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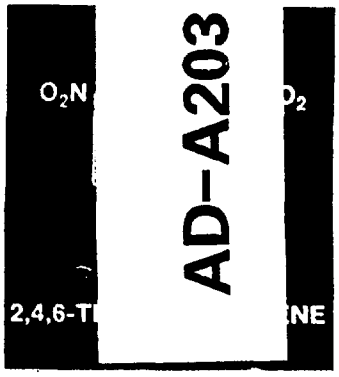

TECHNICAL REPORT EL-88-20

PLANT UPTAKE OF 2,4,6-TRINITROTOLUENE, 4-AMINO-2,6-DINITROTOLUENE, AND 2-AMINO-4,6-DINITROTOLUENE USING ^{14}C-LABELED AND UNLABELED COMPOUNDS

by

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Final Report

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Plant uptake of 2,4,6-trinitrotoluene (TNT) and two of its principal degradation products, 4-amino-2,6-dinitrotoluene (4ADNT) and 2-amino-4,6-dinitrotoluene (2ADNT), were investigated. TNT, 4ADNT, and 2ADNT were added to two soils: Tunica silt and Sharkey clay. The addition rate was 80 ug of the respective treatment compound per gram of soil on an oven-dry weight basis. TNT- and 4ADNT-treated soils received both ^{14}C-labeled and unlabeled compounds. 2ADNT-treated soils received only unlabeled 2ADNT.

Each treatment compound was dissolved in acetone and applied to a 360-g aliquot of soil containing sufficient water to produce an easily mixed slurry. Once well mixed, the treated soil aliquots were poured into flat pans and allowed to air dry. When dry, the caked soil surface was broken up by grinding with a mortar and pestle before the soil was used. Larger soil batches (15,000 g) were prepared in a twin-shell dry soil blender

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by adding fertilizers, lime, and the treated soil aliquot and allowing the soil to mix for 20 min. Controls of both soil types were prepared in the same way except that treatment compounds were omitted.

Treated and control soil batches were potted in the standard (US Army Engineer Waterways Experiment Station) plant bioassay in five replicates per treatment. Soil was moistened to 0.03 to 0.05 MPa. After a 20-day equilibration period, each pot was planted with three sprouted tubers of *Cyperus esculentus*. Plants were allowed to grow in a greenhouse for 45 days before harvesting. Soils were sampled at the time of planting and at the time of harvesting. Soils and plants were extracted and analyzed by gas liquid chromatography and liquid scintillation. The ^{14}C-treated soils were also analyzed by complete combustion in a carbon train.

Results indicated that TNT was degraded in the soil to 4ADNT and, to a lesser extent, to 2ADNT. 4ADNT and 2ADNT remained predominant in 4ADNT- and 2ADNT-treated soils, respectively. Plant uptake was greatest from 4ADNT-treated silt, but also occurred from TNT-treated silt and clay. No plant uptake of 2ADNT was observed.

Percent recoveries of treatment compounds from soils by solvent extraction were much lower than expected. Recoveries obtained by complete combustion analysis were higher. Values were 37.62 and 68.85 percent for TNT-treated silt and clay, respectively, and 32.47 and 34.34 percent for the 4ADNT-treated silt and clay, respectively. Low recoveries by extraction are at least partially explained by adsorption, which limited the amount of the treatment compound that was extractable. However, total mass balance over all treatments at the time of plant harvest averaged only slightly over 50 percent. Another mechanism that possibly explains low recoveries is loss of treatment compounds by photodecomposition or microbial degradation followed by volatilization of decomposition products from the soils. Evidence from the literature supports this possibility.

Keywords: Cyperus esculentus
Contaminants
Soil Pollution

PREFACE

This report describes a plant uptake study of 2,4,6-trinitrotoluene and two of its degradation products, 4-amino-2,6-dinitrotoluene and 2-amino-4,6-dinitrotoluene, from soils. The study was conducted by the Environmental Laboratory (EL) of the US Army Engineer Waterways Experiment Station (WES), Vicksburg, Miss., for the US Army Biomedical Research and Development Laboratory (USABRDL), Fort Detrick, Frederick, Md. The project was authorized by Intra-Army Order No. 82II2032, Change 4, dated 12 February 1985. The research was conducted during the period March 1985 to August 1987.

The study was conducted by Mrs. Judith C. Pennington of the Plant Bioassay Team at the WES. Technical assistance was received from Team members Mrs. Cynthia L. Teeter and Mr. Mark B. Cooper. Gas liquid chromatography was performed by the Analytical Laboratory Group, Environmental Engineering Division, EL. Assistance with statistics was received from Mr. Dennis L. Brandon of the Ecosystem Research and Simulation Division (ERSD), EL. The report was edited by Mrs. Jessica S. Ruff of the Information Technology Laboratory, WES.

Team Leader for the Plant Bioassay Team during the study was Dr. Bobby L. Folsom, Jr. The study was conducted under the general supervision of Dr. Charles R. Lee, Chief, Contaminant Mobility and Regulatory Criteria Group, Mr. Donald L. Robey, Chief, ERSD, and Dr. John Harrison, Chief, EL. Dr. Howard T. Bausum, USABRDL, was Project Manager.

COL Dwayne G. Lee, EN, was the Commander and Director of WES. Dr. Robert W. Whalin was Technical Director.

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

PLANT UPTAKE OF 2,4,6-TRINITROTOLUENE, 4-AMINO-2,6-DINITROTOLUENE, AND 2-AMINO-4,6-DINITROTOLUENE USING ^{14}C-LABELED AND UNLABELED COMPOUNDS

PART I: INTRODUCTION

Background

1. Since the adoption of 2,4,6-trinitrotoluene (TNT) by the US Army in 1904 as one of two primary bursting charges for ammunitions (Nay, Randall, and King 1974), disposal of TNT waste has been a concern at manufacturing plants and at sites of filling, emptying, and cleaning of bombs and shell casings. Most of the concern has resulted from the past practice of disposing of effluents and other wastes directly into adjacent streams or into unlined ponds. Some effluents contained as much as 50 to 100 ppm TNT (Traxler 1974).

2. Extensive aquatic surveys of streams receiving ammunition wastes were conducted in the 1970s and early 1980s (Fox et al. 1975, Weitzel et al. 1975, Jerger et al. 1976, Sanocki et al. 1976, Stilwell et al. 1976, Sullivan et al. 1977, Putnam et al. 1979). A loss of biological communities downstream from discharges was confirmed. However, TNT could not be implicated exclusively since its breakdown products and other contaminants were also present. The aquatic surveys were limited to water quality, fauna, and algae. Uptake by aquatic macrophytes was not examined. However, toxicity of TNT wastes to duckweed (*Lemna perpusilla*) has been demonstrated by Schott and Worthley (1974), and depression of yields in ryegrass by TNT has been cited by Palazzo and Leggett (1983).

3. The environmental fate of TNT is not well defined. However, photodecomposition and microbial degradation are known to occur in the environment. Burlinson (1980) has proposed a mechanism for photodecomposition of TNT and has identified 1,3,5-trinitrobenzene (TNB) as the principal product forming in natural waters. Microbial decomposition of TNT has been studied with the intention of using microorganisms as a waste treatment alternative for TNT-containing wastes. However, microorganisms were unable to cleave the TNT ring structure. The predominant changes effected by microorganisms were reduction of nitro groups to amino groups, and coupling of rings to produce azoxy compounds (Kaplan and Kaplan 1982).

4. Several of the products of microbial metabolism are environmentally less desirable than TNT (Lee et al. 1975, Ellis et al. 1978). Principal microbial degradation products of TNT found by Burlinson (1980) in natural waters and by Kaplan and Kaplan (1982) in compost were 4-amino-2,6-dinitrotoluene (4ADNT) and 2-amino-4,6-dinitrotoluene (2ADNT). Soil leaching studies have shown that TNT either remained in the soil or was transformed to 4ADNT and 2ADNT (Greene, Kaplan, and Kaplan 1984). Only 4ADNT was detected in leachates.

5. It is possible that TNT and its degradation products may be taken up by plants, enter the food chain, and accumulate in higher animals and man where their toxic effects, like those of many pesticides, may be magnified. The US Army Biomedical Research and Development Laboratory (USABRDL) has sponsored plant uptake studies to develop predictive models for the movement of TNT and other organic compounds in the environment. In 1983, under USABRDL sponsorship, the US Army Cold Regions Research and Engineering Laboratory conducted a hydroponic study to quantify uptake of TNT and two of its degradation products (4ADNT and 2ADNT) by yellow nutsedge (*Cyperus esculentus*) (Palazzo and Leggett 1986). Plant uptake was demonstrated for all three test compounds, and reductions in root weights were attributed to test compounds.

6. In 1984 the US Army Engineer Waterways Experiment Station (WES) conducted a plant uptake study of TNT from soils using *C. esculentus* (Folsom et al., in preparation). Results of this study showed that TNT was taken up by the plant and demonstrated the presence of two common degradation products of TNT (2ADNT and 4ADNT) in the plant. The subject of this report is a study that was designed to determine whether these two degradation products of TNT are taken up from the soil or are produced by metabolism or degradation of TNT within the plant.

Objectives

7. Specific objectives of the study were as follows:

   a. To determine whether *C. esculentus* can take up 2ADNT and 4ADNT from soils.

   b. To determine whether TNT, 2ADNT, and 4ADNT are degraded in *C. esculentus*.

c. To determine whether the TNT, 2ADNT, and 4ADNT become concentrated in the plant.

d. To detect principal known degradation products of the test compounds in the soil and in the plant.

PART II: MATERIALS AND METHODS

Preparation and Treatment of Soils

8. Methods of collection, characterization by chemical and physical tests, and initial preparation of the two test soils, Tunica silt and Sharkey clay, are described by Folsom et al. (in preparation). Initial preparation included air-drying of soils followed by grinding to pass through a 2-mm sieve. Soils thus prepared were sealed in noncorrosive drums and stored in a greenhouse at 21° to 30° C until used.

9. Previous experiments had shown that applying crystalline TNT directly to dry soil and hand-mixing produced an uneven distribution of TNT throughout the soil. When this treatment method was used, the variability in TNT concentrations between samples was unacceptably high (Folsom et al., in preparation). Therefore, an alternate treatment method was developed for the present study in which solutions of treatment compounds (TNT, 4ADNT, and 2ADNT) were added to soils. The entire amount of the respective compounds was applied to a small aliquot of soil. This treated aliquot was then mixed with a larger batch of soil that was distributed into pots for the plant uptake study.

10. Three small aliquots (360 g) of silt and three of clay were treated with water to make a thick slurry that could be mixed readily in a malt mixer. Two hundred millilitres of water was added to each aliquot of the silt, and 400 ml was added to each aliquot of the clay. One aliquot of each type of soil was treated with 100 ml of acetone solution containing [U-^{14}C]TNT (California Bionuclear Corporation, Sun Valley, Calif.) and unlabeled TNT; one aliquot of each soil type was treated with [methyl-^{14}C]4ADNT and unlabeled 4ADNT; one aliquot of each soil type was treated with only unlabeled 2ADNT. Concentration of each solution was 0.012 g of respective compound (labeled plus unlabeled) per millilitre. Only unlabeled 2ADNT was used because ^{14}C-labeled 2ADNT was not available.

11. The acetone solution was dropped slowly (about 10 drops/min) into the soil slurry while it was being mixed. When the desired amount of treatment compound had been added to each soil aliquot, the slurries were poured into individual shallow pans and allowed to air-dry for approximately 2 days on the laboratory bench. During this time, the soils were exposed to intermittent laboratory lighting. Any caked soil that had formed during drying was broken

up by grinding with a mortar and pestle. Treated samples were retained for treatment of the larger soil batches (15,000 g total) required for the plant uptake study. The treatments produced a final activity in the large batches of soil of 4.16×10^{-3} µCi per gram of TNT-treated soil, and 3.8×10^{-2} µCi per gram of 4ADNT-treated soil. Final soil concentrations were 80 µg of TNT, 4ADNT, or 2ADNT per gram of soil on an oven-dry weight (ODW) basis.

12. The large soil batches were fertilized to ensure adequate nutrition for plant growth. Each soil batch received 50 µg N as $(NH_4)_2SO_4$, 25 µg P as NaH_2PO_4, and 25 µg K as KCl per gram of soil. This corresponds to a rate of 56 kg nitrogen, 28 kg phosphorus, and 28 kg potassium per hectare. The silt and the clay required addition of calcium carbonate (i.e., lime requirement as described by Allison and Moodie 1965) to raise the pH to 7.0, prior to conducting the WES plant bioassay procedure (Folsom and Lee 1981). Only reagent-grade chemicals were used.

13. Soil batches of 15 kg were dry-mixed in a twin-shell dry soil blender (Patterson-Kelley Co., East Stroudsburg, Pa.) (Figure 1). Controls were mixed before treatments and received fertilizer and lime only. Mixing of soil and fertilizer was interrupted after 5 min for addition of the soil aliquot containing treatment compound. Mixing was resumed for 15 min.

