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Evaluation of a Field Kit for Detection of TNT in Water and Soils

Thomas F. Jenkins and Patricia W. Schumacher

June 1990

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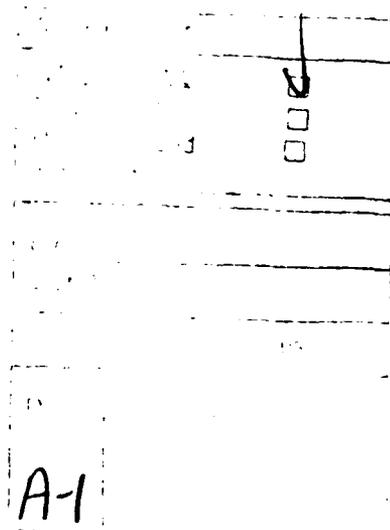


**U.S. Army Corps
of Engineers**
Cold Regions Research &
Engineering Laboratory

Evaluation of a Field Kit for Detection of TNT in Water and Soils

Thomas F. Jenkins and Patricia W. Schumacher

June 1990



Prepared for
U.S. ARMY TOXIC AND HAZARDOUS MATERIALS AGENCY
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PREFACE

This report was prepared by Dr. Thomas F. Jenkins, Research Chemist, and Patricia W. Schumacher, Physical Sciences Technician, Geochemical Sciences Branch, Research Division, U.S. Army Cold Regions Research and Engineering Laboratory.

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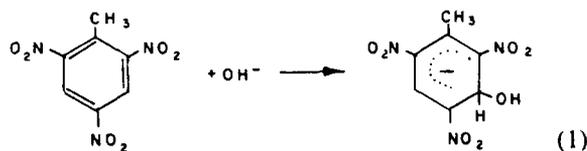
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Evaluation of a Field Kit for Detection of TNT in Water and Soils

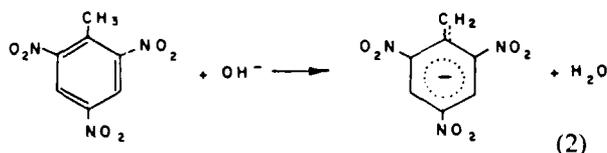
THOMAS F. JENKINS AND PATRICIA W. SCHUMACHER

INTRODUCTION

An indicator tube was developed by Heller et al. (1982) for field detection of TNT in water. The tube consists of two sections. The first, or presection, contains glass beads coated with a basic oxide. The function of this section of the tube is to react with TNT to form a reddish colored anion. The colored species has been attributed to either the classical Meisenheimer anion as shown in eq 1



or to an anion formed by proton abstraction from the methyl group as shown in eq 2 (Erickson et al. 1984).



According to Terrier (1982), the anion shown in eq 2 is more stable, but the anion in eq 1 forms first. The anion is then captured on an anion exchange resin (eq 3), where TNT^- represents the anions produced in eq 1 or 2.



The length of the resulting stain in the anion exchange section of the tube is a function of the inner diameter of the tube, the sample volume, the flow rate through the tube and the TNT concentration in the sample (Erickson et al. 1984). If sample volume and flow rate are held constant for a given tube diameter, the stain length should be a function of TNT concentration.

BACKGROUND

Anion exchange section

The anion exchange resin initially used was Dowex 1 \times 10 (Erickson et al. 1984). This was a highly cross-

linked polystyrene-divinylbenzene copolymer that did not swell upon wetting. Thus, it could be dry-packed in the tubes.

This Dowex resin is no longer available. Many other anion resins are reddish colored in their hydroxyl form and thus are of no use for this application; so, Erickson et al. (1984) selected Bio-Rad AGMP-1 resin to replace the Dowex material. This resin has a pale straw color in the hydroxyl form; however, it does swell upon contact with water. Thus, tubes containing this resin must be wet packed and maintained wet during storage.

Erickson et al. (1984) acknowledge that stain length does not vary linearly with concentration. They feel this is attributable to diffusion of the colored anions into the pores of the resin and suggest that use of a pellicular resin might alleviate this problem. Unfortunately, pellicular indicator tubes are not currently available.

Presection

The presection of the tube is composed of glass beads coated with a strong base. Bases that are strong enough to produce the TNT anions are KOH, NaOH, CaO, $\text{Mg}(\text{OH})_2$, MgO and BaO (Erickson et al. 1984). The current design of the tube calls for a presection composed of a mixture of CaO, $\text{Mg}(\text{OH})_2$ and BaO deposited on glass beads. Initially, Heller et al. (1982) used CaO powder in the presection, but high back pressure developed resulting in leaks. CaO was also found to be rapidly depleted by aqueous solutions containing high amounts of chloride. Erickson et al. (1984) found that a presection containing a combination of oxides with varying water solubilities coated on glass beads alleviated these problems.

Since the pH of the solution after passing through the presection must be very high for the desired reaction with TNT to occur, the acidity of the initial solution is important. Erickson et al. recommend that aqueous samples have a pH of at least 6.5. The degree of buffering present in the sample, however, is as important as the actual pH.

Detection of TNT in soil

These indicator tubes were initially developed for detection of TNT in water. More recently, they have been also used to detect TNT in soil extracts (Erickson

et al. 1984, Mason-Hangar 1989). The choice of solvent for soil extraction was based on two criteria. First, the solvent must provide adequate extraction of TNT from soil, and second, it must provide adequate solubility of the base in the presection of the tube to generate the TNT anions for detection. Erickson et al. (1984) tested several solvents, including acetone, methanol, acetonitrile, propanol, isopropanol, ethanol and a variety of acetone-water solutions with percentages of water varying from 0 to 90%. They reported that methanol gave the best overall extraction efficiency.

In their procedure a 6-g subsample of soil is placed in an extraction flask, 20 mL of methanol is added and the flask is shaken vigorously for 1 minute. The extract is gravity filtered and the filtrate collected. Using soils spiked with TNT in the laboratory, Erickson et al. (1984) reported extraction efficiencies ranging between 87 and 105% for TNT.

The indicator tubes have been used to determine whether soils at Cornhusker Army Ammunition Plant were contaminated above an action criterion of 5 $\mu\text{g/g}$ (Mason-Hangar 1989). At Cornhusker, a 5-g soil sample was extracted with 10 mL of methanol, processed as described above, and a 5-mL aliquot of the filtrate was pumped through an indicator tube. The "stain intensity" (rather than stain length) was directly compared with the stain intensity observed for a standard solution (corresponding to a soil at 5 $\mu\text{g/g}$).

