SERDP Project 1233 Final Technical Report – October 15, 2003

(i) TITLE:

"Development and Application of Flash Pyrolysis – GC/MS Assay for Documenting Natural and Engineered Attenuation of Nitroaromatic Compounds

(ii) PERFORMING ORGANIZATION:

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(iii) PROJECT BACKGROUND (restatement of the problem)

SERDP SON 01-08 indicated that "there exists growing concern about the potential for military training activities leading to groundwater contamination by energetic compounds… an improved understanding of groundwater contaminant/treatment is needed". This project directly addressed this need.

Regardless of their source (e.g., live fire ranges or manufacture and distribution), nitroaromatics and related explosives pose vexing environmental problems. Inadvertent, real-world field experiments on the environmental fate of nitroaromatic compounds have been in progress at various DoD facilities since WWII. Although groundwater plumes of TNT, RDX, and related compounds have been defined and monitored, the standard analytes required by regulatory authorities do not provide sufficient information to discern the mechanisms that may act to bind and/or attenuate these compounds. Nitroaromatic compounds provide a major challenge to environmental chemists and microbiologists addressing chemical- and bio-treatment

based site management plans. The challenge arises because, unlike petroleum hydrocarbon contaminants that can serve as carbon and energy sources for robust microbial reactions, the cometabolic reactions of nitroaromatics leave few obvious geochemical fingerprints in contaminated sites. Without field-based signatures proving that attenuation reactions have actually occurred in the field (for petroleum hydrocarbons, these measures include depletion of oxygen and production of carbon dioxide), the case for nitroaromatics is weak. Even rigorous geochemical modeling (that may suggest quantitative loss of mass of initially-released nitroaromatics) is seldom convincing because the parameters in mass-balance computations are subject to error.

Chemical and biological treatment would be a welcome solution to contamination problems. Despite decades of laboratory, enrichment-based biodegradation studies, clear criteria do not yet exist for seeking field evidence for attenuation of nitroaromatics or enhancing the process. Thus, at the beginning of this project there were at least three choices: (1) concede that the methodological challenges of documenting attenuation of nitroaromatics were insurmountable; (2) accept the argument that the persistent, decades-old plumes at DoD facilities nation wide preclude the possibility of attenuation (if it is going to happen, why hasn't it yet?); or (3) mount new efforts using a broad, hypothesis-based spectrum of procedures and analyses at a variety of DoD sites. This project was designed to address the latter approach by seeking attenuation criteria via a novel suite of chemical, and microbiological measures applied to laboratory experiments and field samples. In the long run, this project sought to serve as the foundation for a comprehensive plan for assessment, classification, implementation, and enhancement of the attenuation of nitroaromatics in contaminated DoD sites.

(iv) OBJECTIVE

This project sought to establish clear chemical criteria for field evidence documenting the attenuation of nitroaromatic compounds and related explosives. The approach relied on a combination of laboratory-based biogeochemical inquiry and contaminated-site-derived data. It applied a novel suite of chemical, biochemical, physiological, and microbiological measures to controlled laboratory experiments and to real-world field samples. These measures included: HPLC analysis of reduced nitroaromatics; assessment of redox, carbon, nitrogen and other potential geochemical factors that may govern irreversible binding and polymerization reactions for nitroaromatics; and flash pyrolysis GC/MS analyses of the bound, polymerized nitroaromatics. The latter received the primary emphasis.

The project's fundamental tenet was that knowledge about chemical “fingerprints” of nitroaromatic-derived polyamines in humus needs to be developed and extended. These should quantitatively and qualitatively provide a measure of the fate of nitroaromatics in field sites. Once such fingerprints have been established, careful inspection of long-contaminated field sites and sites undergoing engineered treatment could provide clues about the key microbiological and chemical processes that govern the fate of nitroaromatic compounds. The prior knowledge base describing the impact of microorganisms and soil constituents on nitroaromatics suggested that immobilization, not mineralization, should dominate attenuation processes. We sought to couple to geochemical characterization data with the FP-GC/MS assay to reveal the field conditions (engineered and natural) that foster immobilization reactions and how these reactions can be recognized, managed and enhanced.

All told, results of this project aimed to substantially augment current knowledge of the field behavior of nitroaromatics. Resultant information was hoped to provide new criteria for

documenting and enhancing natural and engineered attenuation of nitroaromatics and to contribute to efforts directed toward establishing "environmentally acceptable end points" for explosives-contaminated sites.

In the end, however, technical problems led to early project termination. The third year was cut because of lack of analytical sensitivity in the FP-GC/MS instrumentation. The principles of the project were borne out—analytical criteria were found for defining and detecting covalently bound derivatives of TNT in soils from DoD field sites. Unfortunately, the amino moieties on reduced derivatives of TNT [e.g., aminodinitrotoluene (ADNT) and diaminonitrotoluene (DANT)] interact strongly with inner surfaces of the FP-GC/MS instrument—thus diminishing sensitivity of the measurements. More than 30 samples from different contaminated DoD sites were analyzed; however, the FP-GC/MS unit was only able to detect TNT and/or its derivatives in only 2 samples (Louisiana Army Ammunition Plant and the Picatinny Arsenal). The TNT concentrations in these samples was 4500 and 2600 ppm, respectively. While data produced from these DoD soils using the FP-GC/MS procedure allowed project goals to be pursued, an unexpected interim goal was added: discover a means to boost analytical sensitivity of the FP-GC/MS. Despite a sound effort, our attempts to achieve greater sensitivity were unsuccessful. Thus, the scientific goals underpinning potential technology were reached with 2 soils, but the project's practical technological goals could not be extended broadly to DoD sites.

(v) TECHNICAL APPROACH

This project sought to build on existing knowledge of the chemical and microbiological processes that influence nitroaromatic compounds in the laboratory and in real-world field sites. The objective was to implement a novel assay that would provide new information about relationships between attenuation and geochemical conditions that may prevail or be established at contaminated DoD sites. The resultant information sought to answer the questions "Does attenuation of nitroaromatics occur?" and "How can it be converted into an effective, reliable site-remediation technology?"

As originally planned this project was to last 3 years and proceed via 7 logical steps.

Step 1. Conduct a survey compiling existing data on the microbiological and geochemical characterization of several contaminated DoD field sites. This was a preliminary step designed to insure that the conditions chosen for Step 2 realistically spanned a broad range of climatic, redox, soil characteristic, and other geochemical parameters of field relevance to DoD. The criteria were: broad spectrum of geochemical conditions, accessibility for later new sample acquisition, accessibility of archived sediments, and extent of existing data base on site conditions.

Step 2. Laboratory incubations for synthesis of explosives-derived polymeric materials. Varying aqueous concentrations of TNT, RDX, and HMX were to be incubated under a wide variety of controlled laboratory conditions (pH, Eh, geochemistry) in situations ranging from defined chemical solutions to slurried groundwater and subsurface sediments. Hypotheses were to be developed and refined to address chemical and microbiological processes influencing the fate and behavior of the nitroaromatic compounds. These were to be designed to allow single-variable manipulations to isolate, clarify, and test the

influence of key parameters (e.g., biomass, temperature, biotic factors, abiotic factors, redox,) on processes that foster or prevent attenuation of nitroaromatics via polymerization and binding to sediments.

Step 3. Assessing key traits of the polymer reactivity. The degree to which chemical and biological treatments (Step 2) altered the leachability, sorption, and (most critically) irreversible binding of the explosive compounds were evaluated and rated based on HPLC and GC/MS analysis of post-treatment solutions and soil extracts.

Step 4. Polymer characterization by Flash pyrolysis – GC/MS to form a library of GC/MS signatures. The many insoluble and soil-bound polyamine-type molecules formed in Step 2 were to be extracted. After flash pyrolysis-GC/MS, molecular fragment fingerprint patterns were to be assembled, compared for distinctiveness, and matched to their distinctive conditions (chemical and biological) of formation. The fingerprint library relating particular chemical signatures to degree of nitroaromatic immobilization was the key goal of project.

Step 5. Site surveys for natural attenuation. The results of Steps 2-4 were to provide 2 linked key pieces of information: optimal biochemical conditions for nitroaromatics attenuation and a flash pyrolysis-GC/MS fingerprint indicative of the attenuation. Armed with this, we planned to return to the contaminated sites seeking confirmation of correlation between laboratory assays of nitroaromatics attenuation and its occurrence in the field.

Step 6. Models of enigneered remediation. Fresh field site samples were planned to be used in laboratory incubations to refine, improve and test the biochemical processes identified in Steps 4 and 5. Using site-specific fresh field samples, these assays were to verify the

accuracy and robustness, attenuation processes, their effectiveness, and their flash pyrolysis-GC/MS means of verification.

Step 7. Synthesis of field and laboratory investigations. The end product was planned to be a matrix of field and laboratory facts, principles, and criteria for designing chemical and physiological subsurface processes for the attenuation of nitroaromatics.

(vi) SUMMARY (final project deliverables)

This project began with a highly ambitious 7-step technical approach (see above) that that sought to establish a sound scientific foundation for a technology and then sought to pursue the technology.

Project personnel successfully achieved "proof of principle" of the science. After developing a collaborative approach with Dr. Kevin Thorn (USGS; an investigator on an earlier SERDP-sponsored project), we used chemically synthesized complexes between model soil organic matter and TNT-related compounds to validate the FP-GC/MS technique. We then applied this technique to contaminated DoD soils. Using highly contaminated soil samples from LAAP, covalent complexes between soil organic matter and both ADNT and DANT were documented. Using a highly contaminated soil sample from Picatinny arsenal; a soil organic matter-ADNT complex was documented. As this progress was being made, we encountered analytical obstacles that prevented extending the project's scientific findings to allow SERDP's practical technological needs to be met. For this reason, the project was terminated early. Thus, the project deliverables are manifest as a manuscript detailing the scientific achievements. This manuscript has been submitted to Environmental Science and Technology (see Appendix A).

(vii) PROJECT ACCOMPLISHMENTS

See Summary (Section vi), and the bold-font type at the end of Objectives (Section iv)

(viii) CONCLUSIONS

See Summary, Section (vi), the bold-font type at the end of Objectives (Section iv), and Recommendations (Section x).

(ix) TRANSITION PLAN

See Summary, Section (vi), the bold-font type at the end of Objectives (Section iv), and Recommendations (Section x).

(x) RECOMMENDATIONS

Understanding the complex interactions of contaminants and microorganisms in real-world geochemical settings has very important site-management implications for DoD. This project sought to develop an array of technical criteria (flash pyrolysis-GC/MS fingerprints vs. degree and type of polymeric immobilization) that documented attenuation of explosives in natural and engineered settings. The pyrolysis-GC procedures and the resultant library of chemical signatures were hoped to serve as criteria for nitroaromatic attenuation, thereby constituting a new technology serving as the foundation for a comprehensive plan for assessment, classification, implementation, and enhancement of nitroaromatic treatment in contaminated DoD sites. Project logic and promise remain sound. IF the analytical barriers that thwarted this project can be eliminated, perhaps it should be taken up again in the future.

(xi) APPENDIX A (manuscript currently under review at Envrionmental Science and Technology)

Development and Application of Pyrolysis-Gas Chromatography/Mass Spectrometry for the Analysis of Bound Trinitrotoluene Residues in Soil

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Abstract

To date, only differential extraction-based approaches have been able to determine the presence of covalently bound contaminants such as the reduced forms of trinitrotoluene (TNT) in field soils. Here, we employed thermal elution, pyrolysis, and gas chromatography/mass spectrometry (GC/MS) to distinguish between covalently bound and non-covalently bound reduced forms of TNT in soil. Model soil organic matter-based matrices were used to develop an assay in which non-covalently bound (monomeric) amino-dinitrotoluene (ADNT) and

diamino-nitrotoluene (DANT) were desorbed from the matrix and analyzed at a lower temperature than covalently bound forms of these same compounds. A thermal desorption technique, evolved gas analysis, was initially employed to differentiate between covalently bound and added ^{15}N-labeled monomeric compounds. A refined thermal elution procedure, termed "double shot analysis" (DSA), allowed a sample to be sequentially analyzed in 2 phases. In phase 1, all of an added ^{15}N-labeled monomeric contaminant was eluted from the sample at relatively low temperature. In phase 2 during high temperature pyrolysis, remaining covalently-bound contaminants were detected. DSA analysis of soil from the Louisiana Army Ammunition Plant (LAAP; ~5000 ppm TNT) revealed the presence of DANT, ADNT, and TNT. After scrutinizing the DSA data and comparing them to results from solvent-extracted and base/acid hydrolyzed LAAP soil, we concluded that the TNT was non-covalently bound "carry over" from phase 1. Thus, the pyrolysis-GC/MS technique successfully defined covalently bound pools of ADNT and DANT in the field soil sample.

