Organic gunshot residues: observations about sampling and transfer mechanisms

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Abstract

This work aimed at studying the sampling, storage, transfer and persistence of organic gunshot residue (OGSR), mainly stabilizers, using liquid chromatography hyphenated to mass spectrometry. Collection using swabs and stubs was compared through sequential sampling in terms of amount of residues left on the hand of a shooter. While stubs collected nearly all residues, swabs left about 50% of the residues on the hands. Moreover, the study of storage conditions after sampling showed that stubs were more stable than swabs and could be held at room temperature without significant compound loss up to two weeks. Then, shooting experiments were performed to evaluate transfer of OGSR. It was not possible to differentiate different brands of ammunition based on a single compound concentration. Moreover, a memory effect was identified when different ammunition was shot using the same firearm. Finally, various exposed skin surfaces and hair as well as clothing were sampled to estimate what surfaces might be the best targets for OGSR sampling by comparing results just after discharge and two hours after discharging a pistol. The results indicated that OGSR were more rapidly lost from hands than from clothing. Moreover, it was shown that the face and hair of a suspect might be contaminated through secondary transfer. Thus, OGSR might remain longer on other skin surfaces, hair and clothing than on the hands of a suspect. As a consequence, sampling should also include clothing, hair and face.

Keywords

Firearm discharge residue; sample collection; swab; stub; LC-MS; persistence

1. Introduction

Sensitive and robust analytical techniques are essential to face an increasing number of incidents related to firearms, for example to link an individual to an incident or to estimate the firing distance. Gunshot residues (GSR) originate from the primer, propellant, lubricants and metals present in the bullet, cartridge and firearm [1, 2]. A distinction is drawn between inorganic (IGSR) and organic gunshot residues (OGSR) [3]. While in practice the analysis of IGSR using Scanning Electron Microscopy Energy-dispersive X-ray spectroscopy (SEM-EDX) is well established in forensic laboratories, complementary information could be obtained from OGSR. Indeed, the introduction of heavy metal-free or "non-toxic" ammunition on the market presents an additional challenge. Furthermore, the composition of the particles produced by this type of ammunition can also be generated by alternative sources [4]. Therefore, the development of a sensitive and robust method able to detect OGSR without compromising the analysis of IGSR by SEM-EDX is of great interest.

The main contribution to OGSR is from propellants that are made up of explosive compounds, as well as a number of additives such as stabilizers, plasticizers or flash inhibitors that confer specific properties to the powder [3]. Based on their explosive content, gunpowders can be single base containing only nitrocellulose (NC), double base containing NC together with nitroglycerine (NG) or triple base containing NC, NG and nitroguanidine [1]. A wide array of analytical techniques were applied to the analysis of OGSR, each with advantages and drawbacks. Nevertheless, no general agreement has been reached on the best technique and research carries on all fronts. Among potential instrumentation, ion mobility spectrometry (IMS) seems promising, because it is a very rapid field technique [5-7]. However, even with considerably improved sensitivity, further confirmatory analysis is required. The past few years have seen the advent of spectroscopic techniques such as Raman [8-12] and Fourier transformed infrared spectroscopy [13, 14] for the analysis of gunpowders and OGSR. However, these instruments do not permit formal compound identification. Furthermore, no quantitative data were obtained and applicability in practice was not demonstrated yet. Mass spectrometry (MS), more particularly desorption electrospray ionization [15, 16] provides fast identification, though matrix effects are a considerable issue and strongly impact sensitivity when no previous separation is performed. Gas chromatography (GC) was found to be highly selective and various detector types were evaluated, namely thermal energy detection (TEA) [17-19], flame ionization detection (FID) [20, 21], nitrogen phosphorous detector (NPD) [22] and MS [6, 17, 19, 23, 24]. However, thermolabile compounds such as NG and *N*-nitrosodiphenylamine are degraded by the high temperatures used in GC, even if detection of NG remains possible due to the high concentrations found in gunpowders and OGSR. Capillary electrophoresis [25-30] was also assessed, but as a result of the low volumes injected and small capillary diameter, it appears not to be sensitive enough to be implemented in casework. Finally, liquid chromatography (LC) [31-37] is more suitable for

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thermolabile analytes, and provided adequate separations. Its coupling with MS detection has largely improved sensitivity, enabling detection of OGSR just after discharge without preconcentration.

To date, irrespective of the instrumentation used, most of the studies focused on the development of a method that was applied to the detection of OGSR sampled just after discharge from the shooter's hand or from a target made of cloth or paper. Regarding sampling, some data is available in recent publications that compared sampling materials [34, 36, 37]. All results indicated a preference towards stubs. Techniques applied to OGSR collection were summarized in a recent review [38]. If analytical techniques and sampling are relatively well documented, little is known of specific forensic questions such as transfer and persistence. As expected, the amount transferred on a target depends on the shooting distance [19]. The amount of GSR escaping in the firearm vicinity is a function of the weapon type, revolvers producing normally more residues than pistols [39]. Arndt *et al.* did not observe OGSR secondary transfer, suggesting different interaction mechanisms with skin in comparison to IGSR [5]. Pertaining to persistence, early studies by Lloyd showed that it was possible to detect NG up to 2.5 hours after discharge of several rounds of ammunition, either from the hand [40] or from a garment [41]. In another study, he stated that NG was detected up to seven hours on hands, face and throat, and even up to five days on clothing [42]. King reported average NG values of 9 ng and 42 ng for swabbing of hands and vacuuming of clothing in casework between April 1991-April 1992 [43]. Northrop did not observe OGSR on the hand of a shooter one hour after shooting three rounds [26]. In 2003, Zeichner *et al.* detected NG on clothing six hours after firing one round [17]. More recently studies from Bell *et al.* indicated that diphenylamine (DPA) might persist on skin around four hours [5]. They also evidenced absorption of OGSR compounds by the skin due to their lipophilic properties [6]. The authors predicted that with the actual limits of quantification of LC-MS, OGSR detection might be possible for nearly 24 hours after a firing event and that the ideal target analytes for recovery from the skin were ethylcentralite (EC), 2- and 4-nitrodiphenylamine.

So far, data was mainly obtained from hand samples. However, some studies also investigated other surfaces and successfully detected NG in the hair of the shooter using either a modified comb or tape-lifting [18, 29]. NG, DPA and EC [32] as well as NG and dinitrotoluenes [17] were also found on clothing sampled by vacuum. Another question of interest relates to the stability of OGSR after sampling, and a study from Yeager *et al.* using swabs indicated a limited storage time from a few days to a few weeks depending on the storage temperature (room temperature and fridge/freezer, respectively) [7]. Finally, the issue of cross-contamination linked to the use of different ammunition in the same firearm might arise, phenomenon sometimes also called memory effect [44]. This phenomenon was already observed for IGSR with particles having mixed composition [45]. In the field of OGSR, Andrasko indicated no contamination from propellant flakes from previous shootings when analyzing OGSR particles recovered on clothing used as target [20]. On the contrary, Northrop

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observed carryover when using different ammunition [26]. As a consequence, additional research is necessary to get more insight into the issues mentioned above.

