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Installation Restoration Research Program

Comparison of Commercial Colorimetric and Enzyme Immunoassay Field Screening Methods for TNT in Soil

by Karen F. Myers, Erika F. McCormick, Ann B. Strong, WES

Philip G. Thorne, Thomas F. Jenkins, U.S. Army Cold Regions Research and Engineering Laboratory

Prepared for Headquarters, U.S. Army Corps of Engineers

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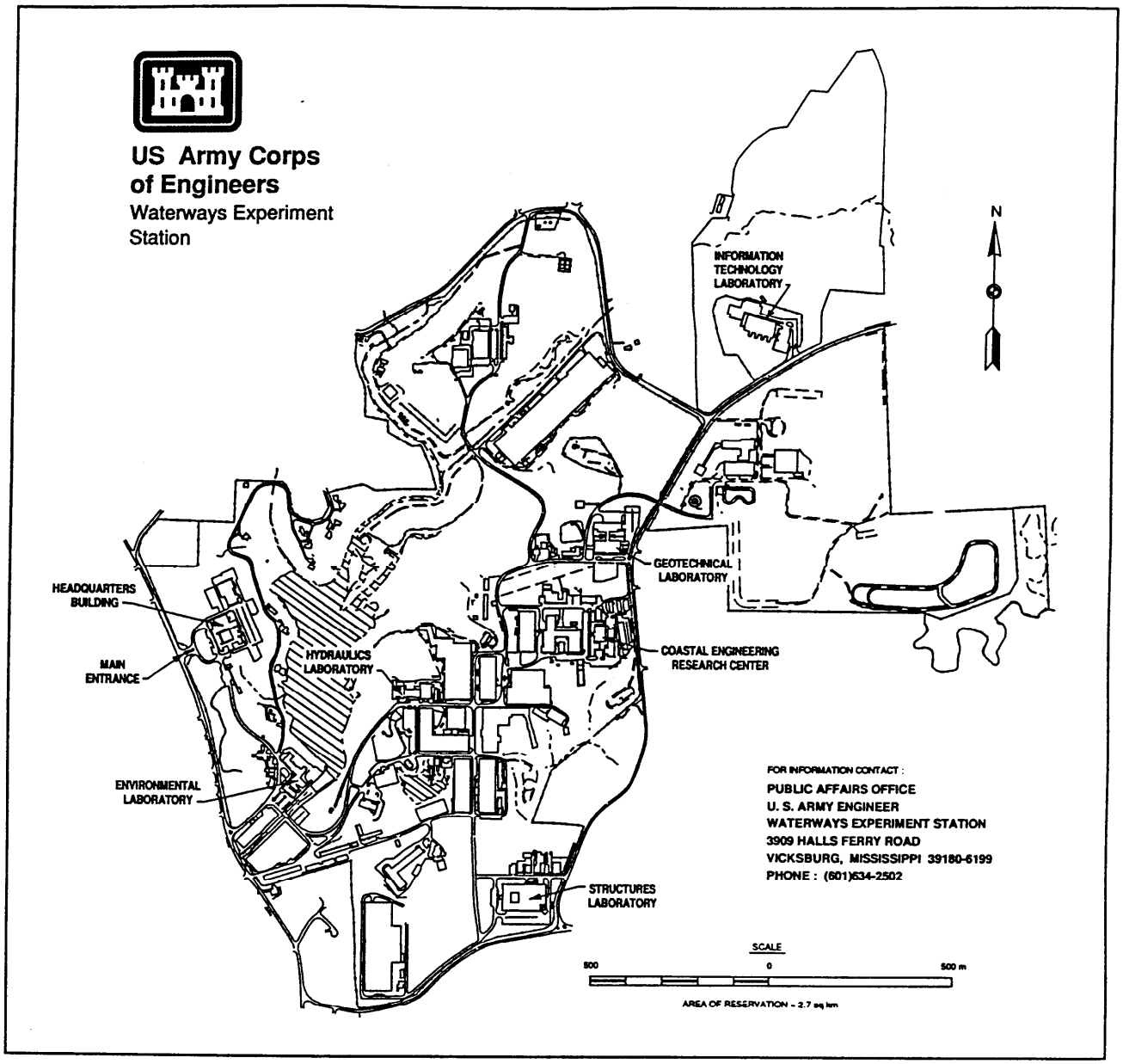

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Preface

The work reported herein was conducted by the U.S. Army Engineer Waterways Experiment Station (WES) as part of the Installation Restoration Research Program (IRRP). This report was prepared by Mses. Karen F. Myers, Erika F. McCormick, and Ann B. Strong, Environmental Chemistry Branch, Environmental Engineering Division (EED), Environmental Laboratory (EL), WES, Vicksburg, Mississippi, and Mr. Philip G. Thorne and Dr. Thomas F. Jenkins, Geological Sciences Branch, Research Division, U.S. Army Cold Regions Research and Engineering Laboratory (CRREL), Hanover, New Hampshire. Funding was jointly provided by WES, Environmental Quality Technology Project AF25, Ms. Ann B. Strong, Project Monitor, and by the U.S. Army Environmental Center (formerly the U.S. Army Toxic and Hazardous Materials Agency), Aberdeen Proving Ground, Maryland, R-90 Multianalytical Services Project, Mr. Martin H. Stutz, Project Monitor. Dr. John Cullinane, WES, was the IRRP Program Manager.

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This study was conducted under the general supervision of Dr. Raymond L. Montgomery, Chief, EED, and Dr. John Harrison, Director, EL. At the time of publication of this report, Dr. Robert W. Whalin was Director of WES. COL Bruce K. Howard, EN, was Commander.

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1 Introduction

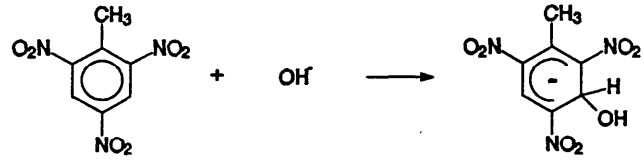
Background

For the greater part of this century, the U.S. Army has manufactured munitions at Army facilities throughout the United States. Disposal practices for wastes generated in these processes were not always farsighted when judged by current standards. Thus, Army lands are known to be contaminated with residues of a wide variety of toxic and hazardous substances. In addition to the types of pollutants common to industries, the Army also has sites contaminated with residues of secondary explosives. The sources of these residues were the manufacture of secondary explosives, loading of these explosives into various types of munitions, and demilitarization of off-specification or out-of-date material. The first step in remediation of this problem is to define the extent and degree of explosives contamination.

Site characterization at explosives-contaminated areas has traditionally been conducted by soil sample collection and analysis at offsite commercial laboratories. Most of these laboratory analyses are conducted using SW846 Method 8330 (United States Environmental Protection Agency (USEPA) 1992), and the results generally appear to have been satisfactory in terms of accurately identifying the various contaminants and their concentrations. Sometimes, however, the turnaround time for obtaining these results has been inadequate for optimum onsite decision making. In addition, per sample analytical costs have sometimes limited the number of samples that could be analyzed, resulting in insufficient spatial resolution of the boundary between contaminated and clean areas. This problem is further compounded by the cost of analyzing samples that are devoid of residues. Onsite field screening has been suggested as a means of addressing some of the shortcomings resulting from exclusive reliance on analysis at offsite laboratories (Jenkins and Walsh 1992). A recent study using information from a large number of laboratory analyses of explosives-contaminated soils has indicated that at least 95 percent of the soils found to be contaminated with residues of secondary explosives contained TNT (2,4,6-trinitrotoluene) and/or RDX (1,3,5-hexahydro-1,3,5-trinitrotriazine) (Walsh et al. 1993). If soils could be screened for these two analytes, most secondary explosives-contaminated soils could be identified.

Colorimetric Methods for TNT

The first screening method for TNT in environmental matrices was reported by Heller, Grenl, and Erickson (1982). This method was developed for water analysis and utilized a detection tube containing two sections. The first section contained a basic oxide which converted TNT to its Meisenheimer anion (Equation 1) which was retained by the anion exchanger in the second section of the tube. Since the Meisenheimer anion for TNT is highly colored, detection was achieved visually, and quantitation was made by measurement of the length of the colored region of the tube. The use of these tubes for detection of TNT in soil was reported by Erickson et al. (1984). An evaluation of the methods of Heller and Erickson indicated that the methods were very effective at detecting the presence of TNT in both soil and water, but that the ability to precisely and accurately estimate the TNT concentration was poor (Jenkins and Schumacher 1990).



