Evaluating Methods for Removing Radioactive Contamination from Traditional Forensic Evidence: Moths

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Abstract

In 2009, Victoria, Australia police found 300 grams of a uranium oxide compound in a storage property. After initial analysis by the Australian Science & Technology Organization (ANSTO), aliquots of the material were sent to Lawrence Livermore National Laboratory (LLNL) for further analysis. While aliquoting the sample for analysis, researchers at LLNL found the head and body of a moth. Analysis of the nuclear material indicated that it could not have originated within Australia. Entomological study of the moth could prove useful for understanding the history of the material from production to interdiction within Australia, a type of signature referred to as a "route attribution" signature in nuclear forensics. However, before the moth could be sent to an entomological laboratory, it would need to be decontaminated, a process that could prove destructive.

To determine an effective and nondestructive method for decontamination of the evidence moth, exemplar moths were collected and contaminated with a uranium ore concentrate. The contaminated moths were ultrasonicated in eleven solvent systems chosen for their potential decontamination properties. Four of the solvents (5% Radiacwash™, 5% Decon® 90, acetone, and 1% nitric acid) provided promising results. They removed a significant mass of the uranium ore concentrate without extensive

damage to the moth. However, using mass difference to determine the amount of uranium ore concentrate removed from each moth by the solvent proved to be imprecise and sometimes difficult to interpret. For example, mass loss was sometimes greater than expected because of incomplete initial desiccation of the moth followed by a more complete desiccation after decontamination. In addition, the loss of wing scales during solvation seems to be unavoidable in all solvent systems. The moths decontaminated with the most promising solvents were ashed and analyzed for remaining uranium content by inductively coupled plasma – mass spectrometry (ICP-MS). Future work may include DNA analysis of the moths to determine if DNA can be cleanly extracted from radioactively contaminated evidence.

Introduction

On April 1, 2009, police in Victoria, Australia conducted drug raids on an alleged amphetamine laboratory. In a nearby storage facility, they found approximately 300 grams of uranium oxide. A man was arrested in connection to the sample but refused to say why he had obtained the uranium. The sample was collected by a team from the Australian Department of Human Services and sent to the Australian Nuclear Science and Technology Organization (ANSTO) in Lucas Heights, Australia, as legal evidence for nuclear forensic analysis (1,2).

Nuclear forensics attempts to answer questions related to where the sample originated, the type of mine and processing that produced the material, and whether it is related to a previous seizure (3,4). It has been defined as "*the analysis of intercepted illicit nuclear or radioactive material and any associated material to provide evidence*

for nuclear attribution. The goal of nuclear forensics analysis is to identify forensic indicators in interdicted nuclear and radiological samples or the surrounding environment, e.g., the container or transport vehicle. These indicators arise from known relationships between material characteristics and process history." (5) By comparing the chemical characteristics of an unknown sample to the known characteristics of individual uranium mines and/or the unique chemical signatures produced by specific processing operations, it is possible to find connections relevant to a criminal investigation. Nuclear forensic analysis of the material suggested that it is not a product of an Australian mine. Based on known processing practices, the sample likely originated in the former Soviet Union.

As part of US-Australian bilateral cooperation in nuclear forensics, ANSTO sent aliquots of the material to Lawrence Livermore National Laboratory (LLNL) for concurrent analysis and confirmation of the results. During aliquoting of the sample for chemical characterization, LLNL found the body, leg, head, and scales of a moth mixed in the uranium oxide powder (Figure 1). ANSTO confirmed that the sample they had received for investigation likely contained more of these moths but that they had focused on the analysis of the nuclear material and had, therefore, discarded the moths as extraneous material. Entomological analysis of the moth could provide information necessary to narrow down possible geographical locations of where the evidence had been located (6,7).

