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Research Paper

Organic and inorganic gunshot residues on the hands, forearms, face, and nostrils of shooters 30 min after a discharge

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ABSTRACT

During the investigation of firearm-related incidents, gunshot residues (GSR) can be collected on the scene and individuals (e.g., shooters or bystanders). Their analysis can give valuable information for the reconstruction of the events. Since GSR collection on persons of interest generally occurs a few minutes to hours after discharge, knowledge is needed to understand how organic (O), and inorganic (I) residues are transferred and persist. In this research, the quantities of OGSR and IGSR were assessed on the right and left hands, forearms, face, and nostrils of four shooters. Specimens were collected immediately before the discharge (shooter's blank specimens) and shortly after (30 min) using carbon adhesive stubs. Organic compounds were first extracted from the collection device and analysed using ultra-high-performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS). Subsequently, IGSR particles were detected on the same stub using scanning electron microscopy coupled with energy-dispersive X-ray spectrometry (SEM/EDS). Shooter's blank specimen analysis revealed background contamination of both O and IGSR in the shooter's environment, predominantly attributed to the presence of an indoor shooting range. However, the background quantities generally remained below the associated 30-minute specimen. Thirty minutes after a discharge, higher quantities were generally detected on the shooter's right and left hands than on other collection regions for both GSR types. Forearms and face emerged as interesting collection alternatives, especially in cases where a person of interest may have washed their hands in the interval between the discharge and collection. In contrast, very low amounts of GSR were detected in the nostrils. Furthermore, the results indicated that OGSR and IGSR have different transfer and persistence mechanisms.

1. Introduction

In events involving firearm use, gunshot residues (GSR) can be transferred to various persons or objects within the shooting environment (i.e., shooter, bystanders, targets, surrounding surfaces) [1–4]. Given that the collection of GSR from a person of interest typically occurs minutes to hours after a discharge, it becomes imperative to understand the transfer and persistence of these residues. Such knowledge is crucial for effective collection and adequate interpretation. However, limited research exists on the subject [5–8], partly due to the complexities associated with conducting such experiments (considering numerous factors to take into account and regulations associated with the use of firearms). The existing studies mainly focused on one single type of GSR, predominantly inorganic residues (IGSR), although some recent studies have explored the fate of organic residues (OGSR).

Studies investigating primary transfer reported that GSR were mainly transferred to the hands, particularly on the area of the thumb and index, generally located on the side adjacent to the ejection port of the firearm [9–12]. Several factors were identified as influencing the GSR transfer, including the types of firearms, the ammunition used, the environmental conditions, and the receptor's affinity for GSR [10,13–17]. Conversely, persistence studies of IGSR as well as OGSR on the shooter's hands showed that most of the target inorganic elements and organic compounds were lost within the initial hours after the discharge, following an exponential decrease pattern [18–23]. Persistence is notably affected by activities performed between discharge and collection, with actions such as running, walking, handwashing, and the application of hand sanitiser described as limiting GSR persistence on the hands. This led to the exploration of alternative collection regions such as the hair, face, forearms, nostrils, ears, shoes, or clothing [24–30].

A recent study examining the persistence of both GSR types on

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different skin regions of shooters found [31] that, in contradiction to previous studies [19,32], no significant loss of most OGSR was observed on the shooters' hands as the time between discharge and collection increase. The analysis of OGSR and IGSR residues was conducted separately in this study. While this discrepancy may be attributed to different experimental designs (e.g., firearm, ammunition, activity, and instrumentation), it underscores the need for further research into the transfer and persistence of both types of GSR to better understand the underlying mechanisms and influencing factors.

The purpose of the present research was to evaluate the presence and quantities of both types of GSR in specimens collected from different regions of a shooter shortly after a single discharge (30 min). This research introduces a novel approach by analysing both types of GSR in a single specimen and by collecting specimens not only from the hands but also from other regions of the shooter. This allows for determining the relevance of each region for GSR collection shortly after discharge rather than then their persistence over longer periods. Although the collected data may not be directly applicable to casework (as each case is specific), they will provide valuable insights into the persistence of GSR shortly after discharge and the dependency between OGSR and IGSR.

Each stub was initially extracted and analysed to detect OGSR compounds using ultra-high-performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS), and then for IGSR particles using scanning electron microscopy coupled with energy-dispersive X-ray spectrometry (SEM/EDS). The obtained results were used to compare the quantities of OGSR and IGSR detected between different regions, to evaluate the relevance of these regions, and to determine if complementary information can be collected through the analysis of both GSR types.

2. Material and methods

2.1. Shooting session

The tests were performed with a semi-automatic 9 mm Parabellum SIG Sauer P226, dismantled, cleaned, and lubricated with WD-40® before the experiments. The ammunition used was Geco Sinoxid® (Full Metal Jacket (FMJ) and batch no. 41 NM 069). To reduce memory effects (i.e., residues from previous ammunition in the weapon), 10 consecutive shots were discharged before the tests [33]. The external parts of the firearm and magazine were cleaned with methanol before each discharge.

Four shooters discharged the firearm and continued their activity for 30 min until GSR collection. The experiment was repeated 4 times on different days, resulting in a total of 16 shots. The shooters worked in the same building and had no access to the indoor shooting range and firearm laboratory. Additional precautions were taken to minimise the risks of contamination (mainly cross-contamination between experiments). The shooters' offices (e.g., keyboard, mouse, computer, desk, chair, pens, and pencils), as well as all objects used or worn by the shooters (e.g., mobile phone, watch, glasses, water bottle), were cleaned with pre-saturated isopropyl alcohol wipes from Electrolube (France) before and after each shot. The shooters washed their hands, forearms, and face with water and soap before the discharge. During the shot, the firearm was held with both hands, and the trigger was pulled with the right index finger (even for the one left-handed shooter, S3 in Table 1). The ventilation of the shooting range was turned off during the discharge to simulate indoor shooting conditions. Each shooter performed a single shot, and all experiments (i.e., shots) were scheduled on different days. After the discharge, the shooters removed the magazine and left without touching any surface within the shooting range. GSR were collected 30 min after the discharge. After each experiment, the shooters were instructed to take a shower and wash their clothes to avoid the risk of contamination between experiments. A minimum period of 72 h was also respected between each experiment.

2.2. Specimens' collection

Five regions were targeted for collection on the shooters: the right and left hands, the forearms (right and left together),[1] the face,[2] and the entrance of the nostrils (see Table 1). Aluminium stubs mounted with two double-sided carbon adhesives inserted in a plastic holder with a cap were used for collection (from Plano, Germany). Two adhesives (No. G3347, from Plano, Germany) were carefully placed on top of each (with cleaned gloves and tweezers) others to ensure that they would not lift after OGSR extraction due to solvent evaporation. The collection device was adapted for the nostrils with one double-sided carbon adhesive mounted on a plastic rod (see Fig. 1).[3] After collection, the "nostril" adhesive was deposited on an aluminium stub mounted with one double-sided carbon adhesive.

Blanks were taken on each of the targeted regions before the experiments (immediately after the shooters had washed their hands, forearms, and face). During the period between the shot and the collection (i.e., 30 min), the shooters were informed to neither wash their hands nor wear laboratory gloves. They were asked to write down a list of their activities (mainly office work such as typing or meeting, as well as instrumental work in the laboratory not requiring gloves). Between 150 and 200 dabbings were applied for collection on each hand, forearms, and face. Fewer dabbings were performed at the entrance of the nostrils (15 in each for a total of 30) due to the smaller collecting surface and the unpleasantness of the dabbings for the volunteers. All collected specimens (80 discharge specimens and 80 shooters' blank specimens) were stored in a fridge at 3 °C until OGSR extraction.

2.3. Combined analysis of IGSR and OGSR

After collection, IGSR and OGSR were analysed in sequence from the same collection device [34–38]. OGSR were extracted first and analysed using UHPLC-MS/MS. Then, IGSR were analysed using SEM/EDS.

Before and during the experiments, the laboratory environment was tested to ensure that there was neither IGSR nor OGSR contamination. Three blank stubs were placed for 72 h in three distinct locations of the laboratory and then analysed (two on the laboratory bench and one under the chemical fume hood). If the environment was contaminated, the entire laboratory was cleaned and re-tested. Only the fume hood needed to be cleaned between some experiments, mainly after the preparation of the calibration solutions.

2.3.1. OGSR extraction and analysis

OGSR were extracted one specimen at a time to avoid cross-contamination. Organic compounds were recovered by adding 100 µL of acetonitrile (grade ULC-MS from Biosolve, France) to the double-sided carbon for approximately 30 s [38,39]. Slight twisting

Table 1
Four shooters discharged a semi-automatic 9 mm Parabellum SIG Sauer P226 four times, loaded with Geco Sinoxid® ammunition.

Shooter	Regions collected	Shooter's blank	Discharge experiment	Total specimens
S1 S2 S3 S4	1) Right hand Left hand Forearms Face Nostrils	4 shooters x 5 regions x 4 experiments = **80 stubs**	4 shooters x 5 regions x 4 discharges = **80 stubs**	**160 stubs**

[1] The shooters wore short-sleeved t-shirts allowing GSR collection on their forearms' skin.

[2] The entire facial surface was collected (including the eyebrows, moustache and small beard).

[3] The collection device penetration at the entrance of the nostrils was less than 5 mm.

