A Field Method for Quantifying Ammonium Picrate and Picric Acid in Soil

Philip G. Thorne, Thomas F. Jenkins
U.S. Army Cold Regions Research and Engineering Laboratory, 72 Lyme Road, Hanover, NH 03755

Received 20 September 1996; revised 21 October 1996; accepted 24 October 1996

Abstract: A simple field method for the determination of ammonium picrate and picric acid in soil was developed. Picric acid is a strong acid with a $pKa = 0.80$, and is colorless when dissolved in an organic solvent, whereas its anion (picrate) is bright yellow. Picric acid and picrate ions were extracted from undried soil by shaking with acetone; any picric acid extracted was rapidly converted to picrate in the wet acetone. Picrate was extracted from the acetone soil extracts by passing the solutions through a solid-phase anion exchanger to remove interferences. Acidified acetone was used to convert the picrate to picric acid and elute it from the ion exchanger. The absorbance of the solution at 400 nm was measured; then the picric acid was converted to the colored picrate ion by diluting the eluent with water. Absorbance at 400 nm was measured again and the concentration of picrate was obtained from the difference in the absorbance measurements, corrected for dilution. The method detection limit is 1.3 $\mu g\ g^{-1}$ of soil. Field-contaminated soils were assayed, and the results compared favorably to those from HPLC analyses in the range of 10–4400 $\mu g\ g^{-1}$. The method is simple to use, can be implemented under field conditions, and complements on-site methods for TNT, RDX, and 2,4-DNT.

Field Analyt Chem Technol 1: 165–170, 1997.

Keywords: picric acid; ammonium picrate; explosives; field screening

Introduction

Ammonium picrate (ammonium 2,4,6-trinitrophenoxide, Explosive D, Yellow D) was used in armor-piercing shells, bombs and rocket warheads by the U.S. military from the turn of the century until after World War II. Although it is no longer manufactured, ammonium picrate is now 8% of the demilitarization inventory. Picric acid (2,4,6-trinitrophenol) was used in grenades and mine fillings.[1] Unlike many of the other high explosives that are no longer manufactured and present environmental clean-up problems unique to the military, picric acid is a common industrial chemical, used widely for metal etching and as feedstock in many processes in the dye, leather, and glass industries.[2] Picrate is also an environmental transformation product of tetryl (trinitro-2,4,6-phenylmethylnitramine), another obsolete military explosive.[3] Picrate was detected in a leachate from soil columns spiked with tetryl[4] and was detected as a transformation product of tetryl in water.[3,5]

The production, toxicology, and environmental fate of ammonium picrate and its parent compound, picric acid, have been reported.[6] Most of the toxicological work reported has been on skin adsorption and inhalation of ammonium picrate dust.[6] The most recent research[2] deals with lethal-dose determinations. The EPA has not set an action level for ammonium picrate or picric acid in soil or water. An allowable daily intake (ADI) of 1–37 μg (kg-day)$^{-1}$ has been suggested.[6] Because the estimated ADI is similar to those of other secondary explosives, similar field detection limits were sought in this research (i.e., low $\mu g\ g^{-1}$ in soil).

There is potential for picric acid to be transformed to picramic acid (2-amino-4,6-dinitrophenol) by adapted bacteria under the anaerobic conditions that are found at some waste sites. This compound has 10 times the mutagenicity of picric acid,[2] and its toxic effects on aquatic organisms are also greater than that of picric acid.[7] Picramic acid is also a mammalian metabolic transformation product of picric acid[2,8] and is excreted in urine. It would be introduced into the environment if picric acid were ingested by grazing animals.

When dissolved in water, both ammonium picrate and picric acid dissociate to the picrate ion. Aqueous solubilities of both compounds are over 10 g L^{-1}, and both compounds appear to be extremely mobile environmental contaminants. Partitioning of picrate from estuarine water to organic sediment is very low.[7] This follows from the low octanol-water partition coefficient of picric acid ($\log K_{ow} = 1.6$).[6]

This publication reflects the personal views of the authors and does not suggest or reflect the policy, practices, programs, or doctrine of the U.S. Army or government of the United States. The contents of this report are not to be used for advertising or promotional purposes. Citation of brand names does not constitute an official endorsement or approval of the use of such commercial products.

