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Environmental Technology Verification Report

TNT Detection Technology

Texas Instruments Spreeta™ Sensor

Environmental Security Technology Certification Program

Oak Ridge National Laboratory

ETV ETV ETV

THE ENVIRONMENTAL TECHNOLOGY VERIFICATION PROGRAM

U.S. Environmental Protection Agency

Environmental Security Technology Certification Program

Oak Ridge National Laboratory

ETV Joint Verification Statement

TECHNOLOGY TYPE:	**SURFACE PLASMON RESONANCE**		
APPLICATION:	**MEASUREMENT OF TNT IN CONTAMINATED SOIL**		
TECHNOLOGY NAME:	**Spreeta™ Sensor**		
COMPANY:	**Texas Instruments**		
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The U.S. Environmental Protection Agency (EPA) has created the Environmental Technology Verification Program (ETV) to facilitate the deployment of innovative or improved environmental technologies through performance verification and dissemination of information. The goal of the ETV Program is to further environmental protection by substantially accelerating the acceptance and use of improved and cost-effective technologies. ETV seeks to achieve this goal by providing high quality, peer-reviewed data on technology performance to those involved in the design, distribution, financing, permitting, purchase, and use of environmental technologies.

ETV works in partnership with recognized standards and testing organizations and stakeholder groups consisting of regulators, buyers, and vendor organizations, with the full participation of individual technology developers. The program evaluates the performance of innovative technologies by developing test plans that are responsive to the needs of stakeholders, conducting field or laboratory tests (as appropriate), collecting and analyzing data, and preparing peer-reviewed reports. All evaluations are conducted in accordance with rigorous quality assurance protocols to ensure that data of known and adequate quality are generated and that the results are defensible.

The Department of Defense (DoD) has a similar verification program known as the Environmental Security Technology Certification Program (ESTCP). The purpose of ESTCP is to demonstrate and validate the most promising innovative technologies that target DoD's most urgent environmental needs and are projected to pay back the investment within 5 years through cost savings and improved efficiencies. ESTCP demonstrations are typically conducted under operational field conditions at DoD facilities. The demonstrations are intended to generate supporting cost and performance data for acceptance or validation of the technology. The goal is to transition mature environmental science and technology projects through the demonstration/validation phase, enabling promising technologies to receive regulatory and end user acceptance in order to be field tested and commercialized more rapidly.

The Oak Ridge National Laboratory (ORNL) is one of the verification organizations operating under the Site Characterization and Monitoring Technologies (SCMT) program. SCMT, which is administered by EPA's National Exposure Research Laboratory, is one of six technology areas under ETV. In this verification test, ORNL evaluated the performance of explosives detection technologies. This verification statement provides a summary of the test results for Texas Instruments' (TI's) Spreeta™ Sensor for 2,4,6-trinitrotoluene (TNT) detection. This verification was conducted jointly with the DoD's ESTCP.

VERIFICATION TEST DESCRIPTION

This verification test was designed to evaluate technologies that detect and measure explosives in soil. The test was conducted at ORNL in Oak Ridge, Tennessee, from August 21 through 30, 2000. Spiked samples of known concentration were used to assess the accuracy of the technology. Environmentally contaminated soil samples, collected from DoD sites in California, Louisiana, Iowa, and Tennessee and ranging in concentration from 0 to approximately 90,000 mg/kg, were used to assess several performance characteristics. The primary constituents in the samples were 2,4,6-trinitrotoluene (TNT); isomeric dinitrotoluene (DNT), including both 2,4-dinitrotoluene and 2,6-dinitrotoluene; hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX); and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX). The results of the soil analyses conducted under field conditions by TI's Spreeta Sensor were compared with results from reference laboratory analyses of homogenous replicate samples determined using EPA SW-846 Method 8330. (Note that the TI sensor is a bioassay for TNT only.) Details of the test, including a data summary and discussion of results, may be found in the report entitled *Environmental Technology Verification Report: Explosives Detection Technology—Texas Instruments, Spreeta™ Sensor,* EPA/600/R-01/064.

TECHNOLOGY DESCRIPTION

Spreeta is an integrated, miniaturized sensor platform which employs surface plasmon resonance (SPR) to detect changes in refractive index within a few thousand angstroms of the active gold surface. Specificity is provided by placing a thin biofilm on the sensor surface. For example, by placing an antibody to fluoroscein on the sensor surface, the binding of fluorosceinated proteins, seen as a local increase in refractive index, is simply performed. SPR has been used in this way to study biomolecular binding events for more than a decade, but Spreeta is the first miniaturized SPR platform. TNT detection is most efficiently performed by methods other than direct binding. This is because on a molecule-for-molecule basis, small molecules are much less effective than large molecules at changing refractive index; thus, any direct SPR assay can detect large molecules at a lower concentration than it can detect small molecules. For this reason, Texas Instruments has developed a robust inhibition assay in which the presence of two TNT molecules (228 daltons) effectively inhibits the binding of one antibody molecule (150,000 daltons). To analyze a sample, 0.5 g of soil is extracted in an aqueous solution. The assay starts with a conjugate of trinitrobenzene (TNB) and bovine serum albumin on the gold sensing surface. Assays are then performed by exposing that sensing surface to an anti-TNT antibody solution which may or may not contain free TNT. When free TNT is present, it binds to anti-TNT antibodies in solution and thereby keeps them from binding to the surface-bound TNT analog. This inhibited binding is compared to a reference run where the antibody solution did not contain free TNT. Results from this assay are reported as interval data (i.e., the concentration of TNT is between 0.3 and 0.9 mg/kg). The lowest reporting interval was 0 to 0.3 mg/kg.

VERIFICATION OF PERFORMANCE

The following performance characteristics of the Spreeta Sensor were observed.

Precision: Precision was assessed by the percentage of combined sample sets where all four replicates were reported as the same interval. For all data, 41% of the 27 data sets were reported consistently (i.e., all four replicates were reported as the same interval). Another 44% had three of four replicates reported consistently, and the remaining 15% had two of four replicates reported consistently.

Accuracy: Accuracy was assessed using the performance evaluation (PE) soil samples, which were spiked to nominal TNT concentrations of 0, 10, 50, 100, 250, and 500 mg/kg by an independent laboratory. Accuracy, defined as the percentage of the Spreeta Sensor interval results that agreed with the nominal (i.e., spiked) TNT concentration, was 75%. In the remaining samples, 21% of the results were biased low and 4% of the results were biased high. For each of the samples that were biased low, the upper limit of the reported Spreeta Sensor interval was within 10% of the nominal concentration (e.g., TI reported the result as 3 to 9 mg/kg, and

the nominal concentration was 10 mg/kg). Further, when comparing the Spreeta Sensor interval to the acceptance ranges provided by the preparation laboratory for the PE soils, the agreement was 96%.

False positive/false negative results: Of the 20 blank soil samples, TI reported TNT as 0.3 to 0.9 mg/kg in two samples (10% false positives). False positive and false negative results were also determined by comparing the Spreeta Sensor result to the reference laboratory result on environmental and spiked samples (e.g., whether the Spreeta Sensor reports a result as a nondetect that the reference laboratory reported as a detect, and vice versa). For TNT, none of the results were false positives relative to the reference laboratory result. TI reported two samples as 0 to 0.3 mg/kg when the laboratory reported a detection at 0.8 mg/kg; these results were considered false negatives (3% rate).

Completeness: The Spreeta Sensor generated results for all 108 soil samples for a completeness of 100%.

Comparability: Comparability, like accuracy, was defined as the percentage of results that agreed with, was above, or was below the reference laboratory result. The percentage of samples that agreed with the reference laboratory results was 65% for all soils (excluding two suspect reference laboratory values). Approximately 3% of the TI results were above the reference laboratory results, but more (32%) were below. One-third of the TI samples that were below the reference laboratory result were for samples with very high (>10,000 mg/kg) TNT concentrations. Of the sample results that did not agree with the reference laboratory, 79% were within ±10 mg/kg of the reference laboratory result.

Sample Throughput: Operating out of a motor home, the TI team accomplished a sample throughput rate of approximately 12 samples per day for the soil analyses. Two instruments were used for the TNT analyses. Two operators analyzed samples in tandem to accomplish a higher sample throughput rate, so the technology can be run by a single trained operator. A mean of four tests per sample was required to generate a reported result.

Overall Evaluation: The verification team found that the Spreeta Sensor was relatively simple for the trained analyst to operate in the field, requiring less than an hour for initial setup. The overall performance of the Spreeta Sensor for the analysis of soil samples was characterized as precise and unbiased for TNT less than 10,000 mg/kg. As with any technology selection, the user must determine if this technology is appropriate for the application and for the project data quality objectives. For more information on this and other verified technologies, visit the ETV web site at http://www.epa.gov/etv.

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Notice

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Acknowledgments

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Abbreviations and Acronyms

2-Am-DNT	2-amino-4,6-dinitrotoluene
4-Am-DNT	4-amino-2,6-dinitrotoluene
BSA	bovine serum albumin
CRREL	U.S. Army Cold Regions Research and Engineering Laboratory
2,4-DNT	2,4-dinitrotoluene
2,6-DNT	2,6-dinitrotoluene
DNT	isomeric dinitrotoluene (includes both 2,4-DNT and 2,6-DNT)
DSP	digital signal processor
DoD	U.S. Department of Defense
DQO	data quality objective
EPA	U.S. Environmental Protection Agency
ERA	Environmental Resource Associates
ESTCP	Environmental Security Technology Certification Program
ETV	Environmental Technology Verification Program
FA	false acceptance error rate
fn	false negative result
fp	false positive result
FR	false rejection error rate
HMX	octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine
HPLC	high-performance liquid chromatograph
LAAAP	Louisiana Army Ammunition Plant
MLAAP	Milan Army Ammunition Plant
ORNL	Oak Ridge National Laboratory
PE	performance evaluation sample
QA	quality assurance
QC	quality control
RDX	hexahydro-1,3,5-trinitro-1,3,5-triazine
RSD	relative standard deviation
SCMT	Site Characterization and Monitoring Technologies
SD	standard deviation
SPR	surface plasmon resonance
TI	Texas Instruments
TNB	1,3,5-trinitrobenzene
TNT	2,4,6-trinitrotoluene

Section 1 — Introduction

The U.S. Environmental Protection Agency (EPA) created the Environmental Technology Verification Program (ETV) to facilitate the deployment of innovative or improved environmental technologies through performance verification and dissemination of information. The goal of the ETV Program is to further environmental protection by substantially accelerating the acceptance and use of improved and cost-effective technologies. ETV seeks to achieve this goal by providing high-quality, peer-reviewed data on technology performance to those involved in the design, distribution, financing, permitting, purchase, and use of environmental technologies.