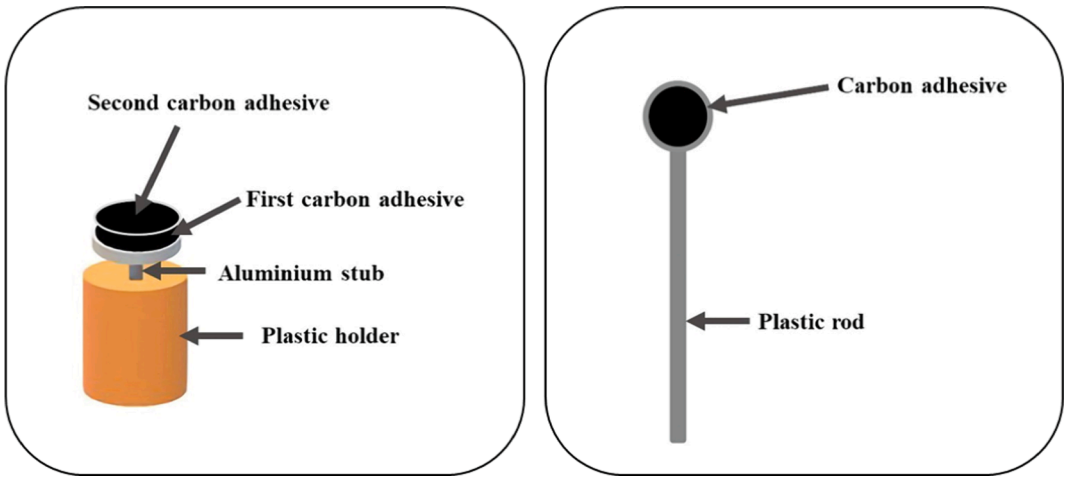

Fig. 1. Schematic representation of the collection devices: (left) Aluminium stubs mounted with two double-sided carbon adhesives inserted in a plastic holder for collection on the hands, forearms, and face; (right) One double-sided carbon adhesive mounted on a plastic rod for the collection from the nostrils. The "nostrils" adhesive was also placed on an aluminium stub after collection. The first carbon adhesive on the aluminium stub was necessary to prevent the second one from lifting after solvent was applied for OGSR extraction.

movements of the stub allowed an even distribution of the solvent on the adhesive, and the plastic holding the stub was closed during the 30 s to limit evaporation. Blank stubs were placed under the fume hood for every OGSR extraction series to control the level of contamination. The extract was then carefully recovered with a pipette so as not to touch the surface of the adhesive and transferred in a 250 µL insert placed in a 2 mL vial. The vials were stored in the freezer at −24 °C until OGSR analysis. The stubs were dried for 3 to 5 min under the fume hood, re-capped, and stored on a rack in the fridge at 3 °C until IGSR analysis.

OGSR were analysed using a UHPLC-MS/MS instrument from AB Sciex. In total, eight organic compounds identified in the ammunitions used in these experiments (SI – Figs. 1 and 2) were targeted (and exhibiting a low prevalence in the environment [24,40,41]): nitroglycerin (NG), methylcentralite (MC), ethylcentralite (EC), akardite II (AK-II), 4-nitrodiphenylamine (4-nDPA), *N*-nitrosodiphenylamine (*N*-nDPA), 2-nitrodiphenylamine (2-nDPA), and diphenylamine (DPA) (Table 3) [31,35,39]. Standards containing 100 µg/mL of the target compounds either in methanol or acetonitrile were procured from NEOCHEMA (Germany). Calibration solutions were prepared at the following concentrations (10 levels in duplicate):

- 1 to 40000 ng/mL for NG
- 0.5 to 100 ng/mL for AK-II, *N*-nDPA, DPA, and EC;
- 0.1 to 5 ng/mL for MC, 4-nDPA, and 2-nDPA.

Chromatographic separation was carried out using an ExionLC™ AD system equipped with a Kinetex Core-Shell C18 LC column (2.6 µm x 2.1 mm x 100 mm) from Phenomenex. The temperature of the LC oven was maintained at 40 °C during the analysis. Table 2 provides a summary of the parameters for the two ionisation modes. The solvents and buffer used (i.e., acetonitrile, methanol, water, and formic acid) were ULC-MS grades from Biosolve (France).

The QTRAP 6500 + mass spectrometer was configured for operation in multiple reaction monitoring (MRM). The MS/MS parameters of each organic compound were summarised in Table 3. In the positive mode, MC, AK-II, EC, *N*-nDPA, DPA, 2-nDPA, and 4-nDPA were ionized using an electrospray Turbo V Ionization Source probe (ESI). This process involved a voltage setting of 5500 V, a desolvation temperature of 500 °C, a curtain gas of 25 psig, and a turbo gas of 50 psig. On the other hand, for NG, which is a more unstable compound, a softer ionisation technique known as atmospheric pressure chemical ionisation (APCI) was employed. The analysis was performed in negative mode with a source temperature of 137.5 °C, a curtain gas of 30 psig, and an ion source gas of 36 psig.

Table 2
UHPLC parameters.

Ionisation	*ESI+*		
Flow rate		0.25 mL/min	
Injection volume		5 µL	
Gradient method	Time [min]	Mobile phases	
		Water + 0.1 % v/v formic acid [%]	Acetonitrile + 0.1 % v/v formic acid [%]
	0	65	35
	0.5	65	35
	6	20	80
	7	0	100
	7.5	0	100
	8.1	65	35
	10	65	35
Ionisation		*APCI-*	
Flow rate		0.40 mL/min	
Injection volume		6 µL	
Gradient method	Time [min]	Water	Methanol
	0	80	20
	1	80	20
	6	50	50
	8	50	50
	9	0	100
	10	0	100
	10.5	80	20
	14	80	20

The limit of quantification (LOQ) was determined from the baseline noise of the adhesive blanks instead of the solvent blanks as a matrix effect was observed for some OGSR compounds (i.e., 4-nDPA and 2-nDPA) (Table 4):

$$LOQ = AverageC_{adhesiveblanks} + 10 \cdot STD \quad (1)$$

Unfortunately, two peaks of DPA and EC were systematically observed in the chromatograms of the blank adhesives. A LOQ was calculated based on the baseline section adjacent to the retention times of these compounds, and the calculated concentration of the detected peak exceeded this LOQ. This slight contamination of carbon adhesive was previously reported [39,42]. Thus, all the adhesive batches were tested before the experiments to assess the levels of contamination (5 replicas/

Table 3
Target OGSR compounds and MS/MS parameters.

Compounds	Source of ionisation	Parent ion [m/z]	Declustering potential [V]	Product ion [m/z]	Collision Cell Exit Potential [V]	Collision energy [V]
Nitroglycerin (NG)	APCI-	227.0 $[M]^-$	−5	107.8	−10	−7
				62	−7	−9
Akardite II (AK-II)	ESI+	227.0 $[M+H]^+$	61	170	20	33
				91	10	23
Ethylcentralite (EC)	ESI+	269.1 $[M+H]^+$	40	148	16	29
				120	10	19
Methylcentralite (MC)	ESI+	241.1 $[M+H]^+$	31	134	14	19
				106	12	33
Diphenylamine (DPA)	ESI+	170.1 $[M+H]^+$	51	93	10	25
				92.1	10	31
N-nitrosodiphenylamine (*N*-nDPA)	ESI+	199.0 $[M+H]^+$	21	66	8	29
				169	20	15
2-nitrodiphenylamine (2-nDPA)	ESI+	215.0 $[M+H]^+$	91	180	20	19
				198	20	23
4-nitrodiphenylamine (4-nDPA)	ESI+	215.0 $[M+H]^+$	191	198	20	43
				167	18	21

Table 4
LOQ and threshold values for each OGSR compound.

Compounds	LOQ [ng/mL]	Threshold [ng/mL]
NG	1	
AK-II	0.04	
EC	0.005	0.06
MC	0.3	
DPA	0.1	0.3
N-nDPA	0.02	
2-nDPA	0.03	
4-nDPA	0.3	

batch) and select the least contaminated adhesives (contamination consistently remained within the same magnitude, below 0.3 ng/mL for DPA and below 0.1 for EC). A threshold was then calculated to determine a limit above which OGSR concentrations (C) was attributable to the shooter rather than the adhesive (Table 4):

$$Threshold = MaximumC_{adhesiveblanks} + 3 \cdot STD \quad (2)$$

This observed contamination underscores the importance of adhesive selection, highlighting the imperative of finding adhesives free from contamination to facilitate the successful implementation of OGSR analysis in forensic laboratories. Additionally, it is noteworthy to mention that the low LOQ values obtained in this study may differ from those laboratories with higher LOD values, potentially resulting in undetected contamination.

2.3.2. IGSR analysis

The adhesives were coated with a layer of carbon using a Carbon coater *108 carbon/A* manufactured by Cressington Scientific Instruments (United Kingdom). A vacuum pressure of more than 0.1 mbar was applied and a current between 100 and 150 A was utilised. The detection of inorganic elements was conducted using a Σigma SEM/EDS with GEMINI technology® by Zeiss, equipped with a 60 mm2 X-Max detector from Oxford Instruments. The software utilised was AztecGSR. During the analysis, an accelerating voltage of 20 kV and a working distance of 8.5 mm were applied. The brightness and contrast settings were calibrated using an Au/Co/C calibration standard. The search for inorganic particles with a minimum size of 0.5 µm was carried out by targeting the three classes of particles defined by the ASTM E1588-20 [43] (Table 5).

The entire surface of the adhesive was scanned. After the analysis, no manual confirmation (obligatory and necessary in practice) of the elemental composition and morphology of the detected particles was performed due to time constraints. While this step may be considered less critical from a research perspective, it should always be performed in practice. Given the amount of GSR collected (see section Results and Discussion), it was judged that manual confirmation would not significantly alter the observations and conclusions. The focus was on generating knowledge about GSR traces, their transfer, and persistence, rather than strictly adhering to the standard protocol. Consequently, the data cannot be directly applied to casework but should be considered as informative regarding GSR trace. The particle count was checked to ensure that a particle was not recorded more than once by the software. Indeed, since the GSR sampling was carried out shortly after discharge, relatively large particles could be observed and were sometimes counted multiple times by the software [20].

Table 5
Classification of the inorganic particles according to the ASTM E1588-20 guidelines [43].