14. During the dry-mixing of ^{14}C-treated soils, all precautions were taken to minimize contamination of greenhouse surfaces and exposure of

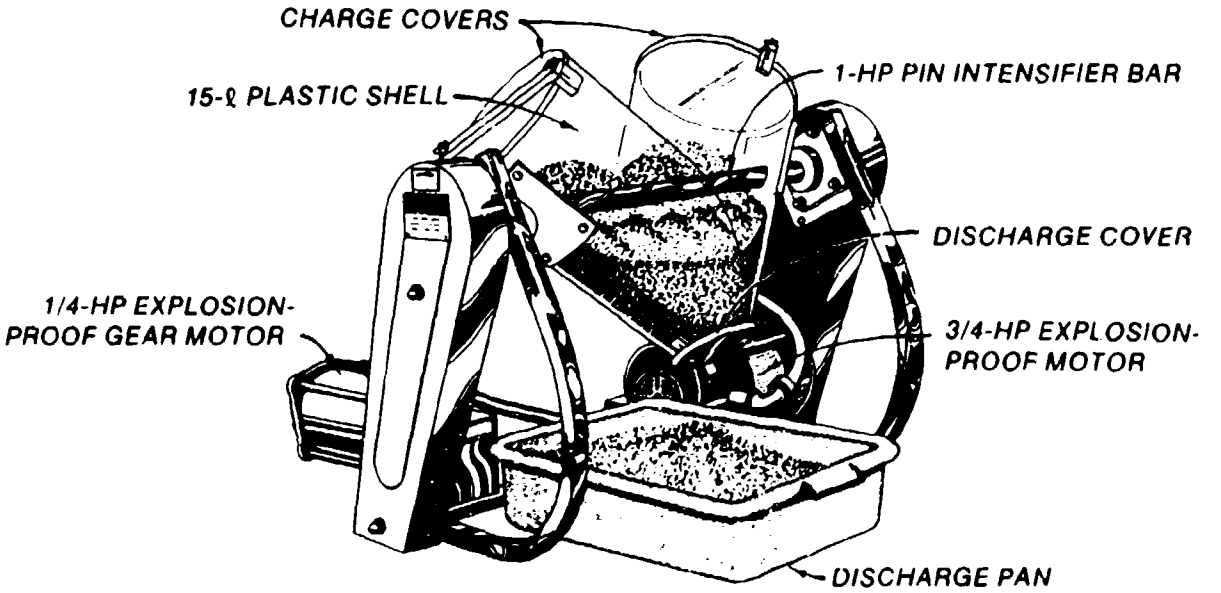


Figure 1. Twin-shell dry soil blender

personnel to treated soils. Access to the greenhouse was limited to individuals actually involved in the conduct of the study. Laboratory coats, shoe covers, gloves, respirators, and film badges were worn by all individuals in the greenhouse. All greenhouse fans were turned off while dry soils were being handled and remained off during the following 24 hr. When potting of treated soils was completed, the air disperser on the greenhouse fan jet was removed and disposed of in a radioactive waste container. When initial soil treatment and potting were completed, all greenhouse surfaces were thoroughly cleaned and subjected to wipe tests to detect radioactivity.

15. Five replicates containing 2.5 kg of each treated soil on an ODW basis were potted in a modification of the standard WES plant bioassay apparatus (Figure 2) (Folsom and Lee 1981). The standard apparatus was modified to accommodate a 3.5-ℓ plastic Bain Marie pot inside a 7.6-ℓ Bain Marie pot rather than the standard 7.6-ℓ inside a 22.7-ℓ. Soils were moistened to 0.03 to 0.05 MPa (30 to 50 percent of field capacity, i.e., field capacity equals 0.00 MPa) by filling the outer bucket with deionized water and monitoring tensiometers (Model 506M, Irrometer Company, Inc., Riverside, Calif.) placed in the soil of each pot. Excess water was siphoned from outer pots when tensiometer readings reached 0.00 MPa.

16. To detect any labeled compound that may have leached from the soil as plants were watered, all of the water siphoned from each treatment was combined, filtered (Whatman No. 5) to remove any incidental soil, and evaporated to 1 ml using a low-temperature hot plate. The 1 ml of water remaining after

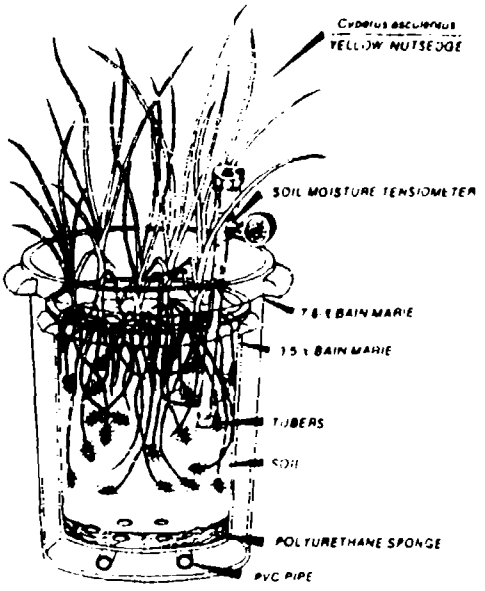


Figure 2. Plant bioassay experimental unit

concentration was diluted with 20 ml of liquid scintillation cocktail (PCS, Amersham Corp., Arlington Heights, Ill.) and counted in a Beckman LS-100 Liquid Scintillation System (LS) (Beckman Instruments, Inc., Fullerton, Calif.). The LS was equipped with a plug-in, fixed, optimum window module for counting ^{14}C and an external reference standard module.

17. One replicate from each treatment and control was randomly selected and designated to provide material for the development of analytical procedures.

18. Pots were randomly located on greenhouse benches, using a computer-generated random numbers table, and allowed to equilibrate for 20 days prior to planting. The temperature of the greenhouse was maintained at a daytime maximum of 30° C and a nighttime minimum of 21° C. Since natural day length during the test period (December to February) varied from slightly more than 10 hr to slightly more than 11 hr, supplemental lighting was used to maintain a 16-hr day length. A 16-hr day produces optimum vegetative growth of *C. esculentus* (Folsom and Lee 1981). A photosynthetic active radiation level of 1,300 $\mu E/m^2/sec$ was maintained during the 65-day period of the experiment.

Soil Sampling and Planting at 20 Days (T20)

19. After a 20-day incubation period (T20), the soil in each pot was sampled. Three soil cores 2 cm in diameter and 10 to 12 cm long were taken from each pot. The three cores were combined, mixed well, and retained for analysis by LS, gas liquid chromatography (GLC), and combustion. A 5-g sample was oven-dried overnight at 104° C to determine oven-dry weight. Immediately after sampling of soils, three sprouted tubers of *C. esculentus* were planted in each pot. Methods for generating and sprouting tubers were given by Folsom and Lee (1981). Plants were watered when tensiometer readings exceeded 0.05 MPa. Moisture levels were monitored daily to maintain 0.03 to 0.05 MPa, as previously described.

Plant and Soil Sampling at 65 Days (T65)

20. Sixty-five days after potting the soils (45 days after planting), plants were harvested. Plants from each pot were clipped 2 cm above the soil level, weighed, chopped into 2-cm segments, and the segments mixed well. Each

sample was divided into two approximately equal subsamples, one for ^{14}C analysis and the other for GLC analysis. Subsamples from each replicate were placed into plastic Ziploc bags. Subsamples for GLC analysis were frozen until the time for analysis. Percent moisture was determined by oven-drying (70° C overnight) 2 g of plant material from each of the ^{14}C subsamples. The remainder of the subsamples for ^{14}C analysis were stored in the dark at 4° C until extracted (within 4 days).

Soil Homogeneity Test

21. A soil homogeneity test was conducted to check for uniformity in the distribution of ^{14}C-labeled compounds throughout the batches before [^{14}C]TNT- and [^{14}C]4ADNT-treated soils were removed from the twin-shell blender (Figure 1). A sample (ca. 25 g) was taken from each of the following positions with regard to the "V" of the blender: the left side, the right side, and the bottom. Three 5-g aliquots of the ^{14}C-treated soil from each position were extracted once with 5 ml of acetone. Extraction was accomplished by shaking at maximum speed (280 excursions per minute) for 10 min on a reciprocating box shaker followed by centrifuging at 17,369 × gravity for 10 min. One millilitre of the extract was diluted with 20 ml of PCS and analyzed by LS counting. Equivalent concentrations of TNT and 4ADNT, i.e., the concentration assuming that all ^{14}C detected was from original ^{14}C-labeled treatment compounds and not from ^{14}C-labeled decomposition products, were determined by consulting standard curves of the respective treatment solutions (see Appendix A). Standard curves were prepared by plotting counts per minute (CPM) per millilitre against micrograms per millilitre of original treatment solutions of respective treatment compounds.

Analysis of Soils

^{14}C analysis

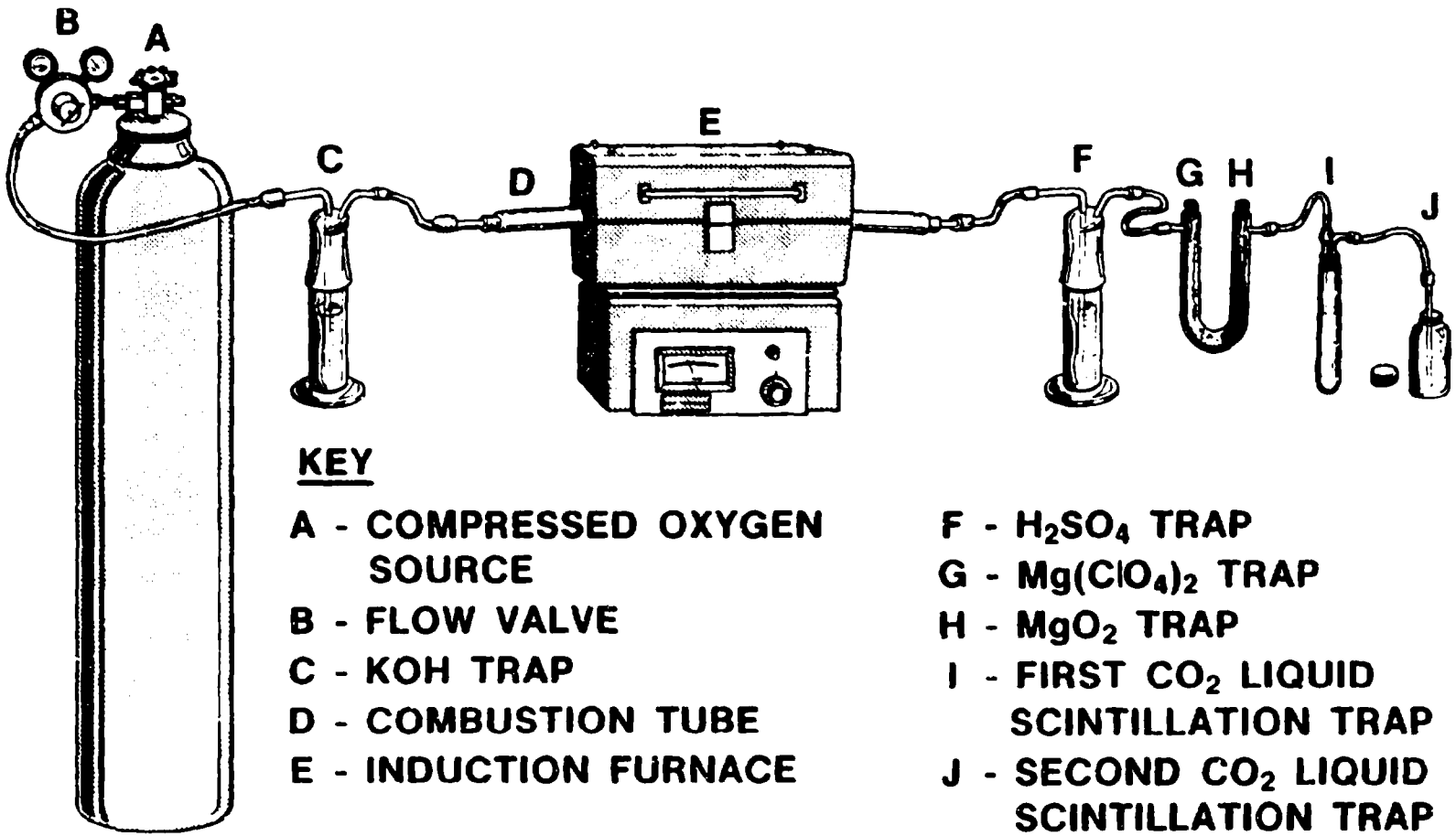
22. Preliminary soil extraction test. A preliminary soil extraction test was conducted to determine which of the following solvents was the most efficient for extracting [^{14}C]TNT from the silt and clay: acetone, benzene, methanol, and methylene chloride. Four 5-g replicates of [^{14}C]TNT-treated soil were extracted once with 5 ml of solvent in a 50-ml stainless steel

centrifuge tube. Extraction and analysis by LS were accomplished as described above for the soil homogeneity test. Five grams of untreated soil in four replicates was extracted in the same manner. One millilitre of the extract was diluted with 20 ml of PCS and counted by LS for 20 min.

23. Extraction of soils from plant uptake study. Soil extraction for analysis of the soils sampled during the plant uptake study was performed in the same way as for the soil homogeneity test, except that samples were extracted three times using acetone, the solvent selected on the basis of results of the preliminary soil extraction test. The three extracts were combined and concentrated under a stream of air to 5 ml. One millilitre of the concentrate was counted by LS. Standard curves were consulted to relate CPM per millilitre to micrograms of TNT or 4ADNT per millilitre (Appendix A). Micrograms per millilitre of soil extract were then related to micrograms per gram of soil (ODW).

24. Carbon train. Two carbon trains for the complete combustion of soil samples were set up according to Nelson and Sommers (1982) with certain modifications. Modifications were made to quantify $^{14}CO_2$ by LS counting instead of determining total carbon gravimetrically. A diagram of the carbon train is shown in Figure 3. Commercially supplied compressed oxygen, regulated by a flow valve, was purified by passage through a 10-percent potassium hydroxide (KOH) trap. The oxygen flow rate was adjusted to approximately 100 ml/min. The purified oxygen then passed through a quartz glass combustion tube housed in a medium-temperature induction furnace (950° C). A porcelain combustion boat containing the weighed soil sample was placed in the center of the combustion tube, and the tube was sealed immediately with a stopper through which the oxygen flowed. Before exiting the tube, excess oxygen and the gases evolved from the burned sample were passed over platinized asbestos, which acted as a catalyst to ensure the complete oxidation of CO and any other volatile C compounds to CO_2. The gases were then freed of most water vapor by passage through a washing bottle, or trap, of concentrated sulfuric acid (H_2SO_4). Remaining moisture, as well as oxides of nitrogen and sulfur and the halogens, was removed by passage through a U-tube filled with anhydrous $Mg(ClO_4)_2$ on the first side and MgO_2 on the other. Samples were burned for 10 min. The CO_2 was trapped in a sealed glass test tube containing 20 ml of Oxifluor-CO_2 (complete oxidizer cocktail for the absorption of radioactive CO_2) (New England Nuclear Research Products, Boston, Mass.). Ten millilitres

CARBON TRAIN



KEY

A - COMPRESSED OXYGEN SOURCE
B - FLOW VALVE
C - KOH TRAP
D - COMBUSTION TUBE
E - INDUCTION FURNACE
F - H_2SO_4 TRAP
G - $Mg(ClO_4)_2$ TRAP
H - MgO_2 TRAP
I - FIRST CO_2 LIQUID SCINTILLATION TRAP
J - SECOND CO_2 LIQUID SCINTILLATION TRAP

Figure 3. Carbon train

of Oxifluor-CO_2 will incorporate 14 millimoles, or 0.60 g, or 300 ml (at standard temperature and pressure) of CO_2. The trapping tube was vented into a vial, also containing 20 ml of Oxifluor to ensure that no $^{14}CO_2$ would be lost if the first trap were exhausted. Oxifluor from both tubes was counted three times for 10 min by LS. The train was continuously flushed with oxygen between successive uses.

