CASE REPORT

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Evaluation of Exhibits from a Murder Case Using the Lead Isotope Method and Scanning Electron Microscopy

ABSTRACT: We have used a combination of lead isotopes and scanning electron microscopy to determine the relationships between different exhibits in a murder case. Samples involved lead projectiles removed from the deceased's head and a pillow, lead-rich scrapings and particles (gunshot residues) from spent cartridges and a silencer, and particles from a pillowcase. The lead projectiles had the same isotopic composition with the lead being derived from the same dominantly geologically old source(s). The lead smear from the silencer had the same isotopic composition as the projectiles, and the lead was probably from the same source. The particles from the spent cartridges had varying elemental compositions ranging from PbO to PbCuZn \pm Ba with or without Si and are consistent with derivation from the primer. The lead isotopic compositions of the particles from the spent cartridges show some variations, but these are markedly different from those of the projectiles, indicating lead from a mixture of geologically old and geologically young lead. The particles from the cartridges. As the exhibits had been handled extensively prior to the present investigation, including some being sent to North America, there is a high likelihood that handling was not done in clean room environments and may have been contaminated. In this instance, as we were concerned about contamination, especially of the pillowcase, we felt contamination negated use of the results for assistance in proving the innocence or guilt of the accused. A combination of high-precision lead isotope measurements with scanning electron microscopy provides a powerful tool for forensic investigations if precautions are taken in handling the exhibits.

KEYWORDS: forensic science, lead isotopes, SEM, homicide, contamination

The case involved a female who was murdered as she slept beside her husband in their family home. Two bullets were fired into the deceased's head, and two bullets were fired into the pillow on which the husband was allegedly sleeping. The alleged murder weapon, a cut-down rifle with no stock and with a bullet stuck in the breach, was recovered from a nearby river about two weeks after the murder. Three silencers were tendered in evidence, but only one was supplied for testing. Our investigation was undertaken *pro bono* for the legal team acting on behalf of the accused.

A re-evaluation of exhibits was undertaken using the lead (Pb) isotope fingerprinting method, in combination with scanning electron microscopy/energy dispersive spectrometry (SEM/EDS), to determine the geological (and hence potentially the manufacturing) source of lead in selected exhibits from the murder case. Scanning electron microscopy has been used routinely in forensic investigations, but the lead isotope method has been used only rarely (1–5), probably because its main use has been in the earth and environmental sciences and more recently in the health field. A summary of past uses is described in Andrasko et al. (4). Previous forensic

³ CSIRO/DEM, POB 136, North Ryde New South Wales 1670, Australia. Received 26 Sept. 2001; and in revised form 10 Jan. 2002 and 28 Feb. 2002; accepted 1 March 2002; published 10 July 2002. studies using lead isotopes involving firearms were restricted to analyses of projectiles and, in one case, lead smears on clothing (4).

The lead isotope technique uses the four isotopes of lead. Three are the stable end products of radioactive decay of uranium and thorium: ²³⁸U to ²⁰⁶Pb, ²³⁵U to ²⁰⁷Pb, and ²³²Th to ²⁰⁸Pb. The abundance of the fourth isotope, ²⁰⁴Pb, has been essentially constant since the Earth began, and this isotope is commonly used as a reference isotope. As three isotopes of lead are produced by radioactive decay, the amounts (abundances) have changed over geological time, and this is reflected in the geological source of the lead. The abundances are usually expressed as ratios so that lead from the geologically old (\sim 1700 million years old) lead-zinc-silver deposits of Broken Hill in NSW Australia-which is used in many products including a dominant component of tetraethyl lead for gasoline—has an abundance ratio of the ²⁰⁷Pb isotope to the ²⁰⁶Pb isotope (²⁰⁷Pb/²⁰⁶Pb ratio) of 0.961. This contrasts with the ratio of 0.861 for geologically young deposits (500 to 400 million years old) on the same continent or from the northern hemisphere and even lower from some lead deposits in the U.S. (6). As it is possible to measure the difference in ratios between 0.961 and 0.960 (see Methods section), these natural differences are easily distinguishable. It is the isotopic differences that are used to evaluate the source of lead in the environment, humans, and animals. In the present study, the ²⁰⁷Pb/²⁰⁶Pb ratio is used as the "indicator" ratio as it is more sensitive to small variations, although the ²⁰⁸Pb/²⁰⁶Pb ratio can also be used and, for lead-rich samples, the 206 Pb/ 204 Pb ratio.

