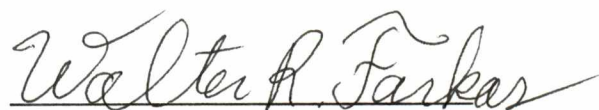


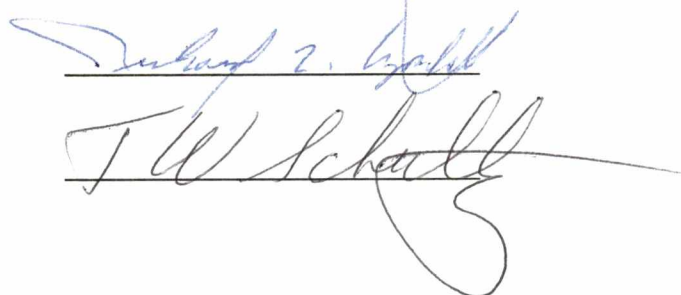
To the Graduate Council:

I am submitting herewith a thesis written by Kevin S. Ironside entitled "Reduction in Genotoxic Effects of Explosives-Contaminated Soil by Composting as Determined by the Salmonella Typhimurium Microsomal Mutagenicity Assay." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science with a major in Life Sciences.



Walter R. Farkas, Major Professor

We have read this thesis
and recommend its acceptance:



Accepted for the Council:



Associate Vice Chancellor
and Dean of the Graduate School

**REDUCTION IN GENOTOXIC EFFECTS OF EXPLOSIVES-CONTAMINATED
SOIL BY COMPOSTING AS DETERMINED BY THE
SALMONELLA TYPHIMURIUM MICROSOMAL MUTAGENICITY ASSAY**

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Kevin S. Ironside

May, 1994

DEDICATION

This thesis is dedicated to my wife
Pamela Lynn Ironside
whose love, support, patience, and prayers
enabled me to complete this endeavor.

ACKNOWLEDGEMENTS

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ABSTRACT

Composting of soils contaminated with the explosives 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetraazocine (HMX) was evaluated in a field study for its ability to detoxify and decontaminate the soil. Soil volumes of 7, 10, 20, 30, and 40% were used in separate compost piles to determine the loading efficiency of the compost. Organic extracts and synthetic precipitation leachates of the various compost piles were subjected to the *Salmonella typhimurium* microsomal mutagenicity assay (Ames test) to monitor reduction in mutagenicity as a result of composting. Composting effectively lowered the mutagenicity for the composts piles containing up to and including 30% soil. Results from the Ames test were compared to results obtained from chemical analysis and *Ceriodaphnia dubia* toxicity tests. The results correlated well as these tests also showed that 30% soil was the maximum loading volume to lower chemical concentration and leachate toxicity. However, an attempt to predict mutagenicity based on chemical concentration showed that the majority of mutagenic activity is unaccounted for by chemical concentration. A risk assessment of the final concentrations of explosives in the composts showed that TNT concentrations were effectively reduced in the composts containing up to and including 30% soil such that the soil could be returned to the environment under an industrial use scenario. However, the RDX concentrations for all but the 7% and 10% soil composts exceeded industrial use scenario risk numbers.

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CHAPTER 1

INTRODUCTION

STATEMENT OF PROBLEM

Soil, sediments, and groundwater have become contaminated with high explosives and related compounds as the result of manufacturing, loading, assembling, and packing of explosives by United States Army munitions facilities (Kaplan and Kaplan, 1982a). The Department of Defense has 26 explosive-contaminated sites on the Superfund list, with more than 1 million tons of soil requiring remediation (Rubin, 1992). The most extensively-used explosive has been 2,4,6-trinitrotoluene (TNT), often found in combination with hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetraazocine (HMX), which were used as propellants. The structures of TNT, RDX, and HMX can be seen in Figure 1.1. TNT is an environmental concern because of its toxicity as well as its explosive nature.

The Umatilla Army Depot Activity (UMDA) in Hermiston, Oregon, is one of the installations with large-scale explosives contamination of sediments and soils, for which it has been placed on the National Priorities List for environmental restoration.

At UMDA, aqueous effluents containing explosives were generally emptied into holding lagoons often called "pink lagoons" because of the color of the TNT-saturated water. Although these pink lagoons are no longer in use, the TNT-

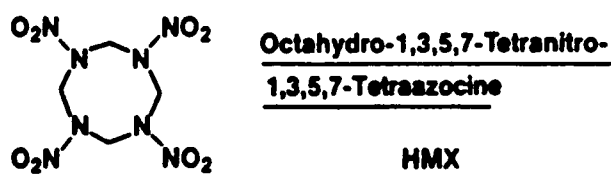
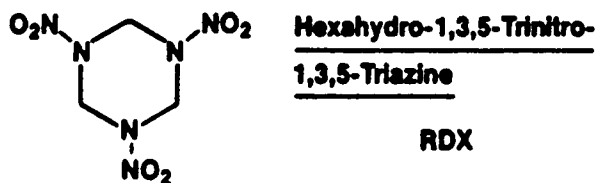
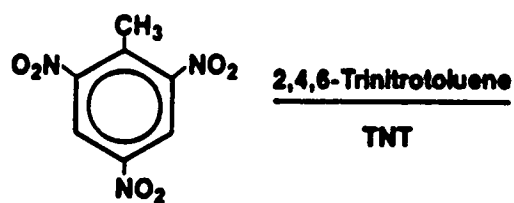


Figure 1.1. Structures of TNT, RDX, and HMX.

contaminated soils and sediments still remain and require remediation. Incineration has been used most often to remediate explosives-contaminated media; however, incineration is time consuming and costly. Because of its ease of implementation and relatively high benefit-to-cost ratio, a number of researchers are currently testing composting as a potentially attractive bioremediation alternative to incineration. UMDA was the site chosen for the second phase of a two-phase composting field study. The first phase of the project took place at the Louisiana Army Ammunition Plant (LAAP) (Griest, 1990).

The LAAP project evaluated the effectiveness of composting on explosives-contaminated lagoon sediments under mesophilic (35°C) and thermophilic (55°C) conditions. Chemical characterization by High Performance Liquid Chromatography (HPLC) was conducted on Toxicity Characterization Leaching Procedure (TCLP) and Synthetic Precipitation Leach Test (CCLT for Clean Closure Leach Test) leachates and organic solvent extracts of the composted material. Toxicity testing of leachates and extracts was conducted using *Ceriodaphnia dubia*, fathead minnow larvae (*Pimephalas promelas*), and the *Salmonella typhimurium* microsomal mutagenicity assay (Ames test). The soil loading volume for the compost piles was 3%, and the amendments consisted of horse manure, alfalfa hay, horse feed, and fertilizer and were chosen because of their availability from local sources. The duration of the composting was 153 days. Samples used for all of the tests were taken at the beginning and end of the 153-day period. The results of the study showed a marked decrease in TNT concentration and in toxicological response, with the thermophilic pile performing slightly better than the mesophilic pile. However, Phase I was originally designed as an engineering study, and the toxicological study

was included as an afterthought. Hence, the toxicological study did not include proper controls (such as uncomposted soil or an uncontaminated soil compost pile) and was performed on samples that had been stored for approximately 10 months. Phase II of the study (Griest, 1991) was designed to incorporate proper controls into the toxicological study and to further expand upon the knowledge gained during Phase I.

PURPOSE OF STUDY

The work performed for this thesis was done as part of the Phase II project, which was a broad-based collaborative study for the United States Army Medical Research and Development Command. This thesis is an evaluation of the reduction in genotoxic effects of explosives-contaminated soils by composting as determined by the Ames test. The collaborative work included analytical chemistry and *Ceriodaphnia dubia* testing (Griest, 1991), and the results of the forementioned were included in this thesis for comparative purposes. A risk assessment of the explosives-contaminated soil has also been included in this thesis, which was performed separately from the Phase II project.

The purposes of the Phase II project were to (1) evaluate the effectiveness of composting in the remediation of explosives-contaminated soils and sediments, and (2) evaluate the loading capacity of the selected compost mixture and optimize the process to bioremediate the greatest quantity of soil possible per unit time.

Determining the effectiveness of composting by measuring the reduction in genotoxic effects was necessary to both demonstrate the actual degradation and/or transformation of TNT and to determine whether breakdown produced

transformation products that could be as toxic, only slightly less toxic, or perhaps more toxic than the starting material. Comparison of the Ames test results with the results of high performance liquid chromatography (HPLC) analysis and *Ceriodaphnia dubia* toxicity testing provided a mode by which the effectiveness of composting could be further verified.

The Ames test was used to determine the toxicity of the acetonitrile extracts and the CCLT leachates as a measure of mutagenicity. HPLC was used to measure the concentration of explosives in the compost mixture by analysis of CCLT leachates and organic solvent (acetonitrile) extracts. The *Ceriodaphnia dubia* fecundity test was used to measure the toxicity of the CCLT leachates.

The Ames tests results were compared with HPLC results to determine if measurable toxicity decreased with time in relation to decreases in the explosives concentrations. Comparison of Ames test results with *Ceriodaphnia dubia* results was used to determine if there was a correlation between different toxicity tests.

Evaluation of the loading capacity of the compost mixture was accomplished by using different amounts of contaminated soils and sediments [hereafter referred to as soil(s)] in the various static compost piles.

Additionally, the study also allowed the evaluation of the composting project under different climatic conditions and with a different set of amendments. Preliminary studies determined the optimal composting amendment mixture which consisted of 40% saw dust, 15% apple pumice, 20% chicken manure, and 25% chopped potato waste. The loading percentages of soil used were 7, 10, 20, 30, and 40% by volume. The amendments were held constant throughout the project. The positive control consisted of 100% contaminated soil (i.e., containing no

amendments), uncomposted. The negative control was a compost mixture with 10% uncontaminated soil. The various compost piles were evaluated over a 90-day composting period, with each of the piles sampled at two or more time points.

LITERATURE REVIEW

Toxicity

The toxicological properties of TNT have been studied since the early 1970s. TNT has been reported to be toxic to bluegill fish and fathead minnows at concentrations of 2 to 3 mg/l (Nay, 1974). It has also been found to be highly toxic to aquatic forms including freshwater unicellular green algae, tidepool copepods, and oyster larvae, at concentrations of 5 mg/l and less (although metabolites of TNT were not toxic at concentrations reaching 100 mg/l) (Won, 1976).

TNT inhibits the growth of many fungi, yeasts, actinomycetes, and gram-positive bacteria (Klausmier, 1973). In addition, it is mutagenic to *Salmonella typhimurium* in the Ames test (Dilley, 1979) (Kaplan and Kaplan, 1982b).

Acute oral toxicity, primary skin irritation, and dermal sensitization have been observed in rats and mice exposed to TNT. Skin contact with absorption of explosives can result in simple discoloration of the skin, dermatitis, headaches and poisoning in humans (Bongiovanni, 1984). Exposure of humans and other mammals to TNT is known to cause pancytopenia, a disorder of the blood-forming tissues characterized by a pronounced decrease in the number of leukocytes, erythrocytes, and reticulocytes (Harris, 1970). Liver damage and anemia have been

reported in workers engaged in large-scale manufacturing and handling practices (Sax, 1963). Inhalation of the dusts or vapors of TNT can be fatal (Bridge, 1942). EPA has classified TNT as a Group C, possible human carcinogen (EPA, 1991c).

The toxic effects of RDX and HMX are less well studied than for TNT.

Acute human exposure to RDX produces dermatitis, nausea, and vomiting.

Convulsions, insomnia, amnesia, and renal damage are chronic effects associated with RDX. In mammalian studies, acute exposure to RDX causes anemia, decreased food intake, dermatitis, and death. Mammalian chronic exposure results in testicular atrophy, prostate inflammation, and liver and kidney toxicity. RDX has been classified as a Group C carcinogen. Research is limited as to the effects of human exposure to HMX, but has been reported to cause skin irritation during patch testing. Mammalian testing of HMX causes convulsions, and liver and kidney damage (EPA, 1991c).

Bioremediation Studies

Biotransformation of TNT by a microbial community has been demonstrated in a laboratory culture by Won and McCormick; in activated sludge by Carpenter; in compost by Doyle, by Isbister, and by Kaplan and Kaplan; and in aqueous culture by Spanggord. Except in the investigations by Spanggord and Won, most of this biotransformation appeared to occur through the reductive pathway of TNT in which the nitro group is first reduced to an hydroxamate then to an amino group. The nitroamine is converted to the hydroxyl compounds that are presumably activated for the subsequent ring cleavage (McCormick, 1976).