Introduction

Trinitrotoluene (TNT) was the most widely produced explosive during World Wars I and II, and many former production sites are highly contaminated with nitroaromatic compounds (1). Due to the toxicity, mutagenicity, and potential carcinogenicity of TNT and its reduced derivatives, remediation of contaminated soil has been deemed necessary (2). Incineration is the most effective, yet expensive, remediation technology applied to TNT-contaminated soils. Technologies including phytoremediation and bioremediation have also been investigated to clean-up TNT-contaminated soils (3-5), but no method has established itself for the treatment of real-world, contaminated soils *in situ* (5, 6).

The sequential, cometabolic reduction of TNT's nitro groups by both biotic and abiotic factors is a common observation in laboratory assays (3, 7-9). Reduced forms of TNT are capable of binding to soil by several mechanisms including: hydrogen bonding, van der Waals interactions, hydrophobic interactions, and covalent bond formation (10). Radiotracer studies have shown that covalently bound amino-dinitrotoluene (ADNT) and diamino-nitrotoluene (DANT) are resistant to extraction by water, organic solvents and by acid/base hydrolysis (11). Experiments using ^{15}N NMR have also clearly demonstrated the irreversible, covalent binding of ADNT and DANT to various soil fractions and constituents (12, 13). These newly formed, high molecular weight nitroaromatic compounds have been deemed non-toxic and non-bioavailable (11, 12). Therefore, reduction followed by covalent binding of TNT contaminants to a soil matrix is viewed as an effective and acceptable form of remediation (3).

Formation of covalent bonds between soil organic matter and reduced forms of TNT under real-world (non-laboratory, non-engineered) conditions is undoubtedly an important natural attenuation process, yet facile procedures for directly distinguishing between covalently and non-covalently bound compounds in field samples have not been developed. Pyrolysis (Py)-GC/MS is a technique that has been employed by chemists in the analysis of compounds ranging from soil-bound pesticides to industrial polymers (14-16) and also to the study of soil organic matter (17, 18). In pyrolysis, a sample (liquid or solid) is placed in an inert atmosphere, heated to a temperature up to or greater than 800°C, and subsequently analyzed by GC/MS. Pyrolysis temperatures are high enough to break the covalent bonds of polymers, and the resulting low molecular weight fragments can be separated and individually analyzed. Recent work by Nakamura et al. has established the use of Py-GC/MS for the separation and analysis of waterborne paints (19). The strategy employed evolved gas analysis (EGA), a thermal

desorption technique linked to MS, to guide the development of a biphasic procedure, here called double shot analysis (DSA), that defined, separated, and analyzed both monomeric and polymeric paint components. Thus, Py-GC/MS was shown to be effective in documenting high molecular weight compounds in complex mixtures based on the thermal characteristics of the individual components in the mixture.

This investigation was designed to develop analytical criteria for defining non-covalently bound (henceforth referred to as monomeric) and covalently bound reduced forms of TNT in contaminated soils. Py-GC/MS was initially used to determine the thermal characteristics of ADNT and DANT bound to model soil components. Py-GC/MS was subsequently employed to analyze the TNT contaminants in a field site-derived soil.

Materials and Methods

Chemicals, standards, and soils

TNT was purchased from Chemservice (Westchester, PA). 2- and 4-Amino-dinitrotoluene (ADNT) were purchased from Sigma (Milwaukee, WI). 2,6-Diamino-nitrotoluene (DANT) was a gift from Dr. Lee Krumholz (U. of Oklahoma, Norman, OK). All ^{15}N-labeled compounds (>98% isotopic purity) and covalently bound standards, in which reduced forms of TNT were linked to model soil matrices, were generously supplied by Dr. Kevin Thorn (USGS, Denver, CO) (12). TNT ^{15}N labels were on all three NO_2 groups. ADNT (4-amino-^{15}N-2,6-dinitrotoluene or 2-amino-^{15}N-4,6-dinitrotoluene) and DANT (2,4-diamino-$^{15}N_2$-6-nitrotoluene or 2,6-diamino-$^{15}N_2$-4-nitrotoluene) compounds only contained ^{15}N labels on their NH_2 groups. Humic acid (Elliott soil standard) was purchased from the International Humic Substances Society (St. Paul, MN). Contaminated soil (~5000 ppm TNT), originally obtained from a "load

and pack" area at the Louisiana Army Ammunition Plant (LAAP) (Doyline, LA), was a gift from Dr. R. Boopathy (Nicholls State University, Thibodaux, LA). The soil, Mhoon silt loam, was analyzed by the Department of Crop and Soil Sciences (Cornell) and found to feature 8.2% organic carbon, 1% total N, a pH of 7.0, and KCl-extractable ammonia and nitrate of 32 and 20 $mg \cdot Kg^{-1}$, respectively. Prior to pyrolysis, LAAP soil was sieved through a 0.5 cm mesh, dried at 70°C overnight, and ground to uniformity with a porcelain mortar and pestle. Solvents were purchased from Mallinkrodt (Phillipsburg, NJ) and were of HPLC grade. High purity helium was supplied by Airgas (Elmira, NY).

Preparation of ^{15}N-labeled amines bound to model soil organic matter

The labeled compounds 4-amino-^{15}N-2,6-dinitrotoluene (4ADNT) and 2-amino-^{15}N-4,6-dinitrotoluene (2ADNT) were custom synthesized by Dr. Ron Spanggord, SRI International, Menlo Park, CA (12). The labeled diamines 2,4-diamino-$^{15}N_2$-6-nitrotoluene (2,4DANT) and 2,6-diamino-$^{15}N_2$-4-nitrotoluene (2,6DANT) were purchased from ISOTEC.

<u>2,6DANT Naphthoquinone Dimer</u>. Separate solutions of 70 mg of 2,6-diamino-$^{15}N_2$-4-nitrotoluene dissolved in 700 ml distilled and deionised water and 360 mg 1,2-naphthoquinone-4-sulfonic acid sodium salt dissolved in 100 ml water were combined. The solution was stirred until precipitate formation was complete. The precipitate was filtered, washed with water, air dried and then desiccated. The major product in the precipitate is 4-(3-amino-2-methyl-5-nitrophenyl)amino-1,2-naphthoquinone.

<u>Reactions of IHSS soil humic acid with amines</u>. Approximately 180-200 mg of the monoamines 2ADNT or 4ADNT were dissolved in 4L deionised and distilled water and 200 mg of the diamines 2,4DANT or 2,6DANT dissolved in 2 L water. Humic acid solutions were prepared by adjusting 500 mg of the H^+-saturated IHSS Elliot soil humic acid in 400 mL H_2O to pH 6.4 with

1 N NaOH. The solutions were stirred open to the atmosphere and at room temperature 14 to 24 days. The samples were then re H^+-saturated by passing the solutions through a Dowex MSC-1 cation exchange column (Dow Chemical), and freeze dried.

Reactions of Pahokee Peat with amines. Two grams of IHSS Pahokee peat were added to solutions of 200 mg each of 2,4DANT or 2,6DANT dissolved in 2.5 L of distilled deionized water, 200 mg of 4ADNT in 3 L water, and 150 mg 2ADNT in 3 L water, respectively. The solutions were sonicated for approximately 30 minutes to disperse the peat, stirred for 3 months open to the atmosphere but protected from exposure to any light source, then freeze dried. The freeze dried peat samples were then dialyzed in 1000 Dalton MW cutoff tubes to remove the unreacted free amines, which are highly colored. The dialyzed peat samples were then re-freeze dried.

Sawdust. Approximately 2 g sawdust (particle size less than 10 mm; mixture of hard- and softwood) was added to a solution of 150 mg 2,4DANT dissolved in 1.5 L H_2O. The slurry was stirred for 17 days open to the atmosphere. The sawdust was collected on a sintered glass funnel and washed with acetonitrile until free of the unreacted yellow 2,4DANT, air dried, then desiccated.

Instruments and analysis

A Hewlett-Packard HP5973 GC/MS (Wilmington, DE) equipped with a double-shot pyrolyzer, model PY-2020iD (Frontier Laboratories Ltd., Saikon, Koriyama, Fukushima, Japan) was used for the analyses. Evolved Gas Analysis (EGA), a thermal desorption technique, employed an unpacked, deactivated, stainless steel, 2.5 meter capillary tube (0.15mm ID) (Frontier Laboratories Ltd.). All covalently bound ADNT and DANT standards (naphthoquinone, humic acid, peat, and pure compounds) were desorbed from 50-600°C at

15°C/min. Contaminated soil was desorbed from 100-600°C at 12°C/min. GC/MS settings for EGA were as follows: oven at 300°C, He carrier gas at a flow of 13.7 mL/min. and a split ratio of 10. The MS detector was operated at 2100 V with a scan range of 50-550 *m*/*z*.

Single-shot analysis (SSA): All samples were pyrolyzed at 400°C for 0.1 minutes then swept into the GC/MS. An RTX-200 30m x 0.25mm ID (Restek, Bellefonte, PA) capillary column was used for separation. The GC oven was initially at 80°C (2 minutes) and increased to 250°C at a rate of 8°C/min., where it was held for 11 minutes. The carrier gas was He with a flow of 8.6 mL/min., and a split ratio of 5 was used. The MS was operated at 2100 V and set to a scan range of *m*/*z* 50-550.

Double-shot analysis (DSA): Naphthoquinone-D(^{15}N)ANT dimer was desorbed (phase 1) from 100-340°C at a ramp of 20°C/min. The sample was raised from the furnace and GC/MS separation and analysis was done on the pyrolysate. Volatilized compounds, condensed at the head of the column, were separated with GC/MS settings similar to those described for SSA, with the exception of a 10:1 split ratio. After phase 1, the same sample was subjected to flash pyrolysis (phase 2) for 0.1 minutes at 440°C. GC/MS separation and analysis, as above, was conducted on the pyrolysate. Humic acid-(^{15}N)ADNT polymer samples were desorbed from 100-225°C at 15°C/min. in phase 1. Phase 2 pyrolysis and analysis was similar to the naphthoquinone dimer. TNT-contaminated soil was desorbed (phase 1) from 100-230°C at 12°C/min. and 100-210°C at 12°C/min. for the TNT/ADNT and DANT analyses, respectively. Phase 2 pyrolysis was conducted at 400°C for 0.1 minutes for all soil samples. GC/MS analysis settings were similar to those described for SSA.

The masses of samples added to deactivated, stainless steel sample cups (Frontier Laboratories, Ltd.) were, ~0.3 mg of naphthoquinone-DANT, ~0.3 mg of complexed humic acid-

ADNT, and 5.0 mg of contaminated soil. Monomeric forms of the DANT and ADNT (e.g., 10ul of a 100 ppm 2,4-DANT (^{14}N) solution for the naphthoquinone-DANT dimer and 5ul of a 10 ppm 2-ADNT (^{14}N) solution for the humic acid-ADNT complex when appropriate) were added from methanolic stock solutions directly to the pyrolysis sample cups. ^{15}N-labeled monomeric forms of TNT, ADNT, and DANT were added to LAAP soil. The methanol was allowed to evaporate at room temperature before naphthoquinone, humic acid, or soil was added to the sample cup. Quartz wool (Shimadzu Scientific Equipment) was layered on top of all samples to prevent spillage within the instrument.

