Agency: National Institute of Justice

Award number: 2018-DU-BX-0193

Project Title: Post-Blast Explosives Attribution

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Submission date: 10/29/2021

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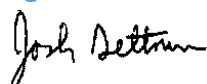
EIN: 042103594

Recipient Organization: MIT Lincoln Laboratory
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Award Period: 05/01/2019 to 10/31/2021

Reporting Period End Date: 10/31/2021

Final Report

Signature of Submitting Official:

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Project Summary

1) Major Goals and Objectives

The purpose of this project was to address the need detailed in the OSAC Research Needs Assessment titled "Source Attribution of Post-Blast Residues" (NIST Organization of Scientific Area Committees, 2018). Currently, no capability exists to link an explosive charge to its manufacturing source from signatures acquired from post-blast trace residues. There is some limited capability to compare signatures from an unexploded device to manufacturer reference samples (including those developed at MIT Lincoln Laboratory), and this knowledge was leveraged to attempt to develop the capability to source signatures from post-blast material. The primary goal of this project was to determine whether relevant conserved signatures of an explosive source can be recovered post-blast and matched to pre-blast signatures. This two-year project was a collaboration between Massachusetts Institute of Technology Lincoln Laboratory (MITLL), which performed study design and execution of all field tests and experiments, and researchers at South Dakota State University (SDSU), who contributed with data and statistical analyses.

In Year 1, analytical methods for trace analysis of signatures were developed and validated. Standards of explosive compounds were purchased and used for optimizing extraction procedures by depositing on sample coupons and soil, with extraction methods adapted from relevant literature and operational forensic procedures, and measuring for quantitation and profiling of trace signatures. University of California at Davis' Stable Isotope Facility developed methods for isotope ratio mass spectrometry (IRMS) analysis of RDX and TNT. A preliminary field test to obtain relevant post-blast samples was conducted at NEWTEC Services Group, Inc. in Edgefield, South Carolina to validate and further optimize extraction procedures and analytical methods.

In Year 2, the samples obtained in the preliminary field test were analyzed via multiple instrumental methods including high performance liquid chromatography – mass spectrometry (HPLC-MS) for analysis of organic signatures, and inductively-coupled plasma – mass spectrometry (ICP-MS) for trace element analysis. Statistical methods for analysis of the data were developed and conducted through a collaboration with Prof. Chris Saunders at SDSU. A second, larger field test was conducted with an improved experimental design using lessons learned from the preliminary field test in Year 1. The resulting signatures from post-blast samples were statistically compared to pre-blast signatures. For results dissemination to the forensic community, multiple oral and poster presentations were given at academic conferences including the American Academy of Forensic Sciences (AAFS) conference and PittCon, and a manuscript is currently being prepared for publication.

2) Research Questions

As described above, the key research question that this study sought to answer was: are chemical signatures for sourcing explosive materials to their manufacturing source preserved after detonation, and which signature types are best preserved?

3) Research Design, Methods, Analytical and Data Analysis Techniques

Introduction

        The purpose of this study was to determine if relevant conserved signatures characteristic of the origin/source of an explosive can be recovered post-blast and matched to pre-blast signatures. In order to test this hypothesis, a field experiment was designed to conduct replicate detonations of multiple types of explosive materials, followed by collection of post-blast residue. The samples of collected post-blast residue were then processed and analyzed via multiple analytical chemistry techniques to acquire signatures specific to the explosive source. Using data and statistical analysis approaches, the signatures from post-blast samples were compared with signatures from pre-blast samples to determine if they were preserved.

Materials and Methods

        Three explosive types were chosen as part of this study including 1,3,5-Trinitroperhydro-1,3,5-triazine (RDX), 2,4,6-trinitrotoluene (TNT), and ammonium nitrate – aluminum (AN-AL). MITLL has prior experience both in detecting trace levels of these explosives (Kunz & Clow, 2007), and examining signatures from them (Campbell, et al., 2017) (Campbell, et al., 2016), so building off this knowledge was helpful in selecting the explosives to use in this study. Furthermore, discussions with members of FBI Research in the early stages of the study development also influenced the selection of RDX, TNT, and AN-AL since these explosives are among the operationally relevant materials found at post-blast sites.

        Significant effort was undertaken to design the test to be as operationally relevant as possible by conducting the explosive detonations and sample collections in a controlled open, outdoor environment as opposed to a laboratory setting. The experiment was conducted at Newtec Services Group, LLC, an explosives test range in Edgefield, SC, owned and operated by Keith Williams, a former Navy EOD technician with extensive experience in working with explosives. All explosive devices used in the test were prepared and handled by Mr. Williams. Multiple sample collection concepts of operation (CONOPS) were executed for each detonation in order to capture relevant scenarios that post-blast site investigators would encounter, including swabbing from a surface, and extracting from soil. All methods for handling and collecting post-blast samples were adapted from the environmental or forensic science literature including the 'clean hands, dirty hands' approach described in EPA Method 1669 (U.S. Environmental Protection Agency, 1996), the field swabbing approach detailed in the FBI Explosives Unit Standard Operating Procedures (FBI Explosives Unit, 2007), as well as support from other sources in the academic literature (DeTata, Collins, & McKinley, 2013) (Hewitt, 2001). Methods for extracting explosives and associated signatures from collected samples were also adapted from forensic science literature to include recovery of organic explosives from swabs (Thompson, Fetterolf, Miller, & Mothershead II, 1999) and soil (Thomas, Donnelly, Lloyd, Mothershead II, & Miller, 2018).

Chemical Signature Selection

        The chemical signatures were chosen based on previous work at MITLL and elsewhere, as well as considering the likelihood of recovering certain signatures after a post-blast event. Some of the prior work at MITLL mentioned above examined multiple signatures from ammonium nitrate fertilizer prills such as trace elements, color, and morphology (Campbell, et al., 2017). Of these, only trace element signatures would likely be preserved after a detonation since the explosion itself and contamination

with the environment would compromise both the color and morphology of the prills. Other work has also demonstrated the use of ICP-MS for trace element analysis of signatures in pre-blast investigations (Brust, et al., 2015) (Fraga, Mitroshkov, Mirjankar, Dockendorff, & Melville, 2017). Most published applied research efforts for post-blast explosives attribution have focused on using isotope ratio mass spectrometry (IRMS) to differentiate the same substance from different manufacturing sources. This technique has been used to differentiate commercial/military grade explosives such as TNT (Nissenbaum, 1975), RDX and HMX (Howa, Lott, Chesson, & Ehleringer, 2014), as well as improvised explosives and their precursors such as ammonium nitrate (Benson, et al., 2009) (Benson, Lennard, Hill, Maynard, & Roux, 2010) (Benson, et al., 2010) (Brust, et al., 2015) (Grimm, Stern, & Lowe, 2018). Also, HPLC-MS has been used extensively for the identification of organic explosive compounds including TNT, RDX, HMX, and PETN from post-blast sites (Borch & Gerlach, 2004) (Anilanmert, Aydin, Apak, Avci, & Cengiz, 2016) (Avci, Anilanmert, & Cengiz, 2017). In addition to the explosive compound itself, other organics found in certain explosive materials such as binders, plasticizers, and other additives (Chapter: 4 Chemical Characteristics of Bombs, 2004) can potentially act as useful signatures detectable by HPLC-MS. Based on the rationale described above, the signatures and associated analytical techniques chosen for this study included trace elements by ICP-MS, isotope ratios by IRMS, and small molecule organics by HPLC-MS.

Test Design

Test Grid Preparation

The test grid was divided into four quadrants where each quadrant was designated for a specific explosive type (Figure 1).

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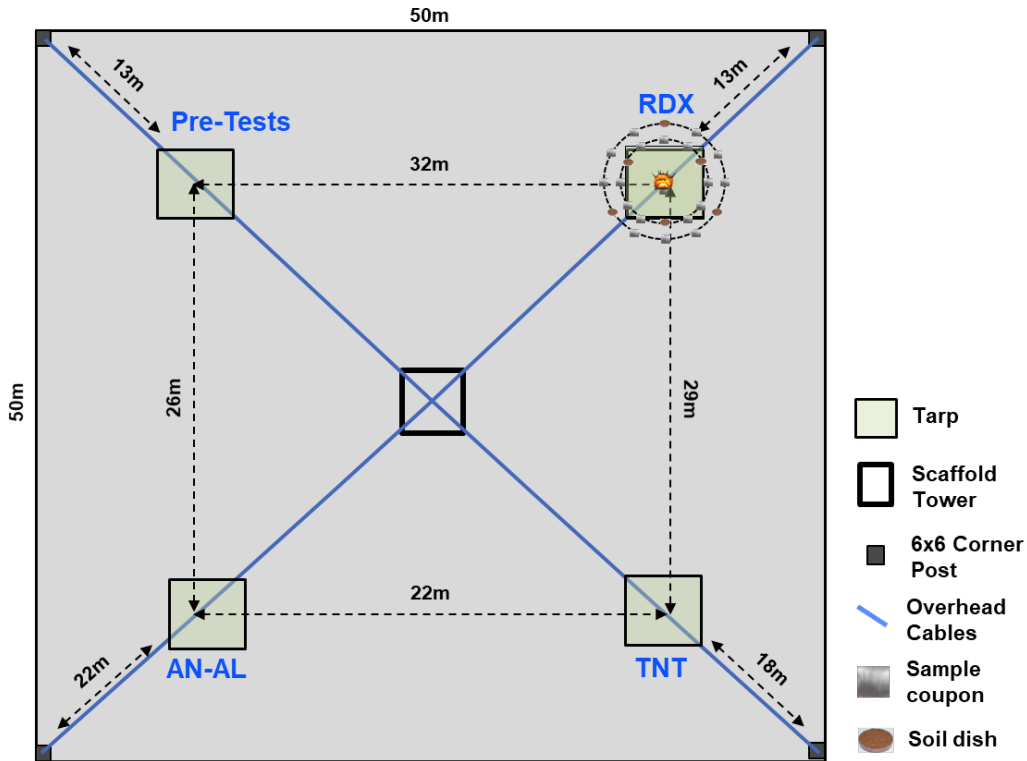
Figure 1: Top-down view of full 50m x 50m testing grid with example grid set up noted for the RDX test area

Replicate detonations for each explosive type were conducted in the same locations to reduce cross-contamination of signatures from other explosive types. All explosive charges were raised 2 meters off the ground to reduce the amount of gravel and dust kick up, and to prevent sample coupons and dishes from getting disturbed from the blast. In order to accomplish this, a scaffold tower was placed in the center of the test grid with cables extending diagonally across each quadrant and connecting to posts on the opposite ends. Each charge was then suspended from a string connected to the cable at the specified location for each explosive type seen in Figure 1. A 10m x 10m tarp was centered below the explosive charge and replaced after each blast to reduce cross-contamination. In an additional effort to reduce cross-contamination, the gravel in the blast area was doused with water from a garden hose between each detonation to further reduce the amount of dust kick up.

The sampling grids for all detonations were arranged in concentric circles at 3 meters and 5 meters from the center Figure 2.

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Figure 2: Example sample grid for a RDX or TNT detonation with sample coupons and soil dishes arranged in concentric circles at 3 and 5 meters from the center. AN-AL sample grids were identical in terms of sample positions and distances, but had polystyrene dishes for sample collection as opposed to coupons and soil dishes.

Sample collection devices were spaced every 30° in each of the circles to maximize the probability of capturing post-blast debris while keeping the overall number 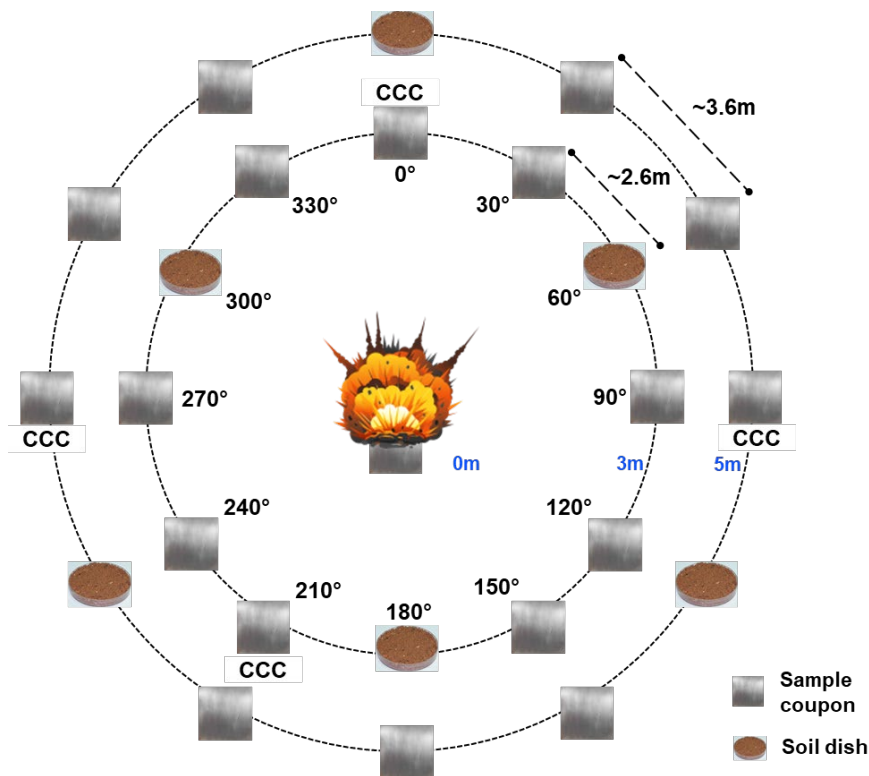of samples to a manageable quantity. In addition to post-blast samples, there were four cross-contamination coupons (CCC) or dishes (CCD) on the grid, which were placed at the start of each grid setup or sampling session, and collected at the end of each session to determine if any explosive was kicked up by personnel during setup or sampling. For RDX and TNT detonations, both sample coupons and soil dishes were used as sample collection devices to simulate different sampling CONOPs as mentioned in the introduction, and were placed in the corresponding positions illustrated in Figure 2. Prior to the test, both coupons and soil dishes were cleaned thoroughly by wetting with deionized water and wiping them with a Kimwipe. This process was then repeated with isopropyl alcohol and methanol. Once cleaned, they were stored in Ziploc bags. For AN-AL, large (6") polystyrene dishes, along with smaller (2") polystyrene dishes containing a circular glass fiber filter (Whatman) were placed at each sample collection site shown in Figure 2. Small magnets were used to hold the filter in place in the small dishes in order to prevent them from getting disturbed during the blast. The purpose of the filters was to provide an alternative means to collect ammonium nitrate residue for IRMS analysis since in previous preliminary tests, large amounts of debris contaminated the samples.

