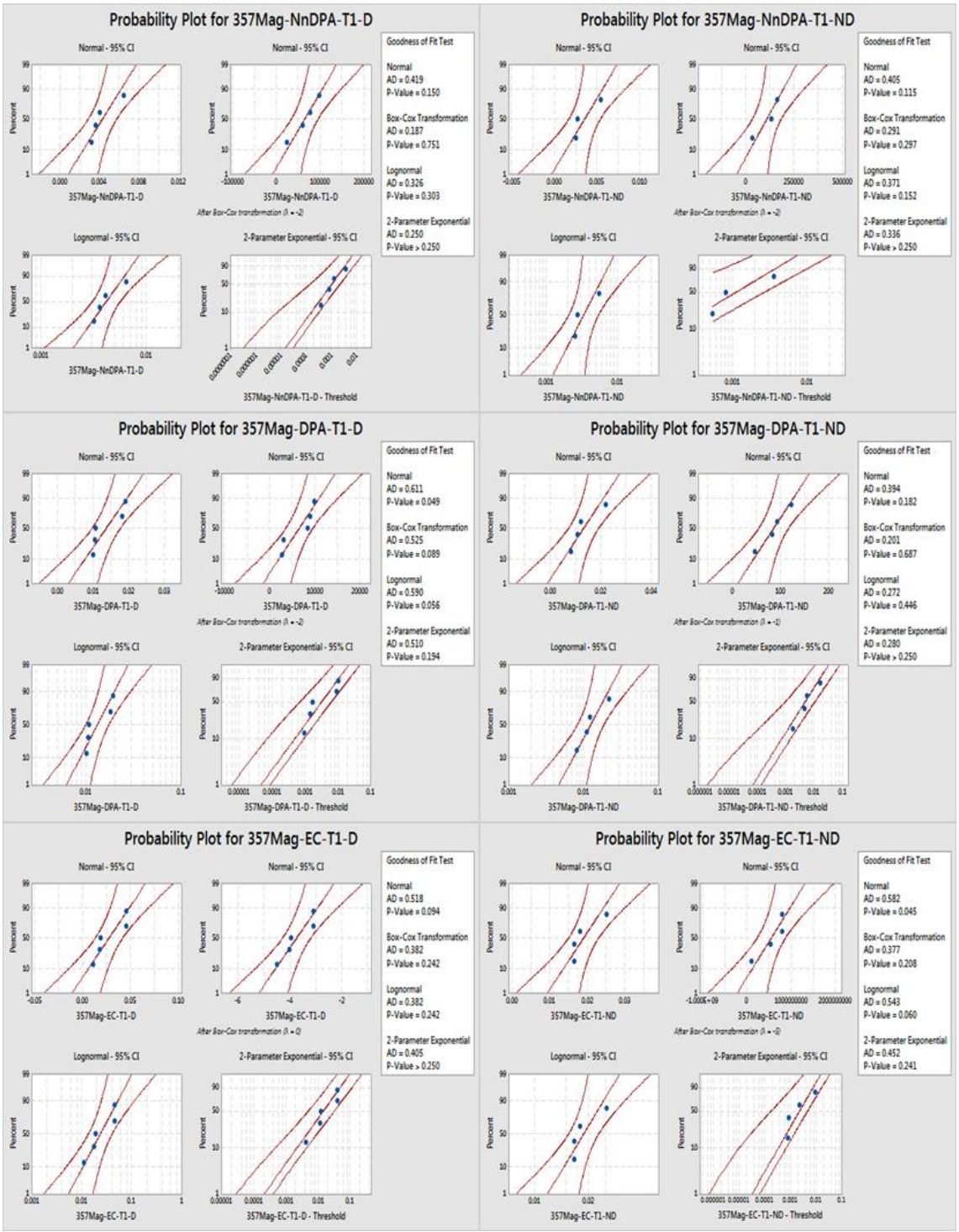
Each graph displays the four fittest distributions **at T0** for each compound, each hand for the calibre **.357 Mag**. The AD and P-value statistics can be found in the table on the right-hand side.