Extraction and base/acid hydrolysis of soil

LAAP soil was rigorously extracted with methanol to remove non-covalently bound forms of TNT, ADNT, and DANT. Homogenized soil (see above) was dried overnight at 70°C, and 30 mg was dispensed into 1.5 ml eppendorf tubes. Five microliters of a methanolic solution containing TNT, 2- and 4-ADNT, and 2,4- and 2,6-DANT (all ^{15}N labeled), approximately 50 ppm each, was added to the soil that was then incubated at 70°C for 2 hours to evaporate the solvent. Each tube received 1 ml of methanol, was vortexed for 30 seconds, and centrifuged at 14,000 rpm. Supernatant was removed with a glass pipette and collected. The extraction procedure was repeated 9 times, after which the soil was dried at 70°C and subjected to double shot analysis. Loss of soil during extraction was accounted for on a weight basis. The soil sample was also subjected to acetonitrile and solid phase extraction/HPLC analysis before and after base/acid hydrolysis by Applied Research Associates (South Royalton, VT) using the technique of Thorne and Leggett (20).

Results

Figure 1 displays a single-shot analysis chromatogram of the 1,2-naphthoquinone-2,6-D(^{15}N)ANT dimer. Distinct peaks corresponding to pyrolysis-regenerated 1,2-naphthoquinone and 2,6-D(^{15}N)ANT monomers are seen. An EGA pyrogram produced from the napthoquinone-2,6-D(^{15}N)ANT dimer with added, monomeric 2,4-DANT (^{14}N) is shown in Figure 2A. Early-eluting and late-eluting humps were examined using selected ion monitoring (Figures 2B and 2C). The monomeric form of DANT (*m*/*z* = 167) eluted maximally at 189°C, while the dimeric, ^{15}N-labeled DANT (*m*/*z* = 169) eluted maximally at 312°C. Although desorption characteristics of covalently bound and monomeric forms of DANT in the mixture were distinctive, a zone of overlap between the two forms was seen. The data in Figures 2B and 2C provided a basis for refining the analysis of the napthoquinone-D(^{15}N)ANT dimer using double-shot analysis (DSA). In DSA, the logic involves selecting a phase 1 temperature program that mobilizes all non-covalently bound analytes from the sample. Thus in the phase 2 (high temperature) analysis, only covalently bound analytes are detected. The minimum phase 1 temperature that meets these criteria will henceforth be termed the "transition temperature". Figure 3 graphically illustrates this strategy. For the naphthoquinone-D(^{15}N)ANT dimer, the EGA-derived transition temperature was determined to be approximately 310°C. Further refinement of the transition temperature was conducted using double-shot analysis. In DSA, a transition temperature of 340°C allowed for the complete desorption of the added monomeric and for a partial desorption of the covalently bound forms of DANT. Data in Figure 4 clearly demonstrate the ability of DSA to distinguish between added monomeric 2,4-D(^{14}N)ANT and covalently bound 2,6-D(^{15}N)ANT. Phase 1 (desorption) was conducted from 100-340°C, and the chromatogram displayed peaks corresponding to both added, monomeric and covalently bound forms. The phase 2 (pyrolysis)

chromatogram (Figure 4) only showed a peak corresponding to covalently bound 2,6-D(^{15}N)ANT.

Table 1 shows EGA results obtained from the analyses of reduced forms of TNT covalently bound to model soil compounds (12). Desorption temperatures for the added monomeric and synthesized covalently bound compounds are shown. Background levels of key ions made it difficult to ascertain the exact start and end points of desorption of ADNT and DANT in some of the more complex matrices. EGA results were used to develop DSA chromatograms for other covalently bound standards. Results from their analysis, specifically the complex between humic acid and 4-(^{15}N)ADNT, further confirmed the applicability of DSA to the analysis of monomeric and covalently bound ADNT and DANT in complex matrices (see Supporting Information).

A variety of soil samples from TNT-contaminated sites were analyzed by Py-GC/MS. Low efficiency of DANT and ADNT elution impaired their detection in many instances. However, principles established with our model complexes were successfully applied to a highly TNT-contaminated soil from the Louisiana Army Ammunition Plant (LAAP). Single-shot analysis of the soil is shown in Figure 5, where significant peaks corresponding to TNT and its reduced forms (including: 2- and 4-ADNT, and 2,4-DANT) were seen. Clearly, portions of the original TNT contamination had undergone nitro- group reduction by naturally occurring biotic and/or abiotic processes. As a preparatory step in determining whether the reduced TNT molecules formed covalent bonds with the soil organic matter EGA was conducted, and the results are shown in Table 2. The EGA data showed, similar to results seen in Table 1, that added, monomeric forms of ADNT and DANT eluted at lower temperatures than contaminant compounds. Similar to more complex model matrices (e.g., humic acid; Supporting

Information), the ubiquitous presence of ions characteristic of ADNT and DANT in soil occasionally led to incomplete information on the thermal desorption of the contaminating as well as added, monomeric forms of ADNT and DANT.

Based on EGA data for TNT in the absence (line 1, Table 1) and presence (line 4, Table 2) of soil, we expected TNT to elute from the LAAP soil in the range of 122 to 143°C. Surprisingly, the TNT contaminant pool (unlabeled) eluted at nearly twice this temperature (line 4, Table 2). For this reason, we tentatively categorized the contaminated pool of TNT as "polymeric" (Table 2). We strongly suspect that this appearance of a bound, non-thermally labile pool of TNT is an artifact of the high ambient concentrations of TNT in the sample. Elution from the matrix may have been kinetically constrained under the chosen experimental conditions. Interpretation of Py-GC/MS data, especially carry over from one analytical phase to another is discussed below.

Double-shot analysis was employed to determine the status of DANT in LAAP soil. The transition temperature for DANT was determined to be 210°C. The phase 1 chromatogram in Figure 6 displayed a 2,6-DANT peak containing ions characteristic of added ^{15}N-labeled 2,6-DANT. No ion abundances characteristic of the 2,6-DANT (^{14}N) contaminant were seen above background levels. Furthermore, 2,4-DANT (with a retention time of 18.5 min.) was below detection in the phase 1 assay. In contrast, the phase 2 chromatogram showed a 2,4-DANT peak containing ions characteristic of the contaminant and no significant levels of key ions characteristic of ^{15}N-labeled 2,6-DANT. Therefore, our analysis suggested the entire pool of 2,4-DANT in this soil had undergone covalent bond formation under field conditions. These data show that field conditions at the LAAP fostered TNT reduction followed by covalent bond formation to soil organic matter.

Double-shot analysis was also used to determine the status of TNT (Figure 7) and ADNT (Figure 8) in this contaminated soil. A transition temperature of 230°C was found to be appropriate for both of these compounds in this soil. Phase 1 chromatograms revealed elution of both the laboratory-added ^{15}N-labeled and the non-labeled (^{14}N) monomeric forms of the contaminants. In contrast, phase 2 chromatograms showed no traces of peaks containing the added ^{15}N-labeled compound ions above background levels. Instead, the phase 2 peaks only contained ions characteristic of the contaminants, TNT (Figure 7) and both 2- and 4-ADNT (Figure 8). The peak areas recovered in phase 2, relative to phase 1, were 1.6%, 340%, and 25%, for TNT, 4-ADNT, and 2-ADNT, respectively.

Two extraction- based procedures were aimed at scrutinizing the phase 2 results of DSA of the contaminated LAAP soil. In the first procedure, double-shot analysis of methanol-extracted soil was performed. Solvent extraction of unbound TNT-derived compounds has previously been reported (18). We confirmed that our procedure (10 sequential extractions) successfully removed non-covalently bound TNT, ADNT, and DANT added to uncontaminated soil. Results (not shown) from the double-shot analysis of the methanol-extracted LAAP soil were consistent with results from the DSA of unextracted soil (Figure 7): TNT and ADNT were found in both phases, while DANT was only observed in the phase 2 chromatogram. Because there is no known mechanism for covalent bond formation by TNT moieties, we suspected that the small amount of TNT present in the phase 2 chromatogram of Figure 7 was simply carry over resulting from imperfect thermal elution of the large mass of TNT present in phase 1. To investigate this possibility, and to augment our data with information about hydrolyzable TNT derivatives, we sought a second analysis of the soil from an independent laboratory (P. Thorne, Applied Research Associates). The second analytical procedure used HPLC to determine TNT,

ADNT, and DANT in acetonitrile-extracts of the soil before and after base/acid hydrolysis. The base/acid treatment is able to release ADNT and DANT after an initial stage of covalent bond formation, but not after formation of second-stage nonhydrolyzable bonds (20). Results confirmed a total solvent-extractable TNT concentration of approximately 5000 ppm. Trace amounts of ADNT and TNT were found after hydrolysis (data not shown); the latter was interpreted to be residue from the first extraction. The HPLC assay failed to detect DANT in any treatment. Because the hydrolysis/HPLC assay was not able to access the pool of non-hydrolyzable residues, the results neither supported nor conflicted with conclusions from Py-GC/MS that ADNT and DANT in the LAAP soil were covalently bound.

Discussion

Soil organic matter contains significant concentrations of quinone groups. These are one of the many types of condensation sites for aromatic amines, and serve as excellent matrices for modeling compound binding to soil (20, 21). For this reason, the synthetic naphthoquinone-DANT dimer was employed as a standard to test the applicability of double-shot Py-GC/MS for the detection of covalently bound compounds in soil. Both Table 1 and Figure 2 show the overlap in the desorption profiles of monomeric and covalently bound compounds. The problem of overlap has not been addressed in previous reports using Py-GC/MS. Published studies conducted with EGA have either used relatively simple mixtures with compounds that have distinct desorption profiles or the occurrence of compound overlap was not a cause for concern (16, 19). Our goal was to conclusively demonstrate the existence of covalently bound, reduced forms of TNT in soil. Therefore, we designed transition temperatures for our double-shot analyses that allowed all monomeric compounds and some covalently bound compounds to elute

in phase 1. The result of this strategy allowed phase 2 of the DSA to document covalently bound species if they were present (Figure 4).

Evolved gas analysis is a sound initial step in determining the thermal desorption and pyrolytic characteristics of contaminants in a variety of matrices. Perusal of the temperature values in Tables 1 and 2 reveals apparent matrix and kinetic effects on the thermal elution of monomeric compounds. Generally, pure TNT, ADNT, and DANT evolved at lower temperatures than when the compounds were added to model matrices. It is possible that monomeric forms of TNT, ADNT, and DANT, when added to standard matrices, underwent some form of reversible, non-covalent binding (e.g., Table 2). Prior research into bound residues in soil has revealed that reactive compounds can exist in four different forms: completely unbound, sequestered, non-covalently bound, and covalently bound (23, 24). It is likely that these forms can be individually analyzed by the thermal desorption/pyrolysis approach described here. Non-covalently bound and sequestered forms of TNT, ADNT, and DANT are likely to have higher desorption temperatures than completely unbound forms.

Complex matrices such as humic acid and soil offer multiple sites for covalent binding reactions by aromatic amines to occur. In the absence of catalysts, aromatic amines undergo nucleophilic addition reactions with quinone and other carbonyl groups in soil organic matter to form both heterocyclic and nonheterocyclic condensation products. Of primary importance are the 1,4-nucleophilic addition reactions of aromatic amines with quinones to form aminohydroquinone and aminoquinone adducts, and the 1,2-addition of aromatic amines with quinones to form imines. Heterocyclic nitrogen structures can result from a number of both inter- or intramolecular condensation reactions of aromatic amines with carbonyl groups. Phenol oxidase enzymes and metals can catalyze covalent binding by effecting free radical coupling

reactions between aromatic amines and soil organic matter, or by creating additional substrate sites within soil organic matter for subsequent nucleophilic addition by the amines (21, 25). It is possible that a variety of binding sites and diversity in the formation of covalent bonds has an effect on both desorption and pyrolysis temperatures.

