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Transmission Electron Microscopy Study of Gunshot-Residue Nanoparticles Collected in Air Samples

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KEYWORDS

Air sampling, energy dispersive X-ray spectroscopy (EDS), nanoparticles, gunshot residue (GSR), transmission electron microscopy (TEM)

ABSTRACT

Traditional gunshot residue (GSR) is usually defined as opaque, individual particles having a characteristic spheroidal shape and composed of the elements lead (Pb), barium (Ba) and antimony (Sb). Routine GSR analysis is performed by scanning electron microscopy (SEM) coupled with energy dispersive X-ray spectroscopy (EDS) and concentrates on particles that are 0.5 μm or larger with diameters less than 5 μm. The purpose of this study was to determine if GSR particles with diameters in the nanometer-size range (10 nm–100 nm) are released into the atmosphere during the discharge of a firearm, and whether transmission electron microscopy (TEM) coupled with EDS is suitable for the detection and analysis of GSR nanoparticles in air samples.

INTRODUCTION

Experiments were performed using TEM-EDS to collect and characterize the GSR particles released in the air during the discharge of a firearm. A total of nine samples of GSR were generated by firing five rounds of Browning High Powered, full metal jacket (FMJ) and Sellier and Bellot (S&B) ammunition from a 9 mm handgun and three rounds of Winchester WinClean ammunition, also from a 9 mm handgun, in an enclosed facility. Air samples were collected simultaneously with the firing of each round of ammunition. The air pumps and filters were strategically set up at varying distances from the muzzle and ejection port of the handgun for each sample that was collected. An ambient air sample was collected 12 hours after firing the initial five rounds of ammunition.

METHODS

Sample Collection

The GSR samples were collected on 0.45 μm pore size, 25 mm diameter, mixed cellulose ester (MCE) filters and prepared according to procedures described in the NIOSH 7402 method for TEM. Each GSR sample was collected by pulling 10 liters (L) of air through each MCE filter with a high volume pump. The samples were collected with a 25 mm cassette in the open face position. The ambient air sample was collected by pulling 150 L of air through each MCE filter with a high volume pump. The description and location for each air filter in relation to the firearm is listed in Table 1.

Sample Preparation

Each sample was prepared according to NIOSH Method 7402, where a portion of the filter is cut, placed on a glass slide and collapsed using acetone in a Jaffe washer (1). The collapsed air filters were then coated with carbon using a Denton vacuum evaporator, and portions of the carbon-coated filter were cut and

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Table 1. GSR Sample Descriptions and Locations

Sample No.	Ammunition	Sample Description	Air Volume (Liters)
1	S&B	Level with muzzle, 4" to right and 20" from muzzle	10
2	S&B	6" right of ejection port, 4" lower than ejection port	10
3	S&B	36" from muzzle, 5" to the right	10
4	S&B	2" from muzzle, 1" down and 1" to the right	10
5	S&B	By ejection port but closed after firing, to the right of port	10
6	Not available	Ambient sample taken 12 hours after shots 1–5.	150
7	WinClean	6" to the right, open ejection port, 1" down (open breeze)	10
8	WinClean	36" in front of the muzzle	10
9	WinClean	24" to the front of the ejection port, 32" to the right	10