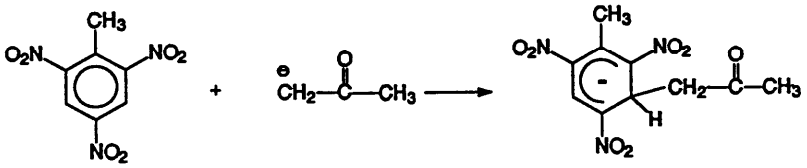
(1)

Haas and Stork (1989) reported a field screening method for TNT in soil. In this procedure, soils were extracted with acetone and the extracts reacted with 1N sodium hydroxide. Absorbance measurements at 460 nm were used to estimate TNT concentration. A detection limit of 1 µg/g was reported.

Another colorimetric method for TNT in water was reported by Stevanović and Metrović (1990). In their method, TNT was adsorbed on a porous silica gel disk; the disk was then air dried and reacted with a solution of o-toluidine. Detection was achieved by reflectance measurements from the colored surface.

A field screening method for TNT in soil was also reported by Jenkins (1990). In this method, undried soils were extracted with acetone by shaking them manually for 3 min; then the extract was filtered and reacted with potassium hydroxide and sodium sulfite to form the highly colored Janowsky anion (Equations 2 and 3), and the TNT concentration was estimated from absorbance measurements at 540 nm. A detection limit of 1 µg/g was reported. A similar method was developed by Medary (1992) at about the same time. Medary's method utilized methanol for TNT extraction. The extracted TNT was reacted with a 10-percent aqueous solution of sodium hydroxide, and TNT was estimated from the absorbance of the colored anion produced at 516 nm. A detection limit of 4-8 µg/g was estimated.

(2)



(3)

A commercially available field screening method for TNT in soil was issued in 1993 by EnSys Inc. This method was patterned after the method reported by Jenkins (1990) and utilized acetone for extraction of TNT from soil. Unlike the Jenkins method, in the EnSys method, soils are air dried prior to extraction. After extraction, the acetone extract is reacted with a solution containing a strong base (tetrabutylammonium hydroxide), which converts TNT to its highly colored Janowsky anion. TNT concentration is estimated from the absorbance at 540 nm. A detection limit of 1 µg/g is reported.

Enzyme Immunoassay Methods for TNT

Enzyme immunoassays are analytical methods based on highly selective binding reactions of antibodies with specific target analytes. In these assays the enzyme acts as a tracer since it undergoes a color change when the appropriate substrate is added. Antibodies are proteins produced in response to foreign substances as part of the vertebrate immune response system. Many environmental contaminants are small molecules that cannot induce antibody production by themselves. These molecules must be covalently bound to larger carrier proteins in order to stimulate antibody production when injected into an animal. These small molecule-protein conjugates are called "haptens." The specificity of an antibody to a target analyte can be influenced by the design of the hapten. This is done by controlling the orientation and spacing between the analyte and carrier protein used to induce the immunological effect. Through careful selection of antibodies it is possible to design immunoassays that can distinguish an analyte from a related family of compounds or a parent compound from its metabolites. For molecules with limited numbers

of functional groups, specificity becomes more difficult, and cross reactivity with other structurally related molecules becomes more likely.

Enzyme immunoassays developed for small molecules are usually formatted as competitive enzyme-linked immunosorbent assays (ELISAs). In one common form of ELISA (Figure 1), the target analyte is bound to an enzyme through a spacer molecule to form an enzyme-analyte conjugate. Antibodies are bound onto the surface of a solid such as the walls of a microtitre well or test tube or onto small spheres held by membrane supports. When a known amount of enzyme-analyte conjugate and sample containing the free target analyte are mixed with the antibodies, they compete with each other for binding sites on the antibodies. Upon the addition of the appropriate substrate, the enzyme undergoes a measurable color change. Quantitation is accomplished by comparing color intensity to a standard curve. The amount of enzyme conjugate retained on the antibodies (i.e., color change) is inversely proportional to the amount of target analyte in the sample (Vanderlaan, Stanker, and Watkins 1990). In other words, the more intense the color development, the lower the concentration of free analyte in the sample. A less intense color indicates higher concentrations of free analyte. Since many immunoassays are cross reactive with other compounds of similar structure, manufacturers generally provide a list of analytes exhibiting 50-percent inhibition of the antibodies on a dose-response curve ($CR_{50\%}$). Positive results may be due to the target analyte, to cross reactive analytes, or to a combination of analytes. For this reason, results of ELISA analyses are often expressed in target analyte equivalents.

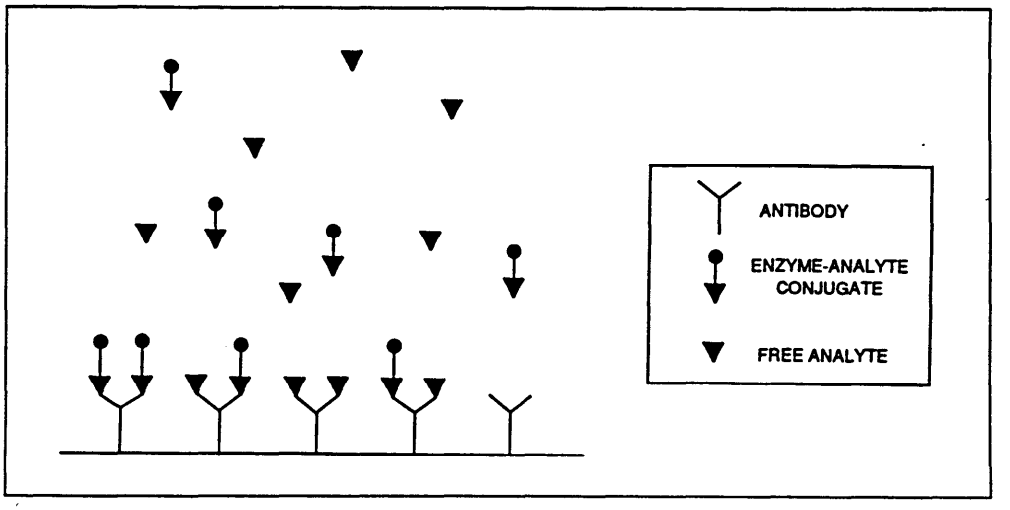


Figure 1. Enzyme-analyte conjugate immunoassay

Enzyme immunoassays have been used successfully in clinical laboratories for the past 20 years. Clinical uses range from testing blood and urine for drugs or infectious diseases, to home pregnancy tests. A number of enzyme immunoassays have been developed in recent years for the detection and analysis of pesticide residues (atrazine, metolachlor, benomyl, carbofuran, 2,4-D, etc.) and hazardous environmental chemicals such as polychlorinated

biphenyls (PCBs), pentachlorophenol (PCP), polyaromatic hydrocarbons (PAHs) and fuel component tracers (BTEX: benzene, toluene, ethlybenzene, and xylenes).

Keuchel, Weil, and Niessner (1992a, b) were the first to report the development of a competitive ELISA to detect TNT in water. The immunoassay used polystyrene microtitre plates coated with antibodies. Enzyme-analyte conjugate was synthesized from 2,4,6-trinitrobenzene sulfonic acid conjugated to the enzyme horseradish peroxidase. Hydrogen peroxide-tetramethylbenzidine was used as the substrate to induce color change. During development of the assay, the importance of spacer length between the sulfonic acid and the enzyme was examined, and its effect on assay sensitivity was evaluated. A three carbon spacer produced the most sensitive immunoassay. Specificity of the antibody was examined in two ways. Cross reactivities of the ELISA with six other structurally related nitroaromatics evaluated at the center point of the assay were all below 2 percent. Cross reactivities measured at different concentrations of the analytes within the working range of the assay were much higher. The authors found antibody inhibition to be evident at low concentrations of analyte due to the sigmoidal shape of the standard curve. Using the ELISA, 16 TNT-spiked drinking water samples were processed in quadruplicate in 1 hr with little matrix effect. The detection limit was estimated to be 0.02 µg/L.

Natural waters often contain large concentrations of humic substances. Keuchel, Weil, and Niessner (1992c) also examined the effect of humic acids on the ELISA by spiking TNT into water prepared with humic acid salts (total organic carbon (TOC) 1.1 mg/L) and into natural bog water (TOC 1.2 mg/L). They found that humic acids adsorb unspecifically to the antibodies, reducing the availability of binding sites for free TNT and enzyme conjugate. This can cause a decrease in enzyme color intensity and lead to erroneously high values at low concentrations of TNT and to false positives in humic waters free of TNT. Bovine serum albumin was found to reduce the adsorption interaction between humics and the antibodies and reduce the occurrence of false positives.