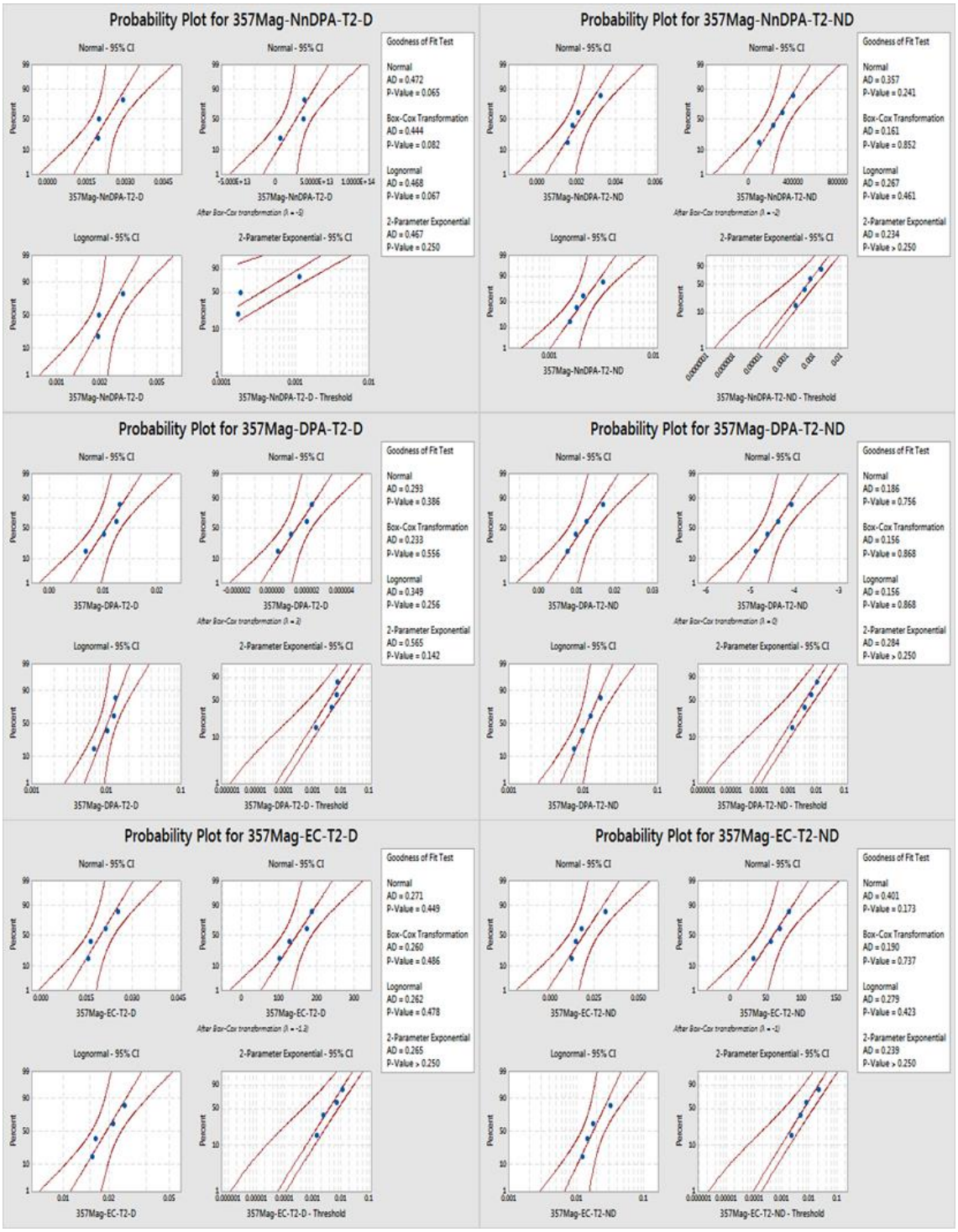
Each graph displays the four fittest distributions **at T0.5h** for each compound, each hand for the calibre **.357 Mag**. The AD and P-value statistics can be found in the table on the right-hand side.