Py-GC/MS (like all other sequential extraction-based procedures) has the potential for "carry over" between steps when analytes are abundant. The peak area for TNT found in phase 2 of the DSA (Figure 7) was <2% of that in phase 1. Given the high TNT content of the LAAP soil, a 100% efficiency of thermal elution in phase 1 should not be expected. It would seem prudent to establish a standard (perhaps 4% of the phase 1 peak area) below which any recovered analyte should be considered “noise”. In this regard, it is crucial to consider that ADNT and DANT found in phase 2 of DSA may be improperly interpreted as evidence for covalent binding to soil. We rule out carry over from phase 1 to phase 2 for 2,4-DANT (Figure 6) because the compound was entirely absent in phase 1. We rule out carry over from phase 1 to phase 2 for ADNT (Figure 8) because the mass of 2-AD^{15}NT added to the soil that fully eluted during phase 1 was large relative to the contaminant ^{14}N-2-ADNT pool detected in phase 2. Thus, when uniform criteria are applied, Py-GC/MS appears able to distinguish between TNT related compounds that can and cannot form covalent bonds with soil organic matter.

The presence of two amino moieties on the DANT molecule provides a highly reactive nucleophilic character as well as two sites available for covalent bond formation (22). This would predict a relatively high degree of binding of DANT to soil. Double-shot analysis (Figure 6) supported this notion. The data clearly demonstrated the entire pool of 2,4-DANT, eluting exclusively in phase 2 of the LAAP soil, was completely covalently bound. Data supporting this conclusion on the fate of DANT in this soil was seen in the methanol extracted soil experiments.

No DANT was found in phase 1 of the DSA whereas significant amounts were seen in the second phase. Absence of DANT in the base/acid hydrolyzed soil extracts suggests that, over the decades, the DANT has entered a nonhydrolyzable state. Extractions of the soil more rigorous than base/acid hydrolysis, possibly including silylation, may help to explain the results seen here. Silylation of soil has been employed to disrupt the bonds between hydroxyl and carboxyl groups in soil organic matter (11). These bonds form the cage-like structures that are the microsites involved in sequestration (11, 14). Achtnich et al. employed a silylation procedure to determine the fate of TNT in a highly contaminated, anaerobic/aerobic composted soil (11). Subsequent to a methanol extraction, composted soil was more rigorously extracted. Thirty to 73% of the radioactivity (initially added as ^{14}C TNT) remaining in the soil after the methanol extraction was released upon silylation of the soil with trimethylchlorosilane.

We also found ADNT in both phases of the double-shot analysis of the LAAP soil. The single amino group on the ADNT molecules only makes them weak nucleophiles and less likely to form covalent bonds (22). Covalent binding of ADNT to soil organic matter (SOM) is possible, however, and is consistent with published results (12). When the methanol extraction procedures were applied to remove non-covalently bound TNT metabolites from the soil, double-shot analysis found ADNT in both phase one and two.

Even though quartz and metallic surfaces within the pyrolyis instrument had been chemically deactivated, recovery of TNT and its reduced analytes (i.e., DANT) was inefficient. In addition to LAAP, several other explosives-contaminated soils were examined in our laboratory. Levels of TNT, ADNT, or DANT were apparently below the Py-GC/MS's detection limit (~40 ug/mg soil) because ions characteristic of TNT, ADNT, or DANT could not be confidently resolved. High background ion abundances and/or reactive surfaces in the pyrolyzer

were the probable causes of impaired sensitivity. Derivatization of amino moieties may lead to their improved analysis by our system. Additionally, frequently cleaning and replacement of liners were required to maintain a properly operating pyrolyzer. Over time, pyrolysis of high organic matter-containing matrices left a residue on the quartz pyrolysis tube and in the injector needle. This residual carbon may have also contributed to the decreased sensitivity of the system. Therefore, data presented here cannot be considered quantitative.

In addition to pyrolysis studies of covalently bound and monomeric forms of reduced TNT , ongoing research in our laboratory is focusing on structurally more complex compounds formed from the incorporation of TNT into the SOM. Recent studies by Bruns-Nagel et al. (13), Achtnich et al. (28), and Thorn et al. (27), employing ^{15}N NMR, have observed TNT reduction products incorporated into anaerobic/aerobic digests of amended soils and aerobically composted soils. In the case of aerobically composted soil, the reduced TNT amines became incorporated into the organic matter via aminohydroquinone, aminoquinone, heterocyclic (e.g. indole), and imine bonds, among others (27). Bruns-Nagel et al. (13) found the ^{15}N-labeled TNT was reduced and transformed into heterocyclic compounds, including: imidazoles, indoles, pyrroles, carbazoles, quinolones, anilides, amides and enaminones. Thirty percent of the ^{15}N was associated equally (15% each) with amino functions (i.e. aniline and phenylamines) and covalently bound structures (i.e. nitroaniline derivatives, anilinohydroquinones, phenoxazones and hydrazines). The data presented by Thorn et al. (27) confirmed and further elaborated on these findings. In particular, Thorn et al. (27) observed 28-29% of the (^{15}N) nitrogen in the fulvic acid and humin fractions occurred as imine nitrogens. GC/MS analysis of pyrolyzed TNT-contaminated soil may be able to identify peaks corresponding to these products of

transformation and incorporation. In this way, additional information about the fate of TNT in the environment may be discovered.

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Table 1. Evolved Gas Analysis (EGA) showing the temperature at which percentages of monomeric and/or polymeric forms of TNT, ADNT, and DANT were released from various model matrices. ^{15}N-labeled ADNT and DANT[1] covalently bound to model matrices with and without added (^{14}N) monomeric forms of ADNT or DANT were analyzed[2].

	"Monomer" Elution Temp. (°C)					"Polymer" Elution Temp. (°C)				
	1.00%	**10%**	**50%**	**90%**	**100%**	**1.00%**	**10%**	**50%**	**90%**	**100%**
Individual Compounds										
TNT	71	77	94	119	143	SA	SA	SA	SA	SA
2-ADNT	106	115	139	157	182	SA	SA	SA	SA	SA
2,6-DANT	104	110	130	169	221	SA	SA	SA	SA	SA
Naphthoquinone (Matrix)										
Blank + 2-ADNT Spike	142	nd	183	nd	235	SA	SA	SA	SA	SA
Blank + 2,6-DANT Spike	139	nd	170	nd	225	SA	SA	SA	SA	SA

Bound 2.6-DANT	SA	SA	SA	SA	SA	248	271	313	335	404
Bound 2,6-DANT + 2,4-DANT Spike	145	nd	189	nd	308	175	272	320	341	433
Humic Acid (Matrix)										
Blank + 2-ADNT Spike	119	nd	138	nd	189	SA	SA	SA	SA	SA
Blank + 2,4-DANT Spike	116	nd	128	nd	176	SA	SA	SA	SA	SA
Bound 2-ADNT	SA	SA	SA	SA	SA	210*	nd	331*	nd	382*
Bound 4-ADNT	SA	SA	SA	SA	SA	200*	nd	nd	nd	453*
Bound 2,4-DANT	SA	SA	SA	SA	SA	364*	nd	nd	nd	402*
Bound 2-ADNT + ADNT Spike	142	nd	171	nd	SL	142	nd	298	nd	SL
Bound 4-ADNT + ADNT Spike	123	nd	134	nd	218	118*	nd	414*	nd	513*
Bound 2,4-DANT + DANT Spike	117	nd	124	nd	165	324*	nd	nd	nd	436*
Peat (Matrix)										
Blank + 2-ADNT Spike	130	nd	145	nd	228	SA	SA	SA	SA	SA
Blank + 2,4-DANT Spike	127	nd	141	nd	185	SA	SA	SA	SA	SA
Bound 2-ADNT	SA	SA	SA	SA	SA	138	141	185	323	SL

Bound 4-ADNT	SA	SA	SA	SA	SA	143	148	181	221	266
Bound 2,4-DANT	SA	SA	SA	SA	SA	190	nd	227	nd	SL
Bound 2,6-DANT	SA	SA	SA	SA	SA	159	171	253	285	SL
Bound 2-ADNT + ADNT Spike	117	nd	138	nd	207	125	131	168	231	302
Bound 4-ADNT + ADNT Spike	132	138	155	203	229	130	144	181	216	269
Bound 2,4-DANT + DANT Spike	123	nd	156	nd	259*	122	nd	142	nd	SL
Bound 2,6-DANT + DANT Spike	132	nd	156	nd	SL	153	174	277	330	SL
Sawdust (Matrix)										
Blank + 2-ADNT Spike	122	nd	135	nd	228	SA	SA	SA	SA	SA
Blank + 2,4-DANT Spike	125	nd	132	nd	186	SA	SA	SA	SA	SA
Bound 2,4-DANT	SA	SA	SA	SA	SA	284	nd	362	nd	457
Bound 2,4-DANT + DANT Spike	125	nd	140	nd	SL	258	nd	362	nd	SL

Abbreviations: TNT = trinitrotoluene; ADNT = amino-dinitrotoluene; DANT = diamino-nitrotoluene; SA = signal absent; SL = signal lost in the background noise; nd = not determined. * = ions characteristic of ADNT and DANT not well observed; temperature values were estimated. “Blank” signifies uncomplexed (no bound ADNT or DANT) matrix.

[1] NMR spectra of all ^{15}N-labeled materials were previously reported (12).

[2] ”Monomer” elution was tracked by monitoring the abundance of key molecular ions above background levels. Key ions for TNT correspond to *m*/*z* = 210, 89, 63; ADNT *m*/*z* = 180, 197, 104; DANT *m*/*z* = 167, 121, 94. Key ions for $T^{15}NT$ correspond to *m*/*z* = 213, 89, 63; (^{15}N)ADNT *m*/*z* = 181, 198, 105; D(^{15}N)ANT *m*/*z* = 169, 123, 95.

Table 2. Evolved Gas Analysis of a TNT-contaminated soil showing the temperature at which percentages of monomeric and/or polymeric forms of TNT, ADNT, and DANT were released from the matrix. Soil was analyzed with and without added ^{15}N-labeled monomeric forms of TNT, ADNT, and DANT. [1]

	"Monomer" Elution Temp. (°C)					**"Polymer" Elution Temp. (°C)**				
	1.00%	**10%**	**50%**	**90%**	**100%**	**1.00%**	**10%**	**50%**	**90%**	**100%**
Compounds										
TNT	SA	SA	SA	SA	SA	147	168	211	252	277
ADNT	SA	SA	SA	SA	SA	170	SL	211	SL	283
DANT	SA	SA	SA	SA	SA	172	nd	213	nd	296
TNT + TNT monomer	105	nd	110	nd	122	108	nd	179	nd	272
ADNT + ADNT monomer	111	nd	123	nd	155	110	SL	181	SL	250
DANT + DANT monomer	118	nd	128	nd	165	132	nd	181	nd	235

Abbreviations: TNT = trinitrotoluene; ADNT = amino-dinitrotoluene; DANT = diamino-nitrotoluene; SA = signal absent; SL = signal lost in the background noise; nd = not determined.

[1] ”Monomer” elution was tracked by monitoring the abundance of key molecular ions above background levels. Key ions for TNT correspond to *m/z* = 210, 89, 63; ADNT *m/z* = 180, 197, 104; DANT *m/z* = 167, 121, 94. Key ions for $T^{15}NT$ correspond to *m/z* = 213, 89, 63; (^{15}N)ADNT *m/z* = 181, 198, 105; D(^{15}N)ANT *m/z* = 169, 123, 95

Figure legends

Figure 1. Single-shot analysis of dimeric 1,2-naphthoquinone-2,6-di(^{15}N)amino-nitrotoluene complex. Pyrolysis at 400°C for 0.1 min. Structure of dimer is shown at right.

Figure 2. Evolved Gas Analysis (EGA) profile of the 1,2-naphthoquinone-2,6-di(^{15}N)amino-nitrotoluene dimer with added, monomeric 2,4-diamino-nitrotoluene. A) Total ion chromatogram showing low-temperature and high-temperature elution of monomer and dimer. B) Selected ion monitoring of *m*/*z* = 167, parent ion of added, monomeric 2,4-di(^{14}N)amino-nitrotoluene. C) Selected ion monitoring of *m*/*z* = 169, parent ion of dimeric 2,6-di(^{15}N)amino-nitrotoluene.