A commercially available enzyme immunoassay for TNT in water and soil was issued in 1993 by D TECH Environmental Detection Systems (Hutter, Teaney, and Stave 1993). This assay makes use of a competitive reaction between enzyme-labeled TNT and free TNT for binding sites on antibody-coated latex particles. The particles are trapped on a membrane, washed clean of unbound enzyme conjugate, and treated with a substrate to induce a color change inversely proportional to the amount of free TNT in the sample. A negative control reference sample is processed with each sample. Homogenized, field-moist soils are analyzed by the same procedure after an acetone extraction. Results are quantitated with a hand-held, dual-beam reflectometer that measures the difference between the sample and the reference control. Detection limits are 5 µg/L and 0.2 µg/g for water and soil, respectively.

After the bulk of the study was completed, a prototype TNT immunoassay became available from Millipore. The EnviroGard TNT kit is intended to be a laboratory assay for semiquantitative analysis of TNT in both soil and water. Water samples or diluted methanol extracts are incubated with TNT-enzyme conjugate in microtitre wells coated with antibody. Upon completion of the incubation, the unbound analytes are rinsed away, substrate is added, and the developed color is measured with an ELISA plate reading spectrophotometer at 450 nm. Detection limits are 0.2 µg/L for water and 0.5 µg/g for soil.

2 Experimental Methods

Preparation of Soils

Soils used in this study came from the Ammunition Burning Grounds at the Naval Surface Warfare Center, Crane, Indiana. Ninety-nine soil samples were collected in precleaned jars and shipped to WES over a period of 16 days. The samples were shipped and stored at 4 °C. Soon after arrival, each sample was emptied onto an aluminum foil tray and homogenized with wooden spatulas. Rocks, large pieces of plant material, and other foreign objects were removed during the mixing process. Each soil was subsampled for analysis by reversed-phase high performance liquid chromatography (RP-HPLC), the EnSys colorimetric field method, and the D TECH enzyme immunoassay field method. Subsamples for the D TECH immunoassay were taken from the field-moist, homogenated soil just prior to analysis. Two subsamples of approximately 40 g each were placed in aluminum weighing dishes and dried overnight in a Blue-M oven (Blue-M Electric, Blue Island, Illinois) using ambient room air at approximately 25 °C. Air-dried percent solids were determined on the EnSys subsamples. The air-dried subsamples were ground separately with a mortar and pestle. Aggregates of the subsamples intended for analysis by the EnSys method were broken up to simulate field grinding conditions. Small pieces of plant material and stones smaller than 2 mm were left in each of these samples. Subsamples intended for analysis by RP-HPLC were ground to a finer texture with plant material and stones removed as completely as possible. All air-dried soils were stored in the 40-mL glass vials at room temperature in the dark until analyzed. The remaining field-moist sample was stored at 4 °C in the dark.

Laboratory Analysis by RP-HPLC

Extraction and analysis of the soils by RP-HPLC was accomplished as described in SW846 Method 8330 (USEPA 1992). Portions (approximately 2.00 g, weighed to two decimal places) from the air-dried subsamples designated for RP-HPLC were extracted with 10.0 mL of acetonitrile for 18 hr in an ultrasonic bath maintained below 30 °C. Extracts were diluted 1:1 with 5.0 g/L aqueous calcium chloride ($CaCl_2$) and filtered through a Millex SR disposable membrane filter. Extracts were stored at 4 °C in the dark until analyzed.

Aliquots (50 µL) of the soil extracts were analyzed independently on two RP-HPLC systems (Millipore/Waters Chromatography Division, Milford, Massachusetts). The primary system consisted of a Waters model 600E MS System Controller, a 712 WISP Auto Injector, a 486 UV Variable Wavelength Detector monitored at 245 nm, and a Maxima 820 chromatography workstation. The columns used were Supelco 25 cm x 4.6 mm LC-18 for the primary separation and 25 cm x 4.6 mm LC-CN for the confirmation separation. Analytes were eluted with 1:1 methanol/water at flow rates of 1.0 mL/min and 1.2 mL/min, respectively. Retention times obtained for the two columns are given in Table 1. Data obtained from both RP-HPLC systems were quantified using peak heights.

Table 1
Retention Times for Nitroaromatics and Nitroamines Using Two RP-HPLC Separations

Analyte	Retention time, min	
	LC-18[1]	LC-CN[2]
HMX	4.0	13.2
RDX	5.7	8.5
TNB	7.6	5.4
Unknown[3]	8.1	6.2
DNB	9.2	5.4
TETRYL	9.9	10.9
TNT	12.3	6.6
4ADNT	12.9	7.0
2ADNT	12.9	7.5
2,6-DNT	14.8	6.0
2,4-DNT	14.8	6.2

[1] LC-18 (25 cm x 4.6 mm, 5 µm) eluted with 1:1 methanol/water at 1.0 mL/min.
[2] LC-CN (25 cm x 4.6 mm, 5 µm) eluted with 1:1 methanol/water at 1.2 mL/min.
[3] Unknown compound demonstrating positive results on both field screening methods.

D TECH Enzyme Immunoassay Field Screening Method

Soils from the immunoassay subsamples were extracted and analyzed according to the instructions provided with the D TECH TNT/RDX soil extraction pack and the D TECH TNT explosives test kit. Field-moist, homogenized

soil was firmly packed into previously weighed, 3-mL sampling tubes supplied with the soil extraction pack. The filled tubes were reweighed and the air-dried weight of soil in each extraction was calculated using the percent solids obtained for the EnSys subsamples. Each soil plug was dispensed into an extraction bottle containing 9.0 mL of acetone and stainless steel ball bearings and was extracted by mixing on a reciprocating shaker for 3 min. The 3 min of manual shaking described in the D TECH instructions proved to be insufficient to disaggregate firmly packed plugs of soil with high clay content. Vigorous mixing provided by the reciprocating shaker followed by visual confirmation that the soil plug was indeed dispersed was needed to ensure the complete extraction of all soil types. Samples were removed from the shaker and allowed to settle for at least 5 min. A 1.0-mL aliquot of clear extract was transferred into a bottle of buffer solution (bottle 2 in the soil extraction pack). For this study, the immunoassay and the colorimetric field screening methods were compared across the same concentration range. The lower detection limit of the D TECH immunoassay method was raised from 0.2 µg/g to 1.0 µg/g (the lower limit of the EnSys method) by diluting the buffered solution in bottle 2 by a factor of 1 part in 5 parts. This dilution was accomplished (after consulting the manufacturer) by adding volumetrically 200 µL of solution from bottle 2 and 800 µL of Milli-Q water to the dilution bottle (bottle A) found in the TNT explosives test package.[1]

The prescribed volume of the diluted soil extracts was added to vials containing enzyme-labeled TNT and antibody-coated latex particles. This mixture was allowed to incubate for 2 min to allow the TNT molecules to compete for binding sites on the antibodies. One negative control reference sample (supplied with the kit) was processed for each individual analysis. The sample and reference sample received identical treatment. Both solutions were poured onto a porous membrane on either side of a plastic cup assembly. The TNT-antibody conjugates retained on the surface of the membrane were washed and treated with a color-developing reagent. The reference side of the cup assembly was used to determine the end point of D TECH color development. The end point was determined to have been reached when the reference color matched the reference bar on the color card supplied with the kit. The time required for color development was less than 10 min and depended on the ambient temperature of the room.

Results from the test kit were determined by two methods. TNT equivalent concentrations were read directly by comparison of the color development on the test side of the cup assembly to the color card. TNT equivalent concentrations were also measured with the DTECHTOR environmental field test meter (EM Science). This device uses the difference in the amount of light reflected from the surfaces of the color-developed test and reference sides of the cup assembly. Readings are in percentages which translate into TNT equivalent concentration ranges. Ranges corresponding to the dilutions used in this study are found in Table 2.

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[1] Personal Communication, 1993, George B. Teaney, Strategic Diagnostics Inc., Newark, Delaware.