Classification	Elemental composition for a Sinoxid-type primer
Characteristic particles	PbSbBa
Consistent particles	BaCaSi, BaSb, PbSb, BaAl, PbBa, PbBaCaSi
Commonly associated particles	Pb, Sb or Ba

3. Results and discussion

3.1. OGSR results

3.1.1. Shooter's blanks

In 71 % of the shooter's blank specimens, at least one OGSR compound was detected in concentrations above the defined limits (Fig. 2 and SI – Table 1). Two shooter blanks contained more than 5 OGSR compounds (i.e., 6 on the forearms of shooters 1 and 7 on the face of shooter 4, respectively). The slightly higher number of OGSR compounds detected on the face's stub may be due to the fact that individuals frequently touch their face, and cleansing procedures for the face are typically less thorough compared to those for the hands and forearms. In contrast, the lower proportion of contaminated stubs for the nostrils might be explained by the smaller sampling surface and number of dabbings (Fig. 2 and SI – Table 1). These findings suggest that the research environment exhibited background contamination despite the cleaning step (washing the hands, forearms, and face with water and soap).

Most organic compounds (i.e., MC, AK-II, DPA, *N*-nDPA, 2-nDPA, and EC) were either detected individually or in combination with others in the blank specimens. However, exceptions were noted for 4-nDPA, the only organic compound detected in a single nostril blank, and NG, systematically detected in combination with other compounds.

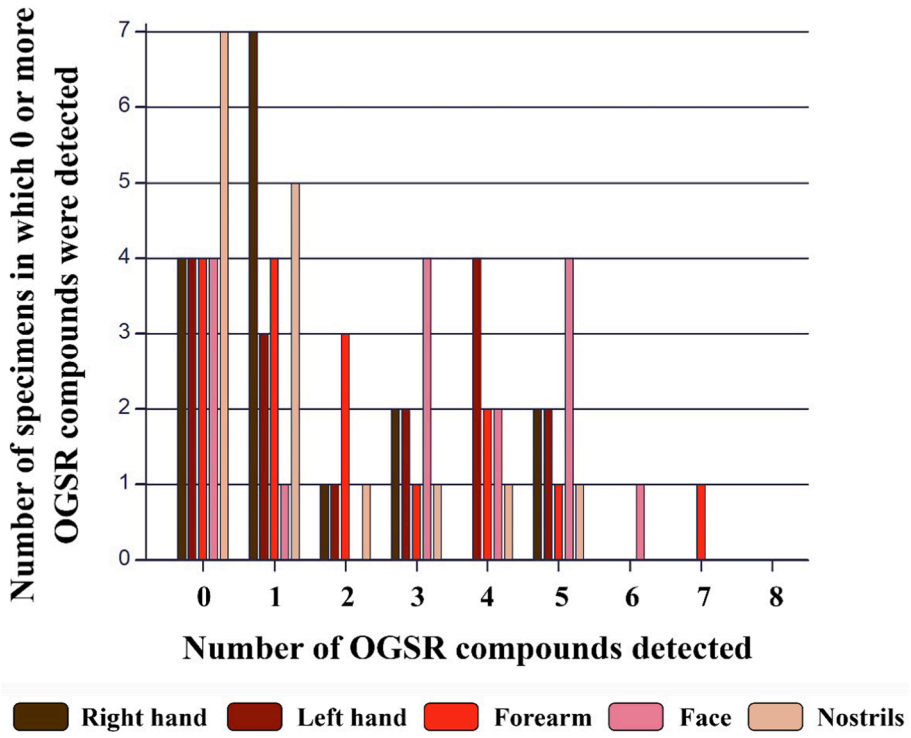

Fig. 2. Histogram representing the number of specimens per collected region in which 0 or more OGSR compounds were detected immediately before the discharge (n = 16 experiments).

Among the targeted OGSR compounds, *N*-nDPA, DPA, and 2-nDPA were more frequently detected in the blanks (i.e., between 43 and 48 % of the blanks), while MC and 4-nDPA were detected in 5 % or less of the blanks **(**Fig. 3**).**

The recovered concentrations were comparable among the shooters, with no difference in contamination rates per compounds. All median values for the shooter's blank specimens were 0.00, as the compounds were each quantified in less than 50 % of the blank specimens **(SI** — Table 2**).** Overall, the detection of these compounds may be due to either inadequate cleaning of the skin or contamination occurring after the cleaning (e.g., through secondary transfer). As for each experiment and region, a specimen was collected before discharge (i.e., shooters' blanks) and 30 min after, the contaminations were discussed along with the obtained results.

3.1.2. *Qualitative discharge results*

Almost all discharge specimens (96 %) contained at least one OGSR compound, except for 3 nostril specimens that contained none **(**Fig. 4 **and SI** — Table 3**).** Eight compounds were detected in 21 out of 80 specimens (10 from the right hand, 7 from the left hand, 2 from the forearms, and 2 from the face). At least six organic compounds were detected in more than half of the specimens (54 %), particularly in those collected on the hands (i.e., six or more organic compounds were detected in 15 and 12 out of the 16 specimens collected on the right and left hands, respectively). No hand specimens contained less than four compounds. These results confirmed that the hands are privileged

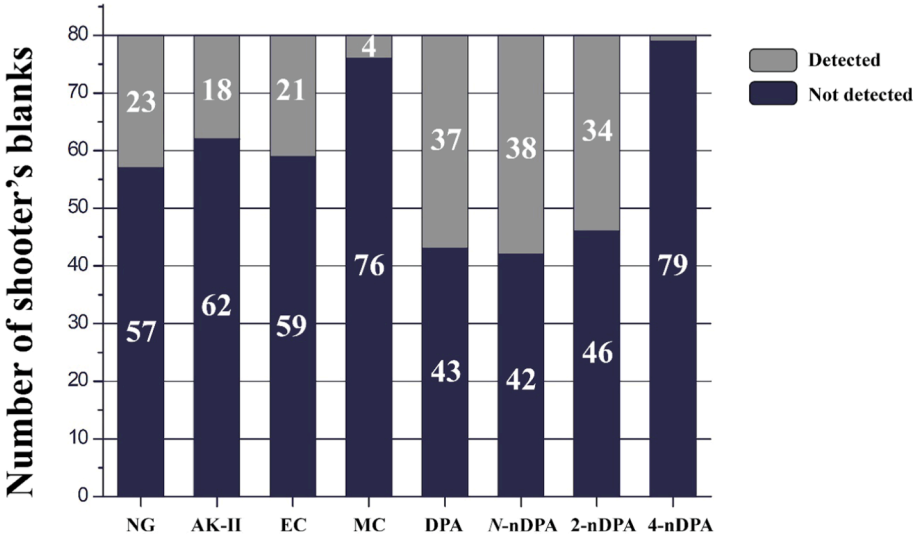

Fig. 3. Histogram representing the number of the shooter's blanks in which OGSR compounds were either detected or not on the shooters, immediately before the discharge (n = 80).

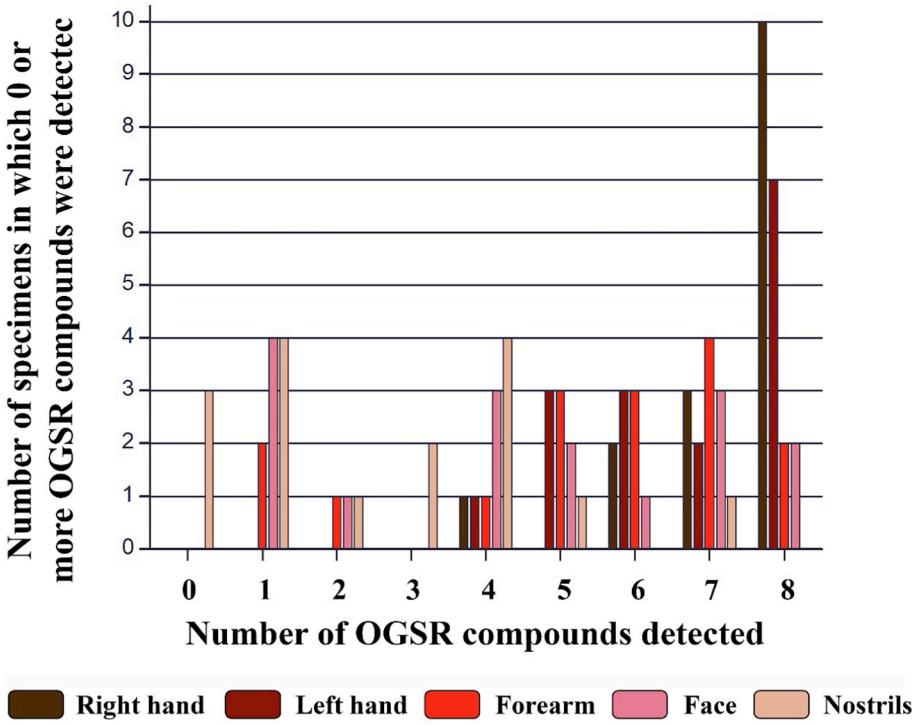

Fig. 4. Histogram representing the number of specimens per collected region in which 0 or more OGSR compounds were detected 30 min after a discharge (n = 16 shots, 80 stubs).

receipting GSR regions and, therefore, interesting for OGSR collection at least 30 min after a discharge with a semi-automatic pistol. For the forearms and face, 9 and 6 specimens were respectively positive for six or more organic compounds, while this was the case only for one nostril specimen out of 16 (Fig. 4). These results support the hypothesis that more OGSR are transferred close to the pistol (on the hands) than further away (forearms, face, and nostrils). The lowest number of OGSR compounds detected in the nostrils may also be explained by the smaller collection surface and a lower number of dabbings. Therefore, nostrils could be collected together with the face specimens rather than separately. The forearms and face may be particularly interesting alternative regions for GSR collection in cases where it is suspected that the persons of interest washed their hands before collection.

The most frequently detected organic compound on the shooters was AK-II (found in 69 out of the 80 collected specimens), while 4-nDPA was the least frequently encountered compound (found in 28 out of the 80 stubs) (Fig. 5 and SI – Table 4). The other remaining compounds (i.e., NG, EC, MC, DPA, *N*-nDPA, and 2-nDPA) were detected in more than half of the specimens.