Correspondence to: P. G. Thorne

Contract grant sponsor: U.S. Environmental Center, Aberdeen Proving Ground, MD.

On the other hand, it has been predicted that picrate will act like phenolic pesticides and become incorporated into or bound to humic substances.[6] Flocculation of clays by picric acid depends on the nature of the clay and associated ions. When picric acid was mixed with solutions containing calcium ions and calcium clays, flocculation occurred rapidly and completely, removing picrate from solution. Mixtures containing sodium ions and sodium clays formed stable suspensions, with picrate remaining in solution. Mixtures of sodium ions with calcium clays or calcium ions with sodium clays produced intermediate effects.[9] These experimental studies suggest that transport of picrate in the environment will be highly variable, depending on the organic and mineral composition of each soil. Previous reports of contamination by picrate showed movement through soil in some cases[4,6] and retention by soil in others.[10]

Ammonium picrate and picric acid are not degraded in the environment, either biologically or photochemically;[6] however, some strains of adapted organisms may make bioremediation a possibility.[11,12]

Previous Analytical Methods

Detection of picric acid has been a goal of analytical chemists since the early 20th century, when malingerers ingested picric acid to mimic the symptoms of jaundice to avoid military service. Early detection schemes used colored precipitates,[8] colored-solvent interfaces,[13] or colored solutions[14] to identify picric acid and its metabolic product, picramic acid. The color-changing behavior of the picric acid–picrate system in aqueous and strongly acidic organic solutions was discovered in 1923.[15]

Forensic analysts have been required to identify and quantify picric acid in complex mixtures of other nitroaromatic explosives. Paper chromatography[16,17] or thin-layer chromotagraphy[18,19] was used to separate picric acid from other explosives; then the picric acid was detected by color-forming reagents. Quantification was done with a photodensitometer.[11]

Contemporary methods for the analysis of picrate in environmental samples have focused on extractions, separation from matrix components, and determinations in the laboratory. A United States Geological Survey method[20] uses benzene for extraction from soil, followed by concentration, solvent exchange, and reverse-phase high-performance liquid chromatography (RP-HPLC) with eluents containing an ion-pairing reagent. One method developed for the U. S. Army Environmental Center[21] uses a 10% aqueous methanol solution for extraction from soil, followed by RP-HPLC with an aqueous methanol eluent. A Midwest Research Institute method[22] extracts picrate from soils with an acidic methanol-water mixture; this is followed by RP-HPLC with the use of ion-pairing conditions. Picrate and other high explosives in forensic samples have been analyzed by RP-HPLC with buffered acidic aqueous-methanol eluents.[23] Less routine analyses have used thermospray HPLC mass spectrometry with chemical ionization to analyze acetone wipes of skin,[24] or capillary supercritical fluid chromatography.[25]

Ammonium picrate and picric acid are not currently target analytes in U.S. Environmental Protection Agency SW846 Method 8330.[26] When the standard conditions for extraction and analysis of explosives in soil from Method 8330 are used, picrate is extracted from soil by overnight sonication in acetonitrile. However, the picrate is not retained by the recommended RP-HPLC column when a 50% aqueous-methanol eluent is used.

Objectives

The major goal of this effort is to develop a field estimation method for ammonium picrate and picric acid that can be used in conjunction with other field methods already established for the high explosives RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine), TNT (1,3,5-trinitrotoluene), and 2,4-DNT (2,4-dinitrotoluene).[27,28] The intent is to use the color change that occurs when a colorless solution of picric acid in acidified acetone is diluted with water. The water reduces the acidity and forms the intensely yellow picrate anion. The presence of picrate is established visually and the concentration estimated by measuring the absorbance with a portable spectrophotometer.

Experiment

The picric acid used to prepare spiked soils was Standard Analytical Reference Material from the U.S. Army Environmental Center, Aberdeen Proving Ground, Maryland. Ammonium picrate was military grade, obtained from the Hawthorne Army Ammunition Plant (HAAP), Hawthorne, NV. Field-contaminated soils from HAAP; the Naval Surface Warfare Center (NSWC); Crane, IN; and the Nebraska Ordnance Plant (NOP), Mead, Nebraska were used to validate the methods. The sodium salt of humic acid was reagent grade, from Aldrich, and the picramic acid was reagent grade, from Sigma.