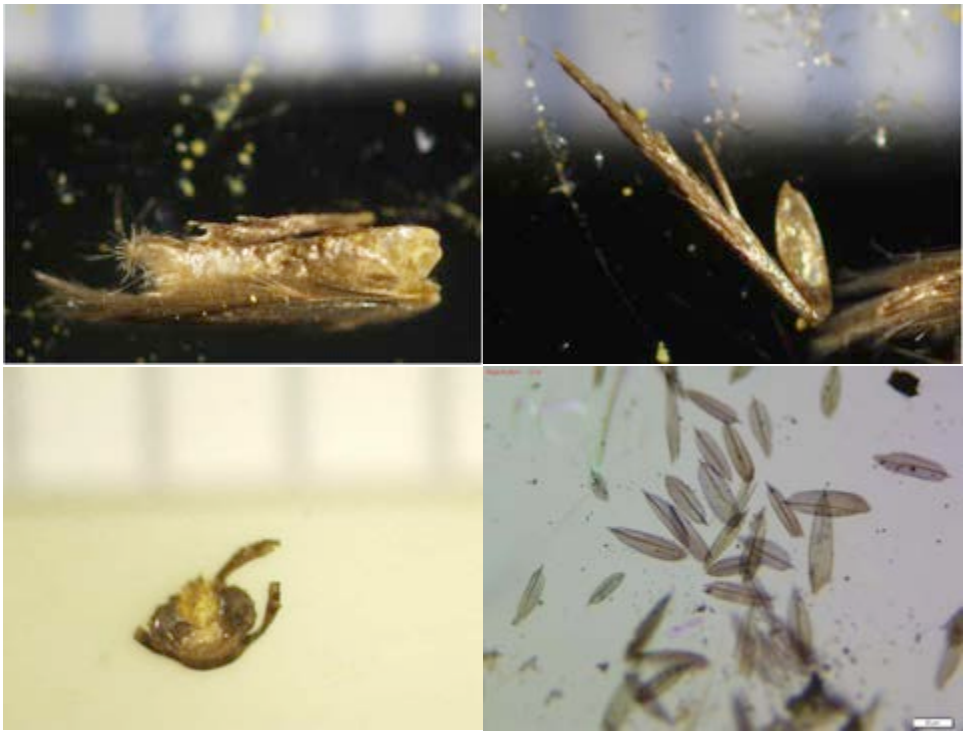

Figure 1. The body (upper left), leg (upper right), head (lower left), and scales (lower right) of a moth found in the uranium oxide sample.

Traditional forensic evidence can become contaminated by dispersible radioactive material when it is associated with interdicted nuclear material, like this moth, or an attack using a radiological dispersal device (RDD or “dirty bomb”). If so, such evidence must either be analyzed by a forensic laboratory capable of handling nuclear material or decontaminated prior to entering a traditional forensic science laboratory. Limits set by the United States Nuclear Regulatory Commission (NRC) state that a license is required for any person that intends to handle evidence contaminated by a radioactive source that is more than 0.05% its weight (8). In order to handle contaminated evidence, a laboratory would have to maintain this license and have the forensic capabilities to analyze the evidence. Therefore, decontamination of the evidence with a method that does not

destroy the evidentiary value at a facility with the proper licensing and then transfer to a traditional forensics laboratory would be preferred.

Very little research has been done combining nuclear chemistry and forensic evidence. ANSTO has previously partnered with the University of Technology Sydney and the Forensic and Technical Services division of the Australian Federal Police in Canberra to determine the effect of ionizing radiation on fiber evidence and the recovery of latent fingerprints (9,10). Only one study has been performed on the effects of decontamination techniques on forensic evidence. ANSTO added a decontamination procedure to the work they had previously begun on documents and fingerprints to see how chemical and physical decontamination techniques affected the forensic value of the documents as well as collection and examination of fingerprints (11). They chose to attempt both physical removal of the contamination by scraping with a razor blade and chemical removal by ultrasonicating the documents in DEZ-1 (Raddez Chimmed^{TM}), a decontamination product.