3.1.3. Quantitative discharge results

NG, as an energy carrier compound, was recovered in the highest median concentrations on the hands and forearms, followed by the stabilisers AK-II, DPA, *N*-nDPA, EC, and 2-nDPA (Table 6, Fig. 6 and SI – Fig. 3). MC and 4-nDPA exhibited the lowest median concentrations. These two compounds were indeed rarely detected in ammunition in

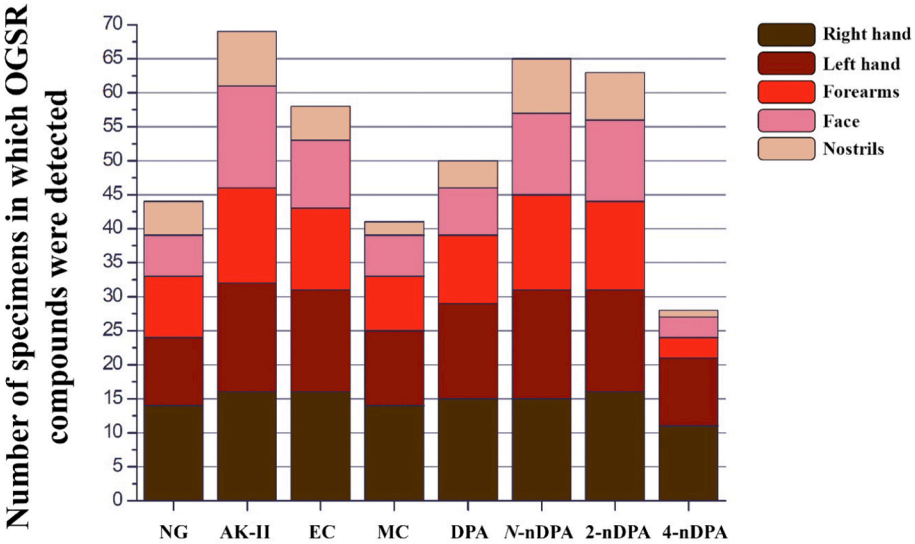

Fig. 5. Histogram representing the number of specimens in which the OGSR compounds were detected on the shooters 30 min after a single discharge (n = 80 stubs).

Table 6
Median concentrations and RSD values of the eight OGSR compounds recovered on the shooters 30 min after a single discharge (n = 16 discharge).

Median concentration of OGSR in ng/mL *RSD*	Right hand	Left hand	Forearms	Face	Nostrils
NG	14 *245 %*	22 *220 %*	2 *226 %*	0* *397 %*	0* *240 %*
AK-II	7.94 *199 %*	2.13 *250 %*	0.77 *336 %*	0.25 *387 %*	0.00* *199 %*
EC	0.41 *200 %*	0.37 *199 %*	0.12 *231 %*	0.12 *341 %*	0.00* *181 %*
MC	0.37 *136 %*	0.34 *96 %*	0.00* *111 %*	0.00* *135 %*	0.00* *274 %*
DPA	1.33 *206 %*	1.17 *184 %*	0.42 *139 %*	0.00* *388 %*	0.00* *216 %*
N-nDPA	0.85 *208 %*	1.00 *192 %*	0.32 *230 %*	0.09 *390 %*	0.00* *173 %*
2-nDPA	0.21 *239 %*	0.09 *208 %*	0.07 *229 %*	0.05 *387 %*	0.00* *120 %*
4-nDPA	0.35 *222 %*	0.35 *201 %*	0.00* *242 %*	0.00* *360 %*	0.00* *400 %*

*The median value is "0.00" since the compound was not detected in most of the specimens, with concentrations below the defined limits). All median values for the shooter's blank specimens were 0.00.

previous studies [24,31,39]. OGSR were recovered in much higher median concentrations on the shooters' hands than on other collection regions **(**Table 6**).** For AK-II, EC, MC, DPA, and 2-nDPA, slightly higher concentrations were generally detected on the right hand than on the left hand, while for NG, *N*-nDPA, and 4-nDPA, similar or slightly lower concentrations were obtained on the shooter's right hand compared to the left hand. However, no statistically significant differences were observed between the two hands for any of the OGSR compounds. Several hypotheses may explain that comparable concentrations were detected on both hands despite the ejection port being on the right side of the firearm. The OGSR cloud produced by the discharge may be large enough to incorporate both hands (primary transfer), the right hand may lose OGSR more quickly during the 30 min after the discharge (persistence), or the OGSR quantities on both hands homogenise through hands rubbing or touching the same surfaces (secondary transfer). On the forearms and face, recovered concentrations were generally significantly lower than on the hand (except for MC and 4-nDPA, which were less detected in the specimens). However, although the forearms were closer to the firearm discharge than the face, similar concentrations were obtained for both regions **(**Table 6**).** A face specimen from shooter 1 contained much higher concentrations than most of the specimens for most compounds (except MC) (SI – Fig. 3 and Fig. 6). The higher concentration might be explained by a secondary transfer, likely occurring through the hands touching the face. This hypothesis is supported by the fact that the concentrations of the associated right-hand specimen (from the same discharge experiment) were 2 to 8 times smaller than on those obtained for the face. The concentrations in the left-hand specimen from the same experiment contained even lower concentrations. For the nostrils, the concentrations were the lowest. This confirms that either the collection surface or the number of dabbings limits the potential of this region 30 min after the discharge. When comparing the data collected 30 min after a discharge to the data from the shooter's blanks, generally higher or sometimes comparable concentrations were detected in the specimens than in the blanks (SI – Fig. 3). None of the blank concentrations were substantially higher (twice as higher) than the associated 30-minute discharge specimens for the EC, MC, 2-nDPA, and 4-nDPA. However, for NG, AK-II, DPA, and *N*-nDPA, one blank concentration, either from the nostrils or left-hand stubs, was twice as high as the associated specimen.

It is essential to highlight that the results exhibited large variations from one discharge to another **(**Table 6 **and SI** – Fig. 3**).** The average RSD value for the different OGSR compounds and targeted regions was 204 %. A closer examination of the results for each of the shooters revealed a large variation both within and between shooters **(**Fig. 7 **and SI** – Fig. 4**).** The highest concentrations were generally recovered on the

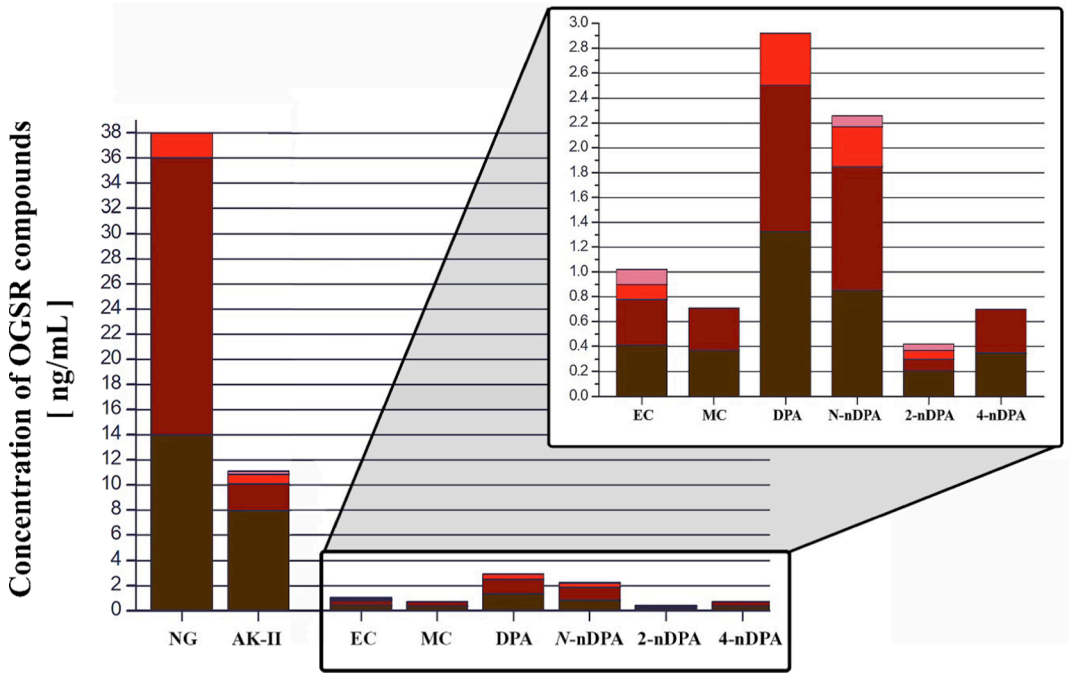

Fig. 6. Histogram representing the median quantities for the eighth OGSR compound detected on the shooters, 30 min after a single discharge (n = 16 shots).