Prior to conducting any tests, pre-tests for each explosive type were conducted to evaluate the spread of the blast debris and determine the optimal distances from the center of the sample grid to place the samples. GoPro cameras were set up on posts near the sample grid to take video recording of

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the detonation. Sample coupons were placed at 3, 5, and 7 meters from the center. After the shot, the sample coupons were visually inspected for debris and the GoPro videos were analyzed to determine the relative spread of the plume. From this, the concentric rings of 12 samples each were placed at 3 and 5 meters from the center for all following tests. In addition, since the explosive device itself was suspended from a cable above the sample grid, 3 additional coupons (RDX/TNT) or dishes (AN-AL) were placed in the center directly below the device. In total, there were 27 sample collection sites for each detonation, with 4 replicate detonations for each explosive type.

A typical sample grid setup began with wetting the blast area and centering a tarp beneath the suspended explosive charge. Metal plates with Velcro were placed at each of the sample locations shown in Figure 2, followed by placement of the four cross-contamination samples. For RDX/TNT tests, sample coupons or dishes for post-blast residue collection, with Velcro on the bottom, were then placed on the metal plates in the corresponding locations in Figure 2. As mentioned before, AN-AL tests used polystyrene dishes for all samples, which were also placed at the same locations in the figure. All samples were placed using the clean hands, dirty hands method described in EPA Method 1669 (U.S. Environmental Protection Agency, 1996). The 'dirty hands' sampler (DH) handles all materials that may potentially be contaminated. The 'clean hands' sampler (CH) dons a clean pair of gloves between every sample and places the appropriate sample collection device. Once sample grid setup was completed, the cross-contamination samples were collected before leaving the grid. Also, located off the sample grid was a small table for positive and negative control samples, which were placed after the sample grid was set up. For RDX/TNT, positive controls were spiked with 250 µL of 8 µg/mL explosive standard for a total of 2 µg. For AN-AL, positive controls were made by placing approximately 5 mg of explosive on a dish. Negative controls were prepared by placing clean coupons or dishes next to the positive controls.

Sample Collection

After detonation, sampling began by collecting the positive and negative control samples and placing cross contamination coupons or dishes at the locations according to Figure 2. Sample collection was performed using the clean hands-dirty hands method as mentioned previously. The DH hands all of the necessary materials to the CH while not contaminating important areas of the sampling materials. For RDX/TNT sample coupons, both CH and DH don a clean pair of gloves. DH opens the individual sampling kit Ziploc which contains a 15 mL conical tube, sampling swab, and a disposable pipette. CH removes both the swab and disposable pipette from the bag. DH opens a vial of isopropanol and CH uses to the pipette to wet the sampling swab with about 0.5 mL of isopropanol. CH swabs the top of the coupon using as much of the swab's surface as possible in a variety of swabbing directions similar to that illustrated in Figure 3. DH then removes the 15 mL conical tube from the Ziploc and opens it. CH breaks the swab tip off into the tube. DH seals the tube and labels the sample with the appropriate sample label.

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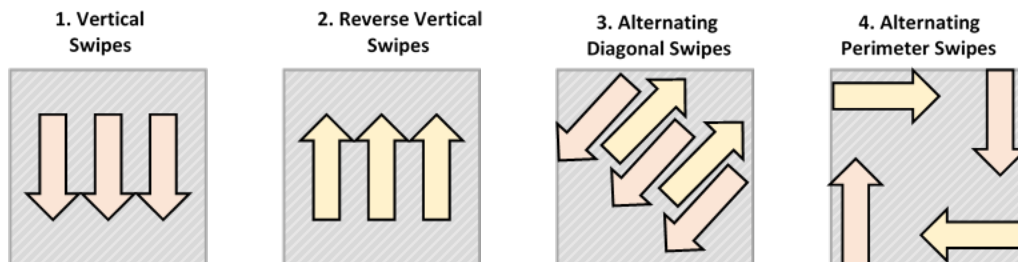
Figure 3: Sample coupon swabbing technique

For RDX/TNT soil dishes, CH removed the soil pan from the metal plate being careful not to lose dirt when pulling it off. DH opens a soil jar and CH pours the soil in. DH caps the jar and labels the sample. Both sample coupons and soil dishes were stored in freezers at -20 C. For AN-AL dishes and filters, the lids were placed on top on the sample dishes, removed from the metal plates, and sealed with tape. The samples were labeled appropriately and stored in Ziplock bags and stored at room temperature. Once all samples were collected, the cross-contamination samples were collected, followed by removal the tarp and setting up for the next detonation.

Sample Processing

All samples were transported back to MITLL in coolers with ice packs. Once samples were returned to the lab, they were stored in -20 C freezers until processing.

RDX and TNT: Swab Extraction

RDX and TNT post-blast samples were extracted from swabs by adding 2 mL of acetonitrile to the 15 mL conical tube containing the sample swab. Sample tubes were transferred to a shaker plate for 15 minutes at 1000 rpm to agitate the swabs and allow for faster dissolving of the explosive and other relevant components. Once dissolved, 2 mL of deionized water was then added to the tube followed by another 15 minutes on the shaker at 1000 rpm. The solvent, now containing extracted sample, was transferred to a 50 mL conical tube while keeping the swab tip behind in the 15 mL tube. Next, 8 mL of deionized water was added to the 15 mL tube and mixed briefly and transferred to the 50 mL tube. This was repeated one more time making sure to press the swab tip against the side of the tube with a pair of forceps to extract as much sample as possible.

RDX and TNT: Soil Extraction

RDX and TNT post-blast samples were extracted from soil by adding 18 mL of deionized water and 2 mL of acetonitrile to jars containing recovered soil. Jars were then vortexed for 30 seconds before transferring the supernatant to a fresh 50 mL conical tube. The sample was then centrifuged at 3000 rpm for 5 minutes to pelletize any solid material.

RDX and TNT: Solid Phase Extraction

Once RDX and TNT samples were extracted from either swabs or soil, they were passed through a solid phase extraction (SPE) process to remove any remaining solid particulates and prepare the samples for instrumental analysis. A manifold was prepared with a pump and SPE cartridges with stopcocks in place to control the flow of reagents and sample through the cartridge. During washes, a

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waste container was placed in the manifold to collect the filtrate as waste. During sample collection, HPLC vials were placed in the manifold to collect samples for analysis. To conduct SPE, 5 mL of acetonitrile was added to each cartridge to prime them. The pump was turned on at the appropriate pressure to allow for solvent flow through the cartridge. The pump remained on for the duration of SPE and flow was controlled using the stopcock valves at the base of each cartridge. This step was repeated twice more with acetonitrile, and once with deionized water, to equilibrate the cartridges. Once primed and equilibrated, 10 mL of extracted sample (in 1:9 acetonitrile to water) was added to each cartridge (1 sample per cartridge). Sample solution was continuously added until all of it had passed through the cartridge. Once sample was loaded onto the cartridges, 1 mL of deionized water was added to each cartridges to wash. After washing was completed, the waste container was replaced with labelled HPLC vials in the slots below each sample's guide needle. To extract sample off the cartridge, 0.5 mL of acetonitrile spiked with 5 ng/mL trinitrophenol (internal standard) was added, passed through the cartridges, and collected in the HPLC vials.

AN-AL: Dish Extraction

AN-AL samples were extracted from the large polystyrene dishes by adding 5.6 mL of deionized water to the dish containing explosive residue. The dish was rotated to capture as much residue in the water as possible. In some cases, a pipette was used to help spread the water across the surface of the dish to capture as much residue as possible. The water was then transferred to a 15 mL tube and vortexed for 30 seconds. Next, 1 mL of each sample was transferred to HPLC vials for shipment to UC Davis for nitrate IRMS analysis. For analysis by ICP-MS, 200 µL of nitric acid and 200 µL of hydrochloric acid were added to the remaining sample in the 15 mL conical tube. The samples were then allowed to digest at room temperature overnight, followed by the addition of 8.6 mL deionized water.

AN-AL post-blast sample recovered on filters in the small polystyrene dishes were set aside since minimal contamination was observed for the large dishes. The filter samples were ultimately not processed and analyzed as the samples from the large dishes provided the necessary data for the study.

Instrumental Methods for Chemical Signature Collection
HPLC-MS Quantitation of RDX and TNT

Extracted RDX and TNT samples in 100% acetonitrile were analyzed for total explosive by HPLC-MS to quantify the amount of explosive in each sample and prioritize samples for sending to UC Davis for IRMS analysis. Samples were run on an Agilent 6520 LC-MS quadrupole time-of-flight (QTOF) with a reversed-phase C18 column. The parameters for the HPLC-MS quantitation method are listed in Table 1, with the HPLC gradient in Table 2.

Table 1: HPLC-MS Quantitation Method Parameters

Mobile Phase A	Acetonitrile
Mobile Phase B	10 mM ammonium nitrate in water
Injection Volume	10 µL
Needle Wash	1x with Acetonitrile
Column Temperature	35.0 °C
Ion Source	Dual ESI
Polarity	Negative
Gas Temperature	200.0 °C

Drying Gas	12 L/min
Nebulizer Pressure	40 psig
Capillary Voltage	4500 V

Table 2: HPLC-MS Quantitation LC Gradient

Start Time (min)	End Time (min)	A (%)	B (%)	Flow (mL/mn)	Pressure Limit (bar)
0.00	12.00	50.0	50.0	0.400	400
12.00	14.00	5.0	95.0	0.400	400
14.00	17.00	50.0	50.0	0.400	400

ICP-MS Quantitation of AN-AL

Extracted and digested AN-AL samples in dilute nitric and hydrochloric acids were analyzed for total aluminum content by ICP-MS. First, all samples were diluted 100x to dilute the amount of aluminum entering the ICP-MS. Nitric and hydrochloric acid were added to bring the diluted sample up to 2% for both acids. For calibration of aluminum, aluminum standard was dissolved in 2% hydrochloric acid and 2% nitric acid at the following part per billion concentrations: 0, 0.05, 0.1, 1, 10, 20, 50, 100, 200, 1000, 5000, 10000, 100000.

Compound-specific stable isotope analysis (CSIA) of TNT and RDX

RDX and TNT (1mg/mL in acetonitrile) were obtained from both Sigma-Aldrich (St. Louis, MO) and Spectrum Chemical (New Brunswick, NJ) for use as laboratory reference materials. Elemental analysis/isotope-ratio mass spectrometry (EA/IRMS) was used to calibrate the δ^{13}C and δ^{15}N values of the explosives prior to their use in calibration and evaluation of gas chromatography/combustion/isotope-ratio mass spectrometry (GC/C/IRMS) measurements. Multiple determinations for each explosive source were made with an Elementar CUBE elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Langenselbold, Hessen, Germany) interfaced to a VisION isotope ratio mass spectrometer (IsoPrime, Stockport, England, U.K.). EA/IRMS results were evaluated using secondary reference materials calibrated against NIST Standard Reference Materials (Gaithersburg, MD, U.S.A.; IAEA-N1, IAEA-N2, IAEA-N3, USGS-40, and USGS-41). The long-term standard deviation for these measurements is ±0.2‰ and ±0.3‰ for δ^{13}C and δ^{15}N values, respectively.

All GC-IRMS measurements were made with a Thermo Trace 1310 GC configured with a PTV inlet and interfaced to a ThermoFinnigan Mat 253 via a GC IsoLink II combustion/high-temperature conversion interface. Optimized conditions for GC-IRMS analysis of two explosive charges (1) 2,4,6-trinitrotoluene (TNT) and (2) hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) are as follows. All measurements were made using a 5m, 0.25mm ID, 0.25μm film DB-5 capillary column (Agilent Technologies) with a constant flow rate of 3mL/min. Initial PTV inlet settings for both TNT and RDX were 60°C; splitless time, 1 min.; purge, off 1 min; split flow, 10mL/min.; purge flow, 50 mL/min. Large volume injections were used, up to 8μL, with an injection speed of 3μL/sec. The PTV program for RDX was as follows: 60°C, hold 0.2min., flow 50mL/min; 5°C/sec. to 180°C, hold 5min.; 14°C/sec. to 270°C, hold 2 min., flow 50mL/min, while the oven program for RDX was 50°C, hold 0.4min; 15°C/min. to 85°C; 20°C/min. to 135°C; 15°C/min. to 280°C, hold 1 min. Initial: 60°C, hold 0.2min., flow 50mL/min. The PTV program for TNT was as follows: 60°C, hold 0.2min., flow 50mL/min; 14.5°C/sec to 260°C, hold 15min.;

14°C/sec to 270°C, hold 2 min., flow 50mL/min. The optimal oven program for TNT was 60°C, hold 1min.; 15°C/min. to 180°C; 90°C/min. to 290°C, hold 6min.

Limit-of quantification of these methods, for both $\delta^{13}C$ and $\delta^{15}N$ of TNT and RDX, was found to be approx. 1.4 µmoles on-column. For TNT, mean accuracy and precision was within ±0.12‰ and ±0.17‰ for $\delta^{13}C$ and within ±0.73‰ and ±0.86‰ for $\delta^{15}N$ measurements. For RDX, mean accuracy and precision was within ±0.28‰ and ±0.31‰ for $\delta^{13}C$ and within ±0.96‰ and ±0.72‰ for $\delta^{15}N$ measurements.

Stable isotope analysis of ^{15}N and ^{18}O from ammonium nitrate

Purified nitrate samples were prepared for stable isotope analysis using the bacterial denitrifier method (Sigman, et al., 2001) (Casciotti, Sigman, Galanter, Bohlke, & Hilkert, 2002) to convert sample nitrate to nitrous oxide. Following conversion of sample nitrate to nitrous oxide, isotope ratios of ^{15}N and ^{18}O were measured using a ThermoFinnigan GasBench/PreCon trace gas concentration system interfaced to a ThermoScientific Delta V Plus isotope-ratio mass spectrometer (Bremen, Germany). Gas samples were purged from vials through a double-needle sampler into a helium carrier stream (25 mL/min). The gas sample passed through a CO_2 scrubber (Ascarite) and N_2O was trapped and concentrated in two liquid nitrogen cryo-traps operated in series such that the N_2O was held in the first trap until the non-condensing portion of the sample gas had been replaced by helium carrier, then passed to a second, smaller trap, for cryofocusing. Finally, the second trap was warmed to ambient temperature and the N_2O was carried by helium to the IRMS following resolution of N_2O from residual CO_2 on an Agilent GS-Q capillary column (30m x 0.32 mm, 40°C, 1.0 mL/min).

A reference N_2O peak was used to calculate provisional isotope ratios of the sample N_2O peak. Final $\delta^{15}N$ values were calculated by adjusting the provisional values such that calibrated $\delta^{15}N$ values for laboratory reference materials were obtained. All laboratory reference materials were directly traceable to the international reference scale for ^{15}N (Air) and ^{18}O (V-SMOW) through regular calibration using standard nitrates USGS 32, USGS 34, and USGS 35, supplied by NIST (National Institute of Standards and Technology, Gaithersburg, MD). Additional laboratory reference materials were included in each batch to monitor and correct for instrumental drift and linearity. Analytical precision and accuracy was better than ±0.4‰ for $\delta^{15}N$ and ±0.5‰ for $\delta^{18}O$ for nitrate concentrations from 4-7000 µM.