25. Two standard curves were prepared to assess the efficiencies with which the carbon trains were able to recover ^{14}C spikes from the soils. Silt was used with one train exclusively and clay with the other to minimize variability. Direct spiking of soils with [illegible] the samples were placed into the combustion tube produced unacceptable variations in recovered $^{14}CO_2$. This may have been due to rapid volatilization of samples before the combustion tube could be sealed. To reduce the variation and improve recovery of spikes, the operating efficiencies of the carbon trains were determined by burning soil samples onto which [^{14}C]TNT had been adsorbed.

26. A stock solution containing 16.0 µg/ml total TNT (^{14}C-labeled plus unlabeled) and 0.023 µCi/ml [^{14}C]TNT was diluted with reverse osmosis water to produce six concentrations of TNT. The total TNT concentrations used (^{14}C-labeled plus unlabeled) were 0.0, 1.28, 3.2, 6.4, 9.6, 12.8, and 16.0 µg/ml. Five-gram samples of each soil type (silt and clay) for each test concentration were weighed into 50-ml stainless steel centrifuge tubes in three replicates. Twenty-five millilitres of [^{14}C]TNT solution were added to each tube, and the tubes were sealed and placed on a reciprocating box shaker operated at maximum speed. Three replicates of tubes prepared in the same manner, but containing only [^{14}C]TNT solution (no soil), were run simultaneously with the soil samples to measure any adsorption of solution to walls of the centrifuge tubes. After 2 hr, all samples were removed from the shaker and centrifuged at 17,396 × gravity for 10 min. Three 1-ml aliquots of solution were removed from each tube and counted in 20 ml of PCS by LS for 20 min. Soil samples containing adsorbed [^{14}C]TNT were frozen until the time for analysis (not more than 2 weeks).

27. After thawing, six 0.5-g soil samples from each centrifuge tube were weighed to the nearest 0.0001 g into porcelain combustion boats. The wet soil in each boat was overlaid with a thin covering of burnt soil to prevent effervescence or flashing (incomplete combustion). A 1-g sample from each tube was weighed into an aluminum pan and placed in a forced-draft oven at

104° C overnight for determination of oven-dry moisture. Moisture loss from the wet soils during weighing was fairly rapid; therefore, the first boat weighed was paired with the last boat weighed, the second with the fifth, and the third with the fourth for combustion in the carbon train. This procedure was used to compensate for differences in moisture between weighings. Each boat of a pair was combusted in a separate run of the train, but $^{14}CO_2$ from both boats was trapped in the same set of Oxifluor traps. Counts from both sets of Oxifluor traps were combined after subtraction of solution background counts. The sum was corrected to oven-dry weight to obtain CPM per gram of combusted soil.

28. To obtain an expected CPM in the soil phase, total CPM in the solution phase were added to total CPM adsorbed to the centrifuge tube and the sum subtracted from the total CPM initially added to each tube. Efficiency curves were prepared by plotting the expected versus the actual CPM found for each soil type and its respective carbon train. A regression analysis was performed on the curves to determine whether their slopes were significantly different from one another.

Gas liquid chromatographic analysis

29. US Environmental Protection Agency Standard Method 3540 for extraction of organic compounds from solid wastes (USEPA 1982) was used to extract soil samples. Analyses were performed by the Analytical Laboratory Group, Environmental Laboratory, WES. Twenty-gram soil samples were extracted by Soxhlet for 17 hr in hexane-acetone (1:1 by volume). Approximately 20 g of anhydrous sodium sulfate was added to each extract as a dehydrating agent. Prior to GLC analysis, extracts were concentrated and transferred to 1 ml of benzene in Kuderna-Danish tubes with condensers.

30. A dual-column Hewlett-Packard Model 5880 GLC was employed for analysis of soil and plant extracts. The instrument had two 30-m fused silica capillary columns. One column (0.329-mm internal diameter) was coated with DB5 (J and W Scientific, Folsom, Calif.), while the other (0.310-mm internal diameter) was coated with SP2100 (Supelco, Inc., Bellefonte, Pa.). The columns were of widely separated polarities. Helium (pressure, 110 kPa) was the carrier gas. A nitrogen-phosphorus detector at a temperature of 300° C was used. The injection port temperature was 250° C. A lower temperature, 200° C, was tried in an attempt to minimize degradation of injected compounds,

but no improvement was achieved. The instrument was programmed for a temperature gradient of 100° to 200° C in 5° C per minute increments.

Analysis of Plants

Plant yields

31. All freshly harvested plant material from each replicate was weighed to the nearest 0.1 g (total fresh weight). Oven-dry weight was determined to the nearest milligram by drying (70° C overnight) a 2.0-g subsample of the fresh plant material harvested as described previously. Yields for all plant material in each pot were calculated from the dry weight of the 2-g subsample.

^{14}C analysis

32. Preliminary plant extraction test. A preliminary extraction test was conducted on plant material to determine which of the following solvents was the most efficient extractant of [^{14}C]TNT: acetone, benzene, hexane/acetone (1:1 by volume), or methanol. Two grams of plant material (fresh weight) from control and TNT-treated replicates that had been designated for investigation of analytical procedures was extracted in 50-ml stainless steel centrifuge tubes. Three replicates were extracted for each test solvent. Extraction was performed by homogenizing plant material in 20 ml of solvent with a Polytron (Brinkmann Instruments, Westbury, N. Y.) operated at maximum speed. Homogenates were centrifuged for 10 min at 17,369 × gravity and the extracts removed with a pasteur pipette. One millilitre of the extract was diluted with 20 ml of PCS in a scintillation vial and counted for 20 min by LS using the internal standard method described by Wang, Willis, and Loveland (1975). Each vial was spiked with [^{14}C]TNT (internal standard) and recounted for 20 min. The counting efficiency (CE) for each vial was calculated using the following equation:

$$CE = \frac{\text{(CPM of internal standard + sample) - (Net CPM of sample)}}{\text{Disintegrations per minute of internal standard}}$$

33. Extraction of 2-g plant samples. Extraction of plant material was performed in the same way as described above for the preliminary plant extraction test, except that samples were extracted three times using benzene. This was the solvent selected by comparing ^{14}C counting efficiencies for spikes by each solvent in the plant extraction test. Three extracts of the

same sample were combined, concentrated to 1 ml under a stream of air, and counted in 20 ml of PCS by LS.

34. Extraction of all remaining plant material. Since ^{14}C counts detected in the initial extracts of 2-g plant samples were very low, all remaining plant material in ^{14}C subsamples was extracted with benzene to increase the chances of detecting ^{14}C. Five-gram samples were weighed until all material for each replicate had been used. An equal weight of anhydrous Na_2SO_4 and 20 ml of benzene were added before the samples were homogenized in the Polytron. Extraction was performed as above, except that only one extraction was done. Extracts from the same replicate were combined, concentrated, and counted by LS.

35. Standard curves were prepared for [^{14}C]TNT and [^{14}C]4ADNT using extracts of untreated plant material. Plant material was prepared as described above for extraction of 2-g plant samples. Extract was measured into scintillation vials containing 20 ml of PCS, spiked with various dilutions of ^{14}C-labeled compound (8, 4, 2, 1.6, 0.8, 0.4, and 0 µg/ml), and counted for 20 min by LS. Micrograms of TNT or 4ADNT per millilitre of extract were determined from a standard curve relating CPM per millilitre to micrograms of TNT or 4ADNT per millilitre of extract (see Appendix A). Oven-dry plant material was calculated as micrograms per gram from micrograms per millilitre of solvent and ODW of plant material extracted.

Gas liquid chromatographic analysis

36. Five grams of fresh plant material was homogenized in the Polytron with 40 ml of benzene and approximately 5 g of anhydrous sodium sulfate. Extracts were filtered, concentrated to 1 ml, and analyzed by GLC.

Statistical Analyses

37. Analysis of variance (ANOVA) using a completely randomized experimental design was performed on the data to test for difference among treatment means (F Tests). The ANOVA was conducted using the procedures available with Statistical Analysis System (SAS Institute, Inc. 1985). When the ANOVA showed that the null hypothesis must be rejected, linear contrasts (Steel and Torrie 1980) or the Waller-Duncan K-Ratio T-Test was used to separate differences between means. The probability of a Type I error was 0.05 in the F Tests and in each contrast. In comparing percent recoveries of ^{14}C by

extraction and by carbon train, the T-Test procedure available with SAS was employed. Carbon train efficiency curves data were subjected to linear regression analyses.

PART III: RESULTS AND DISCUSSION

Chemical and Physical Characteristics of Test Soils

38. Results of chemical and physical characterization tests for the silt and clay are presented in Table 1. Data obtained in all preliminary studies, as well as results of all analyses of the soils and plant materials from the principal study, are presented in Appendix B. Unless CPM data were specifically pertinent, microgram data are given.

Table 1

Chemical and Physical Characteristics of Test Soils

Parameter	Clay	Silt
pH	5.71	4.54
Particle size		
Percent sand	8.70	9.37
Percent silt	36.90	73.13
Percent clay	54.40	17.50
Electrical conductivity (dS/m)	2.45	0.72
Percent organic carbon	2.40	0.57
Cation exchange capacity (meq/100 g)	134.9	17.2
Extractable metals (µg/g)		
Iron	1,252	252
Aluminum	160	196
Manganese	59.6	152
Calcium	0.954	1.10

Soil Homogeneity Test

39. Results of the test for soil homogeneity are shown in Table 2. Sampling was not replicated; therefore, the data could not be subjected to statistical analysis. However, examination of the data showed an average variation among the means of all treatments and soil types of almost 20 µg of TNT and 4ADNT per gram of soil. A higher degree of homogeneity was observed in the silt than in the clay for both treatments. Percent recoveries across treatments were highly variable. Therefore, the treatment could not be considered homogeneous. With the exception of the TNT-treated clay, recoveries of treatment compounds from the soils were less than half of what was added.

Table 2

Percent Recoveries of ^{14}C and Equivalent Concentrations* of TNT and 4ADNT in Silt and Clay from the Soil Homogeneity Test

Blender Position**	Concentration of Constituent							
	TNT-Treated				4ADNT-Treated			
	Silt		Clay		Silt		Clay	
	Percent	µg/g	Percent	µg/g	Percent	µg/g	Percent	µg/g
Left	46.58	37.86	88.60	71.37	29.59	22.85	41.32	32.12
Middle	46.83	38.07	86.12	69.39	29.84	23.04	41.29	32.10
Right	48.45	39.36	79.76	64.32	31.06	24.00	38.21	29.70

* Equivalent concentrations determined by consulting standard curves to relate CPM/ml to µg/ml of soil extract and calculating µg/g of oven-dry soil. This procedure assumes that all ^{14}C detected was from the respective ^{14}C-labeled treatment compounds, i.e., no decomposition to other compounds had occurred.

** See discussion, paragraph 21.

Percent of original ^{14}C treatment recovered by extraction of soils. Means from extractions of three subsamples from each blender position.

These unexpected results provided the first indication that significant amounts of treatment compounds could not be accounted for in soil extracts. Possible mechanisms responsible for this result are explored in subsequent sections of this report.

Analysis of Soils

^{14}C analysis

40. Preliminary soil extraction test. Results of the preliminary soil extraction test showed that acetone and methanol were more efficient extractants of [^{14}C]TNT from both soils (silt and clay) than either methylene chloride or benzene (Table 3). These results are not surprising because TNT is slightly polar and should be more soluble in the more polar solvents. According to Urbanski (1964), the solubility of TNT in acetone is 132 g/100 g of solvent and in benzene is 88 g/100 g of solvent. (Solubilities of TNT in methylene chloride and methanol were not found in the literature.) On the basis of these extraction results, acetone was selected as the extractant for soils.

Table 3

Percent Recoveries of ^{14}C and Equivalent Concentrations* of TNT Extracted from [^{14}C]TNT-Treated Soil and Clay with Four Solvents**

	Concentration of TNT			
	Silt		Clay	
Solvent	Percent†	µg/g	Percent	µg/g
Acetone	57.35	46.45 a††	99.92	80.40 a
Methanol	55.35	44.86 a	83.55	67.34 a
Methylene chloride	49.65	40.36 b	42.26	34.43 b
Benzene	45.54	37.04 c	29.16	23.98 b

* Equivalent concentrations determined by consulting standard curve to relate CPM/ml to µg/ml of soil extract and calculating µg/g of oven-dry soil. This procedure assumes that all ^{14}C detected was from the respective ^{14}C-labeled treatment compounds, i.e., no decomposition to other compounds had occurred.

** Values shown are differences between means of four replicates of TNT-treated and untreated soil.

† Percent of original ^{14}C treatment recovered by extraction of soils.

†† Means followed by the same letter within soil types are not significantly different at the $P = 0.05$ level.

41. Siphoned water. Results of ^{14}C analysis of excess water siphoned from outer pots after watering plants are shown in Table 4. Percents of total counts added for all five replicates of the same treatment and soil type are given. Although these data represent detection of ^{14}C, percent recoveries of total ^{14}C added to the soils initially were small. For all treatments and soil types, the average loss was 0.0005 µg/g of soil.

42. Extracted soils. Results from ^{14}C counts of T20 and T65 silt and clay extracted with acetone and counted by LS are shown in Table 5 The data demonstrate a distinct difference in the behavior of the two treatment compounds in the silt and clay. Carbon 14 was detected in significantly greater quantities in the 4ADNT- than in the TNT-treated silt at T65 and in almost significantly greater quantities at T20 ($P = 0.06$). However, this did not occur in the clay. The TNT-treated clay showed significantly more ^{14}C than 4ADNT-treated clay at both T20 and T65. This result suggests strong adsorption of 4ADNT by the clay. This possibility is explored further when results of carbon train analysis of soils are discussed.

Table 4

Recovery of ^{14}C from Siphoned Water*

Treatment	Percent of Total Counts Added to Soils Initially**
Silt, TNT	1.77×10^{-3}
Clay, TNT	9.46×10^{-4}
Silt, 4ADNT	7.17×10^{-5}
Clay, 4ADNT	6.73×10^{-5}

* Water siphoned from outer pots after watering plants. Water samples from all five replicates of the same treatment and soil type were combined for the entire 45-day growing period.