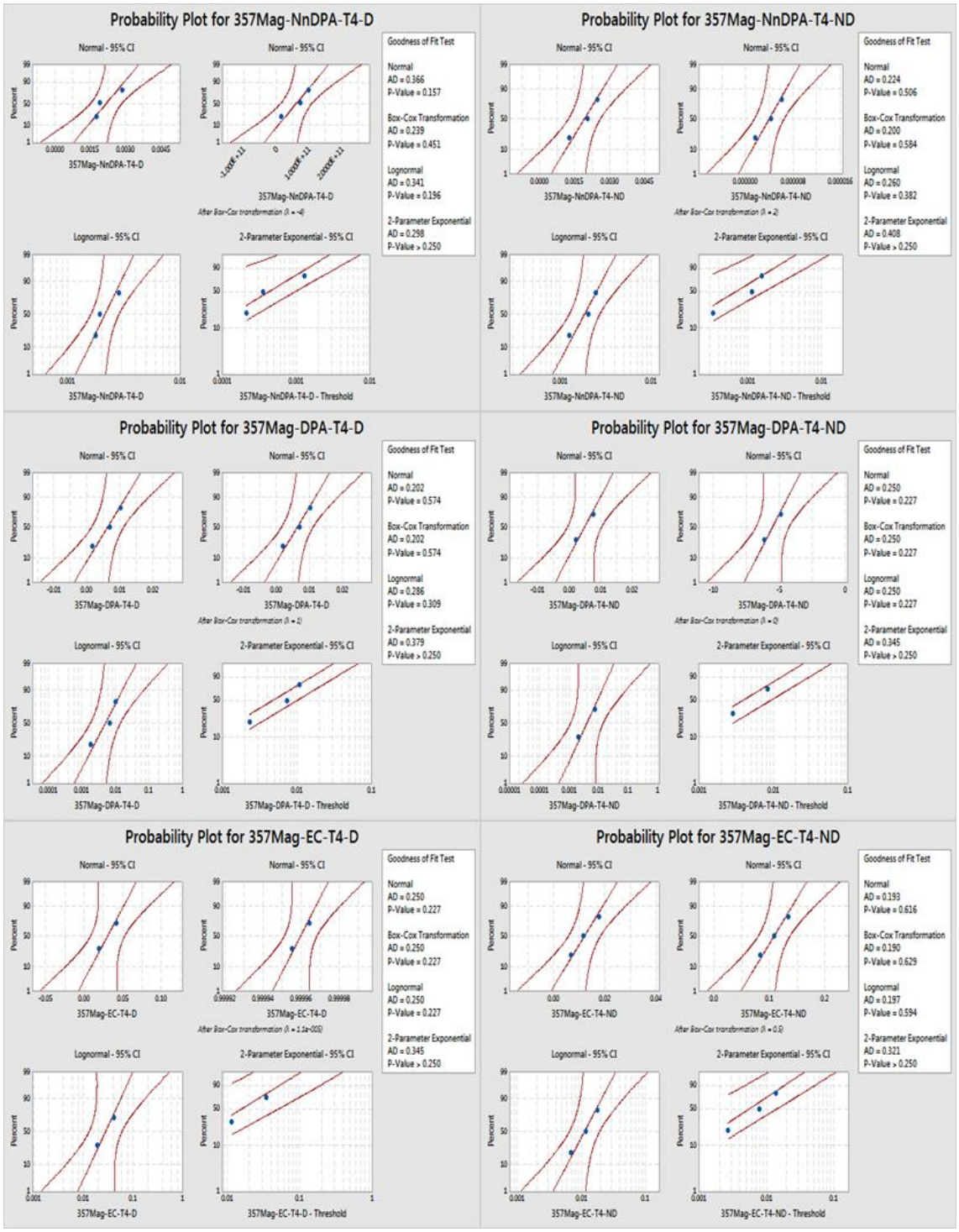
Each graph displays the four fittest distributions **at T1h** for each compound, each hand for the calibre **.357 Mag**. The AD and P-value statistics can be found in the table on the right-hand side.



Each graph displays the four fittest distributions **at T2h** for each compound, each hand for the calibre **.357 Mag**. The AD and P-value statistics can be found in the table on the right-hand side.