Table 2
DTECHTOR TNT Equivalent Concentration Ranges for Soil Extracts

DTECHTOR Reading	TNT, µg/g		
	No dilution	1:5 dilution	1:50 dilution
Lo - 15	<0.2	<1.0	<10
15 - 30	0.2 - 0.5	1.0 - 2.5	10 - 25
30 - 60	0.5 - 1.0	2.5 - 5.0	25 - 50
60 - 75	1.0 - 1.5	5.0 - 7.5	50 - 75
Hi	<1.5	<7.5	<75

Samples with TNT equivalent concentrations exceeding the range of the D TECH immunoassay were diluted only one additional time to an equivalent of 1 part in 50 parts. A 20.0-µL aliquot of buffered solution from bottle 2 (using a Drummond microdispenser) and 980 µL of Milli-Q water were added to bottle A from the TNT explosives test kit to produce a 1 part in 50 parts dilution. In addition, some samples with concentrations less than 1.0 µg/g in the original test were analyzed undiluted later if the RP-HPLC analysis indicated the TNT concentration for that sample exceeded 0.3 µg/g.

EnSys Colorimetric Field Screening Method

Soils from the colorimetric subsamples were extracted and analyzed according to instructions provided with the EnSys TNT test kit. Absorbance was measured at 540 nm on a model 2504 Hach spectrophotometer. A reference standard, provided with the kit, was analyzed before each set of analyses proceeded. Portions (10.0 g) of dried soil were transferred to extraction bottles to which 50 mL of acetone were added using a volumetric dispenser. After mixing on a reciprocating shaker for 3 min, the bottles were allowed to settle for at least 5 min. Thirty milliliters of extract were removed from each bottle with a disposable plastic syringe and filtered through a Gelman Acrodisc (CR-PTFE 0.45µm) directly into a 25-mL spectrophotometer vial. Absorbance was recorded as ABS<initial>. The vial was then removed from the spectrophotometer and placed on a white surface where one drop of developer solution was added. (The developer is of a higher density than the extract.) The developer fell through the acetone to the bottom of the vial before it began to diffuse. Any change in solution color was detectable at this point and was recorded before capping and mixing the vial. The absorbance was read immediately and recorded as ABS<sample>. TNT concentration, expressed as micrograms per gram of soil, was calculated as (ABS<sample> - 4 × ABS<initial>)/0.0323 as directed by the procedure issued by EnSys.

3 Results

Results of Laboratory Analysis

Of the 99 soil samples investigated in this study, 25 had TNT concentrations above the detection limit of RP-HPLC Method 8330, which for this study was rounded to 0.3 µg/g. The TNT concentrations obtained for these samples are given in Table 3. Representative RP-HPLC chromatograms for several of these samples are presented in Figure 2. Since the field screening methods also detect other nitroaromatics in addition to TNT, results of RP-HPLC analysis for eight other samples containing detectable quantities of 2,4-DNT (2,4-dinitrotoluene), TNB (1,3,5-trinitrobenzene), 4ADNT (4-amino-2, 6-dinitrotoluene), 2ADNT (2-amino-4,6-dinitrotoluene), 2,6-DNT (2,6-dinitrotoluene), or an unknown compound not yet identified are also presented (Table 3). This unknown compound is included because it reacts to the EnSys test with a yellow color change that intensifies over time. This compound also reacts with D TECH with a strong positive reaction suggesting a high concentration of TNT. For comparison, the compound was quantitated using peak height against the TNT response factor. For discussion purposes, the samples in Table 3 can be subdivided into three groups. Group 1 (Table 4) consists of the 14 samples with TNT concentrations (by RP-HPLC) above 1.0 µg/g, which is the reported detection limit of the EnSys field screening method and the operational detection limit for the D TECH test as used in this study. Group 2 (Table 5) consists of samples with TNT concentrations between 0.3 and 1.0 µg/g. Group 3 (Table 6) includes those samples with TNT below the RP-HPLC detection limit of 0.3 µg/g, but containing detectable concentrations of other nitroaromatics.

Comparison of Results from the EnSys Colorimetric Method with RP-HPLC

Tables 4-6 present the results of the EnSys field screening method for the samples with TNT concentrations greater than 1.0 µg/g (according to RP-HPLC results), between 0.3 and 1.0 µg/g, and less than detectable, but with other nitroaromatics present, respectively. Numerical results from the EnSys method are included as well as the visual colors observed after addition of the

Table 3
Concentrations of Various Nitroaromatics by Laboratory Analysis (RP-HPLC)

Sample No.	Concentration, µg/g						
	TNT 0.3	2,4-DNT 0.3	TNB 0.3	4ADNT 0.3	2ADNT 0.3	2,6-DNT 0.3	UNK[1] 0.3
36060	3.8	<d[2]	<d	0.8	<d	<d	<d
36154	19.8	<d	1.4	8.2	5.6	<d	<d
36537	18.2	<d	0.5	<d	0.6	<d	0.5
36538	0.9	<d	<d	0.8	0.6	<d	1.0
36539	0.7	<d	<d	0.3	<d	<d	0.4
36541	0.5	0.8	<d	0.5	0.3	0.4	0.9
36542	<d	1.1	<d	0.3	<d	0.6	<d
36543	<d	<d	0.5	<d	<d	<d	<d
36655	<d	1.3	<d	<d	<d	0.3	<d
36656	<d	<d	<d	0.4	0.4	<d	21.3
36657	<d	<d	<d	<d	<d	0.7	<d
36658	0.6	<d	1.6	0.3	<d	<d	<d
36659	1.4	<d	<d	0.4	0.3	<d	<d
36696	136	<d	<d	<d	0.5	<d	0.4
36697	0.4	<d	<d	<d	<d	<d	<d
36699	0.7	<d	<d	<d	<d	<d	<d
36703	3.2	<d	<d	<d	0.3	<d	<d
36929	0.8	4.2	<d	<d	<d	<d	<d
36973	<d	<d	<d	0.4	<d	<d	<d
37019	<d	11.6	<d	<d	<d	0.4	<d
37121	2.8	<d	<d	1.2	1.4	<d	2.2
37122	2,030	<25	37.5	<25	<26	<25	0.7
37158	<d	<d	0.3	<d	<d	<d	<d
37162	0.7	<d	2.3	1.6	1.6	<d	<d
37164	12.3	<d	<d	<d	<d	<d	<d
37165	1.3	<d	<d	<d	<d	<d	<d
37166	0.3	<d	<d	<d	<d	<d	<d

(Continued)

[1] Unknown compound eluting from C 18 at approximately 8.3 min. Concentration was calculated from peak height against TNT response factor.
[2] Concentrations are less than the detection limit given for each compound in SW846 Method 8330.

Table 3 (Concluded)

Sample No.	Concentration, µg/g						
	TNT 0.3	2,4-DNT 0.3	TNB 0.3	4ADNT 0.3	2ADNT 0.3	2,6-DNT 0.3	UNK[1] 0.3
37214	0.4	<d	0.7	<d	<d	<d	<d
37217	0.3	<d	<d	<d	<d	<d	<d
37220	7.6	<d	2.1	2.5	2.2	<d	0.9
37221	60.7	<d	3.0	2.2	1.2	<d	12.2
37222	2.3	<d	<d	<d	<d	<d	0.5
37224	1.6	<d	2.5	<d	0.3	<d	<d

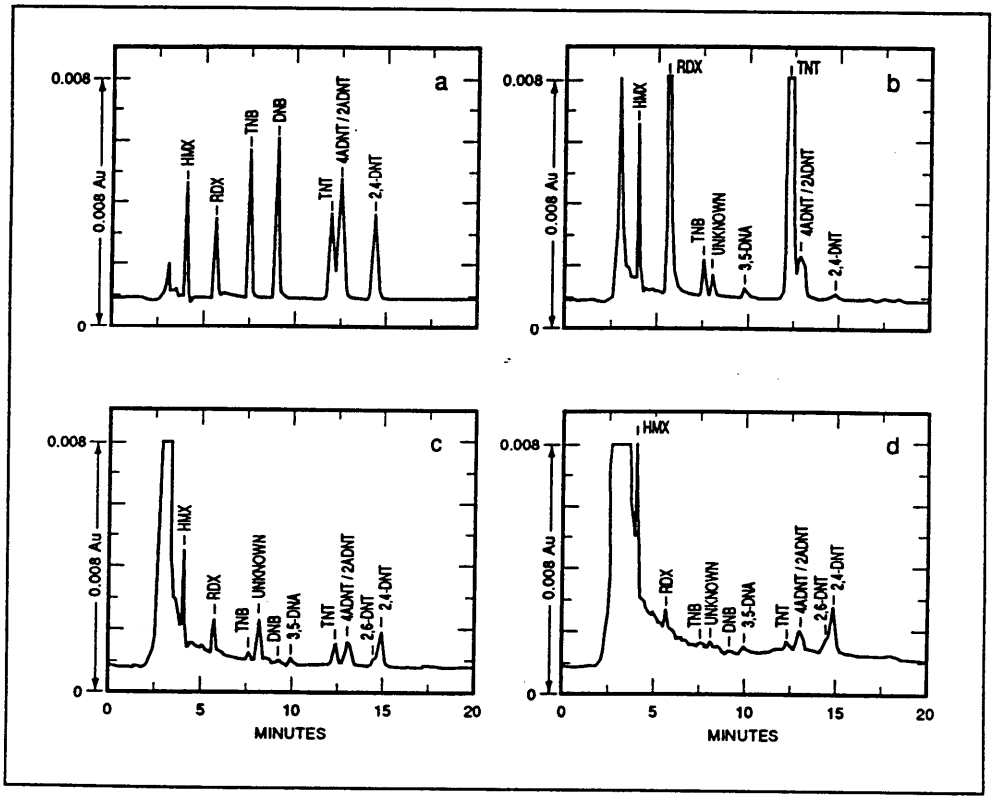

Figure 2. LC-18 chromatograms 2.0-µg/g standard. Sample 36537; TNT concentration > 1.0 µg/g. Sample 36541; TNT concentration 0.3 - 1.0 µg/g. Sample 36542; TNT concentration <0.3 µg/g