Figure 3. Theoretical EGA profile of a complex mixture showing the "zone of overlap" between humps containing "monomeric" and covalently bound forms of the same compound. The transition temperature was designed to ensure phase 1 of the double shot analysis contains all of the "monomer", while phase 2 contains only covalently bound forms of the molecule.

Figure 4. Double-shot analysis of 1,2-naphthoquinone-2,6-diamino(^{15}N)-nitrotoluene dimer with added, monomeric 2,4-di(^{14}N)amino-nitrotoluene. Phase 1: 100-340°C. Phase 2: 440°C.

Figure 5. Single-shot analysis of Louisiana Army Ammunition Plant (LAAP) soil. TNT = trinitrotoluene; ADNT = amino-dinitrotoluene; DANT = diamino-nitrotoluene; DNAB = dinitro-aminobenzene. Lower panel magnifies box shown in upper panel.

Figure 6. Double-shot analysis of LAAP soil with added, monomeric 2,6-di(^{15}N)amino-nitrotoluene [D(^{15}N)ANT]. Phase 1: 100-210°C. Phase 2: 400°C.

Figure 7. Double-shot analysis of LAAP soil with added, monomeric 2,4,6-tri(^{15}N)nitrotoluene ($T^{15}NT$). Phase 1: 100-230°C. Phase 2: 400°C.

Figure 8. Double-shot analysis of LAAP soil with added, monomeric 2-(^{15}N)amino-dinitrotoluene (ADNT). Phase 1: 100-230°C. Phase 2: 400°C.

Figure 1

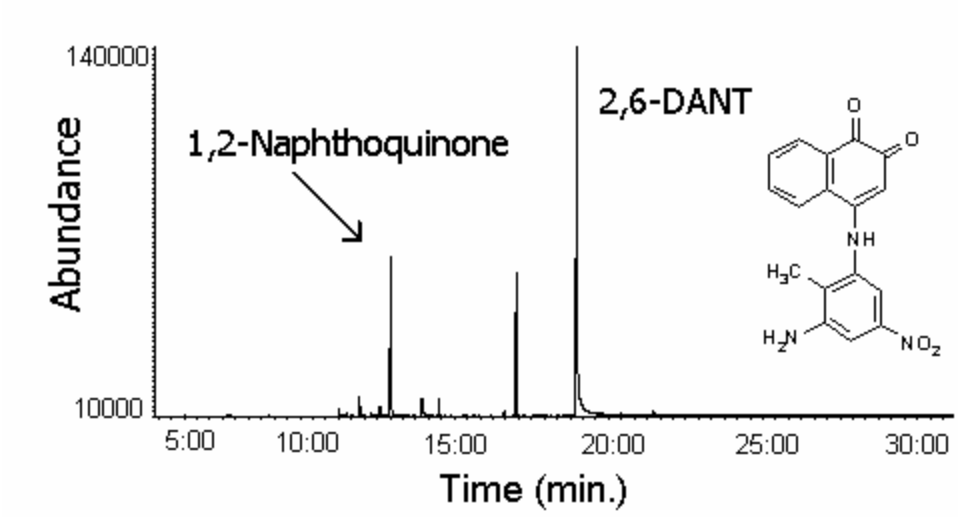
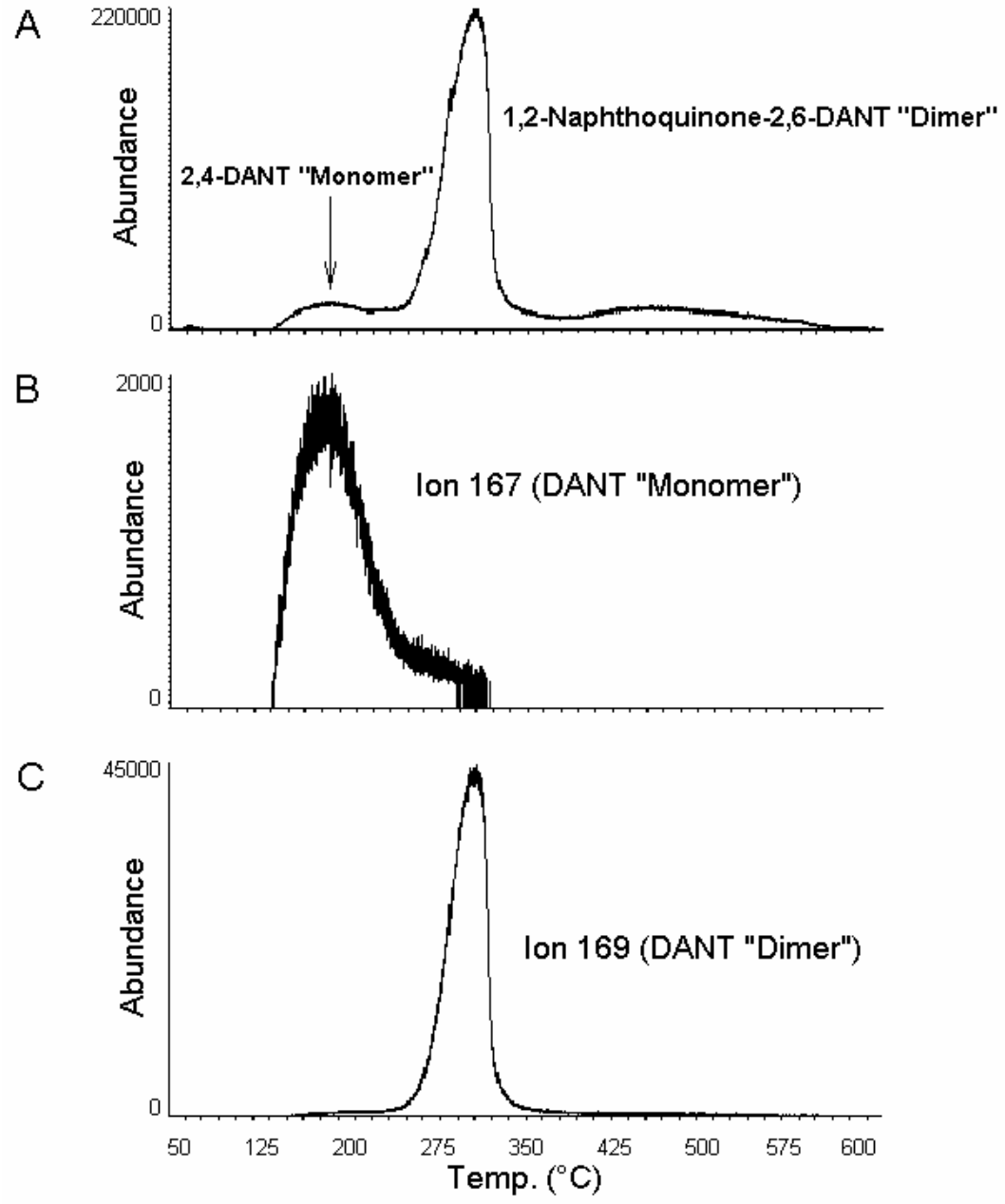

140000
10000
Abundance
1,2-Naphthoquinone
2,6-DANT
5:00
10:00
15:00
20:00
25:00
30:00
Time (min.)
O
O
NH
H3C
H2N
NO2

Figure 2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Abundance
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1,2-Naphthoquinone-2,6-DANT "Dimer"
B
2000
Abundance
0
Ion 167 (DANT "Monomer")
C
45000
Abundance
0
Ion 169 (DANT "Dimer")
50
125
200
275
350
425
500
575
600
Temp. (°C)

Figure 3

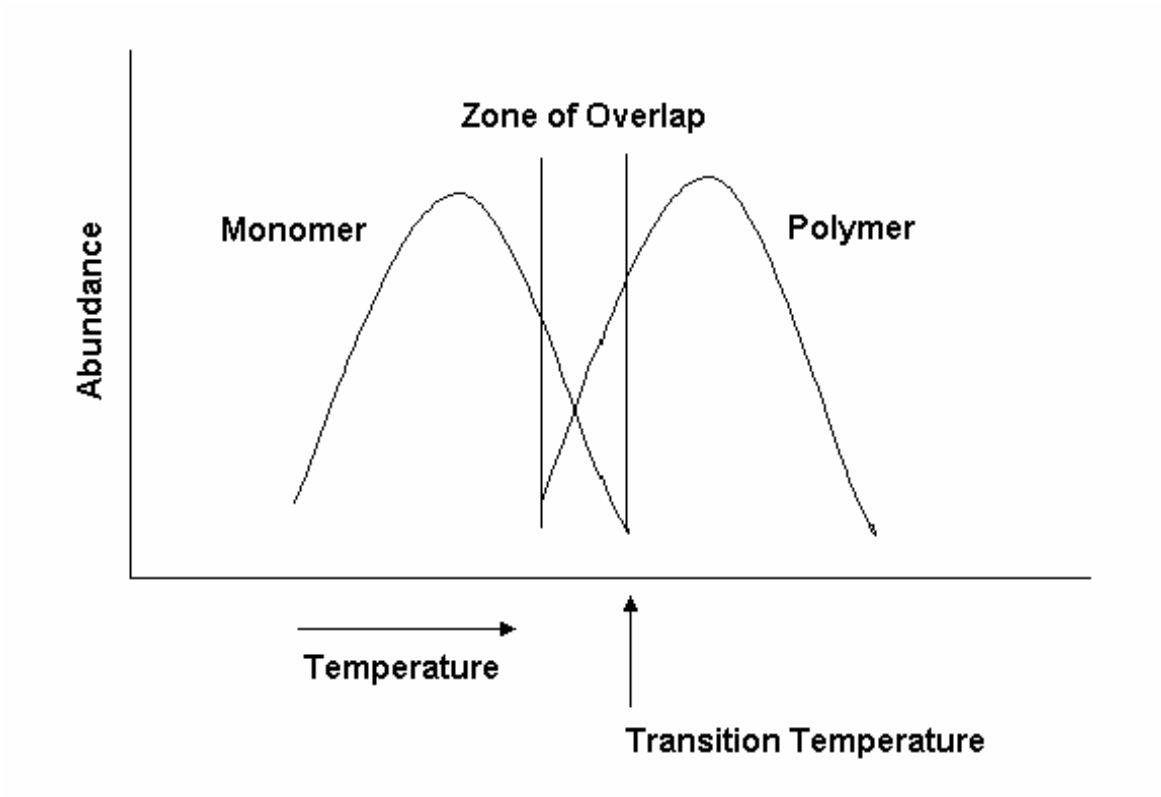
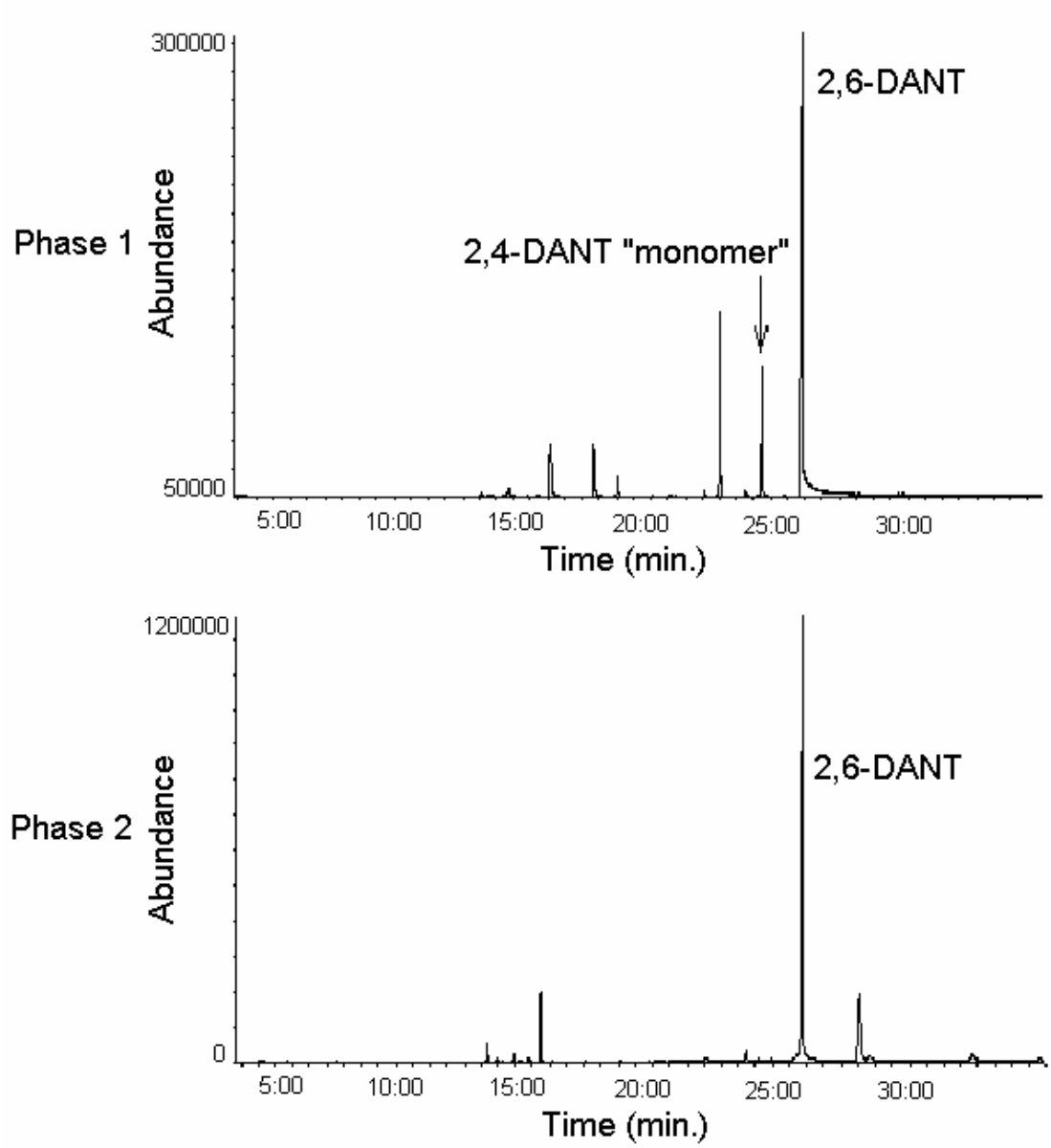