ETV works in partnership with recognized standards and testing organizations and stakeholder groups consisting of regulators, buyers, and vendor organizations, with the full participation of individual technology developers. The program evaluates the performance of innovative technologies by developing verification test plans that are responsive to the needs of stakeholders, conducting field or laboratory tests (as appropriate), collecting and analyzing data, and preparing peer-reviewed reports. All evaluations are conducted in accordance with rigorous quality assurance (QA) protocols to ensure that data of known and adequate quality are generated and that the results are defensible.

ETV is a voluntary program that seeks to provide objective performance information to all of the participants in the environmental marketplace and to assist them in making informed technology decisions. ETV does not rank technologies or compare their performance, label or list technologies as acceptable or unacceptable, seek to determine "best available technology," or approve or disapprove technologies. The program does not evaluate technologies at the bench or pilot scale and does not conduct or support research. Rather, it conducts and reports on testing designed to describe the performance of technologies under a range of environmental conditions and matrices.

The program now operates six centers covering a broad range of environmental areas. ETV began with a 5-year pilot phase (1995–2000) to test a wide range of partner and procedural alternatives in various technology areas, as well as the true market demand for and response to such a program. In these Centers, EPA utilizes the expertise of partner "verification organizations" to design efficient processes for conducting performance tests of innovative technologies. These expert partners are both public and private organizations, including federal laboratories, states, industry consortia, and private sector entities. Verification organizations oversee and report verification activities based on testing and QA protocols developed with input from all major stakeholder/customer groups associated with the technology area. The verification described in this report was administered by the Site Characterization and Monitoring Technologies (SCMT) Center, with Oak Ridge National Laboratory (ORNL) serving as the verification organization. (To learn more about ETV, visit ETV's web site at http://www.epa.gov/etv.) The SCMT Center is administered by EPA's National Exposure Research Laboratory, Environmental Sciences Division, in Las Vegas, Nevada.

The Department of Defense (DoD) has a similar verification program known as the Environmental Security Technology Certification Program (ESTCP). The purpose of ESTCP is to demonstrate and validate the most promising innovative technologies that target DoD's most urgent environmental needs and are projected to pay back the investment within 5 years through cost savings and improved efficiencies. ESTCP responds to: (1) concern over the slow pace and cost of remediation of environmentally contaminated sites on military installations, (2) congressional direction to conduct demonstrations specifically focused on new technologies, (3) Executive Order 12856, which requires federal agencies to place high priority on obtaining funding and resources needed for the development of innovative pollution prevention programs and technologies for installations and in acquisitions, and (4) the need to improve defense readiness by reducing the drain on the Department's operation and maintenance dollars caused by real world commitments such as environmental restoration and waste management. ESTCP demonstrations are typically conducted under operational field conditions at DoD facilities. The demonstrations are intended to generate supporting cost and performance data for acceptance or validation of the technology. The goal is to transition mature environmental science and technology projects through the demonstration/validation phase, enabling promising technologies to receive regulatory and end user acceptance in order to be field tested and commercialized more rapidly. (To learn more about ESTCP, visit ESTCP's web site at http://www.estcp.org.)

EPA's ETV program and DoD's ESTCP program established a memorandum of agreement in 1999 to work cooperatively on the verification of technologies that are used to improve environmental cleanup and protection at both DoD and non-DoD sites. The verification of field analytical technologies for explosives detection described in this report was conducted jointly by ETV's SCMT Center and ESTCP. The verification was conducted

at ORNL in Oak Ridge, Tennessee, from August 21 through 30, 2000. The performances of two field analytical techniques for explosives were determined under field conditions. Each technology was independently evaluated by comparing field analysis results with those obtained using an approved reference method, EPA SW-846 Method 8330. The verification was designed to evaluate the field technology's ability to detect and measure explosives in soil. The primary constituents in the samples were 2,4,6-trinitrotoluene (TNT); isomeric dinitrotoluene (DNT), including both 2,4-dinitrotoluene (2,4-DNT) and 2,6-dinitrotoluene (2,6-DNT); hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX); and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX). Naturally contaminated environmental soil samples, ranging in concentration from 0 to approximately 90,000 mg/kg, were collected from DoD sites in California, Louisiana, Iowa, and Tennessee, and were used to assess several performance characteristics. This report discusses the performance of the Texas Instruments' Spreeta™ Sensor for the determination of TNT in soil samples.

Section 2 — Technology Description

In this section, the vendor (with minimal editorial changes by ORNL) provides a description of the technology and the analytical procedure used during the verification testing activities.

General Technology Description

Spreeta (see Figure 1) is an integrated, miniaturized sensor platform, which employs surface plasmon resonance (SPR) to detect changes in refractive index within a few thousand angstroms of an active gold surface. Analyte specificity is provided by a thin biofilm on the sensor surface. For example, by placing an antibody to fluorescein on the sensor surface, the binding of fluoresceinated proteins is seen as a local increase in refractive index. SPR has been used in this way to study biomolecular binding events for more than a decade, but Spreeta is the first miniaturized SPR platform. TNT and other small molecules are most efficiently detected by methods other than direct binding. For this reason, Texas Instruments (TI) has developed a robust inhibition assay in which the presence of two TNT molecules (228 daltons) effectively inhibit the binding of one antibody molecule (150,000 daltons).

Figure 1. Spreeta Sensor.

The Prototype Spreeta System employed for this test utilized one miniature peristaltic pump, four electromagnetic-actuated valves and a digital signal processor (DSP) driven electronics interface with a keypad and alphanumeric liquid crystal display. Running and regeneration buffers were held in IV bags within the instrument. Waste was also held in a bag inside the instrument. Bags were replaced at intervals of approximately every two days.

The assay used here is a standard inhibition immunoassay. The surface biofilm is a conjugate of trinitrobenzene (TNB) and bovine serum albumin (BSA), which has been attached to the gold sensing surface of the Spreeta sensor. BSA serves both as an adhesion medium for the TNB groups as well as a nonspecific binding reduction layer. The assay is then performed by exposing that sensing surface to an anti-TNT antibody solution, which may or may not contain free TNT, and monitoring surface binding using SPR. When free TNT is present, it binds to anti-TNT antibodies in solution and thereby keeps them from binding to the surface-bound TNT analog. If no TNT is present, the anti-TNT antibodies are not inhibited from binding to the surface and, again, this is detected in real time by SPR. The actual binding for a given sample is compared to a reference run (where the antibody solution did not contain free TNT) to determine the presence or absence of TNT. At the end of any binding test, the surface is regenerated by a brief exposure to an aqueous NaOH/Triton X solution which liberates surface-bound antibody and leaves the biofilm free for the next test.

Figure 2 shows a plot of the refractive index versus time for a reference run, a sample run that was negative, and a sample run that was positive. This illustrates the data the DSP analyses use to provide quantitative results.

Sample Preparation

Soil extracts were prepared using a completely aqueous protocol. Approximately 500 ±1 mg of soil was suspended in 5 mL of phosphate buffered saline and 0.1% Triton X-100 (a non-ionic detergent) in a 10-mL glass vial. The mixture was gently shaken for 3–5 minutes and then allowed to settle for a few minutes. Next, 1.5 mL of the supernatant was removed by pipette and was mixed with 15 µL of antibody solution. This sample was then analyzed for TNT content as previously described. TI has determined that the extraction efficiency of this protocol is approximately 40%.

Calibration and Data Analysis

Reference runs (with no TNT present) were made periodically to verify assay fidelity and biofilm integrity. The antibody used in this assay is completely cross-reactive with trinitrobenzene (TNB), is approximately 10% cross-reactive with dinitrotoluenes (DNT), and is much less cross-reactive with other nitro-aromatic compounds. Therefore, we report an “effective” TNT concentration, which primarily includes contributions from TNT, TNB, and DNT. RDX and HMX do not react with this antibody to an appreciable degree, and therefore their presence is not a factor in this assay. A negative result (with sample-run binding less than 65% of the reference-

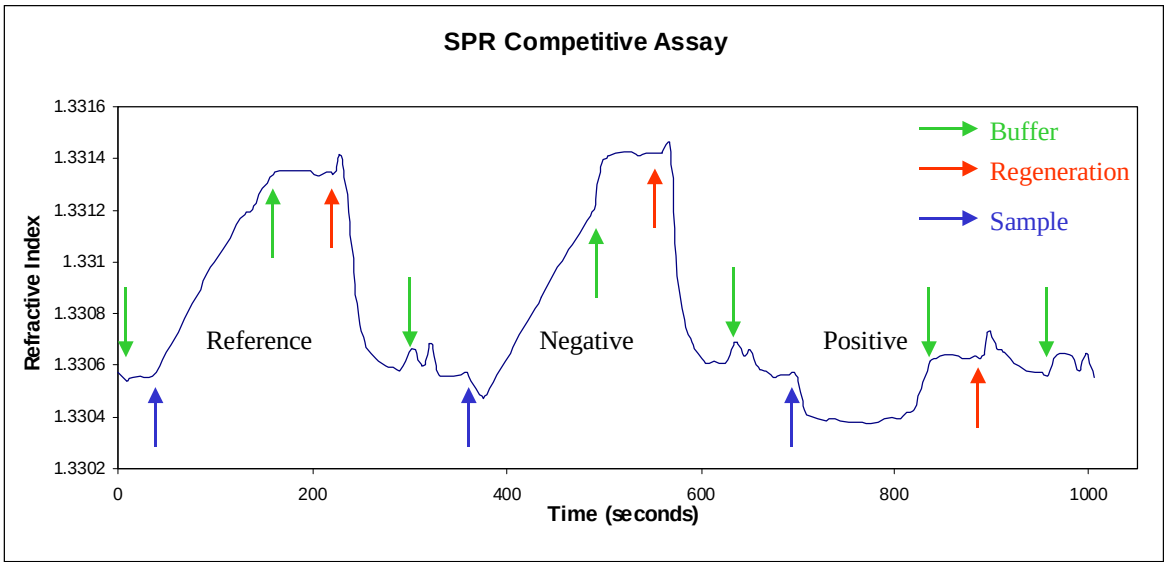


Figure 2. A graphical representation of data used during Spreeta's TNT detection assay.

run binding) with an undiluted sample indicates an effective TNT concentration of less than 0.3 ppm (mg/kg) in soil. A positive result (with sample-run binding greater than 35% of the reference-run binding) with an undiluted sample calls for a dilution and retest of the diluted sample until a negative result occurs yielding a dilution bracket.