HPLC-MS Profiling of RDX and TNT

For HPLC-MS profiling of RDX and TNT, the same HPLC sample vials used for quantitation were also used here. The samples were first spiked with a different internal standard before analyzing by the qualitative method since the quantitative method was done in negative ion mode with an internal standard that is only detected in that mode. Diglyme, an organic compound unlikely to be found in the explosive samples or environment, and is detectable in positive ion mode, was used as the internal standard at a concentration of 500 ng/mL in all samples. Table 3 shows the LC-MS profiling method parameters, and Table 4 shows the LC gradient details. All samples were measured in triplicate.

Table 3: HPLC-MS Profiling Method Parameters

Mobile Phase A	Acetonitrile

Mobile Phase B	10 mM ammonium nitrate in water
Injection Volume	10 µL
Needle Wash	1x with Acetonitrile
Column Temperature	35.0 °C
Ion Source	Dual ESI
Polarity	Positive
Gas Temperature	200.0 °C
Drying Gas	12 L/min
Nebulizer Pressure	40 psig
Capillary Voltage	4000 V

Table 4: HPLC-MS Profiling Method LC Gradient

Start Time (min)	End Time (min)	A (%)	B (%)	Flow (mL/mn)	Pressure Limit (bar)
0	5	95	5	0.4	400
5	35	5	95	0.4	400
35	40	0	100	0.4	400
40	42	95	5	0.4	400

ICP-MS Profiling of AN-AL

Once AN-AL samples were quantified for total aluminum, ICP-MS analysis was conducted again on the samples without any dilution to get the full profile of elements. The following elements were monitored: 7 Li, 9 Be, 11 B, 23 Na, 24 Mg, 31 P, 34 S, 39 K, 44 Ca, 47 Ti, 51 V, 52 Cr, 55 Mn, 56 Fe, 59 Co, 60 Ni, 63 Cu, 66 Zn, 69 Ga, 72 Ge, 75 As, 78 Se, 85 Rb, 88 Sr, 90 Zr, 93 Nb, 95 Mo, 107 Ag, 111 Cd, 118 Sn, 121 Sb, 125 Te, 133 Cs, 137 Ba, 139 La, 140 Ce, 141 Pr, 146 Nd, 147 Sm, 151 Eu, 157 Gd, 163 Dy, 165 Ho, 166 Er, 169 Tm, 172 Yb, 175 Lu, 178 Hf, 181 Ta, 182 W, 205 Tl, 206 Pb, 207 Pb, 208 Pb, 232 Th, and 238 U. Calibration standards for each element listed above were prepared from 0.05 ng/mL to 200 ng/mL. The internal standard elements used were 6 Li, 45 Sc, 85 Y, 115 In, 159 Tb, and 209 Bi. Calibration standards and samples were analyzed by ICP-MS in triplicate with blanks run every three samples.

Data Analysis

HPLC-MS Quantitative Data for RDX and TNT

HPLC-MS data for RDX and TNT quantitation was searched using the Agilent Qual browser software to find the mass to charge ratio of either RDX or TNT in each respective sample. The abundances were exported and the explosive concentration was calculated using calibration curves prepared from standards of each explosive. The samples were then rank-ordered by concentration to prioritize for sending to UC Davis for IRMS analysis. In total, there were 23 TNT and 0 RDX post-blast samples that yielded a quantifiable amount of explosive. Portions of all 23 TNT samples, as well as 3 pre-blast TNT samples, were sent for IRMS analysis. Since RDX was not detected in any post-blast samples, none of them were sent for IRMS analysis. In addition, all samples, including samples that did not have any explosive detected, were measured by HPLC-MS profiling to look for other organic signatures.

ICP-MS Quantitative Data for AN-AL

ICP-MS data for aluminum, exported from the ICP-MS software in ng/mL, was rank-ordered by concentration. All samples yielded a measureable amount of aluminum suggesting that all samples contained at least some collected post-blast AN-AL explosive. Based on this data, the top 30 samples with the highest concentrations of aluminum were selected and the 1 mL portions that were set aside as described above were shipped to UC Davis for IRMS analysis. The quantitative data was not used for any further analysis. All samples were then reanalyzed by ICP-MS for profiling of all measureable elements.

IRMS Data for AN-AL

Of the 30 AN-AL samples (27 post-blast and 3 pre-blast) that were sent for IRMS analysis, 23 of the post-blast samples yielded high enough nitrogen or oxygen concentration for analysis. Since the budget allowed for 30 IRMS measurements, a few of the samples (1 pre-blast and 2 post-blast) were measured in duplicate, with 1 post-blast sample measured in triplicate, to obtain technical replicates for those samples. Data in the form of $\delta 15 N_{Air}$ (‰) and $\delta 18 O_{VSMOW}$ (‰) for nitrogen and oxygen respectively was subsequently analyzed.

For statistical analysis, the samples with two or more technical replicates were averaged within each sample and the averages were included back in the data set. Initial inspection of the scatterplot between oxygen and nitrogen in Figure 4 showed multiple outliers in the post-blast data set. The pre-blast samples (in red) were well-mixed among the post-blast samples (in black). Due to the limited number of samples, Algorithm 1 below was used on the nitrogen and oxygen measurements individually. Algorithm 1 was performed on the post-Blast data only, leaving the pre-blast data to compare with once the analysis was complete. For the atypicality analysis with outlier removal (O'Brien, 2017) (Guharay, Saunders, Su, & Danyliw, 2012) (Ausdemore, Neumann, Saunders, Armstrong, & Muehlethaler, 2021) (Aitchison & Dunsmore, 1976) (McLachlan, 1992), the set of hypotheses are as follows:

- H_0 (the null hypothesis): There is no difference between the removed sample and the average of the remaining sample.
- H_1 (the alternative hypothesis): There is a difference between the removed sample and the average of the remaining sample.

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Algorithm 1: Leave-one-out atypicality algorithm with outlier removal calculated on a univariate score or measurement.

Data: Let S_{n_0} represent the Post-blast data and n_0 represent the number of samples. The data can be a univariate measurement or score.

Result: Two sets of samples, one with no outliers (S_{n_k}) and one only containing the outliers (O_0).

Start

Let $k = 0$ and $O_0 = \emptyset$.

while *There exists at least one $p_i < 0.05$* **do**

    for *i from $1 : n_k$* **do**

        1. Remove sample i ;

        2. With the remaining $n_k - 1$ samples, calculate the sample mean $\hat{\mu}_{-i}$ and sample variance $\hat{\sigma}_{-i}$;

        3. Calculate $d_i = (x_i - \hat{\mu}_{-i})^2/(\hat{\sigma}_{-i})$;

        4. Note that d_i is approximated by a chi-square distribution under the null hypothesis with one degree of freedom ;

        5. Calculate and record p_i, the p-value of d_i under the null hypothesis ;

        6. Add sample i back into the data set ;

    end

    1. Make a new set of $p_i < \alpha$ (we chose $\alpha = 0.05$) ;

    2. Set $k = k + 1$;

    3. Set $n_k = n_0 - k$;

    4. Remove the sample with the lowest p-value from the Post-blast data set. Define this removed sample as $x_{(k)}$;

    5. Set $O_k = O_{k-1} \cup x_{(k)}$;

    Return O_k and $S_{n_k} = S_n \cap O_k^c$;

end

IRMS Data for TNT

      Of the 26 TNT samples (23 post-blast and 3 pre-blast) that were sent for IRMS analysis, only 3 of the post-blast samples yielded high enough nitrogen concentration for analysis, and of those, only 2 yielded high enough carbon concentration for analysis. Each of the post-blast samples were measured in duplicate to obtain technical replicates for those samples. Data in the form of $\delta^{15}N_{Air}$ (‰) and $\delta^{13}C_{VPDB}$

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(‰) for nitrogen and carbon respectively was subsequently analyzed. Due to the limited amount of samples, the averages for each sample were not calculated prior to the atypical analysis.

Initial inspection of the scatterplot between carbon and nitrogen in Figure 9 shows little grouping of the nitrogen measurements on the post-blast data set, and a very narrow range on the carbon measurements for the post-blast data set. The pre-blast samples (in red) grouped among themselves for both elements and separated well from the post-blast samples (in black). Due to the limited number of samples, Algorithm 1 above was used on the carbon and nitrogen measurements individually. This analysis was used on the post-blast data only, leaving the pre-blast data to compare with once the analysis was complete.

HPLC-MS Profiling Data for TNT

Data acquired from HPLC-MS profiling was processed using Agilent ProFinder, a tool for performing untargeted molecular feature extraction of compounds from mass spectra. The analysis began by searching the pre-blast sample data only. This made the process easier since there were fewer pre-blast samples (3 samples x 3 replicates each) compared to the number post-blast samples (26 samples x 3 replicates each). Also, if any potential organic signatures for sourcing exist, they would have to at least be found in the pre-blast samples. A targeted list of potential organic signatures from the pre-blast was generated first and then used to search the post-blast samples. The pre-blast sample data, along with the process blank and negative control sample data, was searched using the parameters detailed in Table 5.

Table 5: Agilent ProFinder Untargeted Compound Search Parameters and Settings

Parameter	Setting
Ion Signal to Noise Ratio	5
Adducts	H, Na, NH$_4$, H$_2$O
Isotope Model	Common organics, no halogens
Charge State	1-2
Ion Count Threshold	2 or more
Retention Time Tolerance Window	5% +/- 0.5 min
Mass Tolerance Window	50 ppm +/- 5 mDa
Molecular Feature Extraction (MFE) Score	>= 70 in 3 replicates per sample
All Other Parameters	Default settings

Once the data were searched, the algorithm yielded a list of 90 potential organic compounds. The results were manually inspected to ensure the resulting entities were real compounds and not artifacts or noise. This involved looking at the overall peak shape and resolution, as well as the relative abundance against the background. While a specific abundance threshold was not included in the above parameters, a notional value of 3000 peak height counts using visual inspection of the chromatograms was used as a minimum threshold. In some cases, if peak resolution against the background was good, compounds below 3000 peak height counts were kept. In addition, any compounds that were detected in either the blank or negative control samples were assumed to be contaminants from the process or

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the environment, and were subsequently removed. These steps eliminated nearly all compounds since only 4 remained. Once the targeted list of 4 compounds was inspected and finalized, the post-blast sample data was imported into the software and searched using the target list. Another round of manual inspection was conducted to ensure the correct chromatographic peaks were integrated for each compound across all samples. Peak areas for each compound were then exported and used as abundance data for further statistical analysis.

As mentioned earlier, only 23 post-blast TNT samples yielded any detectable amount of explosive. The 4 compounds found in both pre- and post-blast samples were also detected in all 23 of the TNT samples where explosive was detected, but not detected in any other sample. Technical replicates were averaged for these 23 post-blast samples prior to any statistical analysis. Initial inspection of the data was done with a pairwise scatterplot Figure 14 to observe any overlap between the pre and post-blast across elements. This revealed several outlier samples, so an atypicality analysis (O'Brien, 2017) (Guharay, Saunders, Su, & Danyliw, 2012) (Ausdemore, Neumann, Saunders, Armstrong, & Muehlethaler, 2021) (Aitchison & Dunsmore, 1976) (McLachlan, 1992) was conducted to identify and remove outliers from the data. Algorithm 2 was used on all four compounds in conjunction. This analysis was used on the post-blast data only, leaving the pre-blast data to compare with once the analysis was complete.

Algorithm 2: Leave-one-out atypicality algorithm with outlier removal calculated on a multivariate set of measurements.

Data: Let S_{n_0} represent the multivariate Post-blast data and n_0 represent the number of samples.

Result: Two sets of samples, one with no outliers (S_{n_k}) and one only containing the outliers (O_0).

Start

Let $k = 0$ and $O_0 = \emptyset$.

while *There exists at least one* $p_i < 0.05$ **do**

    for *i from* $1 : n_k$ **do**

        1. Remove sample i ;

        2. With the remaining $n_k - 1$ samples, calculate the sample mean vector $\hat{\mu}_{-i}$ and sample variance $\widehat{\Sigma}_{-i}$;

        3. Calculate $d_i = (x_i - \hat{\mu}_{-i})^t \widehat{\Sigma}_{-i}^{-1} (x_i - \hat{\mu}_{-i}) \frac{(n_k-1)}{n_k} \frac{(n_k+1-4-1)}{4(n_k+1-2)}$;

        4. Note that d_i is approximated by an F distribution under the null hypothesis with degrees of freedom $df_1 = 4$, $df_2 = n_k + 1 - p - 1$;

        5. Calculate and record p_i, the p-value of d_i under the null hypothesis ;

        6. Add sample i back into the data set ;

    end

    1. Make a new set of $p_i < \alpha$ (we chose $\alpha = 0.05$) ;

    2. Set $k = k + 1$;

    3. Set $n_k = n_0 - k$;

    4. Remove the sample with the lowest p-value from the Post-blast data set. Define this removed sample as $x_{(k)}$;

    5. Set $O_k = O_{k-1} \cup x_{(k)}$;

    Return O_k and $S_{n_k} = S_n \cap O_k^c$;

end

ICP-MS Profiling Data

    Data for all elements measured by ICP-MS was conditioned for statistical analysis in three steps. First, all concentration values that were below 3x LOD were considered not detected and were changed to 0. Second, data were blank subtracted by subtracting 10x the average of the blank (for a given element). All subsequent negative values were changed to 0. Third, out of 12 pre-blast sample

measurements (4 samples with 3 replicates each), if 4 or fewer measurements were below the LOD, the element was removed as a parameter from any further analysis. After applying these conditioning steps, 33 elements remained across 348 samples (4 replicate detonations each with 4 pre-blast samples and 27 post-blast samples, all measured in triplicate).

Technical replicates were averaged for all post-blast samples prior to any statistical analysis. Initial inspection of the data was done with boxplots (see Figure 17 and Figure 18) to observe any overlap between the pre and post-blast across elements. Algorithm 3 was used on all 33 elements in conjunction to calculate the cross-correlation scores; it was applied on both the Pre-blast and the Post-blast data sets separately. This provided a univariate score per sample, reducing the dimensionality allowed for easier visualisation. This revealed several outlier samples, so an atypicality analysis (O'Brien, 2017) (Guharay, Saunders, Su, & Danyliw, 2012) (Ausdemore, Neumann, Saunders, Armstrong, & Muehlethaler, 2021) (Aitchison & Dunsmore, 1976) (McLachlan, 1992) was conducted to identify and remove outliers from the data. Algorithm 1 was applied to the Post-blast cross-correlation scores only, leaving the Pre-blast cross-correlation scores to compare with once the analysis was complete.

Algorithm 3: Calculating a cross-correlation score across a set of samples with multivariate measurements.

Data: The Pre-blast and Post-blast data sets are considered separately. Each are a multivariate set of measurements.

Result: Two sets of samples, one with the cross-correlation scores for all samples from the Pre-blast set, and one with the cross-correlation scores for all samples from the Post-blast set.