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In the current study, we attempted to: (1) analyze for lead isotopes four projectiles removed from the crime scene; (2) identify metallic particles in spent cartridges by scanning electron microscopy and analyze selected grains for lead isotopes; (3) evaluate the source of the lead from the spent cartridge rims and that in projectiles; and (4) evaluate the source(s) of the lead-rich particles found on a pillowcase.

Materials and Methods

Samples and Sample Preparation

Exhibits were examined and sampled under a binocular microscope. The amount of material in the spent cartridges was limited because of previous forensic examinations. The following samples were obtained using microsampling techniques:

- 1. Scrapings from projectiles recovered from the body of the deceased and from the pillow of the husband. To test the homogeneity of lead in the projectiles, two samples from different parts of three projectiles were obtained.
- 2. Scrapings or extraction of particles from four spent cartridges found at the scene.
- 3. Scrapings from a silencer recovered from a nearby river.
- 4. Particulates from the pillowcase of the deceased, identified as gunshot residue (GSR) particles by an earlier forensic expert.

Unfortunately, the bullet from the breech and the other two silencers were unavailable for testing as part of this study.

Isotopic and Lead Concentration Analysis

All sample preparation was performed in purpose-built low-contamination laboratories ("clean rooms") incorporating features such as filtered air intake and laminar flow Class 100 workbench stations. The samples were placed in clean 1 mL Teflon beakers and dissolved in two to three drops of concentrated (16 M) nitric acid that had been purified by sub-boiling distillation in a quartz still. To obtain approximate lead concentrations in all samples, except for the scrapings from the projectiles, a ²⁰²Pb "spike" solution of known isotopic composition and lead concentration (10 ng/g) was added to the aliquot to obtain the concentration of lead and isotopic composition of the unknown sample in the one analysis (this is known as the isotope dilution method). [²⁰²Pb is not naturally occurring and is produced in cyclotrons as a by-product of preparation of thallium used in treatment of thyroid abnormalities. It has a half-life of about 3×10^{5} years, and the mode of decay is by electron capture. The product was supplied through the Canadian Atomic Energy Authority in the mid-1980s]. Because of the extremely small size of the particles, especially those from the pillowcase (commonly $<50 \,\mu m$ across), it was not possible to weigh the samples. The weights were estimated from the dimensions of the particles observed under the binocular or scanning electron microscope. As it was not possible to quantitatively evaluate the relationship between particle dimensions and their weight, especially given the potential varying chemical composition of the particles, there is considerable uncertainty on the precision and accuracy of the lead concentrations of the small particles. However, this is largely irrelevant as the isotopic compositions were the critical parameters in this investigation. Lead was separated from interfering elements, such as Fe and Zn, by anionexchange chromatography in a hydrobromic acid medium.

Fractions of the purified lead samples were loaded onto a rhenium filament using the silica gel technique and analyzed for lead isotope composition on a thermal ionization mass spectrometer or TIMS (VG-ISOMASS 54E) run in fully automatic mode. Isotopic ratios were measured as ²⁰⁸Pb/²⁰⁶Pb, ²⁰⁷Pb/²⁰⁶Pb, and ²⁰⁶Pb/²⁰⁴Pb. The ac-

curacy and precision of the isotopic ratios were monitored by the measurement of an internationally recognized standard, National Institute of Science and Technology SRM 981 Common Lead Standard. The samples and standard were measured during the same spectrometer run. The measured ratios in the samples were normalized to this standard by applying a factor of 0.08% per mass unit. Normalization of the data to this standard allows comparisons of lead isotope data to be made between different laboratories throughout the world. The precision on the 207Pb/206Pb and 208Pb/206Pb ratios for the projectiles and priming mix is $\pm 0.05\%$ (2 sigma) and twice this for the samples from the pillowcase. Over 5,000 analyses of SRM 981 and replicate analyses of geological samples (7) have defined precision estimates on the isotopic ratios. A measurement of the environmental lead acquired by the sample throughout the entire preparation analysis procedure was obtained in the form of a lead "blank" measurement. The amount of contamination detected in blanks was generally around 200 pg. As the blanks contributed negligibly to the lead from the lead-rich samples in projectiles, silencer, and cartridges, no blank corrections to the data were performed. As the weights of the particles from the pillowcase are only qualitative, no blank corrections were made for these data.