Zone of Overlap
Monomer
Polymer
Abundance
Temperature
Transition Temperature

Figure 4


Phase 1
Abundance
300000
50000
2,6-DANT
2,4-DANT "monomer"
5:00
10:00
15:00
20:00
25:00
30:00
Time (min.)
Phase 2
Abundance
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0
2,6-DANT
5:00
10:00
15:00
20:00
25:00
30:00
Time (min.)

Figure 5

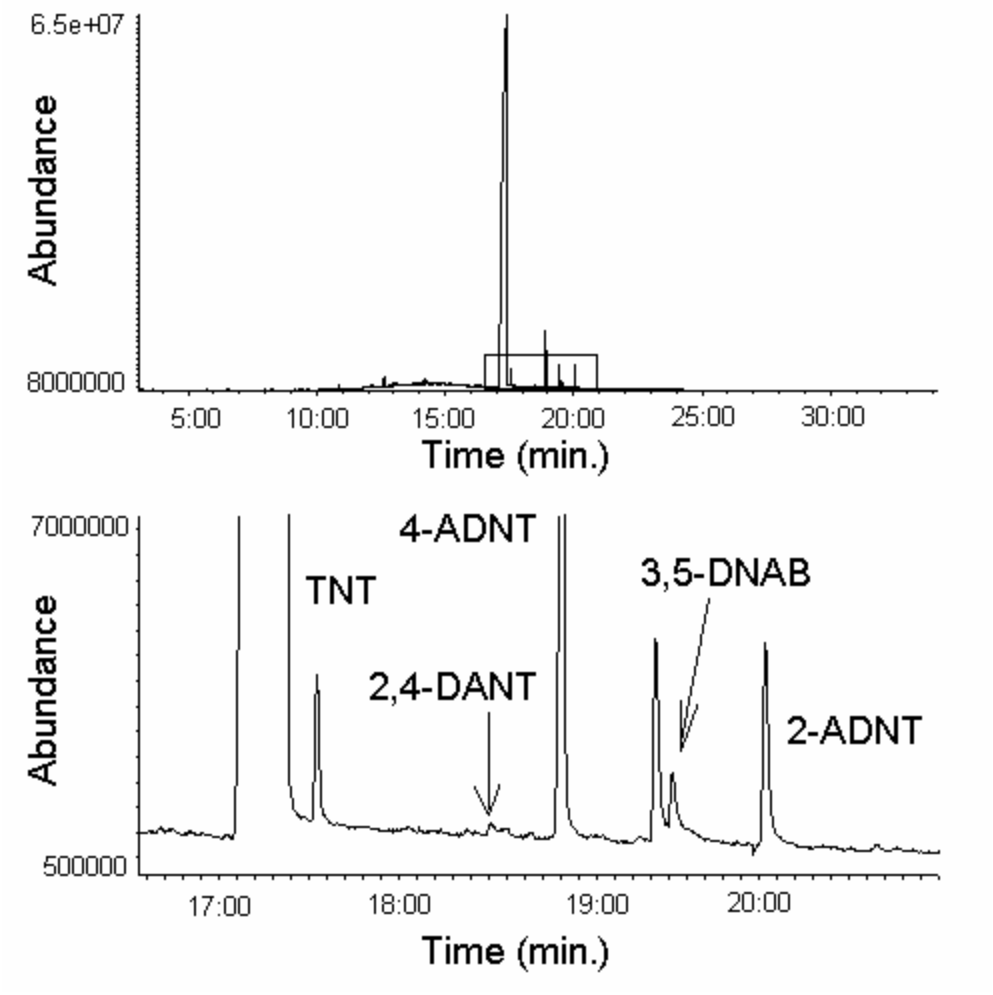

6.5e+07
Abundance
8000000
5:00
10:00
15:00
20:00
25:00
30:00
Time (min.)
7000000
Abundance
500000
4-ADNT
TNT
3,5-DNAB
2,4-DANT
2-ADNT
17:00
18:00
19:00
20:00
Time (min.)

Figure 6

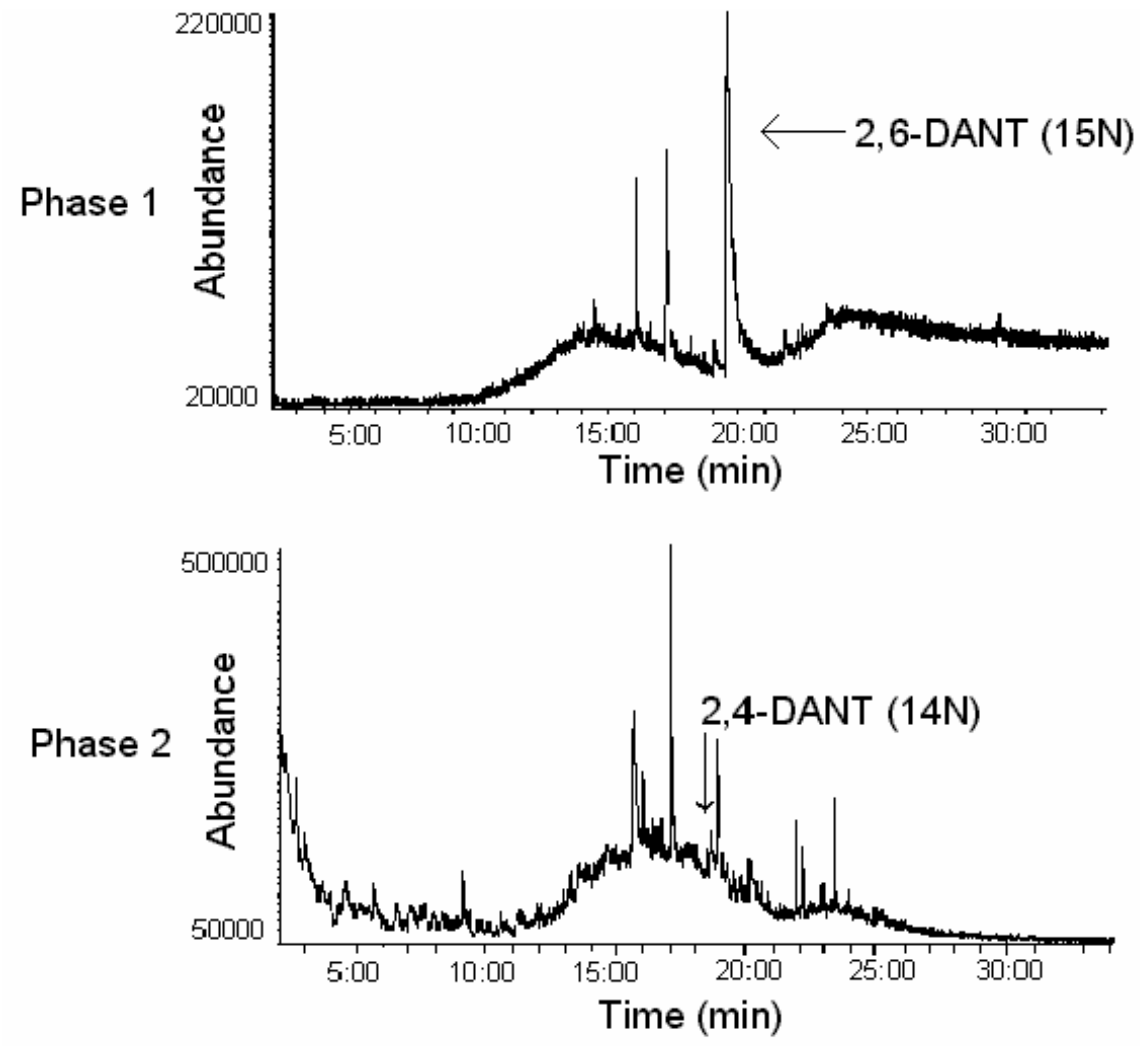
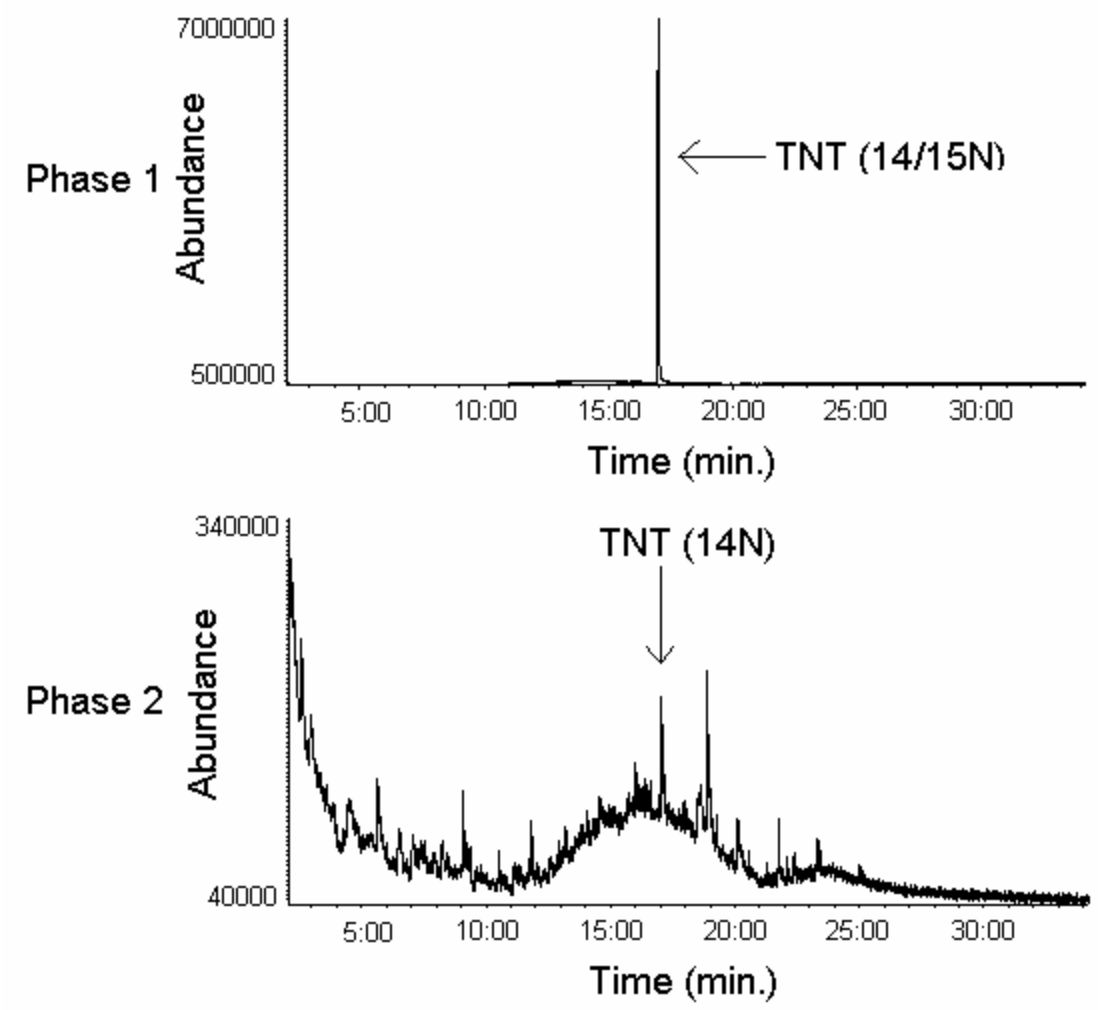