** Values are percents of total ^{14}C CPM added to each soil treatment.

Table 5

^{14}C Analysis of Extracts of TNT- and 4ADNT-Treated Silt and Clay Sampled 20 and 65 Days After Soil Treatment

	Silt		Clay	
Treatment*	T20** µg/g	T65† µg/g	T20 µg/g	T65 µg/g
4ADNT	12.68 Ba††	17.66 Aa	7.88 Cb	5.47 Cb
TNT	9.58 Aa	4.68 Bb	11.26 Aa	10.46 Aa
Control‡	0.75 Ab	0.73 Ac	0.83 Ac	0.76 Ac

* Original treatment levels were 80 µg of respective compound per gram of soil (ODW).

** T20 = 20 days after soil treatment, the time at which tubers were planted.

† T65 = 65 days after soil treatment, the time at which plants were harvested.

†† The equivalent concentrations of treatment compounds given are means of four replicates extracted three times with acetone. Values followed by the same uppercase letter across soil types are not significantly different at the $P = 0.05$ level. Values followed by the same lowercase letter down are not significantly different at the $P = 0.05$ level.

‡ Control soil samples were taken from bioassays that had been handled in the same manner as treatment except that no treatment compound was added.

43. No significant differences were noted between levels of ^{14}C in clay from T20 to T65 for either treatment compound. However, ^{14}C levels changed from T20 to T65 in the silt for both treatment compounds. The ^{14}C level decreased from T20 to T65 in the TNT-treated silt. It is possible that TNT became less extractable through time. There is some evidence in the literature in support of this possibility (Cragin et al. 1985). Volatilization of photo or microbial degradation products is also possible. The ^{14}C level showed a slight, significant increase from T20 to T65 in the 4ADNT-treated silt. This increase may be explained by an increase in extractability of the ^{14}C-labeled compound through time. This possibility is supported by the presence of the ^{14}C-label on the methyl group in 4ADNT. If the methyl group were removed from the molecule by some mechanism, ^{14}C may have become more easily extracted. Carbon-14 was detected in significantly greater quantities in treated soils than in controls, but detectable levels of ^{14}C were present in some controls. It is possible that the low-level contamination in controls resulted from volatilization, or coevaporation with soil moisture followed by cocondensation on the soil surface.

44. Carbon train. An efficiency curve for the carbon train with which the silt was used is shown in Figure 4. Linear regression analysis of the curve data showed a slope of 1.63, which was significantly different from 1 (100-percent recovery) at the 95-percent confidence level. Percent recoveries of added TNT for silt are shown in Table 6. The mean percent recovery of ^{14}C from the silt across test concentrations was 71.30 percent, with a standard deviation of 8.78 percent. However, percent recoveries increased as the concentration of [^{14}C]TNT decreased. Most of the sample values fell into the range of the lower concentrations and, consequently, of greater percent recovery. Mean mass balance for the silt spiked for preparation of the efficiency curve was 89.78 percent, with a standard deviation of 2.25 percent.

45. An efficiency curve for the carbon train with which clay was used is shown in Figure 5. Linear regression analysis of the curve data showed a slope of 1.04, which was not significantly different from 1. Percent recoveries of added TNT for clay are shown in Table 7. The mean percent recovery of ^{14}C from clay across test concentrations was 81.99 percent with a standard deviation of 13.03 percent. Mean mass balance for the clay spiked

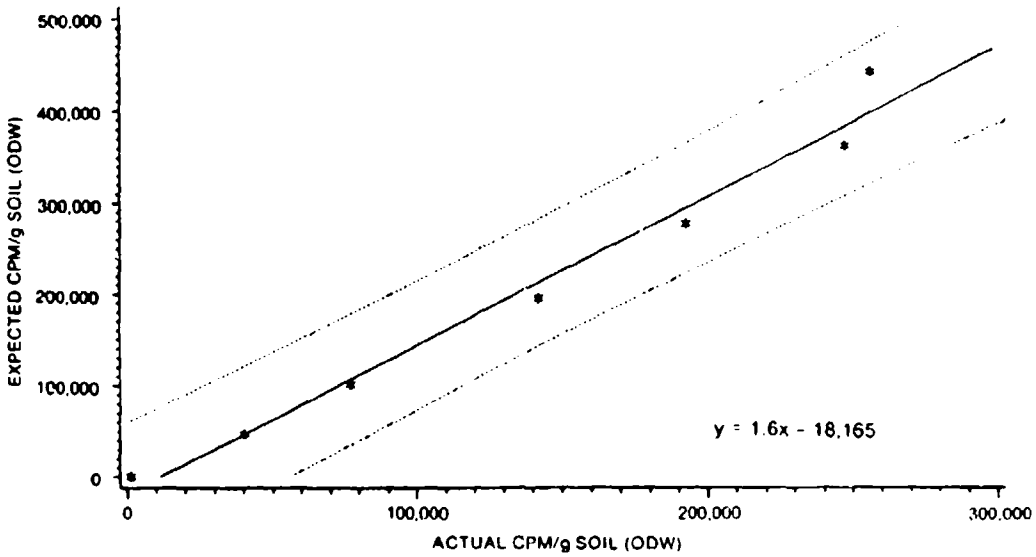


Figure 4. Carbon train efficiency curve for silt soil. Dotted lines represent limits of the 95-percent confidence interval

for preparation of the efficiency curve was 86.09 percent with a standard deviation of 10.85 percent.

46. Carbon train results (Table 8) showed no significant differences between levels of ^{14}C in the 4ADNT- and TNT-treated silt at T20 or at T65. However, the clay exhibited significantly more ^{14}C in the TNT-treated soil than in the 4ADNT-treated soil at both times. Levels of ^{14}C in the TNT-treated clay were also higher than in TNT-treated silt. There were no significant differences between levels of ^{14}C in the 4ADNT treatments at T20 and at T65 in either soil; ^{14}C levels in TNT treatments showed a slight, though significant, decrease from T20 to T65 in the clay, but no difference in the silt.

47. In Table 9, percent recoveries of ^{14}C by extraction and by carbon train analysis are compared. Percent recoveries by carbon train analysis were significantly greater than recoveries by extraction analysis in all soils except controls and the silt 4ADNT treatment at T65, which exhibited no difference. On the average, recoveries by carbon train exceeded recoveries by extraction by a factor of four. If carbon train recoveries were corrected to the efficiencies of the two carbon trains (71.30 percent for the train with

Table 6

Recovery of ^{14}C from Silt Containing Adsorbed [^{14}C]TNT for Determining Carbon Train Efficiency

Amount Added		Amount Recovered						
			Soil				Total	
ml	CPM	Solution CPM*	CPM	Expected**	Percent of Expected	Test Tube CPM	CPM†	Percent of Added
25	1,346,625	789,180	256,102	442,309	57.90	115,136	1,160,418	86.17
20	1,077,300	622,086	246,808	363,105	67.97	92,109	961,003	89.20
15	807,975	461,276	192,329	277,617	69.28	69,082	722,686	89.44
10	536,400	294,852	142,122	195,686	72.63	45,862	482,836	90.01
5	269,325	144,702	76,863	101,596	75.66	23,027	244,592	90.82
2	107,725	50,743	40,289	47,771	84.34	9,210	100,243	93.05

* Values given are means of three replicates.

** Expected CPM in soil were determined by subtracting CPM found in solution plus CPM adsorbed to test tubes from total CPM added initially.

† Total CPM recovered are the sum of CPM in the solution, in the soil, and adsorbed to test tubes.

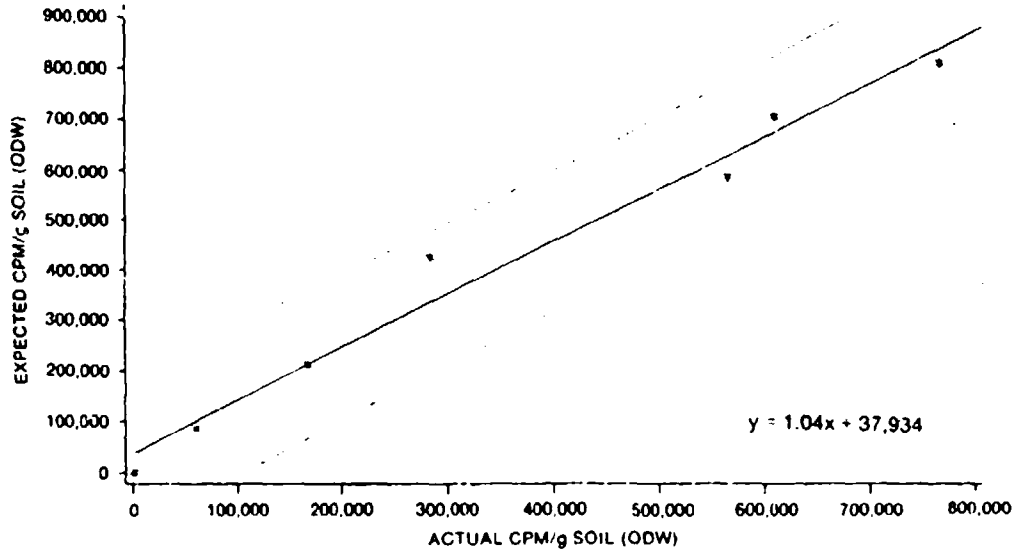


Figure 5. Carbon train efficiency curve for clay soil. Dotted lines represent limits of the 95-percent confidence interval

which silt was analyzed and 81.99 percent for the train with which clay was analyzed), this difference would increase. The carbon train results indicate that the extraction techniques employed did not remove all of the ^{14}C-labeled compounds that were actually present in the soils.

48. Comparison of the extraction data, which showed significantly more 4ADNT in the silt than in the clay, with the carbon train data, which showed no significant difference between amounts of 4ADNT in the silt and the clay, suggests that 4ADNT was more easily extracted from the silt than from the clay. Both methods of analysis showed more TNT in the clay than in the silt. These results support adsorption of both 4ADNT and TNT to the clay. In the silt there was no significant difference between amounts of 4ADNT and TNT (except for significantly more 4ADNT than TNT at T65 by extraction) by either method at either time. However, in the clay there was significantly more TNT than 4ADNT by both methods and at both times. These results suggest that loss of 4ADNT (i.e., loss of the ^{14}C label) from the treated soils was greater than loss from TNT-treated soils. This may be due to stronger adsorption of TNT than of 4ADNT.

Table 7

Recovery of ^{14}C from Clay Containing Adsorbed [^{14}C]TNT for Determining Carbon Train Efficiency

Amount Added		Amount Recovered						
			Soil				Total	
ml	CPM	Solution CPM*	CPM	Expected**	Percent of Expected	Test Tube CPM	CPM†	Percent of Added
25	1,329,271	406,731	766,624	808,888	94.78	113,653	1,287,007	96.82
20	1,063,417	269,042	609,550	703,453	86.65	90,922	969,515	91.17
15	797,563	144,921	564,546	584,450	96.59	68,192	777,659	97.50
10	531,709	64,297	273,279	421,951	64.77	45,461	383,038	72.04
5	265,854	32,298	167,194	210,826	79.30	22,731	222,222	83.59
2	106,342	10,537	60,583	86,713	69.87	9,092	80,212	75.43

* Values given are means of three replicates.

** Expected CPM in soil were determined by subtracting CPM found in solution plus CPM adsorbed to test tubes from total CPM added initially.

† Total CPM recovered are the sum of CPM in the soil, in the solution phase, and adsorbed to test tubes.

Table 8

Carbon Train ^{14}C Analysis of TNT- and 4ADNT-Treated Silt and Clay Sampled 20 and 65 Days After Soil Treatment

	Silt		Clay	
Treatment	T20* µg/g	T65** µg/g	T20 µg/g	T65 µg/g
4ADNT	25.12 Aa†	24.74 Aa	26.61 Ab	21.74 Ab
TNT	30.72 Ca	30.62 Ca	55.62 Aa	41.52 Ba
Control	0.76 Ab	0.74 Ab	0.81 Ac	0.78 Ac

* T20 = 20 days after soil treatment, the time at which tubers were planted.

** T65 = 65 days after soil treatment, the time at which plants were harvested.

† Values shown are means of four replicates extracted three times with acetone. Values followed by the same uppercase letter across soil types are not significantly different at the P = 0.05 level. Values followed by the same lowercase letter down are not significantly different at the P = 0.05 level.

49. Although irreversible adsorption, or extremely slow desorption, may account for low-percent recoveries by extraction, even carbon train analysis recovered an average of only about one half of the treatment levels of ^{14}C. (Extraction analysis at T65 accounted for roughly 12 percent of the treatment level of ^{14}C while carbon train analysis accounted for roughly 40 percent.) The remainder of the original treatment level of ^{14}C must be assumed lost from the soil by some other mechanism.

Gas liquid chromatographic analysis

50. Tables 10 and 11 show results of GLC analysis for T20 and T65 soils, respectively. Results indicate that recoveries of treatment compounds and all potential degradation products were much lower than with either ^{14}C method of analysis. In TNT-treated silt and clay at both T20 and T65, 4ADNT and 2ADNT were present in significantly greater quantities than TNT. This result indicates transformation of TNT to 4ADNT and 2ADNT within 20 days of soil treatment. The TNT concentration exceeded the concentrations of compounds other than 4ADNT and 2ADNT in the TNT-treated silt only. These results suggest that TNT is much less stable or less extractable in the soil than the two degradation products. In both TNT-treated soils, 4ADNT concentrations exceeded 2ADNT concentration at T20 and at T65, an indication that 4ADNT production is

Table 9

Comparison of Percent ^{14}C Recovered by Extraction and by Carbon Train

Treatment	T20* Extraction %	T20* Carbon Train %	T65** Extraction %	T65** Carbon Train %
Clay				
TNT	13.21 B†	68.85 A	12.20 B	51.16 A
4ADNT	10.03 B	34.34 A	6.98 B	28.19 A
Control††	0.13 A	0.09 A	0.04 A	0.06 A
Silt				
TNT	11.11 B	37.62 A	4.96 B	37.48 A
4ADNT	16.09 B	32.47 A	22.38 A	32.05 A
Control	0.02 A	0.04 A	0.00 A	0.01 A

* T20 = 20 days after soil treatment, the time at which tubers were planted.

** T65 = 65 days after soil treatment, the time at which plants were harvested.

† Values shown are means of four replicates extracted three times with acetone. Values followed by the same uppercase letter across and within sampling times are not significantly different at the P = 0.05 level.