All solvents used for extraction, collection, and elution were HPLC grade, from Baker. Reagent-grade water was prepared using a Milli-Q Type 1 System (Millipore). The solid-phase extraction (SPE) material was Alumina-A SPE cartridges (3 ml, 1 g, Supelco). The syringe filters were Millex SR (0.5 μm, 25 mm Millipore).

In the laboratory, picric acid, ammonium picrate, and other explosives were extracted from the soil with acetone and determined by RP-HPLC on a 25 × 4.6-cm, 5-μm, LC-18 column (Supelco). The analytes were eluted with 1.5-ml min-1 of 60:40 (v/v) aqueous buffer (0.05 M KH_2PO_4, pH 3.5): methanol. Acetone extracts required a one-to-four dilution with eluent to achieve reasonable peak shape. The analytes were detected at 365 nm for picrate and 254 nm for all others. The spectrophotometer used for the field method was a battery-powered Hach DR-2000 (Love-

land, CO) with an optional adapter to allow the use of 1-cm square cuvettes (4 ml). Absorbance measurements were made at 400 nm, the lower cutoff wavelength for the DR-2000.

TABLE 1. Retention of picrate on Alumina-A cartridges from various water-acetone mixtures.

% Water	0	10	25	50	75
% Retention	56	14	60	100	100

Results and Discussion

Several polar solvents (acetone, methanol, isopropanol, acetonitrile, and water) were used to extract different portions of a soil from HAAP known to be contaminated with ammonium picrate. All extracts were yellow, but by far the most intensely colored were the acetone and water solutions, indicating that they were the most efficient extraction solvents. The extraction efficiencies of acetone and water were then determined. A 2-g sample of the HAAP soil was placed in a 22-ml glass vial with 10 ml of extractant and shaken manually for 3 min. The vial was then centrifuged for 3 min and the extract was decanted and filtered through a 0.5-μm Millex SR syringe filter. About 9.2 ml of extracting solvent were recovered, and the concentration of picrate was estimated from the absorbance at 400 nm. Further 10-ml aliquots of extractant were added to the soil and the procedure was repeated until no more yellow color was extracted. The total masses of picrate extracted with the use of acetone and water were computed by summing the amounts recovered from the sequential extractions. These results indicated that the first extraction dissolved 100% of the extractable picrate and the residual yellow color in subsequent extracts was due to residual solvent from the first extraction. Thus, the concentration obtained in the first 3-min extraction represents the total extractable picric acid or ammonium picrate for both water and acetone.

Determining picrate concentration by the direct measurement of absorbance in acetone extracts is subject to interference by other yellowish soil components. One way to reduce this problem is to pass the soil extract through a solid-phase ion-exchange column, which retains picrate but not most yellowish interferences, as described below. Then, by passing an acidified eluent through the column, picrate ion is converted to picric acid, which can be eluted from the column with additional solvent.

Initial tests of this concept used Alumina-A solid-phase, anion-exchange extraction cartridges because they are already used in the RDX field method.[27] A solution containing picric acid in aqueous acetone was passed through the Alumina-A cartridges. The sorbent became yellow, indicating that picrate ions were retained and concentrated.

Because acetone extracts of naturally moist soils will contain variable amounts of water, tests were run to determine what effect the amount of water would have on the retention of picrate by the ion exchange material. The breakthrough of picrate was calculated by measuring the absorbances of picrate solutions before and after passing them through an Alumina-A cartridge. The percent retention (100−% breakthrough) was variable when water content was below 50% (Table 1), indicating that extracts of naturally-moist soils would produce unpredictable retention. If, however, acetone extracts are diluted to greater than 50% water, retention should be 100%.