Scanning Electron Microscopy

Each cartridge was separately mounted on a carbon disc, held with double-sided tape and positioned, so as to allow photography and EDS analysis to be performed concurrently.

A number of representative particles were identified as possibly associated with the crime scene. These particles were removed from the bloodstained pillowcase using fine needles under a stereoscopic microscope. The particles were mounted on double-sided tape and gold coated to eliminate possible charging effects which may have been encountered.

The analytical instrument used to perform analysis on the particles was a Cambridge Stereoscan 240 Scanning Electron Microscope operating at 20kV. Qualitative analytical information was obtained using the Noran energy dispersive X-ray fluorescence system (EDS) attached to the SEM. Analysis of the individual particles was performed by rastering the electron beam over the field of particles and monitoring the Pb-La emission characteristic. Once lead-containing phases were identified, the electron beam was changed to spot mode and the phases analysed for other elements using the X-ray fluorescence system with a 50 second count time. Detection of elements greater than approx 1% weight/volume concentrations could be detected by this method.

Results and Discussion

There are three main sources of lead in bullets. The major source is from the projectiles, and these were solid cast lead manufactured in Australia at the time of the homicide (Winchester Australia, personal communication to BLG, May 2001). The propellant consists of nitrocellulose, nitroglycerin, and diphenylamine and was manufactured in the U.S. Analyses of recently produced propellants show that they have low concentrations of lead of about 2 μ g/kg and hence contribute minimally to any lead "budget" (8). The other major source of lead is the primer, which generally consists of lead styphenate, tetracine, barium nitrate, ground glass, and gum arabic and is manufactured in the U.S. The concentration of lead in recently produced primer is about 11% (8). The copper cartridge case containing the primer was made in the U.S.

The different countries of manufacture of the projectiles and primer also probably mean that the sources of lead used in their production came from mines of different geological age and hence of different lead isotopic composition. As there are only two sources of lead in the bullets—projectile and primer—it is possible, if the isotopic compositions of the two are known, to estimate the approximate proportions of each in any samples. The methods are commonly employed in isotope geochemistry (9).

The SEM analyses and photographs of the analyzed particles are shown in Figs. 1 and 2, and lead isotope results, expressed as the ²⁰⁷Pb/²⁰⁶Pb and ²⁰⁸Pb/²⁰⁶Pb ratios and approximate lead concentrations, are listed in Table 1 and illustrated in Fig. 3.

Projectiles

The duplicate analyses for the three projectiles (K575, K576, K577) show that they have a homogeneous lead isotopic composition within each projectile that is within experimental error for this sampling protocol.

The two projectiles (laboratory numbers K577, K578 in Table 1) recovered from the body of the deceased have the same isotopic composition to within experimental error as the two projectiles (K575, K576) recovered from the pillow of the deceased's husband.

These data indicate that the projectiles were manufactured from the same source (batch) of lead. The isotopic compositions indicate that the lead in the projectiles comes mainly from geologically old mines such as the Broken Hill and Mt. Isa mines found in Australia; the approximate proportion of lead from these mines is >85%. In a previous investigation, Andrasko et al. (4) found that several projectiles from the same box of ammunition had essentially the same isotopic composition.

The lead isotopic compositions in the projectiles from this study and in the majority of projectiles from another study (8)—which are indicative of a major component of Australian lead—are different by orders of magnitude from the isotopic compositions measured in projectiles from the U.S. and Sweden (1–4).

Metal "Smears" from Spent Cartridges

All cartridges contained varying amounts of a gray putty material. Scrapings could comprise lead from the projectile and priming mix as well as lead from the cartridge itself, plus lead contamination from previous forensic investigations. An initial examination

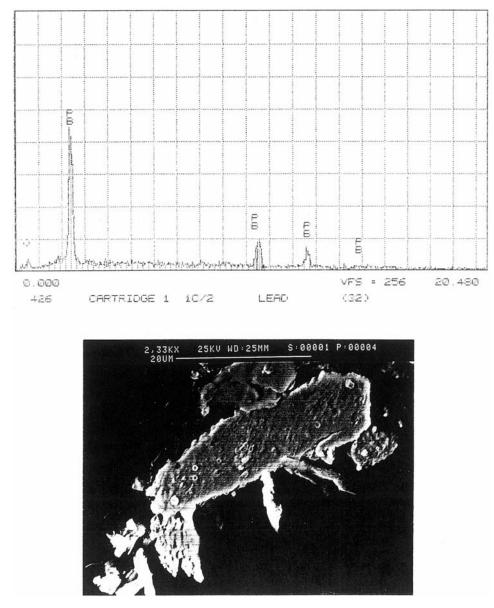


FIG. 1—Particle removed from cartridge No. 1 whose composition was dominantly PbO.