This procedure was subjected to a Class 2 certification procedure as described in USATHAMA (1987). Four blank samples and four extracts at the criterion level of 5 $\mu\text{g/g}$ were analyzed. No stains were observed in the blanks and detectable stains were observed in all four 5- $\mu\text{g/g}$ extracts. Clearly, the tubes can be used to detect the presence of TNT at the 5- $\mu\text{g/g}$ level relative to a blank. However, in practice, the tubes were used to discriminate between levels above or below a 5- $\mu\text{g/g}$ level. No testing of the potential for false positives or false negatives relative to this criterion were reported.

Dynamic range

The indicator tubes are available in two sizes—standard bore and small bore. The authors indicate that for 10-mL samples the standard bore tubes can quantify TNT concentrations over the range of 0.1 to 10 mg/L or, on a soil basis, 0.3 to 30 $\mu\text{g/g}$ (6 g of soil extracted with 20 mL of methanol). Small bore tubes are reported to be useful over a concentration range of 0.02 to 0.2 mg/L or 0.06 to 0.6 $\mu\text{g/g}$ (Erickson et al. 1984).

Interferences

Erickson et al. (1984) also investigated potential interferences for the indicator tubes. A negative interference was discussed earlier for acidic solutions in

which the resulting pH following the presection was too low to form the colored anion. Reddish colored stains, which could result in a positive interference, were observed for 2,4-dinitroaniline, tetryl and 2,4,6-trinitrobenzoic acid. While the shades of red are visibly different from that given by TNT, their coincident occurrence with TNT would undoubtedly result in a positive interference. With the presection removed, 2,4-dinitrotoluene, 2-amino-4,6-dinitrotoluene, ammonium picrate, 3,5-dinitro-*o*-cresol and 4,6-dinitro-*o*-cresol formed either green or yellow stains that could mask the presence of TNT if present at sufficiently high concentrations. Experience indicates that this is very unlikely, however.

The presence of high levels of other anions could displace TNT anions from the exchange sites on the anion exchange resin, thereby increasing stain lengths for a given TNT concentration. Erickson et al. did not identify a common anion that caused this type of interference.

Flow rate

Erickson et al. (1984) recommend flow rates of 2.7 mL/min for the standard bore tubes and 1.0 mL/min for the small bore tubes. Faster flow rates result in longer stains of lower intensity and slower flow rates result in shorter stains of higher intensity. Use of a syringe pump is recommended to obtain a reproducible flow rate.

The Mason-Hangar (1989) procedure for soil analysis recommended a flow rate of 1.0 mL/min for the standard bore tubes and a total sample volume of 5 mL.

TEST OBJECTIVES

The objectives of the tests discussed here were to provide an independent assessment of the utility of the indicator tubes for field measurement of TNT in soil and water. The tubes we used for this assessment were procured commercially and thus should represent the types of indicator tubes currently available for field use. Earlier assessments by Erickson et al. (1984) of the method used tubes they assembled themselves and hence the characteristics could be somewhat different.

Our overall test strategy assumed that the standard bore tubes would be used for detection of TNT in soil extracts, whereas the small bore tubes would be used for detection of TNT in water samples. The latter is ascribable to our experience with analysis of groundwater samples, where TNT concentrations are generally well below 1 mg/L, even in areas subject to substantial pollution by explosive components. For soil, however, levels of TNT vary greatly from sub-microgram-per-gram to percent levels. The criterion level for cleanup at

Cornhusker Army Ammunition Plant was set at 5 µg/g (Rosenblatt 1986), a level expected to be mid-range for the standard bore tubes.

EXPERIMENTAL

Indicator tubes and syringe pump

Standard and small bore indicator tubes were obtained by special order from Supelco, Incorporated (Bellefonte, Pennsylvania). The presections of both tubes were packed with glass beads coated with a mixture of magnesium, calcium and barium oxide. The indicator sections of the tubes contain BioRad AGMP-1 anion exchange resin. The standard bore tubes have a 4-mm id, are 10 cm in length and are packed with 50–100 mesh material. The small bore tubes have a 1.6-mm id, are 10 cm in length and are packed with 100–200 mesh material. Flow rate of sample through the indicator tubes was controlled by a Sage Instrument Syringe Pump (Model 341 B).

Analytical standards

All analytical standards for TNT were prepared from Standard Analytical Reference Materials (SARMs) obtained from the U.S. Army Toxic and Hazardous Materials Agency (USATHAMA), Aberdeen Proving Ground, Maryland. The method of preparation is described elsewhere (Jenkins et al. 1988b).

Aqueous test solutions

Aqueous test solutions of TNT were prepared from recrystallized, reagent grade material from Eastman Chemical. Stock standards were prepared by weighing out the solid material, dispensing it into type I reagent grade water prepared from a Milli-Q system (Millipore Corporation), stirring overnight and filtering through a Whatman glass fiber filter. Individual stock solutions of TNB, 2,4-DNT and 2,6-DNT were prepared in an identical manner from reagent grade material from Eastman Chemical. Individual stocks of tetryl, HMX and RDX and a combined solution of TNT and tetryl were prepared from military grade material, the latter from Tetratol manufactured in 1945.

Test solutions were prepared from these stock standards by volumetric dilution in groundwater obtained from a well in Etna, New Hampshire. The pH and specific conductance of the well water were 5.94 and 360 µmhos/cm respectively.

Methanol test solutions

Test solutions of TNT in methanol were prepared from SARM material as described elsewhere (Jenkins et al. 1988b). Test solutions of 2-Am-DNT and 4-Am-

DNT were prepared from materials obtained from Dr. David Kaplan, Natick Laboratories, Natick, Massachusetts. Identity of these materials was confirmed by GC/MS analysis.

Soils

All soil extraction tests were conducted with field-contaminated soils obtained from the following areas: Chickasaw Ordnance Works, Millington, Tennessee; Raritan Arsenal, Edison, New Jersey; Sangamon Ordnance Plant, Illiopolis, Illinois; and Weldon Springs Training Area, Weldon Springs, Missouri. To simulate field conditions, previously dried soils were rewetted with water (1.0 mL to 2.0 g) prior to extraction.

Instrumental analysis

Test solutions and soil extracts were analyzed by reversed-phase HPLC (High Performance Liquid Chromatography) to establish TNT concentrations. All determinations were obtained on a modular system composed of a Spectra-Physics SP 8810 pump with a Rheodyne 7125 100-µL sample loop injector, and a Spectra-Physics SP 8490 variable wavelength UV detector set at 254 nm. Peak height measurements were made on a Hewlett Packard HP 3393A digital integrator.