This study intends to provide some elements of response to questions regarding OGSR sampling, sample storage, transfer and persistence of OGSR. Using LC-MS in positive electrospray ionization (ESI) mode, nine major stabilizers found in OGSR were targeted. This choice was based on the larger number of relevant compounds detected in positive mode, as only nitroglycerine and dinitrotoluenes are analysed in ESI negative. The study was divided in four parts. In the first section, stubs were compared to swabs in terms of collection efficiency using sequential sampling. Zeichner and Levin proposed this approach to evaluate the efficiency of sampling by evaluating what is left after sampling by sampling the surface a second time using another technique [46]. In the second part of the study, storage after sampling was investigated for both stubs and swabs to determine optimal holding conditions. In the third section, shooting experiments were performed to evaluate transfer of OGSR using different ammunition fired by the same firearm. Finally, various exposed skin surfaces and hair as well as clothing were sampled to estimate what surfaces might be the best targets for OGSR potential detection by comparing results just after, as well as two hours after discharging a pistol.

2. Material and Methods

2.1 *Chemicals*

LC–MS grade water containing 0.1 % formic acid, methanol, formic acid, and acetonitrile were purchased from Sigma-Aldrich (Buchs, Switzerland). Diphenylamine was from Fluka (Buchs, Switzerland). Ethylcentralite, *N*-nitrosodiphenylamine, 4-nitrodiphenylamine, akardite II, 1,3-diphenylurea and *N*,*N*-diphenylformamide were obtained from Sigma–Aldrich (Buchs, Switzerland). 2-nitrodiphenylamine was from Alfa Aesar (Karlsruhe, Germany). Methylcentralite was purchased from MP Biomedicals (Illkirch, France).

2.2 *Instrumentation*

The experiments were carried out using an Agilent Infinity 1290 ultra-high performance liquid chromatography (UHPLC) from Agilent Technologies. The instrument was equipped with a binary pump enabling a maximum delivery flow rate of 5 mL/min, an autosampler, and a column compartment thermostated at 40°C. Separation was performed using a Kinetex core-shell column from Phenomenex (2.6 µm, 2.1 mm × 100 mm), with a C18 stationary phase. A SecurityGuard ULTRA cartridge with C18 selectivity was used to protect the analytical column. The UHPLC system was hyphenated to a triple quadrupole mass spectrometer (5500 QTrap) from ABSciex. Electrospray ionization was operated in positive mode. The $[M+H]^+$ of the target compounds were defined as the precursor ions, and quantification was obtained from the SRM measurements. MS/MS parameters are

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given in Table 1. The source parameters were as follows: the desolvation temperature was set to 500°C, the nebulizer gas to 60 psig, the turbo gas to 50 psig and the curtain gas to 25 psig. The IonSpray voltage was adjusted to 5500 V. Data acquisition, treatment and instrument control were monitored using Analyst software.

Table 1: Target compounds and MS/MS parameters

Compound	Parent ion [m/z]	Product ions [m/z]	Declustering potential [V]	Collision energy [V]
Akardite II **(AK II)**	227.1	170.1 91.9	120	27 36
1,3-diphenylurea **(1,3-DPU)**	213	94 77	100	25 48
Methylcentralite **(MC)**	241.2	134.1 105.9	125	24 36
***N*,*N*-diphenylformamide** **(*N*,*N*-DPF)**	198.1	92 65	130	30 54
Ethylcentralite **(EC)**	269.2	147.9 120	120	20 33
2-nitrodiphenylamine **(2-nDPA)**	215.1	197 180.1	80	14 23
4-nitrodiphenylamine **(4-nDPA)**	215.1	197.8 167.1	60	18 47
Diphenylamine **(DPA)**	170.1	93 66	200	32 58
***N*-nitrosodiphenylamine** **(*N*-nitrosoDPA)**	199.1	169 66	60	15 30

The organic mobile phase consisted of acetonitrile containing 0.1% of formic acid. Water with 0.1% formic acid was used as aqueous phase. The following gradient method was used: 35% B (from 0 to 0.5 min), 35–80% B (in 5.5 min), and 80-100% B (in 1 min). The injection volume was 5 µL and the mobile phase flow rate was set to 0.25 mL/min.

A calibration curve was measured for each sequence of experiments to account for instrument response variation from sequence to sequence. Moreover, to avoid any bias linked to that issue and allow comparison, sample series for sequential sampling and stability were acquired during a single sequence.

2.3 *Shooting experiments*

Shooting sessions were conducted in an indoor shooting range located in a specific building sector with the ventilation turned off. Extraction and analysis of the samples was performed in a separate laboratory in another sector to avoid any contamination of the samples. A semi-automatic 9 mm Parabellum Sig Sauer P226 was used for all experiments. 9 mm Luger cartridges, all containing heavy

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metals, were from Geco (batches 62 QS and 54 G K096), Fiocchi, PMP and RUAG Ammotec (Swiss military ammunition). The shooter was asked to wash his hands before entering the shooting range and was not allowed to touch any surface except for the firearm at the time of firing. Another person was in charge of loading the gun. Then, the shooter was asked to hold the gun with both hands and fire one cartridge. He was then sampled outside the shooting range. After sampling, he washed his hands carefully again before starting the procedure once more. The firearm was not cleaned between shots. In this study, shooters were three women, aged between 27 and 40, having mid-long hair and not using hand cream or hair gel.

2.4 *Sampling of OGSR*

Two sampling methods were compared in the study: swabs and stubs. ESD polyester swabs were from ITW Texwipe (Netherlands). Uncoated aluminium stubs were from Plano (Germany). This collection device consisted of a metal stub 12.5 mm in diameter inserted in a plastic vial with a screwed cap. Carbon tape is commonly used for IGSR collection, but in this study double sided tape 665 from 3M (USA) was used instead. Hand sampling by swabbing was carried out by moistening the swabs with ethanol and scrubbing the right hand surface (back of the hand and surface between thumb and index finger) repeatedly. With the stubs, 50 dabbings were applied to the same part of hand as for swabs and 200 dabbing for sampling hair and clothing following recommendations from Zeichner *et al.* [46]. To avoid any bias when comparing swabs and stubs, the surface area sampled from the hand was the same with both techniques. After sampling, both swabs and stubs (the adhesive was removed from the stub) were transferred to a 1.5 mL vial, extracted using 1 mL MeOH, ultrasonicated during 15 minutes at ambient temperature and finally centrifuged.

