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SOIL SORPTION AND PLANT UPTAKE OF 2,4,6-TRINITROTOLUENE

by

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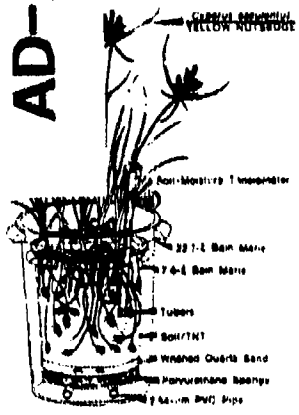

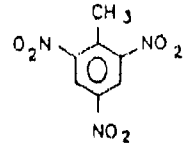

2,4,6 – Trinitrotoluene

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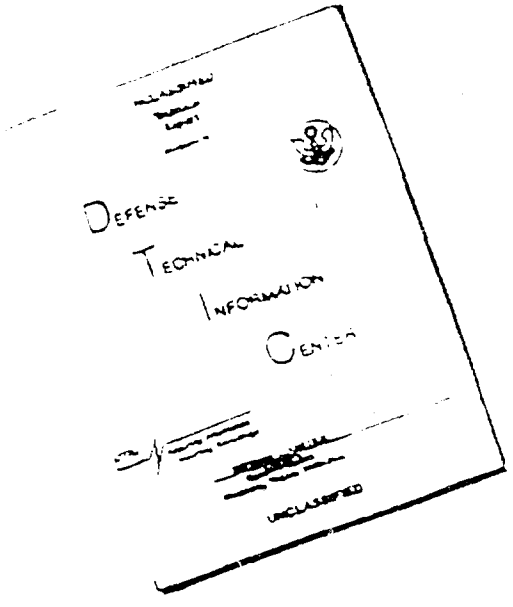

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Potential environmental hazards are posed by the presence of 2,4,6-Trinitrotoluene (TNT) contamination in soils of US Army Ammunition Plants (AAPs). Adsorption and desorption studies were conducted on soil samples collected from 13 AAPs. Soils were found to be primarily silt loams, low in organic carbon. TNT reached a steady state of adsorption with test soils within 2 hr of contact. Desorption also reached steady state within 2 hr with more than half of the adsorbed TNT being removed. Batch adsorption isotherms were best fit by the Langmuir adsorption model. Average adsorption coefficients (soil concentration to solution concentration), or K_d, was 4.0. However, K_d varied with soil type.

TNT adsorption correlated most highly with cation exchange capacity, extractable iron, clay content, and percent organic carbon. Sequential desorption indicated that almost all
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of the adsorbed TNT was desorbed after three sequential desorption cycles. Therefore, soil sorption will not effectively prevent mobility of TNT in the environment unless adsorption increases over extended periods of time or more strongly adsorbing decomposition products are formed. Redox potential and pH exerted no measurable effects on adsorption and desorption. Microbial degradation appeared to be greater in reduced than in oxidized soils, but differences were not significant.

Plant uptake of TNT and two of its principal degradation products, 4-amino-2, 6-dinitrotoluene (4ADNT) and 2-amino-4,6-dinitrotoluene (2ADNT), was also investigated. Results indicated that little TNT and 4ADNT, and no 2ADNT was absorbed by leafy portions of the test plant, yellow nutsedge (*Cyperus esculentus*). Plant uptake was greatest from 4ADNT-treated silt, an indication that 4ADNT is more readily mobilized into the plant than TNT or 2ADNT. Greater plant uptake from silt than from clay indicated that bioavailability is reduced in the clay. The reduction in bioavailability may be due to an increase in soil sorption of TNT and its degradation products over time. Results of the study suggest that plant uptake from soils contaminated with 80 ug of the respective treatment compound per gram will not be environmentally significant. (AUTHOR)

PREFACE

This report describes a study of 2,4,6-trinitrotoluene (TNT) soil sorption and plant uptake. The study was conducted by the Environmental Laboratory (EL) of the US Army Engineer Waterways Experiment Station (WES), Vicksburg, MS. The soils portion of the research was sponsored by the Department of the Army In-House Laboratory Independent Research (ILIR) Program for FY 86 and 87, under ILIR Project No. 4A161101A91D. The plant portion of the research was conducted by EL for the US Army Biomedical Research and Development Laboratory (USABRDL), Fort Detrick, Frederick, MD. The plant uptake research was authorized by Intra-Army Order No. 82II2032, Change 4, dated 12 February 1985. This report was accepted by Louisiana State University in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Marine Sciences in August 1988.

The study was conducted by Dr. Judith C. Pennington of the Plant Bioassay Team at the 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, Ecosystem Research and Simulation Division, and Dr. John Harrison, Chief, EL. Dr. Howard T. Bausum USABRDL, was Project Manager for the plant uptake portion of the study.

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

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PART I: INTRODUCTION

Background

2,4,6-Trinitrotoluene (TNT) was mentioned in the literature as early as 1863 (Urbanski 1964) and industrial poisoning of munitions workers by TNT was mentioned by Hamilton as early as 1921. During World War II, five Army Ammunition Plants (AAPs) manufactured TNT and approximately 25 load, assemble, and pack plants (also called AAPs) loaded bombs and shells with explosives including TNT. Prior to 1968, TNT was manufactured in the US by a batch process that produced excessive volumes of waste effluent. These effluents as well as wash water from munitions loading were discharged directly into local streams or into settling lagoons. In 1968, the Department of the Army (DOA) adopted a modified manufacturing process, the continuous flow method, that more completely utilized raw materials (Nay et al. 1974, Liebel et al. 1978). Even though the new process was more efficient, waste effluents still contained as much as 50-100 ppm TNT (Traxler 1974).

Following the passage of the National Environmental Policy Act in 1969 and after adoption of strong amendments to the Clean Air Act and the Clean Water Act in 1970, several AAPs were cited for violations by the Environmental Protection Agency (EPA) and/or by state and local environmental agencies (Leibel et al. 1978). The DOA established the Army Pollution Abatement Program to address these violations. One product of the Program was a series of aquatic field surveys of streams receiving munitions wastes from direct discharge or from overflow of

lagoons during heavy rains (Cairns and Dickson 1973, Fox et al. 1975, Griffiths et al. 1979, Jerger et al. 1976, Putman et al. 1979, Sanocki et al. 1976, Stilwell 1976, Sullivan et al. 1977, Weitzel et al. 1975). Evidence from these studies showed a definite loss of biological communities downstream from effluent release, but indicated that problem levels were generally confined to times of high TNT production. In these surveys, TNT could not be implicated exclusively in the biological impacts of the effluent because other munitions products were also present.

The Installation Restoration Program (IRP) was initiated by DOA in 1975. The IRP represented a change in policy from one emphasizing containment of contaminants on the installation properties to one emphasizing cleanup of the properties. The new emphasis was due not only to concern for environmental quality and compliance with regulations, but also to emerging consideration for transfer of installations that were no longer needed to non-military government or civilian use (Rosenblatt and Small 1984). The IRP provided for a comprehensive definition of the contamination problem which included surface water, groundwater, and the food chain. The IRP was recently expanded to include formerly used Department of Defense properties with current emphasis on those used in World Wars I and II, but with potential emphasis expanding to include older sites and military installations abroad.

Concern for the environmental effects of TNT wastes are fairly well founded. TNT and many of its degradation products are known to be toxic to fish and other aquatic fauna (Osmon and Klausmeier 1972; Liu,

Spanggord, and Bailey 1976; Nay, Randall, and King 1974; Won, DiSalvo, and Ng 1976), inhibitory to plant growth (Lakings and Gan 1981; Palazzo et al. 1985; Schott and Worthley 1974), and, in some cases, mutagenic to microorganisms (Dilley, Tyson, and Newell 1978; Kaplan and Kaplan 1982a; Kaplan and Kaplan 1982b; Won, DiSalvo, and Ng 1976).

Only a few factors affecting the environmental fate of TNT have been well defined. For example, photodecomposition and microbial degradation are known to occur in the environment. Burlinson (1980) proposed a mechanism for photodecomposition of TNT and 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 affected by microorganisms were reduction of nitro groups to amino groups, and coupling of rings to produce azoxy compounds (Kaplan and Kaplan 1982b). Several of the products of microbial metabolism are environmentally less desirable than TNT (Ellis et al. 1978 and Lee et al. 1975). Principal microbial degradation products of TNT found by Burlinson (1980) in natural waters and by Kaplan and Kaplan (1982b) 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.

The aquatic field surveys mentioned in the previous paragraph were limited to water quality, fauna, and algae. Neither soil sorption nor

uptake by aquatic macrophytes was examined. TNT and/or its degradation products may be irreversibly adsorbed to soils and sediments. Irreversible adsorption has been suggested as the mechanism for loss of TNT from TNT-treated bentonite drilling muds (Leggett 1985), from TNT-treated soils upon subsequent drying (Cragin et al. 1985), and from TNT-treated river sediment (Spanggord et al. 1980b, 1983). Results of these studies suggest that the compound may become adsorbed to soils or to soil organic matter. Therefore, the soils part of this dissertation research was conducted to provide information on soil sorption properties of TNT.

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. Toxicity of TNT wastes to duckweed (Lamna 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). Since no data were available with which to assess uptake of TNT by common plant species, the plant bioassay part of this dissertation research was conducted. The plant bioassay procedure was developed by the USAE Waterways Experiment Station (WES), Vicksburg, Miss for assessment of heavy metal mobility into plants from dredged material. Yellow nutsedge (Cyperus esculentus) was selected for the WES bioassay because it is ubiquitous and can grow under both flooded and upland conditions.

Objectives

Specific objectives of this study were to:

a. Quantify the rate and extent of adsorption and desorption of TNT to soils from 13 AAPs.

b. Determine what soil characteristics correlate most closely with adsorption of TNT.

c. Determine the effects of pH and redox potential on the adsorption and desorption of TNT in soils.

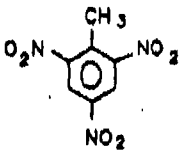
d. Determine whether C. esculentus can take up TNT, 2ADNT and 4ADNT from soils.

e. Determine whether TNT, 2ADNT and 4ADNT are concentrated or degraded in C. esculentus.

PART II: LITERATURE REVIEW

Chemical and Explosion Properties

The chemical structure of TNT is given below.



2,4,6 – Trinitrotoluene

Chemical properties not discussed in this paper can be found in The Merck Index (1976), or Chemistry and Technology of Explosives by Urbanski (1964).

Until the early 1970's TNT was the most commonly used high explosive* derived from aromatic compounds (Urbanski 1964). Its popularity stemmed from the facts that it was simple and relatively safe to manufacture, had high explosive power, and was highly stable by virtue of its relative insensitivity to the impact and friction of handling. Since the Vietnam conflict, more emphasis has been placed on newer munitions compounds. However, TNT is still used, especially in combination with certain of the newer compounds.

In its pure form TNT is a pale yellow crystalline solid having a theoretical melting point of 80.8°C. This number is depressed

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*High explosives are those characterized by extremely rapid chemical transformation accompanied by release of tremendous amounts of energy. Large volumes of hot gases such as CO, CO_2, H_2, and CH_4 result from their explosion. They are not readily detonated by heat, flame, or shock, but require a primary explosive for detonation (Yinon and Zitrin 1981).

proportionately to the quantity of other isomers produced concurrently with the alpha isomer. At 25°C the solubility of TNT is 0.015 g/100 g of water, 88 g/100 g of benzene, and 132 g/100g of acetone (Urbanski 1964). TNT is difficult to ignite. The true ignition temperature (authors differ) is around 300°C and gaseous decomposition has not been detected below 160°C (Urbanski 1964).

Net decomposition by detonation is believed to follow the reaction below:

$$16C_7H_5(NO_2)_3 = 20CO_2 + 47CO + CH_4 + H_2O + 2C_2H_2 + 2HCN + 14.5H_2 + 21.5N_2 + 3NH_4HCO + 29C$$

The explosion of one kilogram of TNT produces 950 kilocalories of heat, 690 litres of gas and a temperature of 2,820°C (Urbanski 1964).

The data of Leggett, Jenkins, and Murrmann (1977) (Table 1) indicate that TNT is not very volatile from the solid phase.

Table 1

Volatility of TNT at Three Temperatures

Temperature (°C)	Vapor Pressure (torr x 10^6)
20.0	1.10
25.5	3.96
40.0	42.4

No data were found addressing volatility of TNT from solution. Data concerning vapor pressures was found for very few of the decomposition or degradation products of TNT.

TNT Manufacture

TNT is produced by the sequential nitration of toluene. A mixture of nitric and sulfuric acids is used as a nitrating medium. The process is completed by removal of the unsymmetrical isomers of TNT as well as other oxidation products by contact with sodium sulfite (sellite). The unwanted isomers and other products are solubilized and removed as an aqueous effluent known as "red water" because of its color. The waste products of the counter-current continuous flow process include excess nitrobodies, spent acids, excess red water and cooling water (Nay et al. 1974, Liebel et al. 1978). The industrial process may be generalized as represented in Figure 1. Each step is typically carried out in a separate explosion proof building and waste products are discharged at each point in the process (Leibel et al. 1978). After sellite purification, the TNT is washed again with water. These wash waters are also high in the waste products mentioned above.

The largest volume of contaminated waste water is produced during TNT finishing processes, i.e. drying, flaking and packaging. The waste water is generated from cleaning equipment and interior surfaces of the finishing plants. As much as half a million gallons of this water can be generated per day at a single installation (Walsh et al. 1973). In a study of waste waters resulting from both production and purification of

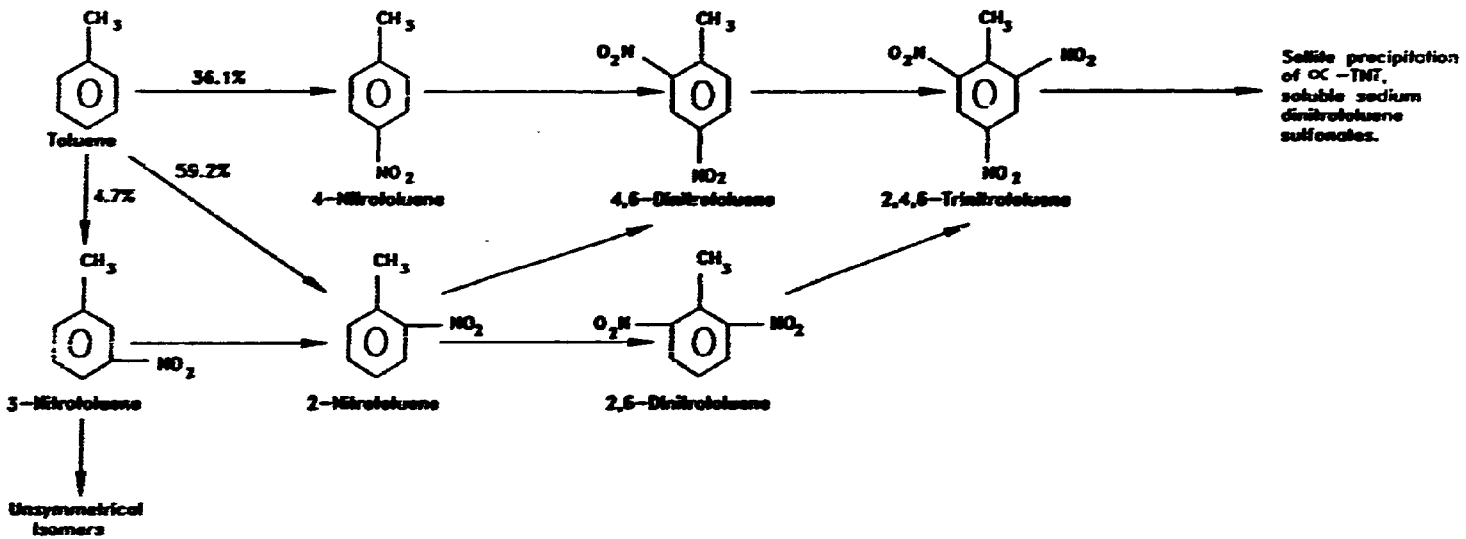

Figure 1. Generealized industrial process for the manufacture of TNT (Adapted from Ubanski 1964)

TNT, Spanggord et al. (1982) identified and quantified over 30 nitroaromatic compounds during one year of sampling.

Degradation Mechanisms and Products

Photodecomposition

Photodecomposition of TNT was probably recognized from earliest days of manufacture because of the conspicuous change of effluents to a red or pink color when exposed to sunlight. The most extensive studies of the photochemistry of TNT have been conducted by Burlinson and his co-workers at the Navy Surface Weapons Center (Burlinson 1980, Burlinson et al. 1973, Burlinson et al. 1979, Kaplan, Burlinson, and Sitzmann 1975) and by Spanggord and his co-workers at the Stanford Research Institute under contract to the US Army Medical Research and Development Command (Spanggord et al. 1980a; Spanggord et al. 1980b; Liu, Spanggord, and Bailey 1976).

Spanggord et al. (1980b) showed that decomposition of TNT was much more rapid in natural sunlight than in darkness. In their study the concentration of TNT in Mississippi River water (pH = 8.2; total organic carbon = 4.48 mg/L) neared zero after 6 - 8 days exposure to sunlight, whereas 30 days in darkness were required to dissipate 90 percent. In the same study photodecomposition was shown to be inversely proportional to pH over a tested range of 1.1 to 11.1 pH units. Aqueous solutions of TNT under neutral or acidic conditions remained very stable in darkness; however, at a pH value of 11.1, TNT decomposed even in darkness. Burlinson (1980) reported that in sunlit Mississippi River waters,

photolysis was more rapid than in distilled water. They attributed this difference to the higher pH of the River water. However, Spanggord et al. (1980b) submitted that the effects of pH on the photolysis rate constant for TNT is insignificant compared with that of natural substances. Their data suggested that light absorption by substances in natural water sensitize TNT resulting in photolytic transformations. They demonstrated that the products of photolysis also accelerate photodecomposition. Conclusions of Mabey et al. (1983) supported the findings of Spanggord et al. (1980b).

Burlinson et al. (1973) reported that the photolysis rate of TNT is affected by the solvent in which it is dissolved. They found the photolysis rate to be nearly the same in cyclohexane and water, but reduced in methanol. Suryanarayanan and Capellos (Spanggord et al. 1980a) found the rate to be faster in nonpolar than in polar solvents. For example, their decay rate constant in benzene was more than 20,000 times greater than the constant in methanol.

A list of all of the photodecomposition products of TNT found in the literature is given in Table 2. Burlinson et al. (1979) provided the most complete list of photodecomposition products produced by laboratory irradiation. (Fewer products have been reported from natural waters.) They reported that only 45-50 percent of the products were recovered in solution. The remainder were present as an insoluble residue that was suggested to be oligomers of azo and azoxy compounds. It should be noted that only soluble compounds were assayed and that the analytical method was thin layer chromatography which does not separate all products. Burlinson's mechanism for photodecomposition of TNT is

Table 2

Photodecomposition Products of TNT

Compound	References
1,3,5-trinitrobenzene	Burlinson et al. 1973; Spanggord et al. 1980; Kaplan et al. 1974; Epstein et al.1975
2,4,6-trinitrobenzaldehyde	Burlinson et al. 1973; Burlinson et al. 1979; Spanggord et al. 1980a; Kaplan et al. 1975; Epstein et al. 1978
4,6-dinitroanthranil	Burlinson et al. 1973; Burlinson et al. 1979; Spanggord et al. 1980a; Epstein et al. 1978
2,4,6-trinitrobenzonitrile	Burlinson et al. 1973; Burlinson et al. 1979; Spanggord et al. 1980a; Kaplan 1975
2,2',6,6'-tetranitro-4,4'-azoxytoluene	Burlinson et al. 1973
4,4',6,6'-tetranitro-2,2'-azoxytoluene	Burlinson et al. 1973
2',4-dimethyl-3,3',5,5'-tetranitro-ONN-azoxybenzene	Burlinson et al. 1973
2,4'-dimethyl-3,3',5,5'-tetranitro-ONN-azoxybenzene	Burlinson et al. 1973
2,4-dinitroisoanthranil	Burlinson et al. 1979
2,2'-dicarboxy-3,3'5,5'-tetranitroazobenzene	Burlinson et al. 1979; Kaplan et al. 1975
2-carboxy-3,3',5,5'-tetra nitro-NNO-azoxybenzene	Burlinson et al. 1979; Kaplan et al. 1975
2-amino-4,6-dinitrobenzoic acid	Burlinson et al. 1979; Spanggord et al. 1980a; Kaplan et al. 1975
4,6-dinitroisoanthranil	Kaplan et al. 1975
4,6-dinitroanthranil	Kaplan et al. 1975
Syn-2,4,6-trinitrobenzal doxime	Kaplan et al. 1975
2,4,6-trinitrobenzyl alcohol	Kaplan et al. 1975; Epstein et al. 1978
3,5-dinitrophenol	Kaplan et al. 1975; Epstein et al. 1978
2,2'-dicarboxyl-3,3',5,5'-tetranitroazoxybenzene	Kaplan et al. 1975
2,4,6-trinitrobenzoic acid	Kaplan et al. 1975
N-(2-carboxy-3,5-dinitrophenyl)-2,4,6-trinitrobenzamide	Kaplan et al. 1975
4,6-dinitro-1,2-benzisoxazole	Epstein et al. 1978

given below. Although this mechanism is commonly accepted, not all of the specific details of the reaction have been elucidated.

TNT---2,4,6-Trinitrobenzaldehyde---(Some undefined nucleophilic complex)---1,3,5-Trinitrobenzene

Microbial Degradation and Complexing

A broad range of microorganisms are capable of growth in the presence of low concentrations of TNT. Klausmeier, Osmon, and Walls (1973) reported that most fungi, yeasts, actinomycetes, and gram positive bacteria grew when TNT concentrations did not exceed 20 mg/L. Many gram negative bacteria grew well in TNT concentrations of 100 mg/L or more. At higher concentrations growth was prevented or inhibited. TNT can serve as the sole source of carbon and nitrogen for some microorganisms (Weitzel et al. 1975; Traxler 1974; Greene, Kaplan, and Kaplan 1985), but most microorganisms require a supplemental carbon and nitrogen source to grow in the presence of TNT (Spanggord et al. 1980b; Jerger, et al. 1976; Klausmeier, Osmon, and Walls 1973). Hudock (1972) reported that Pseudomonas spp. metabolized TNT in a contaminated soil without inhibition only after a period of adaptation. However, Jerger, et al. (1976) showed that reduction of TNT by aquatic and sediment microbial communities proceeded readily without acclimation. They found that indigenous populations isolated from control stations and from stations contaminated with varying concentrations of TNT exhibited similar TNT transformation rates.

One author (Traxler 1974) reported ring cleavage of TNT by a gram negative bacterium. However, the remainder of the literature reported no ring cleavage by microbial degradation (Burlinson 1980; Kaplan and Kaplan 1982d; Jerger et al. 1976; Carpenter et al. 1978; Hoffsommer et al. 1978; and others). Based on the products found, the literature supports a stepwise reduction of the nitro moieties on the TNT molecule yielding amino and hydroxylamino products which further react to form azoxy compounds (Carpenter et al. 1978; Burlinson 1980; Hoffsommer et al. 1978; Kaplan and Kaplan 1982c; Parrish 1977; Won et al. 1974; Spanggord et al. 1983). A biotransformation scheme for TNT in compost (Figure 2) was proposed by Kaplan and Kaplan (1982c). A similar scheme was proposed by McCormick et al. (1976) for mesophilic systems. Para position reduction is favored over ortho position reduction (Kaplan and Kaplan 1982c; Hoffsommer et al. 1978; Parrish 1977; Greene, Kaplan, and Kaplan 1984).

Microbial decomposition has been studied with the objective of using microorganisms as a waste treatment alternative for TNT-containing wastes (Kaplan and Kaplan 1982c; Osmon and Andrews 1978). However, the predominant changes effected by microorganisms, i.e., reduction of nitro groups to amino groups, and coupling of rings to produce azoxy compounds, resulted in products that were environmentally less desirable than TNT (Ellis et al. 1978 and Lee et al. 1975). The fate of TNT in activated sludge has also been investigated (Carpenter et al. 1978; Hoffsommer et al. 1978), but the same undesirable degradation products were found, and the reactions proceeded too slowly to be of practical use as a waste treatment method.

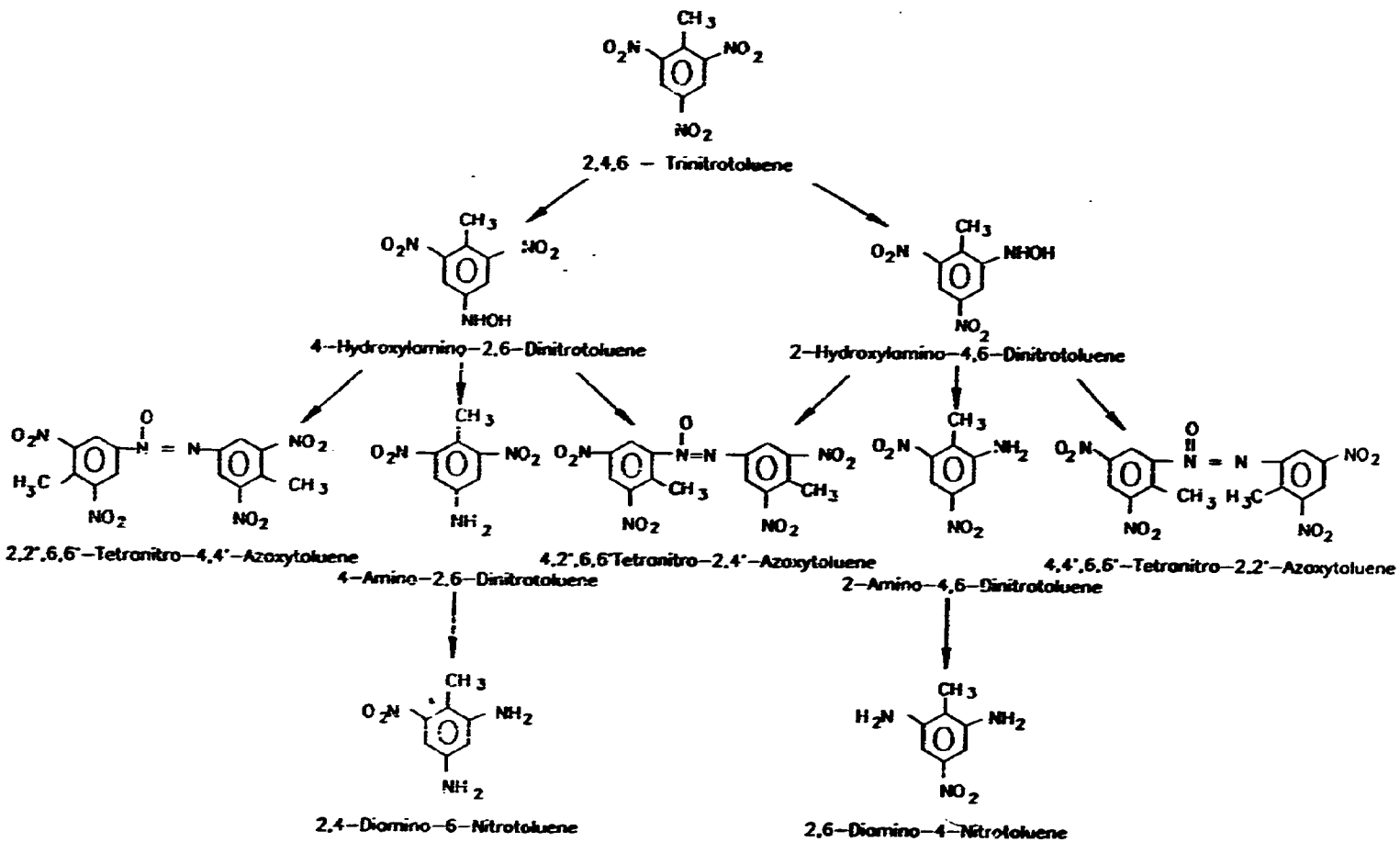

Figure 2. Proposed mechanism for microbial degradation of TNT (Adapted from Kaplan and Kaplan 1982c)

Toxicology

A summary of literature on cases of TNT poisoning to munitions workers through the early 1960's is given by Urbanski (1964). Due to high demand for TNT during World War I, industrial hygiene was widely neglected resulting in numerous cases of poisonings attributable to TNT. For example, during the first seven and one-half months of WWI at a single munitions factory in the United States 17,000 cases of poisoning were recorded, 475 of which were fatal. Statistics for World War II showed marked improvement. About 1,000 cases of mild poisoning, 379 more serious, and 22 fatalities were reported. Current statistics were not found; however, safety standards are now well enforced and reported incidences of poisoning are rare.

The primary modes of exposure of workers to TNT were absorption through the skin (Hamilton 1927), and inhalation. Mild symptoms of poisoning include irritation of the digestive tract, and paleness or purpling of the skin. More severe symptoms are methemoglobinemia, severe jaundice due to liver damage and aplastic anemia caused by disfunction of the bone marrow (Urbanski 1964).

TNT is known to be toxic to rats and mice (Lee et al. 1975, 1977; Ellis et al. 1978), fish (Osmon and Klausmeier 1972), unicellular green algae, copepods and oyster larvae (Won et al. 1974). TNT is also known to inhibit growth of fungi, yeasts, actinomycetes, and bacteria (Klausmeier et al. 1973).

Waste Treatment and Disposal

In the early days of large-scale TNT manufacture, waste effluents were discharged directly into surface water (Liebel et al. 1978, Walsh et al. 1973) or indirectly into surface and/or ground water after only brief periods in settling ponds (Cragin et al. 1985, Spanggord et al. 1982, Walsh et al. 1973, Sanocki et al. 1976, Stilwell et al. 1976, Jerger et al. 1976, Weitzel et al. 1975, Fox et al. 1975). In some cases, natural evaporation was allowed to concentrate the waste residues in lagoons (Spanggord et al. 1983). Screenable solids were sometimes removed from effluents and burned in open areas before water was discharged (Rosenblatt and Small 1981). Today the waste waters are pumped through a carbon adsorption column (Jenkins et al. 1984) or distilled, condensed, and then discharged into streams (Spanggord et al. 1982) or incinerated. Waste treatment of acid effluents often consisted of neutralization with soda ash (crude sodium carbonate) prior to discharge (Cairns and Dickson 1973).