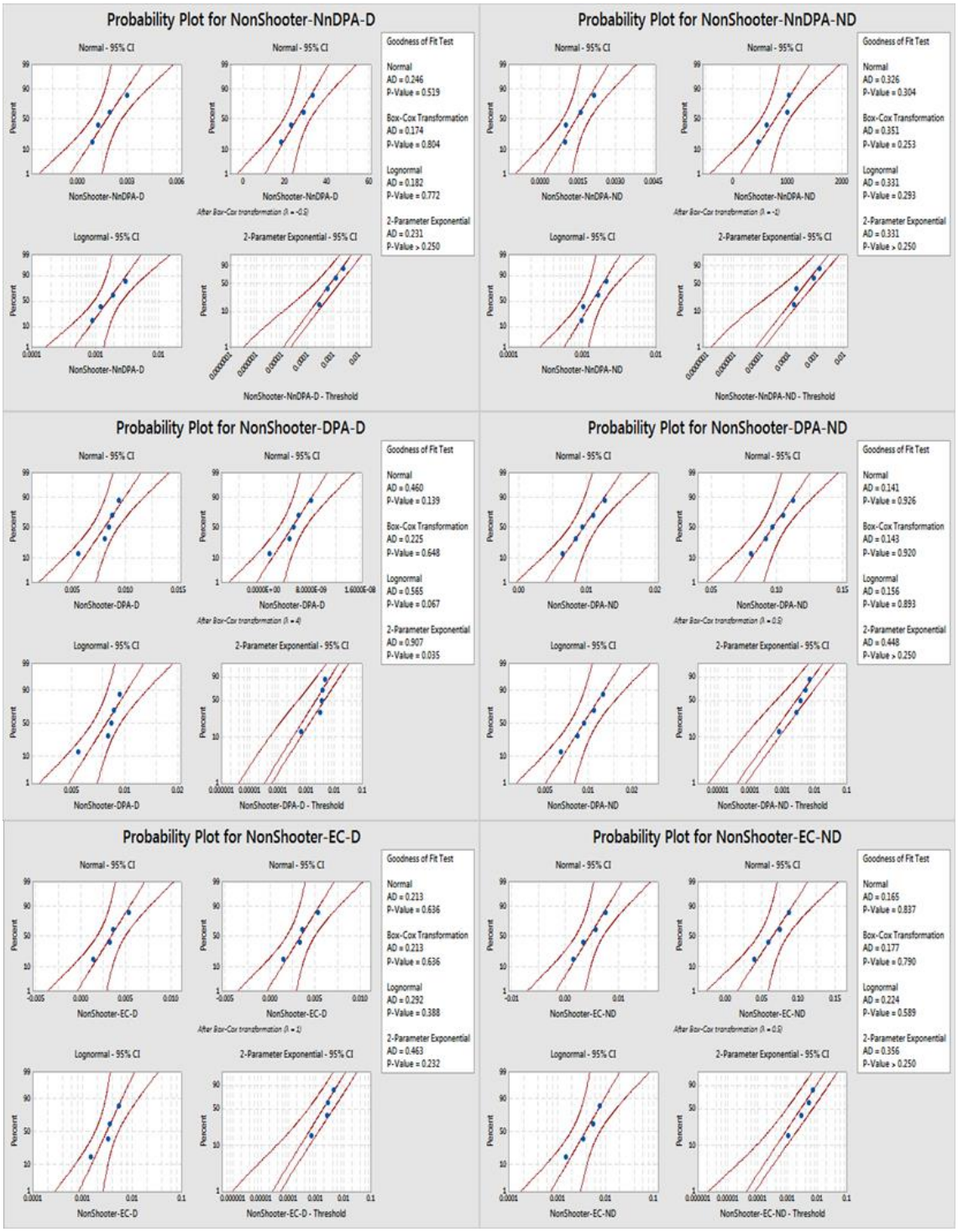
Each graph displays the four fittest distributions **at T4h** for each compound, each hand for the calibre **.357 Mag**. The AD and P-value statistics can be found in the table on the right-hand side.



Secondary transfer

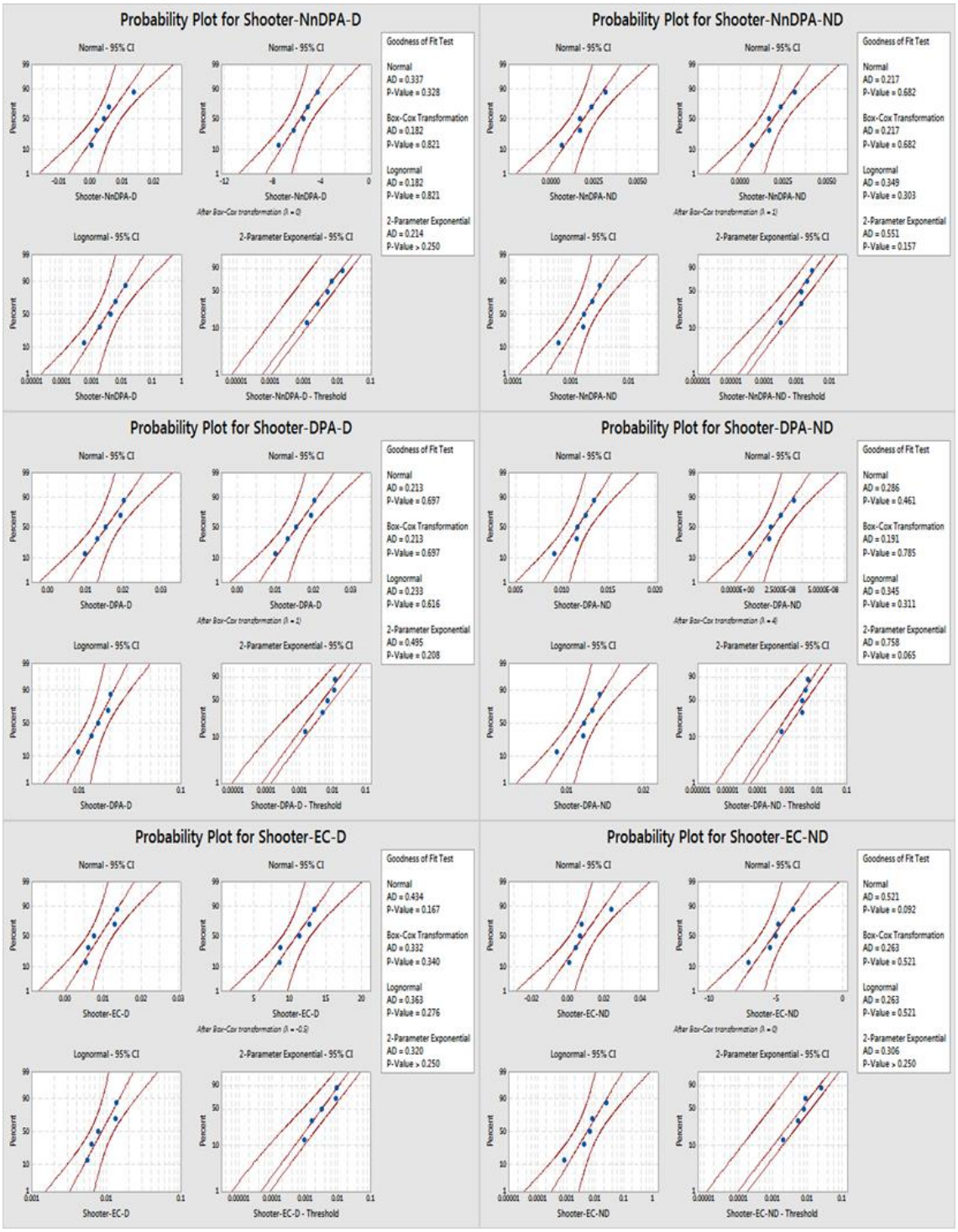
Each graph displays the four fittest distributions **for each participant** for each compound, each hand. The AD and P-value statistics can be found in the table on the right-hand side.