For the purposes of this verification test, 3× dilutions were used, and this resulted in answers that were reported such that the central point of the bracket is approximately 50% above the lower limit and approximately 50% below the upper limit. For example, if the test was positive for a 100× dilution and negative for a 300× dilution, the result was reported as [10–30] mg/kg.

Section 3 — Verification Test Design

Objective

The purpose of this section is to describe the verification test design. It is a summary of the test plan (ORNL 2000).

Testing Location and Conditions

The verification of field analytical technologies for explosives was conducted at ORNL's Building 5507, in Oak Ridge, Tennessee. TI elected to operate their technology in a motor home. The temperature and relative humidity in the motor home were monitored during testing. During the warmer portions of the day, the air conditioner was run. Over the 10 days of testing, the average temperature in the motor home was 73°F, and ranged from 64 to 82°F. The average relative humidity in the motor home was 53%, and it ranged from 32 to 81%.

The samples used in this study were shipped to the testing location for evaluation by the vendors. Explosives-contaminated soils from Army ammunition plants in Iowa, Louisiana, Tennessee, and a former Army base in California (Fort Ord) were used in this verification. Because samples were obtained from multiple DoD sites, the samples represented a reasonable cross section of the population of explosives-contaminated matrices, such that the versatility of the field technology could be evaluated. More specific details about the samples are presented in the following sections.

Primary Analytes in Soil Samples

The primary contaminants in the soil samples were TNT, DNT, RDX, and HMX. The samples also contained trace amounts of 2-amino-4,6-dinitrotoluene (2-Am-DNT) and 4-amino-2,6-dinitrotoluene (4-Am-DNT), which are degradation products of TNT. The total concentration of explosives ranged from 0 to approximately 90,000 mg/kg. The following sections describe the sites from which the samples were collected.

Sources of Samples

Iowa Army Ammunition Plant

Currently an active site, the Iowa Army Ammunition Plant was constructed to load, assemble, and pack various conventional ammunition and fusing systems. Current production includes 120-mm tank rounds, warheads for missiles, and mine systems. During the early years of operation, the installation used surface impoundments, landfills, and sumps for disposal of industrial wastes containing explosives. The major contaminants in these samples are TNT, RDX, and HMX.

Louisiana Army Ammunition Plant

The Louisiana Army Ammunition Plant (LAAAP), near Shreveport, Louisiana, is a government-owned facility that began production in 1942. The facility is currently an Army Reserve plant. Production items at LAAAP have included metal parts for artillery shells; the plant also loads, assembles, and packs artillery shells, mines, rockets, mortar rounds, and demolition blocks. As a result of these activities and the resulting soil and groundwater contamination, EPA placed LAAAP on the National Priorities List of contaminated sites (Superfund) in 1989. The major constituents in the samples from this site are TNT, RDX, and HMX, with trace levels of 1,3,5-trinitrobenzene (TNB), DNT, 2-Am-DNT, and 4-Am-DNT.

Milan Army Ammunition Plant

Currently active, the Milan Army Ammunition Plant (MLAAP) in Milan, Tennessee, was established in late 1940 as part of the pre–World War II buildup. The facility still has ten ammunition loading, assembly, and packaging lines. Munitions-related wastes have resulted in soil contamination. The primary contaminants in these soils are RDX and TNT.

Volunteer Army Ammunition Plant

The Volunteer Army Ammunition Plant, in Chattanooga, Tennessee, was built in 1941 to manufacture TNT and DNT. All production ceased in 1977. Past production practices resulted in significant soil and groundwater contamination. In the samples from this site, concentrations of TNT and DNT ranged from 10 to 90,000 mg/kg, with significantly smaller concentrations of Am-DNT isomers.

Fort Ord Military Base

Fort Ord, located near Marina, California, was opened in 1917 as a training and staging facility for infantry troops and was closed as a military installation in 1993. Since then, several nonmilitary uses have been established on the site: California State University at Monterey Bay has opened its doors on former Fort Ord property, the University of California at Santa Cruz has established a new research center there, the Monterey Institute of International Studies will take over the officer's club and several other buildings, and the post's airfield was turned over to the city of Marina. The Army still occupies several buildings.

An Army study conducted in 1994 revealed that the impact areas at the inland firing ranges of Fort Ord were contaminated with residues of high explosives (Jenkins, Walsh, and Thorne 1998).

Fort Ord is on the National Priorities List of contaminated sites (Superfund), requiring the installation to be characterized and remediated to a condition that does not pose unacceptable risks to public health or the environment. The contaminant present at the highest concentration (as much as 300 mg/kg) was HMX; much lower concentrations of RDX, TNT, 2-Am-DNT, and 4-Am-DNT are present.

Performance Evaluation Samples

Spiked soil samples were obtained from Environmental Resource Associates (ERA, Arvada, Colorado). The soil was prepared using ERA's semivolatile blank soil matrix. This matrix was a 40% clay topsoil that had been dried, sieved, and homogenized. Particle size was 60 mesh and smaller. The samples, also referred to as performance evaluation (PE) samples, contained known levels of TNT and RDX. The concentrations that were evaluated nominally contained 10, 50, 100, 250, and 500 mg/kg of each analyte. Prior to the verification test, ORNL analyzed the spiked samples to confirm the concentrations were within the performance acceptance limits established by the preparation laboratory. The method used was a modified Method 8330, similar to the reference laboratory method described in Section 4. For the verification test, four replicates were prepared at each concentration level.

Blank soil samples were evaluated to determine the technology's ability to identify samples with no contamination (i.e., to ascertain the false positive error rate). The soil was collected in Monroe County, Tennessee, and was certified by ORNL to be free of contamination prior to verification testing. A reasonable number of blanks ($N = 20$) was chosen to balance the uncertainty for estimating the false positive error rate and the required number of blank samples to be measured.

Soil Sample Preparation

All of the soil samples were shipped in plastic bags at ambient temperature to ORNL. The samples were stored frozen (<0°C) prior to preparation. To ensure that the vendors and the reference laboratory analyzed comparable samples, the soils were homogenized prior to sample splitting. The process was as follows. The sample was kneaded in the Ziplock plastic bag to break up large clumps. Approximately 1500 g of soil was poured into a Pyrex pan, and debris was removed. The sample was then air dried overnight. The sample was sieved using a 10-mesh (2-mm particle size) screen and placed in a 1-L wide-mouthed jar. After thorough mixing with a metal spatula, the sample was quartered. After mixing each quarter, approximately 250 g from each quarter was placed back in the 1-L widemouthed jar, for a total sample amount of approximately 1000 g. Analysis by the ORNL method confirmed sample homogeneity (variability of 20% relative standard deviation or less for replicate measurements). The sample was then split into subsamples for analysis during the verification test. Each 4-oz sample jar contained approximately 20 g of soil. Four replicate splits of each soil sample were prepared for each participant. The design included a one-to-one pairing of the replicates, such that the vendor and reference lab samples could be directly matched. To ensure that degradation did not occur, the soil samples were frozen (<0°C) until analysis (Maskarinec et al. 1991).

Sample Randomization

The samples were randomized in two stages. First, the order in which the filled jars were distributed was randomized so that the same vendor did not always receive the first jar filled for a given sample set. Second, the order of analysis was randomized so that each participant analyzed the same set of samples, but in a different order. Each jar was labeled with a sample number. Replicate samples were assigned unique (but not sequential) sample numbers. Spiked materials and blanks were labeled in the same manner, such that these quality control samples were indistinguishable from other samples. All samples were analyzed blindly by both the vendor and the reference laboratory.

Summary of Experimental Design

The distribution of samples from the various sites is described in Table 1. A total of 108 soil samples were analyzed, with approximately 60% of the samples being naturally contaminated environmental soils, and the remaining 40% being spikes and blanks. Four replicates were analyzed for each sample type. For example, 4 replicate splits of each of 3 Fort Ord soils were analyzed, for a total of 12 individual Fort Ord samples.

Table 1. Summary of Experimental Design

Sample source or type	No. of samples
Fort Ord	12
Iowa	4
LAAAP	16
MLAAP	20
Volunteer	12
Spiked	24
Blank	20
Total	108

Description of Performance Factors

In Section 5, technology performance is described in terms of precision, accuracy, completeness, and comparability, which are indicators of data quality (EPA 1998). False positive and negative results, sample throughput, and ease of use are also described. Each of these performance characteristics is defined in this section.

Precision

Precision is the reproducibility of measurements under a given set of conditions. Standard deviation (SD) and relative standard deviation (RSD) are generally used to assess precision for quantitative data. For this evaluation of interval data, the frequency with which the same interval was reported within a set of replicates was used to quantify precision. Examples of how the precision was classified are presented in Table 2. Reporting a higher number of replicates in the same interval for a given replicate set indicates higher precision. In other words, reporting all four replicate results as the same interval indicates the highest possible precision.

Accuracy

Accuracy represents the closeness of the technology's measured concentrations to known (in this case, spiked/PE) values. For quantitative data, accuracy is usually assessed in terms of percentage recovery. For this evaluation of interval data, accuracy was evaluated in terms of the percentage of samples that agreed with, were above (i.e., biased high), and were below (i.e., biased low) the certified value.