Many of the older leaching ponds and settling lagoons were buried when production facilities were rebuilt, modernized, or discontinued. Today, many of these are being excavated because the potential for contamination of groundwater is now recognized. Studies are in progress to determine if they should be completely excavated and disposed of in a more environmentally safe manner (English, Smith and Meuser 1985).

At most installations, concentrated liquid wastes from red water and pink water are incinerated (Cairns and Dickson 1973, Walsh et al. 1973, Cragin et al. 1985). Originally, these wastes were manually

spread on a concrete pad, ignited and allowed to burn in the air. This practice was carried out under temporary waivers in many states where regulations barred open-burning (Forsten 1973). The incineration process produced nitrogen oxides that polluted the air and particulate matter that settled over large areas contaminating the ground and becoming subject to runoff or to leaching into the groundwater (Conley and Mikucki 1976). Under Presidential Executive Order 11507, the government took the lead in developing incineration capabilities that minimized pollution. Forsten (1973) evaluated three incinerator models for this purpose. An underground burning pit with a low air current system has been widely adopted.

For many years expired munitions, primarily TNT and hexahydro-1,3,5-trinitro-1,3,5-triazine, were dumped into the sea. Obsolete liberty ships were filled with munitions and scuttled (Hoffsommer and Rosen 1972, Osmon and Andrews 1978). Some of these ocean dumps have been investigated by the Navy and found negative for explosives contamination (Hoffsommer, Glover, and Rosen 1972; Hoffsommer and Rosen 1972).

Conley and Mikucki (1976) explored the possibility of disposing of liquid and solid TNT wastes in sanitary landfills. Results of their lysimetry studies indicated that TNT did not migrate downward in the soil to any great extent. However, in a subsequent study, Osmon and Andrews (1978) recommended that the concept of disposal by landfilling be abandoned. They found that manipulation of soil moisture, concentration and types of nutrients, and degree of soil aeration were ineffective in causing complete microbial degradation of TNT. They

recommended that large scale composting with domestic or industrial refuse be explored. Kaplan and Kaplan (1982b) conducted a study in which [^{14}C]TNT was composted with horse manure, alfalfa hay, grassclippings, dead hardwood leaves, and garden soils. Their results showed 1) no cleavage of the TNT ring structure, 2) reduction of nitro groups to amino groups, and 3) coupling to azoxy compounds. A significant percentage of the ^{14}C-label (13.9 percent after 91 days) was bound to the humin fraction of the compost. The amount of bound, labeled material increased with time of composting. Therefore, composting did not contribute to decomposition of TNT and was abandoned as an abatement process.

Presently, only one facility actively produces TNT in the United States (Radford AAP, Radford, Va). The facility utilizes carbon adsorption columns for cleanup of effluents. The several AAPs that load and pack TNT-containing munitions utilize incineration for waste disposal.

PART III: MATERIALS AND METHODS

Adsorption and Desorption of TNT by Soils from Selected AAPs

Soil Collection

Locations of AAPs that were sampled for this study are shown in Figure 3. Soil samples were collected from uncontaminated sites at 12 of the AAPs that handle TNT now or have handled TNT in the past. Seven of the 14 installations having documented TNT contamination of ground water or soil in the data base of the US Army Toxic and Hazardous Materials Agency (USATHAMA) (Tucker et al. 1985) were sampled. Five of the AAPs sampled are listed by USATHAMA as potentially contaminated with TNT. The remaining AAP sampled was reported by installation personnel as having handled TNT in the past.

Sampling of soils from all of the AAPs of interest was precluded by budget limitations. Many of the locations were selected because travel by personnel of the WES for other purposes was to proximal areas. However, a special trip was made to Radford AAP, Radford, Va., because it is the only facility currently manufacturing TNT. Holston AAP was sampled on the same trip since it was within practical driving distance of Radford. A special trip was also made to Louisiana, Longhorn, and Lone Star AAPs because they are very close together and within easy driving distance of WES.

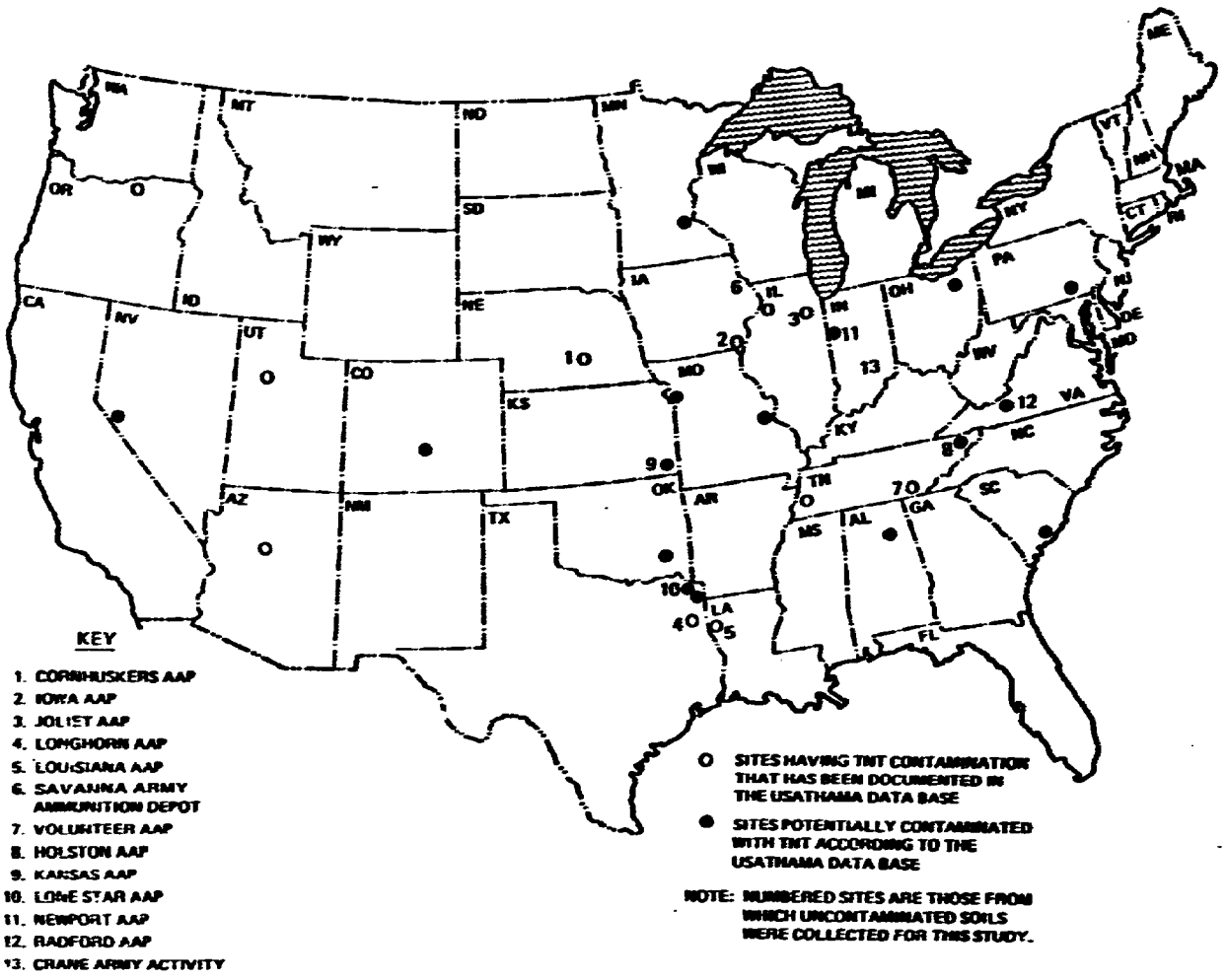

Figure 3. US Army munitions facilities having confirmed or potential TNT contamination (Adapted from Tucker et al. 1585)

Soil survey maps for each of the AAPs to be sampled were obtained from local US Department of Agriculture Soil Conservation Service (SCS) offices. The SCS, in most sampling areas of the country, were preparing new survey maps. Therefore, maps for some areas were not yet updated and were very old. Several counties of interest had no map available. Soil maps were taken to the AAP where personnel familiar with the operations and grounds of the facility were asked to identify areas potentially receiving TNT contamination from past or present activities. Activities mentioned as potential causes of contamination included dumping, burning, or lagoonal disposal of manufacturing effluents or wash waters from load and pack operations. Soil type in potentially contaminated areas was noted on the soil survey map, and areas on the facility having the same soil type, but safely removed from any possible contamination, were located. Test samples were taken from those uncontaminated sites.

Soil samples were taken by removing any vegetative cover or litter from the soil surface and collecting several shovelfuls from the top 15 cm of soil (the A horizon). The same procedure was followed at several spots within a few metres of each other to obtain a representative soil sample. Approximately 40 l of soil were collected from each AAP.

All soils were allowed to air dry, ground to pass through a 2-mm sieve, sealed, and stored at 25°C in 28-l Bain Marie pots until tested.

Physical and Chemical
Characterization of AAP Soils

pH. Four 10-g replicates of soil on an oven dry weight (ODW) basis were weighed to the nearest 0.1 mg into 50-ml glass beakers. The soil samples were mixed with 20 ml of reverse osmosis (RO) water until all

dry particles were thoroughly wet. The resulting suspension was stirred with a magnetic stirrer for 1 min every 15 min until a total of 45 min had passed. The pH of the suspension was then determined with a glass and a reference silver-silver chloride electrode on a Beckman Model SS-3 pH meter (Beckman Instruments Inc., Fullerton, Calif.).

Particle size distribution. The particle size distribution was determined in four replicates by using the method of Day (1956) as modified by Patrick (1958). The method determines the percentage of three size fractions in the soil: sand (2 mm to 50 u diameter), silt (50 to 2 u diameter), and clay (<2 u diameter).

Cation exchange capacity. Cation exchange capacity (CEC) was determined in four replicates using the ammonium saturation method of Schollenberger and Simon (1945).

Electrical conductivity. Electrical conductivity (EC) was determined in four replicates on extracts of saturated pastes made from soils using the method of Rhoades (1982). The conductivity meter was a Model 31 YSI (Yellow Springs Instrument Company, Yellow Springs, Ohio).

Extractable iron, manganese, aluminum, and calcium. An ammonium oxalate/oxalic acid extraction procedure was used to remove hydroxides of iron, manganese, aluminum, and calcium from soils in four replicates (Brannon and Patrick 1985). Extracts were analyzed by the Analytical Laboratory Group, Environmental Laboratory, WES, using a Beckman Spectra Span IIIB Argon Plasma Emission Spectrophotometer (Applied Research Laboratories, Dearborn, Mich.).

Percent organic carbon. Percent organic carbon (OC) was determined by the complete combustion method described by Nelson and Sommers (1982).

Thin-Layer Chromatographic Screening

Selection of extracting solvent. All 13 AAP soils were screened by thin-layer chromatography (TLC) for contamination by TNT and several of its degradation products, even though every effort had been made to avoid soil collection in areas remotely suspected of contamination. A preliminary experiment in which [^{14}C]TNT-treated soils were extracted with four solvents (acetone, benzene, methanol, and methylene chloride) showed acetone and methanol to be the most effective in removing [^{14}C]TNT (or its ^{14}C-labeled degradation products) from the soils. However, to minimize the obscurance of compounds of interest by co-extraction of extraneous compounds, each of the solvents was retested for use with TLC. A clay soil having an organic carbon content of 2.401 percent and a cation exchange capacity of 135 meq/100g was extracted with each of the test solvents listed above. The clay was assumed to be TNT-free because it was collected far from any munitions activities. The soil extracts were chromatographed against 45-50 ul of known standards (listed below).

Standard Compounds for Thin-Layer Chromatographic Analysis

2,4,6-trinitrotoluene	4-amino-2,6-dinitrotoluene
2-amino-4,6-dinitrotoluene	2,4-diamino-6-dinitrotoluene
2,6-diamino-4-dinitrotoluene	2,4-dinitrotoluene
2,6-dinitrotoluene	3,4-dinitrotoluene
2,3-dinitrotoluene	2,5-dinitrotoluene

2,2',6,6'-tetranitro-4,4'-azoxytoluene

A solvent was selected that produced the least visual interference with the known standards when viewed under ultra violet (UV) light (254 nanometers).

Selection of solvent system. A review of the literature revealed that many solvent systems had been used to separate TNT and various related compounds. Based on the work of Jerger et al. (1976), Naumova et al. (1979), and Osmon and Andrews (1978), the following solvent systems were selected for testing: benzene/ethyl acetate (75:25), benzene/hexane/pentane (50:40:10), and benzene/chloroform (75:25). Using known concentrations of TNT, 4ADNT, and 2ADNT, silica gel TLC plates (Redi Plate-Silica Gel GF, Fisher Scientific, Pittsburg, Pa.) were migrated in each of the three solvent systems. Separation of these three compounds was particularly important in this study because of their known prevalence in the environment. Separation of 4ADNT from 2ADNT was especially critical because their markedly similar chemical structures make them difficult, if not impossible, to separate by many analytical techniques. The solvent system most completely separating these three compounds was selected. Any of the 13 AAP soil extracts

exhibiting UV-visible spots, the R_f value of which was not consistent with the three standard compounds, was subjected to a second screening. In the second screening extracts were migrated with all other standard compounds. (Complete list given above.)

TLC of AAP soils. Three five-gram (ODW) replicates of each AAP soil were extracted three times with 10-ml portions of methanol, the solvent selected in the preliminary solvent test. Samples were extracted in 50-ml stainless steel test tubes on a reciprocating box shaker for 30 min, centrifuged at 17,369 x gravity (g), or 12,000 rpm, for 10 min, and the supernatant removed with a Pasteur pipette. Extracts from the same replicate were combined and concentrated under a stream of dry air to a final volume of 0.5 ml before plating on silica gel plates. Plates were migrated in TLC tanks until the solvent front was within a few cm of the top. As the plates were removed from the tank, the solvent front was marked. The plates were allowed to air dry, and then viewed under UV light. Each visible spot was marked and the distance it had migrated from the point of origin was measured so that an R_f value (distance of solvent migration/distance of unknown migration) could be calculated. R_f values of unknowns were compared with R_f values of known standards on the same plate. When R_f values were equal, the possibility that the identity of the unknown was the same as the identity of the standard was acknowledged.

Soil to Solution Ratio

To compare results of tests conducted with different soil to solution ratios, adsorption of TNT using four soil to solution ratios was compared. Since both organic and inorganic surfaces potentially

provide sites for adsorption, and organic carbon is often highly correlated with adsorption of neutral organic compounds, e.g., pesticides (Weed and Weber 1974), a soil high in percent OC and also relatively high in CEC and percent clay was selected. Joliet AAP soil, the soil selected, exhibited the highest percent OC of any of the AAP soils (3.592 percent) and also exhibited a relatively high CEC (102 meq/100 g) and percent clay (23.8 percent). The four ratios tested were 1 to 5, 1 to 10, 1 to 20, and 1 to 30.

Soil samples of 5, 2.5, 1.25, and 0.83 g were weighed (ODW) into 50-ml stainless steel centrifuge tubes in three replicates. To each tube were added 25 ml of a [^{14}C]TNT solution containing 0.023 uCi ^{14}C/ml and 16 ug total (^{14}C labeled plus unlabeled) TNT/ml. Tubes were sealed and placed on a reciprocating box shaker at highest speed (280 excursions/minute) for 2 hr. After shaking, the tubes were centrifuged for 20 min at 17,369 x g. Three 1-ml aliquots of the solution were removed to each of three vials containing 20 ml of PCS liquid scintillation (LS) cocktail (Amersham Corporation, Arlington Heights, Ill.) and counted for 20 min by LS. Standard curves were prepared by plotting counts per minute (CPM) per millilitre against micrograms of TNT per millilitre in the [^{14}C]TNT treatment solution. Micrograms of TNT per millilitre of solution were then related to micrograms per gram of soil (ODW).

Adsorption Kinetics

Adsorption kinetics were determined using soils from two of the AAPs. The two soils, selected on the basis of percent OC, were the Louisiana AAP soil, with a relatively low percent OC (0.367), and the

Joliet AAP soil, with a relatively high percent OC (3.592). Each soil was equilibrated with three concentrations of TNT in aqueous solution (1.0, 4.0, and 16.0 ug TNT/ml). Concentration values included both ^{14}C-labeled and unlabeled TNT. These concentrations were equivalent to 5.0, 20.0, and 80.0 ug TNT/g of soil in the centrifuge tubes. Each of the solutions also contained 0.027 uCi/ml of ^{14}C-labeled TNT. Five-gram soil samples were weighed into 50-ml stainless steel centrifuge tubes in three replicates for each sampling time. Then 25 ml of [^{14}C]TNT solution was added to each tube. The tubes were placed on a reciprocating box shaker and allowed to shake at highest speed. Three tubes were removed at each of the following times: 0.25, 0.50, 1.00, 1.50, 2.00, 5.00, 10.00, and 24.00 hr. As soon as tubes were removed, they were centrifuged for 30 min at 17,369 x g. Three 1-ml aliquots of the supernatant were counted by LS for 10 min. Zero time values were determined by counting 1 ml of solution from each concentration of TNT in three replicates.

Three replicates of each test solution without soil were placed on the shaker and sampled initially and at 2.00 and 24.00 hr. These "no-soil" blanks were included to measure any adsorption of [^{14}C]TNT to the walls of the centrifuge tubes.

A standard curve relating ^{14}C CPM per millilitre to concentration of TNT (micrograms/millilitre) was prepared for each test solution (Appendix A). The TNT concentration in the solution phase, assuming that all ^{14}C activity was due to [^{14}C]TNT and not to radiolabeled decomposition products, was plotted against time to establish an adsorption kinetics curve for each of the soils.

Desorption Kinetics

For comparative purposes, the same soils selected for the adsorption kinetics studies were also used for the desorption kinetics studies. Eighteen 1-g samples (ODW) of Joliet and Louisiana AAP soils were weighed to the nearest 0.1 mg into 50-ml Oak Ridge Type polycarbonate centrifuge tubes (Sybron/Nalge, Rochester, N. Y.). Twenty millilitres of the 16 ug/ml [^{14}C]TNT solution was added to all tubes, the tubes were weighed to the nearest 0.01 g, and the soils adsorbed for 2 hr as described above. Three replicates without soil were run as described above to measure any adsorption of [^{14}C]TNT to the polycarbonate centrifuge tubes. After adsorption, tubes were centrifuged, the TNT solution was removed, and the tubes were brought back to original weight by the addition of RO water. All tubes were returned to the reciprocating box shaker. Three tubes of each soil type were removed at each of the following times: 0.5, 1.0, 1.5, 2.0, 5.0, and 10.0 hr.

Tubes were centrifuged for 20 min at 17,369 x g as soon as they were taken from the shaker. One millilitre of solution was removed for scintillation counting as described for the adsorption test. TNT concentration in the solution phase was plotted against time to establish a desorption kinetics curve for each of the soils.

Batch Adsorption Equilibrium

One-gram soil samples (ODW) from each of the AAPs, plus a Tunica silt and a Sharkey clay, were weighed to the nearest 0.001 g into 50-ml polycarbonate centrifuge tubes in three replicates for each of the following five concentrations of TNT: 1.0, 4.0, 8.0, 12.0, and 16.0

ug/ml. The silt and clay were included because they are routinely used in the standard WES plant bioassay employed later in this study. Twenty millilitres of [^{14}C]TNT solution containing 0.023 uCi [^{14}C]TNT/ml, plus sufficient unlabeled TNT to produce the final concentrations listed above, were added to each tube. All tubes were equilibrated for 2 hr on a reciprocating box shaker operated at maximum speed. At the end of the 2-hr period, tubes were centrifuged at 17,369 x g for 20 min. A 1-ml aliquot of the solution phase was removed and counted three times by LS for 10 min.

Adsorption data were fit to a linear and two classical isotherm models using linear regression. The two classical models were the Langmuir Isotherm Model and the Freundlich Isotherm Model (Weber 1972). Linear regressions were calculated using the procedures available with Statistical Analysis System (SAS Institute Inc. 1985).

Sequential Desorption

Eight soils selected on the basis of average K_d were used in the sequential desorption tests. Soils exhibiting as broad a range in adsorption as possible were selected. Twenty millilitres of 16-ug TNT/ml solution was added to tubes containing 1 +/- 0.001 g of each of the selected soils, and each tube was weighed to the nearest 0.01 g. After 2 hr of adsorption, the solution was removed, and the tubes were brought up to the original weight with RO water. They were returned to the reciprocating box shaker for 2 hr. At the end of the first desorption cycle, the tubes were centrifuged for 10 min at 17,369 x g and the solution removed. One millilitre of the solution was diluted with 20 ml of PCS and counted by LS for 10 min three times. Second and

third desorption cycles were conducted in the same manner. A standard curve was consulted to convert CPM/millilitre to micrograms TNT/millilitre (Appendix A).

Adsorption and Desorption of TNT Under Controlled pH and Redox Potential

Experimental systems for controlling pH and redox potential (Eh) were a modification of that described by Patrick, Williams, and Moraghan (1973) (Figure 4). Modifications included manual manipulation of pH by injection of 1 N HCl or 1 N NaOH through the serum cap, and use of silver-silver chloride (Ag · AgCl) reference and pH electrodes rather than calomel electrodes.

Three pH/Eh test systems (replicates) were set up for each of the following combinations of test conditions: pH 5.0, Eh -150; pH 5.0, Eh +450; pH 6.5, Eh -150; pH 6.5, Eh +450; pH 8.0, Eh -150; and pH 8.0, Eh +450. (Eh values were corrected to the Ag - AgCl electrode according to the procedure given by Jones 1966.) The most adsorptive soil (Joliet AAP soil) was selected for the pH/Eh tests. One hundred thirty g of soil (ODW) which had been ground to pass through a 200 mesh sieve (particle size no greater than 75 microns) was added to 2,600 ml RO water in a 2800-ml Fenback flask to produce a 1:20 soil to solution ratio. The soil was maintained in suspension by magnetic stirring. Each flask was equipped with a no. 13 rubber stopper through which electrodes, gas inlets and outlets, a thermometer, and sampling ports passed (Figure 4).

After equilibration had been achieved (2 - 3 weeks), fifteen 20-ml aliquots of soil suspension were removed via the serum cap using a hypodermic syringe equipped with a long needle. The samples were placed into 50-ml polycarbonate centrifuge tubes (Oak Ridge type). If

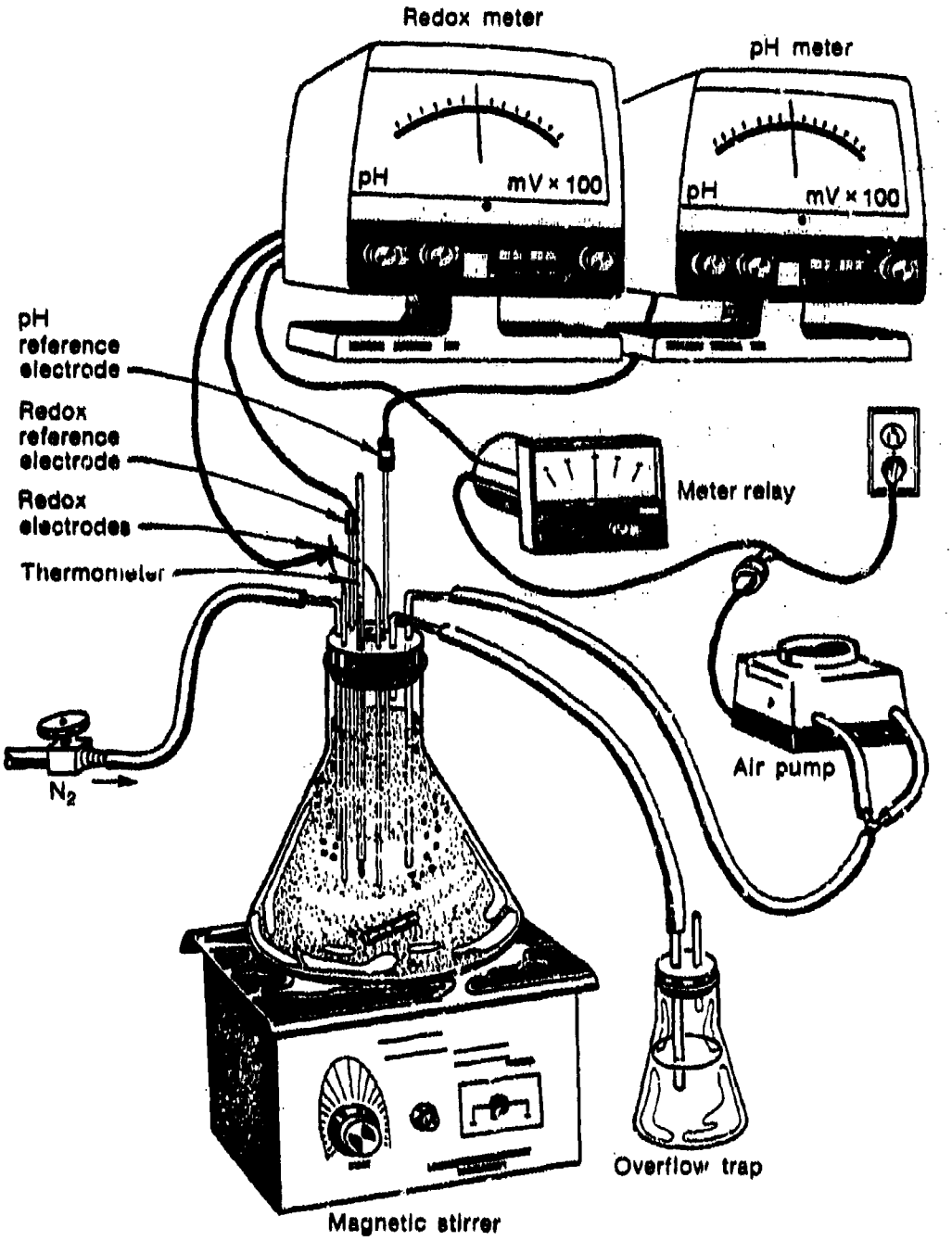

Figure 4. Laboratory apparatus for controlling pH and redox potential in soil slurries

suspensions were from flasks maintained under reduced conditions, each tube was flushed with N_2 prior to filling to maintain a reduced atmosphere. Tubes were spiked with five concentrations of [^{14}C]TNT. The spiking resulted in three replicates of the following TNT concentrations (ug/ml): 1.0, 4.0, 8.0, 12.0, and 16.0. All tubes were placed on a reciprocating box shaker at maximum speed for 2 hr. After shaking, tubes were centrifuged at 17,369 x g for 10 min and one millilitre of the solution was counted in PCS three times by LS for 10 min. After all remaining solution had been removed, each tube from the 12.0 ug TNT/ml treatment was brought up to its original weight with RO water and returned to the shaker for desorption. Desorption was carried out on the 12.0 ug TNT/ml solution because statistical comparison of the TNT desorption data for the 12.0 and 16.0 ug TNT/ml solutions showed no significant differences. These results indicated that the 12 ug/ml solution was sufficiently concentrated to saturate the soil. Therefore, use of the more concentrated solution for desorption was unnecessary. Soil and water were allowed to shake for 2 hr, the tubes were centrifuged and the solutions counted by LS as previously described. Three such desorption cycles were performed in sequence.

Preliminary tests had shown that repeated sampling of flasks through the serum cap using a hypodermic syringe and needle did not produce a consistent ratio of soil to solution. Therefore, the exact soil to solution ratio was determined for each sample withdrawn from the flasks. Centrifuge tubes were weighed before and after receiving the suspension aliquot. At the end of the test, all solution was removed from tubes and the remaining soil allowed to oven dry at 104°C over

night. When cooled, the tubes containing dry soil were weighed again. Since the exact volume of the withdrawn aliquot was known, the soil to solution ratio could be calculated.

Degradation of TNT Under Oxidized and Reduced Conditions

Preliminary Test for the Effectiveness of Mercuric Chloride as a Soil Sterilant

In order to determine whether degradation was biotic or abiotic, a sterile treatment was included in the study. According to Van Cleemput, Patrick, and McIlhenny (1976), a 0.01 M solution of mercuric chloride ($HgCl_2$) is sufficient to inhibit biological reduction without interfering significantly with chemical reduction in a 1:2 soil to water suspension. Therefore, a 200-ml 1:20 soil to water suspension was treated with 10 ml of a 0.2 M $HgCl_2$ solution so that the concentration of $HgCl_2$ in each test was 0.01M. Since the amount of soil in this study was 10 times smaller than in the study reported by Van Cleemput, Patrick, and McIlhenny, the rate used should be more than effective. Nonetheless, a preliminary test was conducted to assess the effectiveness of this rate of $HgCl_2$ as a sterilant under the conditions of the current study.

Sediment from Brown's Lake, a small freshwater lake, was treated in a 1:20 oven dry sediment to water ratio with $HgCl_2$. Three 250-ml polycarbonate centrifuge tubes were treated with $HgCl_2$, and three were left untreated to serve as controls. All six tubes were placed on a reciprocating box shaker operated at maximum speed. All centrifuge tubes were sampled at 10 min, 30 min, 5 hr, 24 hr, and 48 hr. Three

1-ml samples were removed aseptically from each tube and plated on Petri plates containing nutrient agar. Plates were incubated at room temperature for 48 hr in order to ascertain whether or not growth of microorganisms in the suspended sediment had terminated.

Soil Incubation and Treatment

Experimental systems for controlling Eh and pH were prepared using the procedures of Patrick, Williams, and Moraghan (1973). Four replicates of two Eh's (-150 and +450) at pH 7.0 were maintained. Soil from Joliet AAP which had been ground to pass through a 200 mesh sieve was introduced into 2800 ml Fenbach flasks. Sufficient RO water was added to produce a 1:20 soil to water ratio.