†† Control soil samples were taken from bioassays that had been handled in the same manner as treatment, except that no treatment compound was added.

more favored than 2ADNT production, or that 4ADNT is more persistent in the soil than 2ADNT.

51. In 4ADNT- and 2ADNT-treated soils at T20, the treatment compound persisted in significantly greater concentrations than any other compounds with the exception that no compounds predominated in the 2ADNT-treated silt. In the T65 soils, treatment compounds predominated over nonamending compounds in all treatments with two exceptions. The first exception was the failure of TNT to dominate the TNT-treated silt and clay at both times (as discussed in paragraph 50). The second exception was the 4ADNT-treated silt, for which there was no significant difference between the 4ADNT level and the level of 2,4-diamino-6-nitrotoluene (2,4D6NT). These results offer strong evidence that 4ADNT and 2ADNT are the most persistent degradation products of TNT in soils.

Table 10

GLC Analysis of T20 Soils (µg/g Oven-Dry Soil)

Compound Assayed	Treatment							
	Clay				Silt			
	Control	TNT	4ADNT	2ADNT	Control	TNT	4ADNT	2ADNT
2,4,6-trinitrotoluene	0.0220Bc*	0.1343Cbc	0.0368Bc	0.0920Bc	0.0802Ac	0.6413Cab	0.0190Bc	0.8475Aa
4-amino-2,6-dinitrotoluene	0.0060Bb	1.1800Ab	5.3425Aa	0.0410Bb	0.0310Ab	1.8200Ab	5.1350Aa	0.0153Ab
2-amino-4,6-dinitrotoluene	0.0093Bb	0.7350Bb	0.0263Bb	7.1000Aa	0.0170Ab	1.1350Bb	0.0480Bb	0.4576Ab
2,6-diamino-4-nitrotoluene	0.0001Bb	0.0198Cb	0.0905Bb	0.0330Bb	0.0876Ab	0.0103Db	0.4660Ba	0.0171Ab
2,4-diamino-6-nitrotoluene	0.3055Aa	0.0064Ca	0.0818Ba	0.0560Ba	0.0185Aa	0.0194Da	0.1470Ba	0.5575Aa
4-amino-2-nitrotoluene	0.0001Ba	0.0011Ca	0.0151Ba	0.1840Ba	0.0001Aa	0.0208Da	0.0150Ba	0.0613Aa
2,4-dinitrotoluene	0.0001Bb	0.0158Cb	0.0033Bb	0.1080Ba	0.0001Ab	0.0113Db	0.0001Bb	0.0965Aa
2,6-dinitrotoluene	0.0001Bb	0.0318Cb	0.1425Ba	0.0040Bb	0.0001Ab	0.0186Db	0.1130Ba	0.0001Ab
1,3,5-trinitrobenzene	0.1508ABa	0.0248Ca	0.0196Ba	0.0660Ba	0.0548Aa	0.0058Da	0.0200Ba	0.3791Aa

* Values represent means of four replicates. Means having the same lowercase letter across treatments are not significantly different at the P = 0.05 level. Means having the same uppercase letter down the compounds are not significantly different at the P = 0.05 level. Detection limit for all compounds was 0.0001.

Table 11

GLC Analysis of T65 Soils (µg/g Oven-Dry Soil)

Compound Assayed	Treatment							
	Clay				Silt			
	Control	TNT	4ADNT	2ADNT	Control	TNT	4ADNT	2ADNT
2,4,6-trinitrotoluene	0.0001Bb*	0.1570Ca	0.0560Bb	0.0390Bb	0.0001Ab	0.1395Ca	0.0290Bb	0.0458Bb
4-amino-2,6-dinitrotoluene	0.0001Bc	1.1600Abc	3.6275Aa	0.0460Bbc	0.0001Ac	0.4175Abc	2.1326Aab	0.0210Bbc
2-amino-4,6-dinitrotoluene	0.0088Ab	0.7750Bb	0.0283Bb	2.8800Aa	0.0001Ab	0.3000Bb	0.0093Bb	2.4475Aa
2,6-diamino-4-nitrotoluene	0.0001Ba	0.0041Ca	0.0016Ba	0.0011Ba	0.0001Aa	0.0122Da	0.0058Ba	0.0146Ba
2,4-diamino-6-nitrotoluene	0.0001Bc	0.0038Cc	0.0233Bc	0.0198Bc	0.0001Ac	0.0197Dc	0.2400ABa	0.1025Bb
4-amino-2-nitrotoluene	0.0001Ba	0.0016Ca	0.0013Ba	0.0011Ba	0.0001Aa	0.0005Da	0.0011Ba	0.0006Ba
2,4-dinitrotoluene	0.0001Bb	0.0213Cb	0.0008Bb	0.1400Ba	0.0001Ab	0.0013Db	0.0001Bb	0.0265Bb
2,6-dinitrotoluene	0.0001Bb	0.0230Cb	0.0783Ba	0.0008Bb	0.0001Ab	0.0001Db	0.0093Bb	0.0001Bb
1,3,5-trinitrobenzene	0.0001Bb	0.0510Ca	0.0070Bb	0.0023Bb	0.0001Ab	0.0183Db	0.0028Bb	0.0001Bb

* Values represent means of four replicates. Means having the same lowercase letter across treatments are not significantly different at the P = 0.05 level. Means having the same uppercase letter down the compounds are not significantly different at the P = 0.05 level. Detection limit for all compounds was 0.0001.

52. The 4ADNT occurred in significantly highest levels in 4ADNT-treated clay and silt at T20. This result substantiates its stability in soils relative to other degradation products of TNT. 2ADNT was significantly highest in the 2ADNT-treated clay, but was not significantly different from other treatment compounds in the 2ADNT-treated silt at T20. This result suggests greater adsorption of 2ADNT to clay than to silt with consequent stability in the clay.

53. Across soil treatments at T65, TNT predominated in the TNT-treated silt and clay. 4ADNT persisted in significantly highest levels in the 4ADNT-treated silt and clay. However, there was no significant difference between the level of 4ADNT in the silt and in levels of other treatment compounds in both soil types. The 4ADNT level was significantly greater than controls in both soil types. 2ADNT persisted in significantly highest levels in both 2ADNT-treated soils.

54. These results suggest that 4ADNT and 2ADNT do not degrade to significant quantities of any of the other compounds for which soils were assayed in the study. Nevertheless, significant decreases in both 4ADNT and 2ADNT occurred in the soil. Although carbon train results support adsorption as one mechanism reducing the amount of treatment compounds that are extractable, a significant quantity was lost by some other mechanism, e.g., volatilization. Other compounds occurring in concentrations significantly greater than controls were 2,6-diamino-4-nitrotoluene (2,6D4NT) in the 4ADNT-treated silt, 2,4-dinitrotoluene (2,4DNT) in the 2ADNT-treated silt and clay, and 2,6-dinitrotoluene (2,6DNT) in the 4ADNT-treated silt and clay. These results suggest that 4ADNT degrades to 2,6D4NT and 2,6DNT and that 2ADNT degrades to 2,4DNT.

55. The two principal limitations of the GLC analytical method were low recoveries of added known quantities (spikes) and instability of some compounds on the column or at the injection port. Table 12 shows recoveries of spikes added to selected soil samples immediately prior to extraction for GLC analysis. Recoveries of these spikes from soils sampled at T20 and T65 varied with the compound being assayed. However, most recoveries were less than 50 percent. Low recoveries of spikes may have been due to volatilization of compounds or heat degradation of compounds during the Kuderna-Danish concentration step. A change from colorless to pink (an indication of decomposition, or degradation) was observed in solutions of TNT when they were heated

Table 12

Percent Recoveries of Spikes from Selected Soil Samples by GLC Analysis

	Treatment *						
	Clay				Silt		
	T20-	T65			T20		T65-
Compound Assayed	2ADNT	Control	TNT	4ADNT	Control	4ADNT	4ADNT
2,4,6-trinitrotoluene	40	98	34	94	42	42	6.1
4-amino-2,6-dinitrotoluene	40	20	48	--	36	36	--
2-amino-4,6-dinitrotoluene	--	52	22	76	46	46	36
2,6-diamino-4-nitrotoluene	--	--	4.8	--	22	22	16
2,4-diamino-6-nitrotoluene	18	26	2.4	--	9	9	44
4-amino-2-nitrotoluene	14	16	24	--	20	20	30
2,4-dinitrotoluene	92	92	48	104	54	54	36
2,6-dinitrotoluene	70	86	60	134	80	80	58
1,3,5-trinitrobenzene	36	80	--	56	36	36	--

* Treatments not shown (clay control, TNT, and 4ADNT at T20; clay 2ADNT at T65; silt TNT and 2ADNT at T20; and silt control, TNT, and 2ADNT at T65) and those notated "--" received no spikes.

in the laboratory. Samples were not assayed for dimers of TNT, such as the azo and azoxy compounds, because of their ready degradation on the GLC column. TNT and TNB also exhibited some instability at the injection port and on the column.

56. GLC analysis was conducted to detect any of the following compounds: TNT, 4ADNT, 2ADNT, 2,6D4NT, 2,4D6NT, 4-amino-2-nitrotoluene, 2,4DNT, 2,6DNT, and TNB. Except for TNT and TNB, the above compounds were selected because a review of the literature showed that they were the most frequently reported biotransformation products of TNT. Biotransformation was considered to be the most probable transformation mechanism occurring in the soil. The TNB was included because it is a commonly detected photodecomposition product of TNT that could possibly form during treatment, on soil surfaces after potting, or in the plants.

57. Recoveries as a sum of all products detected and based on percentage of original treatment levels are given in Table 13. Recoveries averaged approximately 40 percent of those obtained by ^{14}C extraction analysis and approximately 12 percent of those obtained by ^{14}C carbon train analysis. Spot checks by a high-performance liquid chromatographic (HPLC) method (USATHAMA 1983)* are compared to GLC analysis in Table 14. The HPLC analysis produced higher values than GLC for most samples that exhibited concentrations above detection. However, recoveries were still much lower than with either ^{14}C method of analysis. Recoveries of spikes by HPLC averaged 102 percent, with most values above 100 percent. Extraction for HPLC analysis was by acetonitrile and methanol and did not require application of heat, which could account for higher values if heating were responsible for loss of compounds during sample preparation for GLC. Two disadvantages of the HPLC method were that 4ADNT and 2ADNT could not be separated and that detection limits were higher than with the GLC method.

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* These assays were performed by the Laboratory Branch of the Tennessee Valley Authority, Chattanooga, Tenn.

Table 13

Percent Recoveries of Original Treatment Levels (80 μg/g of Soil) as a Sum of All Compounds Detected by GLC

Soil Type	Treatment	Sampling Time T20	T65
Clay	TNT	2.69	2.75
Clay	4ADNT	7.20	5.92
Clay	2ADNT	9.60	3.91
Clay	Control	0.62	0.00
Silt	TNT	4.60	1.14
Silt	4ADNT	7.45	3.05
Silt	2ADNT	3.05	3.32
Silt	Control	0.36	0.00

Table 14

Comparison of HPLC and GLC Results from Selected T20 Soils

Soil Type	Treatment	TNT GLC*	TNT HPLC**	4ADNT GLC	4ADNT HPLC	2ADNT GLC	2ADNT HPLC
Clay	TNT	0.13†	<1	0.81	4.4	0.50	2
Clay	TNT	0.10	<1	1.7	12	1.2	<1
Clay	4ADNT	0.087	<1	5.9	11	0.027	<1
Clay	4ADNT	0.060	<1	7.6	12	0.028	<1
Silt	TNT	0.51	<1	1.4	2.4	0.92	1
Silt	TNT	0.075	<1	0.85	2.0	0.59	1
Silt	Control	0.091	<1	0.085	3.6	0.025	2

* Detection limit for both TNT and 4ADNT was 0.0001 μg/g.

** Detection limit for both TNT and 4ADNT was 1 μg/g. HPLC was not capable of separating 4ADNT from 2ADNT. Therefore, values given for 4ADNT by HPLC analysis may include 2ADNT.

† Values given are in micrograms per gram of oven-dry soil.

Table 15

Plant Yields (grams)*

Treatment	Soil Type	
	Silt	Clay
Control	5.99a**	7.88a
TNT	6.78a	9.27a
4ADNT	5.63a	8.53a
2ADNT	4.90a	9.71a
Mean of all treatments and controls by soil type	5.824B	8.802A

* Means of four replicates in grams of ODW per pot.

** Means followed by the same lowercase letter within soil types are not significantly different at the $P = 0.05$ level. Means followed by the same uppercase letter across soil types are not significantly different at the $P = 0.05$ level.

Analysis of Plants

Plant yields

58. The data presented in Table 15 show plant yields for each treatment by soil type. There were no significant differences in yield between treatments within soil types. However, ANOVA of means for clay across all treatments and means for silt across all treatments showed significantly greater yields in clay than in silt.

59. Yields for all control and treated pots in this study were significantly lower than (about 28 percent of) those obtained with the standard WES plant bioassay apparatus, which utilizes 7.6-ℓ rather than 3.5-ℓ pots (Folsom et al., in preparation). The reduction in yields may be due to nitrogen limitation. Even though nitrogen was added to the smaller pots at the same rate as in the standard plant bioassay, it is possible that the total quantity of nitrogen available to plants was less in the smaller pots. Nitrogen loss relative to the total added may have been increased due to the greater surface area to volume ratio in the smaller pots.

^{14}C analysis

60. Preliminary plant extraction test. Results of the plant extraction test are given in Table 16. The table shows efficiencies with which the internal ^{14}C standard was recovered from plant extracts. Counts for the benzene extract were significantly higher than counts for the other solvents tested. Since benzene produced the greatest efficiency in counting the internal ^{14}C standard, it was selected as the plant extractant. The internal standard method was used because quenching by chlorophyll was very high in these samples. It is interesting that the same solvent was not selected for the plant and soil extractions. It is possible that acetone, the solvent selected for soil extractions, removed many of the soluble organic compounds from the plants. These compounds may have contributed substantially to quenching of ^{14}C (reduction in scintillation by interference) in the plant extracts. It should be noted that no ^{14}C above-background levels were found in the plant material taken from the TNT-treated clay. This is consistent with results of the 2-g plant analysis discussed below. All of the plant material used in this test was taken from a single TNT-treated clay replicate of the plant uptake study.