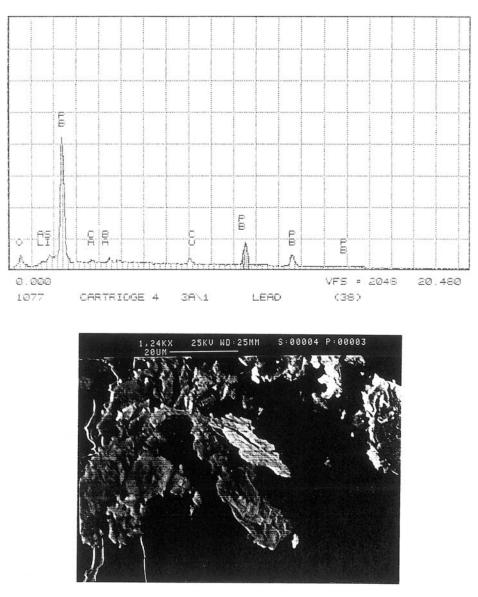


FIG. 2—Particle removed from cartridge No. 4 whose composition was dominantly PbCaCuBaO.

to determine if any lead metal smears from propulsion of the projectiles from the cartridges remained in the cases showed little evidence of smears, especially compared with a freshly fired 0.22 Winchester cartridge, a test carried out by one of the authors (JDD).

Priming Mix from Spent Cartridge Cases

The cartridges found at the scene of the crime were identified as Winchester subsonic Long Rifle 0.22 (Victorian State Forensic Science Laboratory).

In an initial investigation of the cartridges, SEM analyses were not undertaken. However, earlier SEM investigations by the Victorian State Forensic Science Laboratory had identified particles in four cartridges as gunshot residue (GSR). Their composition was PbBa-CaCuZn or PbBaCuZn or PbBaCu (Victorian State Forensic Science Laboratory). The particles still available in cartridges Nos. 1 and 4 examined by us in a follow-up investigation were dominantly PbOCuZn with rarer particles of PbCuZnBa. Trace amounts of Si were found in most particles. The Cu and Zn are thought to be mainly from the cartridge case, although there are trace amounts of Cu in the projectiles (Winchester Australia, written communication, 1994).

Glass-like samples identified by microscopic examination were recovered by sampling tool from the interior base of cartridges Nos. 1, 3, and 4 (K582, K584, K586); they were considered most likely to represent priming mix. They have similar lead isotopic compositions to each other, indicating that the lead was from the same source and could have been from the same manufacturing batch (Table 1, Fig. 3). Cartridge 2 was not sampled in detail because of a concern over contamination as aluminum foil and a gray putty substance were observed in this cartridge.

The lead isotopic composition of the priming mix was different from that in the projectiles—the difference was approximately ten times experimental error for these lead-rich samples. Although the isotopic composition in the primer is very different isotopically to that in the projectiles, it nevertheless is different to the isotopic composition expected of U.S. lead. Much of the U.S. lead comes from so-called Mississippi Valley mines, which have highly unusual and variable isotopic compositions. For example, the ²⁰⁷Pb/²⁰⁶Pb ranges