Phase 1
Abundance
220000
20000
2,6-DANT (15N)
5:00
10:00
15:00
20:00
25:00
30:00
Time (min)
Phase 2
Abundance
500000
50000
2,4-DANT (14N)
5:00
10:00
15:00
20:00
25:00
30:00
Time (min)

Figure 7


Phase 1
Abundance
7000000
500000
TNT (14/15N)
5:00
10:00
15:00
20:00
25:00
30:00
Time (min.)
Phase 2
Abundance
340000
40000
TNT (14N)
5:00
10:00
15:00
20:00
25:00
30:00
Time (min.)

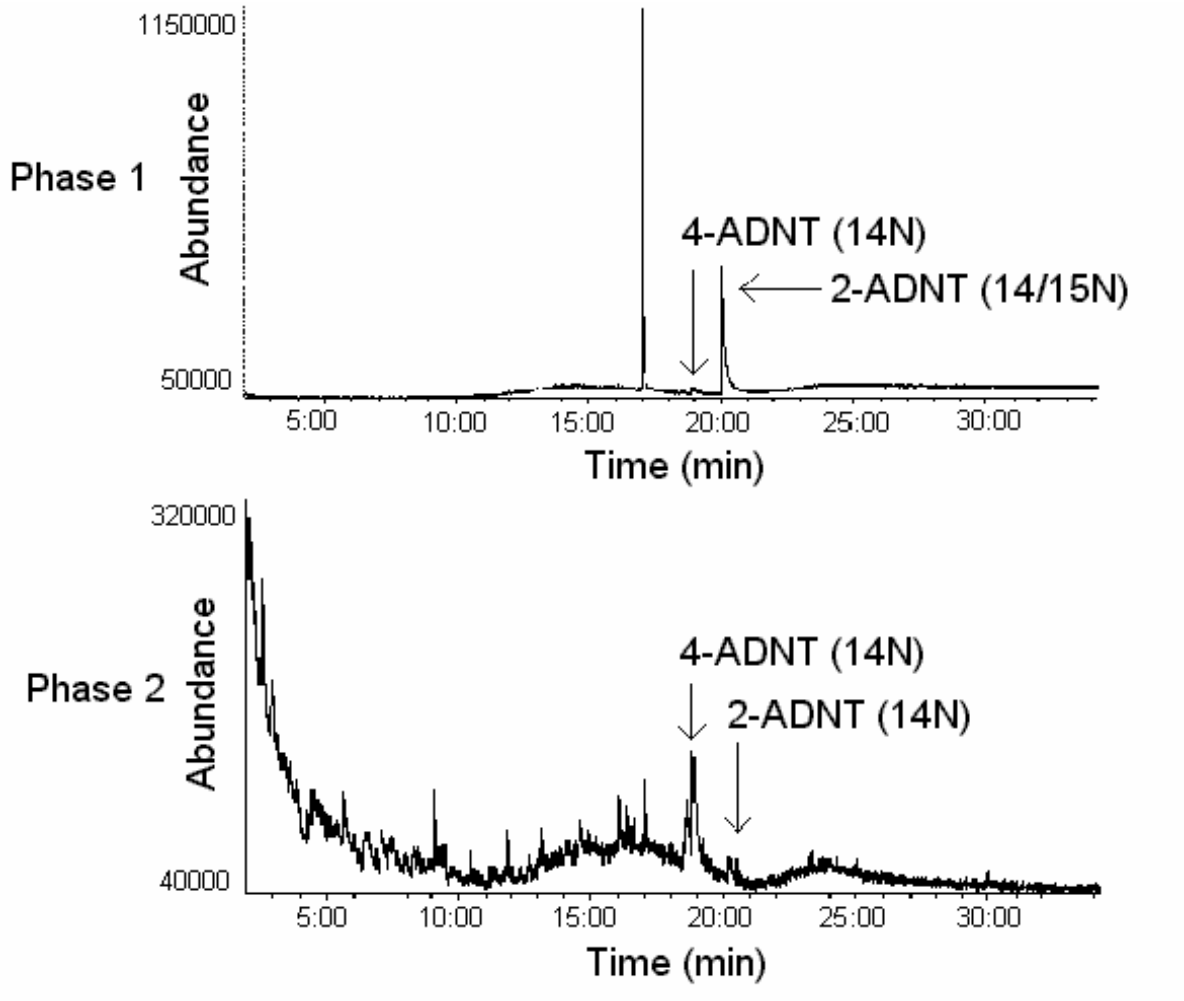

Phase 1
Abundance
1150000
50000
4-ADNT (14N)
2-ADNT (14/15N)
5:00
10:00
15:00
20:00
25:00
30:00
Time (min)
Phase 2
Abundance
320000
40000
4-ADNT (14N)
2-ADNT (14N)
5:00
10:00
15:00
20:00
25:00
30:00
Time (min)

Figure 8

ON-LINE SUPPLEMENTAL MATERIAL FOR THE ARTICLE SUBMITTED TO ENVRIONMENTAL SCINECE AND TECHNOLOGY

Development and Application of Pyrolysis-Gas Chromatography/Mass Spectrometry for the Analysis of Bound Trinitrotoluene Residues in Soil

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Supporting Information

Humic acid, a primary component of soil organic matter reacted with 2-amino(^{15}N)-dinitrotoluene [2-(^{15}N)ADNT] (12), was analyzed by single-shot analysis (SSA), evolved gas analysis (EGA), and double-shot analysis (DSA). Humic acid is more complex and representative of true soil than naphthoquinone (24). Therefore, humic acid provided a more realistic view of the applicability of pyrolysis for the analysis of reduced TNT compounds covalently bound to real-world, explosives-contaminated soil. Figure A displays a SSA-generated chromatogram for the complexed humic acid-2-(^{15}N)ADNT. Discerning a precise transition temperature in results from evolved gas analysis of the humic acid-2-(^{15}N)ADNT

complex was not possible (Table 1). This was due to high background levels of relevant ions in the humic acid matrix. Based on previous results with monomeric ADNT (Table 1), it was expected that the transition temperature would fall between 200° and 250°C. Initial refinement of the transition temperature employed the DSA of clean (no TNT metabolites) humic acid spiked with monomeric 2-ADNT. Results from this analysis (Figure B) revealed a transition temperature of 225°C. This value was confirmed by analyzing a synthetic humic acid-2-(^{15}N)ADNT complex in which 2-(^{15}N)ADNT was previously determined to be covalently bound to the humic acid. Due to the unavailability of the 4-amino isomer of ADNT, we determined the complete removal of added, monomeric 2-ADNT (^{14}N) based on ion abundances. Figure C shows phase 1 results from the double-shot analysis of the humic acid-2-(^{15}N)ADNT complex with added, monomeric 2-ADNT (^{14}N). Key ions for the ^{15}N polymeric and ^{14}N monomeric forms are m/z = 181 and 180, respectively. Mass spectral analysis of the 2-ADNT peak at 21.9 minutes found ions 180 and 181 at levels above those normally seen in the background. The phase 2 chromatogram is seen in Figure D. Examination of the 2-ADNT peak showed that only the parent ion corresponding to the complexed 2-(^{15}N)ADNT (m/z = 181) was seen above background levels. Abundances of the parent ion characteristic of monomeric 2-ADNT (m/z = 180) were at background levels. These results further confirmed the ability of Py-GC/MS to distinguish between monomeric and covalently bound forms of ADNT and DANT in model systems.

Supplemental Data Figure Legends

Figure A. Single-shot analysis of 2-(^{15}N)amino-dinitrotoluene (2-ADNT) covalently bound to humic acid. Pyrolysis at 400°C for 0.1 minutes.

Figure B. Double-shot analysis of humic acid with added, monomeric 2-amino-dinitrotoluene (2-ADNT). Phase 1: 100-225°C. Phase 2: 400°C.

Figure C. Phase 1 of the double-shot analysis of the synthetic humic acid-2-(^{15}N)amino-dinitrotoluene (2-ADNT) complex and added, monomeric 2-(^{14}N)amino-dinitrotoluene. Thermal desorption from 100-225°C was employed. Top panel shows total ion chromatogram. Lower panels display selected ion monitoring of ions 180 and 181, which are characteristic of the (^{14}N) and (^{15}N) 2-ADNT compounds, respectively.

Figure D. Phase 2 of the double-shot analysis of the synthetic humic acid-2-(^{15}N)amino-dinitrotoluene (2-ADNT) complex and added, monomeric 2-(^{14}N)amino-dinitrotoluene. Pyrolysis at 400°C. Top panel shows total ion chromatogram. Lower panels display selected ion monitoring of ions 180 and 181, which are characteristic of the (^{14}N) and (^{15}N) 2-ADNT compounds, respectively.

Figure A

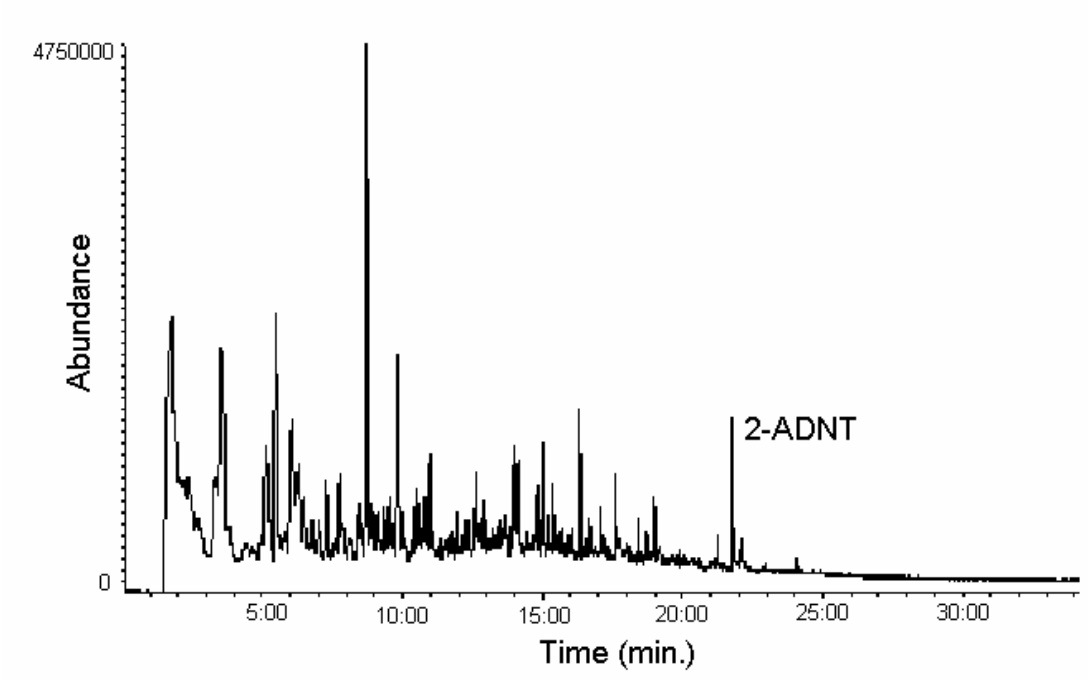

4750000
0
Abundance
2-ADNT
5:00
10:00
15:00
20:00
25:00
30:00
Time (min.)