Table 16

Results from Extraction of Plants Grown in [^{14}C]TNT-Treated and Untreated Clay Using Four Solvents

Solvent	^{14}C Counting Efficiency, percent*	
	TNT-Treated	Untreated
Acetone	19.4c**	20.5bc
Methanol	31.6b	31.3b
Hexane:acetone	15.7c	19.7c
Benzene	47.7a	61.0a

* Values given are means of three replicates. Counting efficiencies were determined by the internal standard method described in the text.

** Means followed by the same letter within columns are not significantly different at P = 0.05 level.

61. Extraction of 2-g plant samples. Results of ^{14}C analysis from extraction of 2-g plant samples are given in Table 17. Carbon 14 was detected in plants grown in 4ADNT-treated silt only. No ^{14}C was detected in any other treatments nor in controls.

62. Extraction of all remaining plant material. Table 18 shows results of ^{14}C analysis of all remaining plant material. No statistical analysis was performed on the data due to the absence of three data cells, two within a single treatment, and because variances lacked homogeneity even after several transformations of the data. Nevertheless, inspection of the means shows that ^{14}C was detected in plants grown in TNT- and 4ADNT-treated silt and in TNT-treated clay. However, uptake levels represented less than 1 percent of the total ^{14}C available in each pot (based on T65 carbon train recoveries from

Table 17

^{14}C Analysis of 2-g Plant Samples*

Silt			Clay		
Control	TNT	4ADNT	Control	TNT	4ADNT
ND** B†	ND B	4.78 A	ND B	ND B	ND B

* Micrograms of treatment compound per gram of oven-dry plant material.
** Denotes none detected. Detection limits were 0.01 µg/g of oven-dry plant material.
† Values given are means of four replicates, each of which was extracted three times. Means followed by the same letter across [illegible] are not significantly different at P = 0.05 level.

Table 18

^{14}C Analysis of All Remaining Plant Material*

Silt			Clay		
Control	TNT	4ADNT	Control	TNT	4ADNT
ND**	44.57†	55.00	ND	13.26	ND

* Micrograms of treatment compound per gram of oven-dry plant material.
** Denotes none detected. Detection limits were 0.01 µg/g of oven-dry plant material.
† Values given are means of four replicates, except for silt control and silt TNT, which contained sufficient plant material for two and three replicates, respectively.

Table 19

GLC Analysis of Plants*

Compound Assayed	Treatment							
	Clay				Silt			
	Control	TNT	4ADNT	2ADNT	Control	TNT	4ADNT	2ADNT
2,4,6-trinitrotoluene	--**	0.099	--	--	--	--	--	--
4-amino-2,6-dinitrotoluene	--	--	--	--	--	--	--	--
2-amino-4,6-dinitrotoluene	--	--	--	--	--	--	--	--
2,6-diamino-4-nitrotoluene	--	--	0.030	--	--	--	--	--
2,4-diamino-6-nitrotoluene	--	--	--	--	--	--	--	--
4-amino-2-nitrotoluene	--	--	--	--	--	--	--	--
2,4-dinitrotoluene	--	--	--	--	--	--	--	--
2,6-dinitrotoluene	--	--	--	--	--	--	--	--
1,3,5-trinitrobenzene	--	--	--	--	0.075	0.049	0.142	--

* Means of four replicates are given in micrograms per gram of plant material on an ODW basis.

** Showed <0.001 µg/g (below detection limit) of the assayed compound.

soils). Nevertheless, these results indicate that the plant did take up labeled compound(s) from the TNT-treated silt and clay and from the 4ADNT-treated silt. Lack of ^{14}C in 4ADNT-treated clay may reflect reduced availability to the plant due to strong adsorption of 4ADNT to the clay. Less plant uptake of ^{14}C from TNT-treated clay than from TNT-treated silt also supports adsorption as a mechanism limiting plant availability of TNT in the clay. Comparison of ^{14}C extraction and carbon train results for 4ADNT-treated clay and silt (Table 9) showed stronger adsorption by the clay at T65.

Gas liquid chromatographic analysis

63. The data presented in Table 19 show results of GLC analysis of plant material. The only compounds detected were TNB, TNT, and 2ADNT. These compounds were detected in plants from the TNT- and 4ADNT-treated silt and clay, but not in those from the 2ADNT-treated soils. TNB was also detected in the silt control. These results are qualitatively consistent with ^{14}C extraction data of all remaining plant material except for the detection of 2ADNT in plants grown in the 4ADNT-treated clay and detection of TNB in silt controls. No ^{14}C was detected in these plants.

64. Recoveries of spikes added to plant samples immediately prior to extraction for GLC analysis were comparable to those obtained with soils, with the exception of the diamino compounds (2,4D6NT and 2,6D4NT). No 2,4D6NT was recovered, and only 4 percent of the 2,6D4NT was recovered. It is probable that these compounds were lost during the concentration step prior to GLC analysis rather than during GLC analysis, since standard preparations of the compounds were stable on the GLC column.

Factors Potentially Limiting Plant Uptake

65. Limited plant uptake of treatment compounds occurred during this study. However, ^{14}C analyses demonstrated uptake of labeled compound(s) by *C. esculentus* from both the silt and clay. Carbon 14 analysis indicated detection of the radioactive isotope only and did not indicate the identity of the compound(s) of which the radioisotope was a part. Therefore, in the absence of GLC detection, the identity of the compound(s) actually present in the plant could not be known.

66. More ^{14}C was taken up from silt than from clay. This result is at least partially explained by the greater adsorption and consequent reduction

in bioavailability of treatment compounds in clay than in silt. Carbon train results indicated that significant adsorption of treatment compounds occurred in both soil types. Comparison of ^{14}C results when soils were analyzed by carbon train and by solvent extraction showed that significant levels of ^{14}C remained in the soils after extraction. The literature also supports adsorption as an explanation for lack of extractability. Cragin et al. (1985) found a decrease in recovery of TNT from soils and sediments over a 7-day storage period. In sediments containing 59 percent moisture, only 5 percent of TNT spikes were recovered by acetone extraction after 2 days. After ruling out volatilization of TNT, the authors attributed this loss to adsorption. It should be noted that volatilization of TNT degradation products was not considered. In the present study, the silt aliquot contained ca. 37 percent water and the clay contained ca. 53 percent water when the acetone treatment solution was applied. Although the treated soil aliquots were allowed to air-dry immediately after treatment, carbon train results indicated that significant adsorption resulted from the treatment method and also occurred between T0 and T20.

67. Plant uptake was also limited by loss of treatment compounds from the soils prior to planting. The first indication of this loss was provided by results from the soil homogeneity test in which percent recoveries for all treatments were much lower than expected. One possible mechanism for loss of treatment compounds is photodecomposition during treatment. Even though efforts were made to protect solutions from exposure to laboratory lighting (there was no natural light in the laboratory) by storage in brown bottles, limited exposure was unavoidable. Acetone, the solvent of choice for application of TNT, 4ADNT, and 2ADNT to the soils is reported by Spanggord et al. (1980) to be a triplet exciter, or photosensitizer. These investigators observed a more rapid loss of TNT from acetone than from aqueous solutions. They reported a half-life of 9 hr for 100 ppm TNT in 0.10-percent acetone solution and 3 hr in a 1.0-percent acetone solution. In the present study, the treated soil aliquots contained 80 µg of treatment compound (e.g, TNT) per gram of soil and a total acetone concentration in the aqueous phase of approximately 0.3 and 0.1 percent for silt and clay, respectively. (The clay required more water to produce a workable slurry and was, consequently, more dilute than the silt.) If photodecomposition occurred at the same rate as reported by Spanggord et al. (1980), significant amounts of the TNT could be

photodecomposed during the treatment period. Corresponding data for 4ADNT were unavailable. However, Burlinson et al. (1979) found in one study that 90 percent of TNT decomposed after 1 hr of irradiation, while only 30 percent of 4ADNT and 20 percent of 2ADNT decomposed.

68. Another possible mechanism for loss of treatment compounds from the soil is volatilization. TNT is not considered a volatile compound because it has a vapor pressure of 1.28×10^{-6} torr at 20.0° C (Coates, Freedman, and Kuhn 1970; Leggett, Jenkins, and Murrmann 1977). However, microbial decomposition products as well as photodecomposition products of TNT may be volatile. For example, Leggett, Jenkins, and Murrmann (1977) reported the vapor pressure of 2,4DNT above solid TNT to be 2.2×10^{-5} torr at 20° C, which is nearly 20 times higher than the vapor pressure of TNT. They also reported that the concentration of 2,4DNT exceeded that of TNT above the solid by at least one order of magnitude. Vapor pressure data on the 20 or so known photodecomposition products of TNT could not be found. However, it is not unreasonable to assume that some of these products, for example the benzenes, would possess higher vapor pressures than TNT. The presence of water in the soil is known to enhance volatilization of pesticides (Guenzi and Beard 1974), many of which exhibit vapor pressures comparable to that of TNT. It is therefore possible that photodecomposition followed by volatilization from the soil during the drying of treated soil aliquots in shallow pans accounts for some loss of treatment compounds and the consequent low recoveries of added compounds.

69. Principal known degradation products of TNT were detected in the soils by GLC analysis, but were found in the plants in extremely limited quantities. Discrepancies between ^{14}C and GLC results indicate that the GLC analytical method was ineffective for plant material. Inability to adequately identify compounds in the plant precluded the drawing of conclusions regarding plant uptake, degradation, or bioconcentration of specific compounds. In the soils, TNT was degraded to 4ADNT and 2ADNT, both of which were more stable than TNT. However, recoveries of ^{14}C by carbon train analysis not only demonstrated significant adsorption of labeled compounds by the soil, but also indicated significant loss of treatment compounds from the soils.

PART IV: CONCLUSIONS

70. Conclusions of the study are summarized below.

a. Little TNT and 4ADNT and no 2ADNT were found in *C. esculentus*. Plant uptake was greatest from 4ADNT-treated silt. Bioavailability of treatment compounds may have been limited by adsorption, or binding, of compounds to soils and possibly by volatilization of microbial and phototransformation products.

b. Since ^{14}C-labeled compounds were present in plants in quantities too low to be detected by gas liquid chromatographic analysis, no conclusion can be drawn concerning degradation of treatment compounds in *C. esculentus*.

c. Neither TNT, 4ADNT, nor 2ADNT became concentrated in *C. esculentus*.

d. In the soils, TNT was transformed to 4ADNT and, to a lesser extent, to 2ADNT. According to GLC results, 4ADNT was more stable and persistent in the soil than either TNT or 2ADNT. Two degradation products, 2,6-diamino-4-nitrotoluene and 2,6-dinitrotoluene, were found in limited quantities in 4ADNT-treated soils, and 2,4-dinitrotoluene was detected in 2ADNT-treated soils.