For the sequential sampling, the procedure was the same as described above with an additional sampling step using a second collection material different from that of the first sampling. Thus, either a swab-stub or a stub-swab sequence was applied. After employing both sampling methods, the shooter washed his hands carefully before repeating the same procedure again.

For spent cartridge analysis, 500 µL of MeOH was poured in the cartridge and the liquid was pipetted a few times to homogenise the solution. It was then decanted in a vial and 500 uL of MeOH were further added to reach the same volume as for swab/stub samples. Finally, it was ultrasonicated and centrifuged following the same protocol as for swabs and stubs.

2.4 *Stability studies*

In order to gain knowledge regarding the storage conditions and degradation of OGSR collected onto swabs and stubs, two stability studies were carried out. The first considered spiked samples and the second used samples obtained from shooting sessions. Spiked samples have the advantage of sample

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standardisation, providing improved repeatability. Nevertheless, spiking a target analyte mixture onto a sampling material does not take into account relative proportions between particulate and vapour deposits of OGSR. It was still considered useful to provide a first estimation of compound losses over time due to evaporation and/or degradation. It was then necessary to evaluate the storage of real samples, even if the variability induced by the firearm discharge cannot be controlled.

The spiking experiments were only performed on swabs, because spiking liquid onto a stub is too different from the real sampling process (*i.e.* dry physical process). A mix of the nine compounds reported in Table 1 was prepared at a concentration of:

- 100 ppb for AK II, EC, MC and 1,3-DPU
- 200 ppb for 2-nDPA, 4-nDPA, *N*-nitrosoDPA and *N*,*N*-DPF
- 5000 ppb for DPA

Ten µL of solution was spiked onto the swab and left to dry for five minutes before cutting the handle, placing the polyester part of the swab inside a 1.5 mL screw vial and closing it tightly. Three conditions, namely ambient temperature (20 °C), fridge (4 °C) and freezer (-20 °C) were evaluated to check for potential influence of the temperature on evaporation/degradation of the compounds. Five samples were prepared for each condition at day 0, 1, 3, 7, 10 and 15. All samples were then extracted as described in section 2.3 and analysed in the same LC-MS sequence.

For the stability experiments with samples acquired during shooting sessions, both swabs and stubs were evaluated. Eight samples per time point were taken from the right hand of the shooter to account for the higher variability compared to spiking experiments. A semi-automatic 9 mm Parabellum Sig Sauer P226 and 9 mm Luger cartridges from the same batch of Geco ammunition were used. Samples were collected at day 0, 7 and 14 for swabs, and day 0, 9 and 16 for stubs. All samples were then extracted as described in section 2.3 and analysed in the same LC-MS sequence.

3. Results and Discussion

3.1 *Collection efficiency of swabs versus tape-stubs: sequential sampling*

As mentioned in the introduction, various studies compared the efficiency of sampling materials for IGSR and OGSR resulting in materials being ranked according to their collection efficiency. Nevertheless, in the case of OGSR, the experiments only estimated the amount of compounds collected and not the remaining residue on the hands after sampling. As the quantity of OGSR produced during discharge is highly variable, it is not possible to determine the absolute recovery. Indeed, the total amount deposited on any surface is unknown and not reproducible. However, by estimating how much is left after sampling using another material/method might give some insight

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into the collection efficiency mechanism. In 1993, Zeichner and Levin used sequential sampling to compare swabbing with stubbing for IGSR sampling from hair and showed that both methods were comparable [46]. Here, after a first sampling using either a polyester swab or a tape stub, a second sampling was carried out using the other method to see how much OGSR could still be collected. The results obtained for EC are presented in Figure 1. Detailed information for other molecules can be found in the Supplementary Information (AK II, DPA, *N*-nitrosoDPA, 2-nDPA and 4-nDPA).

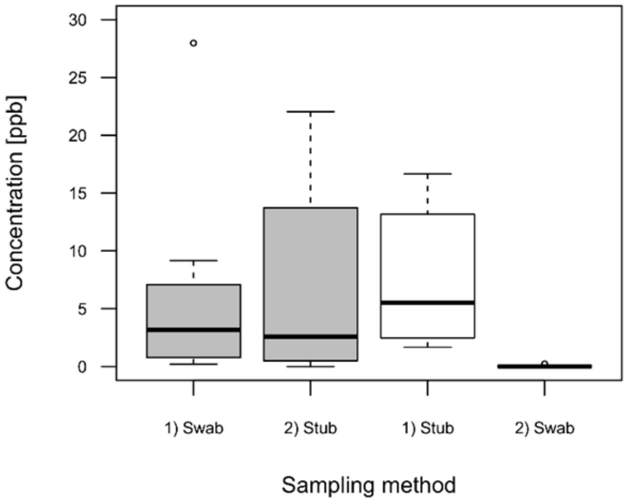

Figure 1: Boxplots illustrating the concentration of EC recovered from the right hand of the shooter after one pistol discharge (n = 8). In grey (left), the sampling sequence is swab and then stub. In white (right), stubbing was used before swabbing. Geco gunpowder was used. Concentration values in ppb are equivalent to amounts in ng.

From Figure 1, it is obvious that when swabbing is performed just after firing, recovery is probably not higher than 50%, as the stub applied to the hand during the second sampling was able to collect about the same amount of EC. However, when tape-stubbing was performed first, the swabs were unable to collect such a high amount of OGSR during the second sampling, as only trace amounts were detected. Results are only shown here for EC, but values obtained for the other compounds detected showed the same trend. While data relative to the physical state in which OGSR are deposited is still missing, it is suspected that part of OGSR is deposited as vapour and part as particles. Therefore, a specific sampling method might be more adapted to one physical state. As swabbing uses a solvent, it is probably better suited to sample vapour deposits because the amount of solvent might not be sufficient to dissolve particles completely. When swabbing is performed, it is possible that the swab moves particles on the hand and does not capture them. That would explain why such a big amount of OGSR can still be sampled with the stub afterwards. In this regard, the stub would be more efficient to collect particles similarly to IGSR particles. The amount collected by the swab after stubbing was very low. This might indicate that the amount of OGSR deposited as vapour is relatively low or that the adhesive collected skin flakes which could have absorbed vapour deposits [46].

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It is also not clear why the total amount recovered from the hand using the sequence swab-stub is higher than the sequence stub-swab (mean 13.2 ppb vs 7.6 ppb and median 9.4 ppb vs 5.5 ppb). Indeed, the median of the first sampling is more or less the same for both swabs and stubs, whereas the medians for the second sampling are significantly different. While it might be explained by the high variability in OGSR production and deposition as illustrated in Figure 2, there is no obvious explanation for this difference.