Non-shooter after the arrest (scenario 1)



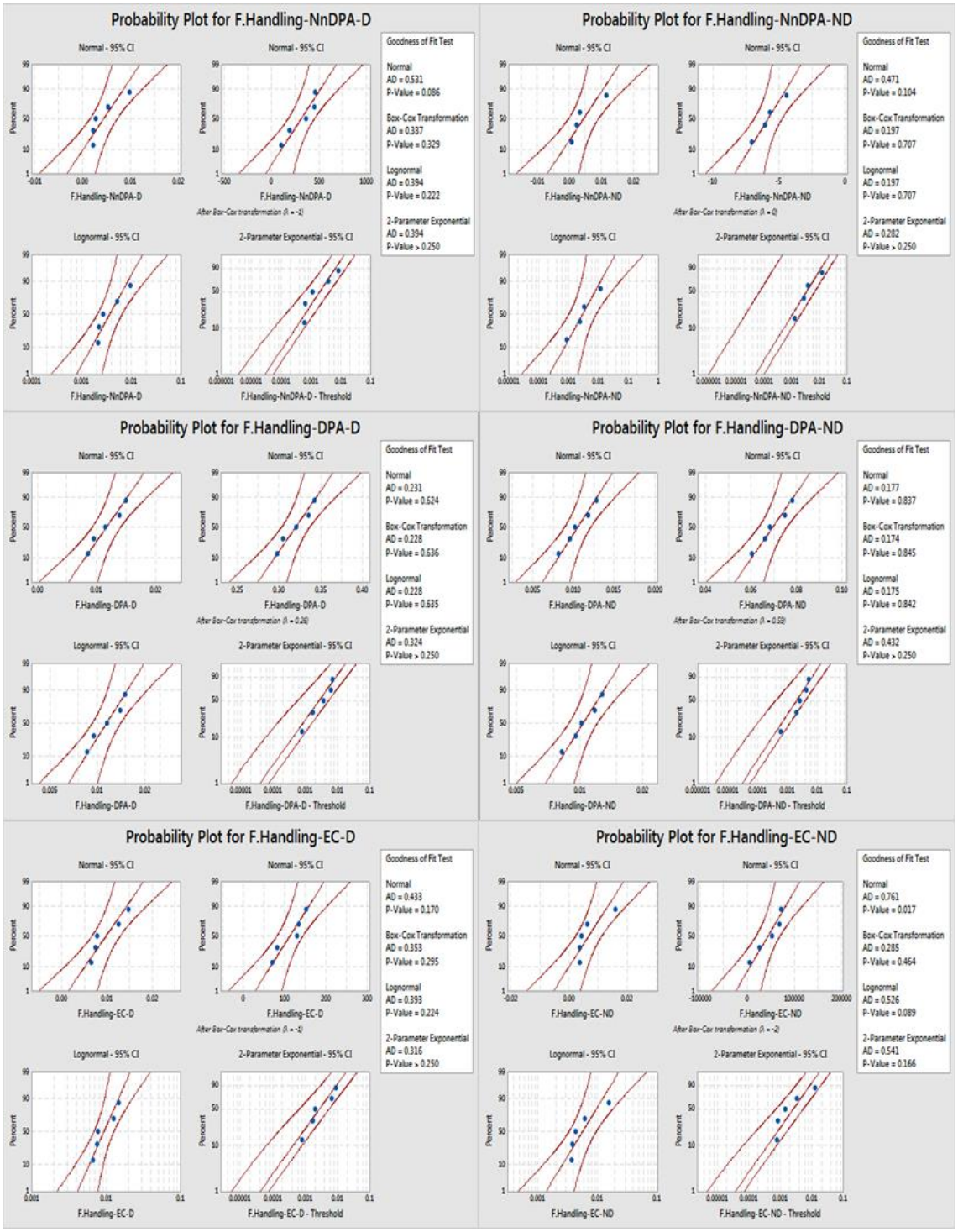
Each graph displays the four fittest distributions **for each participant** for each compound, each hand. The AD and P-value statistics can be found in the table on the right-hand side.