In culture

Unkefer, et al. (1989) were the first to report complete bacterial degradation of TNT to CO_2 in solution. They accomplished this using cultures derived from soils and sediments contaminated with TNT for 30 years. These cultures were comprised of several species of bacteria and were grown initially with succinate and TNT as carbon sources. In the later stages of culture growth, after the succinate was exhausted, TNT served as the sole carbon source. Pure bacterial cultures were also isolated that had the ability to use TNT as their sole source of carbon, although better degradation rates were obtained with consortia. Eighty to 90% degradation was obtained in 8 days at room temperature as determined by measuring radiolabelled CO_2 . Interestingly, these experiments were conducted using 0.22% TNT, which greatly exceeds the solubility of TNT in water (which is approximately 100 mg/l). Under these conditions, the TNT was degraded as it slowly went into solution.

Traxler et al. (1975) reported that several gram-negative bacteria isolated by enrichment culturing from various sources were capable of using TNT as a sole carbon source. They also discovered that the addition of yeast extract enhanced this process, implying that cometabolism was occurring. Sixty-two percent of the TNT was removed in 20 hours from a yeast extract-supplemented medium. When ^{14}C -TNT studies were performed, the incorporation of TNT into the cellular material of two isolates was detected. Small amounts of radiolabelled CO_2 were also detected. This indicates ring cleavage, however, ring cleavage probably accounted for only a small percentage of the decrease in TNT concentration. The rapid removal of TNT from the yeast-enriched cultures may have been due to association

of TNT with the bacterial cell walls, which would explain the detection of radioactivity in the cell mass. In a separate experiment, Traxler found that washed cells effectively removed TNT from their medium with or without organic nutrients. Ninety-four percent of the TNT was removed by a cell mass of 14 mg/ml in 18 hours.

A study by Naumova et al. (1979) reported the transformation of TNT concentrations as high as 200 mg/l to reduced metabolites within four days by a *Pseudomonas denitrificans* strain isolated from soil contaminated with industrial waste.

McCormick et al. (1976) showed that the nitro groups on TNT were reduced by both aerobic and anaerobic microbial enzyme systems. Depending on the reducing potential of the system, one, two, or three of the nitro groups were reduced to amino groups. However, there was no evidence of cleavage of the aromatic ring.

Jerger et al. (1976) conducted laboratory biodegradation studies with benthic microorganisms. His laboratory found that transformation of TNT was rapid, with acclimated and unacclimated populations transforming 70 to 90% of the 10 and 100 mg/l concentrations within 3 to 5 days. Anaerobic degradation also took place, with acclimated organisms tolerating greater than 200 mg/l TNT concentrations. In this microbial population, transformation appeared to require an additional carbon source.

Funk et al. (1993) examined the bioremediation of soils contaminated with 120 ppm of TNT, 30 ppm of RDX, and 3 ppm of HMX by a procedure that produced anaerobic conditions in the soils. Removal of parent TNT molecules from

the soil cultures by the strictly anaerobic microflora occurred within 4 days. HMX, RDX, and TNT metabolites formed by transformation were removed from the cultures within 24 days.

Activated sludge

Carpenter et al. (1978) investigated the fate of ^{14}C -labeled TNT in an activated sludge system. No significant $^{14}\text{C}\text{-CO}_2$ was formed despite the absence of $^{14}\text{C}\text{-TNT}$ in the aerator reactor after 3 to 5 days. The radioactivity was found as ^{14}C -labeled transformation products that were evenly divided between the floc and the supernatant. Evidence indicated that TNT transformation products formed polymeric conjugates with lipids, fatty acids, and proteins. These conjugates were resistant to further biodegradation. No evidence was found to indicate that the TNT aromatic nucleus was cleaved or ultimately degraded.

Aquatic Systems

Spanggord et al. (1985) studied the major transformation and transport processes affecting the environmental fate of TNT in aquatic systems. A variety of microbes possessed a nitroreductase enzyme system that enabled them to reduce the nitro group of TNT to an amino group in rich media, but not when TNT was the sole carbon and energy source. Without additional nutrients, only very slow TNT transformation occurred under either aerobic or anaerobic conditions in populations greater than 10^9 cells/ml. Radiolabelled TNT studies verified that the aromatic nucleus was not cleaved. Biotransformation of TNT in natural waters was found to be 1,000-fold slower than phototransformation.

Waste Lagoons

Spanggord (1983) examined the persistence of TNT in waste disposal lagoons at the Louisiana Army Ammunition Plant. The half-life of TNT resulting from biotransformation was calculated to be 69 days. In experiments using lagoon water, the rate constant for TNT biotransformation at 50 mg/l TNT was only one-third of that observed at 4.2 mg/l.

Kaplan et al. (1982) also studied the influence of TNT concentration, as well as other environmental factors, on biotransformation of TNT. TNT transformation in soil proceeded at the highest rate at 0.1% TNT and was progressively lower at 1.0% and 10.0%. Their results indicated that high concentrations of TNT, low microbial populations, lower temperatures, and dry soil increased the period of time needed to transform the TNT.

Fungal Degradation

Fernando et al. (1990) reported the complete degradation of TNT by the white rot fungus *Phanerochaete chrysosporium*. Soil and water were dosed with TNT at 10,000 mg/kg and 100 mg/l, respectively. Corncobs were used as a supplemental carbon source. Nineteen percent of the initial radiolabelled TNT was converted to $^{14}\text{CO}_2$ in 90 days.

Parrish (1977) found that 183 of the 190 fungi he tested were able to transform TNT in a basal salts medium containing glucose and 100 mg/l TNT. However, only the nitro group in the four position was reduced, and there was no evidence of ring cleavage when radiolabelled TNT was used.

Composting

Klausmeier et al. (1982) examined the composting of TNT on a laboratory scale. During a 74-day composting cycle, the TNT concentration in their composts decreased from 1% to 0.002%.

Kaplan and Kaplan (1982c) emphasized that the transformation pathways of TNT in ambient environments are the same as in thermophilic biotransformation of ^{14}C -TNT under composting conditions (55°C). TNT was found to be transformed under thermophilic conditions, but no $^{14}\text{CO}_2$ was produced. The transformation products appeared to bind to the organic matter fraction, with a progressively greater percentage of the ^{14}C becoming bound or conjugated to the organic matter fractions as compost age and stability increased.

Isbister et al. (1982) studied the degradation of ^{14}C -TNT in laboratory-scale compost systems incubated at 55°C and continuously aerated. The initial concentration of TNT was 1.0%. Solvent extracts were analyzed after 3 and 6 weeks of composting. The TNT concentration decreased by 50% after 3 weeks and 82.6% after 6 weeks. The decrease in extractable TNT was paralleled by a decrease in solvent-extractable ^{14}C and an increase in the residual ^{14}C activity from 6.1% at 0 weeks to 66.5% at 6 weeks. No TNT reduction products were found after 3 weeks, but one of the three replicate composts did contain approximately 1% of the ^{14}C activity as 4-amino-2,6-dinitrotoluene and 2-amino-4,6-dinitrotoluene after 6 weeks. In all test systems, cleavage of the TNT aromatic ring did not appear to take place. Recovery of ^{14}C as CO_2 was less than 1.0%.

The same group also conducted greenhouse-scale composting studies. TNT was mixed with hay, horse feed, and seed compost; additional hay, horse feed, and

moisture were added during the 6-week test period. The temperature within the compost varied but was generally above 55°C and frequently reached greater than 70°C. TNT concentrations were below the detection limit at both 3 and 4 weeks.

HMX and RDX

McCormick et al. (1984) observed gradual reduction of HMX using anaerobic batch cultures, but did not detect any activity using aerobic methods. They inoculated a nutrient broth containing 50 mg/L of HMX with anaerobic sewage sludge and saw a drop in HMX concentration to 25 mg/L after 5 days and a drop to 15 mg/L after 25 days. No further reduction in HMX concentration occurred even after several weeks.

Smith et al. (1982) studied the biological treatability of explosives in waste streams from manufacturing. A rotating biological contractor was tested for as part of technological feasibility study. However, RDX and HMX flowed through the tank essentially unchanged, while TNT photolyzed in the influent tank.

Greene et al. (1985) studied the feasibility of land farming explosives. Simulated pink water containing HMX, RDX, and TNT was continuously applied to a number of soil columns at different flow rates, with and without glucose as a carbon supplement. After 110 days, there appeared to be little degradation of HMX under any of the column conditions.

Doyle et al (1986) conducted pilot scale composting experiments using HMX. Using a manure compost with 10% contaminated sediments, HMX was reduced from an initial concentration of 1,550 mg/kg to 300 mg/kg after 56 days of composting.

Evidence that the absence of oxygen is necessary for RDX biodegradation has been presented by McCormick et al. (1981), who reported the complete disappearance of RDX from an initial concentration of 100 mg/L after only 4 days of incubation in anaerobic sewage sludge.

Bell et al. (1984) reported the degradation of RDX under anaerobic conditions in a bench scale semicontinuous activated sludge treatment system. From an initial concentration of 5 mg/L, RDX decreased to 3 mg/L in only 11 hours. However, no further degradation occurred, apparently because organic nutrients were exhausted. No removal of RDX was observed in a similar system under aerobic conditions.

COMPOSTING PROCESS

Composting is a process by which organic materials are biodegraded by microorganisms, producing organic and/or inorganic byproducts and energy in the form of heat. The heat is trapped within the compost matrix, causing the self-heating characteristic of composting. This nutrient-rich and heated environment creates favorable conditions for the biodegradation of toxic organic compounds.

Composting is initiated by mixing biodegradable organic matter with organic carbon sources and bulking agents, which are added to increase the porosity of the mixture. Bulking agents may also provide additional organic carbon to the composting microorganisms.

Composting can be implemented at various levels of technology. In its least sophisticated form, the material to be composted is simply placed in a pile and allowed to self-heat. Water and nutrients may be added, but air exchange and

temperature control are generally poor. In-vessel composting, the highest technological level, is a continuous treatment process using a system of enclosed composting vessels, automated materials-handling equipment, and aeration and heat removal controls.

This study employed the static pile method, a mid-range technology that uses aeration and heat removal but maintains the simplicity of the basic compost pile. The aeration/heat removal system typically takes the form of a network of perforated pipe underlying the compost pile. The pipe is attached to a mechanical blower, and air is periodically drawn through the compost to control aeration and remove heat.

As stated earlier, one of the goals of this composting field study was to maximize the quantity of soil processed in the composting treatment system per unit time. To achieve this, either a higher percentage of soil would have to be incorporated into the mixture to be composted or the contaminants would have to be transformed at a higher rate. These parameters were investigated by both optimizing the temperature and aeration of the test compost and studying a wide range of soil-to-amendment ratios.

The following parameters were considered in the development and implementation of a composting strategy for this project:

- **Amendments:** Amendments are any materials included in the mixture to be composted in addition to the soil. They may include bulking agents which provide porosity, carbon sources, and specialty materials such as fertilizers. In this project, the amendments were saw dust, chicken manure, fish, and

potato wastes.

- **Temperature control:** The temperature was controlled by a thermostat that activated a blower if the temperature reached 55°C. Temperature was maintained between 50 and 55°C.
- **Moisture:** Moisture content was monitored daily and maintained in the 50 to 55% range by the addition of water when needed.
- **Oxygen:** Oxygen was provided in the course of providing air flow to and controlling temperature of the compost piles.

By maintaining the above parameters consistently throughout the various compost piles, a comparison was made of composting efficiency with the sole variable being the percentage volume of contaminated soil for each of the compost piles. The results of the Ames test and comparisons with the analytical chemistry and *Ceriodaphnia dubia* toxicity studies can be found in the following chapters.

CHAPTER 2

AMES MUTAGENICITY TESTING OF CCLT LEACHATES AND ACETONITRILE EXTRACTS

INTRODUCTION

Bruce Ames (1975) developed a technique in which certain strains of *Salmonella typhimurium* were used as a screening device for chemical mutagens. Since that time, test data on more than 5,000 compounds have been described, establishing this *Salmonella* microsomal mutagenicity assay (the "Ames test") as a major assay in detecting mutagenicity (Maron, 1984). The Ames test employs strains of *Salmonella typhimurium* containing mutations in the *his*-operon that can be reverted by base pair substitutions or frameshift mutations. Strains TA-100 and TA-98 (which revert by base-pair substitution and frameshift mutation, respectively) are the most widely-used test strains. These are grown on a histidine-deficient medium containing various concentrations of the test material. Revertant (hence, mutated) colonies are then counted and the mutagenicity of the test material quantitated.