Start

for M *in* $\{Pre\text{-}blast,\ Post\text{-}blast\}$ **do**

    Let n_M be the number of samples in M ;

    for i *from* $1 : n_M$ **do**

        for j *from* $1 : n_m - \{i\}$ **do**

            1. Let $M^{\{i\}}$ and $M^{\{j\}}$ be the vectors of measurements for the ith and j^{th} samples in M, respectively ;

            2. Let n_e be the number of elements for which there are measurements for in M ;

            3. Calculate and record the cross-correlation between sample i and sample j to get the cross-correlation score:

$$c_{M_{ij}} = \frac{\sum_{p=1}^{n_e}(M_p^{\{i\}} - \overline{M}^{\{i\}})(M_p^{\{j\}} - \overline{M}^{\{j\}})}{\sqrt{\sum_{p=1}^{n_e}(M_p^{\{i\}} - \overline{M}^{\{i\}})^2(M_p^{\{j\}} - \overline{M}^{\{j\}})^2}}$$

        end

        For all cross-correlation scores containing sample i, calculate the average:

$$c_{M_i} = \frac{1}{n_m - 1} \sum_{i=1}^{n_m - \{i\}} c_{M_{ij}}$$

    end

    Return the set C_M which contains c_{M_i} for all $i \in 1, \dots, n_M$;

end

4) Expected Applicability

This study provided the first step in the development of a novel investigative method to associate an explosives attack to a suspect (through a manufacturer) to supplement current post-blast investigative methods. Initial interest in the concept of post-blast attribution of explosives had been expressed by relevant federal government laboratories if the experiments demonstrated recoverable material and suitable analytical/statistical methods. The CSIA method developed for the isotope ratio analysis of organic explosives has been transitioned to a relevant federal forensic laboratory for incorporation in

their workflows. In addition to the developed and transitioned CSIA method, transition of the other developed analytical/statistical methods is possible.

Participants

1) Individual Participants

The participants of this study included:

1. **Josh Dettman** – principal investigator who contributed to the study design and oversight of the project. He also oversaw and assisted in field testing and progress reporting.
2. **Paul Ippoliti** – technical lead on the project who contributed to study design and oversight of the technical activities on the project including method development, field testing, sample processing and analysis, data and statistical analysis, and progress reporting.
3. **Chris Saunders** – co-investigator who oversaw the data and statistical analysis
4. **Jeff Werlich** – chemist who conducted a significant portion of the method development, field testing, and sample processing and analysis.
5. **Cami Fuglsby** – graduate student who worked with Chris Saunders and contributed to much of the statistical analysis of the data
6. **Dennis Hardy** – field technician who oversaw and assisted in much of the field test planning and was involved in the preliminary testing round in Year 1
7. **Kentaro Hansen** – a summer intern who assisted in the method development of sample processing and analysis methods
8. **Kate Digan** – a specialist who assisted with field testing
9. **Keith Williams** – former Navy EOD technician and owner/operator of Newtec Services Group, Inc. who hosted the field test and prepared the explosive devices used in the study
10. **Chris Yarnes** – scientist at UC Davis who conducted the IRMS analysis

2) Collaborating Organizations

The collaborating organizations for this study are as follows:

1. South Dakota State University in Brookings, SD – data and statistical analysis
2. Stable Isotope Facility at University of California, Davis in Davis, CA – isotope ratio analysis
3. Newtec Services Group, Inc. in Edgefield, SC – field testing site

Changes in Approach

A brief initial collection of post-blast samples was added to the plan, with execution in November 2019. This change was made to reduce risk to successful collection of applicable post-blast samples: rather than relying only on analytical standards and a single field test, this initial fielding provided some post-blast samples for method development and testing of the explosive/sampling configurations. Because this was a small effort, there was no significant impact on the budget/timeline.

The field test was originally scheduled for April 2020, but was delayed to October 2020 due to the COVID-19 pandemic. The planning and preparation steps for the field test were more involved to reduce the risk of MITLL personnel contracting COVID while on travel. Multiple contingency plans needed to be put in place and additional precautions were taken which required devoting more time than usual to planning the test. As a result of the delay in the field test, there were shorter time windows to complete the necessary data processing and analysis in time for conference presentations in

early 2021. Fortunately, sufficient data and results to include in the presentations was generated, and there was enough time to complete the remaining data and statistical analysis for the final report and manuscript.

Outcomes

1) Activities/Accomplishments

Summer/Fall 2019:

1. Set up a sub-award to Prof. Chris Saunders at South Dakota State University (SDSU) to conduct data and statistical analysis.
2. Established a subcontract with University of California at Davis' Stable Isotope Facility to develop IRMS methods for explosive compounds and analyze samples recovered from field test
3. Developed procedures for extracting and measuring RDX and TNT from aluminum coupons and soil:
   - Field swabbing method was developed for obtaining signatures from coupon or soil adapted from prior work at MITLL looking at trace explosive background levels in the environment. Also incorporated EPA 1669 Method into the field sampling technique.
   - Solid phase extraction method was adapted from Thomas, et. al., 2018 for removing particulates and extracting organic compounds of interest for analysis by HPLC-MS.
   - HPLC-MS based quantitation method developed to measure relative quantities of RDX and TNT in extracted samples. Calibration curves were prepared from analytical standards of each compound to determine LOD (TNT: 6.4 ng/mL; RDX: 14.2 ng/mL) and perform quantitation of field test samples.
   - Compound stability tests were performed for both RDX and TNT using analytical standards to test for potential degradation during shipping or prolonged sitting (>2 weeks with no change)
   - Recovery tests were performed for both RDX and TNT using analytical standards from both coupons and soil to determine extraction efficiency from a known spiked quantity (TNT: ~80% from coupons, ~95% from soil; RDX: ~75% from coupons, ~70% from soil)
   - HPLC-MS trace organic signatures profiling method developed and tested using pre-blast material acquired from Keith Williams at NEWTEC.
4. Conducted a preliminary field test to collect initial post-blast samples from each of the materials of interest (RDX, TNT, AN-AL) for informing data analysis, further method development, and lessons learned for the primary field test conducted in October 2020.
   - Executed three successful detonations of AN-Al, and one successful detonation each of RDX and TNT
5. Successfully processed samples collected from November field test
   - Quantitation of 53 samples by LC-MS to measure relative quantities of RDX and TNT. Samples were prioritized based on amount of explosive present and 11 samples (including 3 'pre-blast' samples) were sent to UC Davis for IRMS analysis
   - 35 RDX and 18 TNT samples were also analyzed by HPLC-MS for minor and trace organic signatures profiling
   - 33 AN-AL samples were processed and measured by ICP-MS to quantitate aluminum (AL) as a basis for ammonium nitrate (AN) concentration. Samples were prioritized based on amount of Al present and 7 samples (including 3 'pre-blast' samples) were sent to UC Davis for NO_3 analysis by bacterial denitrification followed by IRMS
   - AN-AL samples were also analyzed by ICP-MS for trace element profiling

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6. Conducted data analysis from samples collected from November 2019 field test.
    *   The majority of post-blast isotope ratios are within 0.5 ‰ (analytical variation = 3.13 ‰) of the pre-blast isotope ratios, indicating that isotope ratio signatures of explosive compounds look to have been preserved after detonation in this small sample set
    *   All data was successfully collected with all techniques (e.g. LC-MS, ICP-MS, IRMS) and analyzed by Prof. Chris Saunders at SDSU. Regular biweekly meetings with Prof. Saunders resulted in successful collaboration and significant progress on statistical analysis of the data

Summer/Fall 2020

7. Planned and conducted primary field test in October 2020 at Newtec's test range in Edgefield, SC
    *   Several months were spent planning and preparing the field test which included extensive preparations and contingency plans due to the ongoing pandemic in addition to the necessary technical and logistical preparations for conducting a field test
    *   Field test was executed taking lessons learned from preliminary field test conducted in November 2019
8. Sample processing and analysis of all samples collected on October 2020 fielding successfully completed in November 2020
    *   Samples were processed using sample preparation methods developed and adapted in Year 1 followed by instrumental analysis to include HPLC-MS quantitation of RDX and TNT samples followed by untargeted organic profiling (also by HPLC-MS) of samples with detected levels of explosive. AN-AL samples were processed and analyzed by ICP-MS for quantitation of aluminum followed by trace element profiling
    *   Portions of samples from each explosive type with detected levels of explosive were sent to collaborators at UC Davis for IRMS analysis
    *   Data collected from HPLC-MS and ICP-MS analyses was compiled, post-processed, and sent to Prof. Chris Saunders at SDSU for statistical analysis
9. Abstracts for multiple conferences including the AAFS and PittCon were accepted for oral presentations
    *   Oral presentation and poster were presented at AAFS (poster presented as part of NIJ R&D Symposium)
    *   Oral presentation presented at PittCon
10. IRMS analysis of AN-AL and TNT samples was conducted through the collaboration with the Stable Isotope Facility at UC Davis.
    *   30 AN-AL samples were analyzed for nitrogen and oxygen isotope ratios, with good detection of both nitrogen and oxygen isotopes in all samples.
    *   26 TNT samples were analyzed for carbon and nitrogen isotope ratios, with 3 samples yielding high enough carbon/nitrogen concentration for accurate measurement of isotopes
    *   RDX samples did not yield enough explosive concentration for IRMS detection

Spring/Summer 2021:

11. Further data analysis in collaboration with Prof. Chris Saunders at SDSU

- HPLC-MS and ICP-MS datasets were further refined through manual inspection of chromatographs and mass spectra to improve final signature outputs for statistical analysis
- Outlier detection based set of methods for the analysis of the post blast signatures. The focus on this effort was to select a set of post blast samples that allow for the comparison of the pre-blast samples.

12. Further presentations and publication
    - A full project briefing was prepared for an internal seminar
    - A manuscript on the full work conducted over the last 2 years will be prepared and published

2) Results and Findings

General Results Overview

In total, there were 108 post-blast samples collected (4 replicate shots with 27 samples per shot) and 3 pre-blast samples for each explosive type (see Table 6). For AN-AL, every sample yielded a measureable amount of aluminum by ICP-MS quantitation from which 27 post-blast and 3 pre-blast samples were sent for IRMS analysis. For RDX and TNT, recovery was more challenging with only 23 post-blast TNT samples and 0 post-blast RDX samples yielding a measureable amount of explosive material by HPLC-MS quantitation. RDX and TNT are both high-order explosives which result in detonations that consume all or nearly all explosive material. Of the two, RDX is the highest order explosive, which when detonated, most likely resulted in material residue getting rapidly consumed. Combined with the relatively small size of the devices, it was difficult to obtain any post-blast residue of RDX. Out of 108 post-blast samples collected for TNT, only 23 had any detectable amount of material by HPLC-MS, with only 3 of these samples yielding enough carbon or nitrogen to measure by IRMS.

Table 6: Number of Recovered Post-Blast Samples

Technique	AN-AL	RDX	TNT
Total	108	108	108
ICP-MS	108	N/A	N/A
HPLC-MS	N/A	0	23
IRMS	27	0	3

IRMS Results for AN-AL

For AN-AL, IRMS was used to measure isotope ratios for oxygen and nitrogen. Data was acquired for 3 pre-blast samples and 27 post-blast samples, with one pre-blast sample measured in duplicate. Of the post-blast samples, there were four shots in total with each shot measuring seven, five, five, and six samples respectively. Shot three had one sample with two technical replicates, and another sample with three technical replicates; the remaining three samples each had one technical replicate. Shot four had one sample with two technical replicates, with the remaining five samples having one technical replicate each. The samples with two or more technical replicates were averaged within each sample and the averages were included back in the data set.

The results of Algorithm 1 on the AN-AL post-blast data are summarized by the boxplots in Figures 5-8. Figure 5 shows no overlap between the pre-blast oxygen data and the outlier-removed post-

blast oxygen data, and Figure 6 shows that the pre-blast oxygen data falls entirely within the interquartile range of the outliers of the post-blast data. Figure 7 shows a small amount of overlap of the interquartile ranges of the boxplots of the pre-blast nitrogen data and the outlier-removed post-blast nitrogen data, and Figure 8 that the pre-blast nitrogen data falls entirely within the interquartile range of the outliers of the post-blast data; note that it falls within the tail of the interquartile range.
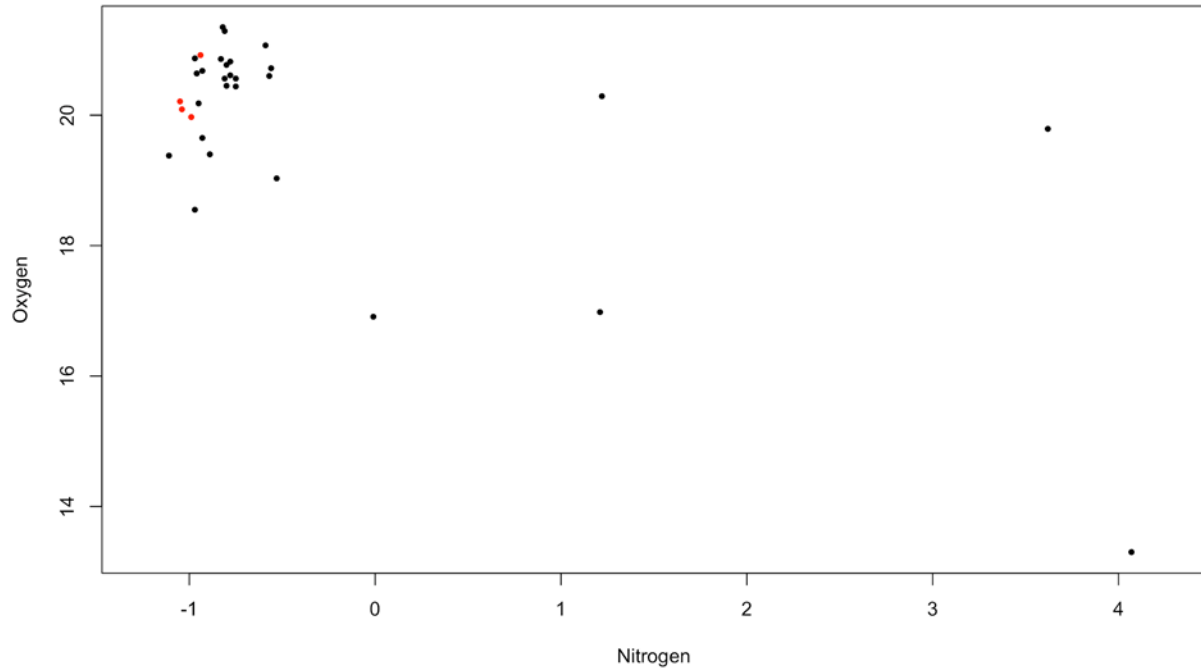
Figure 4: Plot of the IRMS technique on AN - AL pre-blast and post-blast data, oxygen vs nitrogen. Pre-blast measurements are colored red, and post-blast measurements are colored black.