When the desired conditions of Eh and pH were stable for 24 hr, three 300-ml samples of suspended soil were removed from each replicate by hypodermic syringe to 500-ml polycarbonate centrifuge tubes. Efforts to maintain anaerobic conditions consisted of flooding centrifuge tubes with air-free N_2 gas prior to and during sample introduction. Since the exact soil to solution ratio for each sample could not be measured directly, an approximation was made by removing 5 ml of suspended soil from the Fenbach flask before and after removal of test samples. Oven dry weight was determined on these samples and the average was used to calculate the soil to solution ratio in each replicate.

Two of the three samples from each replicate were treated with sufficient TNT to make the final concentration in each tube equal to 16 ug TNT/ml of suspension (equivalent to a treatment of approximately 80 ug TNT/g of soil on ODW basis). One of the TNT-treated samples received 10 ml of 0.2 M $HgCl_2$ as a soil sterilant. The third sample remained

untreated and unsterilized to serve as a control. Efforts were made to keep all samples out of light during and after treatment with TNT.

All samples were placed on a reciprocating box shaker for 24 hr. When removed, sterilized samples were cultured on standard methods agar (BBL Microbiology Systems, Cockeysville, Md) to be sure that they had remained sterile. Samples were cultured by aseptically pipetting one millilitre of suspended soil onto the surface of the media. Cultures were incubated at room temperature (ca. 25°C) for 24 hr.

Extraction and Analysis

Samples were centrifuged at 17,369 x g for 20 min and the solution phase separated from the soil phase by carefully pipetting off the solution. One g (wet weight) of the soil was weighed into a preweighed aluminum pan to the nearest 0.0001 g and oven dried at 104°C over night for determination of percent solids. The remainder of the soil sample was extracted with 50-ml portions of methanol three times using a sonic probe (Fisher Scientific, Springfield, N. J.). In a preliminary study methanol was found to be a more effective solvent for extracting TNT from soils than benzene or methylene chloride. Jenkins and Grant (1987) reported that methanol extraction using an ultrasonic bath was more effective than using Soxhlet, mechanical shaking, or a homogenizer-sonicator for removing TNT from soils. The sonic probe was submersed in the solvent just above the soil surface and activated for three min according to EPA Method 3550 for Sonic Extraction of Solids (EPA 1986). After sonication, samples were centrifuged at 17,369 x g for 20 min. The extracts were pipetted off and the soils were reextracted with fresh portions of methanol. When the final extraction

had been completed, the extract was filtered using a Buckner funnel with Whatman no. 41 filter paper. The three extracts from each replicate were combined, concentrated under a stream of N_2 in the dark, and frozen at -5°C until time for analysis by gas liquid chromatography (GLC).

The solution phase was extracted three times with methylene chloride by shaking in a separatory flask, allowing phases to separate for about 15 min, and removing the extract. Sodium sulfate (Na_2SO_4) was added to each extract to remove any water coextracted by the solvent. The extracts were then filtered to remove the Na_2SO_4. Extracts were concentrated under a stream of N_2 in the dark, and frozen at -5°C until time for analysis by GLC.

Statistical Analysis

Data was subjected to a two factor analysis of variance to test for differences between means. When it was necessary to reject the null hypothesis, Lavene's Test for homogeneity of variances was performed (Brown and Forsythe 1974). If results indicated that variances were different at the 0.05 level of probability, a transformation was applied to the data in an effort to achieve homogeneity of variances. When variances remained unequal after various transformations were applied (square root, $Log_1{}^0$, log^2, ln, and log-log), analysis of data by a nonparametric procedure (e.g., Friedman's Test) was performed. Differences found using nonparametric procedures were separated by conducting multiple comparisons for nonparametric analysis of variance (Zar 1984). When results indicated that variances were homogeneous, a Waller-Duncan K-Ratio Test (Steel and Torrie 1980) was used to separate differences among treatment means.

Plant Uptake of TNT, 4ADNT, and 2ADNT

Preparation and Treatment of Soils

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.

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.

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

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**These two soils were used in the plant bioassay because of a prior agreement with the sponsoring laboratory (US Army Medical Bioengineering Research and Development Laboratory). The agreement was made prior to initiation of tests of AAP soils.

of each type of soil was treated with an 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 (California Bionuclear Corp.) and unlabeled 4ADNT; one aliquot of each soil type was treated with only unlabeled 2ADNT. Only unlabeled 2ADNT was used because ^{14}C-labeled 2ADNT was not available.

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} uCi per g of TNT-treated soil, and 3.8×10^{-2} uCi per gram of 4ADNT-treated soil. Final soil concentrations were 80 ug of TNT, 4ADNT, or 2ADNT per g of soil on an oven-dry weight (ODW) basis.

The large soil batches were fertilized to ensure adequate nutrition for plant growth. Each soil batch received 50 ug N as $(NH_4)_2SO_4$, 25 ug P as NaH_2PO_4, and 25 ug 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.

Soil batches of 15 kg were dry-mixed in a twin shell dry soil blender (Patterson-Kelley Co., East Stroudsburg, Pa.) (Figure 5). 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.

During the dry-mixing of ^{14}C-treated soils, all precautions were taken to minimize contamination of greenhouse surfaces and exposure of personnel to treated soils. Access to the greenhouse was limited to individuals requiring access. 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 dispenser 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 any radioactivity.

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 6) (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

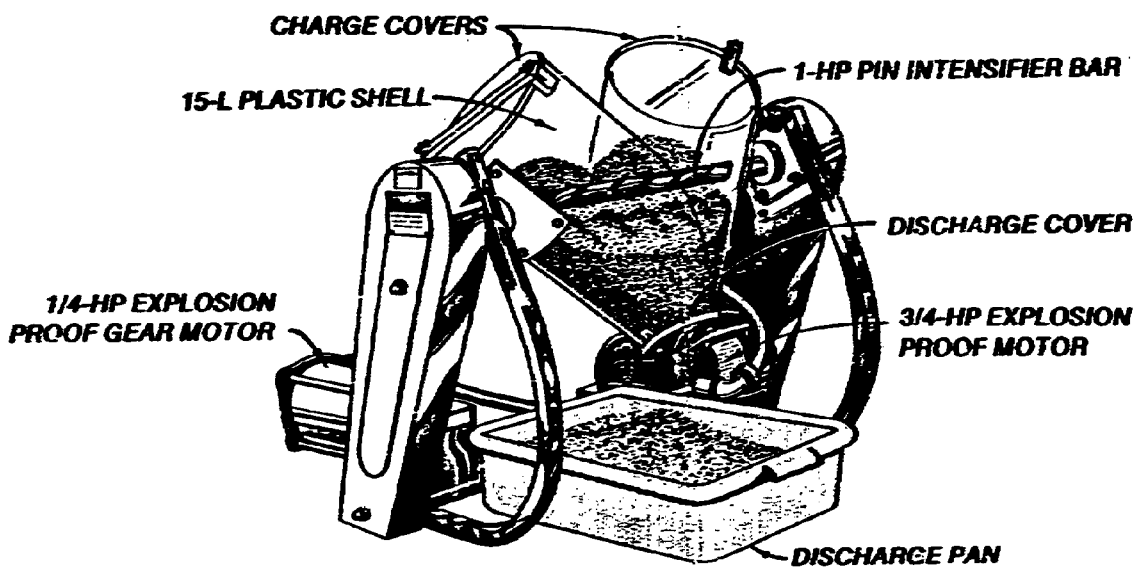


Figure 5. Twin shell soil blender

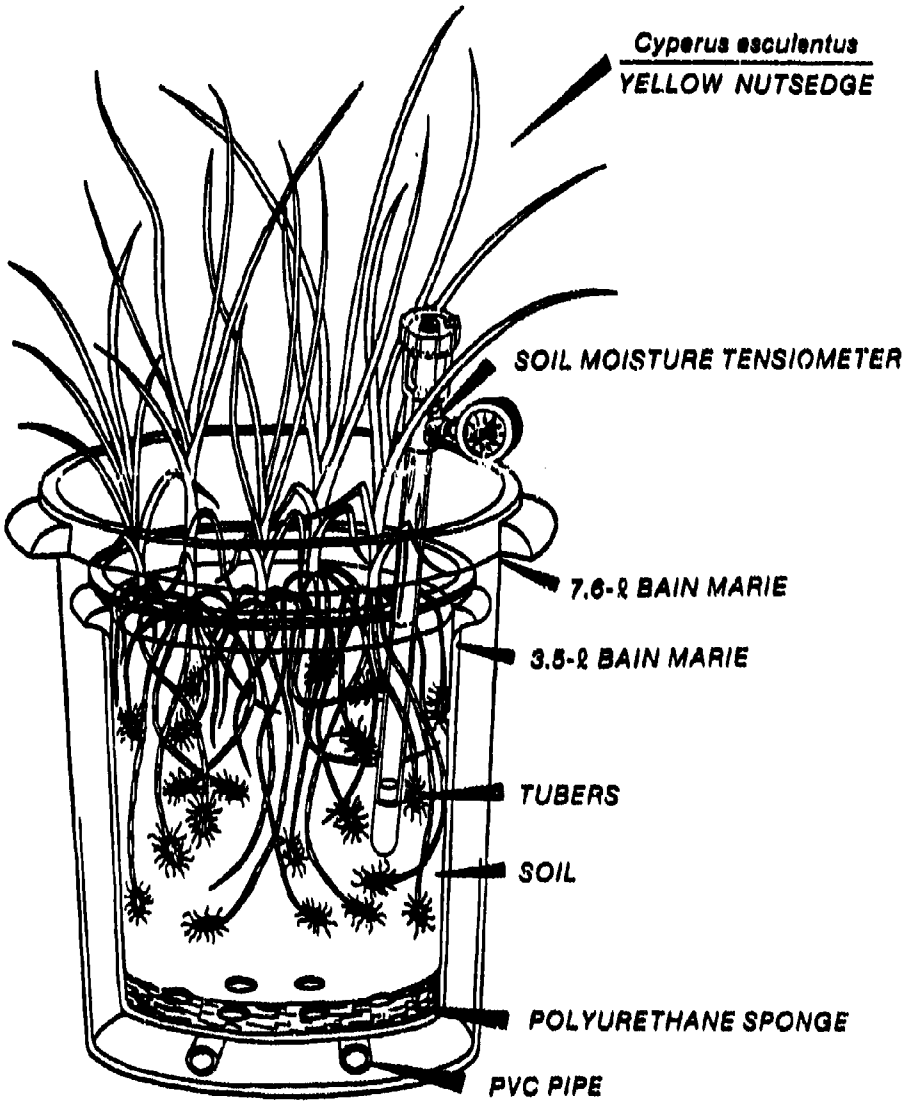


Figure 6. Plant bioassay experimental unit

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.

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 concentration was diluted with 20 ml of PCS and counted by LS.

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

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 uE/m^2/sec was maintained during the 65-day period of the experiment.

Soil Sampling and
Planting at 20 Days (T20)

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, 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)

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 was stored in the dark at 4°C until extracted (within 4 days).

Soil Homogeneity Test

A soil homogeneity test was conducted to check for uniformity in the distribution of ^{14}C-labeled compound throughout the batches before [^{14}C]TNT- and [^{14}C]4ADNT-treated soils were removed from the twin-shell blender (Figure 5). 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 x g 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 (Appendix A).

^{14}C Analysis of Soils

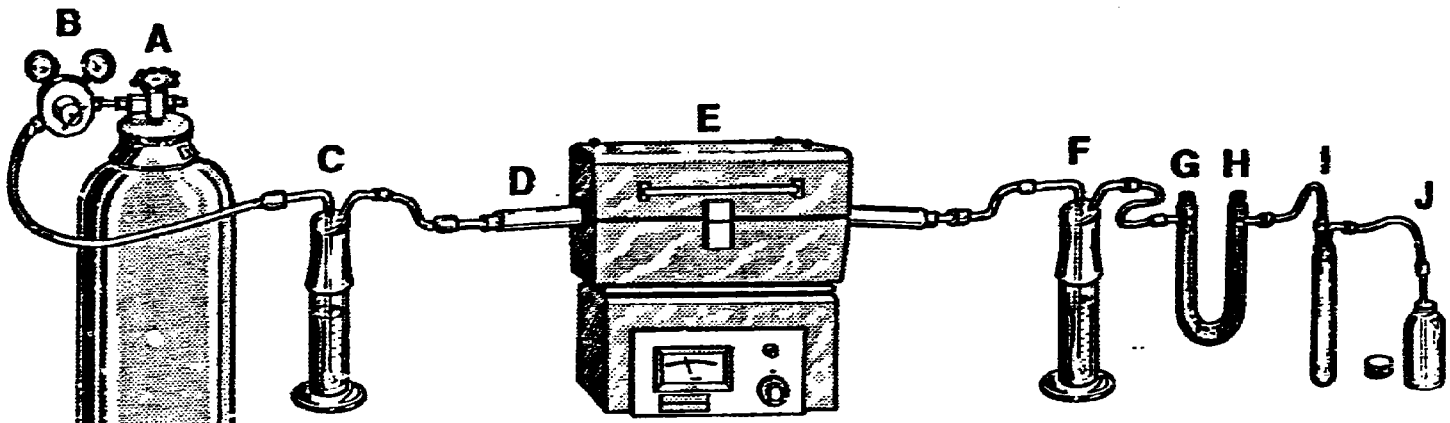
Preliminary soil extraction test. A preliminary soil extraction test was conducted to determine which of the following solvents was the most efficient for extracting [^{14}C]TNT from the silt and clay: acetone, benzene, methanol, and methylene chloride. Four 5-g replicates of [^{14}C]TNT-treated soil 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.

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/ml 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).

Carbon train analyses of soils. 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 7.

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



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 7. Carbon train

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 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.

Two standard curves were prepared to assess the efficiencies with which the carbon trains were able to recover ^{14}C spikes from the soils. [^{14}C]TNT was arbitrarily selected for the efficiency determination. Silt was used with one train exclusively and clay with the other to minimize variability. Direct spiking of soils as 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.

A stock solution containing 16.0 ug/ml total TNT (^{14}C-labeled plus unlabeled) and 0.023 uCi/ml [^{14}C]TNT was diluted with RO 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 ug/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 x g 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).

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 war 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 per minute 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.

To obtain an expected CPM in the soil phase, total CPM in the solution phase were added to rotal 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.

GLC Analysis of Soils

US EPA Standard Method 3540 for extraction of organic compounds from solid wastes (US EPA 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 grams 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.

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. 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.

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 x g 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:

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

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.

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.

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 the following dilutions of ^{14}C-labeled compound (8, 4, 2, 1.6, 0.8, 0.4, 0 ug/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 ug of TNT or 4ADNT per millilitre of extract (Appendix A). Oven-dry plant material was calculated as micrograms per gram from micrograms per millilitre of solvent and ODW of plant material extracted.

GLC Analysis. 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

Analysis of variance (ANOVA) using a completely randomized experimental design was performed on the data to test for differences among treatment means (F Tests). The ANOVA was conducted using the procedures available with SAS (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 IV: RESULTS AND DISCUSSION

Adsorption and Desorption of TNT by Soils from Selected AAPs

Soil Characterization

Table 3 lists the soil types of the AAPs according to Soil Conservation Service maps for the various areas. Results of the soil characterization tests are given in Table 4. In general, the AAP soils represented a wide range in soil characteristics. Average percent OC, CEC, and clay were relatively low, but are not atypical of soils in the eastern and central United States (Buckman and Brady 1969).

Thin-Layer Chromatographic Screening

Selection of extracting solvent. Results of the solvent extract test are given in Table 5. Acetone extracts of the uncontaminated clay soil produced UV-visible spots on the chromatographic plates above the origin, while the other three solvents produced none. R_f values of the two spots corresponded closely with R_f values of two standard compounds (2,3DNT and 4,4'AZOXY). Therefore, acetone extracted potentially interfering compounds and could not be used as the extractant of choice. Since methanol had been shown in a preliminary experiment to be as efficient as acetone and more efficient than the other two solvents tested, methanol was selected as the extracting solvent for TLC screening of the AAP soils.

Table 3

US Soil Conservation Service Soil Types of AAP Soils

Site	Soil Type
Cornhuskers	*
Crane	Loamy Orthents
Holston burning ground	Altavista Silt Loam
Holston roadside	Holston-Urban Land Complex
Iowa	Ladoga Silt Loam
Joliet	Elliott Silt Loam
Kansas	Clayey Orthents
Lonestar	Sawyer Silt Loam
Longhorn	Scotsville Sand Loam
Louisiana	Prentiss and Stough Silt Loams
Newport	Loamy Orthents
Radford	Caneyville Silt Loam
Savanna	*
Volunteer	*
Clay	Sharkey Clay
Silt	Tunica Silt

* Soil map unavailable. Soil type not known.

Selection of solvent system. R_f values for TNT, 4ADNT, and 2ADNT migrated in the three test solvent systems are given in Table 6. The difference between R_f values obtained when all three standards were

Table 4

Chemical and Physical Characteristics of AAP Soils*

Site	pH	Particle Size %sand (>50u)	%silt (50-2u)	%clay (<2u)	EC (dS/m)	%OC	CEC (meq/100g)	Extractable Metals (ug/g) Iron	Aluminum	Manganese	Calcium
Cornhuskers	7.12	52.5	27.5	20.0	2.51	0.826	35.3	124	109	34.0	0.726
Crane	4.79	31.9	47.5	20.6	2.58	2.799	31.2	346	166	76.4	1.16
Holston burning ground	7.23	51.2	30.7	18.1	2.43	2.732	28.8	225	161	40.8	0.924
Holston roadside	6.00	27.5	28.7	43.8	5.35	1.155	35.2	126	140.5	125	0.917
Iowa	5.85	15.0	65.0	20.0	6.58	1.358	44.7	342	126	150	1.14
Joliet	6.77	24.4	51.8	23.8	1.90	3.592	102.0	310	137	44.7	5.06
Kansas	7.13	33.7	40.0	26.3	3.78	2.606	130.4	264	107	93	0.85
Lonestar	4.59	55.6	34.4	10.0	1.26	0.561	15.5	161	80.9	31.5	0.938
Longhorn	4.27	51.9	33.1	15.0	8.78	0.561	20.9	96.4	99.4	29.2	1.03
Louisiana	4.40	50.6	38.8	10.6	13.48	0.367	16.3	166	109	39.8	1.17
Newport	7.72	80.6	13.8	5.6	4.28	3.539	13.4	71.9	10.9	14.9	1.02
Radford	7.21	40.0	35.0	25.0	5.34	1.059	21.5	85.6	109	33.9	0.906
Savanna	5.82	88.7	6.3	5.0	7.65	1.317	13.2	184	191	42.5	1.65
Volunteer	5.60	20.0	75.0	5.0	9.89	1.748	46.4	233.5	280.2	325	1.30
Clay	5.71	8.70	36.90	54.4	2.45	2.401	124.9	1252	160	59.6	0.954
Silt	4.54	9.37	73.1	17.5	0.72	0.567	17.2	252	196	152	1.10

* Values given are means of four replicates.

Table 5

Potential Interference of Compounds Extracted from an Uncontaminated Clay Soil on TLC Analysis of TNT and Its Degradation Products

Extractant	Number of Spots Above Origin	R_f Values
acetone	2	0.66, 0.89*
benzene	0	0
methylene chloride	0	0
methanol	0	0

* Means of three extractions plotted once.

Table 6

R_f Values for TNT, 4ADNT, and 2ADNT Migrated in Three Solvent Systems

Solvent System	R_f Values of Standard Compounds		
	TNT	4ADNT	2ADNT
benzene/ethyl acetate (75:25)	0.74*	0.50	0.42
benzene/hexane/pentane (50:40:10)	0.33	0.08	0.08
benzene/chloroform (75:25)	0.59	0.25	0.23

* Values given represent means of two spots on the same plate: one composed of the standard alone; and one composed of all three standards in combination.

combined and migrated together differed from R_f values of individual standards by no more than +/- 0.004. All three systems separated TNT from the other two compounds. However, the system exhibiting the best separation of 4ADNT from 2ADNT was benzene/ethyl acetate. Once good

separation of 4ADNT and 2ADNT was assured, R_f values for the remaining standard compounds were determined in benzene/ethyl acetate (75:25). Table 7 shows that the R_f values for TNT and 4,4'AZOXY, and for 3,4DNT and 2,3DNT were the same, or nearly the same (i.e., they were not separated). Therefore, adjustments were made in the solvent ratio until all standards were separated. A ratio of 85:15 (benzene/ethyl acetate) produced the best separation of all the standards (Table 7) and was adopted for analysis of the AAP soil extracts.

Table 7

R_f Values of Standard Compounds in Two Benzene/Ethyl Acetate Solvent Systems

Standard Compound	Benzene/Ethyl Acetate Ratio (V/V) 75:25	85:15
2,4,6-trinitrotoluene	0.72*	0.82
4-amino-2,6-dinitrotoluene	0.42	0.49
2-amino-4,6-dinitrotoluene	0.37	0.40
2,4-diamino-6-dinitrotoluene	0.14	0.16
2,6-diamino-4-dinitrotoluene	0.20	0.21
2,4-dinitrotoluene	0.60	0.64
2,6-dinitrotoluene	0.62	0.67
3,4-dinitrotoluene	0.55	0.56
2,3-dinitrotoluene	0.55	0.54
2,5-dinitrotoluene	0.66	0.70
2,2',6,6'-tetranitro-4,4'-azoxytoluene	0.73	0.86

* All values are means of three spots on each of two plates (six spots).

TLC of AAP soils. Only four of the soils exhibited fluorescence on chromatographic plates (Table 8). All others showed no evidence of fluorescence. Each of the four showing potential contamination exhibited a single spot only.

Table 8

R_f Values for Soils Showing Fluorescence on TLC Plates

Soil	R_f Value
Holston burning ground	0.20
Crane	0.84
Lonestar	0.81
Kansas	0.86

Only one of these R_f values corresponds closely to the R_f value of any of the standard compounds. The R_f value for Holston burning ground (0.20) is very close to the R_f value for 2,6-diamino-4-nitrotoluene (2,6D4NT) (0.21). Since this soil sample was collected near an old trash burning ground, it is possible that the spot represents contamination. Therefore, results of studies conducted with this soil must be interpreted with care. However, all other soils were uncontaminated.

Soil to Solution Ratio

Adsorption coefficients for each soil to solution ratio are shown in Table 9. Analysis of variance showed significant differences among the K_d values for the ratios tested. Use of the Waller-Duncan K-Ratio Test for separating differences between means showed a significant

Table 9

Adsorption Coefficients for Each Soil to Solution Ratio

Ratio	K_d
1:5	4.8449 a
1:10	3.9295 b
1:20	3.1473 c
1:30	2.6487 c

* Means of three replicates. Means followed by the same letter are not significantly different at $P < 0.05$ using Waller-Duncan K-Ratio Test.

difference between all ratios except the 1:20 and the 1:30 ($P < 0.05$). The K_d value decreased as the ratio increased. It was desirable to compare results of this study with results of a study of the effects of redox potential on adsorption and desorption of TNT. In the second study it was necessary to maintain an aqueous suspension of soil. The soil to solution ratio that could be most effectively suspended was 1:20. Therefore, the 1:20 ratio was selected for all subsequent tests.

Adsorption Kinetics

Graphs of adsorption kinetics for Joliet and Louisiana AAP soils with three concentrations of TNT are shown in Figures 8 and 9, respectively. Adsorption occurred rapidly. Joliet AAP soil reached a steady state (no significant change in solution concentration) within 1.0 hr. More than half of the TNT was adsorbed within the first hour from all three test solutions. After 2 hr, the solution concentration began to decrease again. A similar decrease was reported by Tucker et al. (1985), who followed the adsorption kinetics of TNT in soil by high

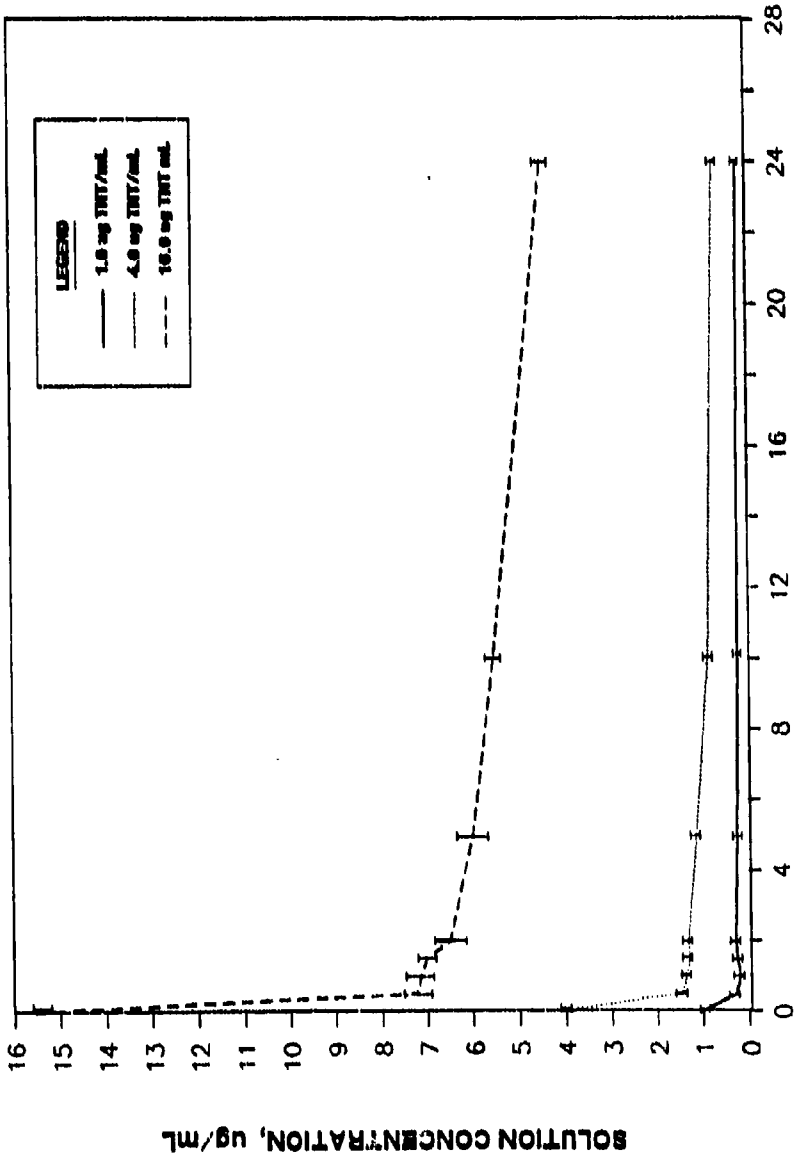


Figure 8. Adsorption kinetics curves for TNT in soil from Joliet AAP using three concentrations of TNT in aqueous solution. (Vertical bars represent +/- 1 standard deviation unit from the mean.)

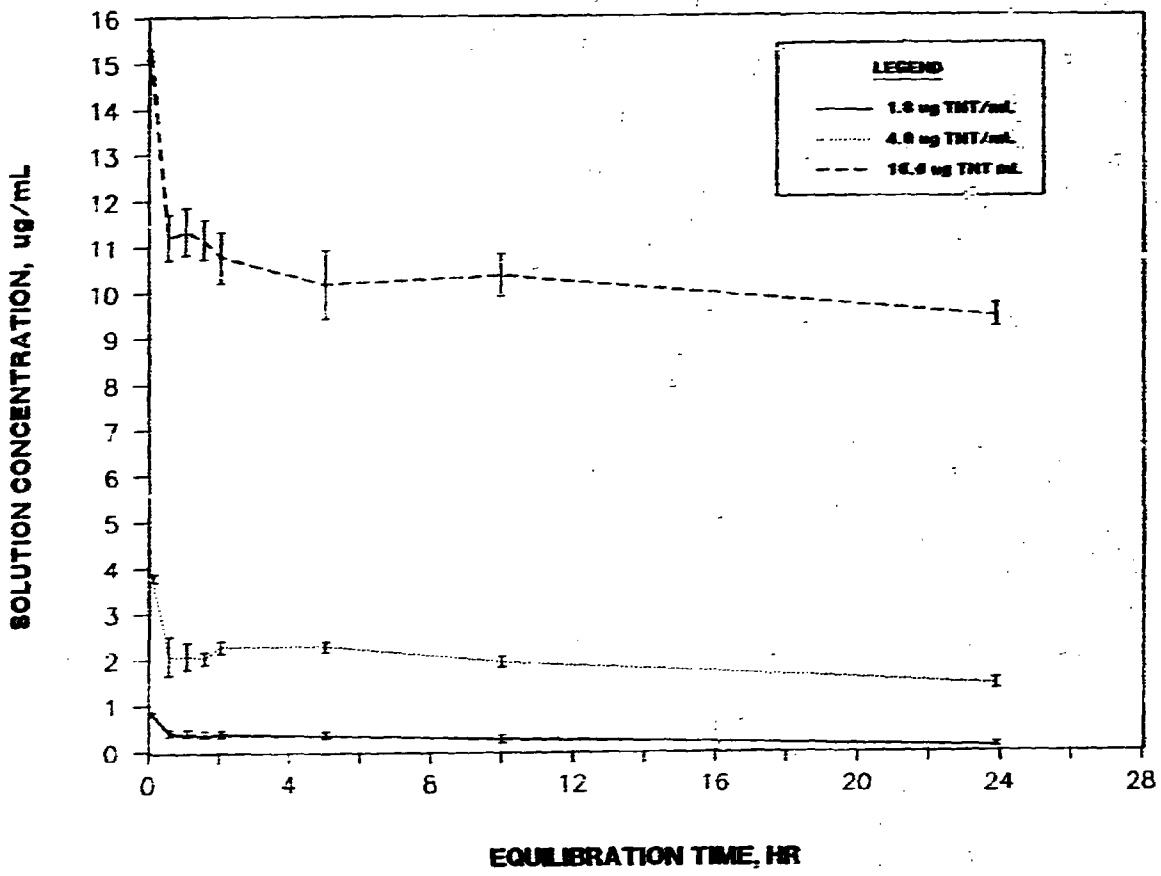


Figure 9. Adsorption kinetics curves for TNT in soil from Louisiana AAP using three concentrations of TNT in aqueous solution. (Vertical bars represent +/- 1 standard deviation unit from the mean.)

performance liquid chromatography (HPLC) of extracts of the solution phase. They attributed this decrease in solution concentration after reaching a temporary steady state to microbial degradation of TNT in the soil phase. Their conclusion was supported by the presence of microbial degradation products in the solution phase. If degradation products are formed, a decrease in solution ^{14}C counts may be due to a shift in the partitioning (equilibrium) caused by the difference between adsorption of TNT and adsorption of the product or products being formed. It is also possible that the heat of friction generated during the test caused some decomposition resulting in a shift in the partitioning.