The CCLT leachates and acetonitrile extracts from this study were subjected to the Ames test to determine the toxicological characteristics of the products resulting from composting. As noted above, this test was used only to monitor reduction of the mutagenicity of the organic extracts of the composted soils with time; it was not intended to evaluate the ability of the Ames test to predict the

environmentally-leachable carcinogenicity of the compost extracts.

Composts Preparation

The composts tested in this study were generated at the UMDA by Roy F. Weston, Inc. Dried and homogenized aliquots of the composts were shipped to the Oak Ridge National Laboratory, where they were stored in the dark at 4°C.

The composts were extracted according to two regimens for this study:

1. Aqueous leaching by the CCLT method. SW-846 method 1312 was followed. The composts were leached for 18 hours using water acidified to pH 5 with a mixture of nitric and sulfuric acids, then pressure-filtered through a 0.7 μm porosity glass fiber medium.
2. Organic solvent extraction using acetonitrile. For analyses of explosives and TNT metabolites, 1 g of compost was extracted with 4 ml of acetonitrile for 18 hours in an ultrasonic bath with cooling, and the supernatant was recovered after the solids settled out. For Ames testing, 4 g of compost were extracted with 20 ml of acetonitrile, and 10 ml of the supernatant were evaporated to dryness in a rotary evaporator.

Because these composted materials will not be disposed of in a municipal landfill, an extraction method was needed that models the leaching of surface-applied, treated wastes by acid rain. Therefore, CCLT was selected instead of the Toxicity Characteristic Leaching Procedure (TCLP). In addition, the acetate in the TCLP interferes with the toxicity tests used in this study.

MATERIALS AND METHODS

The Ames test was performed by mixing the following components in a sterile test tube placed in a 43°C heating block.

- (a) 2.0 ml of top agar.
- (b) 0.1 ml of freshly grown indicator organisms (10^9 organisms/ml) TA-98 or TA-100, and, if metabolic activation was used, 0.5 ml of S-9 solution.
- (c) The appropriate amount of test material.

The contents of the tube were gently vortexed, then poured and evenly distributed over the entire surface of a minimal glucose agar plate, and allowed to solidify. The plates were incubated for 48 hours at 37°C and the number of *his*⁺ revertant colonies counted. Duplicate plates were run for each dilution of each test material. Spontaneous, negative (solvent), and positive controls (sodium azide and nitrofluorene) were run concurrently with each experiment.

Top Agar

The top agar was made by mixing 6 g of Difco-Bacto agar and 5 g NaCl in 1 liter of distilled water. It was then autoclaved and stored in 100-ml bottles at room temperature. Before use, the agar was melted in an autoclave or microwave oven. Ten milliliters of a sterile solution of 0.5mM L-histidine-HCl and 0.5mM biotin was added to the 100 ml of molten agar and mixed thoroughly.

Minimal Glucose Agar Plates

Vogel-Bonner (V-B) medium E with 2% glucose and 1.5% Bacto-Difco agar was used as the medium for the minimal glucose agar plates, and was prepared as

follows:

Vogel-Bonner Salts (50X)

Warm Distilled Water	670 ml
Magnesium Sulfate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$)	10 g
Citric Acid Monohydrate	100 g
Potassium Hydrogen Phosphate (K_2HPO_4)	500 g
Sodium Ammonium Hydrogen Phosphate ($\text{NaH}_2\text{N}_2\text{H}_4\text{PO}_4 \cdot 4\text{H}_2\text{O}$)	175 g

The above salts were added to water at 45°C in the order specified above. Each salt was completely dissolved before the next was added. When the salts were all dissolved, the solution was cooled to room temperature. Approximately 5 ml of chloroform were added to the solution, which was then stored in a capped bottle at room temperature.

Fifteen grams of Difco-Bacto agar were dissolved in 1 liter of water by autoclaving. The mixture was cooled to 60 to 70°C, then 20 ml of 50X V-B salt solution and 50 ml of sterile 40% glucose solution were added. The completed agar was mixed thoroughly and dispensed into 100 x 15 mm plastic petri dishes (20 ml per plate).

Indicator Strains

The indicator *Salmonella* strains were stored at -80°C in 1 ml aliquots of nutrient broth supplemented with 10% sterile glycerol; each aliquot contained approximately 10^9 cells. For each experiment, the 1 ml frozen aliquots were allowed to thaw at room temperature and inoculated into 30 ml of nutrient broth.

The cultures were incubated at 37°C without shaking for 4 hours, after which shaking at 100 rpm for 11 to 14 hours was implemented. Histidine dependency was checked for each strain before an experiment was performed.

S-9 Microsomal Fraction

A rat liver microsomal fraction S-9 with Aroclor activation (Litton Bionetics, Oklahoma City, Oklahoma) was used. The S-9 mix was prepared by mixing the following:

Rat liver S-9	2.0 ml
MgCl ₂ -KCl salts	1.0 ml
1 M Glucose 6-phosphate	0.25 ml
0.1 M NADP	2.0 ml
0.2 M Phosphate buffer	25.0 ml
Sterile distilled water	19.75 ml

The ingredients were added in the reverse order indicated so that the S-9 was added to a buffered solution. Solution was prepared fresh for each day of experiments and was kept on ice until addition with the *Salmonella* strains. Preparation of the ingredients used in the S-9 mix are given in the following.

MgCl₂-KCl salts

Potassium chloride (KCl)	61.5 g
Magnesium chloride (MgCl ₂ •6H ₂ O)	40.7 g
Distilled water	500 ml final volume

The salt solution was prepared by dissolving the ingredients in water,

followed by autoclaving at 121 °C for 20 minutes. The solution was stored in glass bottles at room temperature.

1 M Glucose 6-Phosphate

Glucose 6-phosphate	2.82 g
Sterile distilled water	10 ml

Pre-weighed aliquots of glucose 6-phosphate were prepared by mixing the above and filter sterilized using a 0.22 μ m filter. Solutions were stored in the freezer at -80 °C.

0.1 M NADP Solution

NADP (F.W. 765.4)	383 mg
Sterile distilled water	5 ml

NADP was pre-weighed and put in sterile cryogenic tubes until use. On the day of experimentation, sterile water was added to the NADP for use with the S-9 mix.

0.2 M Sodium Phosphate Buffer

0.2 M Sodium dihydrogen phosphate ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$)	600 ml
(13.8 g/500ml)	
0.2 M Disodium hydrogen phosphate (NaH_2PO_4)	440 ml
(14.2 g/500 ml)	

The above solution was prepared by combining the appropriate volumes of the phosphate solutions and the pH was adjusted to 7.4. The solution was autoclaved for 20 minutes at 121 °C and stored at room temperature.

Confirmation of Indicator Strain Genotypes and Response to Mutagens

Salmonella strains TA-98 and TA-100 have mutations in the *rfa* and *uvrB* genes, as well as the R-factor plasmid pKM101. Prior to testing the compost extracts for mutagenic activity, the genotypes of the *Salmonella* indicator strains were confirmed by demonstrating that they were sensitive to crystal violet and UV irradiation, but were unharmed by ampicillin. The known mutagens nitrofluorene, acetylaminofluorene, and sodium azide were then tested for their mutagenic effects on TA-98 and TA-100 with and without S-9 metabolic activation. The effects of these mutagens are shown in Table 2.1. The indicator strains demonstrated the expected reversion rate in the presence of these compounds.

Having verified the genomic integrity of the indicator strains and the expected reversion in response to known mutagens, tests of leachates and extracts of the composted explosives were undertaken.

RESULTS

CCLT Leachates

Problems arose in the initial tests of the CCLT leachates. Attempts to sterilize the samples by bath and probe ultrasonicators were only successful for the 100% contaminated soil control, which contained no amendments and had not been composted. This suggested that the amendments were the source of the bacterial contamination. Autoclaving the leachates was ruled out because heating could possibly destroy existing mutagenic products or create new ones. Therefore, filtration with a 0.22 μm filter was tested as a suitable sterilization method.

Table 2.1. Results of Ames Tests of Known Mutagens.

Sample	TA-98, Rev./Plate		TA-100, Rev./Plate	
	-S9	+S9	-S9	+S9
CONTROL	25	24	138	141
Nitrofluorene ^a	291	NT	512	NT
Acetylaminofluorene ^a	NT	533	NT	227
Sodium Azide ^b	NT	NT	586	694
Benzo(a)pyrene ^c	NT	165	NT	694

NT = not tested

^a = 10 μ g/plate^b = 2 μ g/plate^c = 5 μ g/plate

Ames testing was conducted on filtered samples of the day 0 leachates from the 7, 10, 20, 30 and 40% soil composts, the 10% uncontaminated soil compost, and a 100% contaminated soil sample. The data from these assays are shown in Table 2.2. No mutagenic activity was observed for any of the day 0 filtered samples except for the highest dose (160 μ l) of leachate from the 100% contaminated soil.

Because the 100% contaminated soil leachate could be sterilized by sonication, filtered vs. unfiltered leachates from this material could be compared. The 100% unfiltered sample produced a slightly higher mutation rate than did the filtered, but activity in both was low, and detectable only at the highest dose. This comparison suggests that the lack of mutagenicity in the filtered leachates was not an artifact of filtration. Subsequently, all remaining leachates were similarly filtered and tested. The results are shown in Table 2.3.

Little or no mutagenic activity was found even when mutagenicity was calculated from the highest dose applied to the plates. The slopes of the dose response curves (as revertants/ml of leachate) were calculated by the method of least squares, using SAS® statistical analysis software. Most of the calculated activities were too low (or negative, because the number of revertants was less than the background) to be considered significant. These results are similar to Phase I findings on the CCLT leachates from composted sediments at the LAAP (Griest, 1990).

Acetonitrile Extracts

Since the CCLT leachates showed only minimal mutagenic activity at day 0,

Table 2.2. Results of Ames Tests of Leachates of Day 0 Composts or Soil Samples.

		Revertants/Plate			
		TA-98		TA-100	
Sample	$\mu\text{L}/\text{Plate}$	-S9	+ S9	-S9	+ S9
Spontaneous	-	23	NT	130	NT
B(a)P ^a	5	19	120	148	490
7% Soil ^b	10	24	28	143	152
	20	20	25	135	145
	40	21	24	134	147
	80	30	19	143	152
10% Soil ^b	10	30	25	149	171
	20	25	26	139	161
	40	27	25	142	152
	80	21	29	137	152
20% Soil ^b	10	22	24	156	158
	20	27	29	143	156
	40	27	23	144	145
	80	35	24	154	159
30% Soil ^b	20	40	23	138	124
	40	30	25	133	122
	80	35	27	149	140
	160	33	24	148	140

Table 2.2 (continued).

		Revertants/Plate			
		TA-98		TA-100	
Sample	$\mu\text{L}/\text{Plate}$	-S9	+ S9	-S9	+ S9
40% Soil ^b	20	37	36	208	219
	40	29	31	230	224
	80	30	31	232	226
	160	42	38	222	205
100% Soil ^b	20	29	36	228	208
	40	27	30	228	245
	80	48	33	265	229
	160	53	32	286	225
100% Soil ^c	20	51	NT	233	NT
	40	48	NT	224	NT
	80	50	NT	262	NT
	160	102	NT	386	NT

^a = Known mutagen

^b = CCLT leachates filtered through 0.2 μm cellulose filter.

^c = CCLT leachates sterilized by ultrasonication.

Table 2.3. Summary of Ames Tests of Compost CCLT Leachates.

Compost or Leachate		Revertants/mL of Leachate*			
		TA-98		TA-100	
		-S9	+S9	-S9	+S9
0	0	NT	47	NT	53
	90	-3	-6	50	81
7	0	13	22	69	41
	90	9	44	19	-13
10	0	19	6	69	22
	10	NT	41	NT	50
	90	19	34	0	44
20	0	3	41	91	75
	90	-3	50	69	69
30	0	22	47	53	75
	90	13	22	84	6
40	0	41	38	59	78
	90	31	28	200	253
100	-	47	94	69	181

* = Data calculated from 80 μ L dose of leachate.

NT = not tested

it was decided to test an analogous set of acetonitrile extracts. Acetonitrile extracts were tested from the 10% soil compost at days 0, 10, 20, 44, and 90, while extracts from the other compost mixtures were tested only for days 0 and 90. The specific mutagenicity was measured in revertants per gram of compost (rev/g) for both the TA-98 and TA-100 strains. As was observed in Phase I, the presence of the S-9 activation system reduces the ability to detect mutagenic activity in the TA-98 and TA-100 strains of *Salmonella*; therefore, the data presented in the figures are only for the assays performed without S-9 activation. The full set of data (with and without S-9 activation) are included in Tables 2.4, 2.5, 2.6, and 2.7.