Separations were achieved on a 25-cm by 4.6-mm (5-µm) LC-18 column (Supelco) eluted with 1.5 mL/min of 1:1 methanol/water. Details of the methods for water and soil extracts are presented in Jenkins et al. (1988a) and Bauer et al. (1989) respectively.

RESULTS AND DISCUSSION

Soil extraction method

The soil extraction method recommended by Erickson et al. (1984) for field use involves the addition of 6 g of soil to 20 mL of methanol, manually shaking for 1 minute and filtering immediately. They report an extraction efficiency for TNT ranging from 87 to 105%. These estimates, however, were based on extraction of soils spiked with TNT in the laboratory. Jenkins et al. (1989) have demonstrated that the rate of extraction of TNT from spiked soil is much more rapid than for field contaminated soil. For this reason we conducted an experiment to determine the extraction efficiency of the method described above using field contaminated soils. For comparison, the soils were also extracted with a more exhaustive method developed in our laboratory (Jenkins and Walsh 1987, Jenkins et al. 1988b, Bauer et al. 1989).

Nine 2-g portions of four air-dried soils, which were known to be contaminated with varying amounts of

TNT, were weighed into glass scintillation vials. The vials were randomly divided into three sets of three vials each. A 1-mL aliquot of Milli-Q water was added to each vial in two of these sets of three and these soils were allowed to equilibrate for 1 hour. To one set of the wetted soils, we added 7.0 mL of methanol, manually shook the vials for 1 minute and gravity filtered the extract through Whatman Phase Separating Paper. The rate of filtration varied from soil to soil and took as long as 30 minutes. A 3-mL aliquot of each filtrate was mixed with 3.00 mL of aqueous CaCl_2 (5 g/L) to cause flocculation of suspended clays, and the samples were refiltered through a Millex SR membrane filter to protect the HPLC column (Jenkins et al. 1988b).

The second set of wetted soils was extracted in an identical manner as described above, except isopropanol rather than methanol was used as the extraction solvent. Isopropanol was chosen for comparison because of its lower volatility and flammability, desirable characteristics for solvents transported to the field.

The third set of three replicates of each soil was extracted as described in Bauer et al. (1989). A 10.0-mL aliquot of acetonitrile was added to each vial, the vials vortex mixed for 1 minute and equilibrated in a sonic bath for 18 hours. The sonic bath was maintained below room temperature throughout this period. The samples were then allowed to settle for 90 minutes and 5.00-mL aliquots of the supernatant were added to 5.00 mL of aqueous CaCl_2 . The vials were allowed to stand for 30 minutes and filtered through disposable Millex SR filters.

All soil extracts were analyzed as described above. To account for differences in peak widths and absorptivities when different types of matrices were analyzed by RP-HPLC, analytical standards were prepared in methanol, isopropanol and acetonitrile. Peak heights for each sample were compared with peak heights obtained for analytical standards prepared in the same solvent as used for extraction.

Mean TNT concentrations obtained for three soils are presented in Table 1 along with the standard deviations among replicates. Results obtained for the Chickasaw soil were too low and variable to allow comparison. For the other three soils, the concentrations of TNT for the methanol extraction procedure were significantly less than those obtained using the acetonitrile-sonic bath method when means were compared using ANOVA at the 95% confidence level. Recoveries in methanol ranged from 58–70% of those obtained using the acetonitrile-sonic bath method, with percent recovery decreasing with decreasing TNT concentration. Concentrations obtained using isopropanol as the extractant were also significantly lower than the acetonitrile-sonic bath procedure and were numerically lower than methanol for each soil tested. The differences between isopropanol and methanol, however, were not significantly different at the 95% confidence level.

The reduced extraction efficiency found for the field method using methanol and isopropanol is undoubtedly attributable to the very short extraction times that must be used in the field. This result is consistent with slow desorption kinetics for this analyte from soil as reported

Table 1. Comparison of TNT concentration ($\mu\text{g/g}$) determined by the field soil extraction method to that determined by the laboratory procedure.

<i>Solvent extraction</i>	<i>Method of equilibration</i>	\bar{X}	S	% Recovery*
<i>Raritan Soil</i>				
Methanol	Shaking, 1 minute	0.087	0.013	58
Isopropanol	Shaking, 1 minute	0.063	0.004	42
Acetonitrile	Sonic bath, 18 hours	0.15†	0.012	100
<i>Weldon Springs Soil</i>				
Methanol	Shaking, 1 minute	145	5.3	70
Isopropanol	Shaking, 1 minute	139	2.2	67
Acetonitrile	Sonic bath, 18 hours	207†	2.3	100
<i>Sangamon Soil</i>				
Methanol	Shaking, 1 minute	16.9	1.29	67
Isopropanol	Shaking, 1 minute	15.4	0.64	61
Acetonitrile	Sonic bath, 18 hours	25.4**	1.41	100

*Relative to laboratory extraction procedure.

†Determined by RP-HPLC analysis.

**Means are significantly different at the 95% confidence level according to ANOVA and least significant difference calculations.

Table 2. Syringe pump settings and corresponding sample flow rates through indicator tubes.

Syringe pump settings	Flow rate (mL/min)	
	5-mL syringe	10-mL syringe
7	0.7	1.0
8	1.0	1.5
9	1.7	2.7

elsewhere (Bauer et al. 1989), and will tend to give the lowest recoveries for soils with low TNT content where the largest proportion of the material is associated with high energy binding sites (Leggett 1985). Thus, the higher recoveries reported by Erickson et al. (1984) will overestimate actual recoveries for field contaminated soils. If comparisons are made to an action criterion, a safety factor is necessary to account for incomplete recovery using the field extraction method.

Initial testing of indicator tubes

Initial testing of the indicator tubes was conducted to gain some general experience with the technique. A syringe pump was used to pump samples through the indicator tubes at a constant rate. The syringe pump settings and corresponding flow rates are presented in Table 2.

A series of solutions of TNT were prepared in both reagent grade water (Milli-Q) and well water covering a concentration range of 0.1 to 10.0 µg/L. A 10-mL volume of each of these solutions was pumped through a standard bore indicator tube at 2.7 mL/min and the stain characteristics (color and stain length) determined independently by four analysts. Erickson et al. (1984) indicate that a "red" colored stain is found from reaction of TNT with the indicator tube. We found that identification is very subjective and differed significantly from individual to individual, particularly as color intensity varies.