Lab Number	Sample type	SEM Composition	Size (µm)	²⁰⁸ Pb/ ²⁰⁶ Pb	²⁰⁷ Pb/ ²⁰⁶ Pb	Pb (%)
K575 -1	Bullet 1-1			2.1933	0.9338	na
K575 -2	Bullet 1-2			2.2006	0.9345	na
K576 -1	Bullet 2-1			2.1947	0.9347	na
K576 -2	Bullet 2-2			2.1953	0.9351	na
K577-1	Bullet 3-1			2.1968	0.9345	na
K577-2	Bullet 3-2			2.1935	0.9340	na
K578 -1	Bullet 4-1			2.1939	0.9348	na
K579	Silencer Scraping			2.1973	0.9359	106 µg/kg
K580	Cartridge No. 2 Scrapings from rim			2.1557	0.8942	na
K582	Cartridge No. 1 GSR			2.1454	0.8823	30
K583	Cartridge No. 3 Scrapings from rim			2.1802	0.9177	0.19
K584	Cartridge No 3. GSR			2.1502	0.8849	0.55
K585	Cartridge No 4 Scrapings from rim			2.1603	0.9023	0.05
K586	Cartridge No 4 GSR			2.1430	0.8818	9
K587*	Pillow case ?GSR			2.1718	0.909	3
K588	Pillow case?GSR			2.1731	0.9087	2
K589*	Pillow case?GSR			2.1522	0.890	0.6
K590*	Pillow case?GSR			2.1762	0.915	0.08
K591	Pillow case?GSR			2.1841	0.9194	na
K609	Cartridge No 3 grains	PbCuZn		2.1895	0.9293	70
K610	Cartridge No 3 grains	PbCuZn		2.1896	0.9292	60
K611	Cartridge No 3 grains	PbCuZn + Ba		2.1571	0.8942	3
K626	Cartridge No 1	PbO minor Si	57×10	2.1977	0.9340	8
K627	Cartridge No 1	PbO	36×10	2.1942	0.9331	48
K629	Cartridge No 1	PbO vvminor Si	32×28	2.1948	0.9346	5
K630	Cartridge No 1	PbO vvminor Si	29×25	2.1918	0.9330	2
K631	Cartridge No 4	PbO vvminor Si	24×8	2.1503	0.8856	18
K632	Cartridge No 4	PbO minor CaBaCuAlSi	10×17	2.1444	0.8824	na

TABLE 1-Elemental and lead isotopic composition results for samples.

vvminor = very very minor amounts

na = not determined because of uncertain dimensions of grain(s) and weights, or in the case of projectiles, too much lead for accurate determination by this method

* Lower quality data than for other samples

GSR = gun shot residue

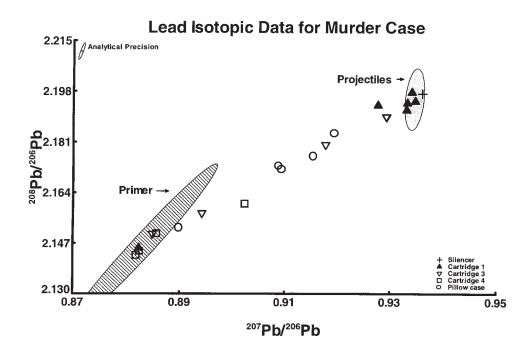


FIG. 3—²⁰⁸Pb/²⁰⁶Pb versus ²⁰⁷Pb/²⁰⁶Pb plot for projectile, primer (gunshot residue) and lead-rich particles removed from spent cartridges and a pillowcase. The ellipses are 95% confidence ellipses around the data points for the projectiles and primer. The analytical precision is valid for most samples except those from the pillowcase.

from 0.711 to 0.803 (6), so the lead in the primer was not produced solely from these mines. Other U.S. sources of geologically older lead with higher ²⁰⁷Pb/²⁰⁶Pb ratios could be from the Coeur d'Alene district in Idaho, where the ²⁰⁷Pb/²⁰⁶Pb ratio is approximately ~0.95 (10). Alternatively, the company could have purchased lead on the open market, sourced from a variety of countries, and some of which may have included a geologically older lead.

Particles in Cartridges

Cartridge No. 3—The data for this cartridge typified the complexity in mixing of lead from the different components in a single cartridge. For example, K584 is representative of the primer, whereas the very high Pb plus Cu Zn of samples K609 and K610 indicate lead that probably derives mainly from the projectiles. The scraping from the rim of the cartridge (K583) has a 207 Pb/ 206 Pb of 0.918, indicating a dominant contribution (~70%) from the projectiles as might be expected by smearing of lead as the projectile is forced from the cartridge. The other particle towards the rim (K611) is composed of PbCuZn + Ba, and its isotopic composition is consistent with a dominant primer source (~80%).

Cartridge No. 1—In spite of their association with trace amounts of Si, which may point to a primer source, all the PbO particles from the rim of cartridge No. 1 have isotopic compositions that are identical to within experimental error of those in the projectiles. The other particle (K582) from the base of the cartridge was considered to represent the primer isotopic composition and was used in the estimation of the primer end member.

Cartridge No. 4—The isotopic composition in the scraping from the rim of this cartridge has a projectile component of >60%, whereas the other three particles are typical of the primer. The two particles identified with the SEM from this cartridge contain CaBaCu in one case (K631) and minor Cu in the other (K632). Calcium and barium are major constituents of the priming mix. As the isotopic ratios in both samples are similar to those measured in the three priming mix samples used to estimate the end member values, they have been allocated to this group.