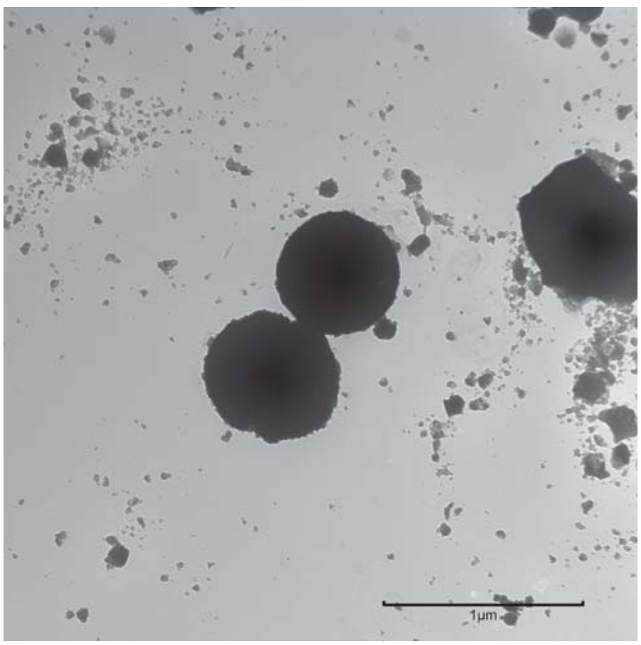

Figure 1. TEM image of particles collected on the filter during the discharge of one round of S&B ammunition. Scale bar = 1 µm.

placed on TEM grids. The filters were dissolved using dimethyl formamide and acetone, leaving only the particles that were left on the grid encased in the carbon thin film. TEM grids come in several different materials such as nickel, aluminum, gold, titanium and stainless steel; however, for this experiment each sample was prepared on copper-mesh TEM grids.

Sample Analysis

For each GSR sample, 10 fields of view were imaged at an instrument magnification of 25,000x. The electron micrographs were generated using a Philips CM 120 TEM operated at 100 kV with a bottom mount 4-megapixel digital camera. The microscope was calibrated using the MAG*I*CAL® calibration reference standard for TEM (2). The images were annotated with a scale bar with the pixel length determined by the MAG*I*CAL®. Each annotated image was opened in ImageJ software, and the magnification scale was set using the scale bar in the image (3). The particles in each image were sized using the "threshold" feature in ImageJ. The "watershed" feature in ImageJ was also used for some images to separate the particles that appeared to be touching each other. The elemental composition of select particles was determined using an Oxford Inca EDS.

RESULTS

Samples 1–5: S&B Ammunition

Samples 1–5 consisted of particles that were both spheroidal and non-spheroidal in morphology, as shown in Figure 1.

For Sample 1, a total of 4,177 particles were counted and sized using ImageJ. Of the 4,177 particles sized, 95.7% are in the range of 5 nm to 100 nm, 4.29% are between 100 nm and 1 µm, and 0.01% are greater than 1 µm in diameter. For Sample 2, a total of 1,082 particles were counted and sized using ImageJ. Of the 1,082 particles sized, 95.1% of the particles sized are between 5 nm and 100 nm, 4.9% are between 100 nm and 1 µm, and 0% are greater than 1 µm in diameter. Sample 3 resulted in a total of 2,326 particles that were counted and sized. Of the 2,326 particles sized, 90.9% range between 5 nm and 100 nm in diameter, 9% range between 100 nm and 1 µm, and 0.1% are greater than

Table 2. Particle Size Distribution of S&B Samples

Particle Diameter Size Fractions	No. of Particles
5 nm–50 nm	6,875
50 nm–100 nm	1,520
100 nm–500 nm	476
500 nm–1 µm	15
>1 µm	4

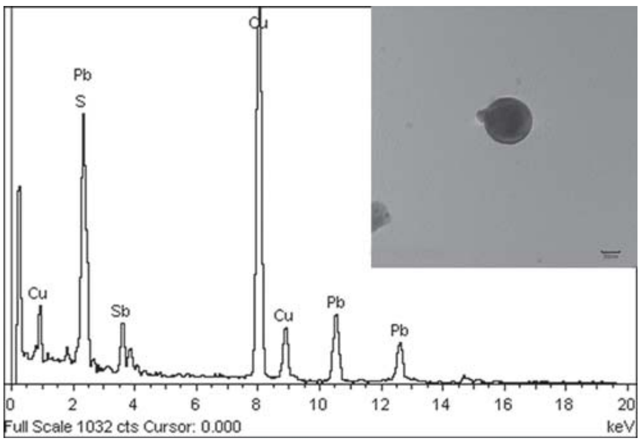

Figure 2. TEM image and EDS spectrum of a nanoparticle composed of Pb and Sb from the S&B samples. Scale bar = 30 nm.