False Positive/Negative Results

A false positive (fp) result is one in which the technology detects explosives in the sample when there actually are none (Berger, McCarty, and Smith 1996). A false negative (fn) result is one in which the technology indicates that no explosives are present in the sample, when there actually are (Berger, McCarty, and Smith 1996). The evaluation of fp and fn results is influenced by the actual concentration in the sample and includes an assessment of the reporting limits of the technology. False positive results are assessed in two ways. First, the results are assessed relative to the blanks (i.e., the technology reports a detected value when the sample is a blank). Second, the results are assessed on environmental and spiked samples where the analyte was not detected by the reference laboratory (i.e., the reference laboratory reports a nondetect and the field technology reports a detection). False negative results, also assessed for environmental and spiked samples, indicate the frequency that the technology reported a nondetect (i.e., [0, 0.3] ppm) and the reference laboratory reported a detection. Note that the reference laboratory results were validated by ORNL so that fp/fn assessment would not be influenced by faulty laboratory data. The reporting limit is considered in the evaluation. For example, if the reference laboratory reported a result as 0.8 mg/kg, and the technology's paired result was reported as [0.3, 0.9] mg/kg, the technology's result was considered correct and not a false negative result.

Completeness

Completeness is defined as the percentage of measurements that are judged to be usable (i.e., the result is not rejected). The acceptable completeness is 95% or greater.

Comparability

Comparability refers to how well the field technology and reference laboratory data agree. The difference between accuracy and comparability is that accuracy is judged relative to a known value, and comparability is judged relative to the results of a standard or reference procedure, which may or may not report the results accurately. Note that the reference laboratory result is not assumed to be the "correct" result. This evaluation is performed for comparison of the field analytical technology result with what a typical fixed analytical laboratory might report for the same sample. A one-to-one sample comparison of the technology results and the reference laboratory results is performed in Section 5. As with accuracy, it is reported as the percentage of samples that agree with, are above, and are below the reference result.

Table 2. Classification of Precision Results

If the replicate results are. . .	. . .then the number reported in identical intervals is. . .	. . .And the precision classification is. . .
[0, 0.3], [0, 0.3], [0, 0.3], [0, 0.3]	4	High
[0, 0.3], [0, 0.3], [0, 0.3], [0.3, 0.9]	3	Medium
[0, 0.3], [0, 0.3], [0.3, 0.9], [0.3, 0.9]	2	Low
[0, 0.3], [0.3, 0.9], [0.9, 30], [30, 90]	1	None

Sample Throughput

Sample throughput is a measure of the number of samples that can be processed and reported by a technology in a given period of time. This is reported in Section 5 as the number of samples per hour or day times the number of analysts.

Ease of Use

A significant factor in purchasing an instrument or a test kit is how easy the technology is to use. Several factors are evaluated and reported on in Section 5.

- What is the required operator skill level (e.g., technician or advanced degree)?
- How many operators were used during the test? Could the technology be run by a single person?
- How much training would be required in order to run this technology?
- How much subjective decision-making is required?

Cost

An important factor in the consideration of whether to purchase a technology is cost. Costs involved with operating the technology and the standard reference analyses are estimated in Section 5. To account for the variability in cost data and assumptions, the economic analysis is presented as a list of cost elements and a range of costs for sample analysis. Several factors affect the cost of analysis. Where possible, these factors are addressed so that decision makers can independently complete a site-specific economic analysis to suit their needs.

Miscellaneous Factors

Any other information that might be useful to a person who is considering purchasing the technology is documented in Section 5. Examples of information that might be useful to a prospective purchaser are the amount of hazardous waste generated during the analyses, the ruggedness of the technology, the amount of electrical or battery power necessary to operate the technology, and aspects of the technology or method that make it easy to use.

Section 4 — Reference Laboratory Analyses

Reference Laboratory Selection

The verification process is based on the presence of a statistically validated data set against which the performance of the technology may be compared. The choice of an appropriate reference method and reference laboratory are critical to the success of the verification test. To assess the performance of the explosives field analytical technologies, the data obtained from verification test participants were compared to data obtained using conventional analytical methods.

The first evaluation of explosives-detection technologies under the ETV program occurred in 1999. Specialized Assays Inc. (SAI), now known as TestAmerica, Inc., of Nashville, Tennessee, was selected as the reference laboratory for that study. A sample holding time study performed by ORNL in May 2000 indicated that the concentration of explosives in the samples had not changed significantly. Therefore, archived soil samples and the reference laboratory data generated in 1999 were used for comparison with the vendor results.

The following describes how SAI was chosen to perform the 1999 analyses. Specialized Assays, Inc. was selected to perform the analyses based on ORNL's experience with laboratories capable of performing explosives analyses using EPA SW-846 Method 8330. ORNL reviewed Specialized Assays' record of laboratory validation performed by the U.S. Army Corps of Engineers (Omaha, Nebraska). EPA and ORNL decided that, based on the credibility of the Army Corps program and ORNL's prior experience with the laboratory, Specialized Assays would be selected to perform the reference analyses.

ORNL conducted an audit of Specialized Assays' laboratory operations on May 4, 1999. This evaluation focused specifically on the procedures that would be used for the analysis of the verification test samples. Results from this audit indicated that Specialized Assays was proficient in several areas, including quality management, document/record control, sample control, and information management. Specialized Assays was found to be compliant with implementation of Method 8330 analytical procedures. The company provided a copy of its QA plan, which details all of the QA and quality control (QC) procedures for all laboratory operations (Specialized Assays 1999). The audit team noted that Specialized Assays had excellent procedures in place for data backup, retrievability, and long-term storage. ORNL conducted a second audit at Specialized Assays while the analyses were being performed. Since the initial qualification visit, management of this laboratory had changed because Specialized Assays became part of TestAmerica. The visit included tours of the laboratory, interviews with key personnel, and review of data packages. Overall, no major deviations from procedures were observed, and laboratory practices appeared to meet the QA requirements of the technology verification test plan (ORNL 1999).

Reference Laboratory Method

The reference laboratory's analytical method, presented in the technology test plan, followed the guidelines established in EPA SW-846 Method 8330 (EPA 1994). According to Specialized Assays' procedures, soil samples were prepared by extracting 2-g samples of soil in acetonitrile by sonication for approximately 16 h. An aliquot of the extract was then combined with a calcium chloride solution to precipitate out suspended particulates. After the solution was filtered, the filtrate was ready for analysis. The analytes were identified and quantified using a high-performance liquid chromatograph (HPLC) with a 254-nm UV detector. The primary analytical column was a C-18 reversed-phase column with confirmation by a secondary cyano column. The practical quantitation limit for soil was 0.5 mg/kg.

Reference Laboratory Performance

ORNL validated all of the reference laboratory data according to the procedure described in the test plan (ORNL 2000). During the validation, the following aspects of the data were reviewed: completeness of the data package, adherence to holding time requirements, correctness of the data, correlation between replicate sample results, evaluation of QC sample results, and evaluation of spiked sample results. Each of these categories is described in detail in the test plan. The reference laboratory reported valid results for all samples, so completeness was 100%. Preanalytical holding time requirements (14 days to extract; 40 days to analyze) were met. A few errors were found in a small portion of the data (~4%). Those data were corrected for transcription and calculation errors that were identified during the validation. One data point, a replicate Iowa soil sample, was identified as suspect. The result for this sample was 0.8 mg/kg; the results from the other three replicates averaged 27,400 mg/kg. This data point was excluded from the evaluation of comparability with the field technology (reported in Section 5) because it was an obvious suspect value. The reference laboratory results for QC samples were flagged when the results were outside the QC acceptance limits. The reference laboratory results were evaluated by a statistical analysis of the data. Due to the limited results reported for the other Method 8330 analytes, only the results for the major constituents in the

samples (total DNT, TNT, RDX, and HMX) are evaluated in this report.

The accuracy and precision of the reference laboratory results are summarized in Table 3. Accuracy was assessed using the PE (spiked) samples, while precision was assessed using the results from both spiked and environmental samples. The reference laboratory results were unbiased (accurate), as mean percentage recovery values were near 100%. The reference laboratory results were precise; all but one of the mean RSDs were less than 30%. The one mean RSD that was greater than 30% (DNT, 56%) was for a limited data set of three.

Table 4 presents the laboratory results for blank samples. A false positive result is identified as any detected result on a known blank. For the soil samples, one false positive detection appeared to be a preparation error because the concentration was near 70,000 mg/kg. Overall, it was concluded that the reference laboratory results were unbiased, precise, and acceptable for comparison with the field analytical technology.

Table 3. Summary of the Reference Laboratory Performance for Soil Samples

Statistic	Accuracy (% recovery)		Precision[a] (% RSD)			
	RDX N = 20	TNT N = 20	DNT[b] N_r = 3[c]	HMX N_r = 13	RDX N_r = 13	TNT N_r = 18
Mean (SD)[d]	102 (17)	100 (23)	56	29	25	29
Median	99	96	32	30	21	25
Range	84–141	76–174	14–123	12–63	4–63	2–72

[a]Calculated from those samples where all four replicates were reported as a detect.
[b]DNT represents total concentration of 2,4-DNT and 2,6-DNT.
[c]N_R represents the number of replicate sets; N represents the number of individual samples
[d] (SD) = standard deviation calculated for the accuracy measurements only. The mean RSD may not be the best representation of precision, but it is reported for convenient reference.

Table 4. Summary of the Reference Laboratory Performance on Blank Samples

Statistic	Soil			
	DNT	HMX	RDX	TNT
Number of data points	20	20	20	20
Number of detects	0	0	0	2
% of fp results	0	0	0	10

Section 5 — Technology Evaluation

Objective and Approach

The purpose of this section is to present a statistical evaluation of the Spreeta Sensor data and determine the technology's ability to measure TNT in contaminated soil samples. The technology's performance verification includes an evaluation of comparability with SW-846 Method 8330 reference laboratory data. Other aspects of the technology (such as cost, sample throughput, hazardous waste generation, and logistical operation) are also evaluated in this section. Appendix A contains the raw data provided by the vendor during verification testing that were used to assess the performance of the Spreeta Sensor. Appendix B contains a data quality objective (DQO) example which uses the performance information generated in this report. This example illustrates the use of the Spreeta Sensor in a real world application.