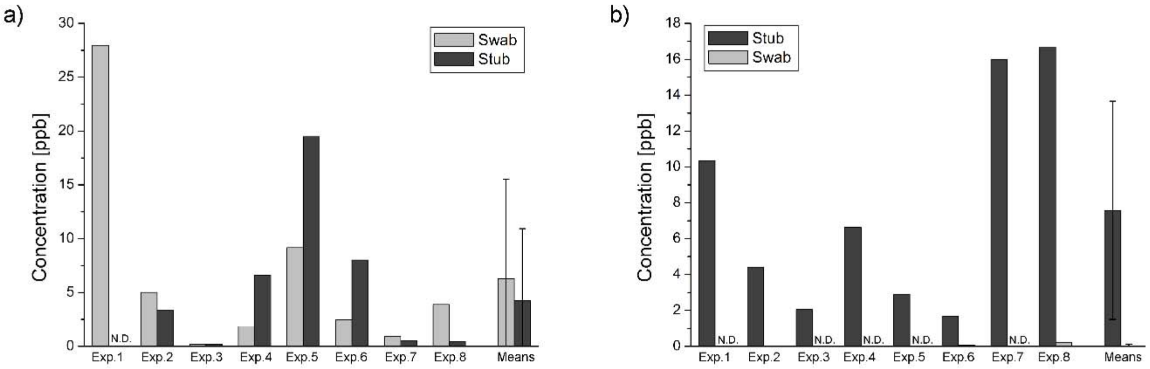

Figure 2: Bar plot illustrating the variation in EC amount recovered from the right hand of the shooter (n = 8). On the left, the sampling sequence is swab and then stub. On the right, stubbing was used before swabbing. Geco gunpowder was used. Exp. is for experiment and N.D. for not detected. Concentration values in ppb are equivalent to amounts in ng.

One could wonder if stubbing might hinder subsequent sampling with a swab. However, an alternative explanation would be that total recovery depends on three experimental parameters: collection efficiency, MeOH extraction efficiency and matrix effects. For each sampling method, each step might be different and thus influences total recovery. Consequently, one must be cautious when comparing average values, as the effects observed are not solely due to collection. However, it is clear that when swabbing is performed first, the amount recovered by stubs later is sometimes lower and sometimes higher than what was collected by the swab, indicating that collection efficiency is lower for swabs than stubs, as the amount collected by swabs after stubbing is always lower when using the stub first.

In the course of a shooting session, the amount recovered during the first sampling varied considerably. Depending on the molecule, the lowest relative standard deviations were about 50% and reached up to 150%. For example, the first experiment in the sequence swab-stub produced an extraordinarily intense peak with a concentration superior to 25 ppb, statistically considered as an outlier (Grubbs' test at 95%, $G = 2.346 > G_{crit} = 2.126$) when constructing the boxplots of Figure 1. The rest of the sequence lead to values lower than 10 ppb. In the stub-swab sequence, no outlier was identified, but the means of the EC concentrations for the whole sequence, illustrated in Figure 2, confirm the high variability of OGSR deposition. It was relatively common during a shooting session

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to observe a large particle on the hands, but its production was completely random. Another way to look at these results is by calculating the following sampling ratio (SR):

$$SR\ [\%] = [EC]_{sampling1} * 100 / ([EC]_{sampling1} + [EC]_{sampling2}) \qquad \text{(Eq. 1)}$$

The values for the sequence swab-stub ranged between 1 and 100% (mean 49% and median 46%), whereas the reverse sequence provided values between 95 and 100% (mean 99% and median 100%). This shows that about 50% of OGSR was recovered when swabbing, whereas nearly 100% was recovered with stubs. These results also highlight the fact that robust statistics should be used when dealing with OGSR data. Means are strongly affected by extraordinary values, while medians are less influenced.

Geco gunpowder produced a lot of residues compared to other tested gunpowders. A number of particles were visible to the naked eye on the hand, inorganic and organic confounded. The experiments were thus repeated with a gunpowder from Fiocchi producing less residues to confirm the results (Figure 3).

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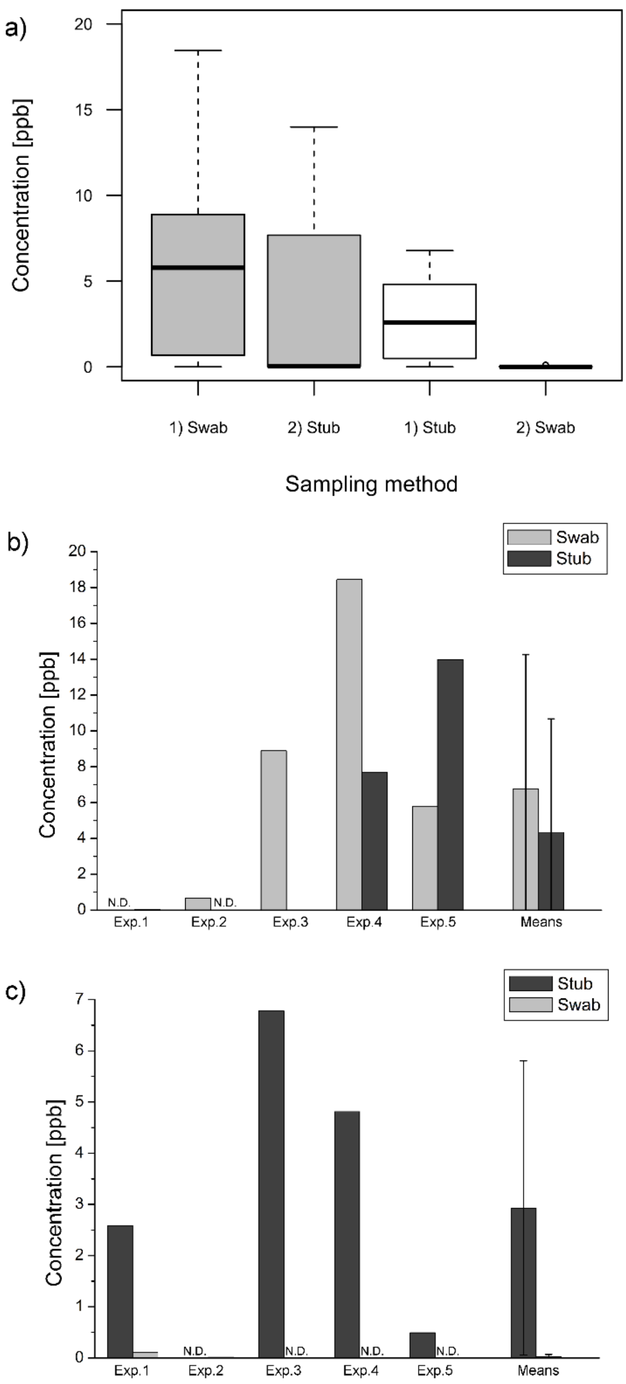

Figure 3: a) Boxplots illustrating the concentration of EC recovered from the right hand of the shooter after one pistol discharge (n = 5). In grey, the sampling sequence is swab and then stub. In white, stubbing was used before swabbing. b) Bar plot illustrating the variation in EC amount recovered from the right hand of the shooter (n = 5) using the sampling sequence swab-stub. c) Same as b) but using the sequence stub-swab. Exp. stands for experiment and N.D. for not detected. Concentration values in ppb are equivalent to amounts in ng. Fiocchi gunpowder was used.