The Louisiana AAP soil reached a steady state within 0.5 hr and maintained the steady state for at least 2 hr at all tested concentrations of TNT. A decrease in solution concentration similar to the decrease observed in the Joliet AAP soil was observed after two hours in the Louisiana AAP soil. However, the decrease proceeded more slowly in the Louisiana AAP soil. It is possible that the higher OC content of the Joliet AAP soil increased the rate of microbial degradation by providing substrate for the microorganisms. Several investigators (Klausmeier, Osmon, and Hoffsommer 1973; Osmon and Klausmeier 1972; Won et al. 1974) have found that although TNT cannot act as the sole carbon source for all microorganisms, microbial degradation of TNT can proceed in the presence of other carbon sources.

Desorption Kinetics

Desorption kinetics curves for Joliet and Louisiana AAP soils are presented in Figures 10 and 11, respectively. Joliet AAP soil reached a steady state in 1.5 hr and Louisiana AAP soil reached a steady state in

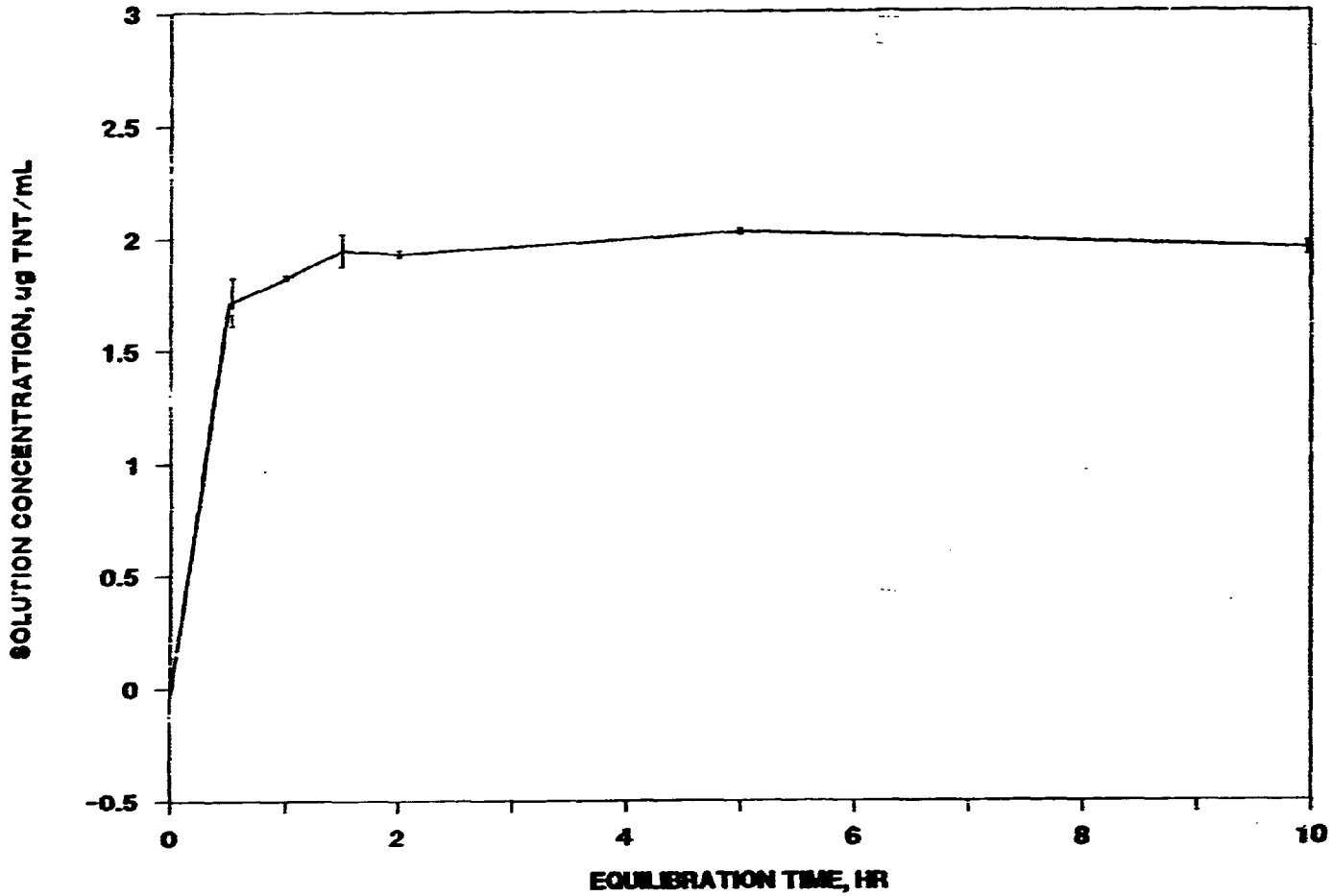


Figure 10. Desorption kinetics for TNT in soil from Joliet AAP.
(Vertical bars represent +/- 1 standard deviation unit from the mean.)

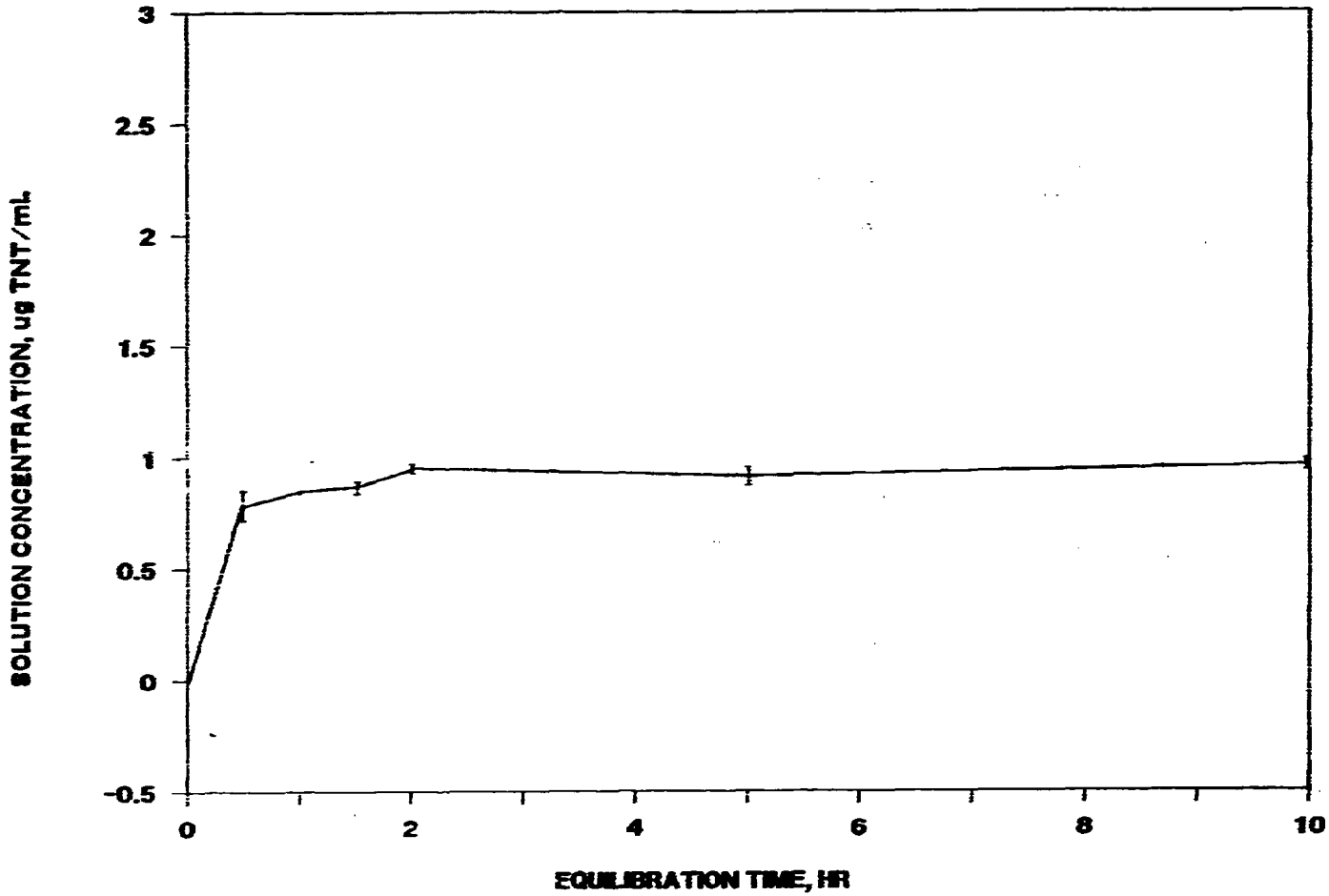


Figure 11. Desorption kinetics for TNT in soil from Louisiana AAP.
(Vertical bars represent +/- 1 standard deviation unit from the mean.)

2 hr. These results indicate that desorption occurs almost as rapidly as adsorption. From 2 to 10 hr, no significant change in the concentration of TNT in the solutions was observed. When a steady state of desorption was reached, Joliet AAP soil still retained an average of 12.5 percent of the added TNT; Louisiana AAP soil retained 6.25 percent.

Batch Adsorption

Batch adsorption data for all of the tested AAP soils are presented in Table 10. Adsorption data were fit to a linear and two nonlinear models that are commonly used to relate solid and aqueous phase contaminant concentrations in soils. The two nonlinear models were the Langmuir Isotherm Model and the Freundlich Isotherm Model. Equations for each Model are presented below (equations 1 - 3, respectively) (Weber 1972).

$$q = K_d C \qquad (1)$$

$$q = QbC/(1 + bC) \qquad (2)$$

$$q = K_d C^{1/n} \qquad (3)$$

where q is the solid phase concentration of contaminant (ug/g), K_d is the adsorption coefficient (ml/g), C is the solution concentration of the contaminant (ug/ml), Q is the monolayer sorption capacity (ug/g), b is the Langmuir constant related to entropy, and n is the Freundlich characteristic constant. Model parameters for the two nonlinear models were determined by fitting the experimental data to the linearized

Table 10

Equivalent Concentrations* of TNT in Soil and Solution After Adsorption of Five Concentrations of TNT

Soil	1 ug TNT/ml		4 ug TNT/ml		8 ug TNT/ml		12 ug TNT/ml		16 ug TNT/ml	
	C**	q+	C	q	C	q	C	q	C	q
Cornhuskers	0.66	6.9	2.8	24.3	6.3	34.4	9.7	45.6	13.7	45.2
Crane	0.70	6.0	2.8	23.2	6.5	29.7	9.8	54.9	13.9	41.1
Holster burning ground	0.66	6.8	2.7	25.5	6.2	36.6	9.7	46.4	13.5	50.0
Holston roadside	0.77	4.6	3.1	18.8	6.7	26.3	10.1	37.4	14.3	33.1
Iowa	0.59	8.1	2.5	29.2	6.0	40.6	9.2	55.8	13.2	55.9
Joliet	0.51	9.8	2.4	31.8	5.5	50.3	8.5	70.1	12.6	68.8
Kansas	0.62	7.5	2.6	27.2	5.9	41.1	8.9	62.3	13.0	60.1
Lonestar	0.77	4.5	3.1	18.2	6.9	22.7	10.4	31.7	14.6	27.4
Longhorn	0.69	6.3	2.8	23.9	6.4	31.44	9.8	43.9	13.9	41.1
Louisiana	0.79	4.2	3.1	18.0	6.8	23.8	10.4	32.7	14.7	26.4
Newport	0.80	3.9	3.1	17.1	6.8	23.9	10.5	29.3	14.8	24.0
Radford	0.73	5.5	3.0	20.8	6.6	28.61	10.0	34.9	14.3	34.8
Savanna	0.82	3.7	3.2	16.7	6.8	24.0	10.3	34.7	14.7	25.9
Volunteer	0.70	6.0	2.8	23.4	6.4	31.5	9.6	48.1	13.8	44.3
Clay	0.43	11.3	1.9	41.2	4.8	64.9	7.3	93.7	10.9	101.7
Silt	0.76	4.9	3.0	20.2	6.7	26.4	10.3	34.3	14.4	31.1

* Equivalent concentrations determined by consulting standard curves to relate CPM/ml to ug/ml of soil extract and calculating ug/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.

** Solution concentration (ug TNT/ml) at steady state. Values given are means of three replicates.

+ Soil concentration (ug TNT/g ODW) at steady state. Values given are means of three replicates.

forms of the Langmuir and Freundlich Models as given below (equations 4 and 5, respectively) (Voice and Weber 1983).

$$1/q = (1/Q) + (1/bQ)(1/C) \qquad (4)$$

$$\ln q = \ln K_d + (1/n)\ln C \qquad (5)$$

Model parameters and statistical information for batch adsorption of TNT onto test soils are presented in Table 11. Examination of R-square values across models for each soil indicated that the adsorption data fit the Langmuir Isotherm Model more closely than the data fit either the Freundlich or the linear model for every soil. There was less difference between R-square values of the Langmuir and Freundlich Models than between the linear model and either of the other two. The fact that the data fit the Langmuir Model indicated that adsorption of TNT reached a maximum as adsorption sites in the soil were filled.

Plots of adsorption isotherm models for two soils (Joliet and Newport) are shown with their respective R-square values in Figures 12 and 13. (See Appendix B for isotherms of remaining soils.) Inspection of the data points revealed that the point for the highest TNT concentration (16 ug TNT/ml) fell below the model curve for all soils except for clay and Joliet. This observation suggests that maximum adsorption (saturation of the TNT-adsorption sites in the soil) had been reached with less than 16 ug TNT/ml. In that case, the final data point would represent no further adsorption and could interfere with fitting of the data to the linear model. Therefore, a regression analysis was conducted omitting the highest concentration data point in order to examine the linearity of the adsorption isotherms prior to the high

Table 11

Estimated Regression Parameters

for Adsorption Data

Soil	Langmuir			Freundlich			Linear	
	R-Square	Q	b	R-Square	K_d	n	R-Square	K_d
Cornhuskers	0.998	72.499	0.160	0.957	10.229	1.574	0.928	4.085
Crane	0.991	76.294	0.122	0.938	8.803	1.508	0.923	3.739
Holston burning ground	0.996	87.572	0.127	0.958	10.261	1.500	0.940	4.423
Holston roadside	0.992	78.748	0.082	0.935	6.593	1.425	0.918	3.002
Iowa	0.996	85.833	0.178	0.956	12.924	1.582	0.930	5.213
Joliet	0.997	93.303	0.229	0.974	16.267	1.570	0.933	6.829
Kansas	0.992	106.945	0.121	0.970	11.592	1.403	0.944	5.696
Lonestar	0.989	54.835	0.119	0.903	6.474	1.578	0.892	2.490
Longhorn	0.990	73.116	0.138	0.939	9.382	1.565	0.919	3.737
Louisiana	0.984	66.909	0.086	0.890	6.087	1.502	0.880	2.494
Newport	0.982	65.403	0.080	0.876	5.710	1.514	0.869	2.281
Radford	0.993	67.763	0.122	0.928	7.937	1.534	0.905	3.224
Savanna	0.972	99.714	0.047	0.891	5.288	1.365	0.878	2.531
Volunteer	0.992	87.062	0.107	0.948	8.896	1.447	0.930	4.051
Clay	0.997	150.392	0.188	0.981	22.149	1.446	0.955	10.983
Silt	0.990	66.597	0.107	0.909	7.196	1.552	0.898	2.826

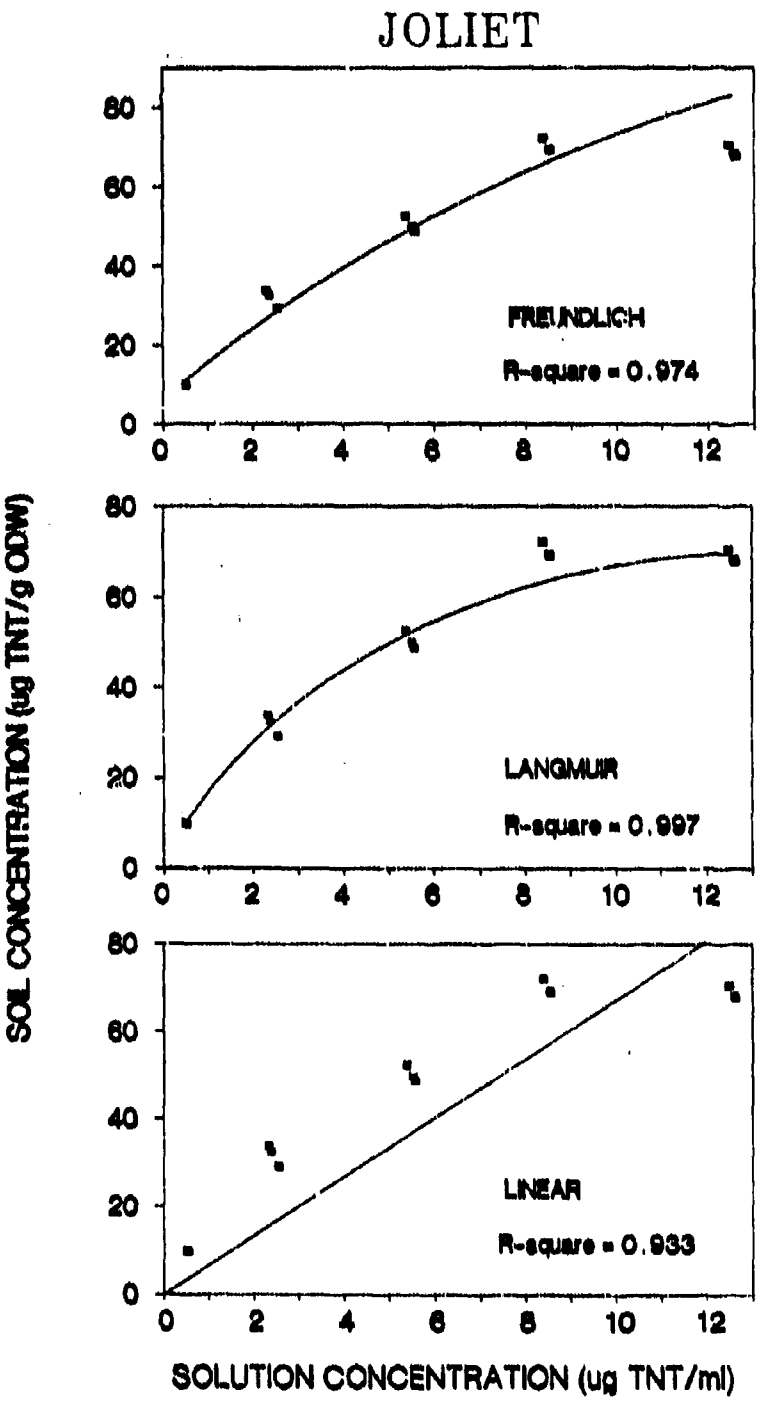


Figure 12. Joliet AAP data plotted with three isotherm models

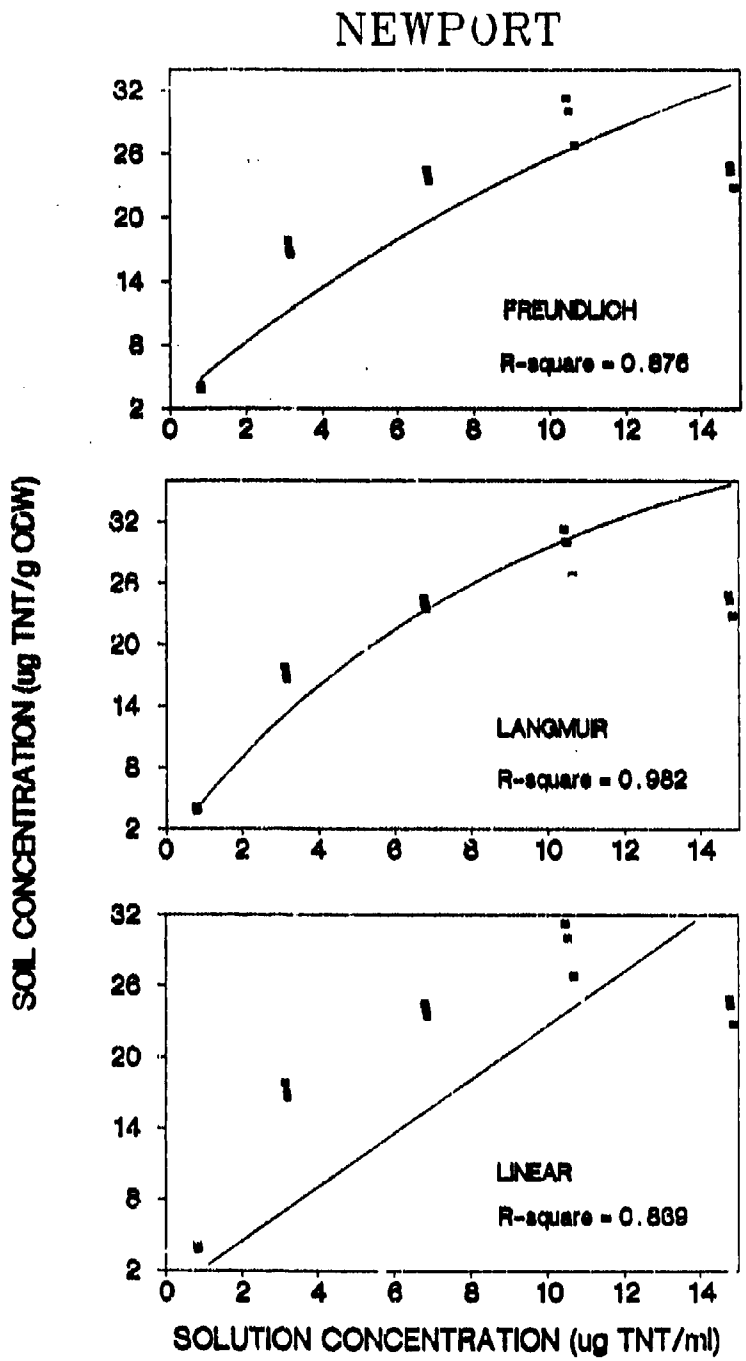


Figure 13. Newport AAP data plotted with three isotherm models

point (Table 12). Comparison of these R-square values with those from the Langmuir Model indicated that the Langmuir Model was still the better fit.

Table 12

R-Square Values for Four-Point Adsorption Isotherms

Soil	R-square	Soil	R-square
Cornhuskers	0.9356	Longhorn	0.9296
Crane	0.9292	Louisiana	0.9077
Holston burning ground	0.9125	Newport	0.8915
Holston roadside	0.9490	Radford	0.9325
Iowa	0.9300	Savanna	0.9588
Joliet	0.9746	Volunteer	0.9452
Kansas	0.9761	Clay	0.9694
Lonestar	0.8701	Silt	0.8839

Results of a Pearson correlation analysis of adsorption K_d values with soil properties are shown in Table 13. Adsorption was most closely correlated with extractable iron ($r = 0.89$) and CEC ($r = 0.87$). Ordinarily, adsorption of hydrophobic compounds that exhibit low aqueous solubilities (less than 10^{-3} M) and that are not susceptible to speciation changes or complex formation is controlled by the organic carbon fraction of the soil (Karickhoff 1981). For example, when the ratio of mineral to organic carbon content is less than 30, mineral contributions to adsorption are usually masked (Karickhoff 1984). The

Table 13

Pearson Correlation Values (R) for Adsorption K_d Values with Soil Properties

Soil Property	R	Soil Property	R
Iron	0.89213	Percent silt	0.16959
CEC	0.86560	pH	0.16798
Percent Clay	0.70079	Manganese	0.04127
Percent OC	0.40174	EC	-0.39643
Calcium	0.34983	Percent sand	-0.55073
Aluminum	0.17950		

ratio of mineral to organic carbon in the AAP soils was less than 30 (except for Holston road side for which the ratio was 38). Therefore, organic carbon should exert a greater affect on adsorption of TNT than the mineral component of these soils. This was not the case. Higher correlation with CEC than with percent OC may be due to the slight polarity of TNT (dipole moment = 1.37 Debye, Merck 1976). It is also possible that decomposition (speciation changes or complex formation) to more polar products, e.g., 4ADNT, occurred during the test. Bowman and Sans (1977) found that some pesticides adsorbed most readily to montmorillonite when the saturating cation was Fe^{+3} They attribute their results to protonation of the $-NH_2$ groups by the acidic clay surfaces. Protonation of $-NH_2$ groups by the acidic clay surfaces may explain the high correlation of TNT adsorption with Fe content in the AAP soils when degradation has produced $-NH_2$ moieties.

Sequential Desorption

Sequential desorption data for all of the tested soils are presented in Table 14. Desorption data were also fit to the linear model, and to linearized Langmuir and Freundlich Models (equations 6, 4, and 5, respectively).

$$q = K_d C + q_r \qquad (6)$$

where q_r is the irreversibly adsorbed concentration in the soil. Model parameters for results of sequential desorption of TNT from seven of the AAP soils and from the clay are presented in Table 15. Comparison of R-square values between models for the same soil indicated that desorption of TNT followed the linear model most closely for all soils except Iowa. Iowa exhibited a slightly higher R-square value with the Freundlich Model than with the linear model. For the clay, Joliet, and Kansas soils, there was little difference between R-square values of the Linear and the Freundlich Models. Even though the linear model was the best fit, none of the three models fit the data very well for the Radford, Newport, or Crane soils (R-square values were 0.5866, 0.6342, and 0.7384, respectively). Extrapolation of the linear model to the Y-axis intercept indicated the amount of adsorbed TNT remaining in the soil after the three sequential desorption cycles (Table 16). Even after three sequential desorption cycles, some TNT remained in the soils. Linear desorption isotherms of two representative soils are shown in Figure 14.

Table 14

Equivalent Concentrations* of TNT in Soil and Solution After Each of Three Sequential Desorption Cycles**

Soil	First Cycle		Second Cycle		Third Cycle	
	C+	q++	C	q	C	q
Clay	2.64	49.0	0.830	32.4	0.306	26.20
Crane	1.45	12.1	0.287	6.4	0.091	4.59
Iowa	1.83	19.3	0.435	10.6	0.164	7.33
Joliet	2.01	28.6	0.529	18.0	0.190	14.20
Kansas	2.02	19.6	0.465	10.3	0.158	7.08
Newport	0.92	5.5	0.146	2.6	0.042	1.73
Radford	1.22	10.5	0.224	6.0	0.067	4.63
Savanna	0.97	6.6	0.183	2.9	0.039	2.14

* Equivalent concentrations determined by consulting standard curves to relate CPM/ml to ug/ml of soil extract and calculating ug/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.

** Sequential desorption was conducted after two hour equilibration with a solution containing a total of 320 ug TNT.

\+ Solution concentration (ug TNT/ml) at steady state.

++ Soil concentration (ug TNT/g ODW) at steady state.

Hysteresis

Slope (K_d) and standard error from linear regression analysis of adsorption and desorption data for each soil are presented in Table 17. Statistical comparison of slopes for adsorption and desorption isotherms within each soil type (difference between two independent regressions, Steel and Torrie 1980) showed no significant differences at the 0.05 level of probability. This result is an indication of absence of

Table 15

Estimated Regression Parameters for Desorption Data

Soil	Langmuir			Freundlich			Linear		
	R-Square	Q	b	R-Square	K_d	n	R-Square	K_d	q_r
Crane	0.5192	10.455	6.768	0.6729	10.210	2.703	0.7384	5.279	4.505
Iowa	0.9272	19.774	3.430	0.9813	15.037	2.477	0.9738	6.871	6.853
Joliet	0.8543	27.647	5.198	0.9543	22.702	3.332	0.9652	7.667	13.255
Kansas	0.8785	18.554	3.742	0.9721	14.470	2.499	0.9811	6.444	6.608
Newport	0.2652	5.352	4.192	0.4683	5.551	2.122	0.6342	4.013	1.776
Radford	0.3426	8.903	10.134	0.5014	9.421	3.201	0.5866	4.780	4.615
Savanna	0.6485	4.898	16.707	0.8327	6.180	2.771	0.9161	4.708	2.019
Clay	0.8829	48.265	3.684	0.9786	36.017	3.431	0.9967	9.595	23.785

Table 16

TNT Adsorbed and Desorbed by AAP Soils

Soil	TNT Adsorbed* ug/g	TNT Adsorbed* % of Total TNT Added	TNT Remaining in Soil After Three Sequential Desorption Cycles ug/g	TNT Remaining in Soil After Three Sequential Desorption Cycles % of Total Adsorbed
Crane	41.08	12.84	4.50	10.95
Iowa	55.89	17.46	6.85	12.26
Joliet	68.81	21.50	13.26	19.27
Kansas	60.06	18.77	6.61	11.01
Newport	23.95	7.48	1.78	7.43
Radford	34.79	10.87	4.62	13.28
Savanna	25.93	8.10	2.02	7.79
Clay	101.73	31.79	23.78	23.38

* Total TNT added was 320 ug/g soil (ODW).