EnSys reagent to each extract. The EnSys method gave a positive result for all 14 samples with TNT concentrations (by RP-HPLC) greater than 1.0 µg/g (Table 4), with no false negatives. For these samples, TNT was the major nitroaromatic present, and the colors observed had a reddish component in all cases. The EnSys method also detected 9 of the 11 samples with TNT

Table 4
RP-HPLC and Field Screening Results for Samples Containing a TNT Concentration (by RP-HPLC) Greater than 1.0 µg/g

Sample No.	RP-HPLC µg/g	EnSys µg/g (color)	D TECH µg/g
36060	3.8	4.4 (red)	0.5 - 1.0[1]
36154	19.8	27 (red)	10 - 25
36537	18.2	15, 22 (red)	>75
36659	1.4	1.5 (brown)	>1.5[1]
36696	136	3.2, 52 (red)	7.5 - 10
36703	3.2	0.5, 0.8, 4.8 (pink)	5.0 - 7.5
37121	2.8	7.5 (red)	5.0 - 7.5
37122	2,030	110, 110, 130 (red)	>75
37164	12.3	3.6 (red)	1.0 - 2.5
37165	1.3	3.6 (pink)	0.5 - 1.0[1]
37220	7.6	21 (red)	50 - 75
37221	60.7	45 (red)	>75
37222	2.3	4.2 (orange)	>7.5, <10
37224	1.6	4.5 (pink)	0.5 - 1.0[1]

[1] Not detected at 1:5 dilution; value obtained from D TECH undiluted analysis.

concentrations between 0.3 and 1.0 µg/g (Table 5) and 5 of the 8 samples with concentrations of other nitroaromatics above 0.3 µg/g (Table 6). For these samples the colors observed were often dominated by blues or purples, indicating that the major nitroaromatic compound reacting with the EnSys reagent was not TNT. This conclusion is supported by RP-HPLC analyses. Of the remaining 66 samples which were nondetects by RP-HPLC, results of the EnSys screening test were 64 nondetects and 2 detects which researchers classified as false positives (Table 7). The quantitative estimates for these two false positives are 1.2 and 2.3 µg/g by EnSys, but the colors indicate that the compounds detected are not all TNT.

Inspection of the numerical agreement of the data in Table 4 indicates that except for the two high concentration samples (by RP-HPLC), the agreement between the RP-HPLC results and those from the EnSys method is quite good. The poor agreement between RP-HPLC results and EnSys for samples 36696 and 37122 was surprising and will be discussed later. The results for the other 12 samples were subjected to regression analysis. The results indicated a slope of 0.72, an intercept of 3.4, and a correlation coefficient of 0.90 which was statistically significant at the 99.9-percent confidence level (Figure 3). These

Table 5
RP-HPLC and Field Screening Results for Samples Containing a TNT Concentration (by RP-HPLC) Greater than 0.3 µg/g but Less than 1.0 µg/g

Sample No.	RP-HPLC µg/g	EnSys µg/g (color)	D TECH µg/g
36538	0.9	1.7 (red)	2.5 - 5.0
36539	0.7	0.8, 2.2 (pink)	>1.5[1]
36541	0.5	10 (blue-purple)	7.5 - 10
36658	0.6	8.0 (blue-purple)	<d[2]
36697	0.4	1.4 (pink)	0.2 - 0.5[1]
36699	0.7	1.9 (red-purple)	<d[2]
36929	0.8	<d (pink)	>1.5[1]
37162	0.7	3.9 (blue-purple)	2.5 - 5.0
37166	0.3	7.1 (blue-green)	1.0 - 2.5
37214	0.4	<d (pink)	0.5 - 1.0[1]
37217	0.3	1.5 (none)	<d[2]

[1] Not detected at 1:5 dilution, value obtained from D TECH undiluted analysis.
[2] Not detected at 1:5 dilution and also not detected from D TECH undiluted analysis.

results indicate that quantitative results from the EnSys method should be usable with a high degree of confidence for decision making onsite. Observation of the colors developed after reaction of the EnSys reagent with soil extracts should be noted and can be used to help distinguish between the presence of TNT and certain other nitroaromatics.

Comparison of the Results from D TECH Immunoassay Method with RP-HPLC

Results for the D TECH field screening test are also presented in Tables 4-6. The D TECH kit detected all 14 samples where TNT concentrations by RP-HPLC were above 1.0 µg/g. Four of these were initially nondetects at the 1:5 dilution but were positive when analyzed undiluted according to the manufacturer's directions. Thus, the D TECH method suffered no false negatives for samples in which RP-HPLC results were greater than 1.0 µg/g. The D TECH kit also detected 8 of the 11 samples with TNT between 0.3 and 1.0 µg/g but only 1 of the 8 samples with detectable concentrations of other nitroaromatics, but no detectable TNT by RP-HPLC. Use of the D TECH kit with the other 66 samples with nondetectable nitroaromatics (by RP-HPLC) resulted in 63 nondetects and 3 false positives. The quantitative values for the

Table 6
RP-HPLC and Field Screening Results for Samples with a Non-Detectable TNT Concentration (by RP-HPLC), but with Other Nitraromatics Above Detection Limits

Sample No.	RP-HPLC Sum of Nitroaromatics, µg/g	EnSys µg/g (color)	D TECH µg/g
36542	2.0	2.5 (blue-purple)	<d
36543	0.5	<d	<d
36655	1.6	1.8 (purple)	<d
36656[1]	22.1	2.0, 2.2 (pink)	2.5 - 5.0
36657	0.7	<d	<d
36973	0.4	2.0 (pink)	<d
37019	12.0	6.0 (blue-purple)	<d
37158	0.3	<d	<d

[1] Sum of nitroaromatics by RP-HPLC includes unknown compound quantitated with TNT response factor.

three false positives were 7.5 - 10, >7.5, and 7.5 - 50 µg/g, respectively, for the D TECH method. It appears that the D TECH kit is also detecting the presence of nitroaromatics other than TNT. While EnSys and D TECH each suffered several false positives, they did not occur for the same samples.

Inspection of Table 4 indicates that the numerical agreement between the RP-HPLC results and those from D TECH was only fair, even after neglecting the results from samples 36696 and 37122. Since the D TECH results are given in ranges rather than as a specific quantity, statistical analysis of the results was not straightforward. To apply regression analysis, as described above for the EnSys results, the RP-HPLC values were regressed versus the midpoint of the range from each D TECH result. A value of 75 µg/g for D TECH was used for samples 36537 and 37221, and a value of 1.5 µg/g was used for sample 36659 because no upper bound was available for these samples. As with the regression for the EnSys data, results for samples 36696 and 37122 were not included in this analysis. The results of this analysis indicated a slope of 1.3, an intercept of 7.2, and a correlation coefficient of 0.70 which was statistically significant at the 95-percent level but not at the 99-percent level (Figure 4). Thus it appears that the quantitative results from the D TECH kit are less reliable than those from the EnSys kit when both are used according to manufacturers' directions.

Table 7
False Positives for Field Screening Methods Relative to RP-HPLC Results

Sample No.	RP-HPLC µg/g	EnSys µg/g (color)	D TECH µg/g
36738	<d	1.2 (pink)	<d
37216	<d	2.3 (orange)	<d
36540	<d[1]	<d	7.5 - 10
37219	<d	<d	>7.5
37223	<d	<d	>7.5 <50

[1] Sample contains ~ 0.7 µg/g of unknown compound based upon TNT response factor.