Tests were conducted to determine the acid strength required to elute the picrate from the Alumina-A cartridge. Results indicated that an acetone solution containing 2% sulfuric acid (by volume) was the mildest solution that would elute the picric acid. Lower concentrations of sulfuric acid in acetone, acetone solutions containing other acids, or acidified methanol were not successful. Acetone solutions containing 2% sulfuric acid converted the picrate to the colorless, undissociated picric acid. The eluted solution was filtered through a Millex SR syringe filter placed on the tip of the cartridge, then diluted with water until the pH was above the *pKa* of picric acid, again giving the colored picrate anion. The formation of this yellow color by water dilution is a good qualitative indication of the presence of the picric ion.[15]

Colorimetric field methods developed at the U.S. Army Cold Regions Research and Engineering Laboratory for TNT, 2,4-DNT, and RDX specify the use of 100 ml of acetone to extract 20 g of soil.[27,28] These methods use about 20 ml each of the common extract for analyses. If the picrate test is to be used in concert with these tests on a common soil extract, about 40 ml is left for the picrate assay. Because it is difficult to remove all of the remaining acetone from the extraction bottle, in subsequent tests we used 30 ml of acetone diluted one-to-one with water.

Further increases in recovery were realized by optimizing extraction and elution conditions. Breakthrough tests were conducted at different extraction rates with the use of 60 ml of 50% aqueous-acetone solutions fortified with picric acid. At a flow rate of 10 ml min^{-} 1 through the cartridge, about 96% of the picrate was retained. Retention decreased to about 87% when the flow rate was increased to 40 ml min^{-1}. Subsequent elution of the cartridges with 10-ml aliquots of 2% sulfuric acid-acetone at 5 ml min^{-1} were sufficient to recover 88 to 91% of the retained picrate.

To achieve the brightest color for quantification, as much extract as possible should be used with sufficient water to produce picrate ion. The absorptivity of equal masses of picrate in aqueous, acidified acetone solutions increases as the water content increases to 25% (Figure 1). For this reason we decided to add water to produce a 25/75 water/acetone solution.

Potential positive interferences in this method can arise from colored substances in the soil that will extract into acetone. These include several environmental transformation products of TNT, a commonly encountered contaminant at military installations, and naturally occurring humic organic matter. Three environmental transformation prod-

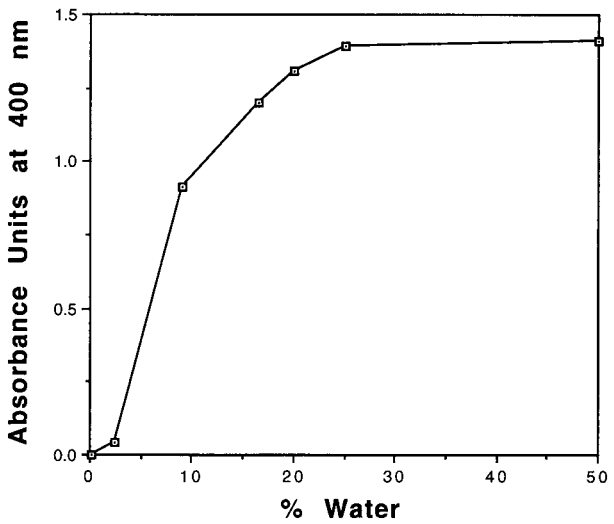

FIG. 1. Absorptivity of 25 μg/ml solutions of picrate in aqueous, acidified acetone as a function of percent water.

ucts of TNT that produce yellow acetone extracts are 3,5-dinitroaniline (3,5-DNA), 2-amino-dinitrotoluene (2-ADNT) and 4-amino-dinitrotoluene (4-ADNT). Maximum reported concentrations of two of these chemicals in explosives-contaminated soils (373 μg g^{-1} for 2-ADNT and 14 μg^{-1} for 3,5-DNA) occurred in samples from sites in the U.S.[29] When an acetone-water solution containing these two compounds at concentrations above their maximum reported levels were extracted with an Alumina-A cartridge, the sorbant retained the yellow color. A rinse with 3 ml of methanol removed all traces of the color, and an additional rinse with 3 mL of acetone removed the methanol, returning the cartridge to the original extraction conditions, ready for the elution step. Neither of these unacidified solvents eluted any picrate, and when the picrate was subsequently eluted with acidified acetone, none of these amino compounds were detectable by RP-HPLC. Thus these compounds will not interfere with picrate detection if the Alumina-A is rinsed with methanol prior to elution of the picrate.