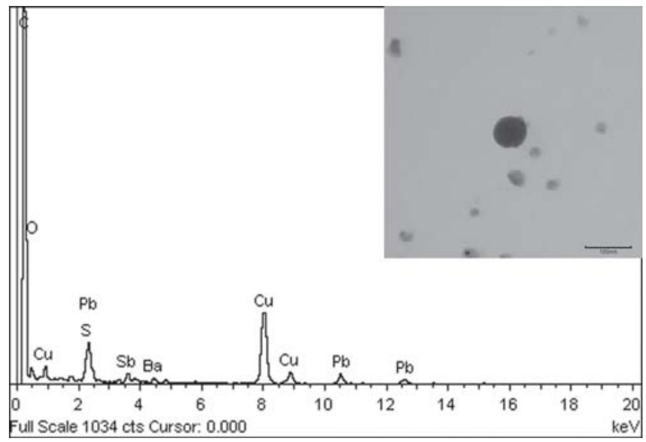

Figure 3. TEM image and EDS spectrum of a GSR nanoparticle found in the S&B samples. Scale bar = 100 nm.

1 µm in diameter. For Sample 4, the filter was damaged during the blast and no data was generated. For Sample 5, a total of 1,305 particles were sized. Of the 1,305 particles sized, 95% range between 5 nm and 100 nm, 4% range from 100 nm to 1 µm, and 1% are greater than 1 µm in diameter. The particle size distribution data for all particles sized in Samples 1–5 is listed in Table 2.

EDS was performed on randomly selected nanoparticles that were detected on the filter media to determine the elemental composition. EDS showed that some particles are composed of lead only. EDS also revealed that other particles contained varying amounts and combinations of Pb, Sb, Ba, sulfur (S), calcium (Ca), iron (Fe), zinc (Zn), chlorine (Cl) and possible copper (Cu), as shown in Figures 2–4. The source of the Cu in these samples could be a combination of the particles and also the copper-mesh TEM grids on which the sample was prepared.

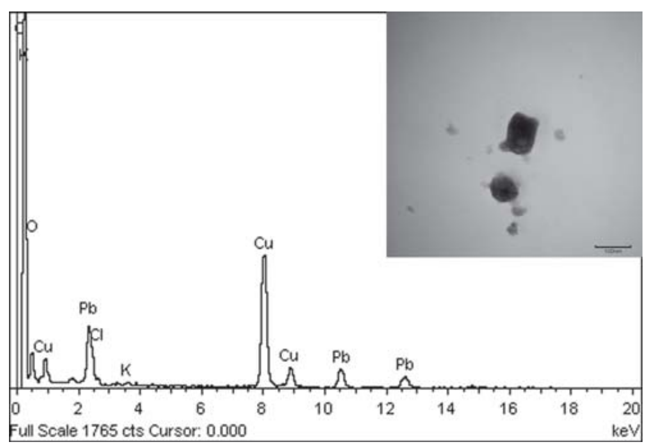

Figure 4. TEM image and EDS of a non-spheroidal lead nanoparticle found in the S&B samples. Scale bar = 100 nm.

Samples 6: Ambient Air Sample Collected 12 Hours After S&B Sample Collection

For Sample 6, a total of 10 grid openings were analyzed. GSR nanoparticles containing Pb, Ba and Sb were

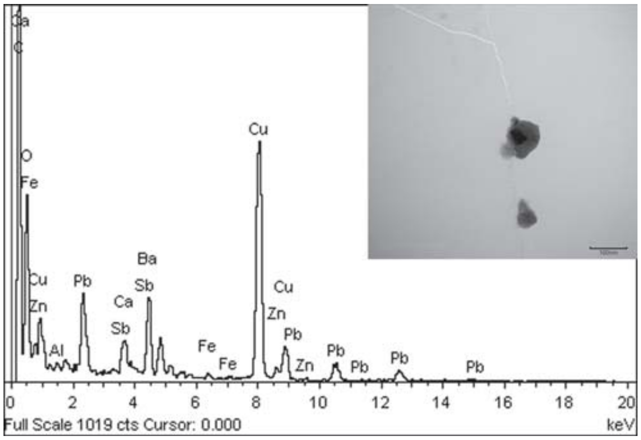

Figure 5. TEM image and EDS spectrum GSR nanoparticles found in the ambient air sample. Scale bar = 100 nm.

detected within the sample, as shown in Figure 5.