The following observations were made. All solutions produced stains that were detectable by all four analysts (0.1 to 10.0 µg/L). Stains were not of uniform length around the circumference of the tube, making it difficult to reproducibly measure stain length. The stain colors were described as pink, rose or peach for low concentration samples and dark red, maroon, orange or plum for the highest concentration samples.

Stains were found to fade rapidly (over a few hours) even when the tubes were maintained in the dark. This instability makes it desirable to make stain length measurements as soon as the tube is removed from the pump. It also requires that calibration be conducted on the same day (perhaps even the same half-day) as sample analysis.

It also appears that stain lengths are much greater for TNT solutions in well water compared to equal concentrations of TNT in reagent grade water (Table 3).

To further pursue whether stain length depends on

Table 3. Stain lengths for TNT in reagent grade water and well water.

Water matrix	TNT concentration (mg/L)	Stain length (mm)					
		Analyst				\bar{X}	S
A	B	C	D				
Reagent grade	0.10	1.5	7	8	5	5.4	2.9
Well water	0.46	14.5	8.5	11	8	10.5	3.0
Reagent grade	0.51	8.5	13	13	12	11.6	2.1
Reagent grade	1.02	10	19	7	14	12.5	5.2
Reagent grade	2.05	13	19.5	16.5	16.5	16.4	2.7
Well water	2.05	40	38.5	33	30	35.4	4.7
Well water	3.58	35.5	34	35	32.5	34.3	1.3
Reagent grade	5.12	17	26	19	18	20.0	4.1
Well water	5.63	—	32	30.5	31	31.2	0.8
Well water	8.19	48.5	49	48	50	48.9	0.8
Reagent grade	10.23	14.5	18.5	12	14	14.8	2.7

Table 4. Stain lengths for 2.05-mg/L TNT solutions in well water, reagent grade water and methanol.

Matrix	Stain length (mm)					
	Analyst				\bar{X}	S
	A	B	C	D		
Well water	35	32.5	32	32.5	33	2.4
Reagent grade water	14	13	15.5	13	14	1.2
Methanol	28	30	45	20	31	10.4

sample matrix, three 2.05-mg/L TNT test solutions were prepared—one in reagent grade water, one in well water and one in methanol. A 10-mL aliquot of each solution was pumped through standard bore indicator tubes at 2.7 mL/min. Four analysts independently measured stain lengths (Table 4). The results confirmed the earlier test that stain length is matrix-dependent. The mean stain length for well water averaged 33 mm, while that for methanol averaged 31 mm and the length for reagent grade water was only 14 mm. While the mean values appear similar for methanol and well water, this may be caused by one high reading for methanol, which increased the mean substantially.

We conducted another test with the small bore indicator tubes to determine if they were subject to the same type of matrix effect. For this test five 0.20-mg/L TNT solutions were prepared in 1) reagent grade water, 2) well water, 3) well water diluted 1:1 with reagent grade water, 4) well water amended with 0.005 M KCl and 5) methanol. A 10.0-mL aliquot of each was pumped through the small bore tube at 1.0 mL/min. The stain lengths obtained along with pH and conductivity for the water matrices are presented in Table 5.

Table 5. Stain lengths for various matrices for 0.20-mg/L TNT solutions pumped through small bore indicator tubes.

Solution matrix	pH	Specific conductance ($\mu\text{mhos/cm}$)	Stain length (mm)
Reagent grade water	6.7	—	12.5
1:1 well water-reagent grade water	6.0	171	15.5
Well water	6.1	337	17
Well water + KCl	5.8	1079	20
Methanol	—	—	15.5

While the matrix effect appears to be somewhat reduced for the small bore tubes, the matrix does seem to affect stain lengths. The major implication of these matrix tests is that calibration standards must be prepared in the same matrix as test samples. For soil extracts this appears to be a minor problem, since standards and samples can both be run in methanol. For water samples, though, this implies that the normal practice of preparing water standards in reagent grade water is inappropriate. Stock standards should be diluted in a water matrix with a specific conductance near to that for the water being tested. If an uncontaminated source from the same groundwater aquifer being tested is available, this would be the best matrix for preparation of standards.

Flow rate test

Heller et al. (1982) indicated that flow rates ranging between 0.74 and 1.5 mL/min produced stains of approximately the same length. At higher flow rates, the stain length increased and the end point became less distinct. At a very slow flow rate (~ 0.08 mL/min), a very intense band was produced but stain lengths were too short to allow for calibration.

Erickson et al. (1984) recommended flow rates of 2.7 and 1.0 mL/min for the standard bore and small bore tubes respectively. Mason-Hangar (1989) used a flow rate of 1.0 mL/min with the standard bore tubes for field analysis of soil extracts.

To assess the effect of flow rate, we prepared two 1.0-mg/L TNT solutions—one in methanol and the other in isopropanol. Three 10-mL aliquots of each solution were pumped through individual standard bore indicator tubes at flow rates ranging from 1.0 to 2.7 mL/min. Stain lengths were measured independently by two analysts (Table 6). The results indicated that stain lengths were relatively independent of flow rate in the flow rate range examined (1.0–2.7 mL/min). Since sample volumes are generally 5 or 10 mL, total pumping time even for the slowest flow rate is only 10 minutes

Table 6. Comparison of stain lengths produced by various flow rates for solution containing 1.05 mg/L of TNT.

Solution matrix	Flow rate (mL/min)	Stain length (mm)		
		Analyst		\bar{X}
		A	B	
Methanol	1.0	8	8	8
	1.5	6	7	6.5
	2.7	6.5	7	6.8
Isopropanol	1.0	8	8	8
	1.5	7.5	8.5	8
	2.7	6.5	7.5	7

per sample. We selected flow rates of 1.5 and 1.0 mL/min for the standard bore and small bore tubes, respectively, for all further tests.

Stain length calibration

The relationship between TNT concentration and stain length forms the basis for quantitation using the indicator tubes. Heller et al. (1982) indicate that stain length varies nonlinearly with TNT concentration. Erickson et al. (1984) confirm the nonlinear relationship, indicating that the relationship is more closely approximated by a logarithmic function (stain length versus the logarithm of the TNT concentration) but that

this empirical function does not make the relationship completely linear.

Erickson et al. (1984) report that stains are dark near the inlet side of the ion exchange resin and become lighter as they approach the outlet size, an observation we can confirm from our study. This progressive decrease in stain intensity toward the outlet end results in stain length measurements that differ substantially from operator to operator as a function of their individual ability to detect a light pink stain on a beige background. This problem is further compounded by the nonuniformity of stain length around the circumference of these commercial indicator tubes.