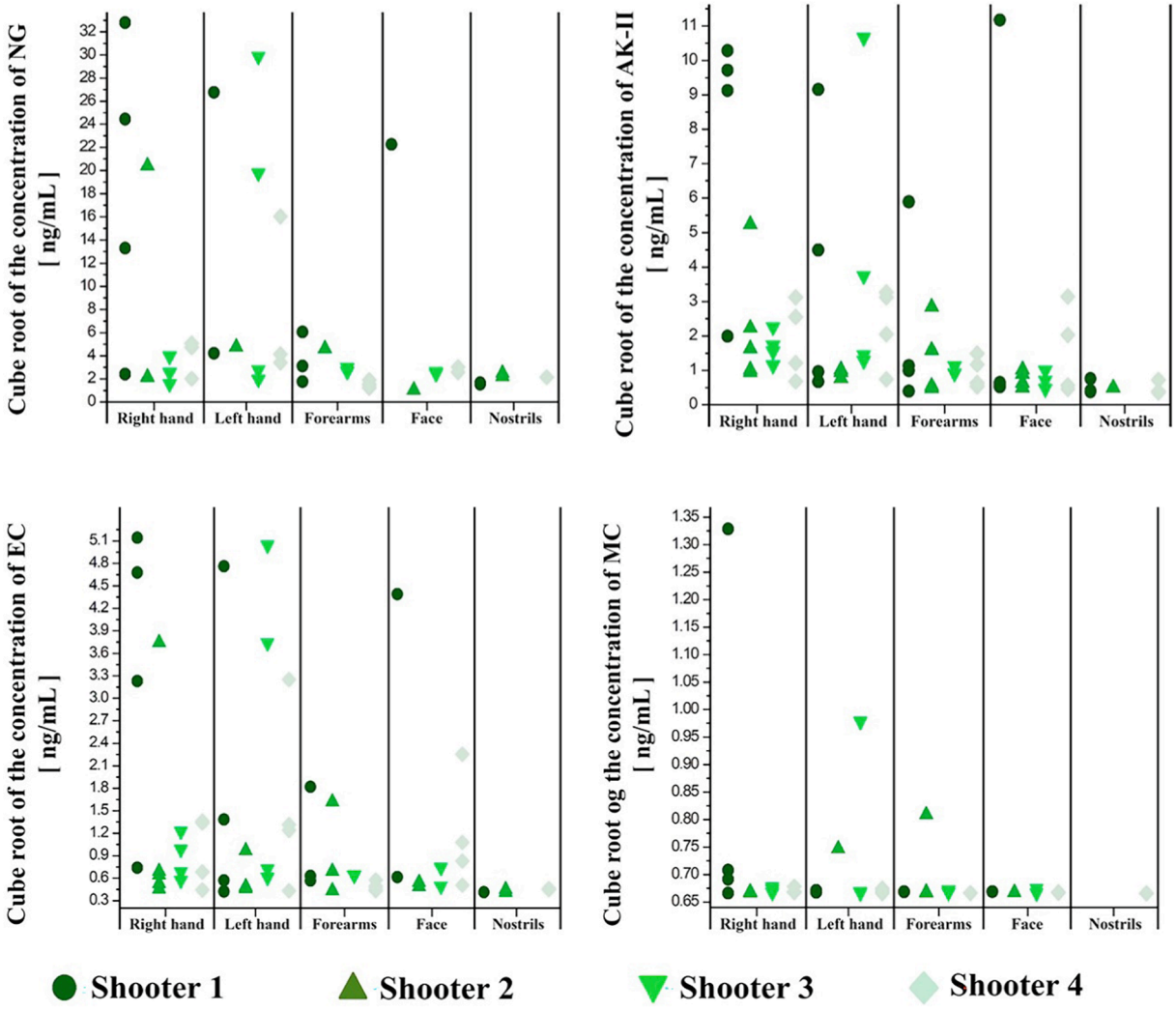

Fig. 7. Scatter plots of the square root of the concentrations of nitroglycerin (NG), akardite-II (AK-II), methylcentralite (MC) and, ethylcentralite (EC), detected in the specimens collected from the 4 shooters, 30 min after a single discharge (n = 4 shots per shooter).

right or left hands, and to some extent, the face, of shooter 1 who reported office tasks during the 30 min before collection (i.e., meeting, typing, reading). Shooters 3 and 4 also reported performing office tasks during the 30-minute delay, while shooter 2 was more physically active and reported several laboratory manipulations. Since there were no major differences between the activities of shooters 1, 3, and 4, the generally higher concentrations on the hands of shooter 1 could be attributed either to some particularity during transfer (e.g., discharge variation, skin properties, firearm initial handling), the subsequent activities (e.g., higher retention, fewer manipulations and movements) or a higher exposure to secondary transfer (e.g., contact with persons or surfaces being contaminated by OGSR). For example, higher concentration values could be attributed to the deposition of very large particles on the shooter. Higher concentrations were also recovered for all the OGSR compounds on the left hand of shooter 3 (which might be explained by the fact that this shooter was the only left-handed). Although left-handed, this shooter held, discharged and secured the firearm (by removing the magazine) in the same manner as the other shooters during the experiments. Therefore, the observed difference may be better explained by subsequent activities during the 30 min delay before collection.

3.2. IGSR results

10 Specimens (from one experiment with shooter 1 and one with shooter 4) could not be analysed after OGSR extraction due to an issue with the carbon adhesive preparation (i.e., only one double-sided carbon adhesive was placed instead of two on the aluminium stub). Consequently, the total number of IGSR specimens was only 70 (14 shots and five collected regions).

3.2.1. Shooter's blanks

In all the shooter's blank specimens, at least one of the target inorganic particles was detected **(SI** — Fig. 5**).** A detailed examination of each of the compositions being targeted reveals that consistent particles of BaSiCa and BaAl, as well as commonly associated with GSR particles of Pb, Ba, or Sb, were detected in nearly all the shooter's blanks (Fig. 8). The median values for those compounds often appeared similar to or even higher than those found in specimens collected 30 min after the discharge **(SI** — Tables 5 and 6**, and SI** — Fig. 4**)**. Consequently, these inorganic particles presented a high prevalence in the experiments' environment and were excluded from the presentation of the results.

For the remaining targeted inorganic particles (PbSbBa characteristic particles and consistent particles with a composition of BaSb, PbSb, PbBa, and CaBaPbSi), 67 % of the blanks were contaminated with at least one of these five targeted inorganic particles. One shooter blank

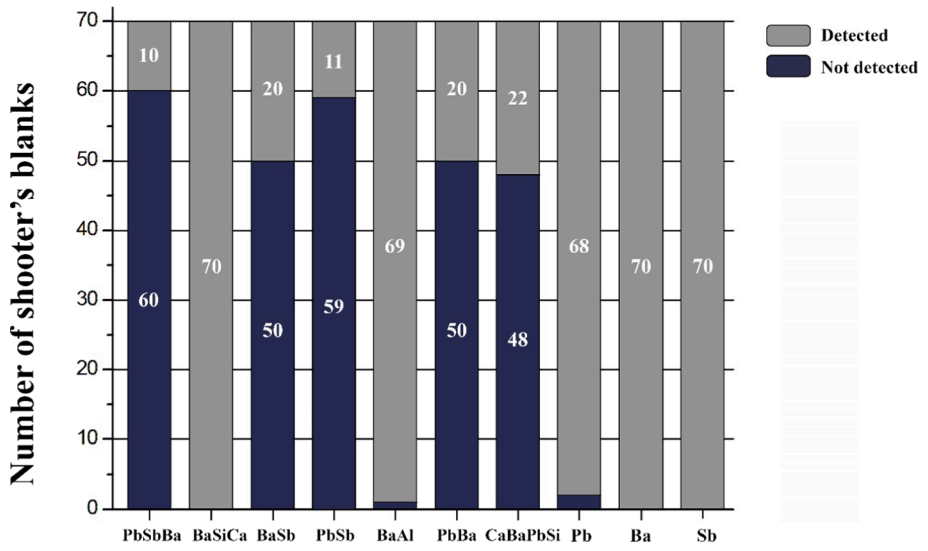

Fig. 8. Histogram representing the number of the shooter's blanks in which IGSR particles were either detected or not on the shooters, immediately before the discharge (n = 70).

contained more than 3 IGSR particles type (i.e., CaBaPbSi, BaSb, PbSb, and PbBa on the right hand of shooter 1) (Fig. 9). A higher proportion of right-hand stubs were contaminated, closely followed by the face and forearms (SI – Table 7**)**. Fewer left-hand stubs were contaminated, and the nostrils stubs were again the least contaminated. The particles BaSb, PbBa, CaBaPbSi were found more often in the shooter's blank (around 30 %) compared to PbSbBa and PbSb particles (around 15 %) (Fig. 8).

The highest number of particles found in a specimen was 30 on the left hand and 13 on the right hand of shooter 3 (during two different discharge experiments). Another specimen, taken from the face of shooter 4, presented 10 target particles, while all other specimens contained fewer than eight particles The contamination of these three stubs was mainly due to the presence of CaBaPbSi and PbBa particles. These three blank specimens also contained zero to one characteristic particle and were associated with 30-minute specimens that showed a high number of characteristic particles (1832, 847, and 363, respectively). These particles might originate from a poor cleaning of the targeted regions, a secondary or subsidiary transfer, or an alternative source in the environment.

All the shooters presented similar rates of contamination. As for the OGSR, the median values for the shooter's blank specimens were consistently at 0.00, as contamination was observed in fewer than 50 % of the blanks for each IGSR particle type (SI – Table 5 for average values detected as well as the maximum and minimum). To account for these

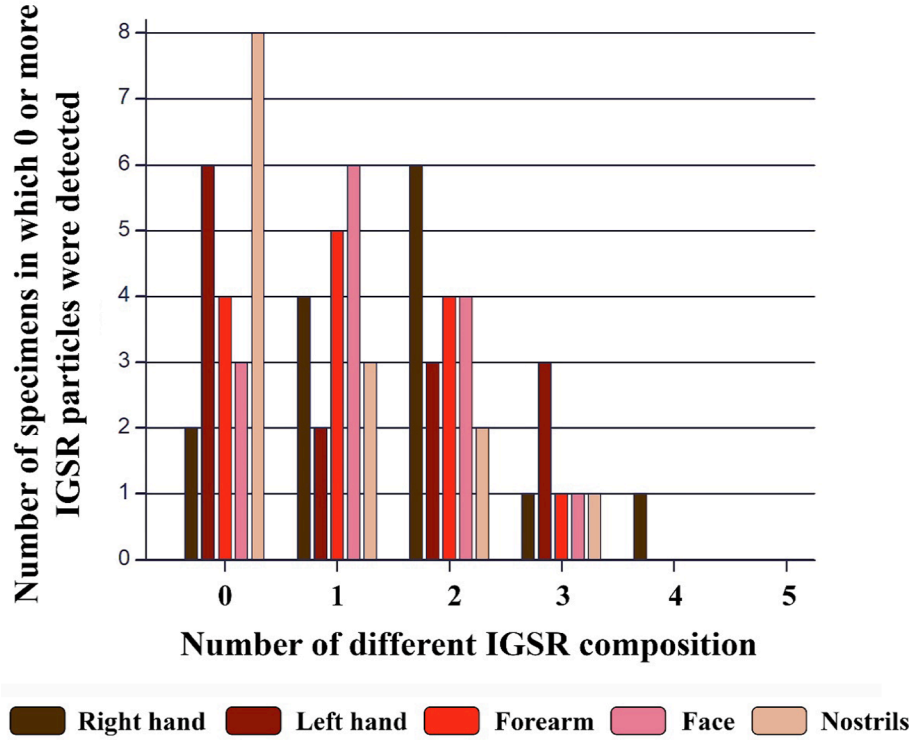

Fig. 9. Histogram representing the number of specimens per collected region in which 0 or more IGSR particles composition (PbSbBa, BaSb, PbSb, PbBa, and CaBaPbSi) were detected immediately before the discharge (n = 14 experiments).

contaminations, data from the shooter's blanks were included in the *quantitative analysis* section alongside data collected 30 min after a discharge.