Precision

Precision is the reproducibility of measurements under a given set of conditions. Precision was determined for this technology by examining the results of blind analyses for four replicates of a sample and evaluating the frequency of all four replicates being reported as the same interval. For example, $N_R = 11$ (11 sets of four replicates) represents a total of 44 individual sample analyses. A summary of the overall precision of the sensor for the soil sample results is presented in Table 5. Some inconsistencies occurred because TI reported intervals that overlapped. In some cases where three of the four intervals were reported consistently, the fourth interval was different, but overlapped the other three (e.g., the four replicates were reported as 3.0 to 9.0, 3.0 to 9.0, 3.0 to 9.0, and 4.0 to 14.0). Overall, 85% of the analyses were precise, as either all four or three of four replicates were reported consistently.

Accuracy

Accuracy represents the closeness of the Spreeta Sensor's measured concentrations to the known content of spiked samples. A summary of the Spreeta Sensor's overall accuracy relative to the nominal spike concentration for the PE soils is presented in Table 6. Note that the PE samples were spiked with both TNT and RDX, but since this is a sensor for TNT, accuracy was only evaluated for that analyte. Of the 24 PE samples, the Spreeta Sensor accurately reported an interval that included the nominal spike concentration for 18 samples (75% agreement). For the remaining samples, most of the intervals were slightly below the nominal concentration (21%), and only one sample had an interval that was reported above (4% of total).

Performance acceptance ranges for the TNT-spiked samples are shown in Table 7. These are the guidelines established by the provider of the spiked materials to gauge acceptable analytical results. Because there is uncertainty in the true concentration of TNT in the samples based on the variability of the preparation method, these acceptance ranges represent a window of results that closely approximate the 95% confidence interval about the nominal value. TI's reported intervals and the reference laboratory results were compared with these acceptance ranges. For all of those PE samples with detectable levels of TNT, TI reported intervals that overlapped with the acceptance ranges, where the reference laboratory reported two samples outside the acceptance ranges. For the four PE samples which contained no spiked TNT, TI reported three samples as 0 to 0.3 mg/kg (acceptable), and one as 0.3 to 0.9 mg/kg. This was the one sample listed in Table 6 as "above."For each of the samples that were biased low, the upper limit of the reported Spreeta Sensor interval was within 10% of the nominal concentration (e.g., TI reported the result as 3 to 9 mg/kg, and the nominal concentration was 10 mg/kg). Further, when comparing the Spreeta Sensor interval to the

Table 5. Summary of Spreeta Sensor Precision

Precision	N_R [a]	Total N_R	%
Frequency of replicate sets where all 4 were reported as same interval	11	27	41
Frequency of replicate sets where 3 of 4 were reported as same interval	12	27	44
Frequency of replicate sets where 2 of 4 were reported as same interval	4	27	15
Frequency of replicate sets where none were reported as same interval	0	27	0

[a] N_R represents the number of replicate sets.

Table 6. Summary of Spreeta Sensor Accuracy: Comparison to Nominal Value

Statistic	No. of samples	Percentage
Agreement with nominal	18	75
Spreeta interval above nominal	1	4
Spreeta interval below nominal	5	21

Table 7. Number of Spreeta Sensor and Reference Laboratory TNT Results within Acceptance Ranges for Spiked Soils

TNT nominal concentration (mg/kg)	Acceptance range (mg/kg)	No. of Spreeta intervals that overlapped range	No. of reference laboratory results in range
0 [a]	Nondetect value [a]	3 of 4 [a]	4 of 4
10	7–13	4 of 4	4 of 4
50	35–63	4 of 4	4 of 4
100	70–126	4 of 4	4 of 4
250	174–315	4 of 4	3 of 4
500	348–630	4 of 4	3 of 4

[a] No TNT was spiked in this sample, so only a nondetect value was acceptable. TI reported one of the four samples as 0.3 to 0.9 mg/kg.

acceptance ranges provided by the preparation laboratory for the PE soils, the agreement was 96%.

False Positive/False Negative Results

Table 8 shows the Spreeta Sensor performance for false positive (fp) results for blank samples. Of the 20 blank soils, TI reported TNT in two samples (10% fp), as did the reference laboratory.

Table 9 summarizes the Spreeta Sensor's fp and fn results relative to the reference laboratory results. (See Section 3 for a more detailed discussion of this evaluation.) For the environmental and spiked soils, none of the TNT results were reported as false positives relative to the reference laboratory results (i.e., the laboratory reported the analyte as a nondetect when TI reported it as a detect). In the case where the laboratory reported a detection and TI reported a nondetect (i.e., false negative), two of the TNT results (3%) were false negatives. The two false negative results were reported on replicate Fort Ord samples that TI reported the sample results as 0 to 0.5 mg/kg, and the reference laboratory reported each as 0.8 mg/kg. It is interesting to note that the other two replicates were reported as 0 to 0.3 mg/kg by TI and <0.5 mg/kg by the reference laboratory. TI reported all four replicates consistently as nondetects, and the reference laboratory reported two of the replicates as slightly over the reporting limits, which accounts for TI having two "false negative" results.

Completeness

Completeness is defined as the percentage of measurements that are judged to be usable (i.e., the result was not rejected). Valid results were obtained by the technology for all 108 soil samples. Therefore, completeness was 100%.

Comparability

Comparability refers to how well the Spreeta Sensor and reference laboratory data agreed. In this evaluation, the laboratory results are not presumed to be the "correct" answers. Rather, these results represent what a typical fixed laboratory would report for these types of samples. A one-to-one sample comparison of the Spreeta Sensor results and the reference laboratory results was performed for all environmental and spiked samples that were reported as a detection. (Please refer to Appendix A to review the raw data. See Section 4 for a complete evaluation of the reference laboratory performance. Recall from Section 4 that the reference laboratory's overall precision was mean RSD = 29% and overall accuracy was mean recovery = 100%.)

Table 8. Summary of Spreeta Sensor False Positive Performance on Blank Samples

Statistic	TNT reported
No. of data points	20
No. of fp results	2
% of total results that were fp	10%

Table 9. Summary of the Spreeta Sensor Detect/Nondetect Performance Relative to the Reference Laboratory Results

Statistic	TNT reported
No. of results lab reported as non-detects	14
No. of fp results by Spreeta	0
% of total results that were fp	0
No. of results lab reported as detects	74
No. of fn results by Spreeta	2
% of total results that were fn	3 [a]

[a] See False Positive/False Negative Results section for details.

As shown in Table 10, most of the TI results (65%) agreed with the reference laboratory, and the majority of the remaining results (32%) were below.

Figure 3 represents graphically the comparison of the Spreeta Sensor and reference laboratory results. TI's results are plotted as the intervals reported. The straight line represents the corresponding reference laboratory results plotted against itself (slope = 1.00). As shown in Figure 3, TI's reported interval generally (65% of the time) included the reference laboratory result. For visual clarity, excluded from the graphs were the higher concentration (>10,000 mg/kg) TNT samples; TI under reported the concentrations of all twelve of these samples.

Table 10. Summary of Spreeta Sensor Comparability [a]

Statistic	No. of samples	Percentage
Agreement	69	65
Spreeta interval above	3	3
Spreeta interval below	34	32

[a] Excludes two reference laboratory suspect result (total N = 106).

Figure 4 represents the absolute difference between the TI-reported interval and the reference laboratory result. This graph includes data from 106 samples, excluding two reference laboratory suspect values (see Section 4 for more information). Figure 4 shows the absolute direction of disagreement that indicates most (79%) of the Spreeta Sensor's measurements were within ±10 mg/kg of the reference laboratory result.

Sample Throughput

Sample throughput is representative of the estimated amount of time required to prepare and analyze the sample and perform the data analysis. Operating out of a motor home, the two-person TI team accomplished a sample throughput rate of approximately 12 samples per day for the108 soil analyses. In order to isolate the reporting interval, several tests had to be run per sample. TI averaged four tests per sample to generate a final result.

Ease of Use

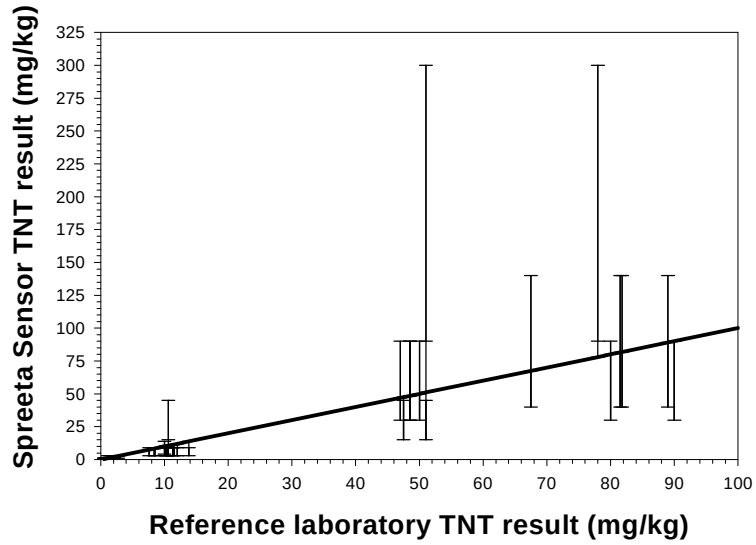
Two operators were used for the test because of the number of samples and working conditions, but the technology can be operated by a single person. The Spreeta instrument does not inherently require any particular skill level. Sample preparations and dilutions do require use of a pipettor.

Cost Assessment

The purpose of this economic analysis is to estimate the range of costs for analysis of explosives-contaminated soil samples using the Spreeta Sensor and a conventional analytical reference laboratory method. The analysis is based on the results and experience gained from this verification test, costs are provided by TI, and representative costs are provided by the reference analytical laboratories that offered to analyze these samples. To account for the variability in cost data and assumptions, the economic analysis is presented as a list of cost elements and a range of costs for sample analysis by the Spreeta Sensor instrument and by the reference laboratory.

Several factors affected the cost of analysis. Where possible, these factors were addressed so that decision makers can complete a site-specific economic analysis to suit their needs. The following categories are considered in the estimate:

- sample shipment costs,
- labor costs, and
- equipment costs.