In contrast to the CCLT leachates, the acetonitrile extracts of the various compost samples yielded considerable mutagenic activity (Table 2.8). Because the acetonitrile extracts produced significant mutagenic activity at day 0, it was possible to show a marked reduction in mutagenicity over the 90-day composting period.

A time course study of the 10% contaminated soil compost was performed. Examination of the data (Figure 2.1) for the five time points taken from the 10% contaminated soil compost reveals that the measurable mutagenicity increased early in the composting period (at day 10 for TA-98, and at day 20 for TA-100), then declined. This phenomenon may have resulted from the creation of biotransformation products in the compost that had greater mutagenic activity than the original starting material. The compost matrix may have created mutagenic products which is suggested by the fact that the negative control showed mutagenic activity at the day 10 time point (Table 2.1) and that this was the only

Table 2.4. Revertants per Plate of Acetonitrile Extracts for Ames Tests without S-9 at Day 0.

OBS	EXTRACT	% Soil	$\mu\text{L}/\text{plate}$	Dose (mg)	Day 1 Rep 1	Day 1 Rep 2	Day 2 Rep 1	Day 2 Rep 2
1	TA 098	7	0	0	24	24	29	29
2	TA 098	7	10	2	208	149	243	272
3	TA 098	7	20	4	273	271	524	449
4	TA 098	7	30	6	386	338	701	751
5	TA 098	7	40	8	423	465	991	902
6	TA 098	10	0	0	20	20	28	28
7	TA 098	10	10	2	394	403	391	425
8	TA 098	10	20	4	661	653	502	655
9	TA 098	10	30	6	.	.	728	771
10	TA 098	10	40	8	906	1014	880	920
11	TA 098	10	80	16	1468	1418	.	.
12	TA 098	20	0	0	25	25	39	39
13	TA 098	20	5	1	295	296	498	461
14	TA 098	20	10	2	640	634	810	790
15	TA 098	20	15	3	643	469	1016	1174
16	TA 098	20	20	4	1112	1204	1540	1586
17	TA 098	30	0	0	39	39	37	37
18	TA 098	30	5	1	295	296	403	354
19	TA 098	30	10	2	518	465	600	534
20	TA 098	30	15	3	643	469	862	890
21	TA 098	30	20	4	842	828	1048	988
22	TA 098	40	0	0	39	39	33	33
23	TA 098	40	5	1	207	252	284	268
24	TA 098	40	10	2	315	306	412	436
25	TA 098	40	15	3	456	502	578	686
26	TA 098	40	20	4	720	604	701	715
27	TA 098	100	0	0	37	37	.	.
28	TA 098	100	5	1	373	414	.	.
29	TA 098	100	10	2	606	600	.	.
30	TA 098	100	15	3	880	834	.	.
31	TA 098	100	20	4	1254	1192	.	.
32	TA 100	7	0	0	134	134	.	.
33	TA 100	7	5	1	300	345	.	.
34	TA 100	7	10	2	514	546	.	.
35	TA 100	7	15	3	700	760	.	.
36	TA 100	7	20	4	890	728	.	.

Table 2.4 (continued).

OBS	EXTRACT	% Soil	μ L/plate	Dose (mg)	Day 1 Rep 1	Day 1 Rep 2	Day 2 Rep 1	Day 2 Rep 2
37	TA 100	10	1	0	98	98	112	112
38	TA 100	10	10	2	350	334	318	323
39	TA 100	10	20	4	520	479	411	474
40	TA 100	10	30	6	.	.	653	706
41	TA 100	10	40	8	760	810	845	861
42	TA 100	10	80	16	1800	1728	.	.
43	TA 100	20	0	0	165	165	178	178
44	TA 100	20	5	1	780	808	680	725
45	TA 100	20	10	2	1134	1132	1320	1320
46	TA 100	20	15	3	2012	2020	1776	1876
47	TA 100	20	20	4	1864	2464	2604	2336
48	TA 100	30	0	0	165	165	134	134
49	TA 100	30	5	1	550	626	533	525
50	TA 100	30	10	2	740	784	830	950
51	TA 100	30	15	3	1226	640	1212	1320
52	TA 100	30	20	4	1768	1466	1662	1620
53	TA 100	40	0	0	165	165	163	163
54	TA 100	40	5	1	443	791	433	415
55	TA 100	40	10	2	804	892	750	694
56	TA 100	40	15	3	1012	1090	8709	919
57	TA 100	40	20	4	1612	1464	1150	1127
58	TA 100	100	0	0	134	134	.	.
59	TA 100	100	5	1	414	432	.	.
60	TA 100	100	10	2	818	758	.	.
61	TA 100	100	15	3	982	986	.	.
62	TA 100	100	20	4	1020	1278	.	.

Table 2.5. Revertants per Plate of Acetonitrile Extracts for Ames Tests without S-9 at Day 90.

OBS	EXTRACT	% Soil	$\mu\text{L}/\text{plate}$	Dose (mg)	Day 1 Rep 1	Day 1 Rep 2	Day 2 Rep 1	Day 2 Rep 2
1	TA 098	7	0	0	23	23	20	20
2	TA 098	7	5	1	.	.	24	32
3	TA 098	7	10	2	36	40	35	43
4	TA 098	7	20	4	40	44	74	74
5	TA 098	7	30	6	48	55	.	.
6	TA 098	7	40	8	80	92	101	97
7	TA 098	7	80	16	.	.	168	200
8	TA 098	10	0	0	20	20	20	20
9	TA 098	10	5	2	26	35	.	.
10	TA 098	10	10	4	65	46	56	48
11	TA 098	10	20	6	93	80	87	85
12	TA 098	10	40	8	125	101	138	144
13	TA 098	10	80	16	250	260	.	.
14	TA 098	20	0	0	23	23	23	23
15	TA 098	20	5	1	39	32	.	.
16	TA 098	20	10	2	67	67	64	68
17	TA 098	20	20	4	96	101	97	100
18	TA 098	20	30	6	.	.	139	149
19	TA 098	20	40	8	205	198	178	202
20	TA 098	20	80	16	358	374	3	.
21	TA 098	30	0	0	26	26	23	23
22	TA 098	30	5	1	79	57	.	.
23	TA 098	30	10	25	130	117	106	91
24	TA 098	30	20	4	224	245	142	136
25	TA 098	30	30	6	.	.	181	183
26	TA 098	30	40	8	444	416	225	252
27	TA 098	30	80	16	919	919	.	.
28	TA 098	40	0	0	26	26	23	23
29	TA 098	40	5	1	140	123	.	.
30	TA 098	40	10	2	230	250	181	171
31	TA 098	40	20	4	447	468	304	304
32	TA 098	40	30	6	.	.	472	412
33	TA 098	40	40	8	783	825	537	478
34	TA 098	40	80	16	1489	1467	.	.
35	TA 098	100	0	0	37	37	.	.
36	TA 098	100	5	1	373	414	.	.
37	TA 098	100	10	2	606	600	.	.
38	TA 098	100	15	3	880	834	.	.
39	TA 098	100	20	4	1254	1192	.	.

Table 2.5 (continued).

OBS	EXTRACT	% Soil	$\mu\text{L}/\text{plate}$	Dose (mg)	Day 1 Rep 1	Day 1 Rep 2	Day 2 Rep 1	Day 2 Rep 2
40	TA 100	7	0	0	120	120	.	.
41	TA 100	7	5	1	144	120	.	.
42	TA 100	7	10	2	147	131	.	.
43	TA 100	7	20	4	143	141	.	.
44	TA 100	7	40	8	144	152	.	.
45	TA 100	7	80	16	174	147	.	.
46	TA 100	10	0	0	120	120	125	125
47	TA 100	10	5	1	.	.	147	153
48	TA 100	10	10	2	186	200	206	179
49	TA 100	10	20	4	220	254	176	184
50	TA 100	10	40	8	260	273	234	249
51	TA 100	10	80	16	.	.	332	340
52	TA 100	20	0	0	175	175	.	.
53	TA 100	20	5	1	238	242	.	.
54	TA 100	20	10	2	238	249	.	.
55	TA 100	20	20	4	293	275	.	.
56	TA 100	20	40	8	324	328	.	.
57	TA 100	20	80	16	416	444	.	.
58	TA 100	30	0	0	120	120	.	.
59	TA 100	30	5	1	166	170	.	.
60	TA 100	30	10	2	219	235	.	.
61	TA 100	30	20	4	281	291	.	.
62	TA 100	30	40	8	388	374	.	.
63	TA 100	30	80	16	568	685	.	.
64	TA 100	40	0	0	120	120	.	.
65	TA 100	40	5	1	198	204	.	.
66	TA 100	40	10	2	283	272	.	.
67	TA 100	40	20	4	439	480	.	.
68	TA 100	40	40	8	736	673	.	.
69	TA 100	40	80	16	1186	1141	.	.
70	TA 100	100	0	0	134	134	.	.
71	TA 100	100	5	1	414	432	.	.
72	TA 100	100	10	2	818	758	.	.
73	TA 100	100	15	3	982	986	.	.
74	TA 100	100	20	4	1020	1278	.	.

Table 2.6. Revertants per Plate of Acetonitrile Extracts for Ames test with S-9 at Day 0.

OBS	EXTRACT	% Soil	$\mu\text{L}/\text{plate}$	Dose (mg)	Day 1 Rep 1	Day 1 Rep 2	Day 2 Rep 1	Day 2 Rep 2
1	TA 098	7	0	0	24	24	29	29
2	TA 098	7	10	2	62	52	57	75
3	TA 098	7	20	4	75	71	103	100
4	TA 098	7	30	6	90	100	144	157
5	TA 098	7	40	8	122	114	214	192
6	TA 098	10	0	0	28	28	.	.
7	TA 098	10	10	2	74	72	.	.
8	TA 098	10	20	4	88	121	.	.
9	TA 098	10	40	6	132	144	.	.
10	TA 098	10	80	8	202	200	.	.
11	TA 098	20	0	0	39	39	.	.
12	TA 098	20	5	1	94	107	.	.
13	TA 098	20	10	2	175	145	.	.
14	TA 098	20	15	3	207	222	.	.
15	TA 098	20	20	4	350	361	.	.
16	TA 098	30	0	0	39	39	.	.
17	TA 098	30	5	1	70	76	.	.
18	TA 098	30	10	2	138	118	.	.
19	TA 098	30	15	3	165	179	.	.
20	TA 098	30	20	4	246	226	.	.
21	TA 098	40	0	0	39	39	.	.
22	TA 098	40	5	1	89	73	.	.
23	TA 098	40	10	2	98	106	.	.
24	TA 098	40	15	3	158	141	.	.
25	TA 098	40	20	4	192	206	.	.
26	TA 098	10	0	0	37	37	.	.
27	TA 098	0	5	1	86	68	.	.
28	TA 098	10	10	2	154	158	.	.
29	TA 098	0	15	3	173	203	.	.
30	TA 098	10	20	4	270	262	.	.
		0						
		10						
		0						
		10						
		0						

Table 2.6 (continued).

OBS	EXTRACT	% Soil	μ L/plate	Dose (mg)	Day 1 Rep 1	Day 1 Rep 2	Day 2 Rep 1	Day 2 Rep 2
31	TA 100	10	0	0	112	112	.	.
32	TA 100	10	10	2	181	150	.	.
33	TA 100	10	20	4	227	206	.	.
34	TA 100	10	30	6	310	280	.	.
35	TA 100	10	40	8	384	348	.	.
36	TA 100	20	0	0	178	178	.	.
37	TA 100	20	5	1	332	408	.	.
38	TA 100	20	10	2	529	514	.	.
39	TA 100	20	15	3	638	788	.	.
40	TA 100	20	20	4	1016	940	.	.
41	TA 100	30	0	0	178	178	.	.
42	TA 100	30	5	1	323	503	.	.
43	TA 100	30	10	2	433	449	.	.
44	TA 100	30	15	3	623	597	.	.
45	TA 100	30	20	4	896	836	.	.
46	TA 100	40	0	0	178	178	.	.
47	TA 100	40	5	1	307	245	.	.
48	TA 100	40	10	2	379	362	.	.
49	TA 100	40	15	3	532	446	.	.
50	TA 100	40	20	4	544	587	.	.
51	TA 100	10	0	0	134	134	.	.
52	TA 100	0	5	1	326	356	.	.
53	TA 100	10	10	2	495	519	.	.
54	TA 100	0	15	3	678	688	.	.
55	TA 100	10	20	4	790	768	.	.
		0						
		10						
		0						
		10						
		0						

Table 2.7. Revertants per Plate of Acetonitrile Extracts for Ames Tests with S-9 at Day 90.