EDS also revealed that other nanoparticles within Sample 6 consist of varying amounts of Pb, Ba, S, Fe, Sb, Ca and possible Cu.

Samples 7–9: Winchester WinClean Ammunition

Samples 7–9 consisted of mostly spheroidal particles that varied in size, as shown in Figure 6. There were significantly less particles collected on each filter during the WinClean sampling.

For Sample 7, a total of 71 particles were counted and sized using ImageJ. Of the 71 particles sized, 74% range from 5 nm to 100 nm, 26% are within 100 nm and 1 µm, and 0% are greater than 1 µm in diameter. For Sample 8, a total of 203 particles were counted and sized using ImageJ. Of the 203 particles sized, 83% are within 5 nm and 100 nm, 17% are within 100 nm and 1 µm, and 0% are greater than 1 µm in diameter. For Sample 9, a total of 42 particles were counted and sized using ImageJ. Of the 42 particles analyzed, 71.4% are within 5 nm and 100 nm, 28.6% are within 100 nm and 1 µm, and 0% is greater than 1 µm in diameter. The particle size distribution data for Samples 7–9 can be found in Table 3. EDS was performed on randomly selected nanoparticles that were detected on the filter media to determine the elemental composition. EDS results illustrated that some particles consisted of a single element such as Pb and Cu, as shown in Figure 7. The Cu peak in Figure 7 could possibly be a result of both the particle and also the Cu-mesh TEM grid on which the sample was prepared. EDS also revealed that other particles contained varying amounts and combinations of Pb, Sb, Ba, S, Ca, Fe, Zn, Cl and possible Cu, as shown in Figures 8 and 9.

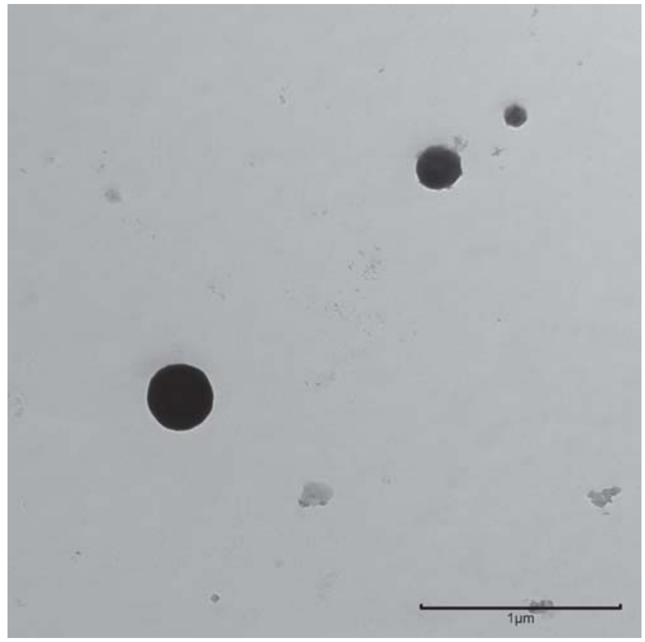

Figure 6. TEM image of particles collected on the filter during the discharge of one round of WinClean ammunition. Scale bar = 1 µm.