While it is not possible to physically remove the powdered contaminate on a small insect, like a moth, it may be possible to decontaminate through ultrasonication in a solvent. Eleven solvents were chosen based on their potential for decontamination. Water was chosen as a solvent control. The International Atomic Energy Agency (IAEA) suggests the use of 3% citric acid, 1.4% sodium bicarbonate, 0.25 M ethylenediaminetetraacetic acid (EDTA) or a 1% diethylene triamine pentaacetic acid solution as possible methods for decontamination of humans (12), a promising place to start to avoid damaging the delicate moth body. EDTA is a chelating agent used for the treatment of heavy metal poisoning; and sodium bicarbonate and citric acid have been

used, previously, as chelating agents for the remediation of contaminated soils (13). Radiacwash™ (Biodex), RBS™ 25 (RBS), and Decon® 90 (Decon Laboratories Limited) are all commercially available products. Radiacwash™ is currently used in LLNL laboratories for the decontamination of laboratory supplies. RBS™ 25, Decon® 90 and nitric acid were used in experiments that assessed the possibility of decontaminating fiber forensic evidence by the Institute of Materials Engineering at ANSTO. (14) Acetone was chosen as an example of a standard organic solvent.

Materials and Methods

Exemplar moths (Miller moths) were collected from northern Colorado where they can be easily found in high abundance. While this resulted in an easy sample procurement, the Miller moths collected were significantly larger than the evidence moth and might prove to be more durable. Initial experiments to determine the amount of time the moths could withstand ultrasonication in the various solvents was performed. A maximum time of 15 minutes was determined before significant heat build-up and solvent effects began to cause sample degradation.

Dry Mass

The moths were placed in individual, pre-weighed glass vials and desiccated at 105 °C for 80 minutes to determine a dry mass.

Mass of UOC Contaminate

The massing was repeated immediately before contamination for an accurate pre-contamination mass. The moths were then contaminated with CUP-2, a Canadian-produced uranium ore concentrate (UOC) by placing a moth in a small glass container of

UOC and gently rolling it in the powder to replicate the coating process experienced by the evidence moth. The newly contaminated moth was carefully removed from the container and placed back in its original glass vial. The vial was then massed again to obtain a post-contamination mass. The difference between pre- and post-contamination masses is the mass of UOC added to each moth.

Solvent Decontamination with Mass Difference Analysis

After contamination, the moths were separated into solvent groups with five moths each; the 5% Radiacwash™ group contained eight samples. The chosen solvent was added to each vial until the solvent covered the moth and was placed in the ultrasonicator for 15 minutes. The moths did float in the solvent causing concern of incomplete solvation; however, the agitation of the ultrasonicator caused some moth movement and occasional manual mixing was used to ensure that the entire moth was exposed to the solvent.

After the ultrasonication step was complete, the moths were removed from their original vial and ultrasonicated for another 15 minutes in water to rinse off any solvent residue. They were transferred to new, pre-weighed vials and desiccated in an oven at 115 °C until a stable mass was obtained. This was assigned as the post-decontamination mass. This mass was compared to the initial dry mass of the moth before the contamination process to determine the mass of UOC remaining after the solvation process. This was used to find the percent of UOC removed, or percent decontamination, by calculating the percent difference between the amount of UOC added to the moths and the amount remaining after the decontamination process.

Solvent Decontamination with ICP-MS Analysis

Samples of each of the following solvents were chosen for further analysis by ICP-MS: water, sodium bicarbonate, Radiacwash™, RBS™ 25, Decon® 90, 1% nitric acid, and acetone. Due to a lack of time remaining in the internship and limited space in the furnace, only three of the original five samples per solvent were analyzed. The moths were ashed in a furnace according to the IAEA's Network of World Analytical Laboratories (NWAL) procedure; the residue was dissolved in approximately 2 mL of aqua regia, heated to dryness, and reconstituted in 1% nitric acid. The resulting solution was analyzed for uranium content by ICP-MS.

Results

Dry Mass

The initial desiccated masses of 58 Miller moths ranged from 25.27 mg to 85.89 mg with a mean moth mass of 54.91 mg and a standard deviation of 15.99 mg.

Mass of UOC Contaminate

The pre-contamination masses of the moths ranged from 30.33 mg to 97.43 mg with a mean mass of 63.76 mg and a standard deviation of 16.52 mg. The post-contamination masses ranged from 33.66 mg to 119.13 mg with a mean mass of 73.71 mg and a standard deviation of 18.56 mg. By subtracting the pre-contamination mass from the post-contamination mass, it was determined that the mean amount of UOC added to each moth was 9.96 mg with a standard deviation of 11.14 mg. The majority of the moths were contaminated with an average of 7.67 mg of UOC with a standard

deviation of 4.20 mg; however, three samples skewed the overall mean: 41.51, 42.86, and 71.24 mg.