OBS	EXTRACT	% Soil	μ L/plate	Dose (mg)	Day 1 Rep 1	Day 1 Rep 2	Day 2 Rep 1	Day 2 Rep 2
1	TA 098	7	0	0	20	20	.	.
2	TA 098	7	5	1	27	36	.	.
3	TA 098	7	10	2	33	24	.	.
4	TA 098	7	20	4	36	26	.	.
5	TA 098	7	40	8	44	50	.	.
6	TA 098	7	80	16	56	63	.	.
7	TA 098	10	0	0	20	20	.	.
8	TA 098	10	5	1	26	24	.	.
9	TA 098	10	10	2	29	32	.	.
10	TA 098	10	20	4	38	34	.	.
11	TA 098	10	40	8	50	34	.	.
12	TA 098	10	80	16	94	75	.	.
13	TA 098	20	0	0	23	23	.	.
14	TA 098	20	20	4	29	22	.	.
15	TA 098	20	40	8	36	26	.	.
16	TA 098	20	80	16	24	20	.	.
17	TA 098	30	0	0	26	26	.	.
18	TA 098	30	5	1	34	41	.	.
19	TA 098	30	10	2	56	44	.	.
20	TA 098	30	20	4	50	68	.	.
21	TA 098	30	40	8	100	84	.	.
22	TA 098	30	80	16	187	193	.	.
23	TA 098	40	0	0	26	26	.	.
24	TA 098	40	5	1	49	54	.	.
25	TA 098	40	10	2	65	68	.	.
26	TA 098	40	20	4	118	114	.	.
27	TA 098	40	40	8	191	208	.	.
28	TA 098	40	80	16	396	413	.	.
29	TA 100	7	0	0	120	120	.	.
30	TA 100	7	5	1	175	159	.	.
31	TA 100	7	10	2	140	176	.	.
32	TA 100	7	20	4	163	161	.	.
33	TA 100	7	40	8	175	186	.	.
34	TA 100	7	80	16	208	199	.	.
35	TA 100	10	0	0	122	122	.	.
36	TA 100	10	5	1	157	162	.	.
37	TA 100	10	10	2	188	163	.	.
38	TA 100	10	20	4	184	181	.	.
39	TA 100	10	40	8	192	184	.	.
40	TA 100	10	80	16	240	267	.	.

Table 2.7 (continued).

OBS	EXTRACT	% Soil	μ L/plate	Dose (mg)	Day 1 Rep 1	Day 1 Rep 2	Day 2 Rep 1	Day 2 Rep 2
41	TA 100	20	0	0	175	175	.	.
42	TA 100	20	20	4	177	179	.	.
43	TA 100	20	40	8	227	196	.	.
44	TA 100	20	80	16	106	188	.	.
45	TA 100	30	0	0	120	120	.	.
46	TA 100	30	5	1	162	174	.	.
47	TA 100	30	10	2	174	181	.	.
48	TA 100	30	20	4	178	186	.	.
49	TA 100	30	40	8	305	245	.	.
50	TA 100	30	80	16	384	302	.	.
51	TA 100	40	0	0	120	120	.	.
52	TA 100	40	5	1	180	200	.	.
53	TA 100	40	10	2	233	247	.	.
54	TA 100	40	20	4	286	309	.	.
55	TA 100	40	40	8	487	411	.	.
56	TA 100	40	80	16	758	759	.	.

Table 2.8. Specific Mutagenicity for Acetonitrile Extracts.

		Specific Mutagenicity, Rev./g (average $\pm$ standard deviation)	
Compost	Days of Composting	TA-98 w/o S9	TA-100 w/o S9
Static Piles:			
0%	0	0	0
	10	37,500	18,800
	20	0	0
	44	0	0
	90	0	0
7%	0	83,200 $\pm$ 12,500	205,000 $\pm$ 5,780
	90	9,820 $\pm$ 610	2,100 $\pm$ 550
10%	0	87,200 $\pm$ 5,390	100,000 $\pm$ 2,750
	10	110,000 $\pm$ 9,200	56,300 $\pm$ 4,970
	20	97,500 $\pm$ 6,750	112,000 $\pm$ 4,920
	44	38,000 $\pm$ 5,400	27,400 $\pm$ 4,380
	90	14,300 $\pm$ 530	12,800 $\pm$ 1,140
20%	0	310,000 $\pm$ 30,700	546,000 $\pm$ 25,200
	90	21,600 $\pm$ 360	14,200 $\pm$ 1,100
30%	0	216,000 $\pm$ 16,100	350,000 $\pm$ 25,000
	90	51,900 $\pm$ 3,700	33,100 $\pm$ 1,030
40%	0	160,000 $\pm$ 9,490	286,000 $\pm$ 19,300
	90	86,900 $\pm$ 4,300	64,800 $\pm$ 2,030

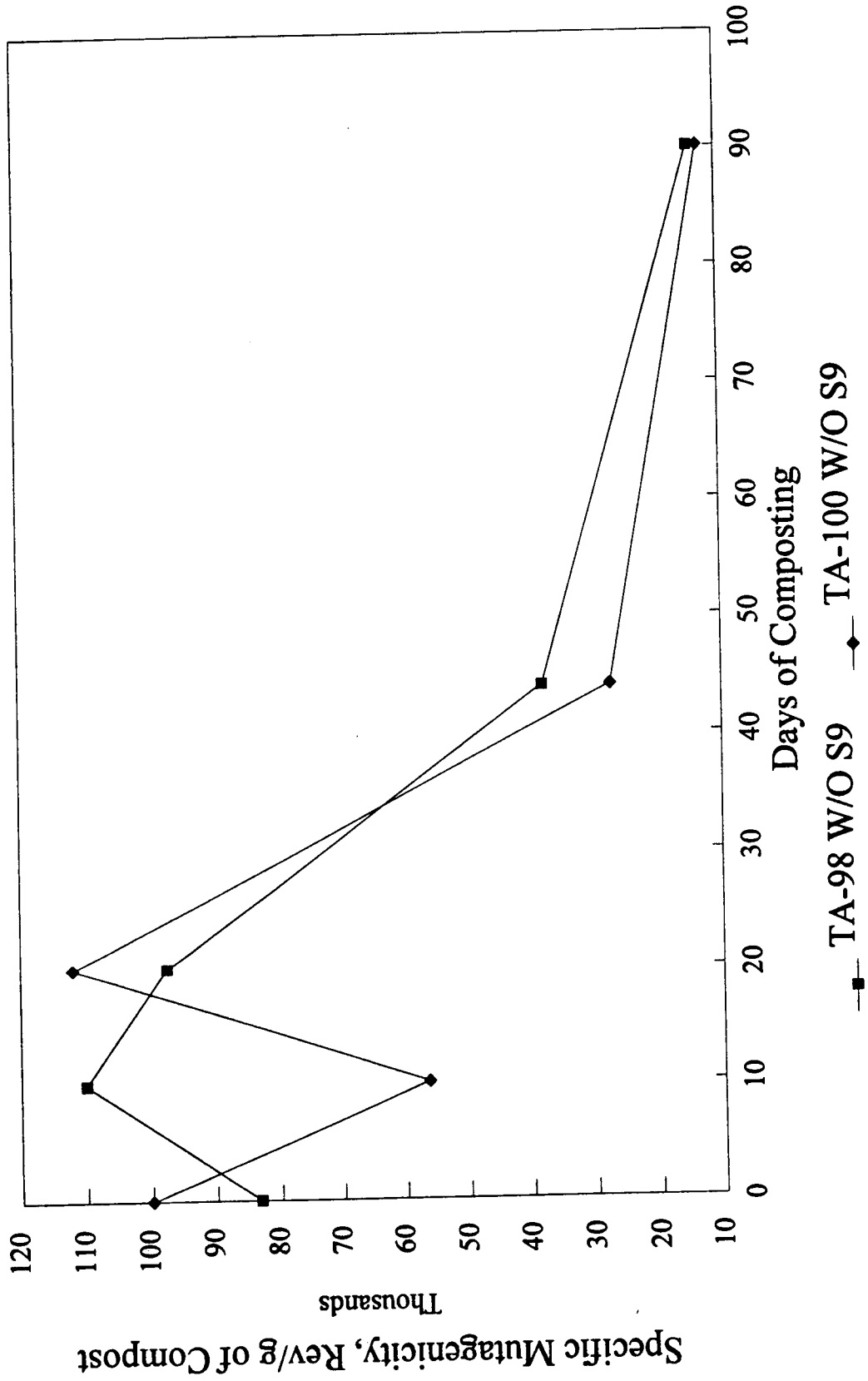


Figure 2.1 Time course plot of mutagenicity of 10% soil compost acetone extracts

time point that demonstrated any activity in the negative control. However, from the 44-day midpoint on, a clear trend in the reduction of mutagenicity was established.

Examination of Figure 2.2 shows that at day 0, the mutagenicity of extracts from the 20% soil compost was greater than that from the 30% soil compost, which was in turn greater than that from the 40% soil compost. Analysis of the data (Figure 2.3) for the day 90 endpoint compost extracts shows that the higher the percentage of soil in the compost mixture, the greater the mutagenicity of the extracts. This discrepancy in beginning and end time correlation of soil percentage and associated mutagenicity, while unexpected, suggests that it is not the amount of explosives that limited composting efficiency in the composts containing higher percentages of soil. More likely, the process was affected by the amount of soil itself and/or the concomitantly reduced percentage of amendments.

The extracts from the 7%, 10%, and 20% soil composts showed over a 90% reduction in mutagenic activity. Reduction of mutagenic activity in the 30 and 40% soil compost extracts was less dramatic. The mutagenic activity of all day 0 compost samples was more pronounced in the TA-100 test strain than in TA-98. However, at day 90 the TA-98 strain showed greater mutagenic response to all of the extracts. The slopes, standard deviations, and degrees of freedom of the dose response curves as calculated by the method of least squares are shown in Table 2.9 and Table 2.10.