3.2.2. Qualitative discharge results

Almost all discharge specimens (97 %) contained at least one IGSR particle type, except for 2 nostril stubs (Fig. 10 and SI – Table 8). All the targeted inorganic particles were detected in 34 out of 70 specimens (10 from the right hand, 9 from the left hand, 7 from the face, and 8 from the face). Therefore, these four regions are particularly interesting for IGSR collection 30 min after a discharge. Similarly, to OGSR, the nostrils seem to be less interesting for IGSR collection, and this may probably be explained by its smaller surface and lower number of dabbings.

The most frequent composition of IGSR was PbSbBa (characteristic particles), which was detected in 61 out of 70 specimens (87 %). While the remaining particle compositions were found in fewer specimens, they were always present in more than 60 % of the stubs (Fig. 11 and SI – Table 9).

3.2.3. Quantitative discharge results

The highest median IGSR number was obtained for the characteristic particles (PbSbBa) in all collected regions (Table 8 **and** Fig. 12). In general, a higher number of particles were recovered from the shooter's right hand when compared to other regions (Table 8 and SI – Figs. 5 and 6). Lower numbers of IGSR were detected on the left hand (with median values at least twice or smaller on the left hand than on the right hand for all composition of the targeted IGSR). Thus, a larger difference was observed in IGSR quantities between the two hands when compared to OGSR. One hypothesis to explain these findings could be the difference in volatility of both types of GSR that influenced the initial transfer. Since inorganic particles are less volatile, they would preferably deposit on the hand next to the ejection port, whereas more volatile organic compounds would be propelled further away from the firearm (or form a volatile cloud around the firearm that deposits more homogeneously). Other explanations may also be related to different secondary transfer and persistence mechanisms for O and IGSR. For IGSR, there might, for example, be less secondary transfer between each hand and when the hands touch the face than for OGSR. Results could also indicate a better persistence (e.g., IGSR loss is mainly mechanical, while OGSR are also known to evaporate or be absorbed by the skin) [13,14,31]. Moreover, lower quantities of PbSbBa and PbBa particles were detected on the forearms followed by the left hand, face, and nostrils, respectively (Table 7). This observation could be explained by the fact that the IGSR is more likely to be deposited on the side of the ejection port (i.e., in this case, right hand and right forearm). As the right and left forearms were sampled together, it was not possible to assess whether a larger quantity of inorganic particles were found on the right forearm compared to the left. Regarding the other IGSR particle compositions (i.e., BaSb, PbSb, and CaBaPbSi) slightly higher (but similar) median values were detected on the left hand than the forearms. The number of IGSR particles collected on the shooter's face was generally lower than those on the hands and forearms (SI – Figs. 5 and 6). For the nostrils, only 1 to 2 inorganic particles of each type were found in the 30-minute specimen, confirming that sampling this region with the proposed protocol is not relevant. Moreover, this was the only region where the summed number of IGSR particles was lower than those collected in the blanks (Table 7). It would not even make sense to include the nostrils in the face specimen as suggested above for OGSR.

While the numbers of transferred particles were also very variable between experiments (average RSD of 154 %), it was less variable than for OGSR results (average RSD value of 204 %). A large variation was also observed within and between shooters' discharges (Fig. 13, and SI – Fig. 9). However, none of the shooters showed a much higher or lower particle number than the others. For instance, more than 1000 particles were found only in five stubs (two right-hand specimens from shooter 1, one left-hand and one forearms specimens from shooter 3 and one right-hand from shooter 4). Similarly, while the left-handed shooter 3 presented more particles on the left hand compared to the right hand for one discharge, the median values were still higher for the right hand compared to other collection regions. For shooter 2, who reported engaging in more physical activities, comparable values were observed between the left and right hands. These observations indicated again a difference in the transfer and persistence of IGSR compared to OGSR.

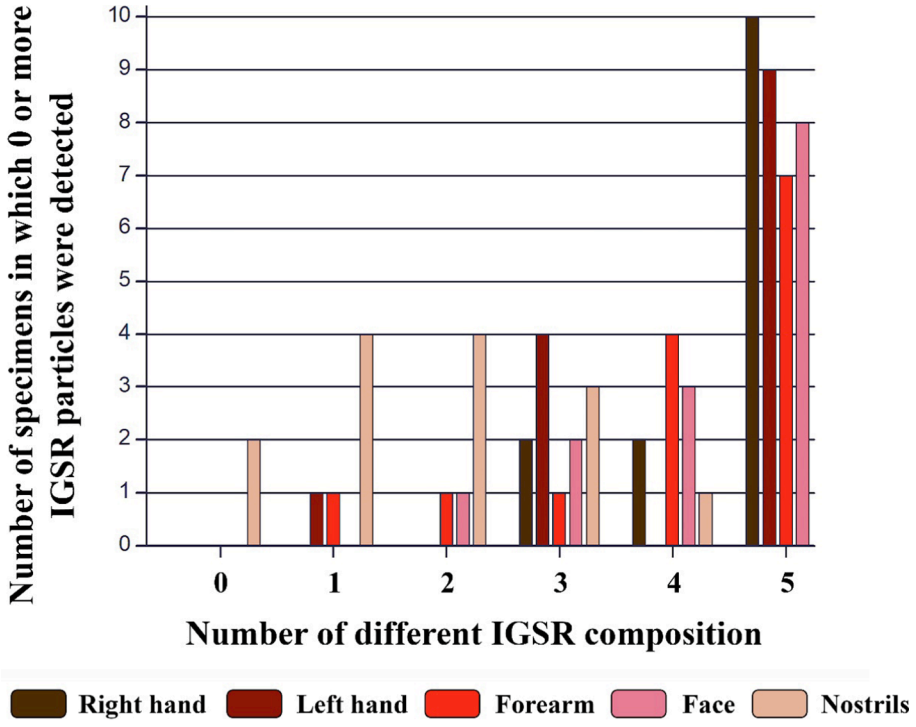

Fig. 10. Histogram representing the number of IGSR particles detected by specimens collected on the shooters, 30 min after a single discharge (n = 14 experiments).

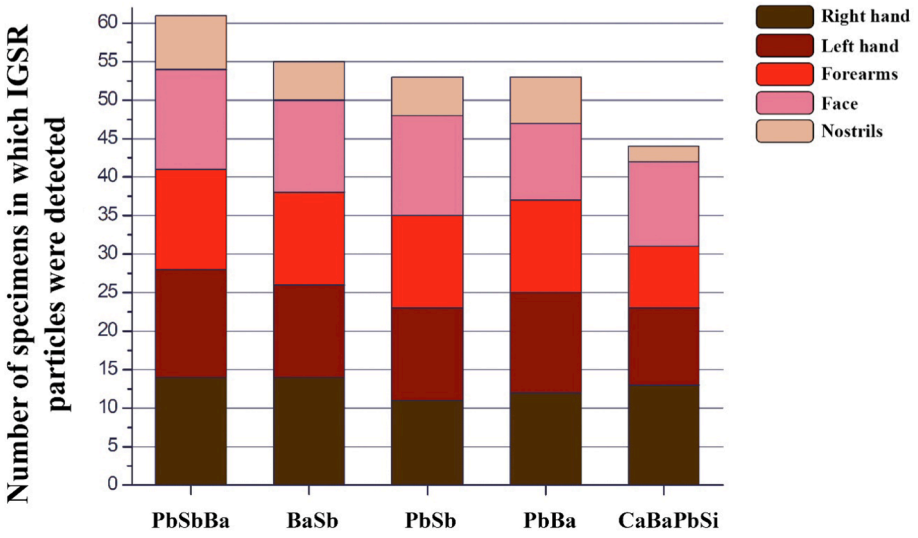

Fig. 11. Histogram representing the number of specimens in which IGSR particles were detected on the shooters, 30 min after a single discharge (n = 14 shots).

Table 7
Median and RSD values of the five compositions of IGSR particles recovered on shooters, 30 min after a single discharge (n = 14 shots and 5 targeted regions).

Median number of IGSR particles *RSD*		Right hand	Left hand	Forearms	Face	Nostrils
Characteristic	Pb Sb Ba	230 *91 %*	38 *219 %*	145 *136 %*	11 *105 %*	1 *136 %*
Consistent	Ba Sb	43 *99 %*	5 *189 %*	4 *125 %*	4 *170 %*	0* *177 %*
	Pb Sb	19 *164 %*	8 *154 %*	3 *136 %*	5 *182 %*	0* *201 %*
	Pb Ba	48 *108 %*	13 *189 %*	20 *103 %*	3 *192 %*	0* *204 %*
	Ca Ba Pb Si	36 *134 %*	5 *143 %*	2 *196 %*	3 *151 %*	0* *151 %*

*A median value of zero was obtained when a composition of particles was not detected in the majority of the specimens. The median values were consistently zero for the shooter's blank specimen too.