IRMS of AN-AL Pre- and Post-Blast w/Outliers Removed
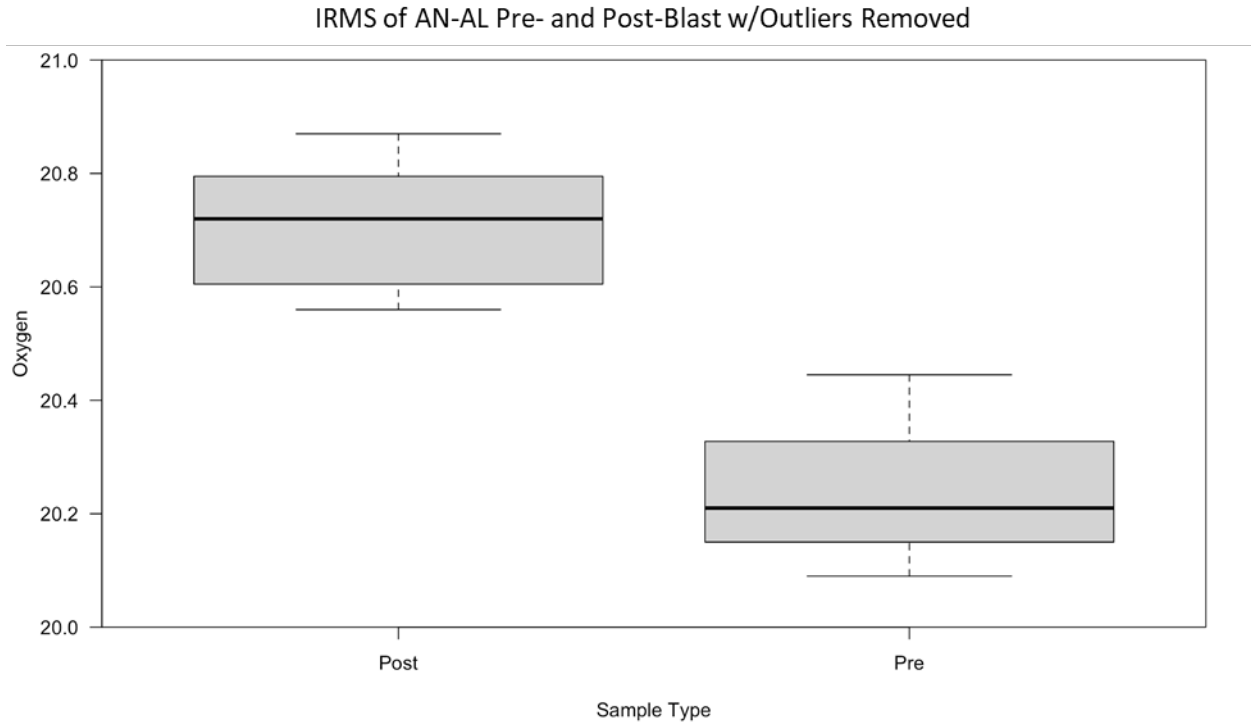
Figure 5: Boxplot of the IRMS technique on AN-AL oxygen data comparing post-blast with outliers removed using the atypical analysis, and pre-blast data.
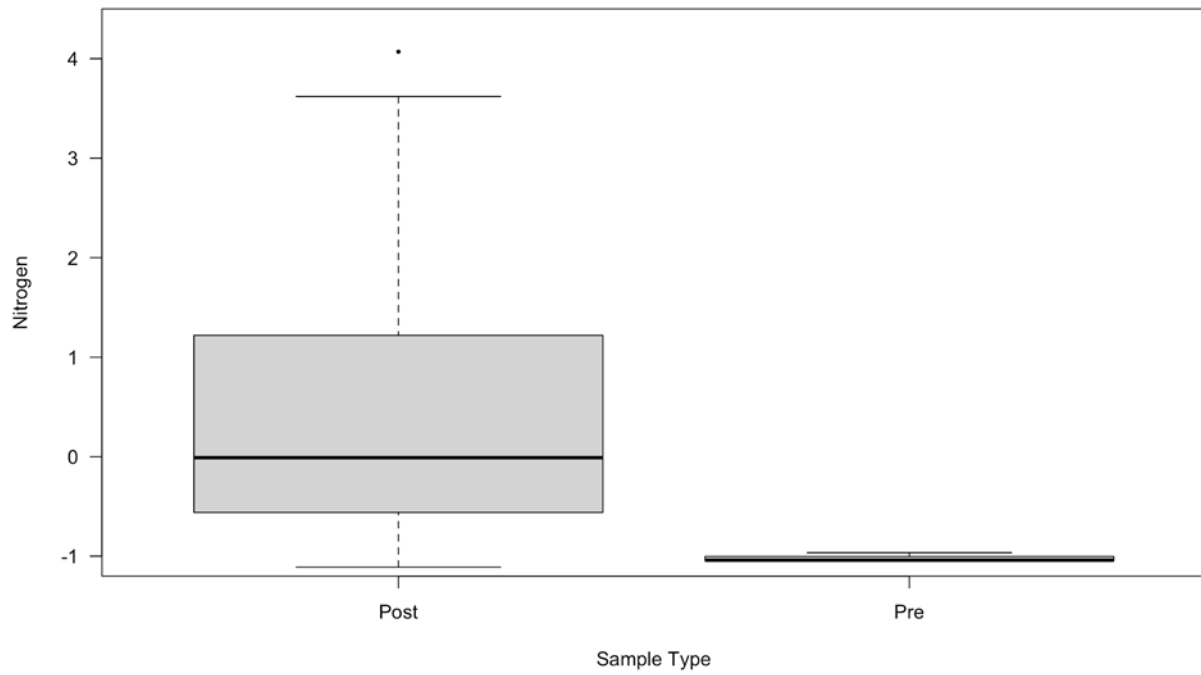

IRMS of AN-AL Pre- and Post-Blast w/Outliers
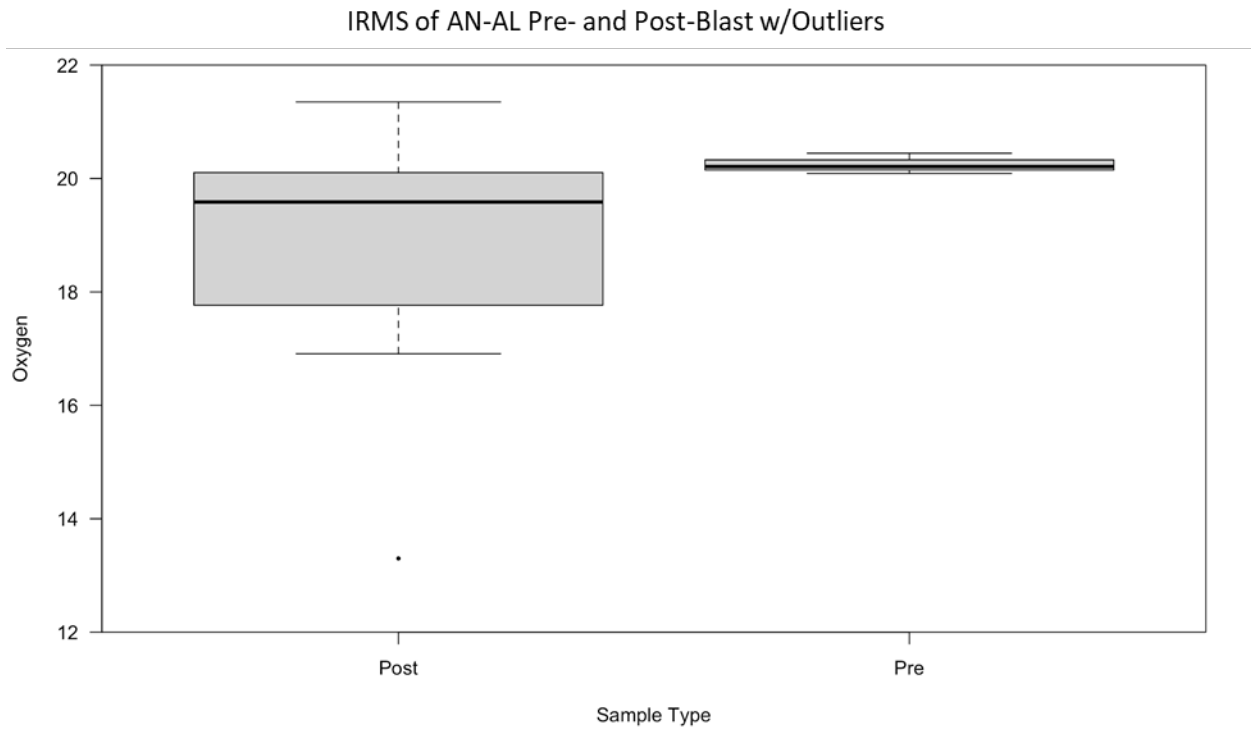
Figure 6: Boxplot of the IRMS technique on AN-AL oxygen data comparing post-blast with outliers, and pre-blast data.

IRMS of AN-AL Pre- and Post-Blast w/Outliers Removed
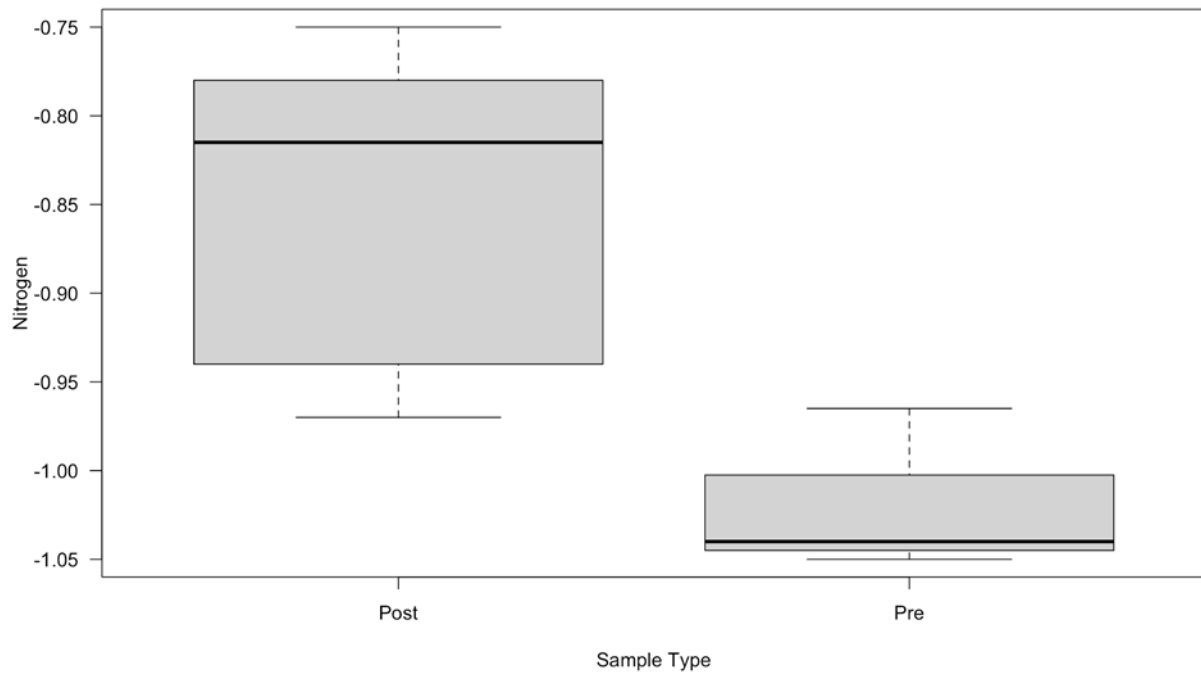
Figure 7: Boxplot of the IRMS technique on AN-AL nitrogen data comparing post-blast with outliers removed using the atypical analysis, and pre-blast data.

IRMS of AN-AL Pre- and Post-Blast w/Outliers

Figure 8: Boxplot of the IRMS technique on AN-AL nitrogen data comparing post-blast with outliers, and pre-blast data.

IRMS Results for TNT

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For TNT, IRMS was used to measure isotope ratios for carbon and nitrogen. Data was acquired for three pre-blast samples and three post-blast samples. Each of the samples in the pre-blast had two technical replicates. For post-blast, data was acquired on shot one, with only two samples. The two samples in the post-blast each had two technical replicates.