Solvent Decontamination with Mass Difference Analysis

Table 1 summarizes the mean results for each solvent from the decontamination process. According to the mean data, the moths decontaminated in 3% citric acid gained mass, while the moths decontaminated in 5% Radiacwash™, 10% RBS™ 25, 5% Decon® 90, 1% and 1 M nitric acid apparently lost over 100% UOC.

Table 1. Mean percent decontamination and the standard error of the mean for each solvent determined by mass difference.

Solvent system	Mean %Decon	Std Error of the Mean
Water	18.7	12.2
3% Citric Acid	-15.2	15.2
1.4% Sodium Bicarbonate	62.0	4.6
0.25 M EDTA	50.2	10.5
1% DTPA	64.9	13.1
5% Radiacwash™	158.5	32.6
10% RBS™ 25	106.7	5.2
5% Decon® 90	170.9	50.1
1% Nitric Acid	147.4	15.5
1 M Nitric Acid	119.6	3.9
Acetone	99.0	6.3

Solvent Decontamination with ICP-MS Analysis

Table 2 summarizes the mean percent decontamination and the standard error of the mean obtained by ICP-MS. These calculations compared the mass of uranium in the UOC contaminate and the mass of uranium remaining in the sample determined by ICP-MS, to calculate a mean percent decontamination. The use of the ICP-MS data should reduce or eliminate the inaccuracies in the calculation of percent decontamination caused by incomplete desiccation on the massing approach.

Table 2. Mean percent decontamination and the standard error of the mean for each solvent determined by ICP-MS.

Solvent	Mean UOC mass remaining (mg)	Mean %Decon	Std Error of the Mean
Water	0.910	70.87	4.56
1.4% Sodium Bicarbonate	0.493	86.53	6.16
5% Radiacwash™	0.114	93.85	0.21
10% RBS™ 25	0.502	93.05	1.24
5% Decon® 90	0.216	91.85	1.16
1% Nitric Acid	1.08	84.13	4.03
Acetone	2.50	63.31	1.88

Discussion

Dry Mass

While performing the initial dry massing in triplicate, it became obvious that, after desiccation in the oven, there was significant reabsorption of water occurring during

the cooling period. The dry masses, therefore, may not be accurate representations of the completely desiccated moths. During the time interval between desiccating the moths and beginning the contamination procedure, the moths gained an average of 8.85 mg of water weight from the humidity in the air.

According to a study performed on butterflies (15), the total body water content varies, depending on species and sex, but ranged between 63.2% and 67.8%. The moths in this study lost an average of 37.1% of their body weight during the initial dry mass process. It is highly likely that they were not completely desiccated and future studies will benefit from greatly increasing the desiccation time and keeping the moths in a dry environment at all times.

Mass of UOC Contaminate

Because the moths were obviously gaining mass from water reabsorption, the masses were recorded immediately prior to and after contaminating each moth with UOC, therefore, the mass of contaminate should be accurate. Most of the moths retained similar amounts of UOC after the contamination procedure. However, three moths retained significantly more, skewing the mean data. Moth shape appears to be the main factor in retention of powdered UOC. Most of the moths were in a bullet shape with their wings tucked back. The moths that retained the most UOC were in scoop-like positions, with their wings pulled forward past their body. This scoop position was able to transfer much more of the UOC out of the contamination glassware and into the individual vial.

Solvent Decontamination with Mass Difference Analysis

Even a cursory glance at the percentages reported in Table 1 show that the use of mass difference is rife with error. According to this data, the moths treated by citric acid

gained mass and the moths treated with Radiacwash™, RBS™ 25, Decon® 90, and both concentrations of nitric acid lost more UOC mass than that with which they were contaminated. It is probable that the moths treated with citric acid were not completely dry when massed after decontamination; therefore, their final mass could be misleadingly high. The moths treated with citric acid were coated with a white residue after decontamination, possibly a citrate salt, accounting for the extra mass. The moths that lost more UOC than is physically possible are easily explained by a high initial dry mass, due to incomplete desiccation.