To estimate concentrations for an unknown sample, the stain length obtained must be converted to a concentration using a calibration curve. An experiment was conducted to gain experience with the shape of these calibration curves and to assess how different these calibration curves are from operator to operator. A series of six TNT calibration standards was prepared in methanol covering a concentration range of 0.15 to 30.0 mg/L. A 10-mL aliquot of each standard was pumped through individual standard bore indicator tubes at a flow rate of 1.5 mL/min on two different days. Stain length measurements were made by five different operators on the first day and four different operators on the second day. To partially eliminate the problem associated with uneven stain penetration, the operators were asked to report an average stain length around the tube's circumference. The results of these stain measurements are presented in Table 7.

Table 7. Stain length measurements for TNT calibration standards.

TNT concentration (mg/L)	Stain length (mm)								
	Analyst					\bar{X}	S	\bar{X}^2	\bar{X}^3
	A	B	C	D	E				
<i>Day 1</i>									
0.15	3.5	2	4	0	4	2.7	1.72	7.3	20
0.60	7	4	10	12.5	9	8.5	3.20	72	614
1.5	16.5	5	12.5	11.5	13.5	11.8	4.24	139	1,643
3.0	24	13	17.5	21	15	18.1	4.45	328	5,930
7.5	34	20	17.5	32	28.5	26.4	7.31	697	18,400
30.0	34	31	30	31	32.5	31.7	1.57	1,005	31,855
<i>Day 2</i>									
0.15	2.5	4	9	3	—	4.6	2.98	21	97
0.60	14	10	14	9.5	—	11.9	2.46	142	1,685
1.5	16.5	12.5	27	11	—	16.8	7.22	282	4,742
3.0	31	20	37	19	—	26.8	8.73	718	19,249
7.5	29	17.5	25	23.5	—	23.8	4.77	566	13,481
30.0	45	40	42	37.5	—	41.1	3.17	1,689	69,427

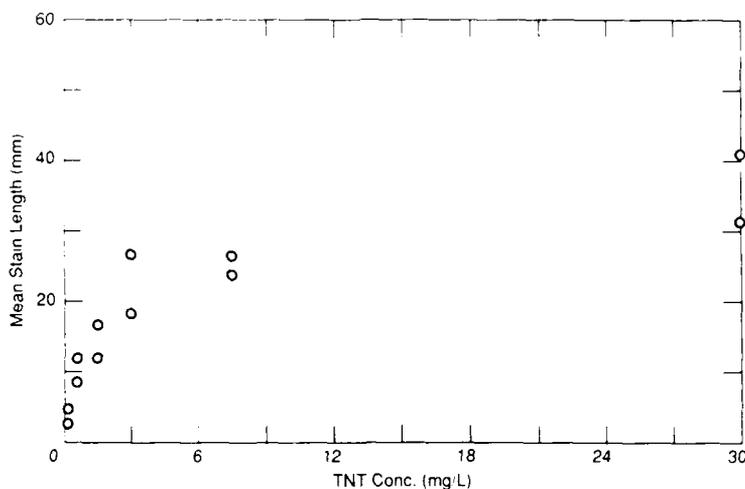


Figure 1. Relationship between mean stain length and TNT concentration.

Clearly, stain length measurements vary significantly from operator to operator for a given tube and for the same analyst for tubes tested on different days. Figure 1 is a plot of the mean stain lengths obtained as a function of TNT concentration. As reported (Heller et al. 1982, Erickson et al. 1984) the relationship between stain length and log TNT concentration (Fig. 2) appears to improve the relationship but some residual curvature remains. A number of other functions were tested to make the relationship linear. The most linear relationship we obtained appears to be mean stain length cubed versus TNT concentration (Fig. 3). Ten of the twelve data points appear to fit this relationship but two values

fall well off the regression line. The regression line shown in Figure 3 was calculated using only the 10 points that visibly fit this relationship.

Calibration curves for individual analysts are shown in Figure 4: Figure 4a presents the relationship of stain length versus TNT concentration and Figure 4b is stain length cubed versus TNT concentration. Clearly, individual calibration curves vary substantially from analyst to analyst. Thus, it is important that the same analyst make all stain length measurements for both standards and unknown samples.

A very important question remains: what is the accuracy and precision of TNT concentration for soil

