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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