Some of the moths lost legs or antennae during transfer between vials or during ultrasonication. These were gathered and continued on in the process with their respective moth. However, it is possible that body parts were lost during the contaminating process. All of the moths, unavoidably, lost some mass from scales that were washed off during ultrasonication. Further experiments would have to be performed to determine if this mass is significant.

Solvent Decontamination with ICP-MS Analysis

The results from the ICP-MS analysis show that ultrasonication in water, the solvent control, removed 70.87% of the uranium in the UOC contaminate. All of the solvents tested proved more effective than water in removing the UOC, except acetone, which only removed 63.31%. Perhaps unsurprisingly, the commercial decontamination products removed the most UOC, well above 90%, with Radiacwash™ showing the best performance. Presented below in Table 3 are the mean mass of UOC remaining on the moths for each solvent and the maximum mass of UOC that would be allowed to remain on the moth per the U.S. NRC regulation (0.05% of the original contaminated mass). As

is obvious from the table, with the current decontamination protocol used, none of the solvents were able to remove enough radioactive material for the moth to be transferrable to a non-radiation laboratory.

Table 3. Mean UOC mass remaining based on ICP-MS results compared to the mean allowable mass of UOC according to the U.S. NRC regulation.

Solvent	**Mean UOC mass remaining (mg)**	**Mean allowable mass of UOC (mg)**
Water	0.910	0.042
1.4% Sodium Bicarbonate	0.493	0.033
5% Radiacwash™	0.114	0.024
10% RBS™ 25	0.502	0.042
5% Decon® 90	0.216	0.044
1% Nitric Acid	1.08	0.036
Acetone	2.50	0.044

Forensic Evidentiary Value

The evidentiary value of the moths depends on the physical state of the insect. Photographs of sample moths using a Leica Microsystems light microscope with a camera attachment was used to assess physical damage to the moth by the ultrasonication in solvent. Figure 1 demonstrates the results seen from most of the solvents, exemplified here by 5% Radiacwash™.

Figure 1. The hindquarters of a moth post-contamination (left) and post-decontamination (right) with 5% Radiacwash™.

Figure 2 shows the physical effects of decontamination in 3% citric acid, which caused the moth's wings to stick to the inside of the vial during the desiccation in the oven post-decontamination, resulting in the loss of parts of its wings. While this is unfortunate, it is probably not as important in identifying the type of moth, and can be easily remedied by more careful placement of the moth in the vial before drying.

Figure 2. The wings of a moth post-contamination (left) and post-decontamination (right) with 3% citric acid.

RBS™ 25 (Figure 3) was the only solvent to show a serious degradation of the moth's appearance. It is possible that this solvent left behind substantial amounts of residue that were baked onto the moth during the post-decontamination desiccation process. All of the other solvents did not have any marked effect on the moth's physical state.

Figure 3. A moth pre-contamination and post-decontamination with 10% RBS™ 25.

Conclusions

The decontamination potential of eleven solvents was analyzed by mass difference and ICP-MS. The analysis by mass difference was more difficult to interpret than initially anticipated as a stable desiccated mass of the moths proved problematic. The ICP-MS results show that the commercial decontamination solvents performed well with Radiacwash™ removing 93.85%, RBS™ 25 removing 93.05% and Decon® 90 removing 91.85% of the contamination. However, none of the solvents were able to remove enough of the radioactive contamination to allow for transfer to a traditional

forensic science laboratory without further processing. Photographic analysis showed that the evidentiary value of these moths was retained for all of the solvents, except for RBS™ 25, which resulted in a charred appearance.

Further studies will have to be performed to optimize the study parameters. First, the drying of the moths should be complete and the moths should be stored in desiccant to produce more accurate masses. Second, exemplar moths that are similar to the evidence moth in size would produce results more easily transferrable. Third, the costs and benefits should be considered of running the moths through a second round of the decontamination procedure. Could a second round remove more UOC or would this lead to more sample degradation? Finally, should the moth's DNA prove useful for the entomological study, the effect of the decontamination procedure on DNA should be tested, as well as the ease of obtaining DNA from such a sample.

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