The same trend was observed for Fiocchi gunpowder, even if the amount of OGSR collected with the stub was lower than for Geco gunpowder. The results obtained for AK II are shown in Supporting

Information as these were the only target compounds detected in this gunpowder (Table 2). These results confirm that stubs might indeed be better suited to collect OGSR. This sampling method did not leave residues on the hand and would moreover be far easier to apply to sampling on clothes and hair. It is also a well-established method for the routine sampling of IGSR within forensic laboratories and police services.

3.2 *Stability studies*

When samples are collected, it is not always possible to analyse them immediately. Thus, the optimal storage conditions have to be determined in order to avoid compromising the sample. A recent study from Bell *et al.* showed that OGSR compounds evaporate relatively quickly [7]. In this work, an exploratory study was first carried out with swabs spiked with a mix of target analytes. Three conditions, namely ambient temperature (20 °C), fridge (4 °C) and freezer (-20 °C) were evaluated to check for potential influence of the temperature on evaporation/degradation of the compounds. Then, with regard to the results obtained in the previous section and the potential physical state in which OGSR are predominantly collected, stability of the target molecules was studied for both polyester swabs and tape stubs.

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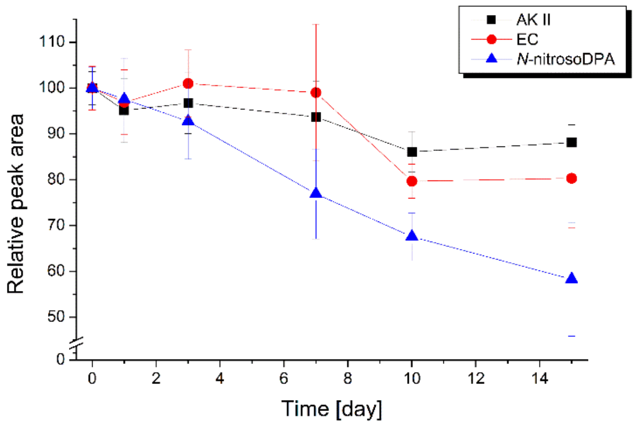

Figure 4: Analyte concentration losses for swabs spiked with a solution of target analyte mix at ambient temperature over a two week period (n = 5).

As illustrated in Figure 4 for samples stored at ambient temperature, the extent of analyte loss on spiked swabs depends on the family of compounds. DPA-related analytes decrease was more marked than for the other molecules. For *N*-nitrosoDPA, the loss reached more than 40% after 15 days, whereas for AK II, this value was limited to about 10%. These results are in accordance with those obtained by Yeager *et al.* [7] in the sense that a trend towards lower values was observed. However, the extent of the loss was lower than reported in their study, indicating losses superior to 50% for

DPA, EC and MC over nine days. At lower temperatures (fridge and freezer), no significant decrease was detected in our samples after 15 days (Table 2).

Table 2: Analyte concentration losses for swabs spiked with a solution of target analyte mix: ratio of the peak area at 15 day to the peak area at time zero for the three temperatures studied.

Temperature	**AK II**	**EC**	***N*-nitrosoDPA**	**1,3-DPU**	**MC**	***N,N*-DPF**	**2-nDPA**	**4-nDPA**	**DPA**
Ambient (20 °C)	88.2%	80.3%	58.3%	102.0%	68.3%	67.7%	66.8%	66.8%	63.6%
Fridge (4 °C)	98.2%	97.9%	97.2%	102.9%	94.9%	106.2%	100.5%	99.7%	108.8%
Freezer (-20 °C)	95.0%	101.2%	99.6%	100.1%	94.9%	105.5%	103.5%	99.8%	108.2%

Indeed, at -20 °C and 4 °C, the ratios between peak areas at day 15 and day zero were mostly superior to 95% with RSD around 5%, so the decrease was not significant. Values larger than 100% are due to measurement variation. Thus, storage in a fridge is recommended for that type of samples. Yeager et al. observed a decrease in concentration even when the sample was stored in a freezer. However, the concentration at which the study was carried out was not the same (lower in the present case in order to be close to amounts that might be encountered in casework). Moreover, in the present work, swabs were stored in the vial where the extraction further took place, whereas in Yeager’s study the samples were stored in a Petri dish. The different protocols might thus significantly influence the results.

A series of shooting experiments was then performed in order to evaluate the stability of real samples containing a biological matrix susceptible to degrade the compounds of interest. Both swabs and stubs were evaluated to check for potential differences. Eight samples were collected from the right hand of the shooter and the samples were stored at room temperature and analysed in the same analytical sequence (Figure 5, Table 3).