Another source of yellow in acetone soil extracts is elemental sulfur.[30] The yellow color in a sulfurous acetone extract from an anaerobic Eagle River Flats, AK, sediment was not retained on Alumina-A.

Humic materials in soil extracts are a problem because these highly colored acids are retained by acidic ion exchangers. Some humic substances were eluted along with the picrate. The quantity of eluted humic color was highly variable (from 14 to 45% of the applied material) and absorbed strongly in the 400-nm region, where picrate absorbance is at its maximum. However, unlike the picric acid/picrate system, the absorptivity of humic acid/humate does not change substantially at different pH values. Hence, its contribution to the final absorbance can be corrected by subtracting the absorbance of the acidified acetone eluent before water is added to convert picric acid to picrate.

Aqueous acetone solutions of reagent-grade humic acid (sodium salt) and extracts of loamy soils were processed according to the method. The absorbance at 400 nm of the yellowish, acidified acetone Alumina-A extract was reduced one-half by adding an equal volume of 50% aqueous acetone. The resulting 25% aqueous acetone would then produce an optimal molar absorptivity for picrate in contaminated samples (Figure 1). Confirmation of picrate is obtained by first diluting the acidified extract with 5 ml of unacidified acetone, then adding 5 ml of water. The addition of 5 mL of unacidified acetone will cause a visible decrease in the absorbance of colored eluents. The subsequent addition of 5 ml of water will cause a visible increase in yellow if picrate is present. Thus the humic acid's initial absorbance, before adding acetone and water, is divided by the factor-of-2 dilution and subtracted from the final absorbance of the 20 ml of acetone-water solution.

A locally acquired sandy soil was fortified by adding aqueous picrate solutions to the air-dried soil such that the resulting moisture content was 10% and the picrate concentrations were 0.0, 4.6, 18, 37, and 54 μg g^{-1} (as picric acid). Three replicates were processed at each concentration. The samples were extracted and processed according to the method described above, and the concentration of picrate recovered was compared to the concentration fortified. Regression analysis indicated a slope of 0.983 and an intercept of 0.312 μg g^{-1}. The standard deviation of the slope and intercept were 0.015 and 0.445, respectively. The intercept was not significantly different from zero and was unimportant from a practical point of view. Hence the recovery (98%) was independent of concentration, and a single daily one-point calibration is adequate.

The linear range of the test was determined by processing simulated extracts that had been fortified with ammonium picrate. The slope was the same as that for the recovery tests and the range was linear up to 150 μg g^{-1}. The absorbance at 400 nm equaled 1.67 absorbance units (A.U.), expressed as picric acid.

To establish the method detection limit (MDL)[31] for this method, seven replicate 22-g samples of locally acquired sandy loam that had been fortified with 5 μg g^{-1} of picric acid were processed according to the method described above. Acetone extracts had a slight yellow color, due to humic materials in the soil. The initial acetone dilution of the Alumina-A extract decreased the color visibly. Then 5 ml of reagent-grade water was added, and the resulting deeper yellow color was measured at 400 nm. The MDL was calculated to be 1.3 μg g^{-1}.

A field analysis method that produces a quantitative result requires a daily check sample for validation. An aqueous, unacidified standard at 10 μg ml^{-1} of picric acid is stable for at least 2 weeks. A solution equivalent to a 50 μg g^{-1} soil extract is prepared by diluting a 30-ml aliquot of the aqueous standard one-to-one with acetone. This mixture is then extracted and eluted according to the method. The absorbance (cm^{-1}) at 400 nm should be 0.56 $\pm$ 0.08.

Performance Evaluations

The method was tested on field-contaminated soils. Two soils from NSWC produced straw-colored acetone extracts. Analyses of the same acetone extracts by HPLC showed that they contained no picrate. The field screening method produced a very light yellow Alumina-A extract, which was reduced 50% by dilution, and gave a field-method result of zero.

The soils from NOP had been analyzed previously by Method 8330. The only detected analyte was tetryl. Both the field screening method and HPLC using the buffered eluent system revealed the presence of picrate. Because picrate is a hydrolysis product of tetryl,[2-4] it is to be expected in environmental samples contaminated with tetryl.