Shooter after the arrest (scenario 1)



Each graph displays the four fittest distributions **for each participant** for each compound, each hand. The AD and P-value statistics can be found in the table on the right-hand side.

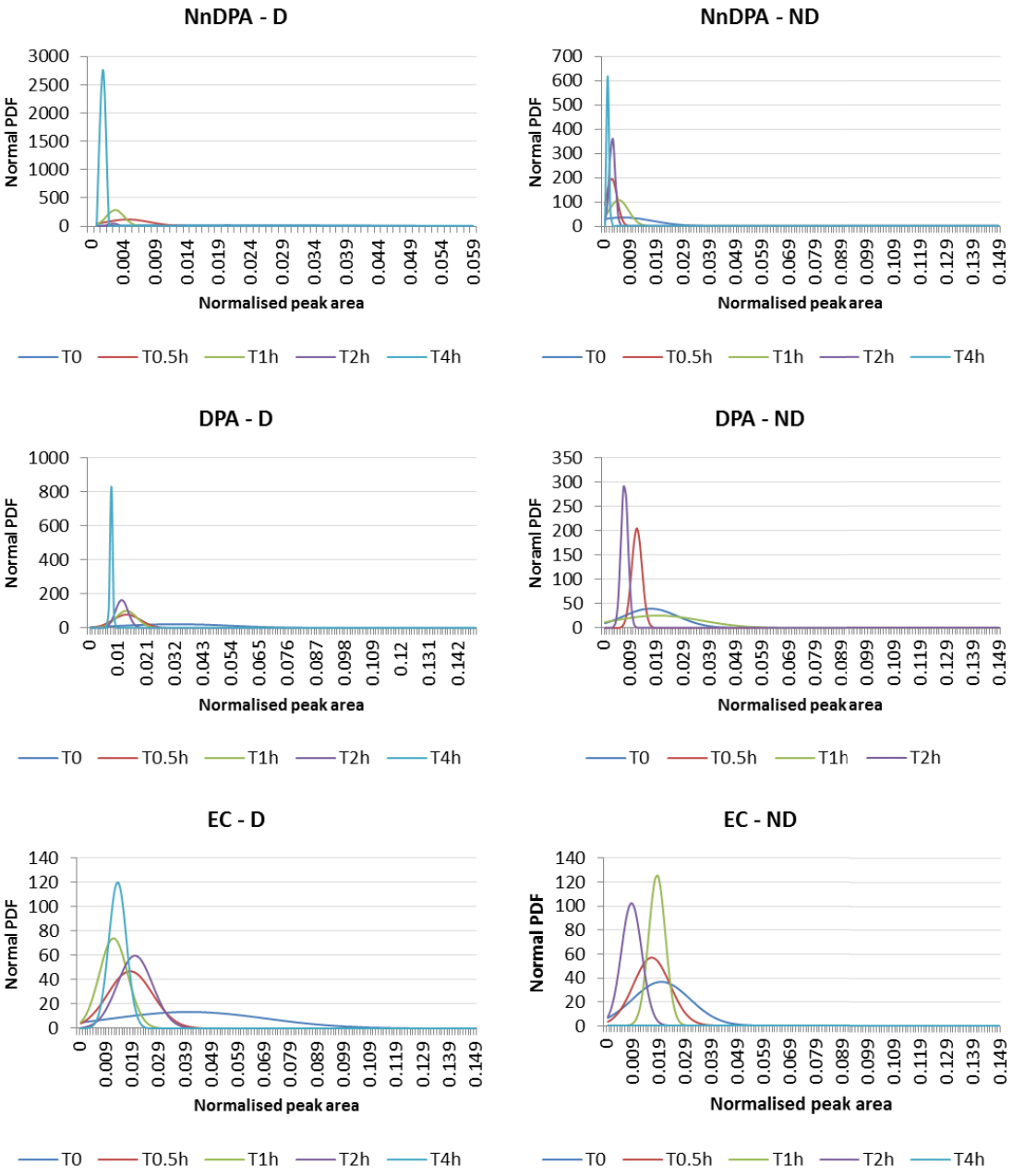
Non-shooter after the firearm handling (scenario 2)