CONCLUSIONS

This study confirms that, depending on the ammunition, thousands of sub-micrometer nanoparticles are ejected into the atmosphere during the discharge of a firearm. In this study, 80%–94.4% of the particles are within the nanometer-size range. This study also shows that the nanometer-size particles can remain suspended in the air for up to 12 hours after they have been released. The presence of GSR nanoparticles can be very valuable because they are much more abundant than particles greater than 1 µm in diameter and are likely to remain in the air for longer periods of time. Therefore, the detection of GSR nanoparticles may be significant to future forensic investigations. TEM-EDS is instrumental in the analysis of GSR nanoparticles because of its high magnification capability and the ability to gather elemental data from nanometer-size particles.

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Table 3. Particle Size Distribution of WinClean Samples

Particle Diameter Size Fractions	No. of Particles
5 nm–100 nm	252
100 nm–500 nm	56
500 nm–1 µm	8
>1 µm	0

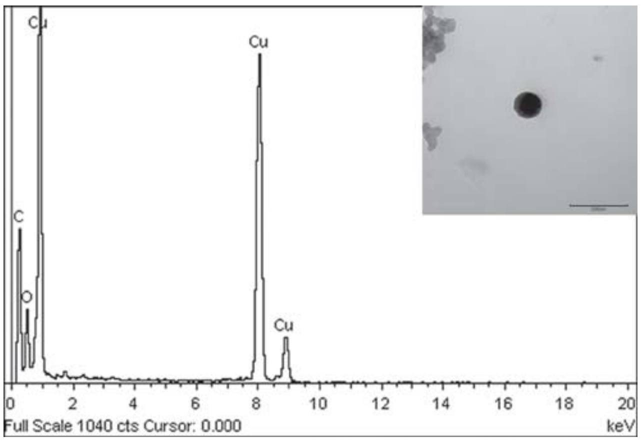

Figure 7. TEM image and EDS of a copper nanoparticle detected in the WinClean samples. Scale bar = 250 nm.

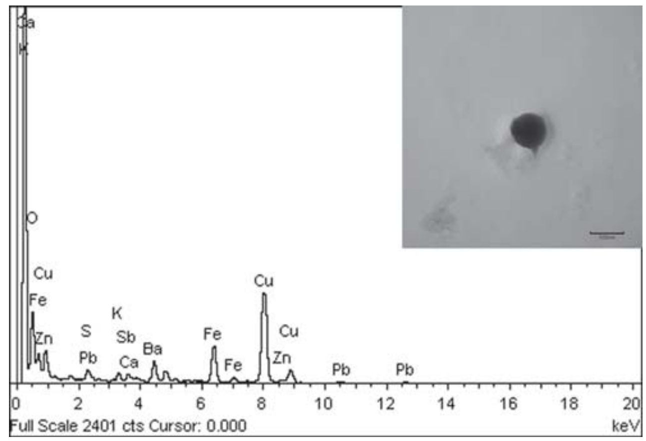

Figure 8. TEM image and EDS of a GSR nanoparticle from WinClean samples. Scale bar = 100 nm.

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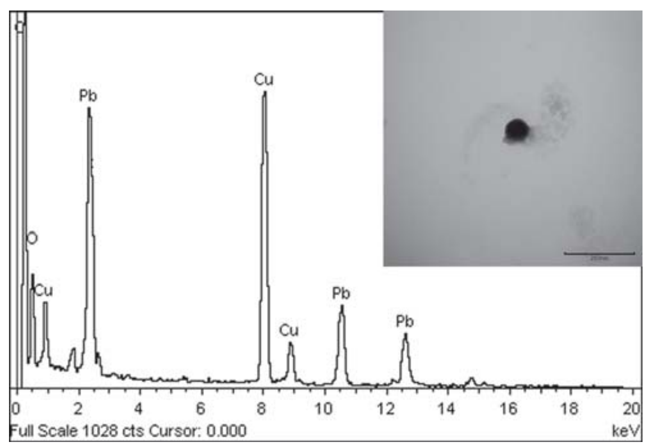

Figure 9. TEM image and EDS of a Pb nanoparticle found in the WinClean samples. Scale bar = 250 nm.