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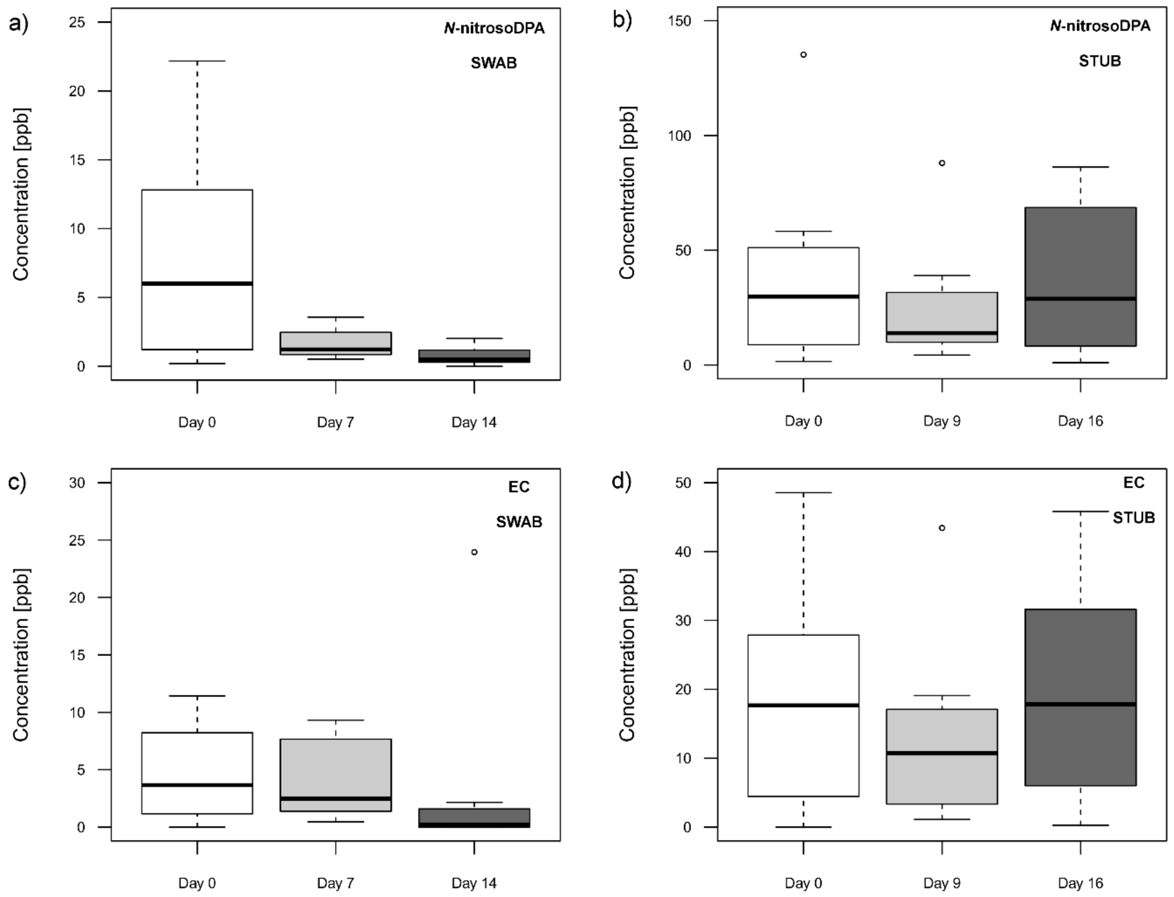

Figure 5: Stability of samples collected from the right hand of a shooter from swabs (left column) and stubs (right column). Data are presented for *N*-nitrosoDPA and EC and were obtained using Geco ammunition (n = 8). Samples were stored at room temperature. Concentration values in ppb are equivalent to amounts in ng.

Table 3: p-values calculated for *N*-nitrosoDPA and EC for data from Figure 5. w/o stands for without.

	***N*-nitrosoDPA swab**	***N*-nitrosoDPA stub**	**EC swab**	**EC stub**
p-value (all points)	0.0098	0.7013	0.9118	0.6898
p-value (w/o outliers)	-	0.2957	0.0338	0.3084

A different trend can be observed for both sampling methods. Indeed, after two weeks, there is a significant decrease in concentration for both *N*-nitrosoDPA and EC in swabs (if not considering the outlier), whereas the concentrations are similar for stubs. Similar results were obtained with the other molecules. The decrease seems to happen faster for *N*-nitrosoDPA than EC, in accordance to what was observed for spiked samples (Figure 4). It is not clear why there is a difference in stability. However, the physical state of the OGSR might explain these results. During swabbing, OGSR are probably dissolved by the solvent and are more prone to evaporation than particles. Studies from Bell *et al.* showed that evaporation is a relatively fast process for some compounds such as DPA and losses occur in a matter of hours [6]. If stubs collect mainly particles, these seem less affected by degradation than what is collected by swabs. In conclusion, storage time should be chosen according to the sampling method. Stubs seem to have a better stability than swabs, at least one week longer. However, as a precaution, all samples shall be stored in the fridge and be analysed relatively rapidly after collection.

3.3 *Transfer of OGSR on the right hand of the shooter using different ammunition*

In order to study the influence of the chemical composition of a gunpowder on detected OGSR, five different gunpowders were used in shooting sessions. Table 4 presents the main target compounds detected in the gunpowders.

Table 4: Target analytes detected in gunpowders used in shooting sessions. Presence is indicated in grey.

	AK II	**EC**	**DPA**	***N*-nitrosoDPA**	**2-nDPA**	**4-nDPA**
PMP		x				
Geco (batch 62 QS)	x	x	x	x	x	x
Fiocchi	x	x				
Geco 2 (batch 54 G K096)	x	x	x	x	x	x
RUAG Ammotec		x	x	x	x	x

A series of five discharges were carried out for each ammunition type, except for the most recent batch of Geco ammunition (Geco 2, n = 8) (Figure 6).

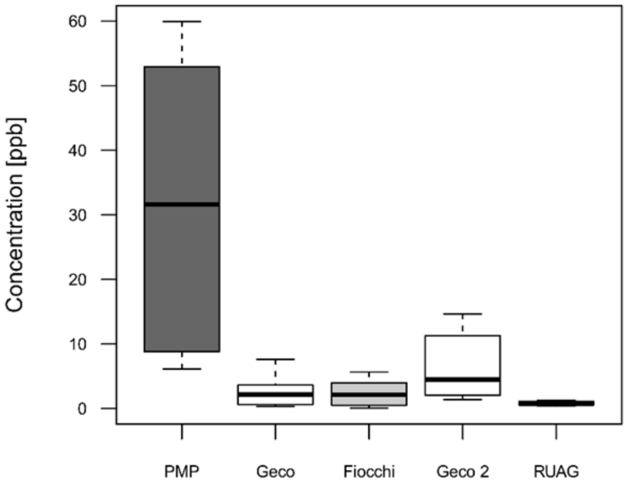

Figure 6: Boxplots illustrating the concentration of EC recovered from the right hand of the shooter after one pistol discharge for five types of ammunition (n = 5 except for Geco 2 for which n = 8). Concentration values in ppb are equivalent to amounts in ng.

For EC, the amounts of OGSR detected for each ammunition were similar, except for PMP. However, the concentration of EC in this gunpowder was much higher than for the other gunpowders. Due to the high variability in OGSR production and recovery, it seems difficult to distinguish OGSR from

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different gunpowders based on the concentration of a specific compound. However, the presence of a specific compound can be a good indication for qualitative comparison with ammunition that might be discovered at a suspect's place for example. Exceptions can arise as results obtained for OGSR produced by PMP ammunition were not consistent with the composition of the gunpowder. Indeed, AK II, DPA, *N*-nitrosoDPA, 2-nDPA and 4-nDPA were detected, whereas these compounds were absent from the gunpowder. Discharges of PMP ammunition were performed after shooting of a series of Geco cartridges. The pistol was not cleaned in-between (Figure 7).