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APPENDIX A: STANDARD CURVES

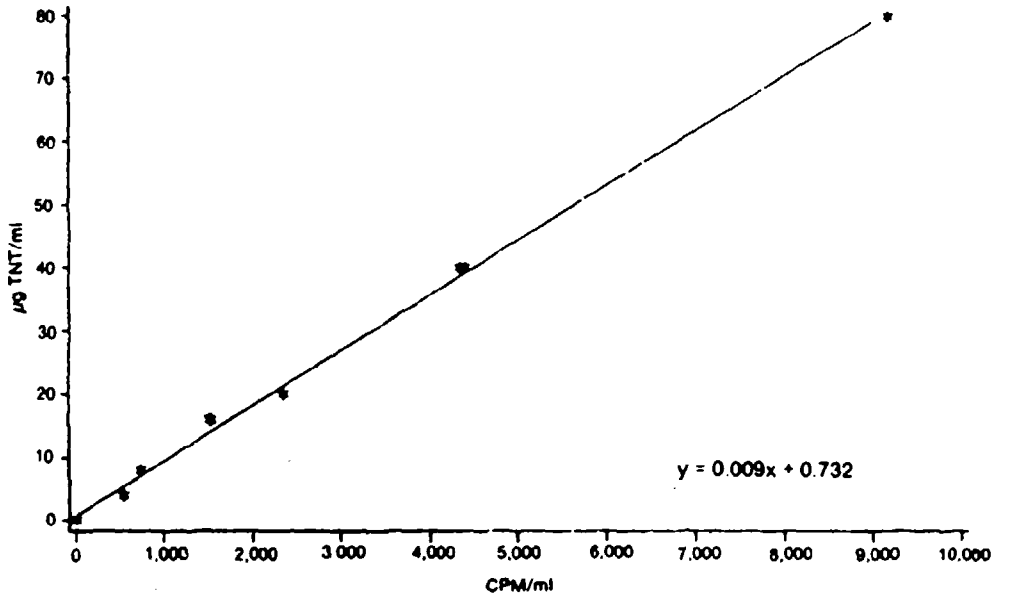

Figure A1. Standard curve for [^{14}C]TNT treatment solution

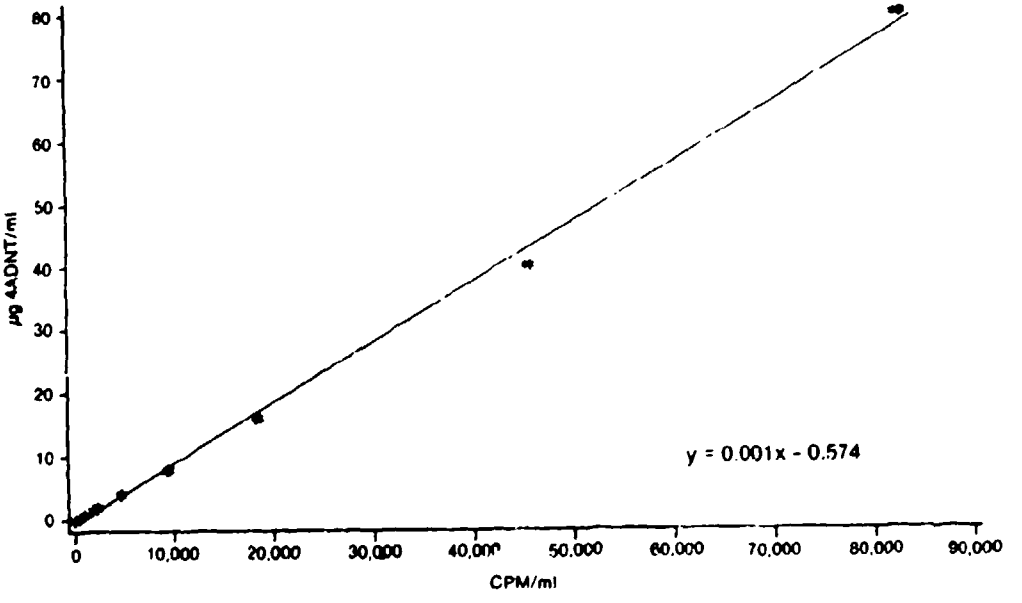

Figure A2. Standard curve for [^{14}C]4ADNT treatment solution

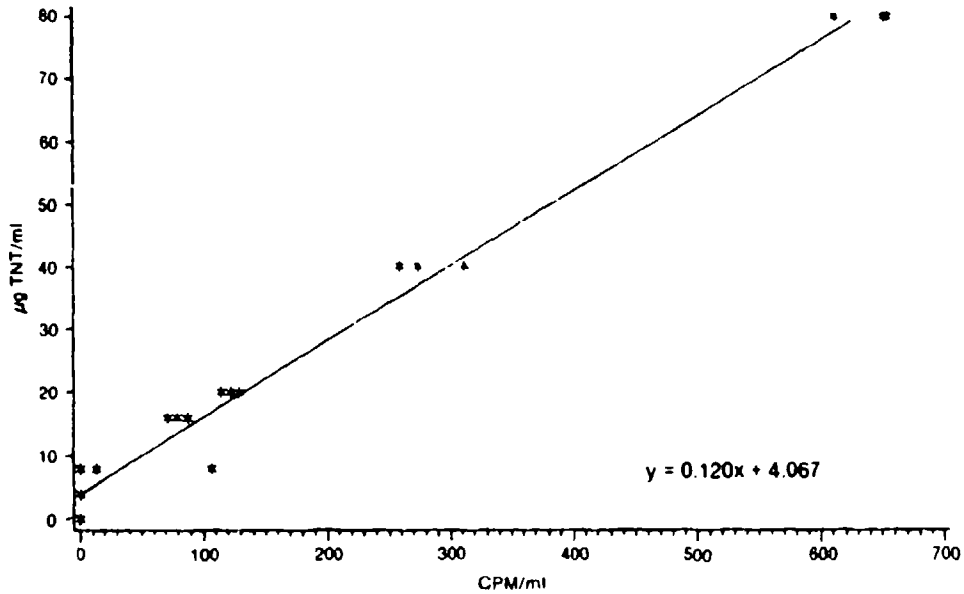

Figure A3. Standard curve for [^{14}C]TNT plant extracts

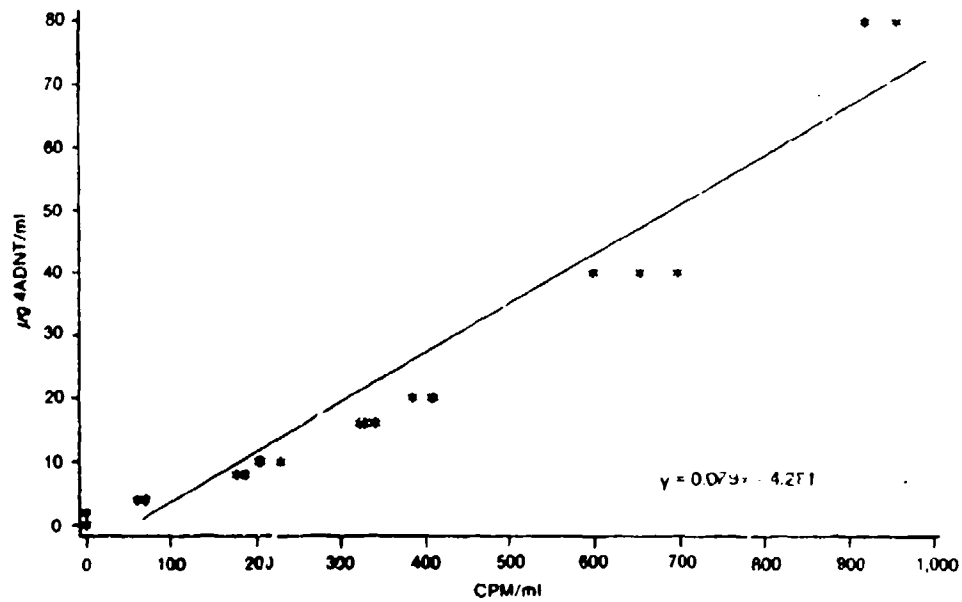

Figure A4. Standard curve for [^{14}C]4ADNT plant extracts

APPENDIX B: DATA

Soil Homogeneity Test

Soil	Treatment	Replicate	Left*	Middle*	Right*
Clay	4ADNT	1	32.1236	31.1816	31.2484
Clay	4ADNT	2	35.1229	33.2136	29.9234
Clay	4ADNT	3	29.1135	31.9020	27.9429
Clay	TNT	1	67.3064	76.9503	74.5300
Clay	TNT	2	82.7184	60.6535	56.2878
Clay	TNT	3	64.0726	70.5750	62.1490
Silt	4ADNT	1	23.3904	22.0485	28.0652
Silt	4ADNT	2	22.0295	23.5461	22.3820
Silt	4ADNT	3	23.1389	23.5126	21.5567
Silt	TNT	1	39.0766	38.0265	41.0350
Silt	TNT	2	35.8942	37.8778	37.4080
Silt	TNT	3	38.6181	38.3041	39.6360

* Expressed in micrograms per gram of soil.

Soil Solvent Test

Soil	Treatment	Replicate	Solvent µg/mℓ
Clay	Acetone	1	83.882
Clay	Acetone	2	79.103
Clay	Acetone	3	108.49
Clay	Acetone	4	50.113
Clay	Benzene	1	22.384
Clay	Benzene	2	22.813
Clay	Benzene	3	26.056
Clay	Benzene	4	24.661
Clay	Methanol	1	71.010
Clay	Methanol	2	60.127
Clay	Methanol	3	74.120
Clay	Methanol	4	64.108
Clay	Methylene chloride	1	30.904
Clay	Methylene chloride	2	35.069
Clay	Methylene chloride	3	35.779
Clay	Methylene chloride	4	35.949
Silt	Acetone	1	43.946
Silt	Acetone	2	46.607
Silt	Acetone	3	46.168
Silt	Acetone	4	49.097
Silt	Benzene	1	38.740
Silt	Benzene	2	37.317
Silt	Benzene	3	35.483
Silt	Benzene	4	36.615
Silt	Methanol	1	45.153
Silt	Methanol	2	45.453
Silt	Methanol	3	43.758
Silt	Methanol	4	45.077
Silt	Methylene chloride	1	38.380
Silt	Methylene chloride	2	37.987
Silt	Methylene chloride	3	41.083
Silt	Methylene chloride	4	43.990

^{14}C Analysis of Siphoned Water

Soil	Treatment	Total CPM
Silt	TNT	2030.56
Clay	TNT	1083.30
Silt	4ADNT	746.75
Clay	4ADNT	699.60
Total TNT added		1.15×10^8
Total 4ADNT added		1.04×10^9

^{14}C Analysis of Extracts of TNT- and 4ADNT-Treated Silt and Clay at T20 and T65

Soil	Treatment	Replicate	Day	µg/g
Clay	4ADNT	1	20	9.8758
Clay	4ADNT	2	20	6.7555
Clay	4ADNT	3	20	6.0868
Clay	4ADNT	4	20	8.8032
Clay	Control	1	20	0.8926
Clay	Control	2	20	0.7889
Clay	Control	3	20	0.7885
Clay	Control	4	20	0.8674
Clay	TNT	1	20	12.5028
Clay	TNT	2	20	11.6976
Clay	TNT	3	20	11.4143
Clay	TNT	4	20	9.4202
Clay	4ADNT	1	65	5.3159
Clay	4ADNT	2	65	6.8236
Clay	4ADNT	3	65	4.3251
Clay	4ADNT	4	65	5.4133
Clay	Control	1	65	0.7391
Clay	Control	2	65	0.7932
Clay	Control	3	65	0.7517
Clay	Control	4	65	0.7662
Clay	TNT	1	65	11.5514
Clay	TNT	2	65	10.6251
Clay	TNT	3	65	9.5259
Clay	TNT	4	65	10.1378
Silt	4ADNT	1	20	15.8579
Silt	4ADNT	2	20	12.2581
Silt	4ADNT	3	20	9.8113
Silt	4ADNT	4	20	12.7888
Silt	Control	1	20	0.7658
Silt	Control	2	20	0.7587
Silt	Control	3	20	0.7454
Silt	Control	4	20	0.7301
Silt	TNT	1	20	9.2059
Silt	TNT	2	20	9.9692
Silt	TNT	3	20	9.2376
Silt	TNT	4	20	9.9121
Silt	4ADNT	1	65	28.0098
Silt	4ADNT	2	65	13.8326
Silt	4ADNT	3	65	14.8368
Silt	4ADNT	4	65	13.9432
Silt	Control	1	65	0.7301
Silt	Control	2	65	0.7301
Silt	Control	3	65	0.7437
Silt	Control	4	65	0.7301
Silt	TNT	1	65	2.6700
Silt	TNT	2	65	4.9853
Silt	TNT	3	65	5.8837
Silt	TNT	4	65	5.1891

Carbon Train Efficiency Curve Using Sorbed Silt

Added		Recovered, CPM			Total Recovered	
ml	CPM	Solution	Soil	Test Tube	CPM	Percent
25	1,346,625	791,291	258,152	115,136	1,162,468	86.3246
25	1,346,625	791,115	254,950	115,136	1,159,266	86.0868
25	1,346,625	785,133	255,205	115,136	1,159,520	86.1057
20	1,077,300	624,231	245,977	92,109	960,172	89.1276
20	1,077,300	619,408	245,586	92,109	959,781	89.0914
20	1,077,300	622,620	248,860	92,109	963,055	89.3952
15	807,975	464,140	191,415	69,082	721,772	89.3310
15	807,975	465,985	193,547	69,082	723,905	89.5950
15	807,975	453,703	192,024	69,082	722,382	89.4064
10	536,400	290,786	142,685	45,862	483,399	90.1191
10	536,400	300,647	141,530	45,862	482,244	89.9039
10	536,400	293,124	142,150	45,862	482,864	90.0193
5	269,325	146,481	76,817	23,027	244,546	90.7997
5	269,325	141,808	76,863	23,027	244,592	90.8167
5	269,325	145,816	76,909	23,027	244,638	90.8338
2	107,725	51,980	40,164	9,210	100,118	92.9380
2	107,725	51,480	40,196	9,210	100,150	92.9677
2	107,725	48,770	40,508	9,210	100,461	93.2572
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0

Carbon Train Efficiency Curve Using Sorbed Cley

Added		Recovered, CPM			Total Recovered	
ml	CPM	Solution	Soil	Test Tube	CPM	Percent
25	1,329,271	419,512	774,767	113,653	1,295,150	97.4331
25	1,329,271	371,128	763,512	113,653	1,283,895	96.5864
25	1,329,271	429,553	761,593	113,653	1,281,976	96.4420
20	1,063,417	273,481	610,434	90,922	970,399	91.2529
20	1,063,417	259,065	608,563	90,922	968,527	91.0768
20	1,063,417	274,580	609,654	90,922	969,619	91.1795
15	797,563	160,357	560,773	68,192	773,886	97.0313
15	797,563	138,538	567,521	68,192	780,634	97.8774
15	797,563	135,868	565,345	68,192	778,457	97.6045
10	531,709	49,660	274,479	45,461	384,237	72.2645
10	531,709	76,510	272,770	45,461	382,528	71.9431
10	531,709	66,721	272,590	45,461	382,348	71.9094
5	265,854	33,656	166,529	22,731	221,558	83.3381
5	265,854	34,708	167,782	22,731	222,811	83.8095
5	265,854	28,530	167,271	22,731	222,299	83.6169
2	106,342	11,837	60,480	9,092	80,109	75.3317
2	106,342	10,833	60,152	9,092	79,781	75.0237
2	106,342	8,941	61,117	9,092	80,746	75.9310
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0

^{14}C Analysis of Carbon Train Results for TNT- and 4ADNT-Treated Silt and Clay at T20 and T65

Soil	Day	Treatment	µg/g
Clay	T20	4ADNT	27.5573
Clay	T20	4ADNT	24.9116
Clay	T20	4ADNT	27.0033
Clay	T20	4ADNT	26.9570
Clay	T20	Control	0.7849
Clay	T20	Control	0.8536
Clay	T20	Control	0.8014
Clay	T20	Control	0.7675
Clay	T20	TNT	54.3386
Clay	T20	TNT	56.1613
Clay	T20	TNT	59.5865
Clay	T20	TNT	52.4037
Clay	T65	4ADNT	21.3404
Clay	T65	4ADNT	18.2605
Clay	T65	4ADNT	21.9235
Clay	T65	4ADNT	25.4156
Clay	T65	Control	0.7591
Clay	T65	Control	0.7368
Clay	T65	Control	0.7664
Clay	T65	Control	0.8388
Clay	T65	TNT	40.3792
Clay	T65	TNT	41.3907
Clay	T65	TNT	45.8115
Clay	T65	TNT	38.4843
Silt	T20	4ADNT	23.5565
Silt	T20	4ADNT	25.0543
Silt	T20	4ADNT	24.8321
Silt	T20	4ADNT	27.0497
Silt	T20	Control	0.7547
Silt	T20	Control	0.7411
Silt	T20	Control	0.7585
Silt	T20	Control	0.7817
Silt	T20	TNT	39.9860
Silt	T20	TNT	33.6581
Silt	T20	TNT	35.2291
Silt	T20	TNT	14.0243
Silt	T65	4ADNT	28.4024
Silt	T65	4ADNT	18.8327
Silt	T65	4ADNT	25.1018
Silt	T65	4ADNT	26.8121
Silt	T65	Control	0.7376
Silt	T65	Control	0.7338
Silt	T65	Control	0.7362
Silt	T65	Control	0.7301
Silt	T65	TNT	25.5656
Silt	T65	TNT	34.1449
Silt	T65	TNT	30.6050
Silt	T65	TNT	32.1453

GLC Analysis of T20 Soils (µg/g Oven-Dry Soil)