The soils from HAAP were collected from a waste lagoon below a munitions washout plant and from an open-burn/open-detonation disposal area. The levels of picrate in some of these samples were so high that the capacity of the Alumina-A cartridge was exceeded and breakthrough was easily visible. The following adaptation of the method corrected this problem. The absorbance of the initial acetone extract was measured at 400 nm. If it was above 1.0 A.U., an aliquot of the extract was diluted until the absorbance was below 1.0. The amount of extract that would account for an absorbance of 1.0 was then taken and diluted one-to-one with water and the method was followed. For the HAAP samples, the volumes of acetone extract that were processed ranged from 0.4 to 20 ml. A correction factor for the difference between the 30 ml of daily check standard and the actual sample volume was applied. The small volumes used in these cases reduced the extraction times; however, the extract could also be diluted in 30 ml of acetone to simplify the calculations.

A comparison between 49 field method and RP-HPLC determinations of picrate in contaminated soils shows very good agreement over three orders of magnitude (Figure 2). The regressed relationship was:

$$\mu g\ g^{-1}\ (\text{field method}) = 0.96\ \mu g\ g^{-1}\ (\text{RP-HPLC}) + 1.56\ \mu g\ g^{-1}.$$

The standard errors of the slope and intercept were 0.007 and 6.40, respectively. The slight positive intercept was not significantly different from zero at the 95% confidence level. Samples from soils contaminated with picrate at levels less than 10 $\mu g\ g^{-1}$ showed a positive bias when compared to RP-HPLC determinations. However, the RP-HPLC chromatograms of these sample extracts had multiple unidentified peaks. Picramic acid was not present in these samples. Because picrate is known to complex readily with various compounds,[6] it is conceivable that the RP-HPLC results underestimated the level of contamination. Samples from soils at NSWC and HAAP that were contaminated with TNT, but not with picrate, produced results that were below detection limits for picrate, as expected. Thus, the field method did not produce false positives.

This soil method can be easily added to accepted screening methods for the military explosives TNT, 2,4-DNT, and RDX in soil.[27,28,32] A single 100-ml acetone extract can be split for the four tests. The Alumina-A cartridges used for this picric acid/picrate method are also required for the RDX test to remove interfering nitrates and nitrites.

Method Summary

The method is performed as follows:

1. Place 20 g of soil in a plastic bottle, add 100 mL of acetone, and shake for 3 minutes.
2. Filter 30 ml of acetone extract and measure the absorbance at 400 nm.
3. If the absorbance is greater than 1.0, dilute the extract with acetone.
4. Mix the filtered or diluted extract with an equal volume of water and pass the mixture through an Alumina-A SPE cartridge.
5. Rinse the cartridge with methanol followed by acetone.
6. Elute the picric acid with 10 ml of acidified acetone and measure the absorbance at 400 nm. Record the value as "Initial ABS."
7. Dilute the acidified acetone eluent with 5 ml of unacidified acetone followed by 5 ml of water. Note any change in color and measure the absorbance at 400 nm. Record the value as "Final ABS."
8. Calculate the quantity of picrate in the soil, expressed as picric acid:

$$\mu g\ g^{-1} = rf \times [\text{final ABS} - 0.5 \times (\text{initial ABS})] \times df,$$

where

$$rf = 50\ \mu g\ g^{-1} \times [\text{final ABS} - 0.5 \times (\text{initial ABS})]^{-1}$$

is the daily response factor and df = the dilution factor, if used in step 3.

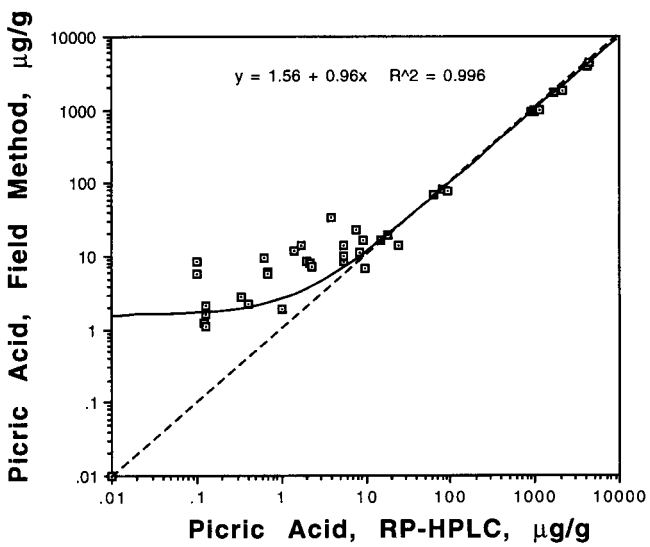

FIG. 2. Correlation of concentration estimates for picric acid, with the use of the field method and RP-HPLC.