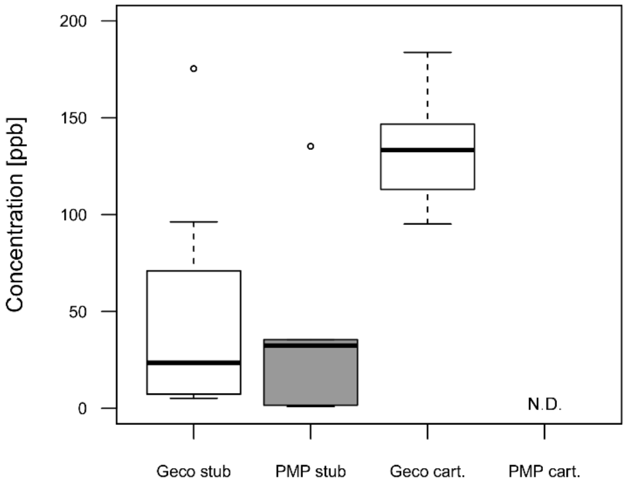

Figure 7: Boxplots illustrating the concentration of DPA recovered from the right hand of the shooter after one pistol discharge when eight cartridges of Geco ammunition were discharged followed by the discharge of five cartridges of PMP ammunition. The spent cartridges were also analysed for comparison. Concentration values in ppb are equivalent to amounts in ng.

From Figure 7, it is obvious that the DPA recovered from the hand of the shooter did not originate from the PMP ammunition, as this molecule was detected neither in the spent cartridge nor in the gunpowder (Table 4). However, this molecule was a major component of Geco gunpowder. This demonstrates that a memory effect between shots cannot be excluded as already observed by Northrop [26].

To estimate if this interaction came from particles present in the barrel, the latter was scrubbed with a cotton bud to remove particles and soot before another experiment was carried out alternating Geco and PMP ammunition (Figure 8).

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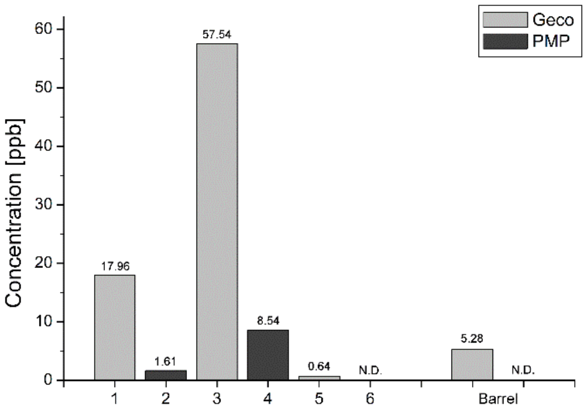

Figure 8: Bar chart illustrating the concentration of DPA recovered from the right hand of the shooter after one pistol discharge when alternating discharge of Geco and PMP ammunition (n = 3). Horizontal numbers on the axis indicate the discharge number. A sample from the end of the barrel was also analysed for comparison after shots 1 and 2. Concentration values in ppb are equivalent to amounts in ng.

By cleaning the barrel in-between discharges, the extent of memory effect was reduced, but did not completely disappear. A thorough cleaning by completely dismounting the firearm might be the only way to get rid of this interaction. However, in real cases, the suspect might not be very fussy about firearm cleaning and potential memory effect must be taken into account when interpreting OGSR evidence. Following these observations, proper identification of the ammunition batch used in a case might be impossible if maintenance of the firearm is not carried out on a regular basis and different types of ammunition are regularly used.

3.4 *Sampling of other exposed skin surfaces, hair and clothing*

To date, most studies used samples collected from the hands of the shooter or from a target. As evidenced by the analysis of IGSR, large particles are rapidly lost from the hands due to activity such as hand washing, lessening the chances to later recover GSR. Moreover, as previously mentioned, evaporation and skin permeation of OGSR will also affect recovery. As a consequence, other exposed surfaces that are less frequently washed, such as face and hair are potentially interesting targets. Some crime scene units already have protocols for IGSR collection from eyebrows and hairline. Clothing is also of great interest, as the fibres might better retain particles than skin. A few experiments were carried out to see where OGSR can be detected at time zero and two hours after discharging three rounds of ammunition. The shooter was wearing a long-sleeve sweater made of cotton and polyester. Sampling was performed on the sleeves up to the elbow and on the part of hair closest to the forehead. For the time interval between discharge and sampling, the shooter was working on her computer. She was allowed to walk inside the building and touch her face/hair, but not to wash her hands. Tables 5 and 6 show detected compounds.

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Table 5: Molecules detected just after discharge of three rounds of Geco ammunition (n = 1). Presence is indicated in grey. The LOD for each compound is also indicated.

	AK II	**EC**	**DPA**	***N*-nitrosoDPA**	**2-nDPA**	**4-nDPA**
LOD [ng/mL]	0.01	0.005	0.5	0.02	0.02	0.02
right hand	x	x	x	x	x	x
left hand	x	x	x	x	x	x
right sleeve	x	x	x	x	x	x
left sleeve	x	x	x	x	x	x
right eyebrow + forehead		x		x		
left eyebrow + forehead	x	x			x	x
hair	x	x		x		

Table 6: Molecules detected two hours after discharge of three rounds of Geco ammunition (n = 1). Presence is indicated in grey.

	AK II	**EC**	**DPA**	***N*-nitrosoDPA**	**2-nDPA**	**4-nDPA**
right hand	x	x	x	x	x	
left hand		x		x		
right sleeve	x	x	x	x	x	x
left sleeve	x	x	x	x	x	x
eyebrows + forehead	x	x	x	x	x	
hair	x	x		x	x	

Just after discharge, all main compounds of the gunpowder were detected on both hands and pullover sleeves. In hair and on the face, fewer compounds were detected. Two hours after discharge, all the compounds were detected on the sleeves only. However, more compounds were detected on the face than just after discharge. Figure 9 presents the concentrations recovered from the different exposed skin surfaces, hair and clothing for EC and 2-nDPA.