Silencer

The interior of this device was severely rusted and most of the particles scraped from the interior were iron-bearing. The amount of lead in the analyzed sample was very low at approximately 100 μ g/kg, but this is probably a "dilution" effect due to the overwhelming bulk of iron-bearing material. The ²⁰⁷Pb/²⁰⁶Pb ratio (K579) is the same as that in the projectiles (Fig. 3), and the source of lead in the silencer and projectiles can be interpreted to be the same. Alternatively, the source of the lead could be from lead-bearing steel as lead is added to steel to facilitate machining and turning (BHP, personal communication to JCE, August 18, 1994).

Pillowcase

Five dark brown to black particles, generally of a size $<50 \ \mu m$ across, suspected to be gunshot residue, were recovered from the pillowcase and measured by the isotope dilution method.

The isotopic ratios were variable (K587 to K591) and lie in the range between the lead found in the projectiles and the priming mix (Fig. 3), suggesting that the lead found in these suspected GSR particles was a mixture of lead from both sources. In four of the five particles, over 50% of the lead is derived from projectile lead, if

projectile and priming mix lead are the two main sources of lead in the GSR particles. However, interpretation of data for the GSR particles is confounded by potential contamination of exhibits from previous investigations and handling.

Assessment of Results

Apart from cartridge No. 2, which was not examined in detail due to potential contamination, there is an overall consistency in the isotopic results for the other three cartridges, which allows some conclusions to be drawn.

- There are major isotopic differences between the lead in the projectiles and priming mix consistent with the lead being derived from different sources. It is assumed that these are the two main sources of lead in the exhibits, and from this the proportions of lead from each source can be estimated.
- 2. The similarity in isotopic composition of the lead particles from cartridge No. 1 and projectiles is consistent with the lead deriving from the same source, i.e., that at least one of the projectiles could have derived from spent cartridge No. 1. Likewise, the two lead-rich particles from cartridge No. 3 have essentially the same isotopic composition as the projectiles, and this isotopic composition is similar to four of the five particles from cartridge No. 1.
- 3. Much of the lead in the other cartridges is probably from the lead styphenate used in the priming mix. The large contribution made by the priming mix lead to the total lead budget in the spent cartridges is rather surprising given that the projectiles are approximately 100% Pb. The potential for mixing (contamination) of lead from the priming mix and projectiles needs to be considered in future investigations. On the other hand, the consistency in the isotopic data for the priming mix lead would have allowed evaluation with the GSR particles on the pillow case if the exhibits had been handled more carefully.
- 4. The lead in the silencer comes from a similar source to that in the projectiles. However, there is no "library" of lead isotope data in projectiles available in Australia from which to make comparisons.
- 5. The suspected GSR particles from the pillowcase have lead that could derive from a mixture of lead from the projectiles and priming mix. However, the particles are extremely small and could derive from other sources in the environment such as paint, petrol, or airborne dust particulates. We have shown that such particles are common in Sydney air (11), and, unless confirmed by SEM/EDS and lead isotopic analyses, some of the suspected GSR particles on the pillowcase may be from exogenous sources. As the pillowcase was examined by other investigators and apparently not under clean room conditions, exogenous particles are a real possibility.

Recommendations for Future Forensic Investigations

SEM/EDS and lead isotopic analyses are potentially powerful tools in forensic investigations, but, because of the ubiquity of lead and possibility of contamination by workers unaware of the precautions necessary when handing lead, especially at low concentrations, it is absolutely essential that strict precautions are taken to minimize contamination during the handling, sampling, and storage of exhibits. For any investigations involving lead (or lead isotopic or SEM/EDS analyses), sampling for these should be undertaken at the earliest stages of the investigations in "clean" laboratories to minimize contamination from endogenous and exogenous sources.

In this case, early sampling of the cartridges and pillowcase under "clean" conditions may have allowed for more definitive conclusions regarding the origin of lead in the GSR particles and lead metal particles on the pillowcase. Similarly, the potential contamination of the cartridges and removal of the majority of lead smear material constrained any conclusions that could be drawn from the available isotopic data. Comparisons would also have been possible between GSR particles extracted from the skin of the deceased and potential sources.

In this instance, as we were concerned about contamination, especially of the pillowcase, we felt contamination negated use of the results for assistance in proving the innocence or guilt of the accused.

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