Table 8
Shooters' blank stubs with the highest contamination.

OGSR (summed concentration above 10 ng/mL)	IGSR (10 or more particles)
S1 (discharge 3): left and right hands, forearms, and face	S3 (discharge 1): left hand
S2 (discharge 1): nostrils	S3 (discharge 2): right hand
S3 (discharge 2): left, and right hands, forearms, and face	S4 (discharge 2): face

*The specimens that exhibited the higher contaminations in IGSR and OGSR were not the same.

3.3. Comparison of OGSR and IGSR results

For both types of GSR, some of the shooter's blanks exhibited contamination with organic compounds and/or inorganic elements despite undergoing the washing procedure (i.e., cleaning the hands, forearms and face with water and soap). Out of a total of 70 stubs analysed for both types of GSR, 40 stubs were contaminated with both types, while 23 and 17 were contaminated only with OGSR or IGSR, respectively. Only 7 were found to be free of any residues. Moreover, the most contaminated blanks for OGSR and IGSR were not the same (Table 8).

This indicates that the contamination levels of these two types of GSR were not necessarily correlated. The results additionally showed slightly higher contamination of OGSR compounds, both in terms of the number of contaminated blanks (i.e., with 6 additional stubs were contaminated compared to IGSR) and relative quantities (i.e., more stubs exhibited higher contamination for OGSR compared to those with a higher number of IGSR).

The effectiveness of washing in removing OGSR and IGSR was also evaluated in previous studies. Two studies reported that some blanks still contained OGSR after washing, but at much lower quantities [31,44]. IGSR research indicated that almost all the characteristics, consistents and commonly associated with GSR particles were removed after the washing step [22,25,31]. The presence of organic compounds and/or inorganic elements despite washing may either be attributed to an insufficient cleaning step or to contamination occurring after the cleaning step. In either case, the shooter's blank contaminations revealed the presence of background noise in the shooter's environment.

As no GSR were detected in all laboratory blanks, the only plausible explanation for the contamination was the background environment. Despite thorough cleaning of all offices before and after each experiment, and the fact that shooters had no access to the indoor shooting range or firearm laboratory, contamination was still detected. Such contamination might also be expected in a police officer's working environment. Operating in a completely GSR-free environment is challenging. Therefore, it is essential to control the collection environment to ensure that detected residues do not originate from contamination in real cases [45–49]. However, in real cases, it is not feasible to collect the shooter's blanks, as GSR are collected form a person of interest after a discharge event. This is why it is imperative to investigate whether the individual possess, handles or has fired a firearm, and to report any potential contamination due to secondary or subsequent transfer, such as by a police office. This knowledge is crucial for the accurate interpretation of a GSR result.

Examining the results obtained from discharge specimens, it becomes apparent that collecting GSR on the hands of the shooters is relevant for both types of residues, as higher OGSR concentrations and IGSR particle numbers were generally detected 30 min after a single discharge. The forearms and face proved to be interesting collection alternatives, especially if it is suspected that the persons of interest have cleaned their hands. Nevertheless, the quantities recovered from the

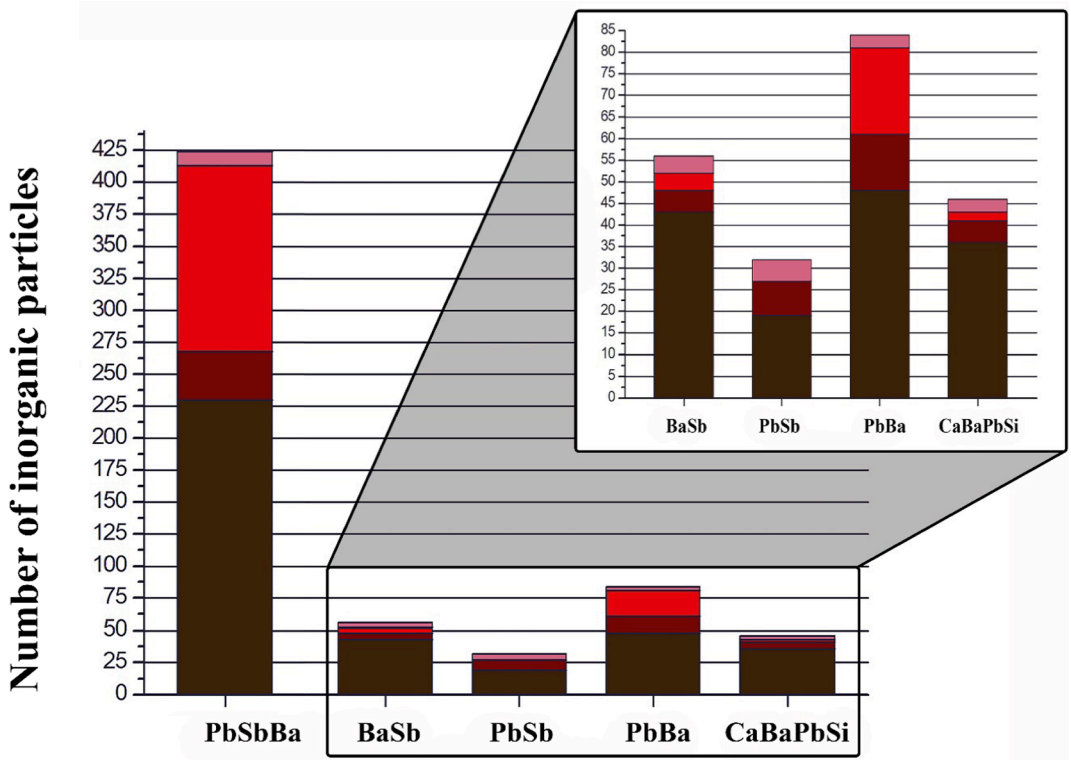

Fig. 12. Histogram representing the median number for each composition of IGSR particles targeted and detected on shooters 30 min after a single discharge (n = 14 shots on 5 collected regions for a total of 70 stubs).

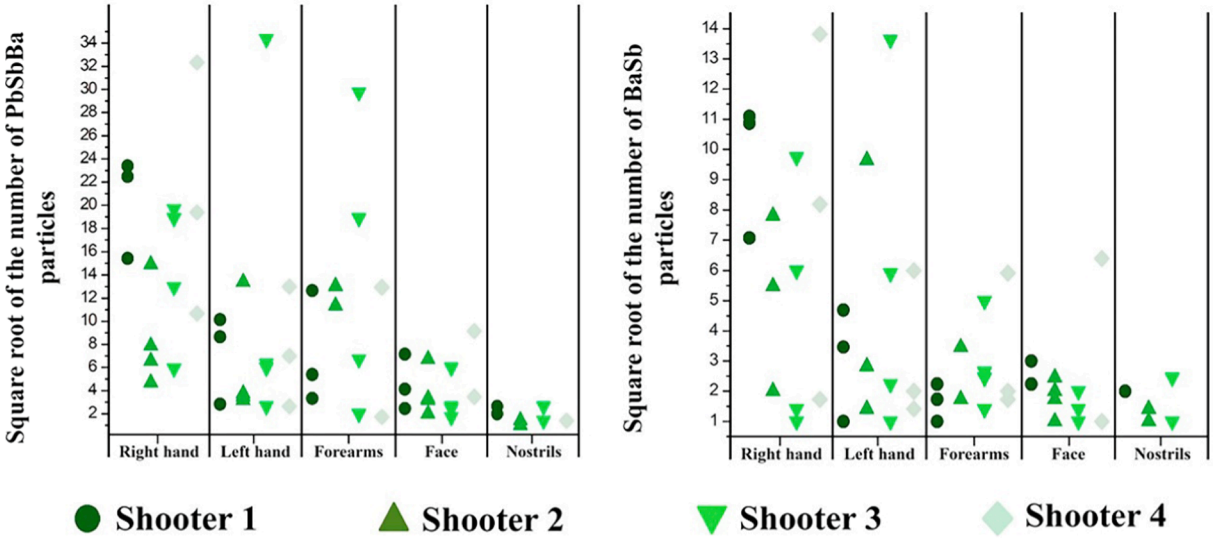

Fig. 13. Scatter plots of the square root of the number of IGSR particles with a composition of PbSbBa (left) and BaSb (right) detected in the specimens collected from 4 shooters 30 min after a single shot (n = 3 shots for shooters 1 and 4, and 4 shots for shooters 2 and 3).

nostrils were generally too low to be interesting. Therefore, the nostrils entrance could be collected together with the face, using the same stub.

From these discharge results, several distinctions were highlighted between the two types of GSR (Table 9). The five targeted inorganic particles were more frequently detected in the specimens than the organic compounds, with all IGSR being detected in 49 % of the specimens as opposed to 26 % for all OGSR. In fact, some organic compounds were often detected (i.e., AK-II, *N*-nDPA, and 2-nDPA were detected in more than 70 % of the stubs), while others were less found (i.e., NG, MC, and 4-nDPA were detected in 55 % of the stubs or less). This may be due to some compounds found in smaller quantities in the ammunition powder (e.g., MC and 4-nDPA) or due to their limited persistence (e.g., NG is known to degrade relatively quickly). Regarding IGSR, characteristic PbBaSb particles were detected in 87 % of the stubs, while consistent particles were detected in less than 80 % of the stubs (but always above 60 %). Another element demonstrating a distinction between IGSR and OGSR was observed when Pearson correlations were calculated between each pair of variables (Fig. 14). The correlations between IGSR and OGSR were less than 0.6 whereas, within OGSR compounds or IGSR composition, the correlations were generally greater than 0.6. These correlation values indicated that there is only a modest correlation (<0.6) between the two types of GSR, whereas a

Table 9
Comparison of key results between OGSR and IGSR illustrating the differences and similarities between both types of residues.