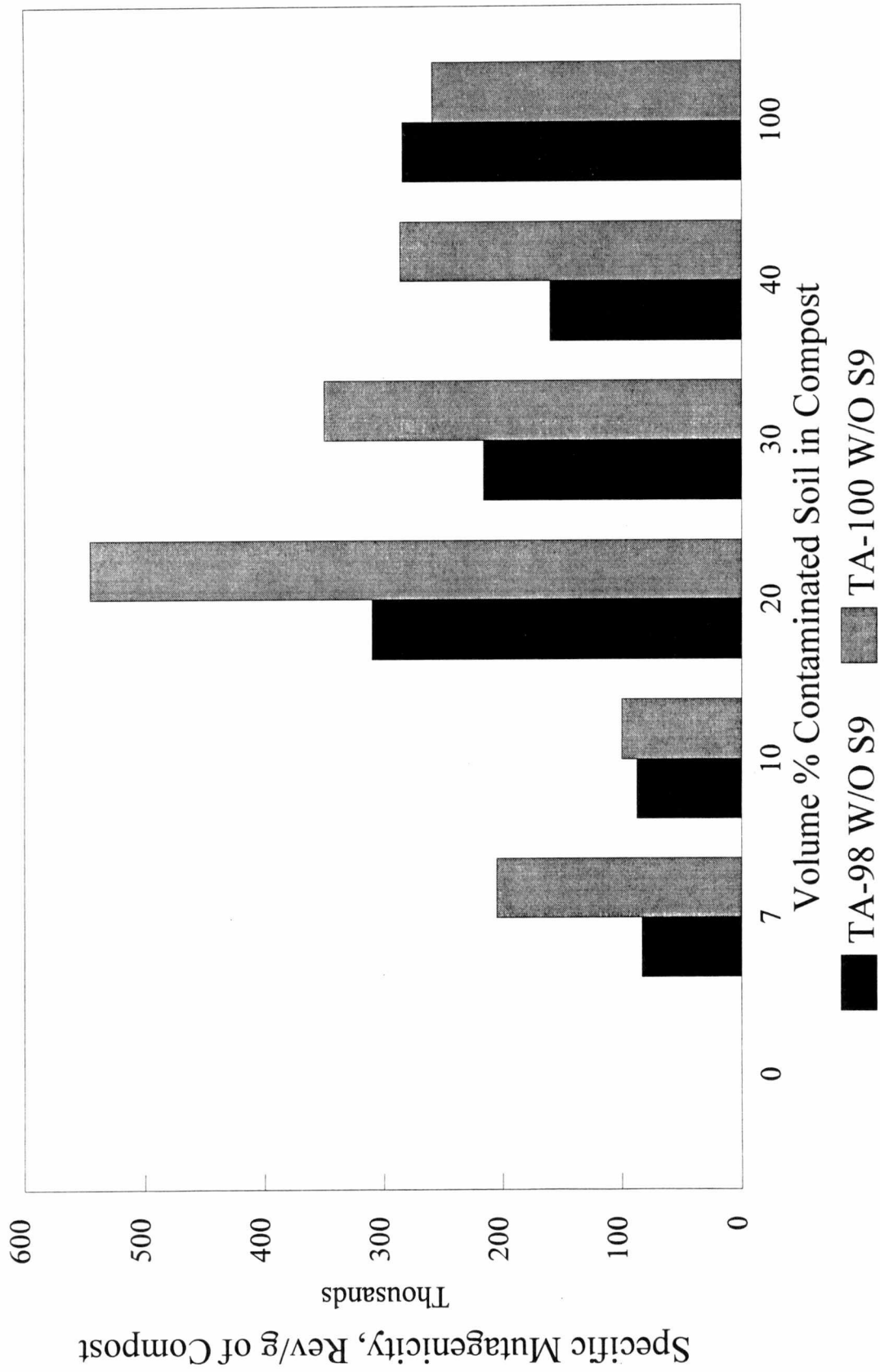


Figure 2.2 Mutagenicity of acetonitrile extracts at Day 0

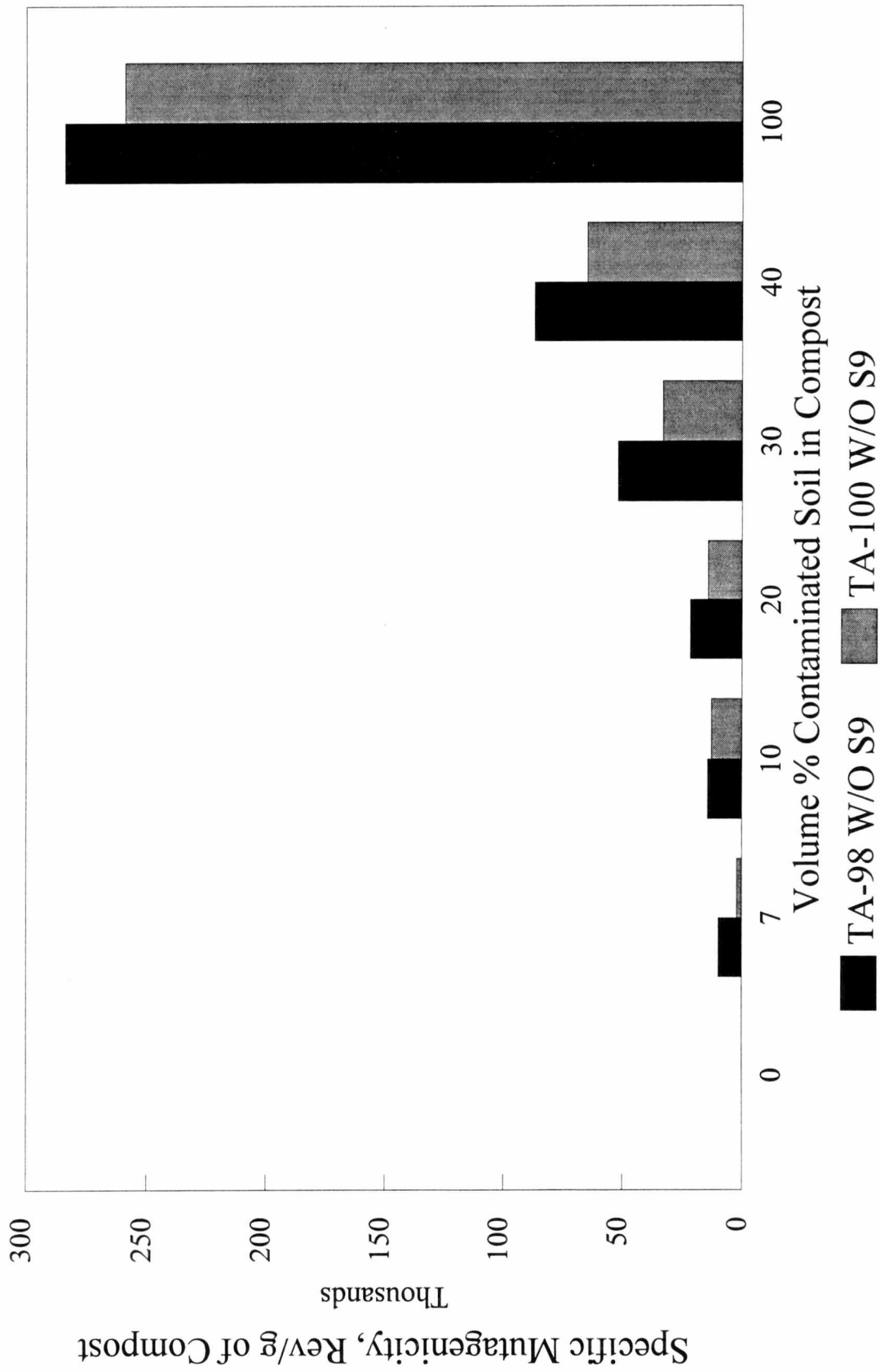


Figure 2.3 Mutagenicity of acetonitrile extracts at Day 90

Table 2.9. Slopes (revertants/mg), Standard Deviation of Slopes, and Degrees of Freedom for Ames Test of Acetonitrile Extracts without S-9.

% Soil	TA-98		TA-100	
	Day 0	Day 90	Day 0	Day 90
7%	83.2 12.5 18	9.8 0.6 20	204.8 5.8 8	2.1 0.6 10
10%	87.2 5.4 18	14.3 0.5 18	100.1 2.8 18	12.8 1.1 18
20%	309.5 30.7 18	21.6 0.4 20	546.4 25.2 18	14.2 1.1 10
30%	215.6 16.1 18	51.9 3.7 20	350.0 25.0 18	33.1 1.0 10
40%	160.1 9.5 18	86.9 4.3 20	286.1 19.3 18	64.8 2.0 10
100%	283.6 10.7 8	- - -	259.1 20.4 8	- - -

Table 2.10. Slopes (revertants/mg), Standard Deviation of Slopes, and Degrees of Freedom for Ames Test of Acetonitrile Extracts with S-9.

% Soil	TA-98		TA-100	
	Day 0	Day 90	Day 0	Day 90
7%	16.5 2.0 18	2.3 0.3 10	- - -	3.9 0.9 10
10%	20.6 1.4 8	3.8 0.4 10	31.9 2.0 8	6.7 0.9 10
20%	74.7 6.1 8	-0.1 0.3 6	194.3 12.4 8	1.6 1.0 6
30%	49.3 2.5 8	10.0 0.5 10	157.3 16.8 8	13.3 1.6 10
40%	38.9 2.4 8	23.5 0.4 10	98.8 6.7 8	38.5 1.2 10
100%	56.7 3.3 8	- - -	163.2 7.2 8	- - -

CHAPTER 3

COMPARISON OF AMES TEST WITH CHEMICAL ANALYSIS

RESULTS OF CHEMICAL ANALYSIS

CCLT Leachates

CCLT leachates of samples from all of the compost piles were analyzed at the beginning (day 0) and ending (day 90) time points. In addition, the 10% contaminated and 10% uncontaminated soil composts were also sampled at days 10, 20, and 44. Data for explosives and TNT metabolites in the leachates are presented in Table 3.1.

A look at the time-course fate of the 10% contaminated soil compost shows that at day 0, the TNT concentration was 35 mg/l, but an increase to 51.5 mg/l was observed at day 10. This increase in TNT may correlate with an observed rise in acidity of the leachate (Table 3.1). The TNT concentration then dropped rapidly with time, and at 90 days, was 9 mg/l.

A plot of the time course of TNT metabolite formation (Figure 3.1) shows that the concentration of 4-amino-2,6-dinitrotoluene (4-A-2,6-DNT) steadily decreased as 2,4-diamino-6-nitrotoluene (2,4-DA-6-NT) slowly increased. The increase in concentration of 2,4-DA-6-NT coincided with decreases in the concentrations of the dinitrotoluene metabolites, which would suggest, as

Table 3.1. Explosives Concentrations and pH of CCLT Leachates.

Sample	Concentration, mg/L				pH
	2,4-DA-6-NT	2-A-4,6-DNT	4-A-2,6-DNT	TNT	
CCLT Blank	<0.15	<1.07	<0.94	<1.17	5.00
10% Uncontaminated Soil	Day 0	<0.15	<1.07	<0.94	<1.17
	Day 10	<0.15	<1.07	<0.94	6.40
	Day 20	<0.11	<0.12	<0.10	7.11
	Day 44	<0.11	<0.12	<0.10	7.64
	Day 90	<0.11	<0.12	<0.10	7.68
7% Contaminated Soil	Day 0	<0.15	6.41	5.45	10.5
	Day 90	≤0.24	1.51 ± 0.80	≤0.16	4.97 ± 0.06
10% Contaminated Soil	Day 0	<0.15	3.38	6.51	35.0
	Day 10	<0.15	3.91	4.92	51.5
	Day 20	<0.38	5.86 ± 0.47	5.94 ± 0.48	32.5 ± 2.92
	Day 44	5.06 ± 0.07	1.68 ± 0.11	<0.29	12.4 ± 1.83
	Day90	2.43 ± 0.06	1.43 ± 0.05	<0.20	9.07 ± 0.13
					7.63

Table 3.1. (continued).

Sample		Concentration, mg < L				pH
		2,4-DA-6-NT	2-A-4,6-DNT	4-A-2,6-DNT	TNT	
20% Contaminated Soil	Day 0	<.015	6.39	4.80	56.1	7.35
	Day 90	0.84 ± 0.06	6.19 ± 0.14	1.59 ± 0.02	15.4 ± 0.13	7.74
30% Contaminated Soil	Day 0	<0.33	3.23	4.16	69.2	6.70
	Day 90	<0.38	4.04 ± 0.03	1.67 ± 0.06	16.2 ± 0.06	7.60
40% Contaminated Soil	Day 0	<0.15	1.81	4.29	92.5	7.20
	Day 90	<0.77	526 ± 0.16	56.75 ± 0.23	68.3 ± 0.98	7.75
100% Contaminated Soil (Not Composted)		0.57	<1.53	<1.63	72.0	8.50

Notes: "<" indicates "not detected"; Differences amount "<±" for given constituent dilutions before HPLC. Standard Deviation shown for samples analyzed in 3 replicates. 2,6-DA-4-NT and other TNT metabolites were not detected in any sample.