Investigation of Differences Between Quantitative Results from Field Screening and Laboratory Analyses

While soil samples that were found to contain detectable concentrations of TNT using the standard RP-HPLC method were also generally detected by both field screening methods, the quantitative agreement was not always up to expectations. For example, as pointed out above, much higher TNT concentrations were obtained for samples 36696 and 37122 by RP-HPLC than by the EnSys colorimetric method or the D TECH immunoassay method (Table 4). Conversely, analysis of samples 36541 and 37220 resulted in somewhat higher TNT concentrations by both screening methods than found by the laboratory procedure (Tables 4 and 5). These differences could be due to actual differences in performance of the screening methods relative to the laboratory procedure, either due to less efficient extraction or interferences from other compounds present in the extracts, or to sample heterogeneity, where the actual analyte concentration varied substantially among the various subsamples analyzed by the various methods.

To further investigate this phenomenon, a set of 11 soil samples, where substantial quantitative differences between the laboratory and field screening methods existed, were extracted and analyzed as follows. A 10.0-g portion of each air-dried soil sample was extracted with a 50-mL portion of acetone by manual shaking for 3 min. For samples 36696 and 37122, after 3 min of shaking and the normal settling time, a 7.0-mL aliquot was withdrawn and the remainder further extracted in an ultrasonic bath for 18 hr in an analogous manner to the laboratory procedure. After the 18-hr extraction, a 7.0-mL aliquot was removed from these two extracts and processed along with the 3-min extracts from all 11 samples. The acetone extract from each sample was

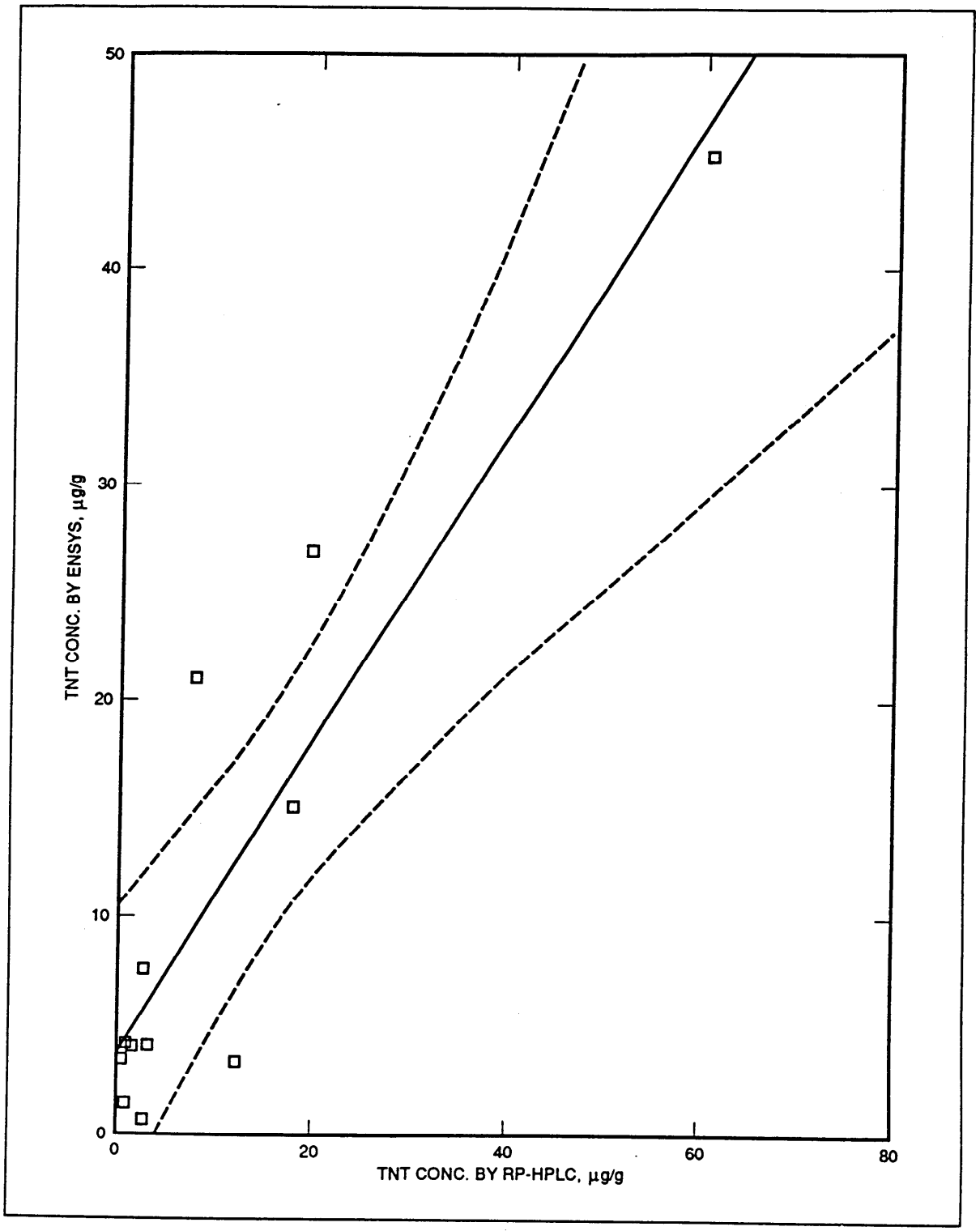

Figure 3. Regression analysis of TNT concentrations by RP-HPLC and EnSys on samples in Table 4. [EnSys] = 0.72 [HPLC] + 3.4, r = 0.90. Confidence intervals are 99 percent

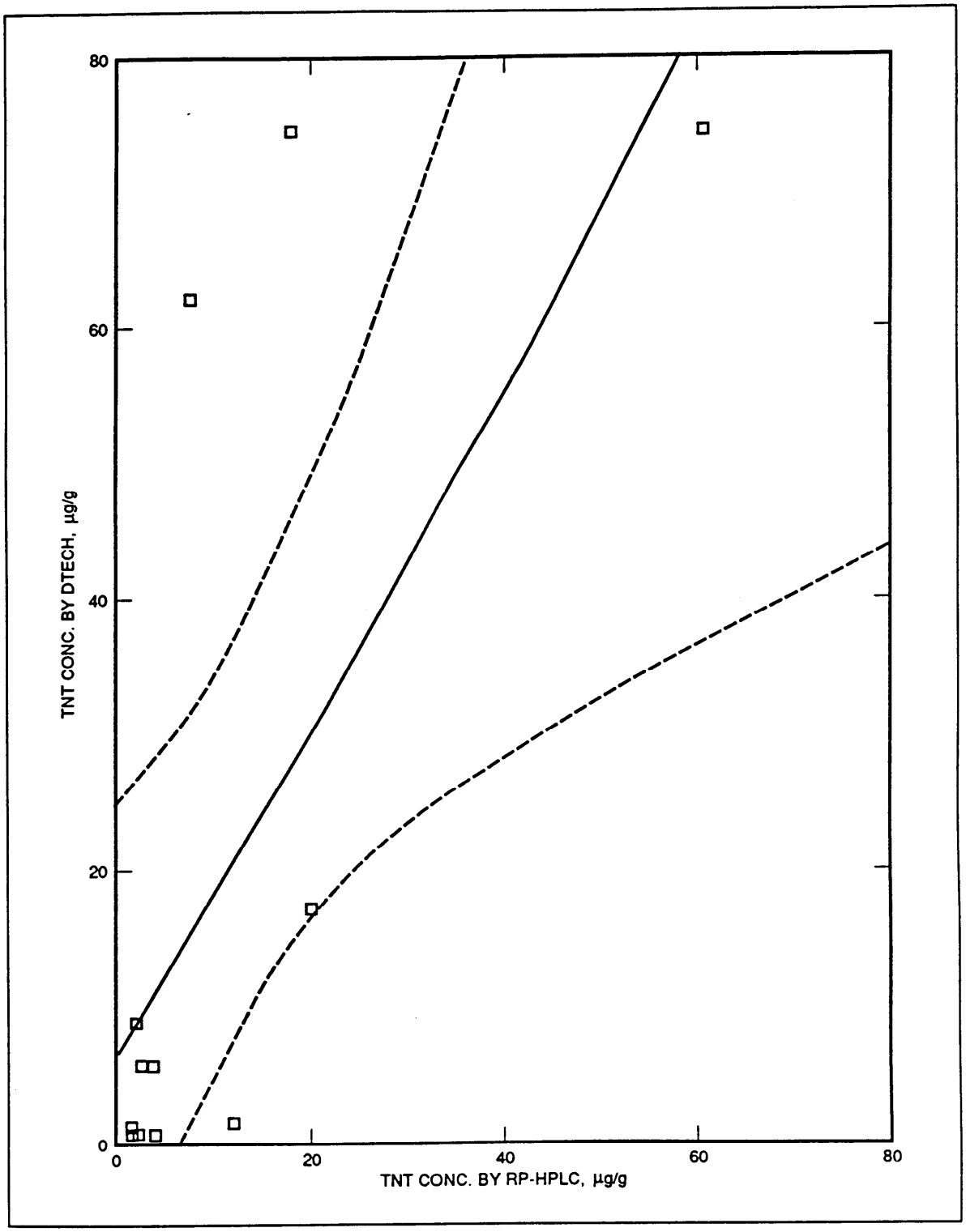

Figure 4. Regression analysis of TNT concentrations by RP-HPLC and D TECH on samples in Table 4. [D TECH] = 1.25 [HPLC] + 7.2, r = 0.70. Confidence intervals are 95 percent.