Conclusions

The proposed method for field screening for picric acid/ammonium picrate in soil is a combination of contemporary solid-phase extraction materials and a 70-year-old qualitative colorimetric assay. The resulting method is both sensitive and relatively free from interference by humic substances or other nitroaromatics that are likely to be found at military sites. A single extract can be used to screen for picric acid/ammonium picrate, TNT, 2,4-DNT and RDX in soils. The estimated cost of a few dollars per sample is very low. A single assay can be run in about 20 min. Multiple samples can be processed in less time per sample with the use of cartridge manifolds.

A method for analyzing water for picrate, with the use of solid-phase anion extraction membranes and a similar detection scheme, is under development.

Acknowledgments

The authors gratefully acknowledge Dr. Clarence L. Grant, Professor Emeritus, University of New Hampshire and Marianne E. Walsh, CRREL, for useful comments on this manuscript.

Funding was provided by the U.S. Army Environmental Center, Aberdeen Proving Ground, Md, Martin H. Stutz, Project Monitor.

References

1. R. Meyer, *Explosives,* VCH Verlagsgesellschaft mbH, Weinheim, Germany, 1980, pp. 269–271.
2. J. F. Wyman, M. P. Serve, D. W. Hobson, L. H. Lee and D. E. Uddin, "Acute Toxicity, Distribution, and Metabolism of 2,4,6-Trinitrophenol (Picric Acid) in Fischer 244 Rats" *Acute J. Toxicol. Environ. Health* **37,** 313, (1992).
3. E. Kayser, N. E. Burlinson and D. H. Rosenblatt, "Kinetics of Hydrolysis and Products of Hydrolysis and Photolysis of Tetryl," Naval Surface Weapons Center, Report No. NSWC TR 84-68, Silver Spring, MD, 1984.
4. E. Kayser and N. E. Burlinson, "Migration of Explosives in Soil: Analysis of RDX, TNT, and Tetryl from 14C Lysimeter Study" *J. Energetic Mater.* **6,** 45 (1988).
5. T. F. Jenkins, P. G. Thorne, K. F. Myers and E. F. McCormick, "Preservation of Water Samples Containing Nitroaromatics and Nitramines," U.S. Army Cold Regions Research and Engineering Laboratory, Special Report No. 95-16, Hanover, NH, 1995.
6. D. Layton, B. Mallon, W. Mitchell, L. Hall, R. Fish, L. Perry, G. Snyder, K. Bogen, W. Malloch, C. Ham and P. Dowd, "Conventional Weapons Demilitarization: A Health and Environmental Effects Data Base Assessment—Explosives and Their Co-Contaminants Final Report, Phase II" Lawrence Livermore National Laboratory, Livermore, CA, 1987.
7. W. L. Goodfellow, D. T. Burton, W. C. Graves, L. W. Hall and K. R Cooper, "Acute Toxicity of Picric Acid and Picramic Acid to Rainbow Trout, *Salmo gairdneri,* and American Oyster, *Crassostrea virginica*" *Water Res. Bull.* **19,** 641, (1983).
8. E. Barral, "Picric acid and malingering" *Chem. Abstr.* **10,** 2100, (1915).
9. C. W. Chang and J. U. Anderson, "Flocculation of Clays and Soils by Organic Compounds" *Soil Sci. Soc. Am. Proc.* **32,** 23–27, (1968).
10. C. Ruchholt and F. Norris, "Estimation of Ammonium Picrate in Wastes from Bomb- and Shell-Loading Plants" *Ind. Eng. Chem.* **18,** 480, (1946).
11. J. F. Wyman, H. E. Guard, W. D. Won and J. H. Quay, "Conversion of 2,4,6-Trinitrophenol to a Mutagen by *Pseudomonas aeroguinosa*" *Appl. Environ. Microbiol.* **37,** 222, (1979).