The results of Algorithm 1 on the TNT post-blast data are summarized by the boxplots in Figures 10-13. Figure 10 is showing no overlap between the pre-blast nitrogen data and the outlier-removed post-blast nitrogen data. Figure 11 shows that the pre-blast nitrogen data falls entirely within the interquartile range of the outliers of the post-blast data. Figure 12 and Figure 13 are showing separation between the boxplots of the pre-blast carbon data and the post-blast carbon data.
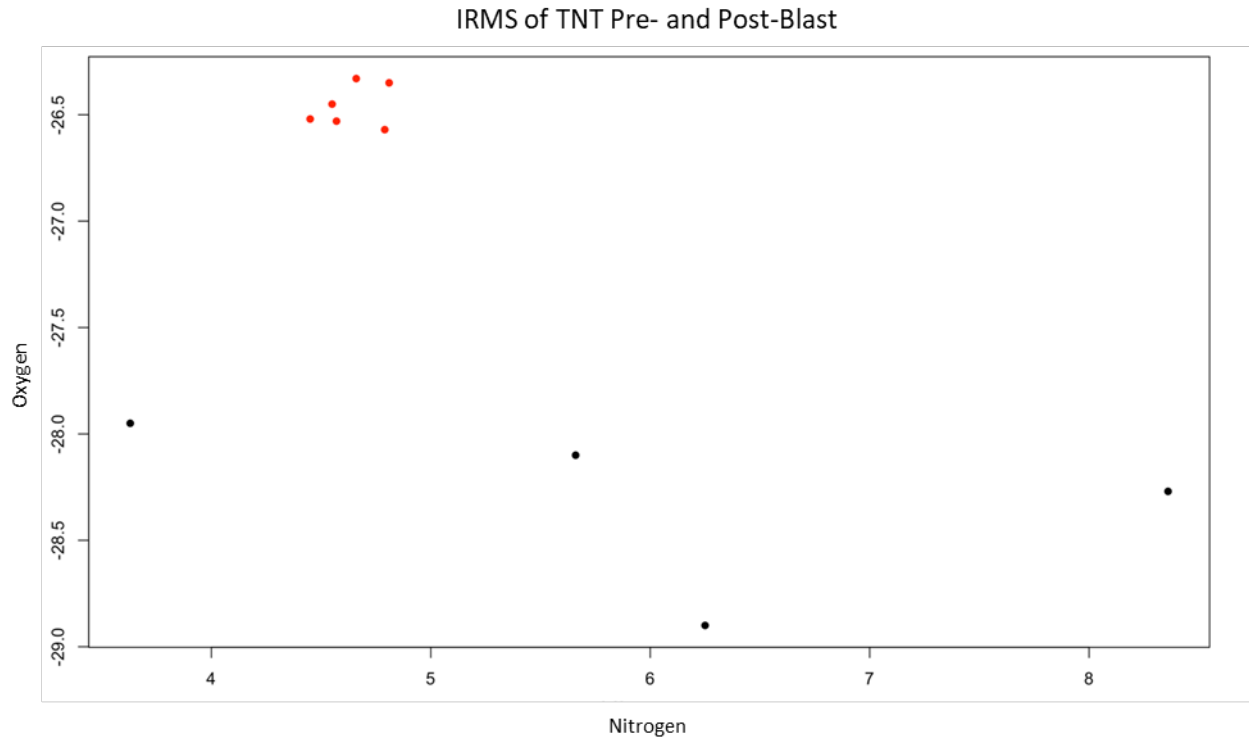
Figure 9: Plot of the IRMS technique on TNT pre-blast and post-blast data, Oxygen vs Nitrogen. Pre-blast measurements are colored red, and post-blast measurements are colored black.
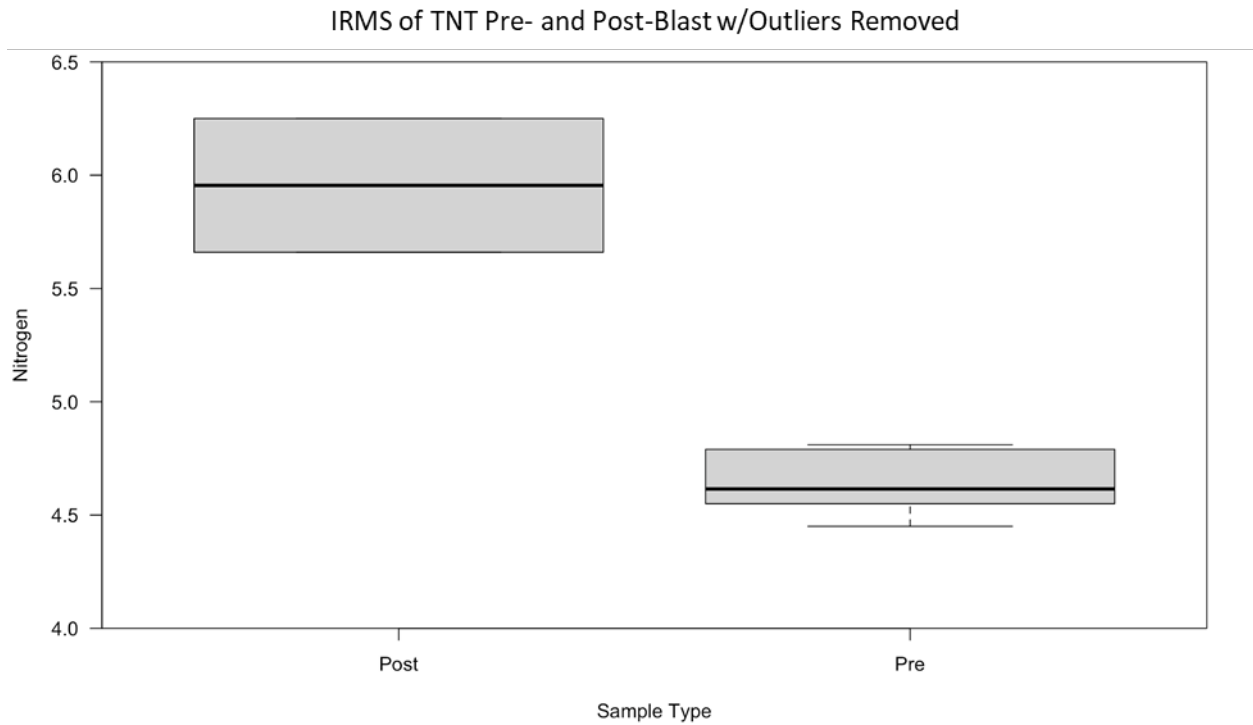
IRMS of TNT Pre- and Post-Blast w/Outliers Removed

Figure 10: Boxplot of the IRMS technique on TNT nitrogen data comparing post-blast with outliers removed using the atypical analysis, and pre-blast data.
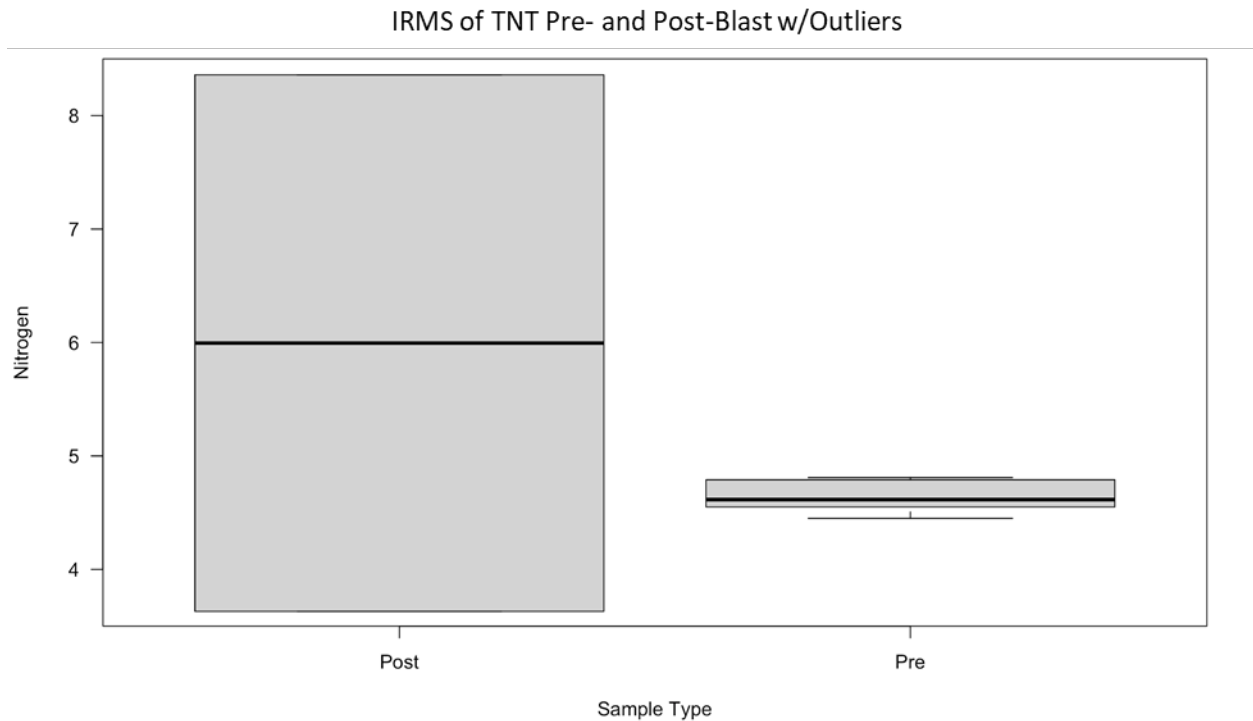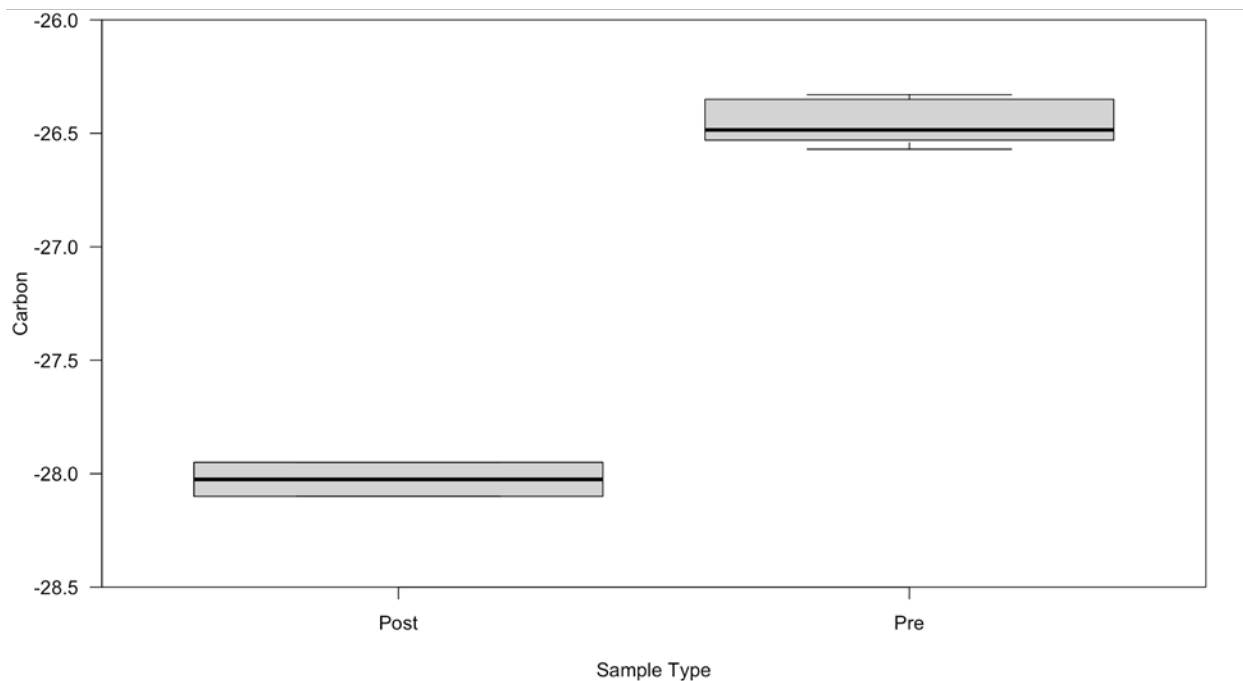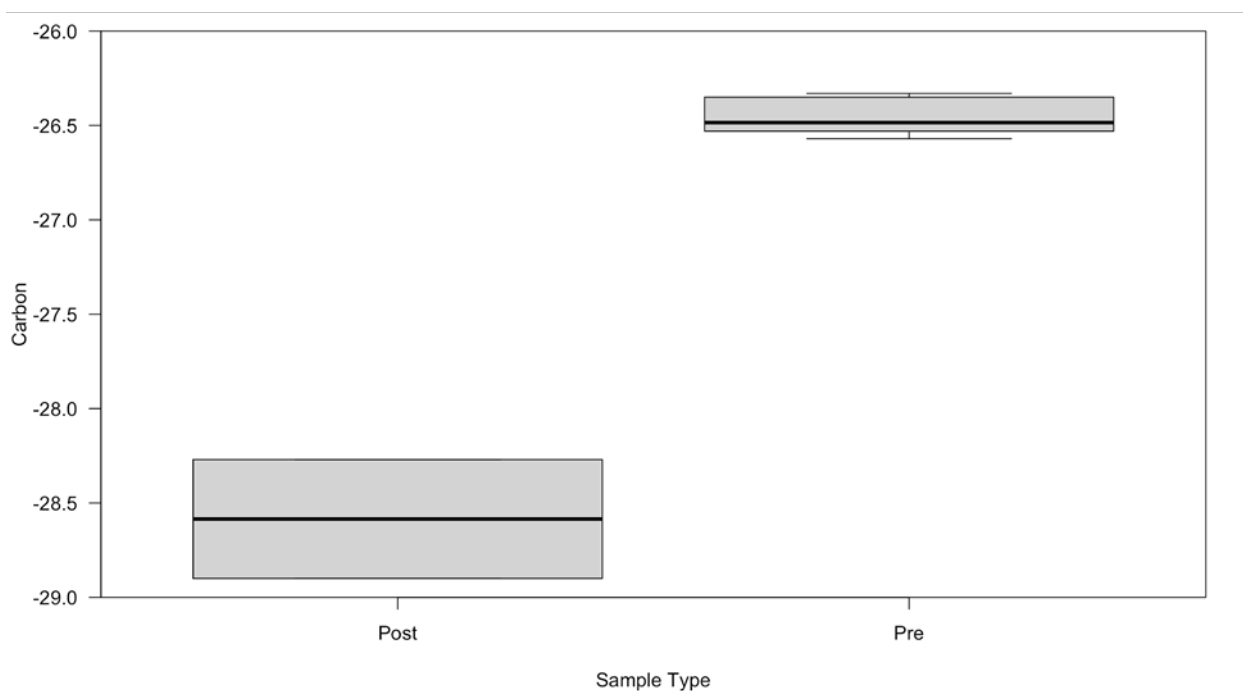

IRMS of TNT Pre- and Post-Blast w/Outliers

Figure 11: Boxplot of the IRMS technique on TNT nitrogen data comparing post-blast with outliers, and pre-blast data.

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Figure 12: Boxplot of the IRMS technique on TNT carbon data comparing post-blast with outliers removed using the atypical analysis, and pre-blast data.

Figure 13: Boxplot of the IRMS technique on TNT carbon data comparing post-blast with outliers removed using the atypical analysis, and pre-blast data.

HPLC-MS Results for TNT

Data from HPLC profiling of TNT resulted in four compounds found in both pre and post-blast samples, and were not found in any blanks or negative control samples. Three samples, each with three technical replicates, were measured pre-blast. For post-blast samples collected from coupons, data was acquired for eight samples from shot one, two samples from shot two, four samples from shot three, and three samples from shot four, with three technical replicates per sample. For post-blast samples collected from soil, data was acquired for two samples from shot one and three samples from shot three, also with three technical replicates per sample.