Environmental Consequences of Adsorption/Desorption Properties

The conditions under which this study was performed were most closely analogous to short-term exposure of surface soils to aqueous TNT contamination in the environment. Sorption properties of TNT photodecomposition products or microbial degradation products were not taken into account. It is likely that soil sorption properties of these compounds differ from those of TNT. In the absence of degradation products, TNT was only slightly resistant to desorption. Almost 20 percent of adsorbed TNT was retained after three sequential desorption cycles of Joliet AAP soil, the AAP soil most recalcitrant to

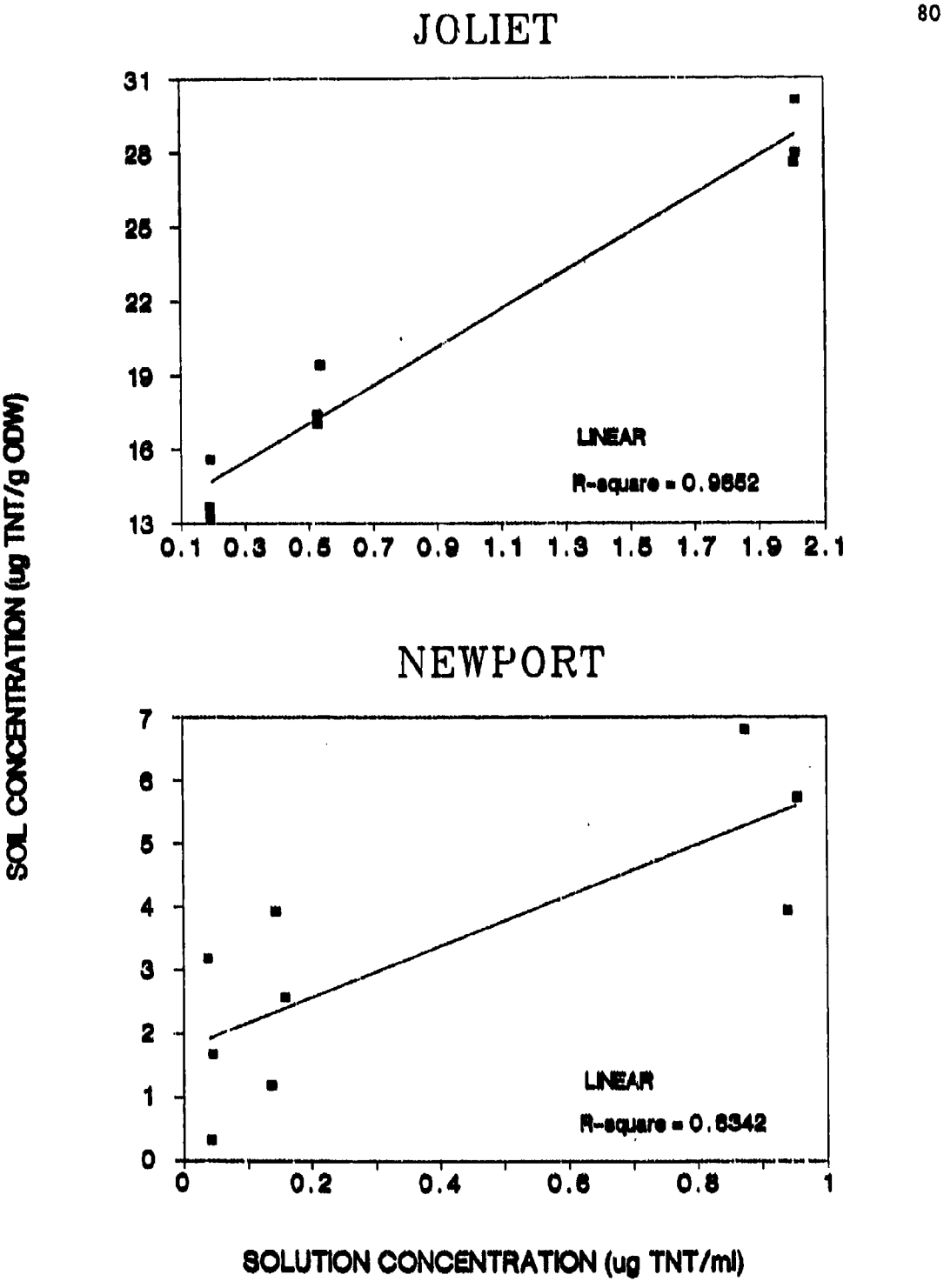


Figure 14. Linear sequential desorption isotherms for Joliet and Newport AAP soils

Table 17

K_d Values for Adsorption and Desorption of TNT on AAP Soils

Soil	Adsorption Slope* (K_d)	Adsorption Std. Error	Desorption Slope (K_d)	Desorption Std. Error
Cornhuskers	4.085	0.3031		
Crane	3.739 a	0.2894	5.279 a	1.188
Holston burning ground	4.423	0.2991		
Holston roadside	3.002	0.2403		
Iowa	5.213 a	0.3833	6.871 a	0.4261
Joliet	6.829 a	0.4890	7.667 a	0.5502
Kansas	5.696 a	0.3695	6.444 a	0.338
Lonestar	2.490	0.2319		
Longhorn	3.737	0.2960		
Louisiana	2.494	0.2463		
Newport	2.281 a	0.2362	4.0129 a	1.152
Radford	3.224 a	0.2793	4.780 a	1.517
Savanna	2.531 a	0.2514	4.708 a	0.5387
Volunteer	4.051	0.2975		
Clay	10.983 a	0.6358	9.595 a	0.2084
Silt	2.826	0.2546		

* Slopes followed by the same letter within soil type across are not significantly different at the $P < 0.05$ level. Values represent means of three replicates.

hysteresis, i.e., adsorption and desorption occurred to the same extent. The mean percentage adsorbed was 13.9 percent. The mean percentage desorbed after three sequential desorption cycles was 88.3 percent. desorption. The average percentage retained by the AAP soils was 12. Lack of hysteresis suggests that continued desorption, or leaching, may remove more, and perhaps all, of the TNT from the AAP soils unless more strongly adsorbed degradation products are formed. In the event of TNT contamination of soils higher in CEC or in OC than the AAP soils tested in this study, greater retention of TNT by the soils can be expected.

Effects of pH and Redox Potential on Adsorption and Desorption of TNT

Batch Adsorption

Batch adsorption data for Joliet AAP soil incubated under six combinations of pH and Eh are given in Table 18. These data were fit to the linear, Freundlich, and Langmuir Models. Estimated regression parameters are shown in Table 19. Examination of R-square values for each model indicated that the Freundlich Model provided the best fit for each combination of conditions except for pH 5.0/Eh +450 for which the Langmuir Model exhibited a slightly higher R-square value. In fact, there was little difference between R-square values of the Freundlich and Langmuir Models for all combinations of pH and Eh. Therefore, these results are not inconsistent with results of the batch adsorption study of other AAP soils. Table 20 shows results of a statistical comparison

Table 18

Equivalent Concentrations* of TNT in Joliet AAP Soil Incubated Under Selected Conditions of pH and Redox Potential After Adsorption of Five Concentrations of TNT

Eh	pH	1 ug TNT/ml		4 ug TNT/ml		8 ug TNT/ml		12 ug TNT/ml		16 ug TNT/ml	
		C**	q+	C	q	C	q	C	q	C	q
-150	5.0	0.513	12.5	2.70	31.1	5.77	51.1	8.83	63.3	11.7	77.4
+450	5.0	0.632	7.72	2.97	20.2	13.1	37.4	9.14	50.0	11.9	67.8
-150	6.5	0.428	13.3	2.44	35.8	5.35	58.7	7.91	91.8	11.0	88.4
+450	6.5	0.670	6.79	3.04	18.9	6.14	36.0	9.09	53.3	12.0	62.3
-150	8.0	0.564	10.3	2.75	29.0	5.81	48.4	8.55	74.5	11.7	73.6
+450	8.0	0.656	7.36	3.06	20.2	6.04	39.3	9.15	50.2	12.0	63.8

* Equivalent concentrations determined by consulting standard curves to relate CPM/ml to ug/ml of soil extract and calculating ug/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.
** Solution concentration (ug TNT/ml) at steady state. Values given are means of three replicates.
+ Soil concentration (ug TNT/g ODW) at steady state.

Table 19

Estimated Regression Parameters for Adsorption of TNT Under Selected Conditions of pH and Redox Potential

		Langmuir			Freundlich			Linear	
Eh	pH	R-Square	Q	b	R-Square	K_d	n	R-Square	K_d
-150	5.0	0.746	60.1	0.549	0.837	18.5	1.81	0.899	7.10
+450	5.0	0.943	62.6	0.220	0.912	10.3	1.41	0.925	5.67
-150	6.5	0.865	82.7	0.458	0.898	22.3	1.68	0.895	9.51
+450	6.5	0.975	77.0	0.142	0.985	8.83	1.28	0.989	5.53
-150	8.0	0.911	83.2	0.246	0.948	15.0	1.48	0.943	7.33
+450	8.0	0.969	72.6	0.169	0.975	9.70	1.34	0.983	5.56

Table 20

K_d Values for Adsorption and Desorption of TNT Under Selected Conditions of pH and Redox Potential

		Adsorption		Desorption	
Eh	pH	Slope (K_d)*	Std. Error of Estimate	Slope (K_d)	Std. Error of Estimate
-150	5.0	7.10 Aa	0.636	11.1 Aa	1.63
+450	5.0	5.67 Aa	0.432	11.3 Aa	1.11
-150	6.5	9.51 Aa	0.873	16.5 Aa	3.86
+450	6.5	5.53 Aa	0.155	9.74 Aa	1.13
-150	8.0	7.33 Aa	0.483	16.5 Aa	1.75
+450	8.0	5.56 Aa	0.197	9.24 Aa	0.975

* Slopes followed by the same uppercase letter for each combination of Eh and pH are not significantly different at the $P < 0.05$ level. Values given are means of three replicates. Slopes followed by the same lowercase letter across a single Eh and pH are not significantly different at the $P < 0.05$ level.

of K_d values between the combinations of test conditions (comparison of two independent regressions, Steel and Torrie 1980). Results indicated no significant differences. Therefore, pH and redox potential exerted no effect on adsorption of TNT by the tested soil. Adsorption isotherms are shown in Figures 15 and 16.

Sequential Desorption

Sequential desorption data for Joliet AAP soil incubated under six combinations of pH and Eh are given in Table 21. Standard errors of the K_d values for each combination of test conditions are given in Table 20. Statistical comparisons of desorption slopes (comparison of two independent regressions, Steel and Torrie 1980) indicated no significant differences. Therefore, pH and Eh exerted no significant effect on desorption of TNT by the tested soil. However, standard errors for this data were great which is not surprising when the length of time during which the soil was in contact with the solution is considered. For generation of adsorption data, tests were conducted for 2 hr. For generation of sequential desorption data, an additional 6 hr was required. Within that additional time, degradation of TNT resulting in variable desorption of ^{14}C-labeled compounds could have occurred. Examination of the results revealed a general trend that suggested greater retention of TNT by the reduced soil at all three pH values.

Examination of the data indicated that variances for one specific flask were consistently greater than that for other replicates. The adsorption count data for solution phase in this flask were consistently lower than for the other two replicates. However, the desorption count data for the solution phase in this flask were consistently higher than

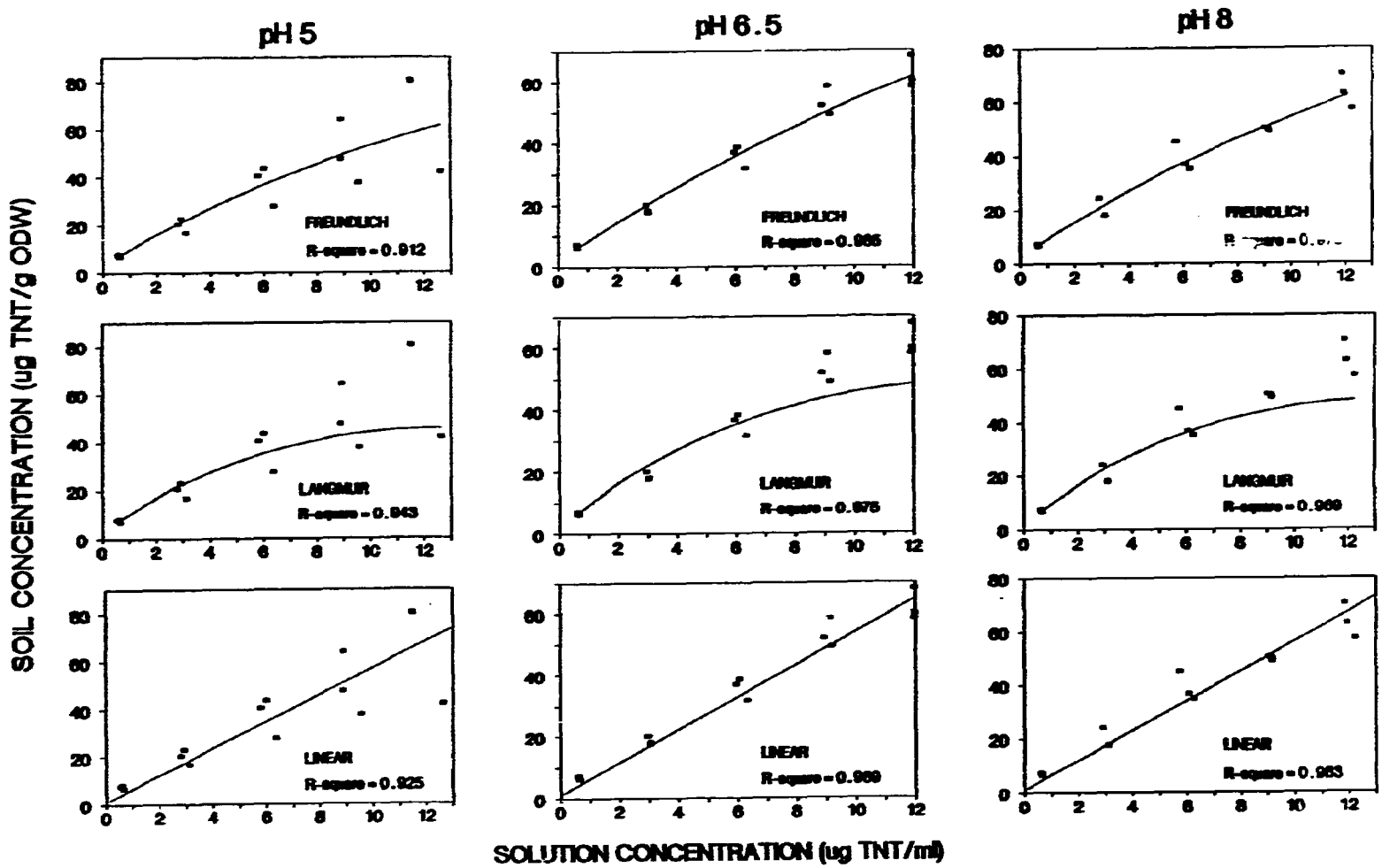


Figure 15. Adsorption isotherms for Joliet AAP soil incubated under oxidized conditions and pH 5.0, 6.5, and 8.0

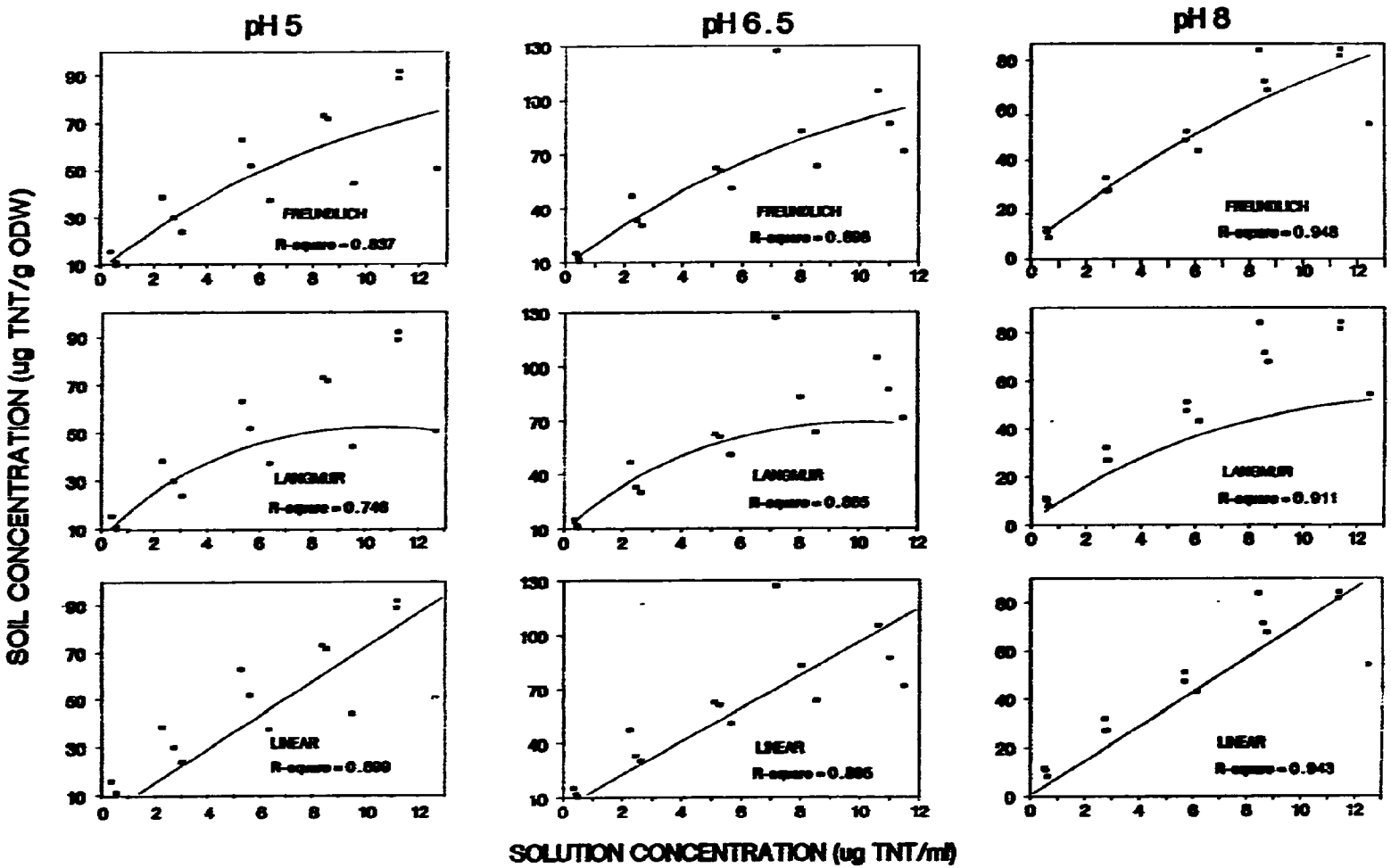


Figure 16. Adsorption isotherms for Joliet AAP soil incubated under reduced conditions and pH 5.0, 6.5, and 8.0

Table 21

Equivalent Concentrations* for TNT in Joliet AAP Soil Under

Selected Conditions of pH and Redox Potential

After Three Sequential Desorption Cycles

Eh	pH	First Cycle		Second Cycle		Third Cycle	
		C**	q+	C	q	C	q
-150	5.0	1.22	61.7	0.345	53.2	0.158	49.3
+450	5.0	1.57	33.1	0.455	21.6	0.186	16.9
-150	6.5	1.26	131.	0.400	120.	0.237	113.
+450	6.5	1.49	42.3	0.374	32.4	0.145	28.6
-150	8.0	1.19	101.	0.405	89.5	0.186	84.1
+450	8.0	1.36	35.8	0.338	27.2	0.125	23.9

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

** Sequential desorption was conducted after two hour equilibration with a solution containing a total of 240 ug TNT.

\+ Solution concentration (ug TNT/ml) at steady state.

++ Soil concentration (ug TNT/g ODW) at steady state.

for the other two replicates. Since each adsorption data point was pipetted independently from the others, the difference could not be attributed to pipetting error. These observations suggested that something in the soil of that particular sample adsorbed abnormally strongly, but very readily (although incompletely) desorbed. Therefore, assuming that the population is normally distributed, Dixon's test for outliers was applied (Sokal and Rohlf 1981). The test indicated that the values could not be considered outliers. Variances for all of the data was too great. Therefore, that data point was not removed from the data set.

Curve fitting for this data set (except for the linear model) was not attempted because of the high variance coupled with the fact that only three points were generated by the sequential desorption cycles. Linear regression analysis was conducted and R-square values for each combination of Eh and pH (Table 22) illustrate that desorption for all conditions was fairly linear.

Table 23 summarizes amounts of TNT adsorbed and desorbed for each set of test conditions. The data showed that only about 25 percent of the added TNT was adsorbed. About half of the adsorbed TNT was removed from oxidized tests, but very little (less than 10 percent) was removed from the reduced tests.

Table 22

R-Square Values for Linear Regression Analysis of Soil at Each Eh and pH

Eh	pH	R-square
-150	5.0	0.979
+450	5.0	0.990
-150	6.5	0.948
+450	6.5	0.987
-150	8.0	0.989
+450	8.0	0.989

Table 23

Amounts of TNT Adsorbed and Desorbed by Joliet AAP Soil Incubated Under Selected Conditions of Eh and pH

		TNT Adsorbed*		TNT Remaining in Soil After Three Sequential Desorption Cycles	
Eh	pH	ug/g	% of Total TNT Added	ug/g	% of Total Adsorbed
-150	5.0	63.3**	26.4	49.8	78.6
+450	5.0	50.0	20.8	20.1	40.2
-150	6.5	91.8	38.2	113.2	123.4
+450	6.5	53.3	22.2	28.6	53.6
-150	8.0	63.9	26.6	84.1	131.5
+450	8.0	50.2	20.9	23.9	47.7

* Data given on adsorption is from the 12.0 ug/ml treatments only since the samples receiving that treatment level were also desorbed.
** Values are mean of three replicates.

Degradation of TNT Under Oxidized and Reduced Conditions

Preliminary Test for the Effectiveness of $HgCl_2$ as a Soil Sterilant

No growth of microorganisms occurred on plates that received $HgCl_2$-treated sediment at any of the sampling times, while plates that received untreated sediments were completely overgrown. Therefore, $HgCl_2$ at the concentration used was an effective soil sterilant.

Degradation of TNT

GLC analysis of degradation samples are shown in Table 24. Lack of homogeneity in variances after several transformations of the data dictated application of nonparametric analysis. Results indicated very few statistically significant differences among the treatments. However, several trends in the data were observed. For example, limited abiotic reduction of TNT was evident. In both oxidized and reduced solution phase, small quantities of 4ADNT, 2ADNT, and 2,6D4NT were detected. In the soil phase, only 4ADNT and 2ADNT were found; however, quantities were much higher in the soil, especially in the reduced soil, than in solution. Biotic reduction of TNT exceeded abiotic reduction even when abiotic reduction was factored out of soil data. (Little difference between biotic and abiotic reduction was evident in the solution data.) Microbial degradation was greater under reduced than under oxidized conditions and was more evident in the soil than in the solution phase.

Table 24

Degradation of TNT Under Oxidized and Reduced Conditions

Compound Assayed	Solution Phase (ug/ml)					
	Oxidized			Reduced		
	TNT	TNT+$HgCl_2$	Control	TNT	TNT+$HgCl_2$	Control
2,4,6-trinitrotoluene	12.5 Aab*	16.2 ABab	--- Ab	9.92 ABab	17.4 Ab	--- Ab
4-amino-2,6-dinitrotoluene	0.552 ABab	0.020 ABab	--- Ab	2.20 ABa	0.014 Bab	--- Ab
2-amino-4,6-dinitrotoluene	0.246 Ba	0.015 Bab	--- Ab	1.18 ABa	0.017 ABab	--- Ab
2,6-diamino-4-nitrotoluene	0.008 Bb	--- Bb	--- Ab	--- Bb	--- Bb	--- Ab
2,4-diamino-6-nitrotoluene	--- Bb	--- Bb	--- Ab	--- Bb	--- Bb	--- Ab
4-amino-2-nitrotoluene	--- Bb	--- Bb	--- Ab	--- Bb	--- Bb	--- Ab
2,4-dinitrotoluene	--- Bb	--- Bb	--- Ab	--- Bb	--- Bb	--- Ab
2,6-dinitrotoluene	--- Bb	--- Bb	--- Ab	--- Bb	--- Bb	--- Ab
1,3,5-trinitrobenzene	--- Bb	--- Bb	--- Ab	--- Bb	--- Bb	--- Ab

* Values represent means of four replicates. Means having the same uppercase letter within treatments (oxidized and reduced) are not significantly different at the $P < 0.05$ level. Detection limit for solution phase was 0.003 ug/ml. Means having the same lowercase letter across treatments (i. e., within analyte) are not significantly different at the $P < 0.05$ level. "---" denotes none detected.

Table 24 (Continued)

Compound Assayed	Soil Phase (ug/g)					
	Oxidized			Reduced		
	TNT	TNT+$HgCl_2$	Control	TNT	TNT+$HgCl_2$	Control
2,4,6-trinitrotoluene	8.50 Aab**	23.5 Aa	--- Ab	2.67 ABab	20.8 Aa	--- Ab
4-amino-2,6-dinitrotoluene	2.45 ABab	0.475 Bab	--- Ab	7.60 ABa	1.52 ABab	--- Ab
2-amino-4,6-dinitrotoluene	1.17 Bab	0.303 Bab	--- Ab	4.40 ABa	0.915 ABab	--- Ab
2,6-diamino-4-nitrotoluene	--- Bb	--- Bb	--- Ab	--- Bb	--- Bb	--- Ab
2,4-diamino-6-nitrotoluene	--- Bb	--- Bb	--- Ab	--- Bb	--- Bb	--- Ab
4-amino-2-nitrotoluene	--- Bb	--- Bb	--- Ab	--- Bb	--- Bb	--- Ab
2,4-dinitrotoluene	--- Bb	--- Bb	--- Ab	--- Bb	--- Bb	--- Ab
2,6-dinitrotoluene	--- Bb	--- Bb	--- Ab	--- Bb	--- Bb	--- Ab
1,3,5-trinitrobenzene	--- Bb	--- Bb	--- Ab	0.028 Bb	--- Bb	--- Ab

** Values represent means of four replicates. Means having the same uppercase letter within treatments (oxidized and reduced) are not significantly different at the P< 0.05 level. Detection limit for solution phase was 0.03 ug/ml. Means having the same lowercase letter across treatments (i. e., within analyte) are not significantly different at the P < 0.05 level. "---" denotes none detected.

Plant Uptake of TNT, 4ADNT, and 2ADNT

Chemical and Physical Characteristics of Test Soils

Results of chemical and physical characterization tests for the silt and clay are presented in Table 25.

Table 25

Chemical and Physical Characteristics of Test Soils

	Clay	Silt
pH	5.71	4.54
Particle Size		
Percent Sand	8.70	9.37
Percent Silt	36.9	73.1
Percent Clay	54.4	17.5
Electrical conductivity (dS/m)	2.45	0.72
Percent organic carbon	2.40	0.57
Cation exchange capacity (meq/100 g)	135.	17.2
Extractable metals (ug/g)		
Iron	1,252	252
Aluminum	160	196
Manganese	59.6	152
Calcium	0.954	1.10

Soil Homogeneity Test

Results of the test for soil homogeneity are shown in Table 26. 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 ug of TNT and 4ADNT per gram of soil. A higher degree of homogeneity was observed in the silt than in the clay for both

Table 26

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

	Concentration of Constituent							
	TNT-Treated				4ADNT-Treated			
	Silt		Clay		Silt		Clay	
Blender Position	%**	ug/g	%	ug/g	%	ug/g	%	ug/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 ug/ml of soil extract and calculating ug/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.

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

\+ Means from extractions of three subsamples from each blender position.

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. 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.

^{14}C Analysis of Soils

Preliminary soil extraction test. Results of the preliminary soil extraction test showed that acetone and methanol were more efficient

extractants of ^{14}C from both soils (silt and clay) than either methylene chloride or benzene (Table 27). 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/100g 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 27

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

	Concentration of TNT			
	Silt		Clay	
Solvent	(%)+	(ug/g)	(%)	(ug/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 ug/ml of soil extract and calculating ug/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 three extractions of TNT-treated and untreated soil.

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

++ Means followed by the same letter which was within soil types are not significantly different at the $P < 0.05$ level.

Siphoned water. Results of ^{14}C analysis of excess water siphoned from outer pots after watering plants are shown in Table 28. 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 ug/g of soil.

Table 28

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 sample 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.

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 29. The data demonstrate a distinct difference in the behavior of the two treatment compounds in the silt and clay. Carbon 14 was present 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 retained significantly more ^{14}C than 4ADNT-treated clay at both T20 and

Table 29

^{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* ug/g	T65** ug/g	T20 ug/g	T65 ug/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

* 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.

T65. This result suggests strong adsorption of TNT by the clay. This possibility is explored further when results of carbon train analysis of soils are discussed.

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 or its ^{14}C-labeled degradation products 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.

Carbon train. An efficiency curve for the carbon train with which the silt was used is shown in Figure 17. 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 30. 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.