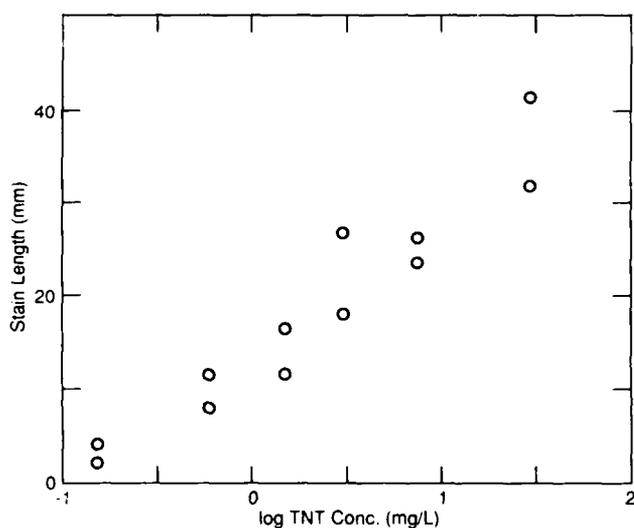
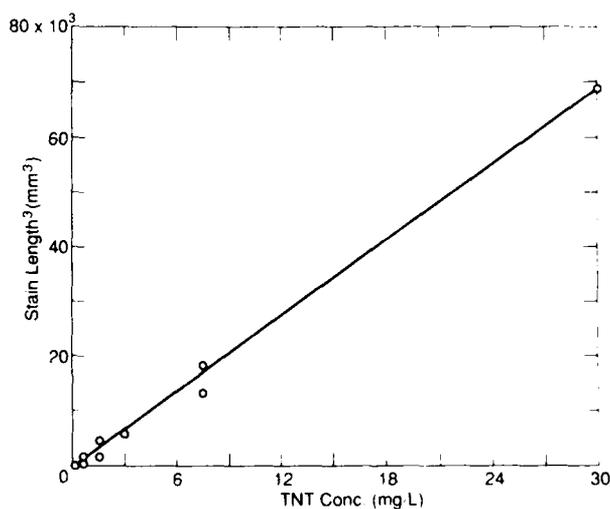
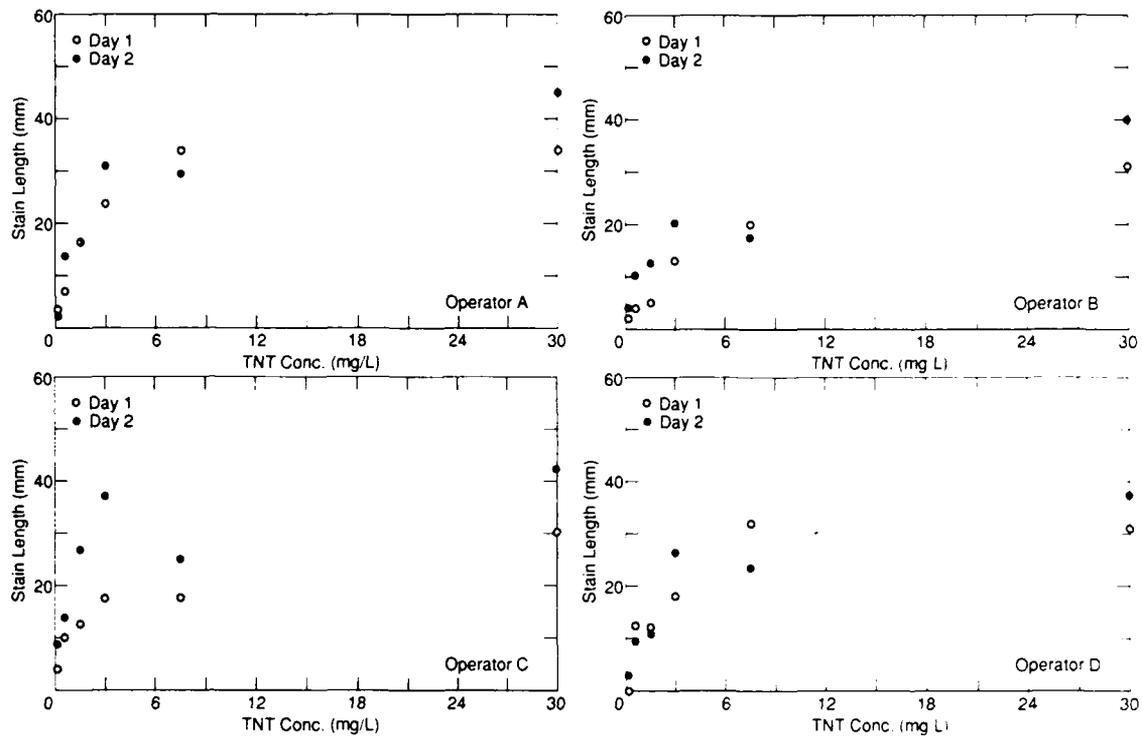
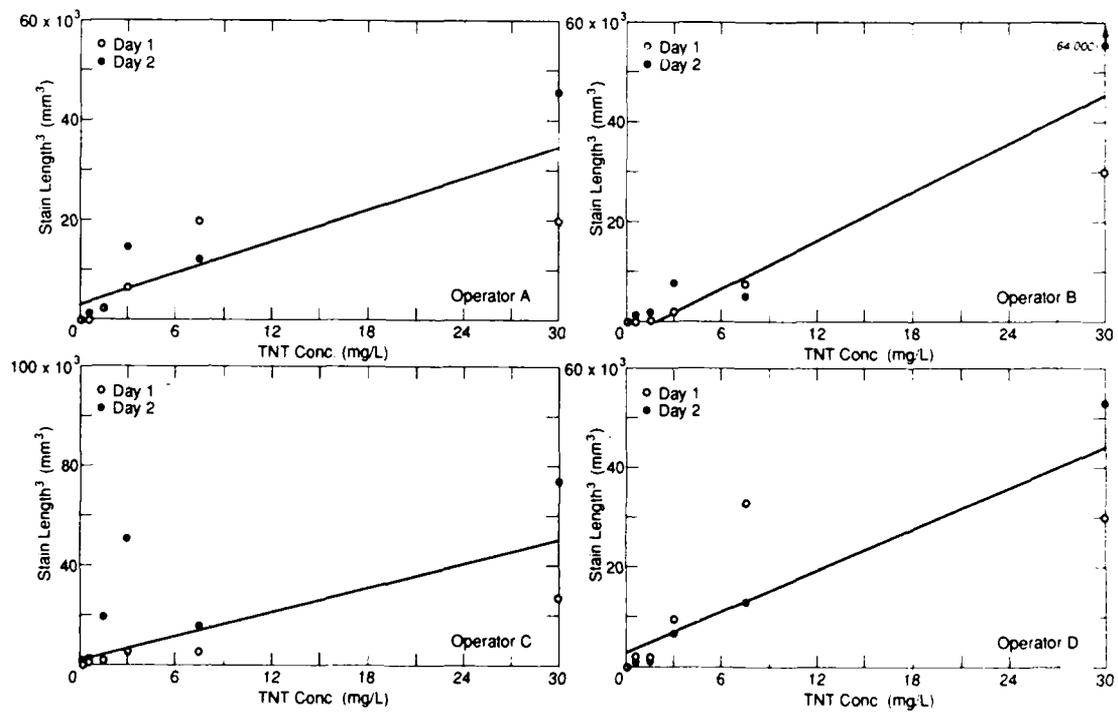


Figure 2. Relationship between mean stain length and logarithm of TNT concentration.





a. Stain length versus TNT concentration.



b. Stain length cubed versus TNT concentration.

Figure 4. Calibration relationships for individual analysts.

extracts estimated from stain length measurements? To answer this, a large-volume methanol extract of field contaminated soil from Weldon Springs, Missouri, was obtained. The TNT concentration in the extract was determined by RP-HPLC (Bauer et al. 1989) to be 2.54 ± 0.03 mg/L (Table 8). Two 10-mL aliquots were pumped through indicator tubes on each of two days and compared to calibration curves obtained on the same day (Fig. 4). The stain lengths were measured by five different analysts and concentrations were estimated from the calibration curves obtained by the same analyst. Results were obtained both using the direct stain length versus TNT concentration relationships shown in Figure 4a and the stain length cubed versus concentration regression equations shown in Figure 4b. Results are presented in Table 8.