The results of Algorithm 2 on the HPLC TNT post-blast data are summarized by the pairwise scatterplots in Figures 15-16. Figure 15 shows the pre-blast samples (in red) and the post-blast samples (coupon samples in black, soil samples in green) with outliers removed. The pre-blast samples are well-mixed in with the post-blast samples with outliers removed. Figure 16 shows the pre-blast samples (in red) and the outliers of the post-blast samples (coupon in black); notice there are no soil samples included in the outliers of the post-blast samples. These scatterplots are showing some separation between the pre-blast and the outliers.
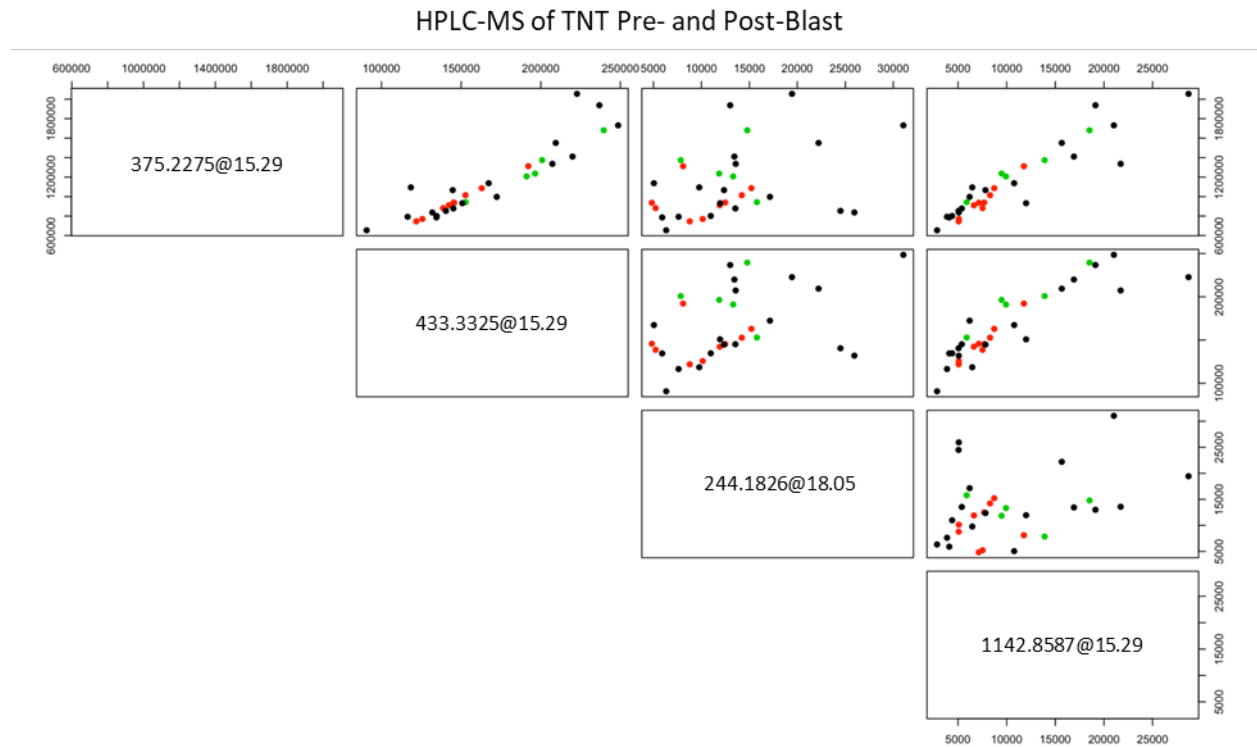

Figure 14: Scatterplot of the TNT blast data measured using the HPLC technique. The red points represent the pre-blast measurements, the black points represent the post-blast measurements on coupons, and the green points represent the post-blast measurements on the soil
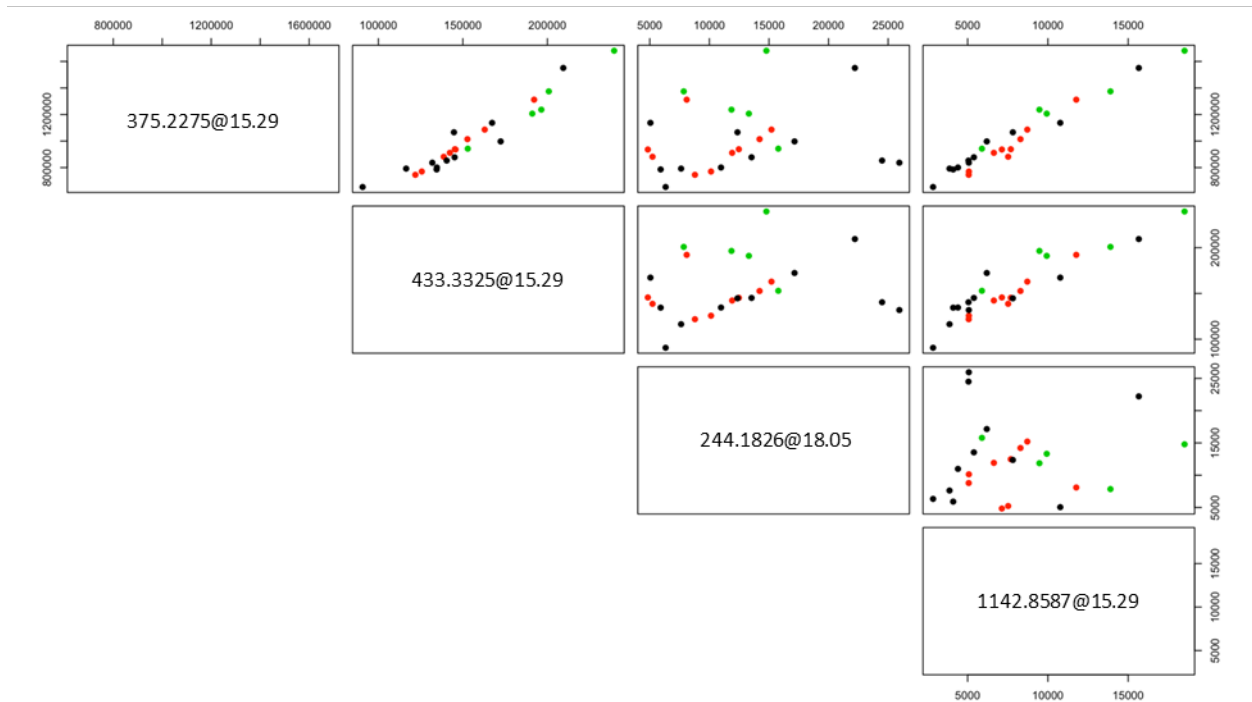
Figure 15: Scatterplot of the TNT blast data measured using the HPLC technique resulting from removing outliers from Algorithm 2. The red points represent the pre-blast measurements, the black points represent the post-blast measurements on coupons, and the green points represent the post-blast measurements on the soil.
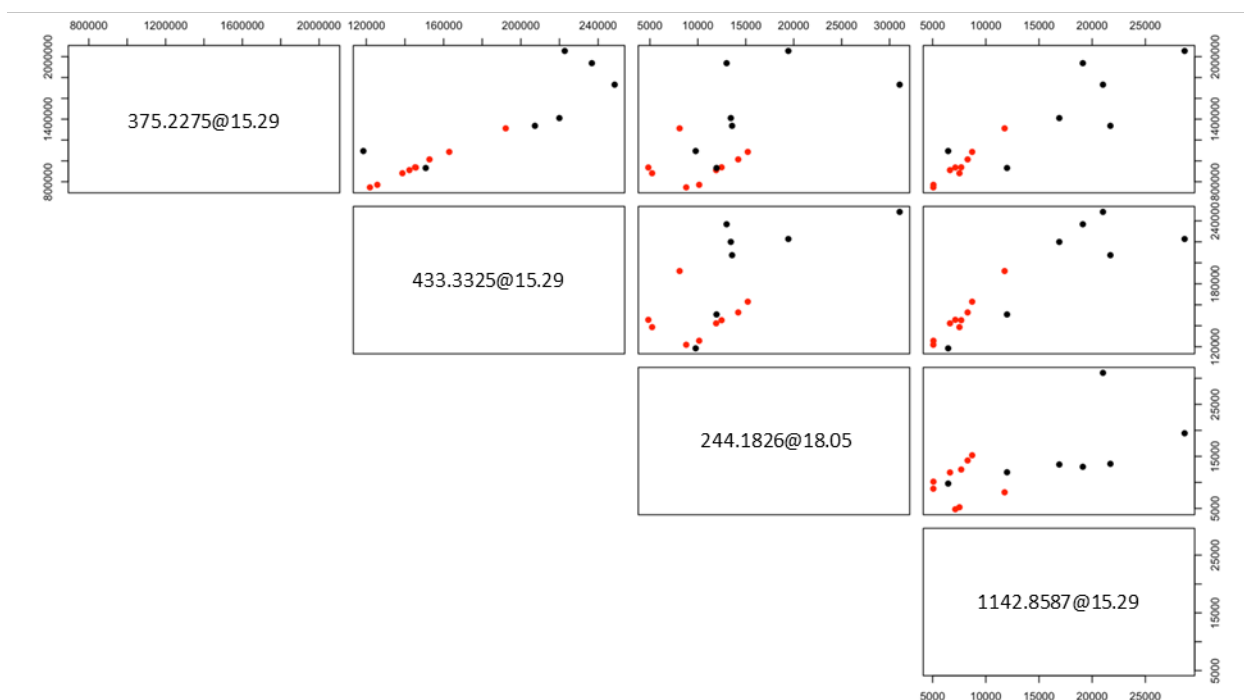
Figure 16: Scatterplot of the TNT blast data measured using the HPLC technique resulting from removing outliers from Algorithm 2. Here we only plotted the pre-blast and the outliers from Algorithm 2. The red points represent the pre-blast measurements, the black points represent the post-blast measurements on coupons. Notice there are no soil measurements in this group.

ICP-MS Results for AN-AL

Thirty-three elements were measured from 4 pre-blast and 108 post-blast AN-AL samples (across 4 shots and 27 samples per shot), each with 3 technical replicates, using ICP-MS. Initial inspection of the boxplots in Figure 17 show that the pre-blast samples (in blue) overlap with the post-blast samples (in red) for certain elements, e.g. copper (Cu) and nickel (Ni). Other elements such as gallium (Ga) and zirconium (Zr) show little to no overlap between pre-blast and post-blast samples. Many of the elements have a number of outliers. Figure 18 is the same data with the outliers (determined by IQR) removed.

The results of Algorithm 3 on the AN-AL ICP-MS post-blast cross-correlation scores are summarized by the boxplots Figures 19-20. Figure 19 shows the boxplots of the cross-correlation scores of the pre-blast samples and the scores of the post-blast samples with outliers (determined by Algorithm 1) removed. The pre-blast scores cover a wider range and contain the post-blast scores with outliers removed. The remaining 27 post-blast scores all had correlations between 0.839 and 0.8403. In Figure 20, the post-blast outliers show some overlap between the pre-blast scores and the post-blast outlier scores. These results show promise in the search for a group of compounds that are consistent pre-blast and post-blast.
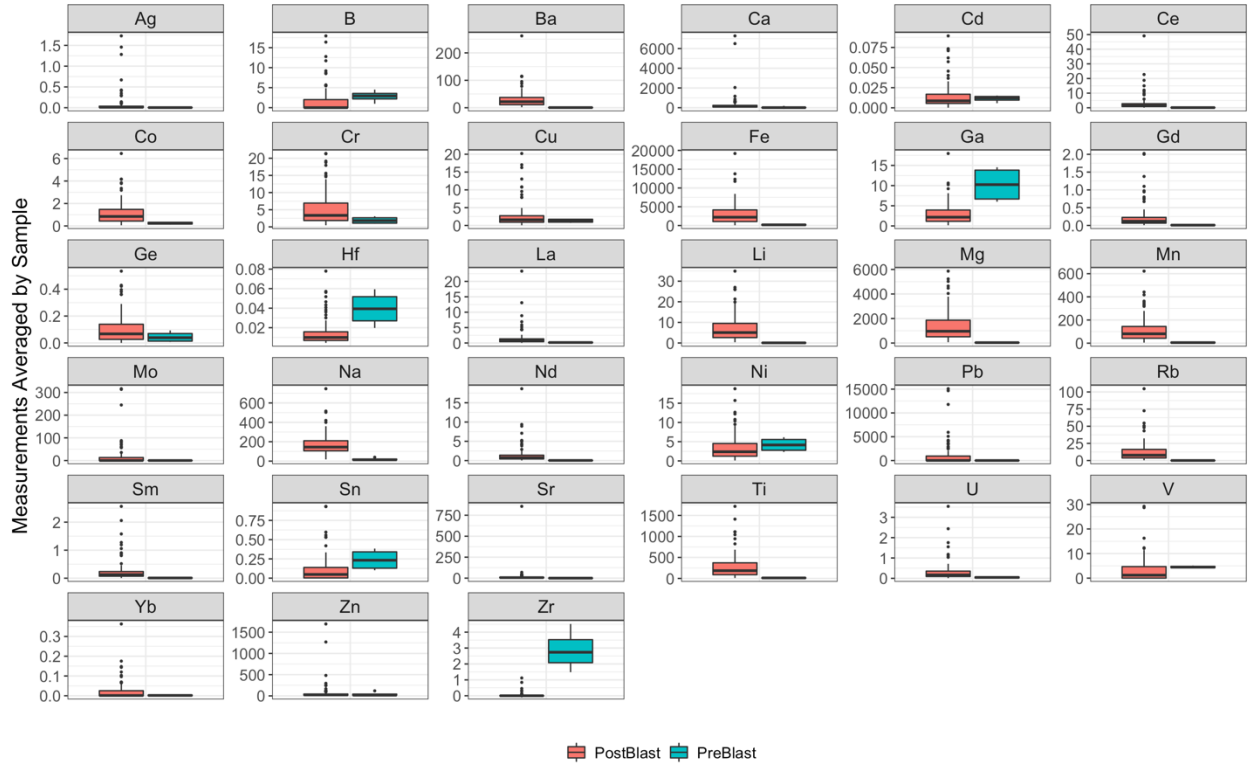
Figure 17: Boxplots of the AN-AL blast data measured using the ICP-MS technique. Each element is represented, and the data split into pre-blast and post-blast measurements. The data were averaged across the technical replicates in each sample.
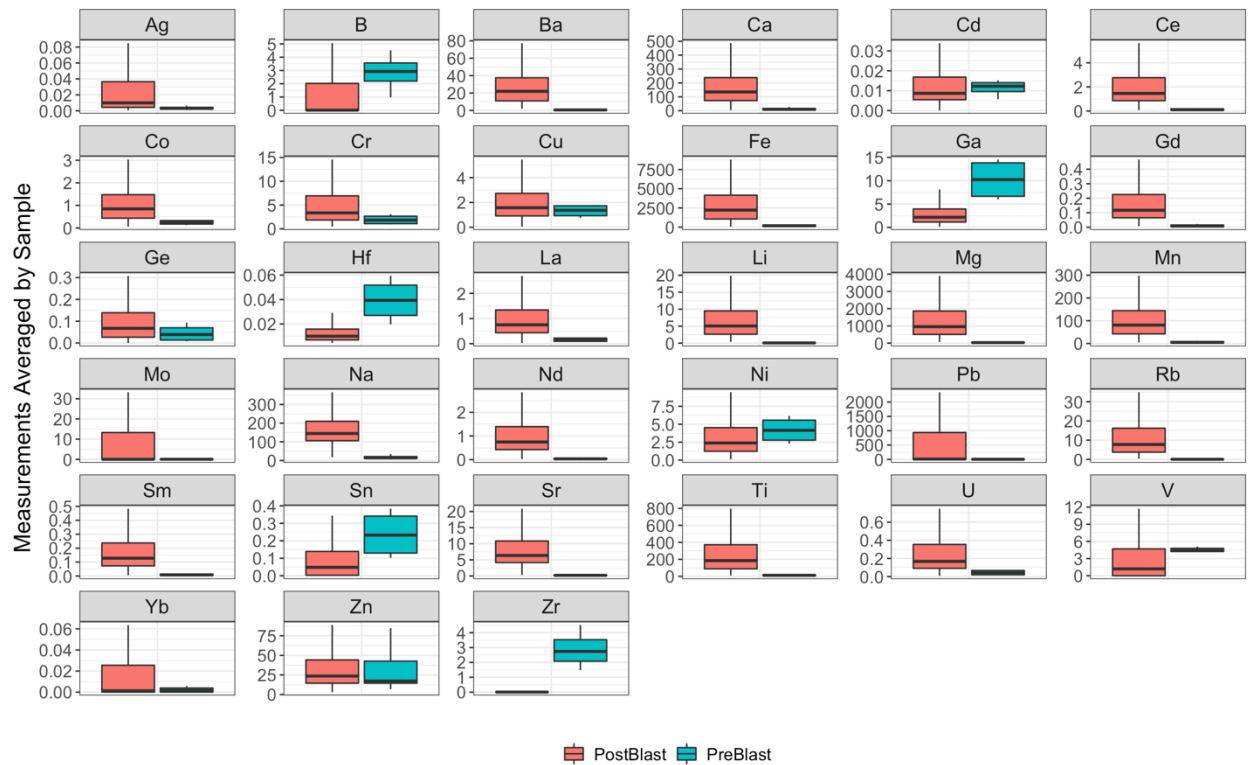

Figure 18: Boxplots of the AN-AL blast data measured using the ICP-MS technique. Each element is represented, and the data split into pre-blast and post-blast measurements. The data were averaged across the technical replicates in each sample.