Table 8
Results of Testing to Identify Reasons for Quantitative Differences Between Laboratory and Field Screening Results for TNT Using a Common Acetone Extract

Sample No.	RP-HPLC	EnSys	D TECH[1]	Env[2]
3-Min Extraction				
36696	243	240	120 - 250	190
37122	986	1300	620 - 1200	1200
36541	0.6	5.6	5.0	5.0
36656	0.3	2.3[3]	2.5 - 5.0	4.0
36658	<d	1.5[3]	<0.5	1.5
36973	<d	1.6[3]	0.5	2.3
37121	1.5	7.3	10	12
37162	0.3	5.8	1.0	6.3
37165	1.2	1.9	0.5 - 1.3	2.0
37216	<d	2.2[3]	<0.5	0.4
37220	4.7	19	10	18
18-Hr Extraction				
36696	238	230	120 - 250	320
37122	1000	1200	620 - 1200	970

[1] D TECH data quantitated by color card.
[2] EnviroGard TNT kit from Millipore.
[3] Colors observed for these samples were beige by the EnSys method.

divided into three portions and analyzed by each of the three methods (RP-HPLC, EnSys, and D TECH). The results are presented in Table 8. Since analyses for the three procedures were conducted from a common extract, these results do not include any contribution from subsample heterogeneity or from differences in extraction efficiencies.

The results of the analysis of samples 36696 and 37122 are very revealing. The concentration estimates for these two samples from all three procedures are in excellent agreement. In addition, the values for the 3-min extraction and the 18-hr extractions are essentially identical. These results indicate that the initial disparity found for these two samples (Table 4) was not a result of either slow extraction kinetics or interferences, but rather due to sampling error (subsample heterogeneity). This problem may be particularly significant for samples with high concentrations where the analyte may be present as discrete particles rather than sorbed on soil components. The D TECH method seems particularly susceptible to this problem since a smaller subsample is used for

analysis and homogenization prior to subsampling is accomplished using field-moist soil. Homogenization with wet soils is much less effective than that achievable after drying and grinding.

This explanation does not resolve the differences observed between the concentration estimates for the RP-HPLC and the two field methods for the other samples shown in Table 8. Even using a common extract, the field results are consistently higher for these low concentration samples than for RP-HPLC. For the EnSys procedure, a positive result was obtained for samples 36656, 36658, 36973, and 37216 although the RP-HPLC results for these samples ranged from nondetect to 0.3 µg/g. The color observed when these extracts were reacted with the EnSys reagent was light beige rather than pink-red, and hence the concentration estimates from the EnSys method are probably a result of interference from humic materials present in the extract. Extracted humics can increase in color when reacted with base (Jenkins 1990). Thus it is believed that users should consider extracts that react to form either a yellow or beige color to be nondetects even if an absorbance change is found using the formula specified by EnSys.

Results from the remaining five samples are also instructive. Even when the concentrations of other nitroaromatic analytes are also added to the TNT concentration by RP-HPLC, the RP-HPLC results are still consistently lower than either the EnSys or D TECH results. This is believed to be due to response of the two field methods to other transformation products of TNT which are not resolved by RP-HPLC, but retain the functionality required to react with the two field methods. These could be monomeric compounds of very different polarity than TNT or transformation products of TNT bound to humic substances. Since a portion of the humic material extracts into acetone, bound residues could interact like free analyte if the requisite functionality is still present.

Subsamples taken from the 11 common extracts were also analyzed by the EnviroGard TNT plate method. Results are included in Table 8 and illustrate the confusion caused by cross-reactivity with other compounds. The antibody used for this kit has different responses to commonly occurring explosives and degradation products than the antibody used for the D TECH kit (Table 9). The positive errors resulting from quantifying these compounds depend upon the exact mix of the compounds present, as was seen with both the EnSys and D TECH tests.

Practical Observations and Concerns

Once a method has been proven analytically, the ease with which it can be implemented and the type of data generated will greatly influence the method's suitability for a given application. The EnSys colorimetric TNT method is well suited in situations in which quantitation is needed to comply with an action level. However, the method is subject to interferences from other

Table 9
Nitroaromatic Compounds Demonstrating Positive Results on the EnSys, D TECH, and EnviroGard Tests

Compound	EnSys	D TECH	EnviroGard
TNT	+	+	+
2,4-DNT	+		
2,6-DNT	+		+
TNB	+	+	+
2ADNT	+	+	
4ADNT	+		+

compounds, such as humic acid and other nitroaromatics that are extracted by acetone and give a positive absorbance at 540 nm. The EnSys method can differentiate between TNT and DNT because of the distinctive color changes observed in the extract upon the addition of base.

The colorimetric method has a dynamic range from 1 µg/g to 30 µg/g. If quantitation is required, dilutions of samples higher than 30 µg/g are relatively easy and do not require the purchase of additional tests. Sufficient base is provided for more than the 20 tests per kit. With adequate precautions, a suitable dilution can be made from the original extract directly into the spectrophotometer vial. All that is needed is a pipetter with disposable tips, a few disposable beakers, and fresh acetone. On the down side however, only one ampule of TNT control is provided per box of 20 tests. Samples cannot be analyzed in small batches of less than 20 without a source of additional TNT control. For this study, the control was supplemented with laboratory stock TNT standard. Before initiating a field study, the user should identify a source of additional TNT control or plan analytical batch sizes around the number of controls available. For ease of use and continuity, it is suggested that EnSys make additional ampules of TNT control available to its customers. Also, in this study, a few samples did not settle sufficiently to allow 25 mL to be filtered with only one filter disk. Users are advised to have an additional supply of filter disks on hand as a precaution.

The good correlation reported between EnSys data and RP-HPLC data can be partially attributed to the large sample size and to the use of dried, homogenized sample. In this study, EnSys subsamples were air-dried overnight and processed the following day. Drying large numbers of samples, however, may be difficult to do in the field and can delay analysis and decision making. Field-moist, unhomogenized soils can be used in this procedure but with some loss of precision due to increased sample heterogeneity. The original screening method uses an extraction of undried soil (Jenkins 1990) and has been used in the field with good results for several years. Thus, the choice of

method of sample preparation depends on the data quality objectives and on the time available for decision making.

Hardware store grade acetone was the extraction solvent of choice in the original field screening method. The use of local sources of acetone is recommended to avoid transporting hazardous substances. Use of a 50-mL bottle-top dispenser is recommended to avoid spillage and waste. If using hardware store acetone, the setup, an empty bottle with dispenser attached, should be included with the supplies. If a bottle-top dispenser is impractical for the job, a Teflon squeeze bottle and a 50-mL graduated cylinder would be a second choice. Users should also familiarize themselves with any portable analytical equipment prior to a field study. In this laboratory study, an older, analog model of the Hach spectrophotometer was used which did not shut off between samples. Researchers did attempt to use a portable balance but found it to be unreliable and inaccurate.