Soil	Treatment	Replicate	TNT	2ADNT	4ADNT	26D4NT	24D6NT	4ANT	24DNT	135TNB	26DNT
Clay	2ADNT	1	0.1100	0.0001	0.0190	0.0001	0.0200	0.0040	0.1700	0.1000	0.0001
Clay	2ADNT	2	0.0620	6.0000	0.0290	0.1300	0.1300	0.7200	0.0710	0.0500	0.0150
Clay	2ADNT	3	0.120	10.9000	0.0500	0.0001	0.0410	0.0050	0.0800	0.0620	0.0001
Clay	2ADNT	4	0.076	11.5000	0.0670	0.0001	0.0320	0.0070	0.1100	0.0500	0.0024
Clay	4ADNT	1	0.0600	0.0280	7.5500	0.1700	0.0830	0.0460	0.0110	0.0630	0.1800
Clay	4ADNT	2	0.0001	0.0170	3.7900	0.0440	0.0800	0.0001	0.0020	0.0150	0.1200
Clay	4ADNT	3	0.0001	0.0330	4.1000	0.0180	0.0640	0.0001	0.0001	0.0001	0.1300
Clay	4ADNT	4	0.0870	0.0270	5.9300	0.1300	0.1000	0.0140	0.0001	0.0001	0.1400
Clay	Control	1	0.0540	0.0060	0.0050	0.0001	0.0001	0.0001	0.0001	0.0400	0.0001
Clay	Control	2	0.0340	0.0310	0.0190	0.0001	0.0020	0.0001	0.0001	0.0430	0.0001
Clay	Control	3	0.0001	0.0001	0.0001	0.0001	0.5700	0.0001	0.0001	0.2100	0.0001
Clay	Control	4	0.0001	0.0001	0.0001	0.0001	0.6500	0.0001	0.0001	0.3100	0.0001
Clay	TNT	1	0.1270	0.5000	0.8100	0.0001	0.0037	0.0001	0.0160	0.0001	0.0200
Clay	TNT	2	0.1000	1.1600	1.7200	0.0540	0.0090	0.0040	0.0230	0.0001	0.0560
Clay	TNT	3	0.1400	0.6100	1.0000	0.0001	0.0068	0.0001	0.0120	0.0001	0.0260
Clay	TNT	4	0.1700	0.6700	1.1900	0.0250	0.0060	0.0001	0.0120	0.0990	0.0250
Silt	2ADNT	1	0.6800	0.0001	0.0001	0.0001	0.2400	0.0280	0.0440	1.4800	0.0001
Silt	2ADNT	2	0.0001	0.0001	0.0610	0.0680	0.1100	0.0260	0.0180	0.0360	0.0001
Silt	2ADNT	3	0.5100	1.8300	0.0001	0.0001	1.7500	0.1500	0.2500	0.0001	0.0001
Silt	2ADNT	4	2.2000	0.0001	0.0001	0.0001	0.1300	0.0410	0.0740	0.0001	0.0001
Silt	4ADNT	1	0.0200	0.0220	4.8900	0.0190	0.1400	0.0001	0.0001	0.0370	0.1700
Silt	4ADNT	2	0.0160	0.0250	0.0001	0.0260	0.1900	0.0110	0.0001	0.0430	0.1500
Silt	4ADNT	3	0.0001	0.0840	4.4500	0.7900	0.0680	0.0001	0.0001	0.0001	0.0210
Silt	4ADNT	4	0.0390	0.061	11.2000	1.0300	0.1900	0.0490	0.0001	0.0001	0.1100
Silt	Control	1	0.0910	0.0250	0.0850	0.3500	0.0610	0.0001	0.0001	0.1000	0.0001
Silt	Control	2	0.0630	0.0160	0.0120	0.0001	0.0040	0.0001	0.0001	0.0460	0.0001
Silt	Control	3	0.1100	0.0110	0.0150	0.0001	0.0050	0.0001	0.0001	0.0270	0.0001
Silt	Control	4	0.0570	0.0160	0.0120	0.0001	0.0040	0.0001	0.0001	0.0460	0.0001
Silt	TNT	1	0.8700	1.4400	2.1800	0.0100	0.0037	0.0420	0.0190	0.0001	0.0250
Silt	TNT	2	0.0750	0.5900	0.8500	0.0001	0.0330	0.0180	0.0110	0.0230	0.0140
Silt	TNT	3	1.1100	1.5900	2.8000	0.0130	0.0087	0.0230	0.0150	0.0001	0.0280
Silt	TNT	4	0.5100	0.9200	1.4500	0.0180	0.0320	0.0001	0.0001	0.0001	0.0073

GLC Analysis of T65 Soils (µg/g Oven-Dry Soil)

Soil	Treatment	Replicate	TNT	2ADNT	4ADNT	26D4NT	24D6NT	4ANT	24DNT	135TNB	26DNT
Clay	2ADNT	1	0.048	0.0001	0.073	0.0001	0.023	0.0040	0.2500	0.0030	0.0030
Clay	2ADNT	2	0.032	4.4300	0.049	0.0001	0.035	0.0001	0.0910	0.0030	0.0001
Clay	2ADNT	3	0.041	3.3200	0.031	0.0001	0.005	0.0001	0.1050	0.0001	0.0001
Clay	2ADNT	4	0.035	3.7700	0.031	0.0040	0.016	0.0001	0.1140	0.0030	0.0001
Clay	4ADNT	1	0.063	0.0350	4.610	0.0001	0.005	0.0010	0.0030	0.0010	0.0120
Clay	4ADNT	2	0.063	0.0390	1.970	0.0001	0.056	0.0001	0.0001	0.0180	0.0420
Clay	4ADNT	3	0.031	0.0090	2.460	0.0001	0.029	0.0001	0.0001	0.0001	0.0890
Clay	4ADNT	4	0.067	0.0300	5.470	0.0060	0.003	0.0060	0.0001	0.0090	0.1700
Clay	Control	1	0.0001	0.0160	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Clay	Control	2	0.0001	0.0190	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Clay	Control	3	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Clay	Control	4	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Clay	TNT	1	0.3350	1.4300	1.9600	0.0160	0.0001	0.0044	0.0450	0.1300	0.0260
Clay	TNT	2	0.1320	0.7500	1.0700	0.0001	0.0040	0.0001	0.0230	0.0270	0.0450
Clay	TNT	3	0.0910	0.5300	0.8700	0.0001	0.0070	0.0017	0.0120	0.0001	0.0160
Clay	TNT	4	0.0700	0.3900	0.7400	0.0001	0.0040	0.0001	0.0050	0.0470	0.0050
Silt	2ADNT	1	0.0460	3.4900	0.0050	0.0001	0.1200	0.0001	0.0091	0.0001	0.0001
Silt	2ADNT	2	0.0300	0.0001	0.0070	0.0001	0.1700	0.0001	0.0040	0.0001	0.0001
Silt	2ADNT	3	0.0460	2.0600	0.0270	0.0580	0.0001	0.0001	0.0980	0.0001	0.0001
Silt	2ADNT	4	0.0610	4.2400	0.0450	0.0001	0.1200	0.0020	0.0040	0.0001	0.0001
Silt	4ADNT	1	0.025	0.0001	0.0001	0.0100	0.2700	0.0001	0.0001	0.0001	0.0090
Silt	4ADNT	2	0.026	0.0370	1.8000	0.0010	0.2100	0.0020	0.0001	0.0001	0.0070
Silt	4ADNT	3	0.049	0.0001	6.7300	0.0110	0.2700	0.0020	0.0001	0.0001	0.0100
Silt	4ADNT	4	0.016	0.0001	0.0001	0.0010	0.2100	0.0001	0.0001	0.0110	0.0110
Silt	Control	1	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Silt	Control	2	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Silt	Control	3	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Silt	Control	4	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Silt	TNT	1	0.220	0.26	0.37	0.004	0.0088	0.0001	0.0001	0.0001	0.0001
Silt	TNT	2	0.038	0.29	0.36	0.007	0.0250	0.0001	0.0001	0.0400	0.0001
Silt	TNT	3	0.150	0.24	0.43	0.031	0.0200	0.0001	0.0001	0.0330	0.0001
Silt	TNT	4	0.150	0.41	0.51	0.007	0.0250	0.0017	0.0050	0.0001	0.0001

Plant Yields

Soil	Replicate	Treatment	Yield g
Clay	1	2ADNT	8.50
Clay	2	2ADNT	8.81
Clay	3	2ADNT	11.15
Clay	4	2ADNT	10.37
Clay	1	4ADNT	10.15
Clay	2	4ADNT	9.55
Clay	3	4ADNT	7.54
Clay	4	4ADNT	6.89
Clay	1	Control	9.35
Clay	2	Control	8.93
Clay	3	Control	8.23
Clay	4	Control	5.00
Clay	1	TNT	6.46
Clay	2	TNT	10.05
Clay	3	TNT	6.38
Clay	4	TNT	14.19
Silt	1	2ADNT	3.97
Silt	2	2ADNT	4.52
Silt	3	2ADNT	5.05
Silt	4	2ADNT	6.06
Silt	1	4ADNT	5.17
Silt	2	4ADNT	4.02
Silt	3	4ADNT	4.84
Silt	4	4ADNT	8.49
Silt	1	Control	7.71
Silt	2	Control	2.07
Silt	3	Control	4.08
Silt	4	Control	10.08
Silt	1	TNT	6.24
Silt	2	TNT	8.71
Silt	3	TNT	7.45
Silt	4	TNT	4.73

Plant Solvent Test

Treatment	Solvent	Replicate	Efficiency percent
Untreated	Methanol	1	25.6
Untreated	Methanol	2	24.2
Untreated	Methanol	3	44.0
Untreated	Benzene	1	62.4
Untreated	Benzene	2	64.3
Untreated	Benzene	3	56.3
Untreated	Hexane:acetone	1	16.2
Untreated	Hexane:acetone	2	21.7
Untreated	Hexane:acetone	3	21.3
Untreated	Acetone	1	20.5
Untreated	Acetone	2	23.4
Untreated	Acetone	3	17.6
TNT	Methanol	1	31.0
TNT	Methanol	2	32.5
TNT	Methanol	3	31.3
TNT	Benzene	1	45.0
TNT	Benzene	2	45.7
TNT	Benzene	3	52.3
TNT	Hexane:acetone	1	16.9
TNT	Hexane:acetone	2	15.6
TNT	Hexane:acetone	3	14.7
TNT	Acetone	1	24.0
TNT	Acetone	2	16.5
TNT	Acetone	3	17.7

^{14}C Analysis of 2-g Plant Samples

Soil	Treatment	Replicate	µg/g
Clay	4ADNT	1	ND*
Clay	4ADNT	2	ND
Clay	4ADNT	3	ND
Clay	4ADNT	4	ND
Clay	Control	1	ND
Clay	Control	2	ND
Clay	Control	3	ND
Clay	Control	4	ND
Clay	TNT	1	ND
Clay	TNT	2	ND
Clay	TNT	3	ND
Clay	TNT	4	ND
Silt	4ADNT	1	ND
Silt	4ADNT	2	6.24
Silt	4ADNT	3	12.12
Silt	4ADNT	4	0.77
Silt	Control	1	ND
Silt	Control	2	ND
Silt	Control	3	ND
Silt	Control	4	ND
Silt	TNT	1	ND
Silt	TNT	2	ND
Silt	TNT	3	ND
Silt	TNT	4	ND

*ND = none detected. Detection limit, 0.01 µg/g.

^{14}C Analysis of All Remaining Plant Samples

Soil	Treatment	Replicate	µg/g
Clay	4ADNT	1	ND
Clay	4ADNT	2	ND
Clay	4ADNT	3	ND
Clay	4ADNT	4	ND
Clay	Control	1	ND
Clay	Control	2	ND
Clay	Control	3	ND
Clay	Control	4	ND
Clay	TNT	1	44.14
Clay	TNT	2	ND
Clay	TNT	3	8.91
Clay	TNT	4	ND
Silt	4ADNT	1	71.53
Silt	4ADNT	2	21.55
Silt	4ADNT	3	126.91
Silt	4ADNT	4	ND
Silt	Control	1	ND
Silt	Control	2	*
Silt	Control	3	ND
Silt	Control	4	*
Silt	TNT	1	ND
Silt	TNT	2	69.74
Silt	TNT	3	*
Silt	TNT	4	19.40

Note: ND = none detected. Detection limit 0.01 µg/g.
* Insufficient plant material remained for test.

GLC Analysis of Plant Extracts

Soil	Treatment	Replicate	TNT	2ADNT	4ADNT	26D4NT	24D6NT	4ANT	24DNT	135TNB	26DNT
Clay	2ADNT	1	--*	--	--	--	--	--	--	--	--
Clay	2ADNT	2	--	--	--	--	--	--	--	--	--
Clay	2ADNT	3	--	--	--	--	--	--	--	--	--
Clay	2ADNT	4	--	--	--	--	--	--	--	--	--
Clay	4ADNT	1	--	--	--	0.120	--	--	--	--	--
Clay	4ADNT	2	--	--	--	--	--	--	--	--	--
Clay	4ADNT	3	--	--	--	--	--	--	--	--	--
Clay	4ADNT	4	--	--	--	--	--	--	--	--	--
Clay	Control	1	--	--	--	--	--	--	--	--	--
Clay	Control	2	--	--	--	--	--	--	--	--	--
Clay	Control	3	--	--	--	--	--	--	--	--	--
Clay	Control	4	--	--	--	--	--	--	--	--	--
Clay	TNT	1	0.394	--	--	--	--	--	--	--	--
Clay	TNT	2	--	--	--	--	--	--	--	--	--
Clay	TNT	3	--	--	--	--	--	--	--	--	--
Clay	TNT	4	--	--	--	--	--	--	--	--	--
Silt	2ADNT	1	--	--	--	--	--	--	--	--	--
Silt	2ADNT	2	--	--	--	--	--	--	--	--	--
Silt	2ADNT	3	--	--	--	--	--	--	--	--	--
Silt	2ADNT	4	--	--	--	--	--	--	--	--	--
Silt	4ADNT	1	--	--	--	--	--	--	--	--	--
Silt	4ADNT	2	--	--	--	--	--	--	--	--	--
Silt	4ADNT	3	--	--	--	--	--	--	--	--	--
Silt	4ADNT	4	--	--	--	--	--	--	--	0.570	--
Silt	Control	1	--	--	--	--	--	--	--	--	--
Silt	Control	2	--	--	--	--	--	--	--	--	--
Silt	Control	3	--	--	--	--	--	--	--	--	--
Silt	Control	4	--	--	--	--	--	--	--	0.300	--
Silt	TNT	1	--	--	--	--	--	--	--	--	--
Silt	TNT	2	--	--	--	--	--	--	--	0.196	--
Silt	TNT	3	--	--	--	--	--	--	--	--	--
Silt	TNT	4	--	--	--	--	--	--	--	--	--

* Value below the 0.001 µg/g detection limit.