An efficiency curve for the carbon train with which clay was used is shown in Figure 18. 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 31. 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

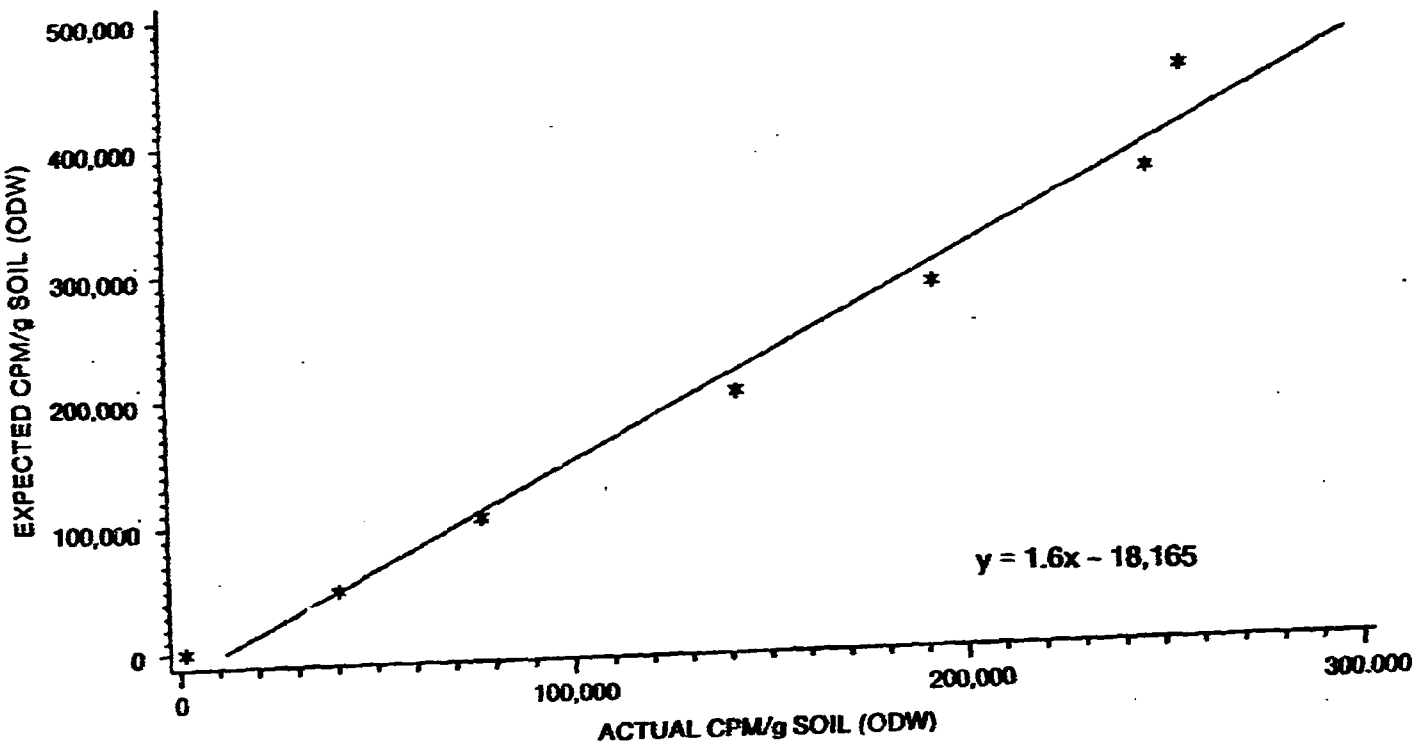


Figure 17. Carbon train efficiency curve of silt soil

Table 30

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 soil, in the solution phase, and adsorbed to test tubes.

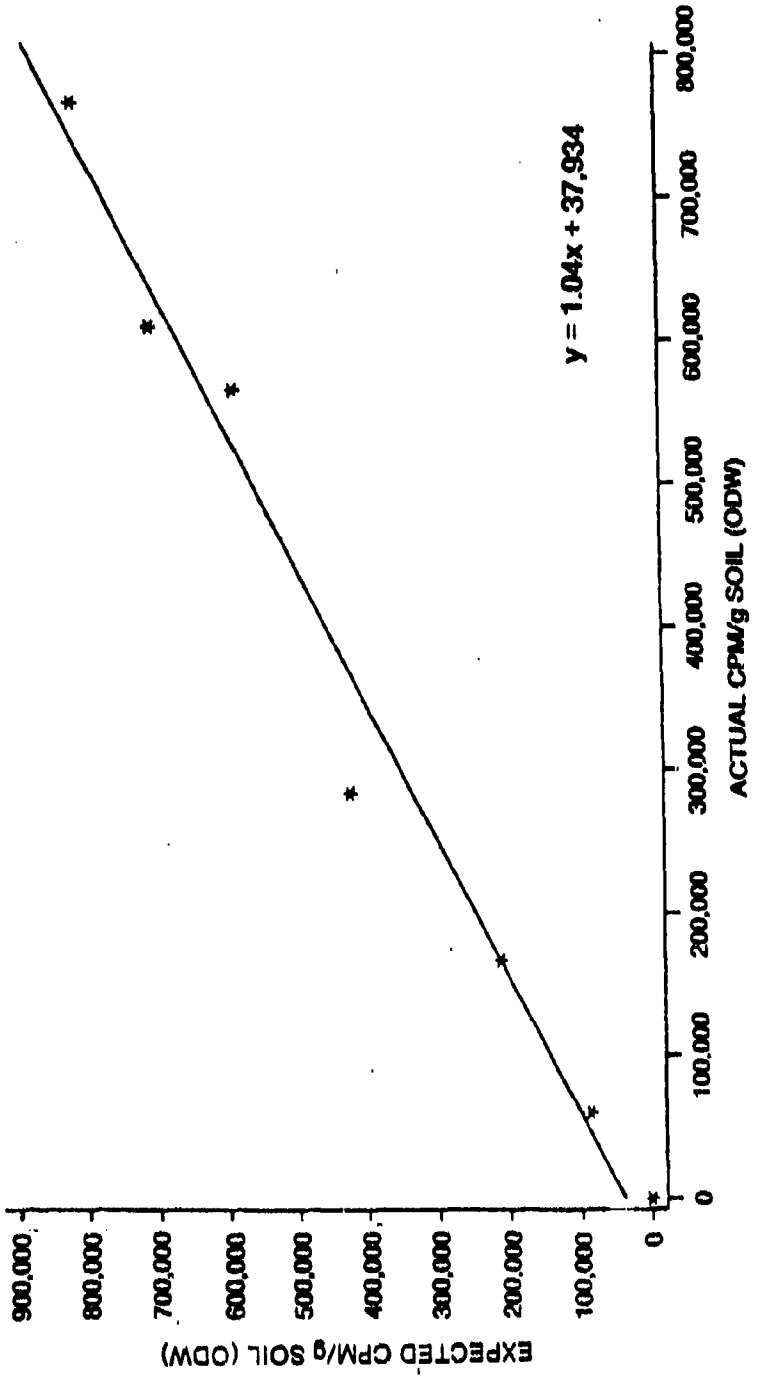


Figure 18. Carbon train efficiency curve for clay soil

Table 31

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.

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

Carbon train results (Table 32) 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-treatment than in the 4ADNT-treatment 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.

Table 32

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* ug/g	T65** ug/g	T20 ug/g	T65 ug/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.

In Table 33, percent recoveries of ^{14}C by extraction and by carbon train analysis are compared. Percent recoveries by carbon train analysis were significantly greater ($P < 0.05$; T-test) 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 those by extraction by a factor of four. If carbon train recoveries were corrected to the efficiencies of

Table 33

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
4DNT	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.

the two carbon trains (71.30 percent for the train with 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.

Significantly more 4ADNT was found in the silt than in the clay by extraction, but the carbon train showed no significant difference between amounts of 4ADNT in the silt and the clay. Comparison of the extraction data with the carbon train data 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 the clay has a greater retentive affinity for TNT than for 4ADNT. This is consistent with results of soil sorption studies which showed highest correlation between adsorption and iron, CEC, percent clay and percent OC. The clay was markedly higher in each of these properties than the silt.

Although adsorption 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.

GLC Analysis of Soils

Tables 34 and 35 show results of GLC analysis for T20 and T65 soils, respectively. 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 biodegradation products of TNT. Biodegradation was considered to be the most probable degradation mechanism occurring in the soil. Some of the compounds (2ADNT, 4ADNT, and 2,4D6NT) had also been prepared by chemical reduction of TNT in the laboratory (Sitzmann 1974). The successful reduction of TNT to these compounds in the laboratory raises the possibility of spontaneous chemical reduction as a mechanism of degradation in the soils, in the plants, or during analytical processing. TNB was included because it is a commonly detected photodecomposition product of TNT that could possibly form during treatment, on soil surfaces after potting, in the plants, or during sample preparation.

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 degradation of TNT to 4ADNT and 2ADNT within 20 days of soil treatment. In the clay, TNT concentration was

Table 34

GLC Analysis of T20 Soils (ug/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	0.0920Bbc	0.0802Ac	0.6413Cab	0.0190Bc	0.8475Aa
4-amino-2,6-dinitrotoluene	0.0060Bb	1.1800Ab	5.3425Aa	0.4010Bb	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	0.03303b	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 ug/g.

Table 35

GLC Analysis of T65 Soils (ug/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 ug/g.

significantly higher than concentrations of compounds other than 4ADNT and 2ADNT. In the silt, TNT concentration was no different from concentrations of compounds other than 4ADNT and 2ADNT. 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 more favorable than 2ADNT production, or that 4ADNT is more persistent in the soil than 2ADNT.

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 either sampling time. 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 and that 2,4D6NT is a degradation product of 4ADNT.

Across soil treatments at T20, TNT occurred in significantly highest levels in the TNT- and 2ADNT-treated silt and in second highest levels in the TNT- and 2ADNT-treated clay. This result suggests some equilibrium between TNT and 2ADNT in the soil unless cross-contamination of 2ADNT-treated soils with TNT occurred. The latter cannot be ruled out because TNT and other compounds were also detected in controls. If

volatilization, or coevaporation with soil moisture followed by cocondensation on the soil surface occurred, such low-level cross-contamination could result. Low-level contamination of controls was also observed with both ^{14}C methods of analysis. 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.

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.

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 in the soil.

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 36 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 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.

Recoveries as a sum of all products detected and based on percentage of original treatment levels are given in Table 37. 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

Table 36

Percent Recoveries of Spikes from Selected Soil Samples by GLC Analysis

	Treatment*						
	Clay				Silt		
Compound Assayed	T20 2ADNT	Control	T65 TNT	4ADNT	Control	T20 4ADNT	T65 4ADNT
2,4,6-trinitrotoluene	40	98	34	94	42	42	6.4
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.

Table 37

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

Soil Type	Treatment	Sampling Time T20	Sampling Time 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

chromatographic (HPLC) method (USATHAMA 1983)+ are compared to GLC analysis in Table 38. 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

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

Table 38

Comparison of HPLC and GLC Results from Selected T20 Soils

Soil Type	Treatment	TNT		4ADNT		2ADNT	
		GLC*	HPLC**	GLC	HPLC	GLC	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 ug/g.
** Detection limit for both TNT and 4ADNT was 1 ug/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.

HPLC method were that 4ADNT and 2ADNT could not be separated and that detection limits were higher than with the GLC method.

Analysis of Plants

Plant yields. The data presented in Table 39 show plant yields for each treatment by soil type. There were no significant differences in yield between treatments within soil types. However, ANOVA for clay across all treatments and means for silt across all treatments showed significantly greater yields in clay than in silt.

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 biossay apparatus, which utilizes 7.6-1 rather than

Table 39

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.

3.5-1 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. Differences between results with the two pot sizes will be further investigated in a later study due to its importance for the standard WES plant bioassay.

^{14}C analysis of preliminary plant extraction test. Results of the plant extraction test are given in Table 40. The table shows efficiencies with which the internal ^{14}C standard was recovered from

Table 40

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.

plant extracts. Counts for the benzene extract were significantly higher than those 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. The same solvent was not selected for the plant and soil extractions. It is probable 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 in the plant extracts. These compounds, e.g., especially the photosensitive chlorophylls, 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.

^{14}C analysis of 2-g plant samples. Results of ^{14}C analysis from extraction of 2-g plant samples are given in Table 41. Carbon-14 was detected in plants grown in 4ADNT-treated silt only. No ^{14}C was detected in any other treatments nor in controls. It is important to recall that the ^{14}C-label was on the methyl group of the 4ADNT-molecule. The methyl group is susceptible to removal by photochemical processes. The possibility of detecting degradation products of 4ADNT are therefore greater.

Table 41

^{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 ug/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 soil types are not significantly different at $P < 0.05$ level.

^{14}C analysis of all remaining plant material. Table 42 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

Table 42

^{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 ug/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.

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 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 33) showed greater retention of 4ADNT by the clay at T65.

GLC analysis. The data presented in Table 43 show results of GLC analysis of plant material. The only compounds detected were TNB, TNT,

Table 43

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 ug/g of plant material on an ODW basis.
** Showed <0.001 ug/g (below detection limit) of the assayed compound. "---" denotes none detected.

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.

The presence of TNB in plants grown in the 4ADNT-treated silt, although in very limited quantity, may support photodecomposition of 4ADNT in the silt.

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. 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 was unknown.

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 (Table 33) showed that significant levels (roughly 30 - 45 percent) of the ^{14}C remained in the TNT-treated soils after extraction. In adsorption studies the clay soil adsorbed 31.8 percent of the TNT added and retained 23.4 percent of the adsorbed amount (or about 7.5 percent of the total amount added) after three sequential desorption cycles (Table 16). Comparison of results of the soil sorption studies with those of the plant uptake study suggest a discrepancy between the short term (1 - 2 days) and the long term (20 - 65 days) adsorption steady state of TNT in soils. Such a discrepancy is discussed by Karickhoff and Morris (1985) for sorption of hydrophobic organic pollutants in sediment suspensions. They assert that when only changes in aqueous phase and/or sorbed phase concentrations are measured, then the process appears complete after a few hours, but in reality it may continue indefinitely. A two-compartment model is used to distinguish rapid or "labile" exchange, requiring at most a few hours to achieve, from highly retarded or "nonlabile" sorption requiring days to weeks to occur. The authors suggest that an intraparticle process, whereby chemical is slowly incorporated into either particle aggregates or sorbent components, is responsible for this apparent dichotomy in behavior. TNT literature also supports increased adsorption over time

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.

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. (1980b) 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 ug 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. (1980b), 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.

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. Furthermore, 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.

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 levels 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.

Environmental
Implications of Plant Uptake

Adsorption of TNT and 4ADNT by leafy portions of C. esculentus is minimal at the soil levels utilized in this study. No adsorption by 2ADNT was detected. Soil characteristics exert an important influence on plant uptake because adsorption to certain soil fractions, e.g., clays or organic carbon, reduces bioavailability. The influence of soil sorption on plant uptake may become less important as soil levels of the compounds increase. That is, once soil sorption reaches a maximum, more compound may become bioavailable.

Limited concentrations of TNT and 4ADNT in the leafy portions of the plant does not preclude accumulation in lipid-rich plant parts, e.g., seeds or tubers. The compounds may be transported to seeds via passive aqueous transport and bioaccumulate there while remaining at relatively low concentrations in stems and leaves. It is also possible that simple partitioning with lipid-rich tubers occurs in the soil. The environmental implications of bioaccumulation in seeds or tubers is significant because these plant parts are important foods for wildlife.

PART V: CONCLUSIONS

Soils from the Army Ammunition Plants sampled exhibited a broad range of physical and chemical characteristics except for their relatively low organic carbon and clay content. Such soil properties are generally consistent with low retention of organic contaminants. Adsorption of TNT to the AAP soils was rapid and followed the Langmuir Adsorption Isotherm Model. Adsorption correlated most highly with extractable iron, cation exchange capacity, percent clay, and percent organic carbon. Desorption was also fairly rapid. After three sequential desorption cycles, an average of 12 percent of the adsorbed TNT remained in the soil. These results indicate that soil sorption will not effectively prevent mobility of TNT in the environment unless adsorption increases over extended periods of time, or more strongly adsorbing degradation products are formed.

Redox potential and pH exerted no measurable effect on adsorption or desorption of TNT. However, a trend in the data suggests greater retention of TNT by reduced than by oxidized soil. If this is the case, TNT would remain somewhat immobilized if buried in reduced sediment, e.g., at the bottom of a disposal lagoon.

Limited uptake of TNT and 4ADNT, and no uptake of 2ADNT by C. esculentus was detected. Availability of treatment compounds was probably limited by loss of compounds from soils by volatilization of microbial and photodegradation products, and irreversible adsorption of compounds and/or their degradation products to soils. Neither TNT, 4ADNT, nor 2ADNT became concentrated in C. esculentus. It is unlikely

that any of these compounds present a problem to the plant at the levels tested. However, if soil levels are sufficiently high to saturate the adsorbing components of the soil, plant uptake and soil mobility may increase. Limited concentrations of TNT and 4ADNT in leafy portions of the plant does not preclude accumulation in lipid-rich plant parts, e.g., seeds and tubers.

Since ^{14}C-labeled compounds were present in plants in quantities too low to be detected by GLC analysis, no conclusion can be drawn concerning degradation of treatment compounds within C. esculentus. However, in the soils TNT was degraded 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. Implications are that 4ADNT is more bioavailable in the soil than TNT, but both are mobilized into the plant to a limited extent.