TNT concentration estimates obtained from stain length measurements on the indicator tubes ranged from 0.0 to 15 mg/L, with mean values of 4.9 ± 2.7 or 5.1 ± 3.5 mg/L, depending on the type of calibration curve used. Thus, TNT concentration estimates for a real soil extract were about twice those obtained by RP-HPLC determination. Initially, we thought that other compounds were probably present in the soil extract that also reacted with base to form colored anions that competed for exchange sites on the anion exchange resin, thereby affecting stain length measurements. RP-HPLC analysis of the extract did indicate that 1,3,5-

trinitrobenzene (TNB) was present at about one-tenth the TNT concentration and that traces of 2- and 4-aminodinitrotoluene (2-AmDNT and 4-AmDNT) were also present. It is possible that formation of Meisenheimer anions for these substances or others compounds not observed by RP-HPLC analysis were responsible for the inaccurate TNT results.

To test this hypothesis, 15-mL aliquots of the soil extract and calibration standards for TNT, TNB, tetryl, 2-AmDNT and 4-AmDNT were placed in individual scintillation vials. A pellet of potassium hydroxide (KOH) was added and the vials vortex mixed to dissolve the KOH. Highly colored solutions resulted from TNT (purple), TNB (orange) and tetryl (yellow), but colorless solutions resulted from the two AmDNTs. Measurements of molar absorptivities from 400–600 nm for the colored anions from TNT, TNB and tetryl were obtained (Table 9, Fig. 5). The visible spectrum for the soil extract was also obtained over the same wavelength region (Table 9). The λ_{\max} and overall shape of the visible spectrum matches that of TNT.

An estimate of TNT concentration in the soil extract from measurements of the absorbance at 510 nm indicated a concentration of 3.1 mg/L. This value is slightly higher than the 2.54-mg/L value estimated by RP-HPLC but is reasonable, considering the presence of TNB in the extract as well. The 3.1-mg/L estimate is much lower than the 4.9- and 5.1-mg/L estimates ob-

Table 8. Concentration (mg/L) estimates for extract of field contaminated soil from Weldon Springs.

Replicate	RP-HPLC analysis	Analyst					\bar{X}	S
		A	B	C	D	E		
<i>Calculated from (length)³ vs concentration regression equation</i>								
1	2.49	3.8	2.9	0.0	3.8	5.9		
2	2.53	5.1	10.7	2.1	6.9	9.1		
3	2.54	3.8	4.1	7.4	2.6	—		
4	2.56	5.6	4.1	7.4	3.0	—		
\bar{X}	2.54	4.6	5.5	4.2	4.1	7.5	4.9	2.65
S	0.03	0.9	3.6	3.8	2.0	2.3		
<i>Obtained from plot of stain length vs concentration</i>								
1	2.49	2.7	4.1	0.9	3.3	4.1		
2	2.53	3.3	15.0	4.7	4.8	5.1		
3	2.54	2.7	6.0	10.2	2.4	—		
4	2.56	3.5	6.0	10.2	3.0	—		
\bar{X}	2.54	3.1	7.8	6.5	3.4	4.6	5.1	3.46
S	0.03	0.4	4.9	4.5	1.0	0.7		

Table 9. Molar absorptivity measurements.

Wavelength (nm)	Molar absorptivity ($\times 10^4$)			Absorbance: Weldon Springs soil extract
	TNT	TNB	Tetryl	
400	0.590	1.01	0.99	0.141
410	0.550	1.40	1.26	0.142
420	0.560	1.75	1.35	0.149
430	0.595	1.80	1.20	0.150
440	0.650	1.80	1.00	0.150
450	0.711	1.75	0.91	0.146
460	0.776	1.60	0.90	0.147
470	0.850	1.50	0.95	0.150
480	0.930	1.50	0.99	0.159
490	0.99	1.47	1.00	0.168
500	1.02	1.45	0.97	0.172
510	1.03	1.40	0.84	0.177
520	0.99	1.30	0.64	0.170
530	0.94	1.10	0.40	0.156
540	0.85	0.765	0.200	0.138
550	0.755	0.459	0.088	0.119
560	0.649	0.230	0.033	0.099
570	0.545	0.099	0.012	0.083
580	0.479	0.044	0.006	0.077
590	0.441	0.021	0.002	0.070
600	0.420	0.012	0.001	0.069

tained from stain length measurements on the indicator tubes. Thus, some other factor must be responsible for these high results.

The most likely reason for high estimated TNT concentrations in the soil extract from stain length measure-

ments is the presence of other compounds that may not form a colored anion but nevertheless compete with the Meisenheimer anions for anion exchange sites. This would result in longer stains relative to the standard for equal TNT concentrations. Since these substances are likely to be present in variable concentrations from soil to soil, percent inaccuracy will likely be different in each case.

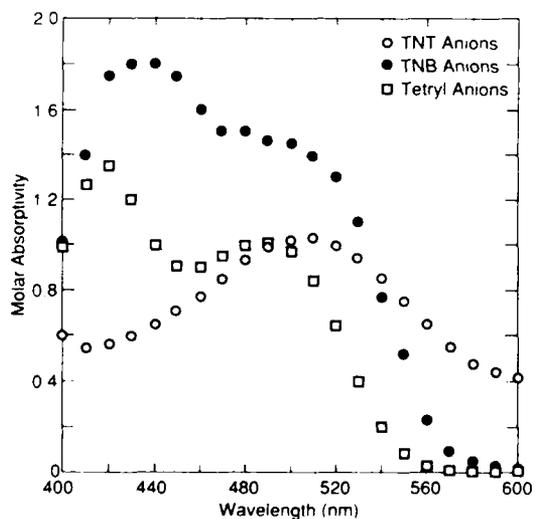


Figure 5. Molar absorptivities for the colored anions from TNT, TNB and tetryl.

Interferences

Erickson et al. (1984) report that red stains that could interfere with TNT estimation are formed for several other compounds. These include N-methyl-N,2,4,6-tetranitroaniline, 2,4,6-trinitrobenzoic acid and 2,4-dinitroaniline. We suspected that TNB would also form a colored Meisenheimer anion under these conditions as well. Standards of several of these compounds and other common components of explosive residues were prepared and pumped through individual standard bore indicator tubes. Results of this test are presented in Table 10.

In addition to TNT, colored stains were observed for TNB and tetryl. No stains were observed for the other compounds tested. The color of the stain produced for TNT was visibly quite different from those for TNB and tetryl. The TNT stain was purplish, while those for TNB and tetryl were orange. While it would be easy to

Table 10. Results of interference tests.