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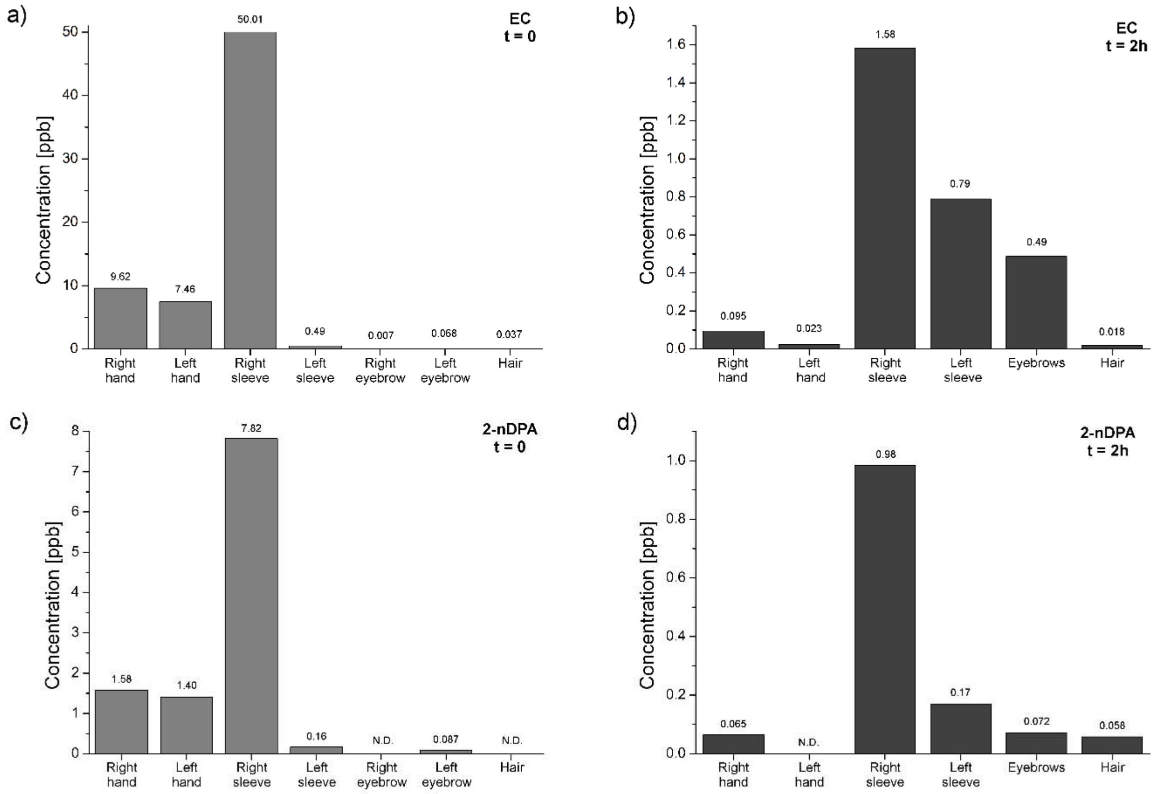

Figure 9: Concentrations of EC and 2-nDPA detected on various exposed skin surfaces, hair and clothing just after (left column) and two hours after (right column) discharge of three rounds of Geco ammunition (n = 1). Concentration values in ppb are equivalent to amounts in ng.

At both time zero and two hours, the highest amount of EC and 2-nDPA was recovered from the right sleeve of the shooter's sweater. Regarding the distribution of concentrations, it can be seen that the concentration on hands strongly dropped, by a factor of 100 for EC and superior to 20 for 2-nDPA. As a comparison, the ratio is only 31 and 8 for the right sleeve. Hands were not washed during these experiments. Thus, results would be worse if hands were washed carefully. It is probable that no more OGR would be detected. The quantities detected on the eyebrows and hair were relatively low, but their concentration did not decrease much after two hours. This indicates that transfer might actually occur after discharge when the shooter touches his face and hair with hands contaminated with OGSR. More data on OGSR transfer is required to determine which surface might be the one to receive most OGSR and what minimal and maximal amounts can be expected. However, from these data, it is clear that OGSR are more rapidly lost from hands than from clothing. Thus, due to longer persistence, there might be more chances to detect OGSR on clothing than on hands. Nonetheless, one must keep in mind that the evidential value associated to OGSR detection also depends on the location where these were discovered. Thus, GSR on clothing is less meaningful for linking a suspect to a discharge than GSR detected on hands, face or hair. Hence, it is essential to sample all relevant surfaces and not only clothing.

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4. Conclusions

This study intended to provide some information regarding OGSR sampling, sample storage, transfer and persistence. In the first part stubbing was compared to swabbing using sequential sampling. The results evidenced a very high variability for both techniques, associated to OGSR production rather than sample collection. When swabbing was performed before stubbing, the swab collected only about 50% of the total amount recovered, whereas this value was close to 100% for the reverse sequence. Stubbing was thus considered a better sampling technique as it left nearly no residues on the hand. In the second part of the study, storage conditions were investigated after sampling using both stubs and swabs. Here again, storage time was dependent on the sampling method. Stubs were more stable than swabs and could be held at room temperature without significant compound loss for two weeks. For longer storage times, it might be advisable to keep samples in the fridge.

Shooting experiments were then performed to evaluate transfer of OGSR using different ammunition. The variability in quantities detected did not enable the distinction between ammunition based on a single compound. Moreover, when shooting various ammunition with the same firearm, a memory effect was detected. In the present case, DPA-related compounds were detected in hand samples even if these compounds were absent from the gunpowder. A quick cleaning of the barrel using a cotton bud was not sufficient to remove all traces from the previous shot and the possibility of multiple ammunition usage should be taken into account if analyzing OGSR with a view to possibly link it to a gunpowder. Finally, various exposed skin surfaces and hair as well as clothing were sampled to evaluate what surfaces would be the best targets for OGSR collection by comparing results just after discharge and two hours after discharging a pistol. The results indicated that OGSR were more rapidly lost from hands than from clothing. Moreover, it was shown that the face and hair of a suspect might be contaminated through secondary transfer. Thus, OGSR might remain longer on other skin surfaces, hair and clothing than on the hands of a suspect. As a consequence, sampling should not be limited to hands and also include clothing, hair and face. Also, OGSR concentrations detected after two hours were relatively low compared to what can be detected just after discharge. As the limits of detection were already reached after two hours for some molecules, it will be necessary to develop a preconcentration technique to evaluate persistence in a thorough study.

Obviously, many variables can modify the transfer and persistence of OGSR. Factors such as gender, skin type (pH, humidity, sweating, and so on), pilosity, age or hair type/length can possibly affect the detection of OGSR. External factors such as cosmetics might also play a role. Moreover, ammunition and firearm type as well as weather might influence transfer. Finally, activity of the suspect as well as passive processes such as evaporation and skin absorption will impact persistence. This work is therefore a first step into the wide panel of factors interacting during transfer and persistence of OGSR. More studies will be necessary to acquire more knowledge about these phenomena.

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Acknowledgements

The authors wish to acknowledge the Swiss National Foundation (no PP00P1_150742) for financial support. They also wish to thank the Bundeskriminalamt (BKA), particularly Dr Rüdiger Schumacher and Dr Dieter Kirsch, for fruitful discussions.

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