The dynamic range of the D TECH test used as recommended by the manufacturer is 0.2 µg/g to 1.5 µg/g. The D TECH test kit is completely self-contained with everything needed to perform a field screening analysis unless dilutions are required to quantitate high-level samples. In these cases, a new TNT test must be used for each dilution and an attempt to quantitate could require several TNT tests. This requirement for multiple tests per sample increases costs and must be included in the design of the study. In this study, dilutions were made from the buffered dilution bottle (bottle 2) in the soil extraction pack. This method of dilution is adequate unless the TNT concentration exceeds the aqueous solubility of 130 µg/mL, a situation which is possible when a site is first characterized for reactive levels (≥10 percent) of TNT. In such cases dilution of the acetone extract into an appropriate portion of fresh acetone might be a better choice with 1 mL of the diluted acetone extract added to the buffer solution (bottle 2). If the aqueous solubility of TNT is exceeded in the buffer during any dilution, TNT can precipitate and inaccurate data can result.

The most appropriate application of the D TECH test is quick, on the spot screening rather than quantitative analysis. The mode of sampling and small sample size factor into the inconsistent numerical correlation with RP-HPLC data. When used with appropriate sampling design, the test appears to be sensitive enough to identify contaminated sites. Discrimination of concentration around an action level may not be possible, however. The screening intent of the kit also negates, to some extent, the usefulness of the reflectometer. This device gives a result in percent of difference to the negative control which must be converted to TNT parts per million in soil using a calibration chart supplied with the kit. This chart breaks the percent values into five ranges, only one more subdivision of the dynamic range than that supplied on the color card. The reflectometer diagnostic intended to instruct the analyst to "wait" for sufficient color development does not adequately judge when color development is complete. Readings taken too early will be inaccurately high. The user must be careful to follow instructions and allow the reference side of the cup assembly to fully develop to the color of the reference strip on the

color card before taking a reading. At this point it is very easy to turn the color card to the appropriate set of color ranges and take a concentration reading. When using the color card, the user must ensure that the colored regions of the cup assembly are not in shadow, or erroneous readings will result. This includes judging when color development is completed. As in other immunoassays, the color development is not stable and will proceed to complete development (i.e., a negative detect) unless stopped with an acidic solution. Readings should be taken as soon as possible after the color is developed. Slight differences were also found in percent data when the reflectometer was calibrated with a calibrator versus calibration with a clean, unused cup assembly, re-emphasizing the fact that the calibration or cup assembly used as a calibrator must be kept clean and white and protected as instructed in the D TECH directions. Data obtained on each sample from comparison to the color card and from the reflectometer were very similar. Use of the reflectometer can eliminate some of the subjective variability from TNT measurements; however, correct use of the reference strip on the color card is still required. In this study up to 20 soils were extracted at one time but actual analysis of the soil extracts was limited to four or five tests per batch. It was difficult to effectively monitor the color development of more cup assemblies than this at one time.

Interference compounds can affect immunoassays in three different ways. An interfering component may bind with the free analyte or enzyme-conjugate, removing it from the competitive binding process. A cross-reactive compound may bind with the antibody, tying up active antibody sites and causing erroneously high results. Or, the interference may alter the structure of the enzyme or antibody, causing loss of function that may also lead to erroneously high results. Interferences from other cross-reactive compounds appear to be a major factor affecting the numerical correlation between D TECH and RP-HPLC data. Humic materials can also cause inaccuracies. But the buffer dilution step, added to remove compounds that would discolor or clog the membrane in the cup assembly, may dilute the humics and lessen this likelihood.

4 Discussion of Results for Various Use Scenarios

It appears that there are two very different ways in which these two field screening methods could be used effectively in conjunction with confirmation by offsite laboratory analysis. In the first scenario, field screening could be used to distinguish between soil contaminated with TNT and clean soil. In this scenario, samples found to be contaminated would be sent to an offsite laboratory when quantitative analysis was needed. The major cost savings would result from the reduced number of samples that needed to be analyzed by the offsite laboratory. If the tests are performed according to manufacturer's directions, the D TECH kit best fits this usage. Since soils are not dried prior to use, the analysis is fast and samples can be sent to the offsite laboratory on the same day they are collected. In a second scenario, the user would want to make decisions onsite relative to whether concentrations are above or below an action level, and a certain percentage of detects would be sent to an offsite laboratory for confirmation. In this use scenario, good quantitative agreement with the standard laboratory method is essential, and the user should be willing to wait a little longer for results. It appears that the EnSys kit better fulfills this usage because a higher correlation with the laboratory method was demonstrated.

These judgments are based on the use of the kits as described by the manufacturer. A better sampling procedure would vastly improve the quantitative agreement of the D TECH method relative to the RP-HPLC procedure. Likewise, the EnSys method could be used with undried soil if a lesser degree of comparability of quantitative results with the laboratory method can be tolerated.

5 Conclusions

Two commercially available screening methods for TNT in soil were evaluated and compared with the standard RP-HPLC laboratory method (SW846 Method 8330). Comparison of samples extracted according to the manufacturers' instructions was difficult because of subsample heterogeneity. The colorimetric method, EnSys, demonstrated a good one-to-one linear correlation with RP-HPLC that can be attributed to the procedure for extraction, i.e., a large sample size of dried, homogenized soil. Comparison of D TECH, the immunoassay method, was more difficult because results are presented in ranges rather than discrete numbers. One-to-one linear correlation with RP-HPLC was poorer for the D TECH method. This was due, in part, to statistical evaluation of the midpoint of the range and, to a greater extent, to the method of sampling and extracting a small volume of homogenized, field-moist soil.

Results also showed that both methods are susceptible to interferences. For the EnSys method, interferences are any compound or mix of compounds that produces a change in color upon addition of base. This includes humic substances and other nitroaromatics, some of which produce distinctive colors that can be used for qualitative identification. The D TECH immunoassay is susceptible to interferences that bind to or inactivate the analytes, the enzyme-conjugates, or the antibodies in some fashion. Although both methods showed strong tendencies to cross react with other nitroaromatics, sometimes resulting in false positives, in a sampling of 99 soils, neither method produced a false negative.

The EnSys colorimetric test is well suited for studies requiring good quantitative agreement with the standard laboratory method, such as compliance with a discrete action level. When used according to manufacturer's instructions, the EnSys method can be used to obtain good numbers with a small delay in response time. The D TECH immunoassay is better suited for quick, onsite screening in situations where all samples above a certain range will be sent forward to a laboratory for confirmation by the standard method.

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13. ABSTRACT *(Maximum 200 words)*

A study comparing two commercially available methods of field screening for TNT in soil, utilized 99 soil samples from the Naval Surface Warfare Center, Crane, Indiana. All soil samples were analyzed using a commercial colorimetric method (EnSys) and a commercial enzyme immunoassay method (D TECH), and the results were compared with those from the standard reversed-phase high performance liquid chromatography (RP-HPLC) laboratory method (SW846 Method 8330). Comparisons were made relative to numerical agreement of screening results with laboratory analysis by RP-HPLC and usage in two distinct scenarios.

Of the 99 soil samples analyzed by the laboratory method, 25 had TNT (2,4,6-trinitrotoluene) concentrations greater than the RP-HPLC detection limit of 0.3 µg/g. Of these 25, 11 had concentrations in the range 0.3 - 1.0 µg/g and 14 had concentrations greater than 1.0 µg/g (the operating field screening detection limit for this study). Results were positive from both field screening methods for all 14 soils with TNT concentrations greater than 1.0 µg/g by RP-HPLC. Thus, no false negatives were observed by either method for samples above the field screening detection limit. Of the 11 samples with TNT concentrations in the range 0.3 - 1.0 µg/g, D TECH failed to

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detect 3 samples and EnSys failed to detect 2 samples. For 66 of the samples, RP-HPLC analysis yielded nondetects for TNT and other nitroaromatics. For these samples, the D TECH kit yielded 63 nondetects and 3 false positives, and the EnSys method resulted in 64 nondetects and 2 false positives.

Quantitative results from both field methods were regressed against the numerical results of the RP-HPLC analyses. Somewhat better agreement was obtained between results from RP-HPLC with the results from the EnSys method. These better results appear to be largely due to a decrease in sampling error achieved by air drying a fairly large portion of soil and homogenizing prior to subsampling. Results suggest that both kits respond to the presence of other nitroaromatics in addition to TNT. For the EnSys kit, different colored solutions are produced when the major nitroaromatic present is TNT (pink-red) or 2,4-DNT (blue-purple).

The authors suggest that the D TECH kit is best suited to use in a pure field screening mode, where quantitative results are taken from laboratory analyses. Quantitative results from the EnSys kit appear to be of sufficient quality to permit rapid decisions in the field as to whether TNT concentrations are above or below an action concentration. When used according to manufacturer's directions, the D TECH kit produces results much more rapidly, since the EnSys kit requires that soils be dried prior to use.