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APPENDIX A: STANDARD CURVES

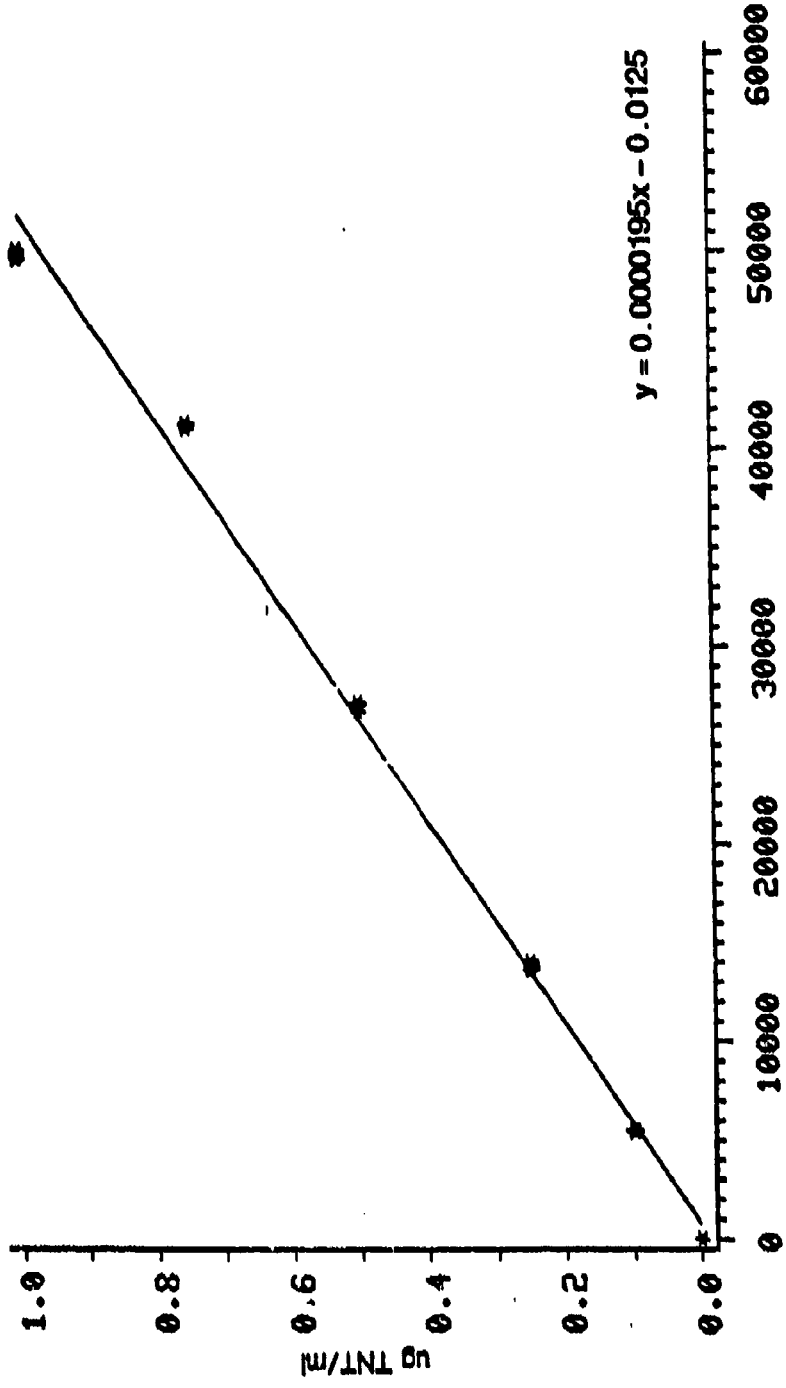

Figure A1. Standard curve for 1 ug TNT/ml treatment solution

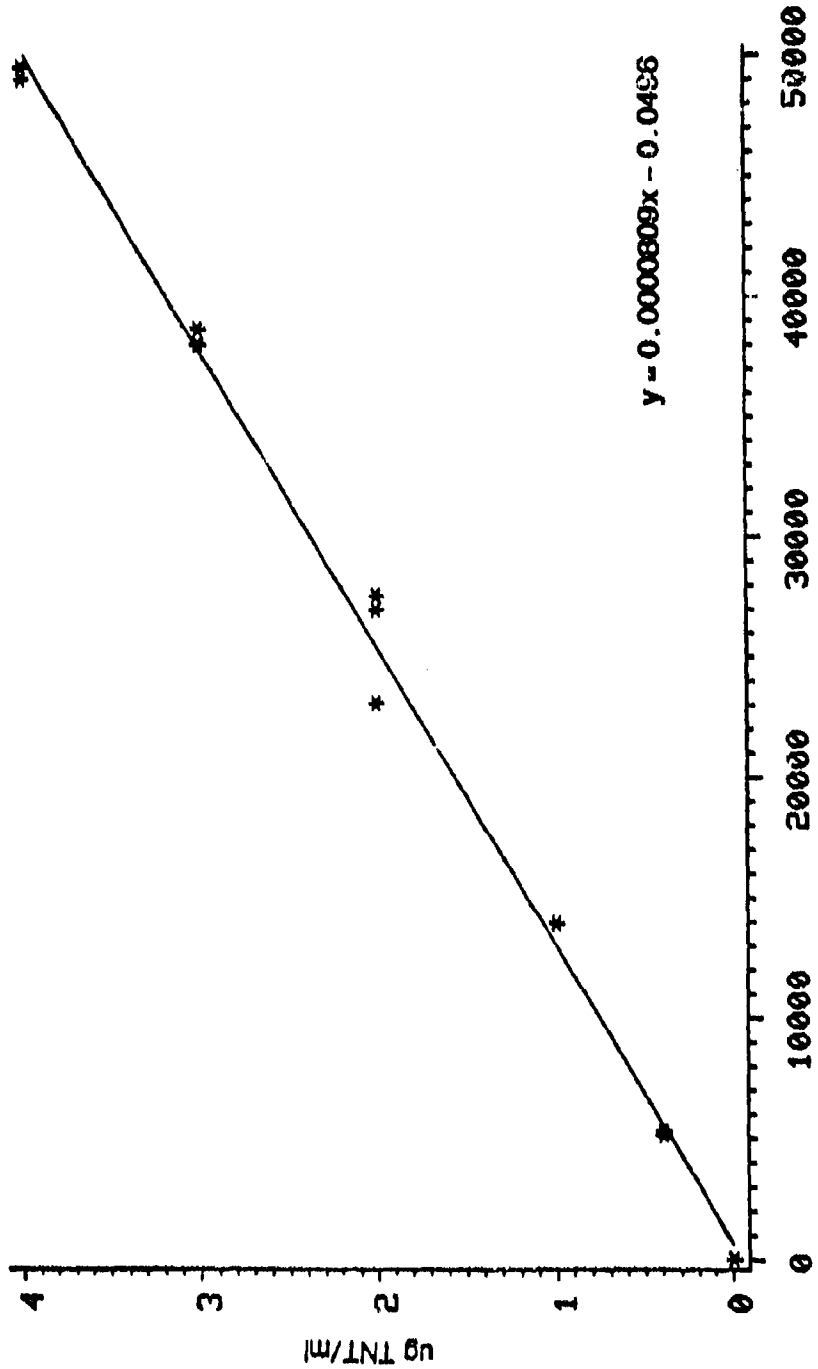

Figure A2. Standard curve for 4 ug TNT/ml treatment solution

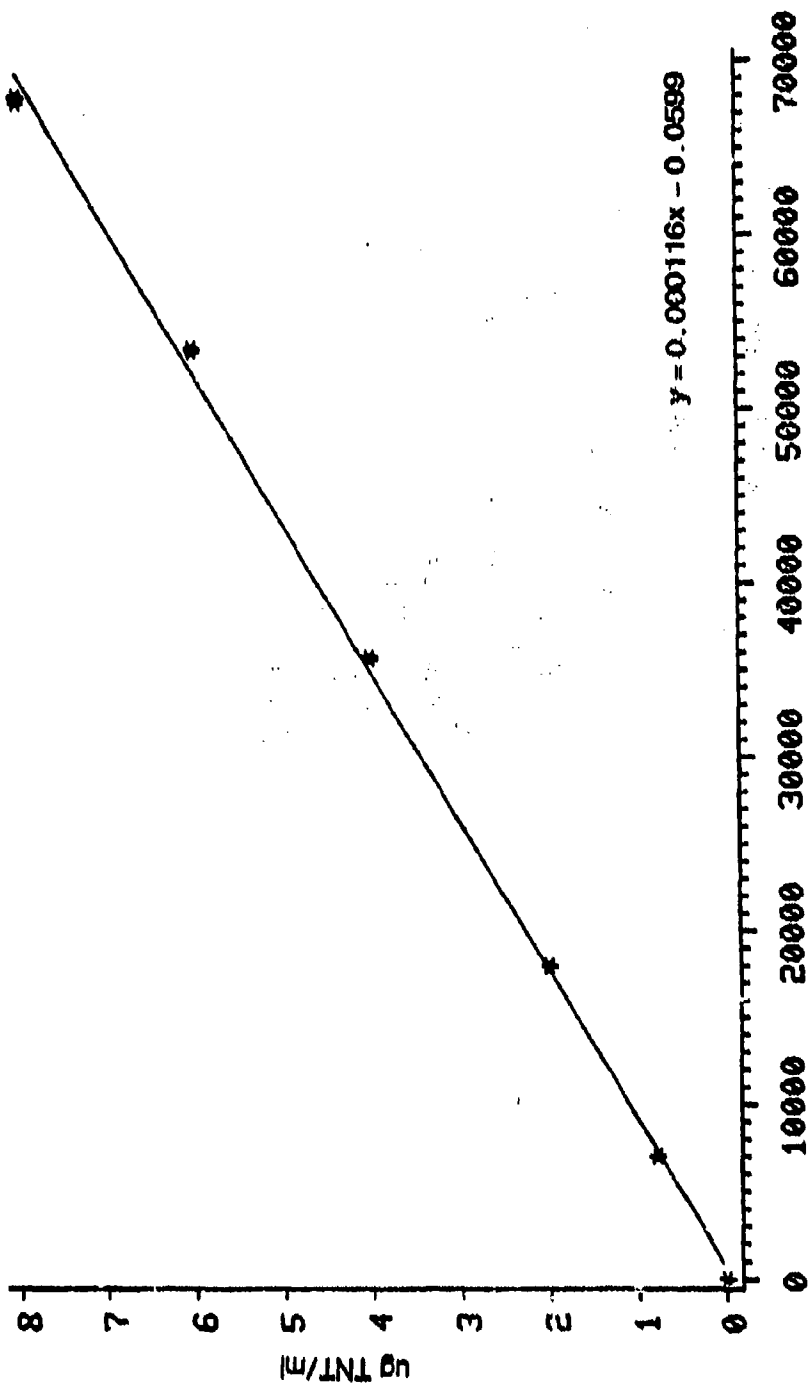

Figure A3. Standard curve for 8 ug TNT/ml treatment solution

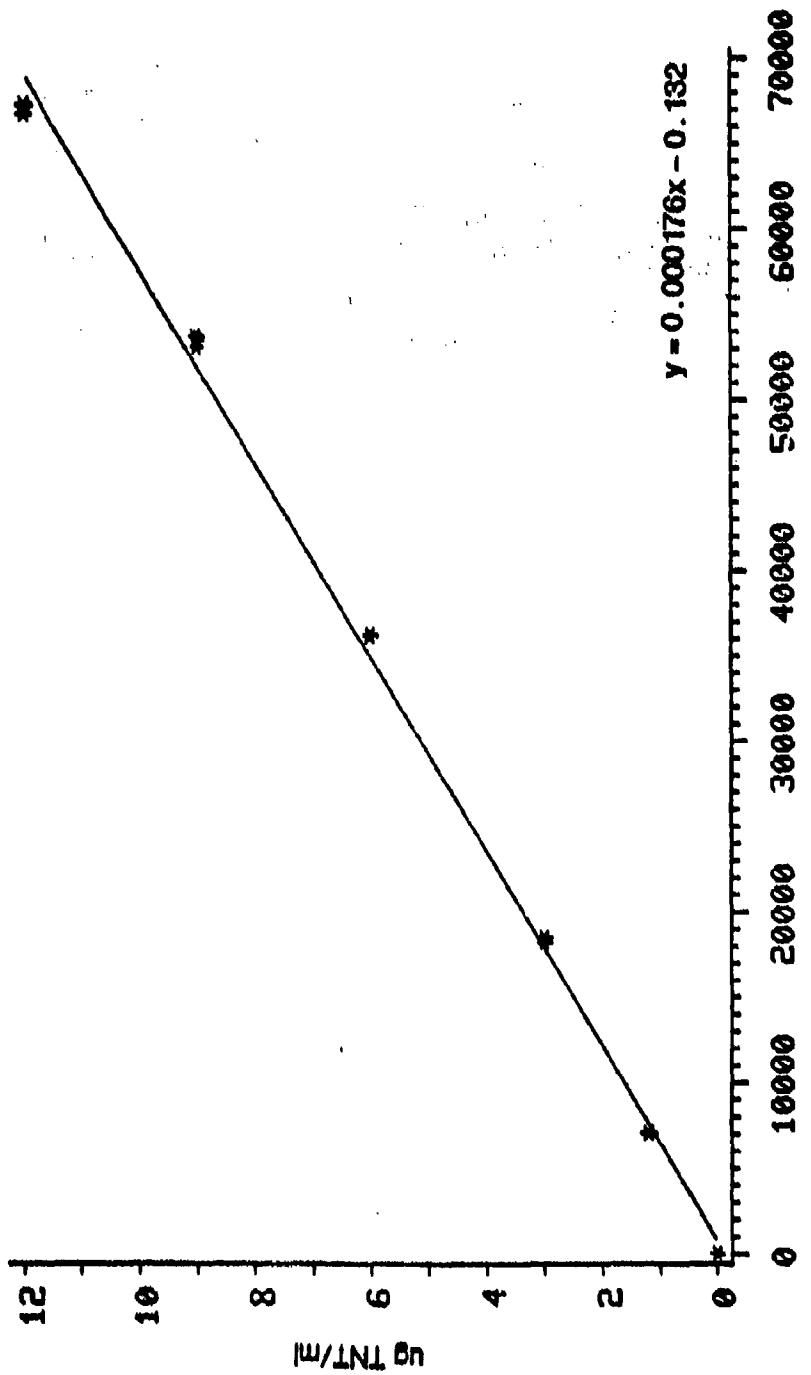

Figure A4. Standard curve for 12 ug TNT/ml treatment solution

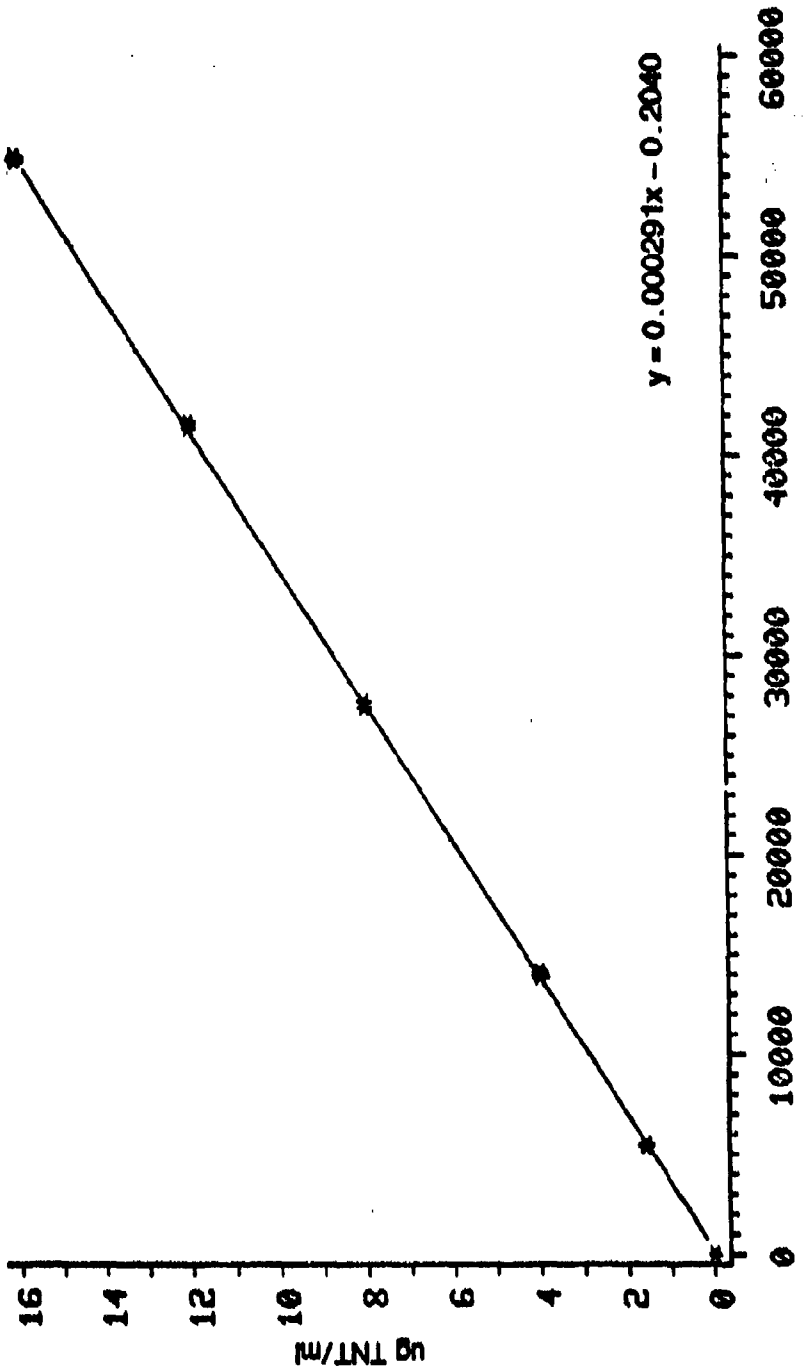

Figure A5. Standard curve for 16 ug TNT/ml treatment solution

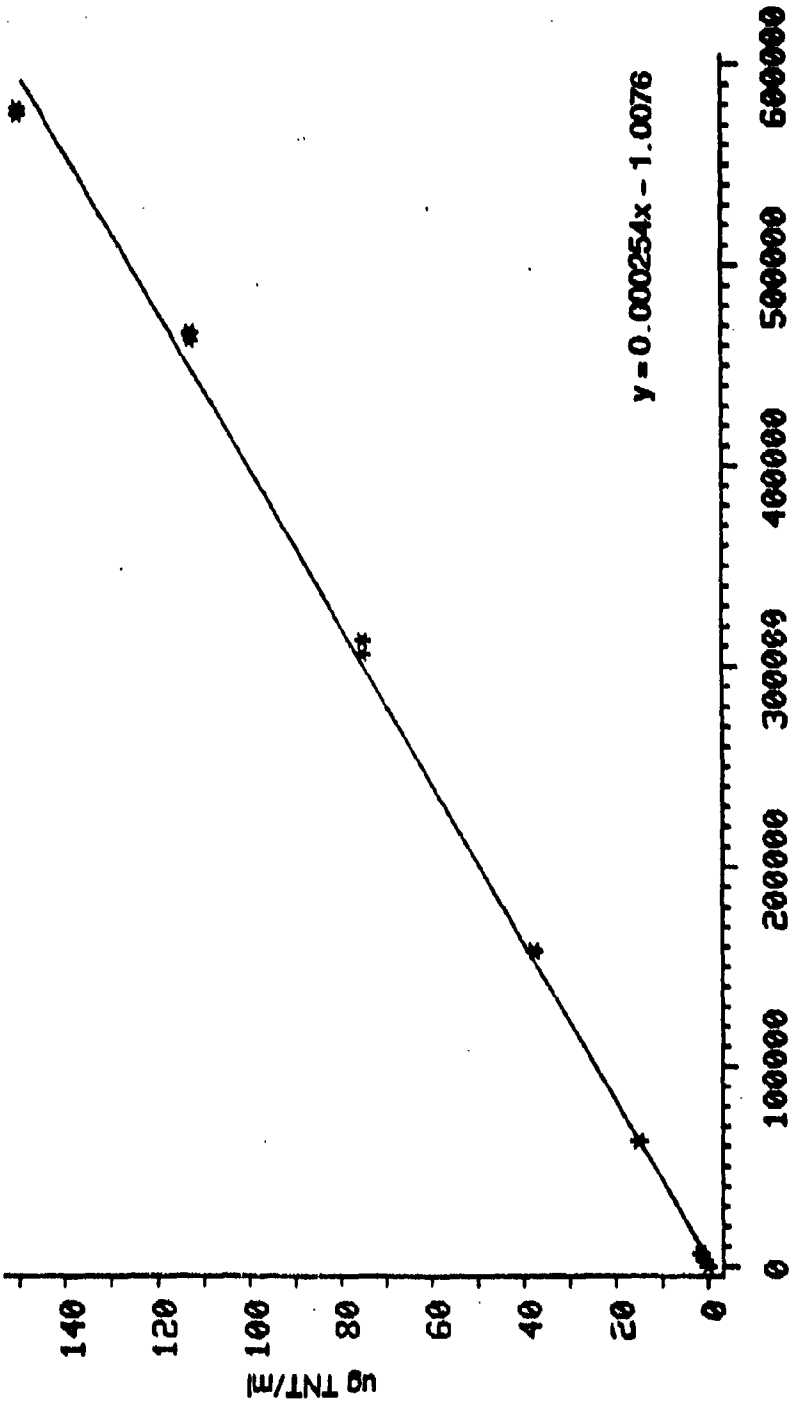

Figure A6. Standard curve for 150 ug TNT/ml treatment solution

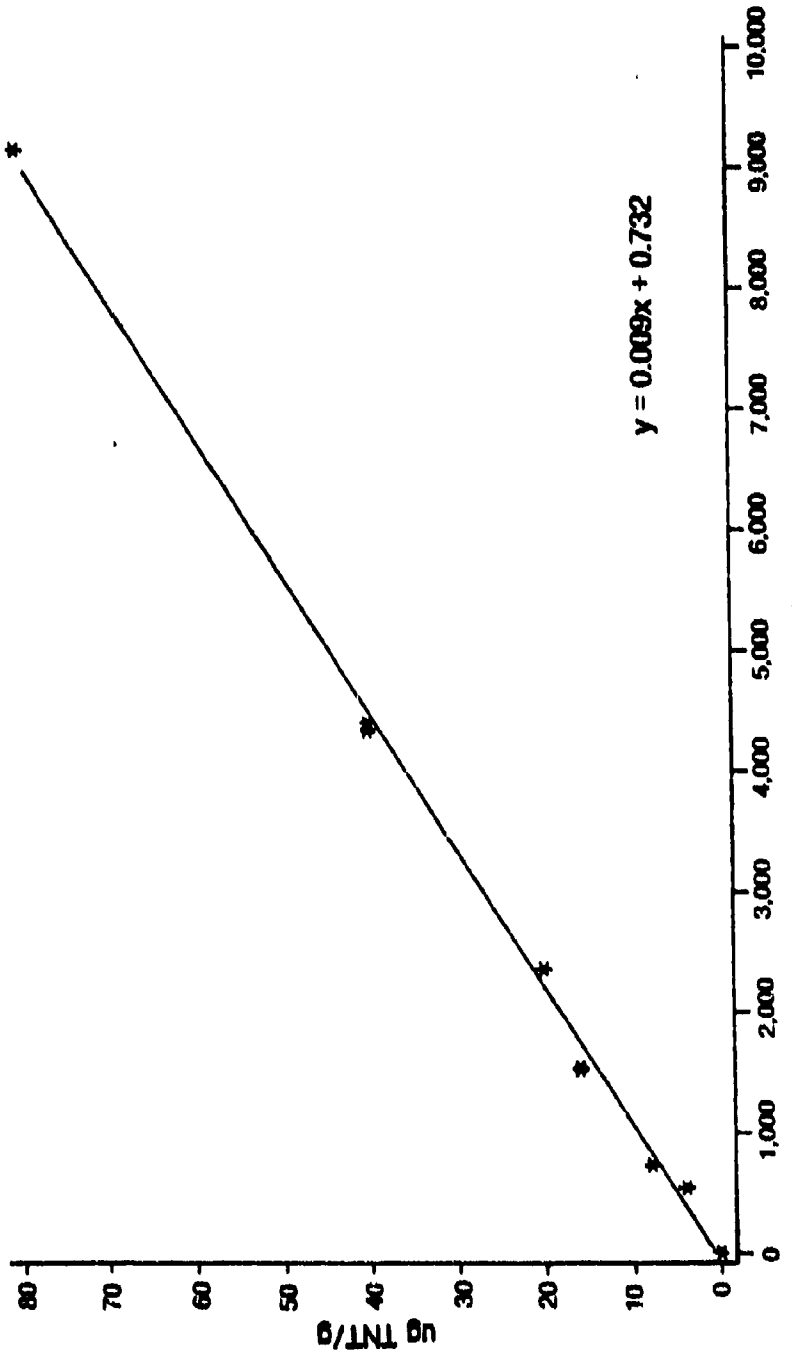

Figure A7. Standard curve for [^{14}C]TNT analysis of soil in plant uptake study

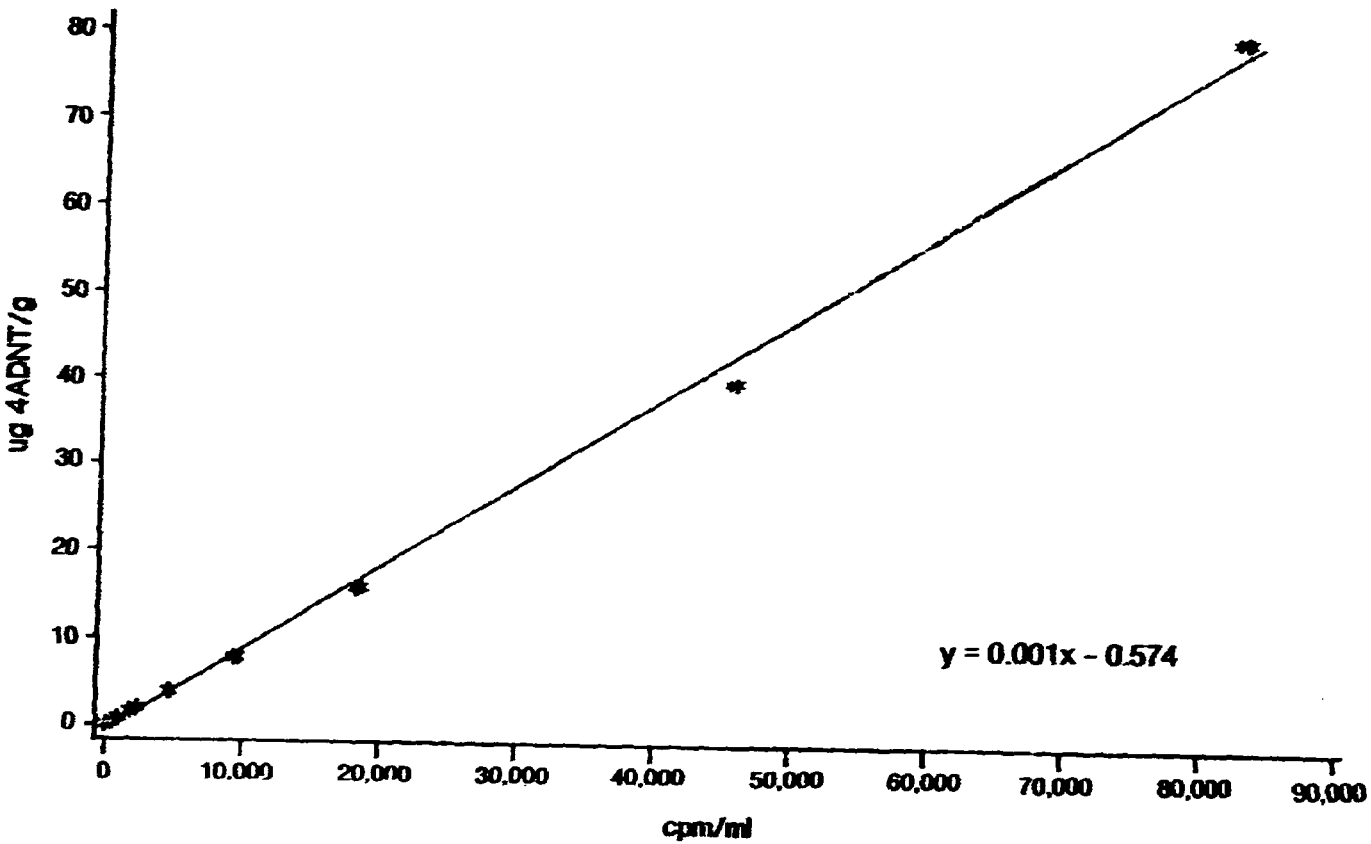

Figure A8. Standard curve for [^{14}C]4ADNT analysis of soil in plant uptake study