Outliers were removed for this plot to better show the range of the boxplots. Outliers were determined by any element measurement greater than the 75th quartile + 1.5 * IQR or less than the 25th quartile + 1.5 * IQR.

Figure 19: Boxplot of the AN-AL blast data measured using the ICP-MS technique. The plot compares the pre-blast scores and the post-blast scores with outliers removed from Algorithm 3.
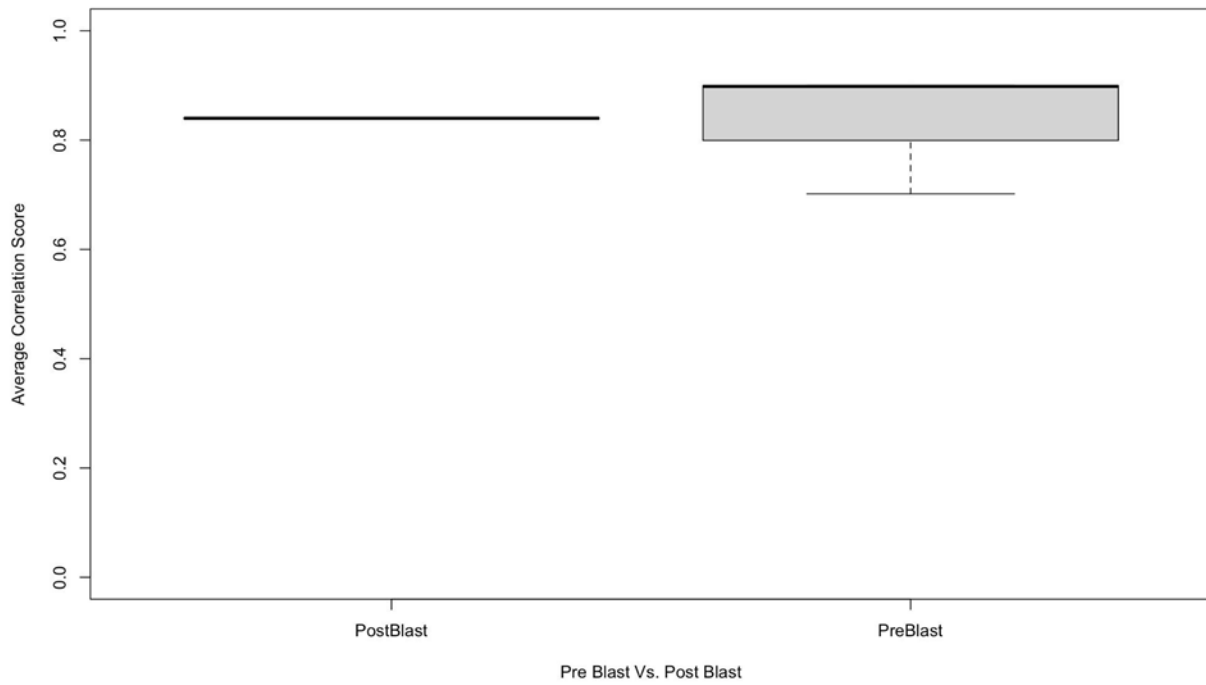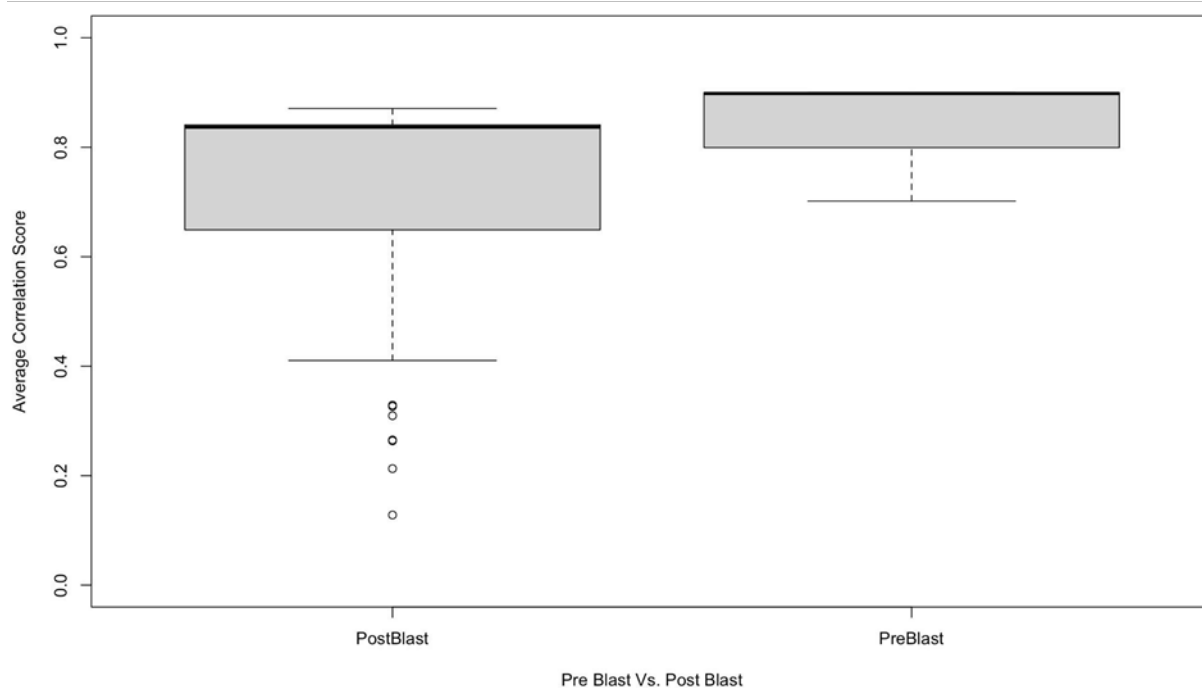
Figure 20: Boxplot of the AN-AL blast data measured using the ICP-MS technique. The plot compares the pre-blast scores and the post-blast scores with outliers.

Discussion and Conclusions

Upon examination of the results, it was determined that the overall results show promise in the ability to detect and identify signatures for attribution in post-blast residues. The explosive type that yielded the most samples with useful signature data by far was AN-AL. Trace element data were collected for nearly all samples measured, and all 30 samples that were sent for IRMS analysis were at detectable levels for both isotopes (oxygen and nitrogen) measured. While the number of samples alone does not provide conclusive information, the greater number of data points does provide for a stronger dataset and therefore greater confidence in the results.

However, while many of the plots of the pre- and post-blast IRMS results do not overlap visually, when the standard deviation of the reference material replicates is considered, there is actually much more overlap. The reference material for nitrogen standard deviation is 0.2 ‰, so the majority of the sample distributions overlap within 1 standard deviation of the reference, and the entire spread of the distributions overlap within 2 standard deviations. Similarly for oxygen, the reference standard deviation is 0.4 ‰, so the pre- and post-blast distributions partially overlap within 1 standard deviation, and completely overlap within 2 standard deviations of the reference. In conclusion, oxygen and nitrogen isotope ratio results show some overlap between pre and post blast for AN-AL when the variability of the IRMS technique is taken under consideration, and therefore may be useful signatures for attribution.

In addition to isotope ratios, trace elements may serve as useful signatures for attribution of improvised explosives such as AN-AL. Several elements in Figure 18 show some overlap between pre and post-blast, including cadmium (Cd), chromium (Cr), copper (Cu), germanium (Ge), nickel (Ni), tin

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(Sn), and zinc (Zn). As described in the results section above, the cross-correlation scores of the post-blast samples overlap with the pre-blast scores, showing that there is a general consistency between the pre and post-blast samples for trace element signatures. Between these results and the isotope ratio results, both approaches show considerable promise for a post-blast attribution capability for AN-AL.

For the organic explosives tested, post-blast RDX samples did not yield any detectable amounts of explosive, and therefore no signature data was obtained to be able to draw any conclusions. Given that RDX is a high-order explosive, and nearly all recoverable explosive material is consumed, it cannot be conclusively determined from this test whether post-blast attribution is possible with RDX. Prior studies mentioned earlier have shown that RDX is recoverable post-blast, so future analysis may reveal potential approaches for post-blast attribution of RDX.

TNT on the other hand, despite being a high-order explosive like RDX, did yield 26 post-blast samples with detectable levels of explosive. HPLC-MS profiling revealed 4 compounds that were present in both the pre-blast and all 26 post-blast samples, and were not present in any blanks or negative controls. The fact that these 4 compounds are present in post-blast residue, and also determined not to be a source of contamination, is important on its own. The presence or absence of certain signatures can either rule out or confirm a particular source, so simply finding one of these 4 compounds may be an indication of the source. Since this test did not incorporate TNT from multiple sources, it is unclear if any one of these 4 compounds is unique to a manufacturing source or may be present in multiple sources. In addition, looking at the pairwise scatterplots in Figure 15, there is good overlap between the pre-blast and post-blast for all 4 compounds, providing further confidence that these compounds may be useful for post-blast attribution.

Of the 26 samples the yielded a detectable amount of TNT, 4 samples managed to have high enough TNT concentration to conduct IRMS for carbon and nitrogen isotope ratios. The results in Figure 10 and Figure 12, for nitrogen and carbon respectively, show no overlap between the pre and post blast with outliers removed. While this may lead to the conclusion that isotope ratio analysis does not work for organic explosives like TNT, it should be noted that only 4 post-blast data points for each element does not constitute a strong enough dataset to draw any significant conclusions. Therefore, no conclusions can be drawn from the IRMS analysis of TNT as there is insufficient data. However, based on the HPLC-MS results for TNT, and the IRMS and ICP-MS results for AN-AL, it can be concluded that the development of a post-blast explosive attribution capability is promising and is worth further study.

3) Limitations

As described in some of the previous sections, a key limitation encountered during the study was obtaining recoverable amounts of the high order explosives RDX and TNT. The rapid consumption of explosive material resulted in zero recovered samples of RDX and only 26 (out of 108) TNT. Other potential factors for poorly recovered material include the placement positions of sample collection devices on the sample grid, and the number of total sample collection devices. Given the budget and number personnel involved in the test, the total number of sample collection devices was limited to only 27 samples per shot. While this was still a high number of samples, more would have been advantageous. Despite efforts to optimize both the size of the explosive device and the placement of sample collection devices on the test grid, recovery of post-blast material for RDX and TNT remained a challenge.

In addition, the amount of recovered sample, particularly for TNT, was also a significant limitation for acquiring good quality IRMS data. With reasons described above, even for the samples that did recover detectable quantities of TNT (by HPLC-MS), the limits of detection for GC-IRMS are approximately 100 fold higher. While detecting and identifying TNT in post-blast samples was challenging by itself, conducting IRMS was near impossible, with only 4 samples yielding any results. The improvements described above e.g. more samples, better placement positions for samples, etc. would potentially allow for better recovery of post-blast sample for IRMS, but other limitations may have resulted in some degradation of samples before they had a chance to be analyzed. It was difficult to recover samples quickly enough off the sample grid and store them in proper temperature controlled storage units in time. Also, due to the COVID-19 pandemic, the samples were transported back to MITLL in the personnel vehicles used to travel to Newtec to conduct the test. The samples were stored in coolers with ice packs which were challenging to keep at the proper temperature (-20 C). Furthermore, because the IRMS work had to be contracted out to a collaborator, samples had to be shipped out for analysis. Similar to difficulties in maintaining the proper temperature during travel back to MITLL, samples may have experienced temperature fluctuations during shipment to UC Davis for IRMS analysis. Overall, in addition to temperature fluctuations, the total time between sample collection and IRMS analysis was approximately 2 months (minimum) due to the fact that samples had to be processed first upon return to MITLL, followed by quantitative analysis to prioritize the most concentrated samples for IRMS analysis. With these factors to consider, it is possible that portions of some samples degraded during this time, reducing the overall concentration yield required for proper IRMS analysis.

Artifacts

1) List of Products

Publications, conference papers, and presentations

An oral presentation for the AAFS 2021 conference as well as a poster presentation for the NIJ R&D symposium were given. An oral presentation for PittCon 2021 was also given. A manuscript detailing the findings from the study will be prepared and submitted to a relevant journal such as the Journal of Forensic Science.

Technologies or techniques

There have been multiple analytical techniques developed and produced both at MITLL and through the collaboration with the Stable Isotope Facility at UC Davis. In particular, a novel GC-IRMS method for the isotope ratio analysis of RDX and TNT has been developed. The method has been transitioned to research scientists at a relevant federal forensic laboratory. Other techniques for profiling organic and inorganic signatures have been developed or have been adapted from previously developed in-house methods. Sample extraction methods have been adapted and improved upon from published methods for the extraction of explosives and relevant explosives signatures.

2) Data Sets Generated

Four datasets of chemical signature data have been generated as part of this study and are listed below. Data spreadsheets for any or all of the datasets can be provided upon request.

- IRMS chemical signature data for AN-AL
  - Nitrogen and oxygen isotope ratio signature data for 27 post-blast and 3 pre-blast AN-AL samples
- IRMS chemical signature data for TNT
  - Nitrogen and carbon isotope ratio signature data for 3 post-blast and 3 pre-blast TNT samples
- HPLC-MS chemical signature data for TNT
  - Peak area abundances of 4 organic compound signatures for 23 post-blast and 3 pre-blast samples
- ICP-MS chemical signature data for AN-AL
  - Concentrations of 33 element signatures for 108 post-blast and 4 pre-blast samples

3) Dissemination Activities

As mentioned in the List of Products section above, in addition to this final report, a manuscript will be submitted to a forensic journal such as the Journal of Forensic Sciences to disseminate this work publically. As also mentioned, this work has been presented at multiple external academic conferences already, and an abstract will be submitted to the NIJ Research and Development Symposium at AAFS 2022. Beyond publication of a manuscript and presentations at academic conferences, this report will be shared with representatives of other stakeholders within the U.S. government including members of FBI Research Laboratories and Navy EXU-1. The report may be distributed to other agencies upon their request. So far, the results generated have been discussed in a few briefings with a Senior Explosives Scientist, Unit Chief of the Explosives Unit, and Research Scientists at the FBI Laboratory in Quantico, Virginia. In addition, we transitioned the GC-IRMS method developed for the isotope ratio analysis of RDX and TNT to the FBI Laboratory for validation and use at their request.

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