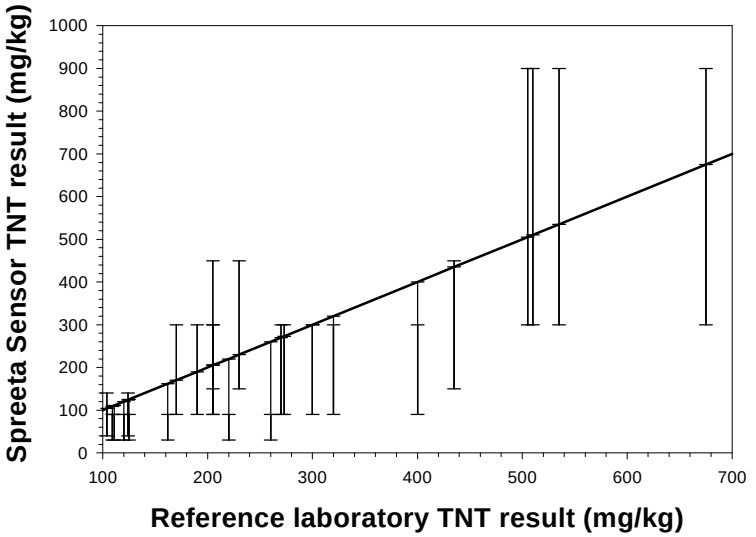


Figure 3. Upper graph represents the comparison of the Spreeta Sensor's results versus the reference laboratory for concentrations <100 mg/kg, while the lower graph represents the comparison for higher concentrations. An extra tick mark in an interval (e.g., at reference laboratory value of 400 mg/kg) indicates TI reported the same lower limit in the interval, but two different upper limits.

Each of these cost factors is defined and discussed and serves as the basis for the estimated cost ranges presented in Table 11. This analysis assumed that the individuals performing the analyses were fully trained to operate the equipment. Costs for sample acquisition and preanalytical sample preparation, which are tasks common to both methods, were not included in this assessment.

Spreeta Sensor Costs

The costs associated with using the Spreeta Sensor instrument included labor, equipment, and waste disposal costs. No sample shipment charges were associated with the cost of operating the instrument because the samples were analyzed on site.

Labor

Labor costs included mobilization/demobilization, travel, per diem expenses and on-site labor.

- *Mobilization/demobilization*. This cost element included the time for one person to prepare for and travel to each site. This estimate ranged from zero (if the person is on site) to 5 h, at a rate of $50/h.
- *Travel*. This element was the cost for the analyst(s) to travel to the site. If the analyst is

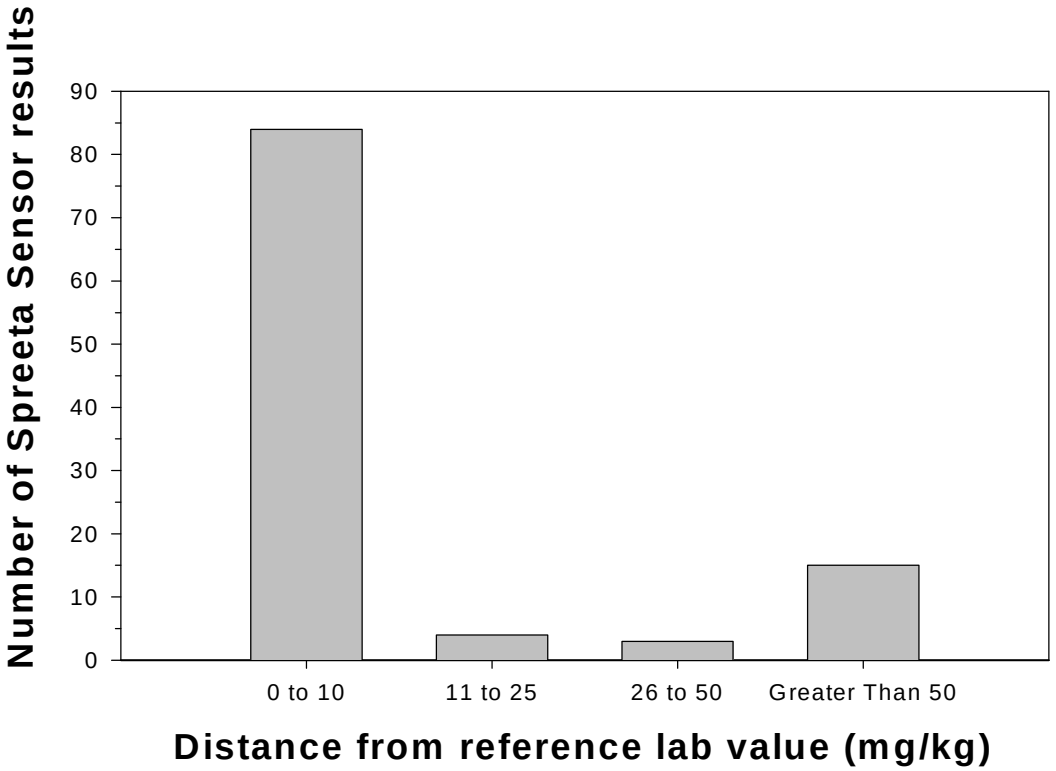


Figure 4. Absolute difference between Spreeta Sensor result and reference laboratory result.

Table 11. Estimated Analytical Costs for Explosives-Contaminated Samples

Analysis method: Spreeta Sensor **Analyst/manufacturer:** Texas Instruments **Sample throughput:** 12 samples/day		**Analysis method:** EPA SW-486 Method 8330 **Analyst/manufacturer:** Reference laboratory **Typical turnaround:** 21 working days	
Cost category	**Cost ($)**	**Cost category**	**Cost ($)**
Sample shipment	0	**Sample shipment**	
		Labor	100–200
		Overnight shipping	50–150
Labor		**Labor**	
Mobilization/demobilization	0–250	Mobilization/demobilization	Included[a]
Travel	0–1,000 per analyst	Travel	Included
Per diem expenses	0–150/day per analyst	Per diem expenses	Included
Rate	30–75/h per analyst	Rate	150–188 per sample
Equipment		**Equipment**	Included
Mobilization/demobilization	0–150		
Instrument purchase price	to be determined		
Reagents/supplies	<$1 per sample (expected)		

[a] "Included" indicates that the cost is included in the labor rate.

located at the site, travel cost to the site would be zero. The estimated cost of an analyst traveling to the site for this verification test ($1000) included the cost of airline travel and rental car fees.
- *Per diem expenses*. This cost element included food, lodging, and incidental expenses. The estimate ranged from zero (for a local site) to $150/day for each analyst.
- *Rate*. The cost of the on-site labor was estimated at a rate of $30–$75/h, depending on the required expertise level of the analyst. This cost element included the labor involved with the entire analytical process, comprising sample preparation, sample management, analysis, and reporting.

Equipment

Equipment costs included mobilization/ demobilization, rental fees or purchase of equipment, and the reagents and other consumable supplies necessary to complete the analysis.

- *Mobilization/demobilization*. This included the cost of shipping the equipment to the test site. If the site is local, the cost would be zero. For this verification test, the cost of shipping equipment and supplies was estimated at $150.
- *Instrument purchase*. The current version of this instrument applicable to TNT detection is at the pre-commercial stage, and TI has not determined the retail price.
- *Reagents/supplies*. These items are consumable and are purchased on a per sample basis. TI estimates that this will be less than $1 per sample, once the instrument is available commercially.

Reference Laboratory Costs

Sample Shipment

Sample shipment costs to the reference laboratory included the overnight shipping charges, as well as labor charges associated with the various organizations involved in the shipping process.

- *Labor*. This cost element included all of the tasks associated with the shipment of the samples to the reference laboratory. Tasks included packing the shipping coolers, completing the chain-of-custody documentation, and completing the shipping forms. The estimate to complete this task ranged from 2 to 4 h at $50/h.
- *Overnight shipping*. The overnight express shipping service cost was estimated to be $50 for one 50-lb cooler of samples.

Labor, Equipment, and Waste Disposal

The labor bids from commercial analytical reference laboratories that offered to perform the reference analysis for this verification test ranged from $150 to $188 per sample. The bid was dependent on many factors, including the perceived difficulty of the sample matrix, the current workload of the laboratory, and the competitiveness of the market. This rate was a fully loaded analytical cost that included equipment, labor, waste disposal, and report preparation.

Cost Assessment Summary

An overall cost estimate for use of the Spreeta Sensor instrument versus use of the reference laboratory was not made because of the extent of variation in the different cost factors, as outlined in Table 11. The overall costs for the application of any technology would be based on the number of samples requiring analysis, the sample type, and the site location and characteristics. Decision-making factors, such as turnaround time for results, must also be weighed against the cost estimate to determine the value of the field technology's providing immediate answers versus the reference laboratory's provision of reporting data within 30 days of receipt of samples.

Miscellaneous Factors

The following are general observations regarding the field operation and performance of the Spreeta Sensor instrument:

- The system, which weighs approximately 2 lb, was easily transported to the field.
- The technology could have been operated outdoors, as there was no AC power requirement (a lantern battery was used), but TI elected to work out of a motor home to simulate a mobile laboratory environment.
- No organic solvents were used for soil extraction, only buffered deionized water. Waste generated during the analyses was rather innocuous.
- An extraction efficiency correction (40%) was applied by TI to all Spreeta results. This extraction efficiency was determined on one soil sample prior to the verification test. The extraction efficiency most likely varies from soil-to-soil and may have effected the results.
- TI had to regenerate their sensors after arriving on-site, as they learned that the sensor surface was changed after shipment by airplane. The problem appeared to be easily corrected.
- TI used approximately 25 sensors to analyze the 108 samples. Over 500 tests were performed during the verification test, and the Spreeta Sensor was replaced after about every 20 tests.
- Although this particular application for TNT detection has not been commercially released, the Spreeta Sensor is currently available in the form of an evaluation kit from TI (www.ti.com.spreeta). In addition, a life sciences R&D instrument, based on Spreeta, is due to be released during the third quarter of 2001 from Prolinx, Inc. (www.plinx.com).

- Other nitroaromatic compounds (such as trinitrobenzene and dinitrotoluene) will respond to the sensor and be quantified as TNT.
- Some scatter in the TI results may have been attributed to the use of a small sample size (0.5 g).
- Early on in the verification test, TI elected to reduce the amount of time that the sensor was rinsed before and after sample exposure, therefore reducing the amount of analysis time for each test from 14 min to 7 min. This did not appear to affect the results.
- Waste generated during the test consisted of 12 L of nonregulated aqueous buffered solutions (i.e., no hazardous waste generated).