APPENDIX B: ISOTHERMS

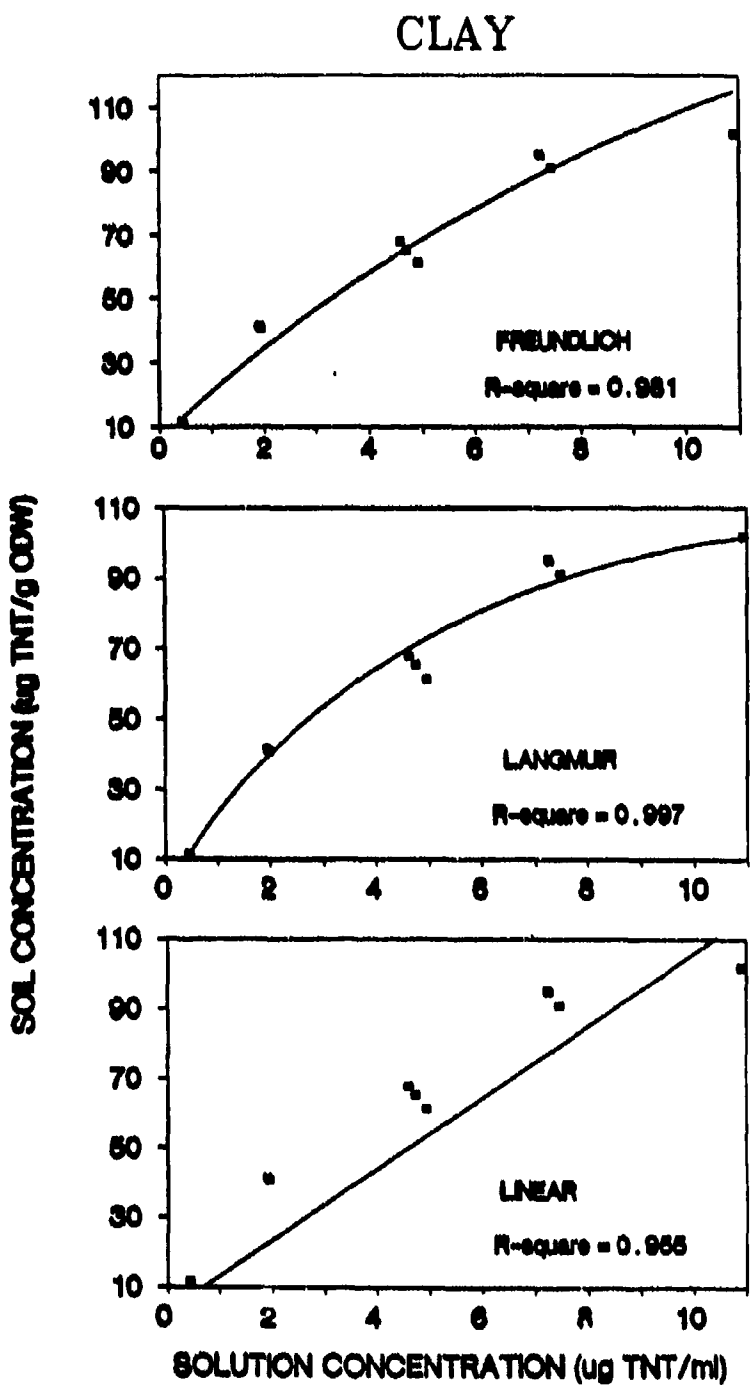

Figure B1. Clay soil data plotted with three isotherm models

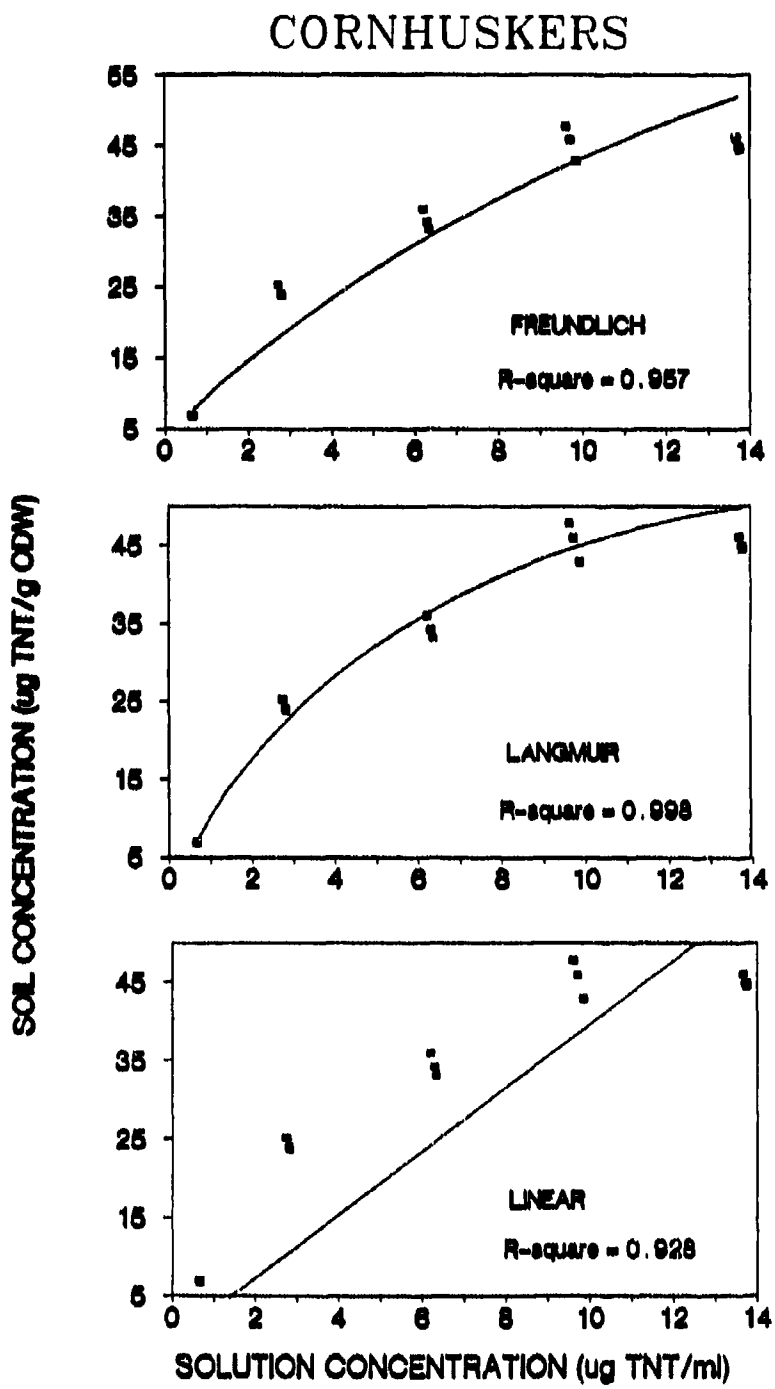

Figure B2. Cornhuskers AAP data plotted with three isotherm models

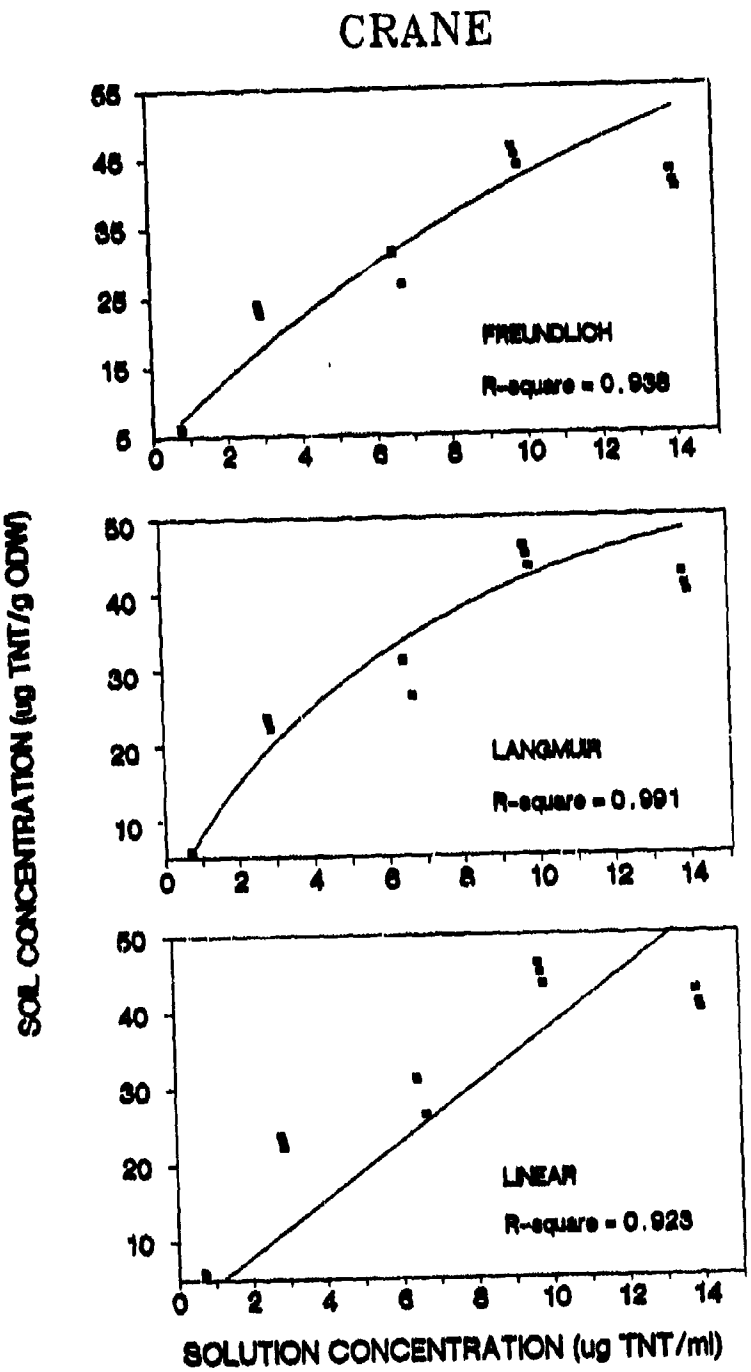

Figure B3. Crane AAP data plotted with three isotherm models

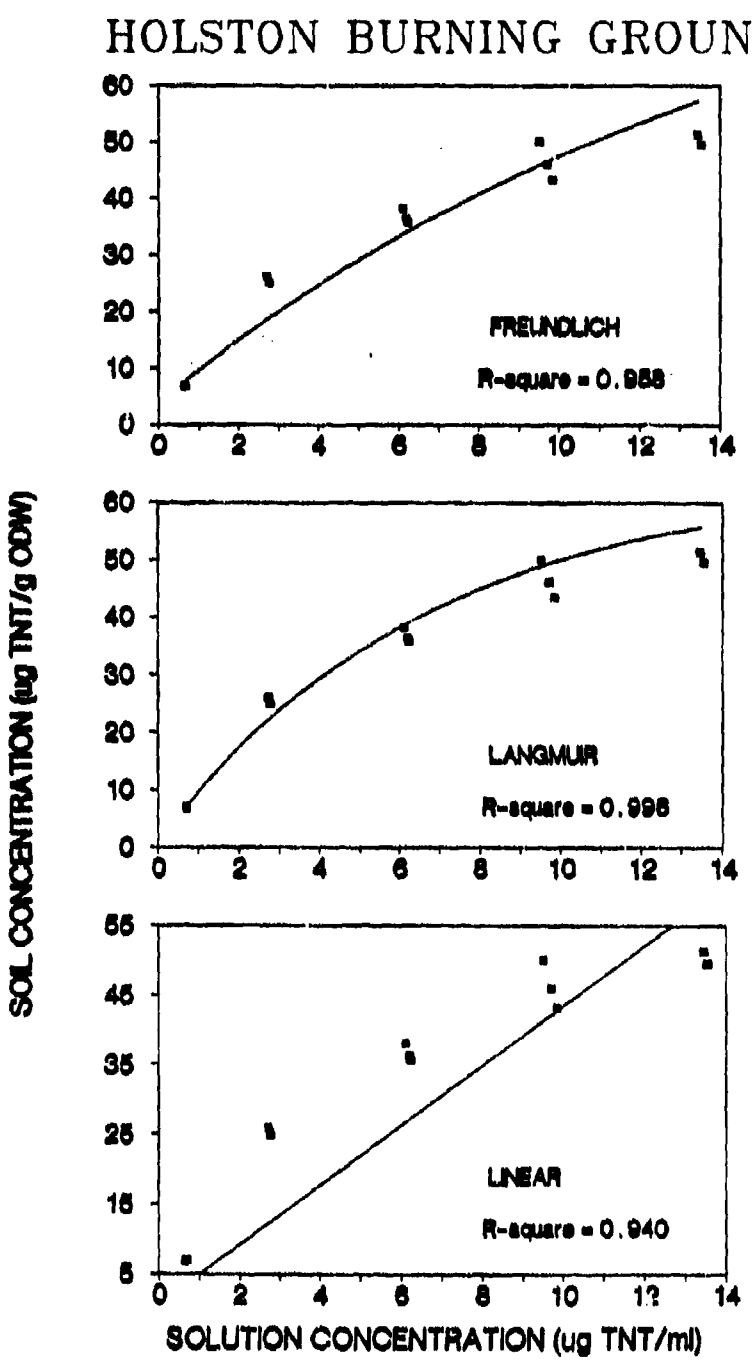

Figure B4. Holston burning ground AAP data plotted with three isotherm models

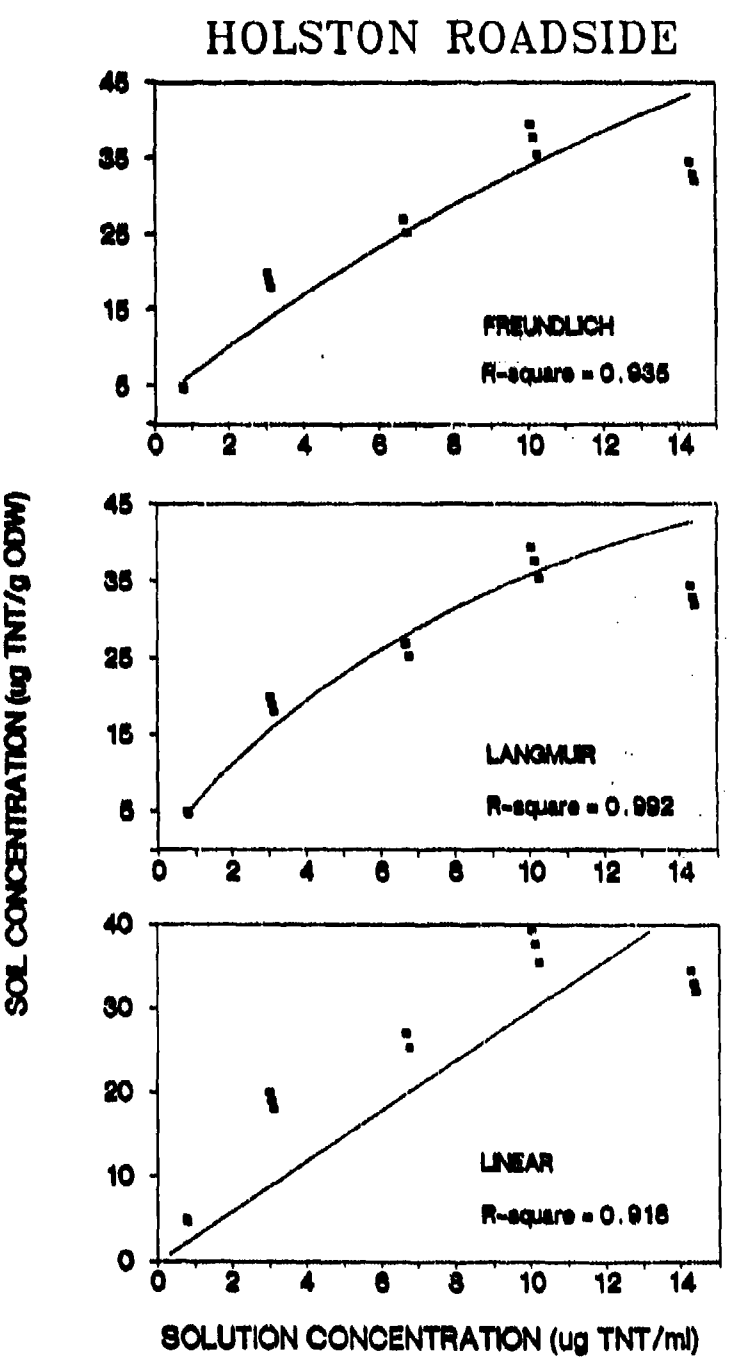

Figure B5. Holston roadside AAP data plotted with three isotherm models

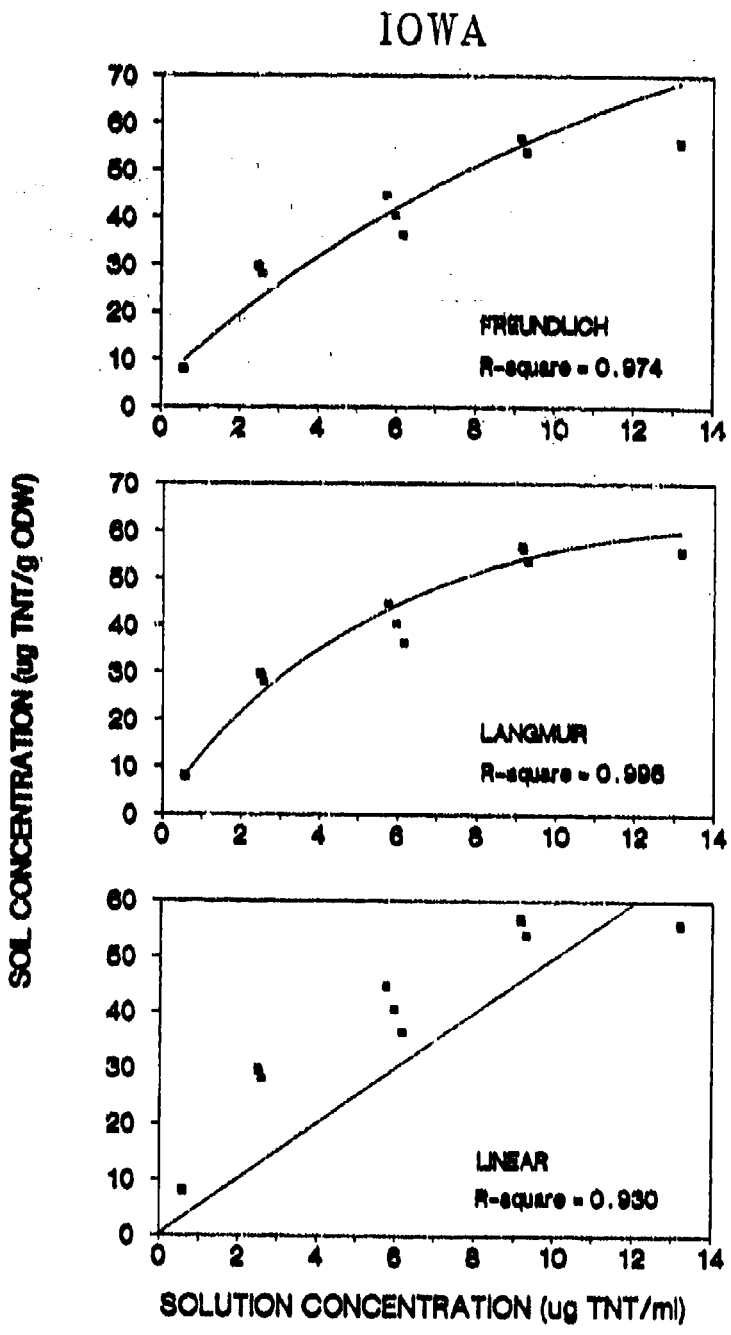

Figure B6. Iowa AAP data plotted with three isotherm models

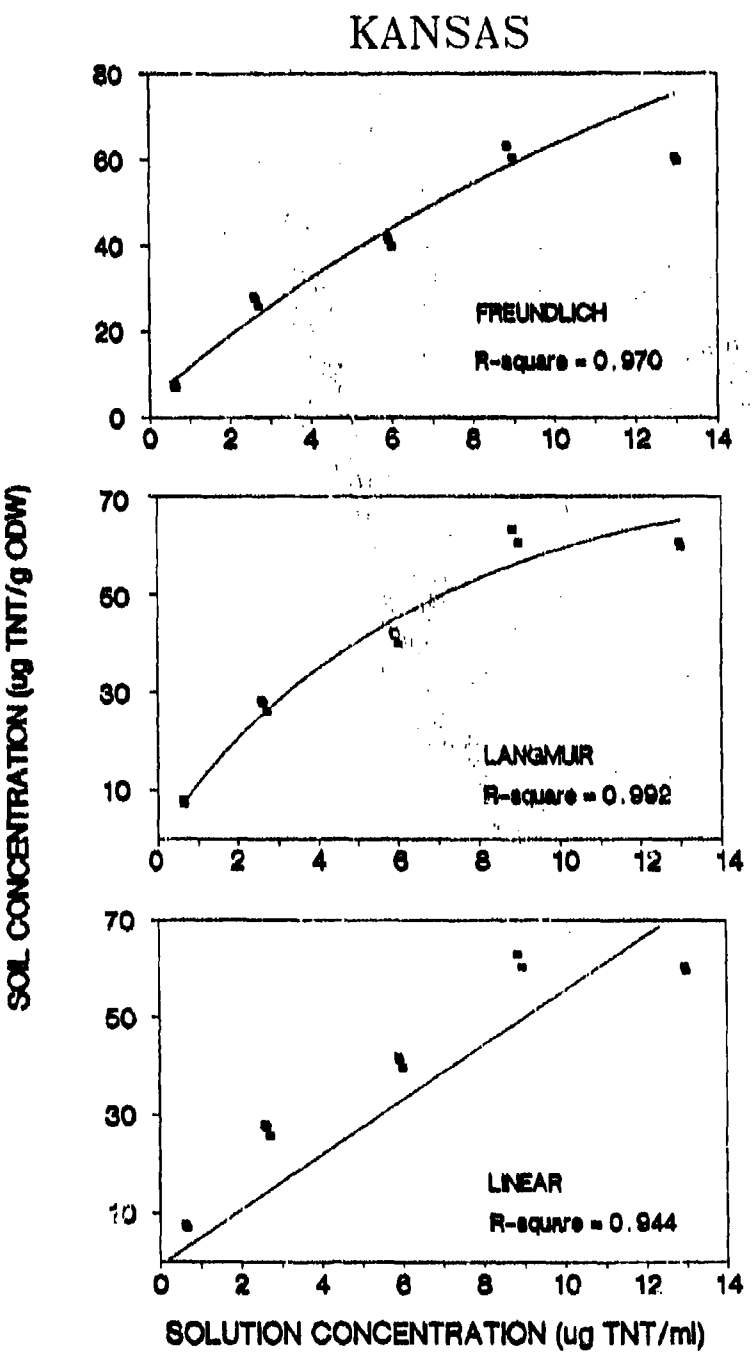

Figure B7. Kansas AAP data plotted with three isotherm models

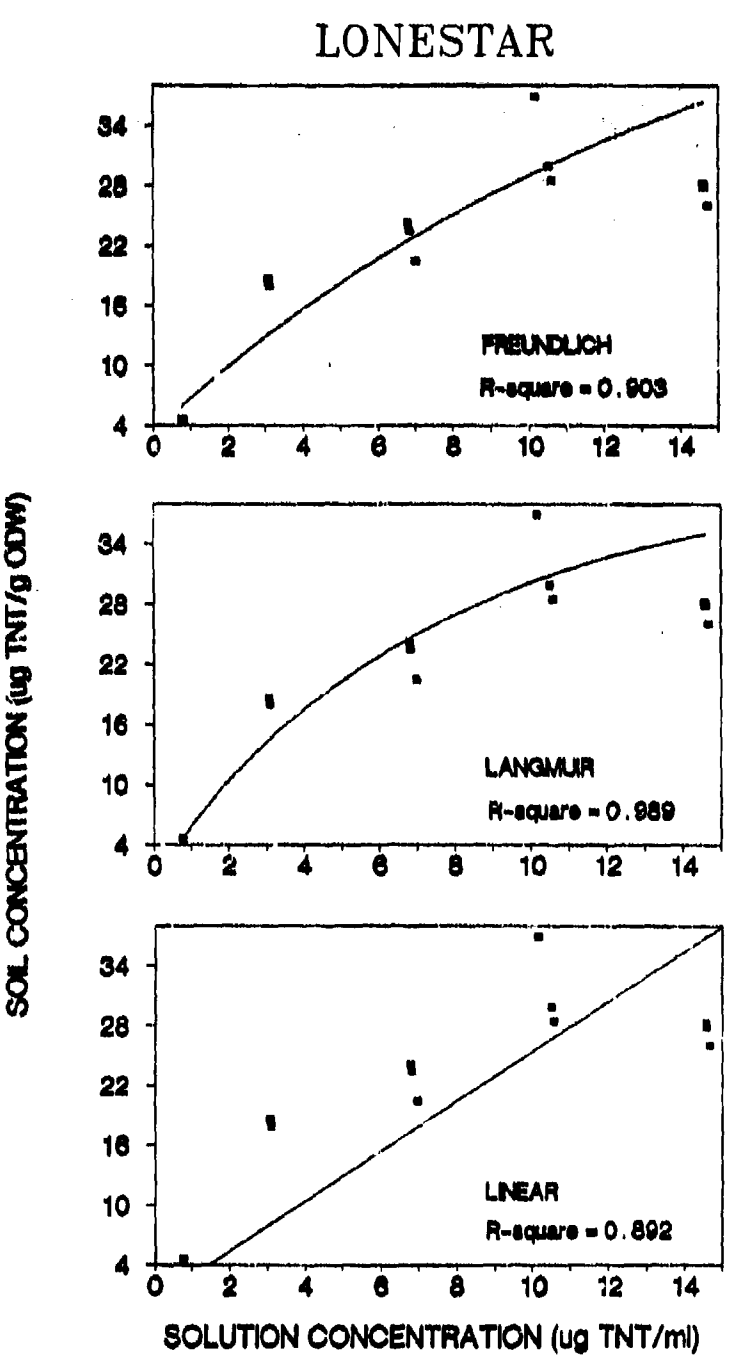

Figure B8. Lonestar AAP data plotted with three isotherm models

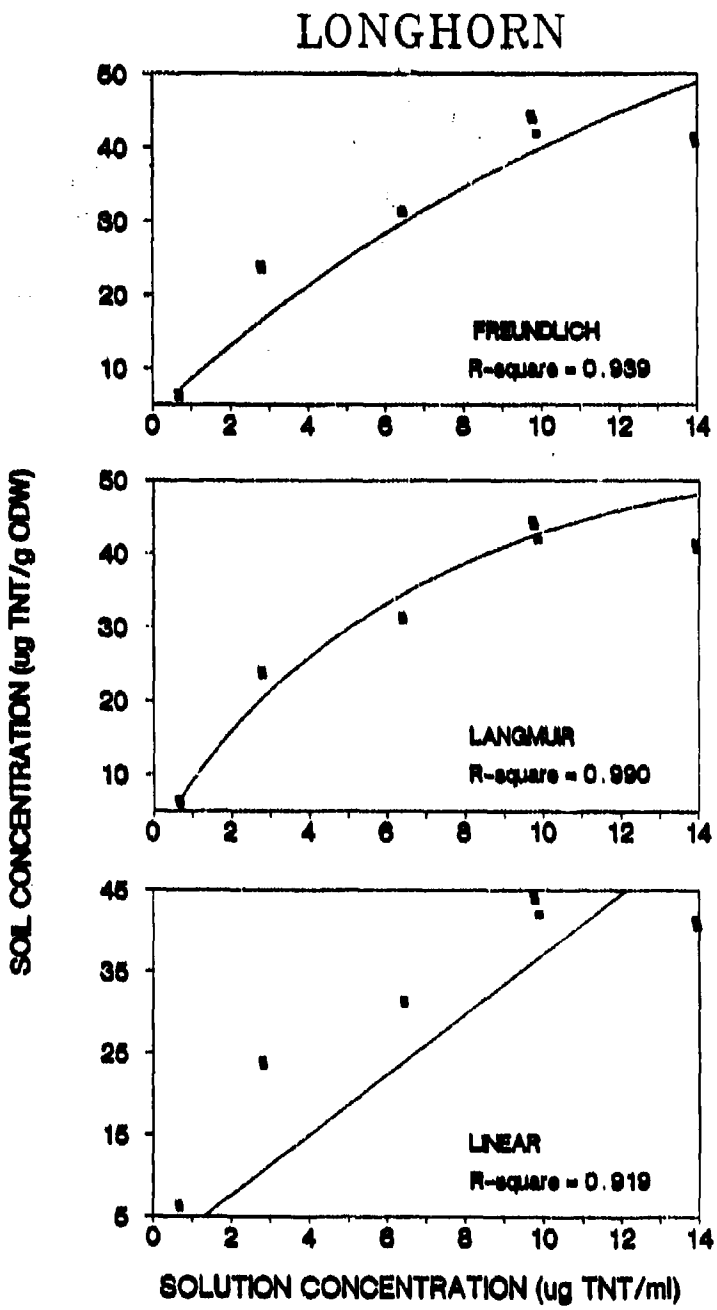

Figure B9. Longhorn AAP data plotted with three isotherm models

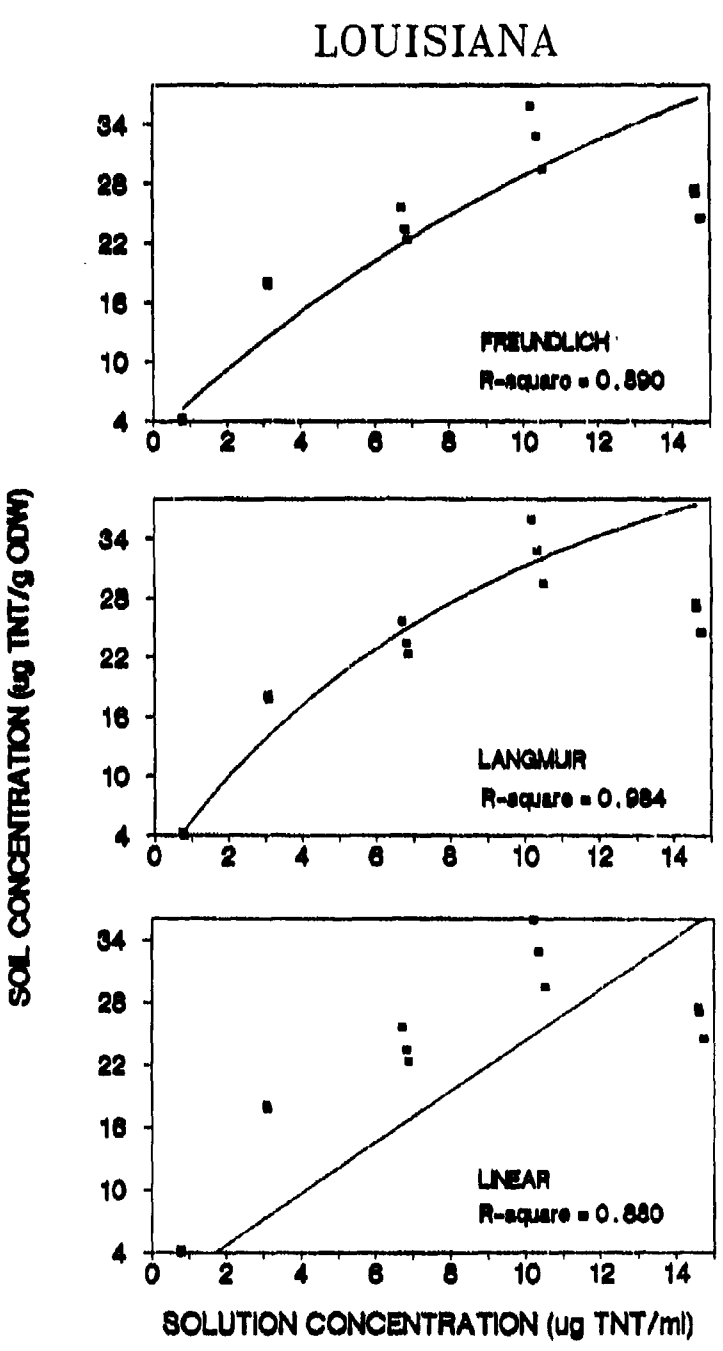

Figure B10. Louisiana AAP data plotted with three isotherm models

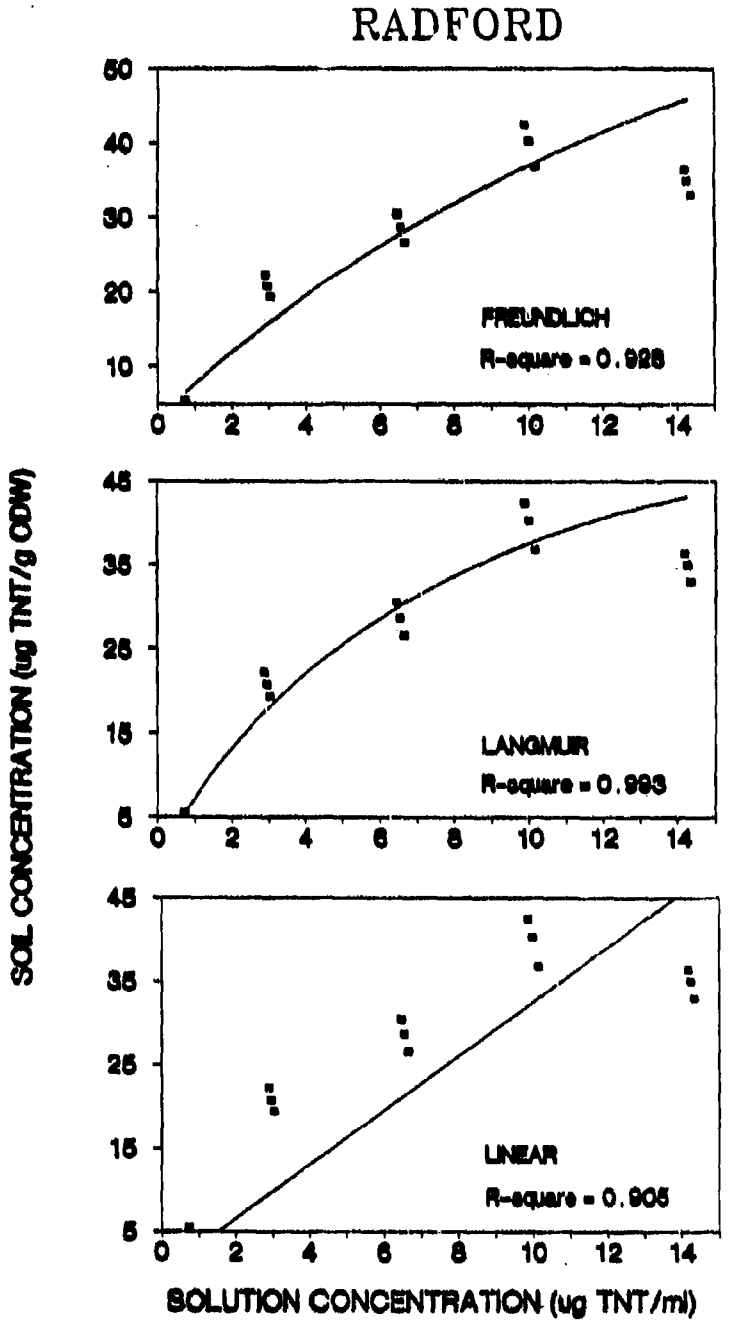

Figure B11. Radford AAP data plotted with three isotherm models

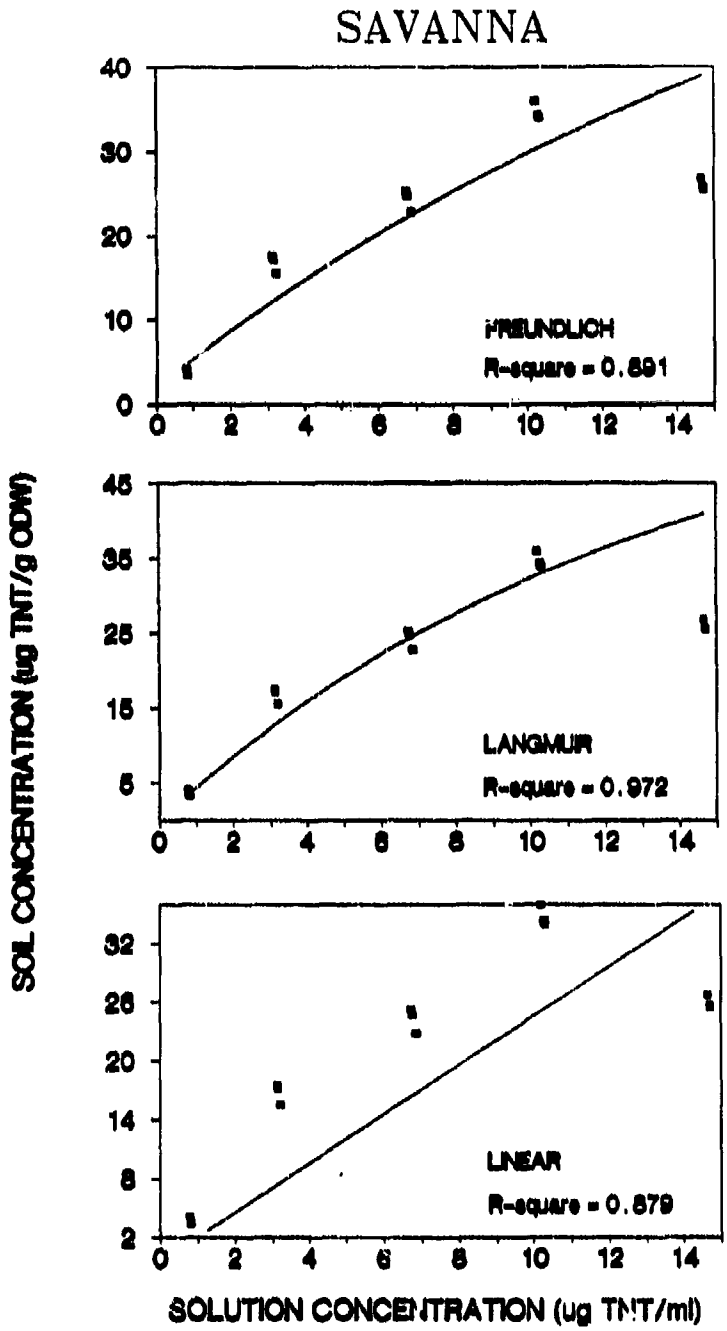

Figure B12. Savanna AAP data plotted with three isotherm models

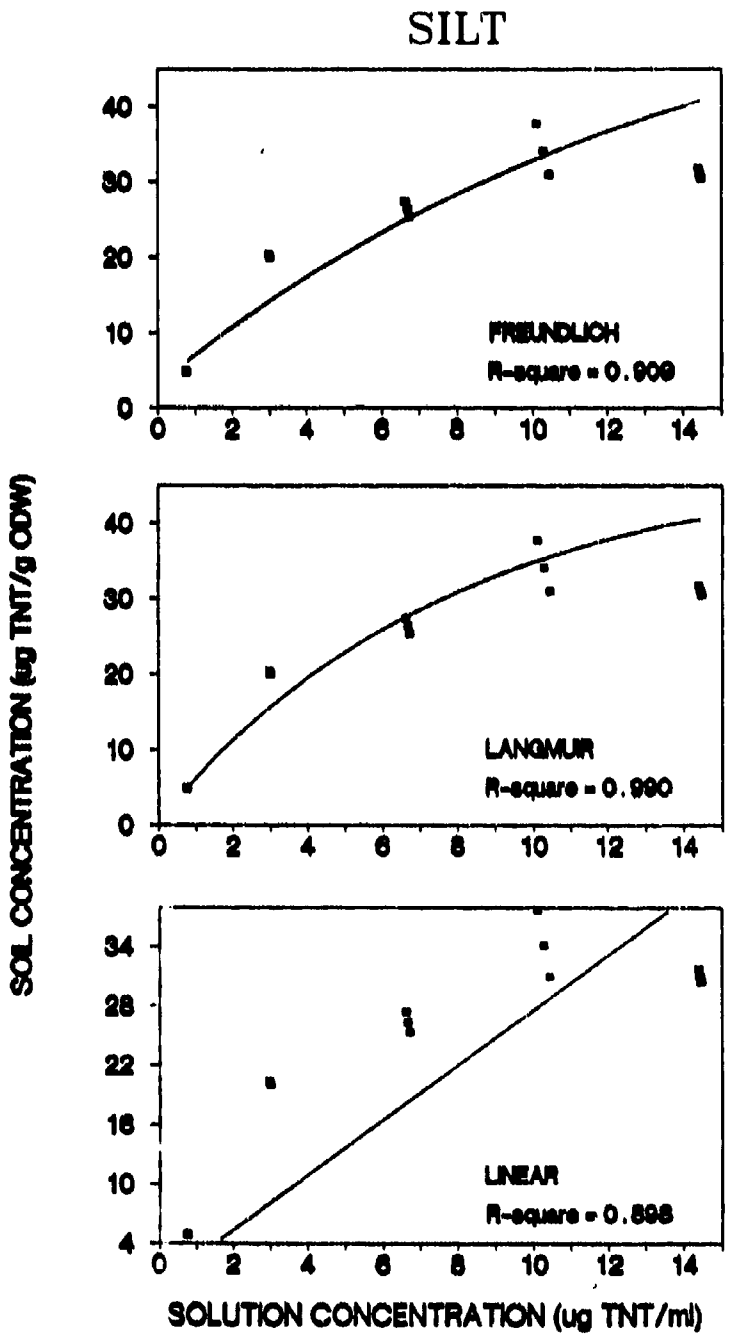

Figure B13. Silt AAP data plotted with three isotherm models

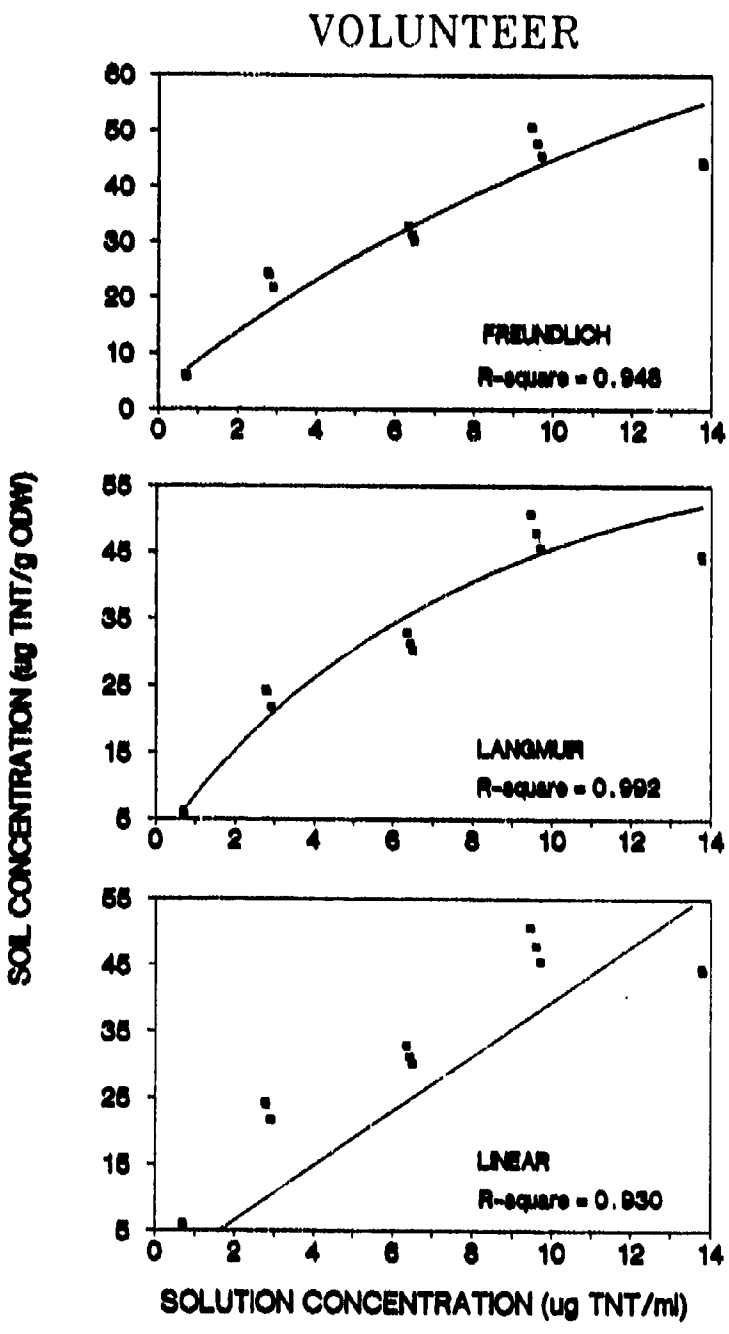

Figure B14. Volunteer AAP data plotted with three isotherm models