<i>Compound</i>	<i>Color of stain observed</i>
TNT	Purple*
HMX	None
RDX	None
TNB	Orange
tetryl	Orange
2,4-DNT	None
2,6-DNT	None
2-AmDNT	None
4-AmDNT	None
Tetrato†	Brick red

* Turns brown upon standing

† Combination of tetryl (80%) and TNT (20%)

differentiate TNT from the other two compounds based on the color of the stain if the compounds were present alone, this would be unusual for explosive residues. Tetryl was generally used with TNT in an explosive called Tetrato (80% tetryl and 20% TNT). When a standard made from Tetrato was pumped through an indicator tube, the resulting stain was brick red. This color was sufficiently different to be recognizable compared to a stain from TNT alone. TNB is present in these residues as a result of photodegradation of TNT. It is generally present at much lower concentrations than TNT. A stain observed from a soil extract with a concentration ratio of TNT/TNB of 10/1, however, was indistinguishable from a stain from TNT alone. Anions formed from TNB or tetryl should compete with TNT anions for exchange sites on the anion exchange resin and thus affect stain length measurements.

Thus, while some interference from other compounds is likely for many residues, it shouldn't affect the ability to qualitatively detect TNT. It will, however, affect stain length and thereby influence quantitative results.

Use of indicator tubes for direct intensity comparisons

Clearly, the use of the indicator tubes for concentration estimation via stain length measurement is questionable. This is primarily attributable to the inability to reproduce stain length measurements (poor precision) and to the influence of other matrix components on the mobility of the stain along the anion exchange resin (poor accuracy). Another approach is to directly compare stain intensity (rather than stain length) for an unknown sample to stain intensity for a standard solution (Mason-Hangar 1989). This approach is particu-

larly useful for soil samples at a specific location where a criterion level has been established (Rosenblatt 1986) and measurements are made to determine if concentrations exceed this action level.

To assess whether concentrations above an action level can be identified, we conducted the following experiment. TNT standard solutions were prepared in methanol at 1.25, 2.5 and 5 mg/L. These concentrations are equivalent to soil extracts from soils containing 2.5, 5.0 and 7.5 $\mu\text{g/g}$ TNT if 2-g soil samples are extracted with 10 mL of methanol. Five 5.0-mL aliquots of each solution were pumped through individual standard bore indicator tubes at 1.0 mL/min. The resulting tubes were randomly numbered, so that analysts comparing stains would not know which tube was associated with which solution. Each indicator tube corresponding to 2.5 or 7.5 $\mu\text{g/g}$ was compared directly to an indicator tube from a 5.0- $\mu\text{g/g}$ solution. Each analyst was asked to select the indicator tube with the greatest stain intensity in each pair. Seven different analysts made all ten comparisons. Five analysts made their comparisons under laboratory fluorescent lighting and two made their comparisons outside under natural light. The results are shown in Table 11. All analysts were able to correctly decide that the stains corresponding to 2.5- $\mu\text{g/g}$ soil extracts were less intense than those from the 5.0- $\mu\text{g/g}$ extracts in all cases. In 34 of 35 comparisons, the analysts were also able to correctly indicate that the indicator tubes corresponding to 7.5- $\mu\text{g/g}$ extracts had a greater stain intensity than those from the 5.0- $\mu\text{g/g}$ tubes. Thus, it appears that the tubes are capable of discriminating between TNT concentrations above or below an action criterion of 5 $\mu\text{g/g}$ if the differences are $\pm 2.5 \mu\text{g/g}$.

Table 11. Comparison of stain intensities for indicator tubes from 2.5-, 5.0- and 7.5- $\mu\text{g/g}$ TNT solutions.

<i>Analyst</i>	<i>Lighting conditions</i>	<i>Correct choices/total choices</i>	
		<i>2.5 vs 5.0 $\mu\text{g/g}$</i>	<i>5.0 vs 7.5 $\mu\text{g/g}$</i>
A	Fluorescent	5/5	5/5
B	Fluorescent	5/5	5/5
C	Fluorescent	5/5	5/5
D	Fluorescent	5/5	5/5
E	Fluorescent	5/5	5/5
F	Natural	5/5	4/5
G	Natural	5/5	5/5
Total		35/35	34/35

CONCLUSIONS AND RECOMMENDATIONS

In general, the results of the evaluation were mixed. The indicator tubes were found to be very effective at detecting the presence of TNT at concentrations as low as 40 µg/L in water samples and 0.5 µg/g in soil samples. These results were obtained using the small bore indicator tubes (1.6-mm id) for water samples and the standard bore indicator tubes (4-mm id) for soil extracts. On the other hand, the ability to precisely and accurately estimate TNT concentration in water or soil extracts is poor.

We feel that the indicator tubes are quite usable under field conditions. The syringe pump, which is necessary to obtain a constant flow rate through the tubes, does require ac power, so operation at present is not totally portable. The poorest aspect of the detection kit, with respect to field usability, is the soil extraction-sample filtration procedure. Some additional thought should be given to configuring an extraction-filtration module for field use.

The soil extraction procedure recommended for use with the indicator tubes was given an independent evaluation using field contaminated soils. The results indicated that TNT was not completely extracted using a 1-minute shaking period in methanol. This would result in an underestimation of the concentration of TNT in soil. Percent extracted was found to be a function of the concentration of TNT in the soil. Recoveries ranged from 58% for a low concentration soil to 70% for a high level soil, compared to a standard laboratory extraction method.

The indicator tubes were also assessed for their ability to discriminate between concentrations above or below an action level using stain intensity as the criterion. In this regard, analysts were able to correctly decide that indicator tubes corresponding to a TNT concentration of 2.5 µg/g were below an action criterion of 5.0 µg/g, while those at 7.5 µg/g were above the action level of 5.0 µg/g. In our opinion it would be difficult to reproducibly discriminate concentrations much closer to the action level.

The indicator tubes were originally developed for detection of low levels of TNT in water. The concentrating effect of the anion exchange resin is quite good at reducing detection levels well below what could be accomplished by direct colorimetric analysis. For soils, however, action criteria for soil cleanup will vary from location to location but probably will be above 1 µg/g in most instances. Based on the molar absorptivity of the Meisenheimer anion for TNT at its λ_{\max} (510 nm), TNT could be easily detected and precisely quantified at concentrations below 1 µg/g using a direct colorimetric procedure. This approach is attractive for two reasons.

First, field portable, battery operated colorimeters are available and their cost is about the same as the price of the syringe pump used to pump samples through the indicator tube. Second, much more precise and accurate TNT concentration estimations would be possible using direct colorimetry because of more reproducible calibration. We recommend a thorough assessment be given to developing a simple colorimetric procedure for this application.

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