Summary of Performance

A summary of performance is presented in Table 12. Precision defined as the frequency that TI reported replicate sets consistently. In 85% of the replicates sets, TI reported either all four as the same interval or three of four as the same interval. Accuracy, defined as the percentage of the Spreeta Sensor results which agreed with the spiked concentration, was 75%, indicating that the soil results were unbiased. Of the 20 blank soils, TI reported TNT in two samples (10% false positives). Additionally, false positive and false negative results were determined by comparing the Spreeta Sensor result to the reference laboratory result for the environmental and spiked samples. None of the TNT results were reported as false positives relative to the reference laboratory results, but 3% of the results were false negatives.

The verification test found that the Spreeta Sensor instrument was relatively simple for a trained analyst to operate in the field, requiring less than an hour for initial setup. The sample throughput of the Spreeta Sensor was twelve samples per day. Two operators analyzed samples during the verification test, but the technology can be run by a single trained operator. The overall performance of the Spreeta Sensor for the analysis of TNT was characterized as unbiased for low concentration (<10,000 mg/kg) samples and precise for soil analyses.

Table 12. TNT Performance Summary for the Spreeta Sensor

Feature/Parameter	Performance summary
Precision	Frequency of replicate sets where all 4 were reported as same interval: 41% Frequency of replicate sets where 3 of 4 were reported as same interval: 44% Frequency of replicate sets where 2 of 4 were reported as same interval: 15% Frequency of replicate sets where 0 of 4 were reported as same interval: 0%
Accuracy	% agreement with nominal concentration: 75% % Spreeta interval below nominal concentration: 21% % Spreeta interval above nominal concentration: 4%
False positive results on blank samples	10%
False positive results relative to reference laboratory results	0%
False negative results relative to reference laboratory results	3%
Comparison with reference laboratory results (all data, excluding suspect values)	% agreement with laboratory result: 65% % Spreeta interval below laboratory result: 32% % Spreeta interval above laboratory result: 3%
Completeness	100% of 108 soil samples
Weight	2 lb
Sample throughput (2 operators)	12 samples per day
Power requirements	250 mA at 6V (A lantern battery was used in the verification test.)
Training requirements	One-half day technology-specific training
Cost	To be determined after commercially-available

Section 6 — Technology Update

In this section, the vendor (with minimal editorial changes by ORNL) provides information regarding new developments with its technology since the verification activities. In addition, the vendor provides a list of representative applications in which its technology has been used.

Technology Update

Spreeta Sensor technology is a low-cost immunoassay platform that can be applied to essentially any biosensing application (Melendez et al. 1996, Melendez et al. 1997, Elkind et al. 1999, Strong et al. 1999). It can be made available for license by suitable equipment manufacturers. The TNT assay demonstrated here could, in principle, be replicated for any small molecule for which antibodies can be generated. Such developments are currently under way.

During this verification test, dilutions of >3000× were inadvertently not performed, and so the reported concentration of TNT in samples over 3000 ppm was accidentally underestimated. This procedural problem adversely affected the accuracy of 12 out of the 108 samples tested here.

The Spreeta Sensor as well as the sample preparation protocols used here were development prototypes and, therefore, not optimized for speed. In the commercial version, the cycle time for this test should be <4 min, including system clean-out and sensor regeneration steps. The number of tests needed to quantify TNT for each soil sample will vary with dilution strategy, but with 3× dilution steps and with a 0.3 to100,000 ppm TNT concentration dynamic range, the number of tests will average between four and five.

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Appendix A

TI's Spreeta Sensor Sample Results Compared with Reference Laboratory Results

Sample site or type	Sample no.	Sample replicate	TNT concentration (mg/kg)			TI analysis order[a]
			TI lower interval	TI upper interval	Reference laboratory	
Blank	1	1	0.0	0.3	<0.5	1057
Blank	1	2	0.0	0.3	<0.5	1010
Blank	1	3	0.0	0.5	<0.5	1072
Blank	1	4	0.0	0.3	<0.5	1044
Blank	2	1	0.3	0.9	<0.5	1030
Blank	2	2	0.3	0.9	70900.0	1066
Blank	2	3	0.0	0.5	<0.5	1048
Blank	2	4	0.0	0.3	<0.5	1065
Blank	3	1	0.0	0.3	<0.5	1076
Blank	3	2	0.0	0.3	<0.5	1101
Blank	3	3	0.0	0.3	<0.5	1040
Blank	3	4	0.0	0.3	<0.5	1089
Blank	4	1	0.0	0.3	0.9	1051
Blank	4	2	0.0	0.3	<0.5	1053
Blank	4	3	0.0	0.3	<0.5	1063
Blank	4	4	0.0	0.3	<0.5	1009
Blank	5	1	0.0	0.3	<0.5	1059
Blank	5	2	0.0	0.3	<0.5	1029
Blank	5	3	0.0	0.3	<0.5	1054
Blank	5	4	0.0	0.3	<0.5	1058
Fort Ord	1	1	0.0	0.3	<0.5	1074
Fort Ord	1	2	0.0	0.5	0.8	1094
Fort Ord	1	3	0.0	0.5	0.8	1005
Fort Ord	1	4	0.0	0.3	<0.5	1020
Fort Ord	2	1	0.4	1.4	0.8	1039
Fort Ord	2	2	0.4	1.4	2.1	1037
Fort Ord	2	3	0.3	0.9	0.8	1069
Fort Ord	2	4	0.4	1.4	0.8	1016
Fort Ord	3	1	0.0	0.3	<0.5	1023
Fort Ord	3	2	0.0	0.3	<0.5	1013
Fort Ord	3	3	0.0	0.5	<0.5	1034
Fort Ord	3	4	0.0	0.3	<0.5	1007

Sample site or type	Sample no.	Sample replicate	TNT concentration (mg/kg)			TI analysis order [a]
			TI lower interval	TI upper interval	Reference laboratory	
Iowa	1	1	900.0	3000.0	20400.0	1041
Iowa	1	2	900.0	3000.0	0.8	1022
Iowa	1	3	1500.0	4500.0	33400.0	1071
Iowa	1	4	900.0	3000.0	28300.0	1004
Louisiana	1	1	30.0	90.0	109.0	1032
Louisiana	1	2	30.0	90.0	120.0	1061
Louisiana	1	3	30.0	90.0	111.0	1019
Louisiana	1	4	30.0	90.0	125.0	1095
Louisiana	2	1	30.0	90.0	50.0	1067
Louisiana	2	2	90.0	300.0	51.0	1006
Louisiana	2	3	15.0	45.0	51.0	1064
Louisiana	2	4	15.0	45.0	10.6	1060
Louisiana	3	1	90.0	300.0	205.0	1015
Louisiana	3	2	90.0	300.0	170.0	1104
Louisiana	3	3	90.0	300.0	300.0	1068
Louisiana	3	4	90.0	300.0	400.0	1108
Louisiana	4	1	40.0	140.0	89.0	1049
Louisiana	4	2	90.0	300.0	78.0	1021
Louisiana	4	3	40.0	140.0	81.5	1090
Louisiana	4	4	40.0	140.0	67.5	1056
Milan	1	1	0.9	3.0	2.7	1045
Milan	1	2	0.9	3.0	1.1	1018
Milan	1	3	0.9	3.0	1.4	1075
Milan	1	4	0.9	3.0	1.7	1087
Milan	2	1	0.0	0.5	<0.5	1096
Milan	2	2	0.3	0.9	<0.5	1036
Milan	2	3	0.0	0.5	<0.5	1028
Milan	2	4	0.3	0.9	<0.5	1002
Milan	3	1	90.0	300.0	190.0	1014
Milan	3	2	90.0	300.0	270.0	1106
Milan	3	3	90.0	300.0	320.0	1077
Milan	3	4	90.0	300.0	273.0	1105
Milan	4	1	30.0	90.0	220.0	1031
Milan	4	2	30.0	90.0	260.0	1008
Milan	4	3	30.0	90.0	80.0	1085
Milan	4	4	30.0	90.0	162.0	1078
Milan	5	1	3.0	9.0	11.5	1046
Milan	5	2	3.0	9.0	10.2	1098
Milan	5	3	3.0	9.0	11.3	1027
Milan	5	4	3.0	9.0	10.6	1035

Sample site or type	Sample no.	Sample replicate	TNT concentration (mg/kg)			TI analysis order [a]
			TI lower interval	TI upper interval	Reference laboratory	
Spike/PE	1	1	40.0	140.0	81.8	1079
Spike/PE	1	2	40.0	140.0	104.0	1091
Spike/PE	1	3	30.0	90.0	90.0	1011
Spike/PE	1	4	40.0	140.0	124.0	1038
Spike/PE	2	1	0.0	0.3	<0.5	1083
Spike/PE	2	2	0.3	0.9	<0.5	1047
Spike/PE	2	3	0.0	0.3	<0.5	1024
Spike/PE	2	4	0.0	0.3	<0.5	1050
Spike/PE	3	1	3.0	9.0	8.4	1017
Spike/PE	3	2	3.0	9.0	7.6	1042
Spike/PE	3	3	4.0	14.0	10.0	1033
Spike/PE	3	4	3.0	9.0	8.5	1003
Spike/PE	4	1	15.0	45.0	47.5	1073
Spike/PE	4	2	30.0	90.0	48.5	1055
Spike/PE	4	3	30.0	90.0	48.5	1097
Spike/PE	4	4	30.0	90.0	47.0	1107
Spike/PE	5	1	150.0	450.0	230.0	1099
Spike/PE	5	2	90.0	300.0	205.0	1093
Spike/PE	5	3	150.0	450.0	435.0	1052
Spike/PE	5	4	150.0	450.0	205.0	1084
Spike/PE	6	1	300.0	900.0	535.0	1062
Spike/PE	6	2	300.0	900.0	505.0	1026
Spike/PE	6	3	300.0	900.0	675.0	1082
Spike/PE	6	4	300.0	900.0	510.0	1001
Volunteer	1	1	1500.0	4500.0	108000.0	1043
Volunteer	1	2	1500.0	4500.0	75500.0	1103
Volunteer	1	3	900.0	3000.0	117000.0	1025
Volunteer	1	4	1500.0	4500.0	61000.0	1080
Volunteer	2	1	1500.0	4500.0	11300.0	1102
Volunteer	2	2	900.0	3000.0	12600.0	1081
Volunteer	2	3	900.0	3000.0	26200.0	1100
Volunteer	2	4	900.0	3000.0	8920.0	1070
Volunteer	3	1	3.0	9.0	12.0	1012
Volunteer	3	2	3.0	9.0	10.3	1092
Volunteer	3	3	3.0	9.0	13.8	1088
Volunteer	3	4	3.0	9.0	10.4	1086

[a] These are the sample numbers from which the analysis order can be discerned. For example, 1001 was analyzed first, then 1002, etc.