Appendix V: Normal probability density functions

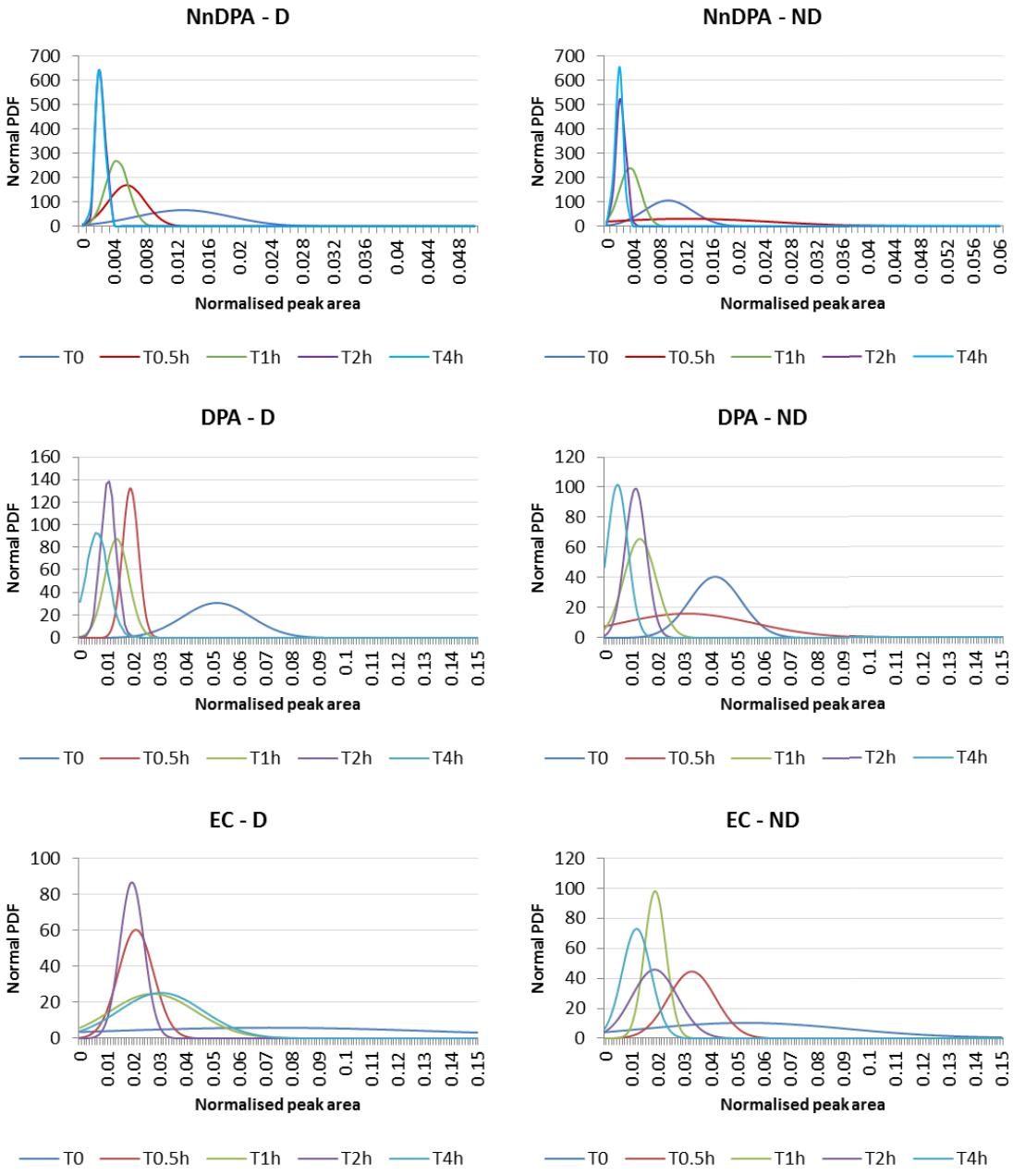
.40 S&SW

Each graph represents the Normal probability density function at each time point for each compound on each hand of the shooter.