	OGSR 8 target compounds 16 discharges 80 stubs	IGSR 5 target particles 14 discharges 70 stubs
Contamination was detected in: (% shooter's blanks)	71 %	67 %
All targets were detected in: (% discharge specimens)	26 %	49 %
No target was detected in: (% discharge specimens)	4 %	3 %
Most common target in: (% discharge specimens)	AK-II (86 %)	PbBaSb (87 %)
Most abundant target: (median)	NG (22 ng/mL on left hand)	PbBaSb (230 particles on right hand)
Most abundant region:	Right and left hands	Right hand

stronger correlation was observed within each type of GSR.

A difference between the different collected regions was also observed between both GSR types. In the case of IGSR, a significantly higher number of inorganic particles was noted on the right hand compared to the left, while for OGSR, the results were comparable between both hands. Additionally, the median OGSR concentrations on the forearms and face were comparable (and generally significantly lower than on the hands). However, higher concentrations were occasionally detected on the face, indicating a potential secondary transfer after the discharge. Concerning IGSR, the number of characteristic particles collected on the left hand was similar to those on the forearms, whereas the face exhibited a lower median count. This confirmed that the two types of GSR exhibit different transfer and persistence mechanisms [39]. IGSR tended to be transferred close to the firearm openings during the discharge (i.e., ejection port on the right side), whereas OGSR may be transferred more homogeneously around the discharge point as similar quantities were found on both hands. However, this could also result from secondary transfer after the discharge between the hands and the face (as indicated by a few very high values obtained from the face specimens). Moreover, some shooter replicas displayed the highest concentrations for OGSR, while no major differences were observed between shooters for IGSR.

Finally, an interesting finding is that the variation from one discharge to another was lower for IGSR (average RSD of 154 %) than for OGSR (average RSD of 204 %). The variation might be explained by various factors. This phenomenon was previously documented in other studies and was attributed to the highly variable discharge process [18–20,31,39]. In this research, several parameters were set for all 16 shots to limit the variation (i.e., firearm and ammunition batch, indoor shooting range environment, cleaning, discharge, and collection procedures). However, certain parameters cannot easily be controlled:

- Firearm handling (e.g., slight vertical or horizontal movements may induce a variation between discharges),

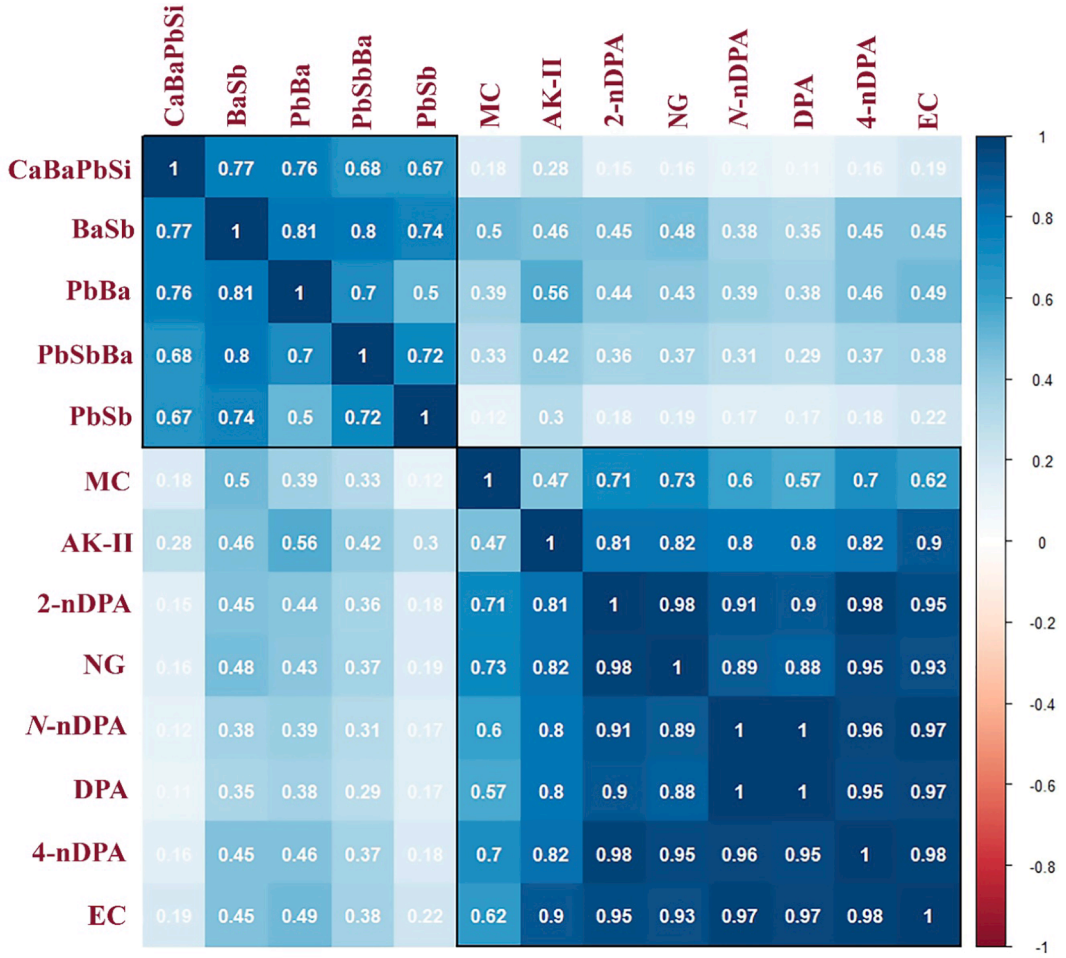

Fig. 14. Corrplot representing the correlation (Pearson) between inorganic and organic compounds detected on the hands, forearms, face, and nostrils of shooters, 30 min after a single discharge.

- Discharge process (e.g., combustion of the smokeless powder, memory effect, cartridge case ejection, mechanisms of IGSR and OGSR formation),
- Shooter receptor affinities (e.g., skin properties, pilosity),
- Activities carried out during the 30 min before sampling (e.g., reading vs. typing).

This underscores the complexity of the GSR formation, transfer, and persistence. Even with the control of numerous parameters, some level of variation in results is inevitable. While further research is required to comprehensively understand GSR transfer and persistence, obtaining representative data applicable to real firearm discharge cases remains particularly challenging. This research demonstrates that each discharge is a one-off event. Therefore, the assessment of the significance of GSR traces can only be specific to a given case context [5,50–53].

Additionally, while OGSR analysis provides complementary information to IGSR, it also requires additional resources and competencies (e.g., extraction procedure, and analysis with a UPLC instrument). Moreover, the extraction procedure may present a risk if not executed correctly. Stubs must be prepared with two carbon adhesives to prevent adhesive lifting, which could hinder subsequent IGSR analysis. This occurred for 10 stubs in this study. Although the additional information provided by OGSR 30 min after a discharge may not seem to justify the added costs and time, it holds promise for the examination of heavy-metal-free ammunition, which may be increasingly encountered on the market due to health and environmental concerns. Such evolution may also impact some of the targeted organic compounds also toxic contained in the ammunition [54–56]. Analysis and evaluation approaches will need to adapt to the future evolution of ammunition formulation.

4. Conclusions

The objective of this research project was to evaluate where and in which quantities IGSR and OGSR persisted 30 min after a discharge. Specimens were collected with carbon adhesive stubs from the right and left hands, forearms, face, and nostrils of four shooters. A stub was collected per region immediately before the discharges (i.e., shooter's blank) and 30 min after (i.e., discharge specimens), for a total of 16 discharges (4 per shooter, each scheduled at least 72 h apart from each other's to minimise the risk of cross-contamination). Each adhesive stub was analysed first for OGSR using a UHPLC-MS/MS, and then for IGSR particles using an SEM/EDS.

The findings revealed that 34 shooters' blank specimens contained both organic compounds and inorganic particles, primarily attributed to the presence of an indoor shooting range in the experimental environment, rather than contamination from poor laboratory practices, as indicated by the negative laboratory blanks. Fortunately, contamination levels in the blanks generally remained below those in the corresponding 30-minute specimens. Considering that conducting such experiments necessitates access to the shooting range and qualified personnel to handle firearms, achieving a contamination-free research environment appears challenging given the restrictions regulating the use of firearms in many countries.

When considering specimens collected 30 min after a discharge, the shooter's right hand generally exhibited the highest amounts of IGSR, while similarly high concentrations of OGSR were detected on both hands. This confirms the relevance of these regions for GSR collection shortly after a shot. In contrast, the nostrils were less informative, exhibiting the lowest quantities for both IGSR and OGSR. Nitroglycerine was the most abundant organic compound, while Arkadite-II was the most frequently encountered. Characteristic PbBaSb particles were the most abundant and frequently encountered IGSR particles.

The results revealed differing transfer and persistence mechanisms for IGSR and OGSR. IGSR particles tended to transfer and persist closer to the ejection port (i.e., right hand), while OGSR compounds exhibited a more uniform transfer and persistence between both hands. This may be explained by a transfer extending to both hands or by a secondary transfer between hands after the discharge. A few face specimens contained a higher amount of OGSR suggesting that secondary transfer can occur after the discharge. The added costs and resources needed to analyse OGSR do not seem to be justified for heavy metal-based ammunition. However, it may become useful when encountering more heavy metal-free ammunition cases.

Finally, the results of this study confirmed the complexity and uniqueness of firearm discharge and GSR production. Although more research is required to gain a deeper understanding of this trace, its highly variable nature requires that evaluation takes into account case-specific contextual information (e.g., used firearm and ammunition, alleged activities before, during, and after the event).

Declaration of generative AI in scientific writing

During the preparation of this work, the authors used ChatGPT-3.5 to improve language, grammar, and readability of the text. After using this tool/service, the authors carefully reviewed and edited the content as needed and take full responsibility for the content of the publication.

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CRediT authorship contribution statement

Virginie Redouté Minzière: Conceptualization, Data curation, Writing – original draft, Writing – review & editing. **Céline Weyermann:** Funding acquisition, Conceptualization, Project administration, Writing – original draft, Writing – review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scijus.2024.08.002.

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