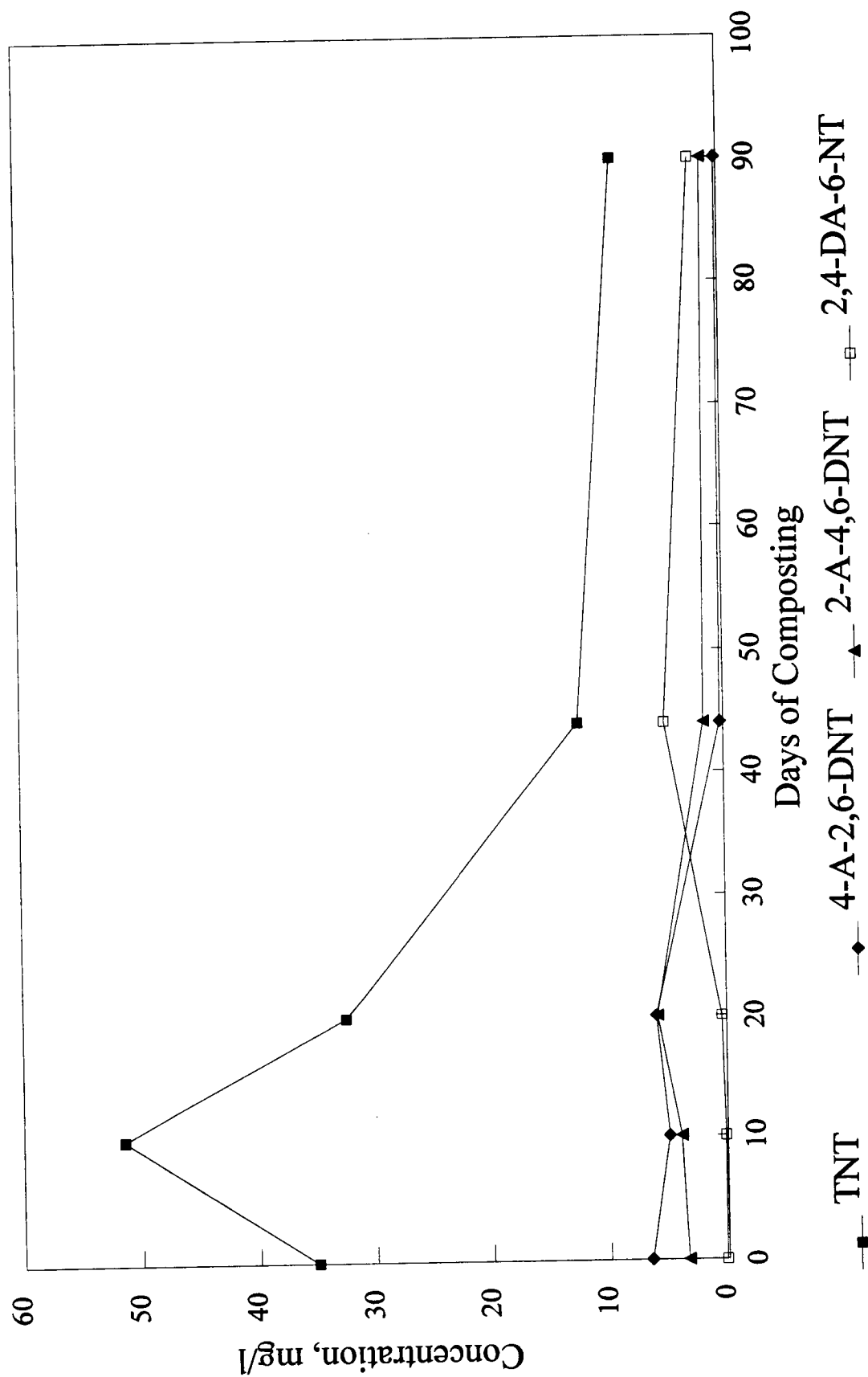


Figure 3.1 Time course plot of TNT and metabolites in 10% soil compost leachates

expected, that nitrotoluene metabolites are a later transformation product of TNT. RDX and the TNT metabolite 2-amino-4,6-dinitrotoluene were not detected; HMX was detected in some samples at low dilution, but was below the reporting limit.

The leachability of the TNT and its solubility in the CCLT leaching solution appear to be the limiting factors because, while the concentration of TNT in the composts was appreciable, its solubility in water is very low (100 mg/l). It seems unlikely that the metabolites were the result of *in situ* transformation in the soil prior to composting because no TNT metabolites were detected in the 100% contaminated soil control. It is possible that the TNT metabolites present in the compost leachates at day 0 arose from microbial action in the piles between the time of mixing and the start of the composting experiment. They may also have been produced during the 18-hour aqueous leaching period, which was conducted at room temperature.

A bar graph comparing the concentrations of TNT and metabolites in the leachates of the composts at day 90 is shown in Figure 3.2. TNT concentrations in the final leachates generally paralleled the percentage of soil in the compost, suggesting that as soil percentage was increased, the concomitantly lower percentage of amendments produced composts that were proportionately less efficient in biotransforming TNT.

Based on the concentrations of leachable explosives and metabolites in the final leachates, 30% appears to be the maximum percentage of soil that can be used in a static pile with this amendment mixture before composting efficiency drops off. This is supported by the observation that for all composts up to and including 30% soil, the final concentration of TNT was 16 mg/l or below, while the

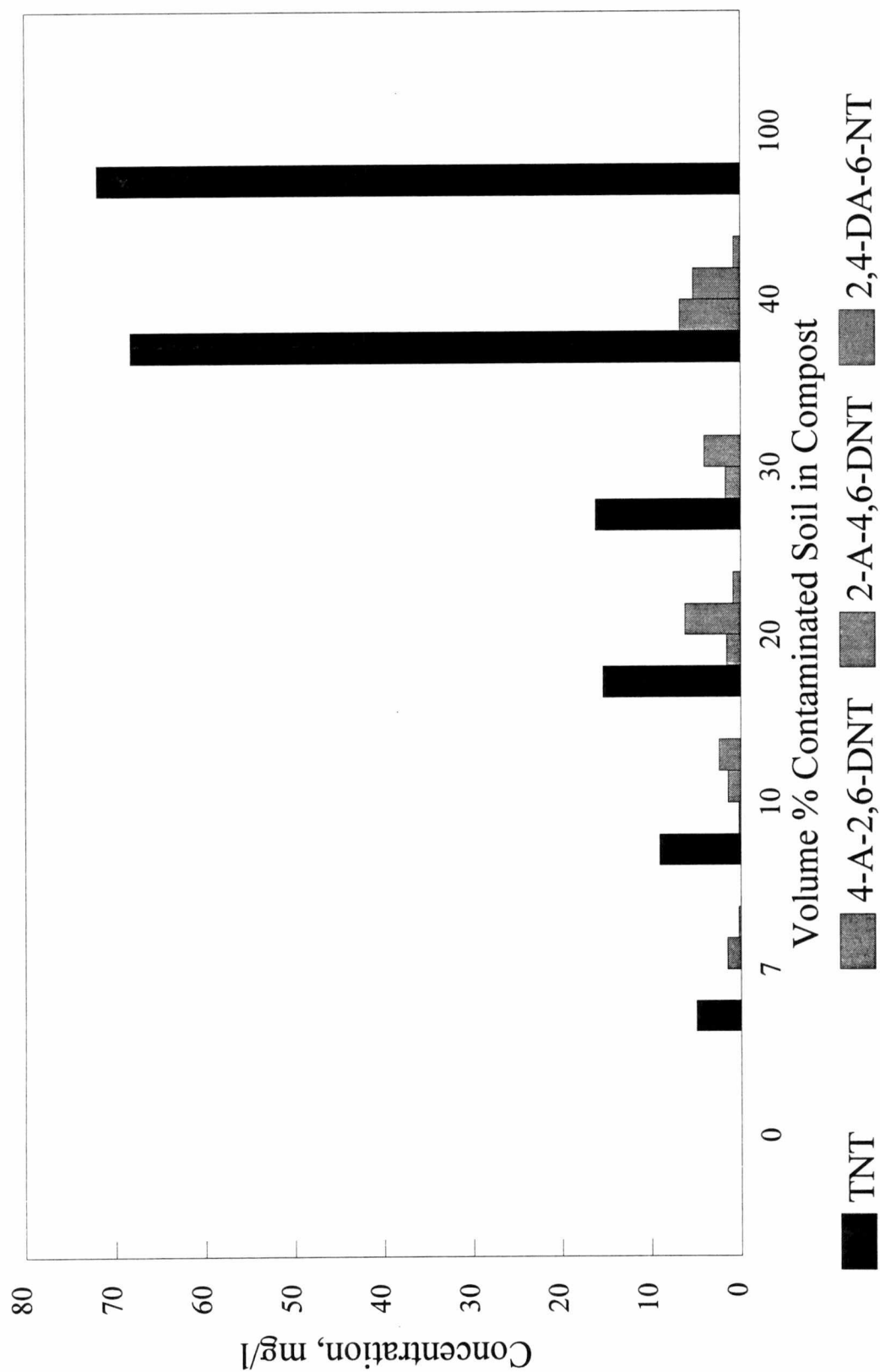


Figure 3.2 Bar graphs of TNT and metabolite concentrations in leachates at Day 90

final TNT concentration in the 40% soil compost was 69.3 mg/l.

Acetonitrile Extracts

A sample set identical to that for the CCLT leachates was collected for acetonitrile extraction. Data for explosives and TNT metabolites in the acetonitrile extracts are shown in Table 3.2.

Acetonitrile extraction allowed greater sensitivity than CCLT in the detection of HMX and RDX as well as the monoaminodinitrotoluene and diaminonitrotoluene metabolites of TNT. As was observed for the CCLT leachates, the greater the percentage of soil in the compost, the less biotransformation of the explosives took place. This reinforces the conclusion that greater volumes of soil decreased the volume of amendments available to enhance biotransformation. Also, as observed for the CCLT leachates, the composting efficiency decreased considerably when the soil percentage reached 40%.

Acetonitrile extracts of the 10% contaminated soil compost pile were sampled at days 0, 10, 20, 44, and 90, which was analogous to the sample set taken for the CCLT leachates. Figures 3.3 A and 3.3 B show the time course fates of TNT and its metabolites over the 90 day composting period. Figure 3.3 A shows the results of TNT and its metabolites on the same scale, and Figure 3.3 B has TNT at 1/100 scale to show greater resolution of the metabolite transformation. The concentration of TNT in the compost decreased from 4,830 mg/kg at day 0 to a final concentration of 97 mg/kg. The concentration of 4-A-2,6-DNT rose initially then fell; 2-A-4,6-DNT concentration declined steadily while the diamino metabolites (2,4-DA-6-NT and 2,6-DA,4-NT) concomitantly increased.

Table 3.2. Explosives Concentrations of Acetonitrile Extracts.

Compost Sample	Concentration ^a , Average ± Standard Deviation, mg/Kg							
	2,6-DA-4-NT	2,4-DA-6-NT	2-A-4,6-DNT	4-A-2,6-DNT	2,4,6-TNT	RDX	HMX	
Uncontaminated Soil	Day 0	<3.9	<9.9	<5.1	<3.0	4.4 ± 6.2	1.9 ± 2.7 ^b	<27
	Day 10	<3.9	<9.9	<5.1	<3.0	8.2 ± 0.7	<6.7	<27
	Day 20	<3.9	<9.9	<5.1	<3.0	1.4 ± 0.2 ^c	<6.7	<27
	Day 44	<3.9	<9.9	<5.1	<3.0	<2.1	<6.7	<27
	Day 90	<3.9	<9.9	<5.1	<3.0	4.2 ± 0.2	<6.7	<27
7% Contaminated Soil	Day 0	<20	<50	494 ± 26	169 ± 14	1240 ± 144	762 ± 32	220 ± 17
	Day 90	2.5 ± 0.3 ^b	3.9 ± 0.2 ^b	39 ± 17	125 ± 69	279 ± 248 ^c	256 ± 45	136 ± 23
10% Contaminated Soil	Day 0	<39	<99	278 ± 17	58 ± 2.6	4830 ± 476	909 ± 38	203 ± 68 ^b
	Day 10	<20	<50	157 ± 7.9	64 ± 2.2	1690 ± 119	799 ± 29	226 ± 17
	Day 20	<13	<33	175 ± 17	155 ± 13	798 ± 39	687 ± 23	193 ± 16
	Day 44	8.3 ± 1.5 ^b	32 ± 8.4	142 ± 33	337 ± 26	197 ± 42	761 ± 30	275 ± 29
	Day 90	6.7 ± 0.2	69 ± 4.9	32 ± 99	110 ± 3.4	97 ± 44	395 ± 9.6	153 ± 62

^aN = 3. " < " means compound not detected at all. Reporting Limit listed varies with sample extract dilution.

^bResult listed is less than reporting limit, and is an estimate.