Figure B

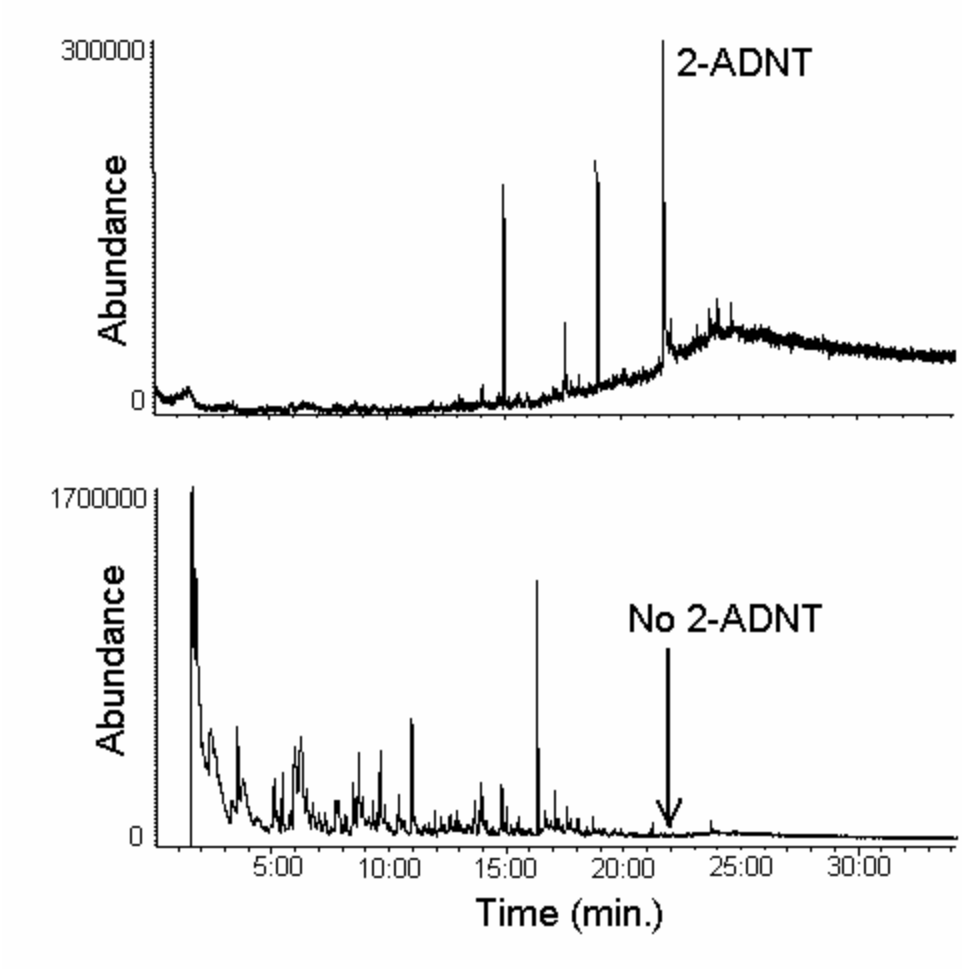

300000
Abundance
0
2-ADNT
1700000
Abundance
0
No 2-ADNT
5:00
10:00
15:00
20:00
25:00
30:00
Time (min.)

Figure C

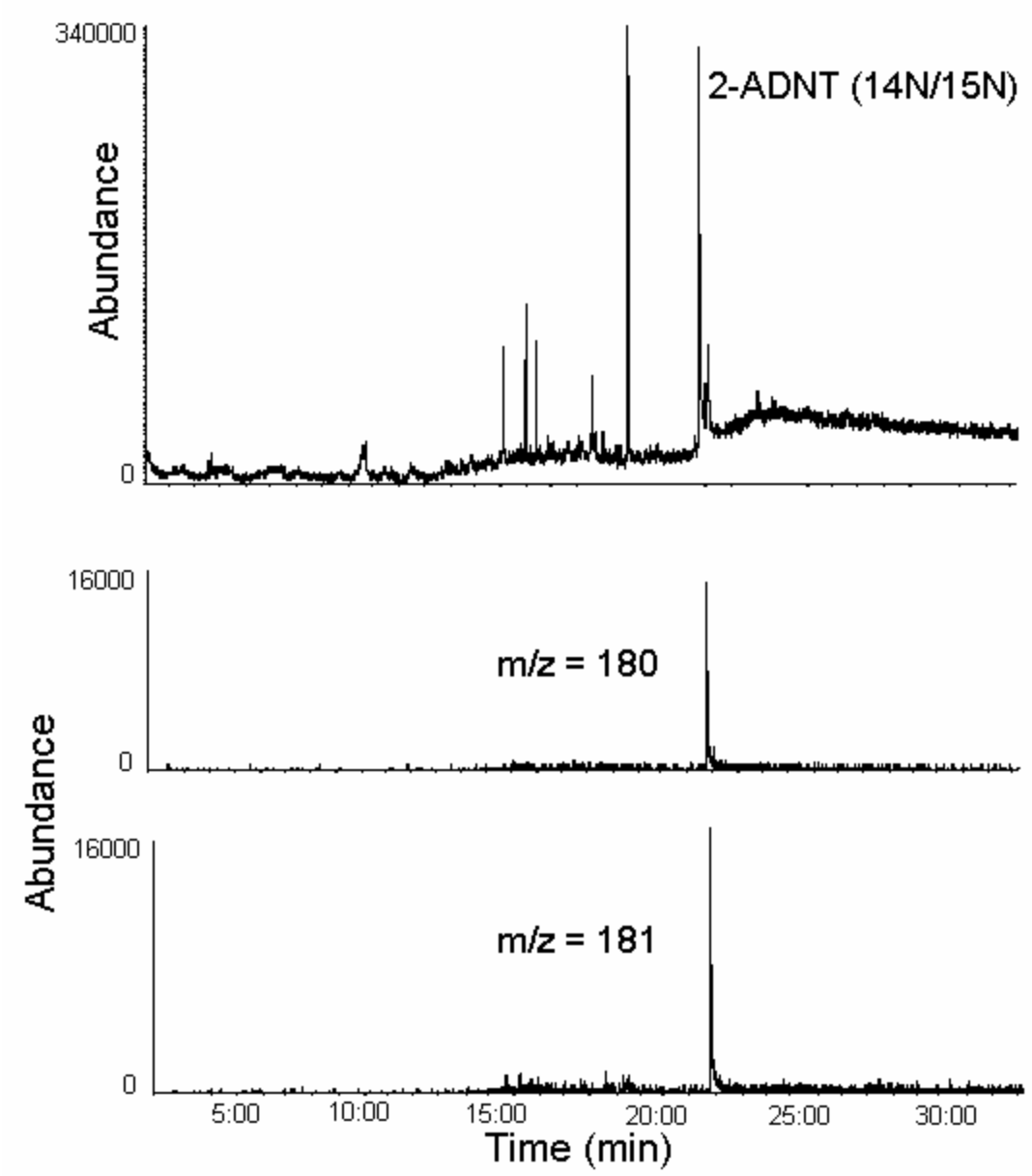
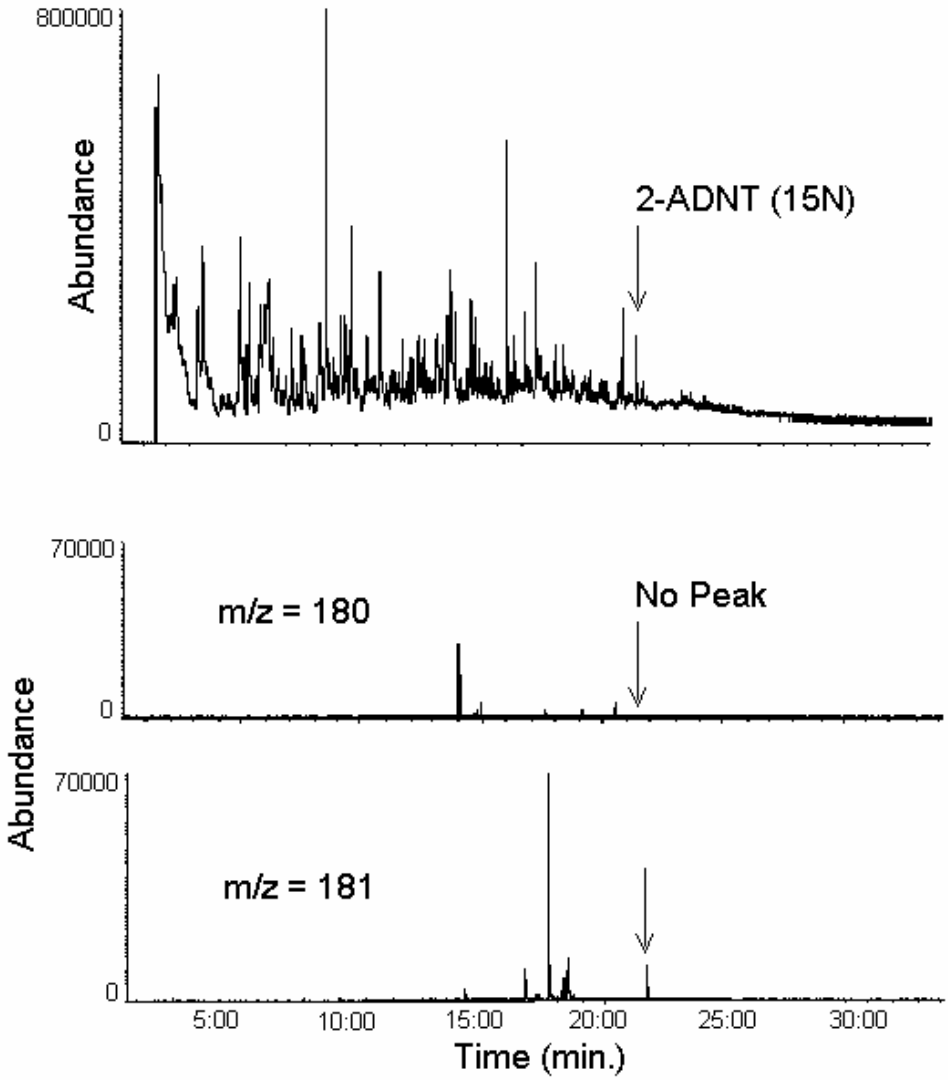

340000
Abundance
0
2-ADNT (14N/15N)
16000
m/z = 180
0
Abundance
16000
m/z = 181
0
5:00
10:00
15:00
20:00
25:00
30:00
Time (min)

Figure D


800000
Abundance
0
2-ADNT (15N)
70000
m/z = 180
No Peak
0
Abundance
70000
m/z = 181
0
5:00
10:00
15:00
20:00
25:00
30:00
Time (min.)

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