Appendix B

Data Quality Objective (DQO) Example

Disclaimer

The following hypothetical example serves to demonstrate how the information provided in this report may be used in the data quality objectives (DQO) process. This example serves to illustrate the application of quantitative DQOs to a decision process, but it cannot attempt to provide a thorough education in this topic. Please refer to other educational or technical resources for further details. Additionally, because the focus of this report is on the analytical technology, this example makes simplifying assumptions (such as the sample is homogeneous and the reference laboratory results represent the true concentration) in the example that may not be valid in the real world.

Background and Problem Statement

An Army Ammunition Plant that produced TNT was recently decommissioned. Past practices had resulted in contamination of four areas around the plant. Soils at each site were mixtures of clay, silt, and organic matter with initial concentrations of about 1500 mg/kg of TNT. Forty cubic yards (40 yd^3) of TNT-contaminated soil were loaded into a bioreactor. After three months of processing, the soil mixture was dewatered and put into drums. The simplifying assumption was made that the soil in each drum was homogeneous based on process knowledge. In agreement with regulators, the treatment goal established for the site was to reduce the soil concentration to < 15 mg/kg of TNT. Soil with < 15 mg/kg of TNT would be returned to the four areas around the plant. Those drums containing soil with TNT concentrations ≥ 15 mg/kg would be stored for additional processing.

The company's DQO team considered using Texas Instruments' Spreeta Sensor to measure the TNT concentration in each drum, based on the data generated in the ETV study. The plan was to randomly select soil samples from each drum and determine the TNT concentration with the TI Spreeta Sensor. In the ETV test, the TNT concentrations measured by the TI Spreeta Sensor were reported in variety of different intervals some of which overlapped. The maximum concentration in seven intervals was < 15 mg/kg, and eight intervals reported a minimum concentration as ≥ 15 mg/kg. The DQO team decided that a drum would be sent to storage if any of the results from the TI Spreeta Sensor indicated a concentration ≥ 15 mg/kg.

General Decision Rule

If *all* of the TNT analyses indicate concentrations of < 15 mg/kg then return the soil to the plant areas.

If *any* of the TNT analyses indicate ≥ 15 mg/kg then send the soil to a storage warehouse.

DQO Goals

The DQO team's primary goal was to calculate how many samples would need to be analyzed by the Spreeta Sensor in order to confidently make a decision about remediating the processed soil, given the uncertainties of the technology's results. Because the team decided that inadvertently returning soil that exceeded 15 mg/kg of TNT was the worst possible mistake, the number of samples measured is primarily related to this false-rejection decision error rate. A secondary decision error would be to unnecessarily store a drum that contained TNT concentrations < 15 mg/kg which would be a false-acceptance decision error. Consideration of both the false-rejection decision error and the false-acceptance decision error was used to determine the final sampling plan.

EPA required that a sufficient number of samples be measured from each drum so that the false-rejection error rate (FR) for the decision rule was 0.05 or less if the true drum concentration was 15 mg/kg or greater. This DQO goal represents a 5% chance of returning a drum containing 15 mg/kg or more of TNT to the plant area.

The DQO team did not want to store and reprocess an excessive number of drums if a drum's TNT concentration was < 15 mg/kg because of the expense. Therefore, the DQO team recommended that the false-acceptance error rate (FA) for the decision rule be 0.10 if the true drum concentration was < 15 mg/kg. That is, there would be a 10% chance of storing and reprocessing a drum if the true TNT concentration for a

drum was < 15 mg/kg.

Determining the Number of Samples

The number of samples needed to satisfy the FR and FA requirements depends on the misclassification error rates of the Spreeta Sensor. Two types of misclassifications have to be considered:

1. Underestimating the TNT concentration (P_U) —classifying a sample concentration to be < 15 mg/kg when the true TNT concentration is ≥ 15 mg/kg.

2. Overestimating the TNT concentration (P_O) —classifying a sample concentration to be ≥ 15 mg/kg when the true TNT concentration is < 15 mg/kg.

The probabilities P_U and P_O are relative to the target value (15 mg/kg) and depend on the true TNT concentration of the sample. These probabilities will decrease with distance of the true concentration from the target value. Ideally, a project-specific experiment should be run with replicate samples having TNT concentrations near the target value but have TNT concentrations that are both below and above the target value. The ETV verification results do not provide sufficient information to make good estimates of P_U and P_O. However, we will use the ETV results to illustrate this example even though the data clearly underestimates the misclassification errors.

The ETV verification results for 108 analyses of performance evaluation soil samples and environmental soil samples will be used to estimate the error rates for the two types of misclassifications as follows. For the 51 samples where the reference values were ≥15 mg/kg, Spreeta underestimated the TNT concentration one time, or 1/51, for an estimated probability of underestimation $P_U = 0.020$. Note many of the TNT concentrations were much higher than 15 mg/kg so P_U is most likely too small. For the 57 samples where the reference values < 15 mg/kg, Spreeta overestimated the TNT concentration two times, for an estimated probability of overestimation $P_O = 2/57 = 0.035$.

The probability distribution of classifying the number of soil samples in different concentration intervals follows a binomial probability distribution (Sachs 1984). This probability distribution and the requirements for FR and FA can be used to determine the number of samples to meet the DQO goals. The FR for the decision rule is related to P_U by

$$FR = Pr[\ All\ Spreeta\ results < 15\ mg/kg\ for\ TNT \geq 15\ mg/kg\ ] = (P_U)^N \qquad \text{(Eq. B-1)}$$

The FR error rate decreases as the sample size increases. The sample size is solved as

$$N = \frac{\log(FR)}{\log(P_U)} \qquad \text{(Eq. B-2)}$$

where

N = number of samples from a drum to be measured
FR = false-rejection decision error rate (e.g., FR = 0.05)
P_U = probability of underestimating the TNT concentration

$$N = \frac{\log(0.05)}{\log(0.020)} = \frac{-1.301}{-1.699} = 0.77 \approx 1 \quad .$$

The sample size was rounded up to the next integer, as fractions of a sample analysis are not possible. Rounding to the higher integer will decrease the FR for the decision rule. Based on the uncertainties in the Spreeta sensor measurements, the DQO team would have to analyze only one sample from each drum to meet the decision rule's false rejection (FR) requirement. The false acceptance (FA) for the decision rule is related to P_O by

$$FA = Pr[\ Some\ of\ Spreeta\ results \geq 15\,mg/kg\ for\ TNT < 15\,mg/kg\ ] = 1 - (1 - P_O)^N \qquad \text{(Eq. B-3)}$$

The error rate of a FA decision (sending a drum to storage and reprocessing) actually increases with increasing sample size because the chance that the Spreeta Sensor will overestimate a concentration increases with continued testing. The sample size required to meet the FA requirement is

$$N = \frac{\log(1 - FA)}{\log(1 - P_O)} \qquad \text{(Eq. B-4)}$$

where

N = number of samples from a drum to be measured
FA = false-acceptance decision error rate (e.g., FA = 0.10)
P_O = probability of overestimating a TNT concentration

$$N = \frac{\log(1 - 0.10)}{\log(1 - 0.035)} = \frac{-0.046}{-0.016} = 2.88 \approx 3 \quad .$$

The sample size must be rounded up to N = 3 (fractions of a sample analysis are not possible). When N = 3, the value of FA percentage is 10.2% which is only slightly higher than the DQO team's goal of 10% . By taking three samples from the drum, the probability with regard to false-rejection results improves. That is, FR percentage decreases to 0.0008%.

Therefore, the DQO team in this example decided that the sampling procedure would be to randomly select three soil samples from each drum and analyze the sample with the Spreeta Sensor. The DQO team would return a soil drum to the excavated area if all TNT concentrations were < 15 mg/kg, and store the soil drum for reprocessing if any of the TNT concentrations were ≥ 15 mg/kg. The DQO team's goals of a 5% for the FR percentage and 10% for the FA percentage would be met by this sampling plan.

Decision Rule for 5% FR Percentage and 10% FA Percentage

If three randomly selected soil sample has a Spreeta Sensor result reported in an interval < 15 mg/kg then return the soil drum to the excavated area.

If one or more of the three randomly selected soil samples has a Spreeta Sensor result in the interval ≥ 15mg/kg then store the soil drum for additional processing.

Worst Case P_U and P_O Estimates

The DQO team used all 108 samples in the EPA ETV verification test to estimate the P_U and P_O because the number of performance evaluation samples were not sufficient in the range of interest (15 mg/kg). Because this analysis is based on the reference laboratory data and not the actual true concentration, the determination for the required number of samples was recalculated using the upper 95% confidence limits (i.e., higher possible values considering the uncertainties on the P_U and P_O values), representing a "worst case" scenario. The values of the upper 95% confidence limits for the two probabilities were 0.090 for P_U and 0.106 for P_O. Calculations with these upper limit values determine the number of samples to be 2, a FR percentage of 0.8%, and a FA percentage of 20.1%. These results show that the regulatory DQO would still be met, but the chance of storing "good" drums increases. If we keep the original number of samples (N =3), the calculated error rates using the upper 95% confidence limits on P_U and P_O would give a FR percentage of 0.07% and FA percentage of 28.5%. Considering the uncertainties in P_U and in P_O, the regulatory DQO will also be met with the original sampling plan but the probability uncertainties will have the greater affect on the FA error rate.