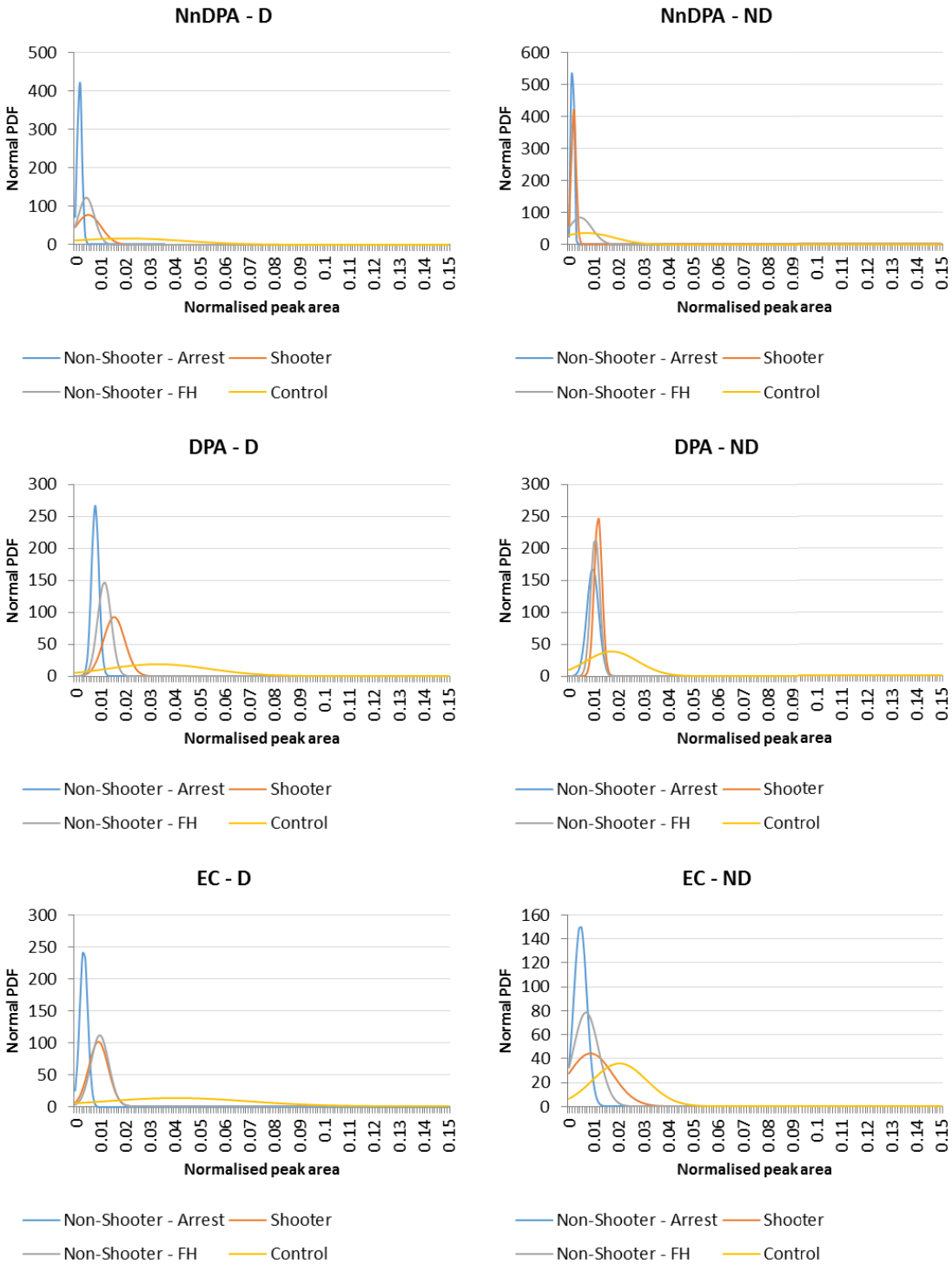
.357 Mag

Each graph represents the Normal probability density function at each time point for each compound on each hand of the shooter.



Secondary transfer

Each graph represents the Normal probability density function for each participant involved in the secondary transfer studies, for each compound on each hand.



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