12. H. Lenke and H. Knackmuss, "Initial Hydrogenation during Catabolism of Picric Acid by *Rhodococcus erythropolis* HL 24-2" *Appl. Environ. Microbiol.* **58**(9), 2933, (1992).
13. G. Rodillon, "A Specific Reaction of Picric Acid" *Chem. Abstr.* **10,** 3268, (1915).
14. Ydrac, "Detection of Picric Acid by the Formation of Potassium Isopurate" *Chem. Abstr.* **11,** 2469, (1916).
15. G. Deniges, "Nature and Application of the Precipitate Formed by Strong Acids in Aqueous Solutions of Picric Acid" *Chem. Abstr.* **18,** 2858, (1923).
16. J. Barnabas, "Identification of Phenols by Circular Paper Chromatography" *Chem. Abstr.* **49,** 10128, (1954).
17. D. M. Colman, "Paper Chromatography of Nitro Compounds. I. Substituted Trinitrobenzenes" *J. Chromatogr.* **8,** 399–403, (1962).
18. D. Parihar, S. P. Sharma and K. C. Tewari, "Thin-Layer Chromatography of Polynitrophenols, Nitrophenols, Nitrohydroquinones and Related Compounds" *J. Chromatrogr.* **24,** 230, (1966).
19. L. Bagnato, G. Grasso, "Two-Dimensional Thin-Layer Chromatography for the Separation and Identification of Nitro Derivitives in Explosives" *J. Chromatogr.* **357,** 440, (1986).
20. D. F. Goerlitz, "Analysis of Picric Acid in Water by High-Performance Liquid Chromatography" U.S. Geological Survey, Open File Report No. 79-207, Menlo Park, CA, 1979.
21. USAEC, "Method LW-13. Picric Acid in Soil Samples," U.S. Army Environmental Center (formerly USATHAMA), Aberdeen Proving Ground, MD, 1989.
22. E. Conrad, "Standard Operating Procedures No. 104, Determination of Munitions, Group B, in Soils" Midwest Research Institute, Kansas City, MO, 1990.
23. J. Lloyd, "Microcolumn Clean-Up and Recovery Techniques for Organic Explosives Compounds and for Propellants Traces in Firearms Discharge Residues" *J. Chromatogr.* **330,** 121, (1985).
24. R. D. Voyksner and J. Yinon, "Trace Analysis of Explosives by Thermospray High-Performance Liquid Chromatography-Mass Spectrometry" *J. Chromatogr.* **354,** 393. (1986).
25. A. Munder, S. N. Chesler and S. A. Wise, "Capillary Supercritical Fluid Chromatography of Explosives" *J. Chromatogr.* **521,** 63, (1990).
26. U.S. EPA, "Nitroaromatics and Nitramines by High Pressure Liquid Chromotography. Revision 1," U.S. EPA Office of Solid Waste and Emergency Response, Draft Method 8330, SW846, Washington, DC, 1990.
27. T. F. Jenkins and M. E. Walsh, "Development of Field Screening Methods for TNT, 2,4-DNT and RDX in Soil" *Talanta* **39**(4), 419, (1992).
28. T. F. Jenkins, P. G. Thorne and M. E. Walsh, "Field Screening Method for TNT and RDX in Groundwater," U.S. Army Cold Regions Research and Engineering Laboratory, Special Report 94-14, Hanover, NH, 1994.
29. M. E. Walsh, T. F. Jenkins, S. Schnitker, J. W. Elwell and M. H. Stutz, "Evaluation of SW846 Method 8330 for Characterization of Sites Contaminated with Residues of High Explosives," U.S. Army Cold Regions Research and Engineering Laboratory, Report No. 93-5, Hanover, NH, 1993.
30. M. E. Walsh, personal communication.
31. U.S. EPA, "Definition and Procedure for the Determination of the Method Detection Limit," Code of Federal Regulations, Part 136, Appendix B, 1984.
32. T. F. Jenkins, "Development of a Simplified Field Method for the Determination of TNT in Soil," U.S. Army Cold Regions Research and Engineering Laboratory, Special Report No. 90-38, Hanover, NH, 1990.