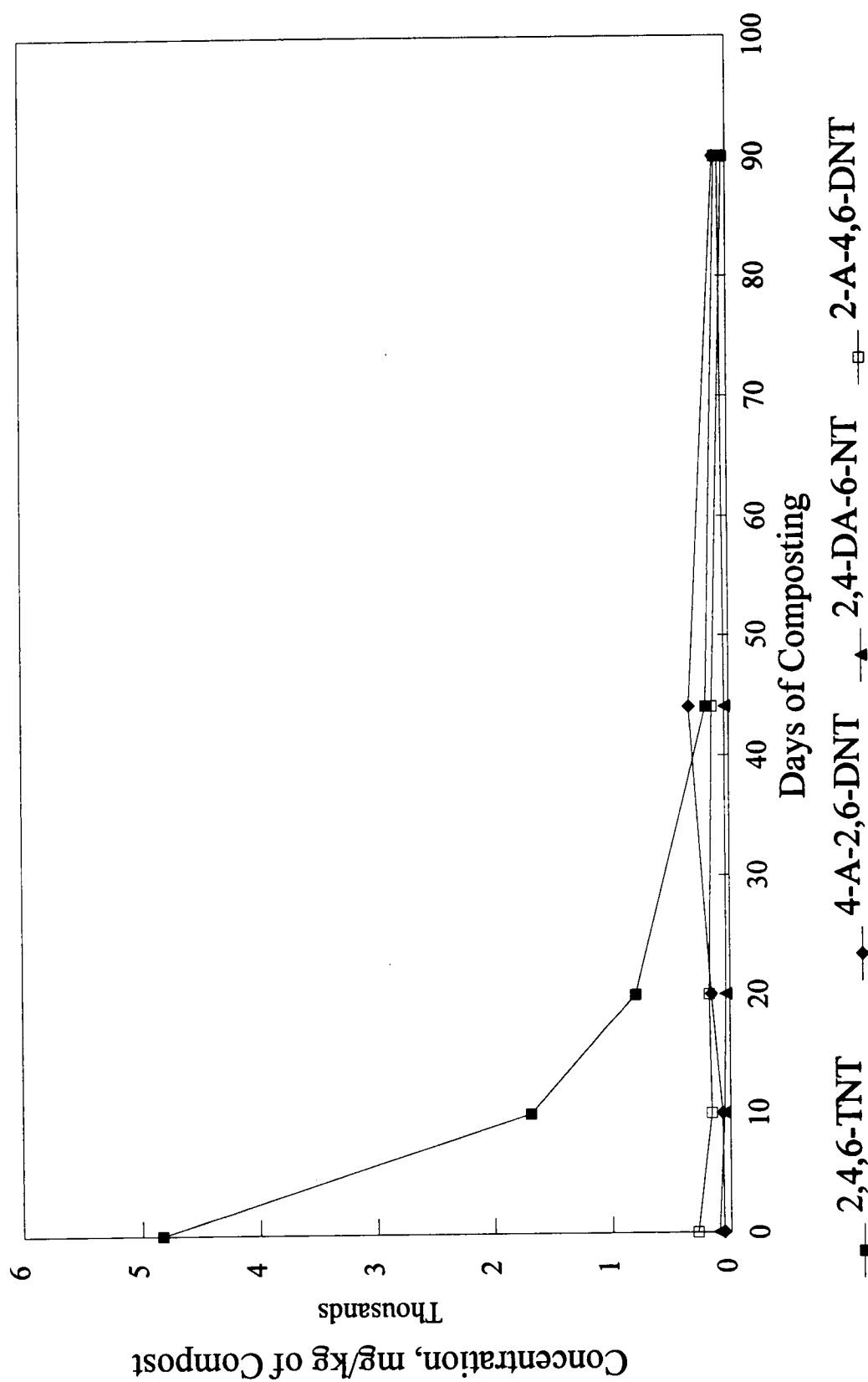
^cResult is 104 if one value (630) is dropped, n = 2.

Table 3.2. (continued).

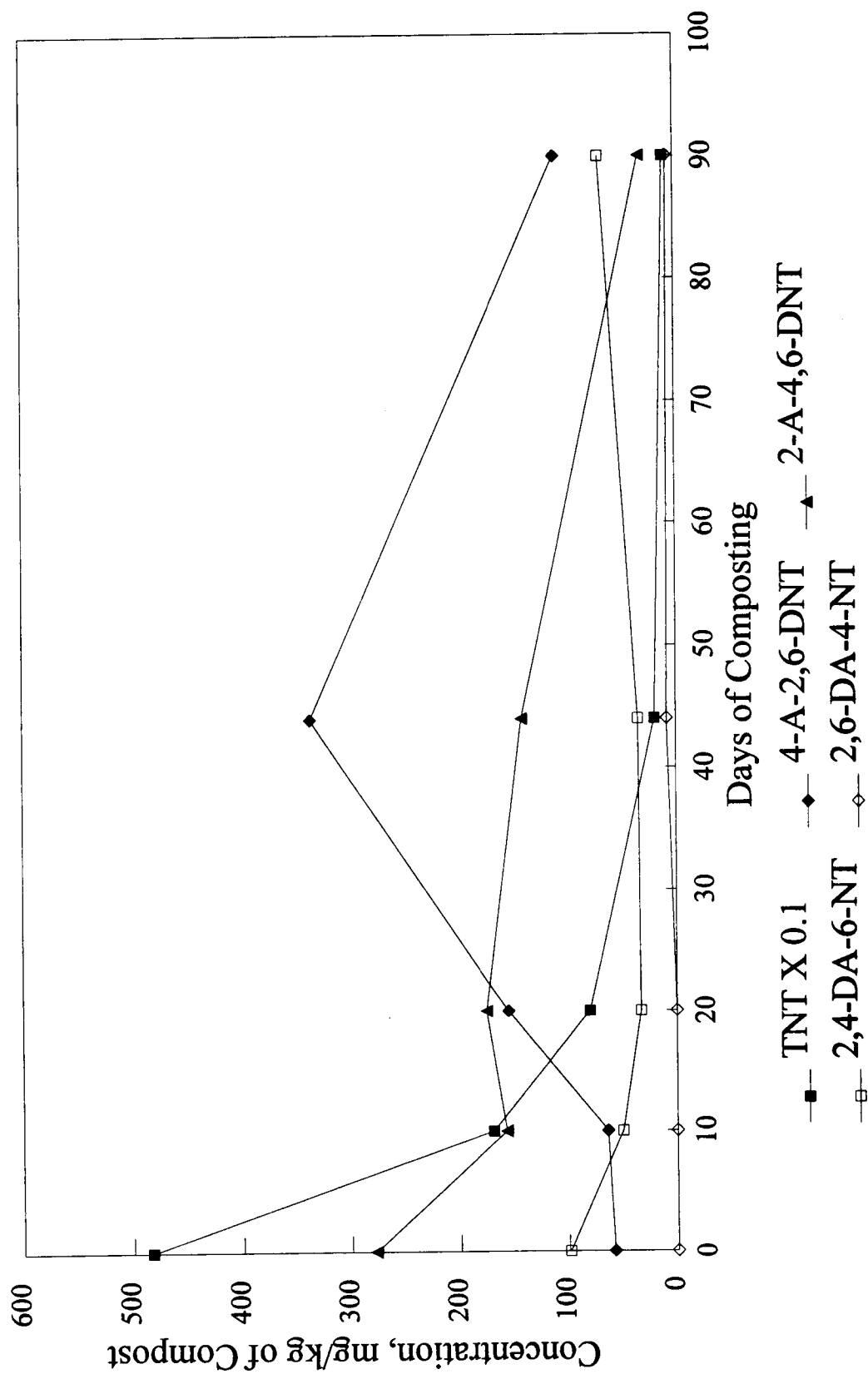
Compost Sample	Concentration ^a , Average $\pm$ Standard Deviation, mg/Kg						
	2,6-DA-4-NT	2,4-DA-6-NT	2-A-2,6-DNT	4-A-2,6-DNT	2,4,6-TNT	RDX	HMX
20% Contaminated Soil							
Day 0	<39	<99	355 $\pm$ 45	133 $\pm$ 13	6550 $\pm$ 363	1100 $\pm$ 77	320 $\pm$ 24
Day 90	6.5 $\pm$ 0.8	21 $\pm$ 2.6	92 $\pm$ 7.0	295 $\pm$ 29	143 $\pm$ 19	647 $\pm$ 11	241 $\pm$ 1.5
30% Contaminated Soil							
Day 0	<39	<99	164 $\pm$ 10	32 $\pm$ 3.0	7950 $\pm$ 199	1030 $\pm$ 43	296 $\pm$ 16
Day 90	6.5 $\pm$.04	90 $\pm$ 1.3	132 $\pm$ 18	232 $\pm$ 27	222 $\pm$ 33	778 $\pm$ 44	319 $\pm$ 12
40% Contaminated Soil							
Day 0	<39	<99	165 $\pm$ 11	34 $\pm$ 6.2	9410 $\pm$ 712	1240 $\pm$ 52	340 $\pm$ 19
Day 90	<26	<66	322 $\pm$ 5.4	156 $\pm$ 1.5	2750 $\pm$ 135	1440 $\pm$ 120	376 $\pm$ 3.4
100% Contaminated Soil (not composted)	<65	<165	<85	<50	12200 $\pm$ 1400	1380 $\pm$ 128	409 $\pm$ 32
THAMA Std. Soil Blank	<3.9	<9.9	<5.1	<3.0	<2.1	<6.7	<27

^aThree replicates from a homogenized composite of individual samples collected at 5 locations in the compost piles. "<" means compound not detected at all. Reporting Limit listed varies with sample extract dilution.

^bResult listed is less than reporting limit, and is an estimate.



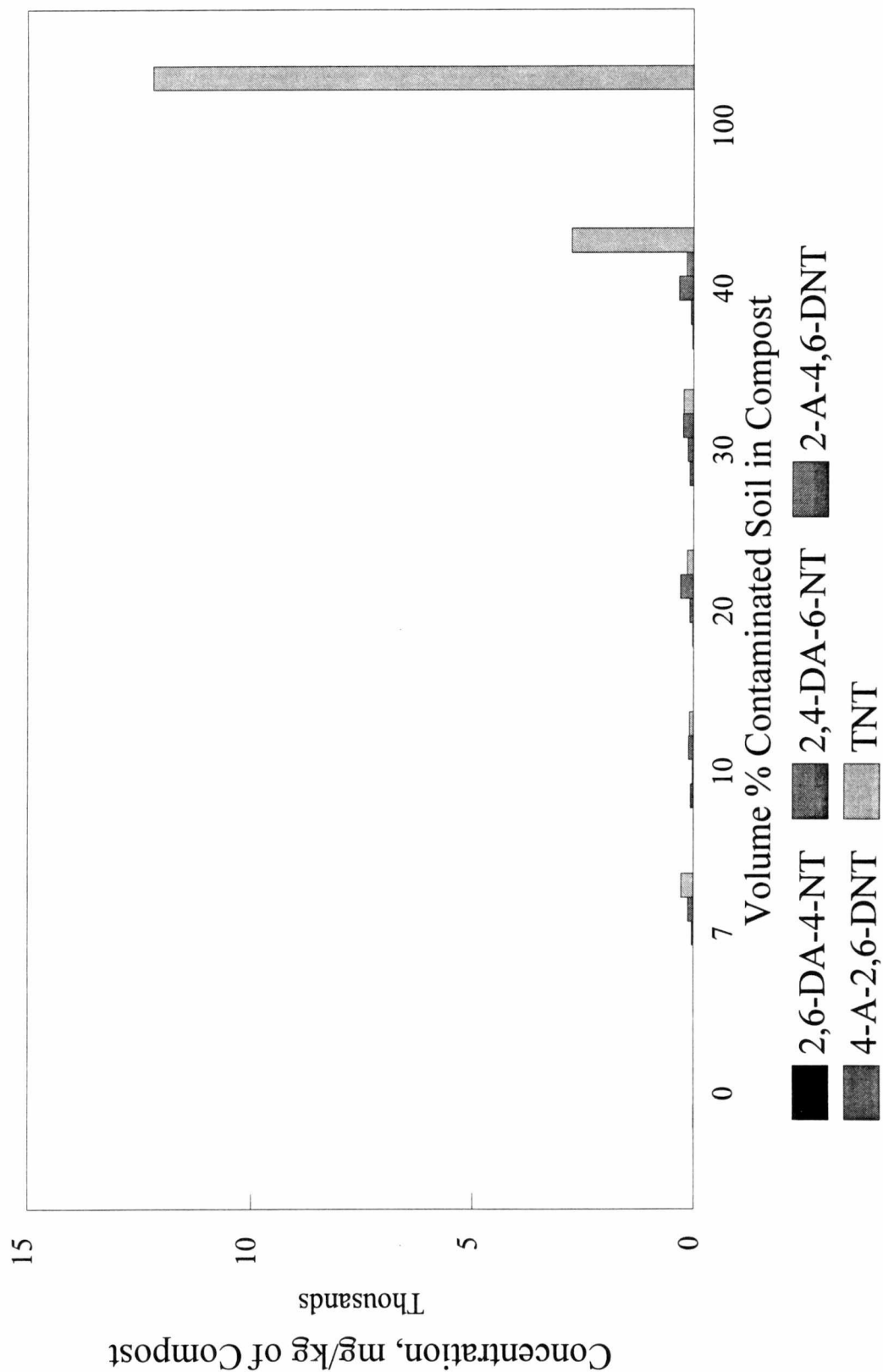
A. TNT at normal scale
Figure 3.3. Time course plot of TNT and metabolites for acetonitrile extracts of 10% soil compost.



B. TNT at 1/10 scale
Figure 3.3 (continued).

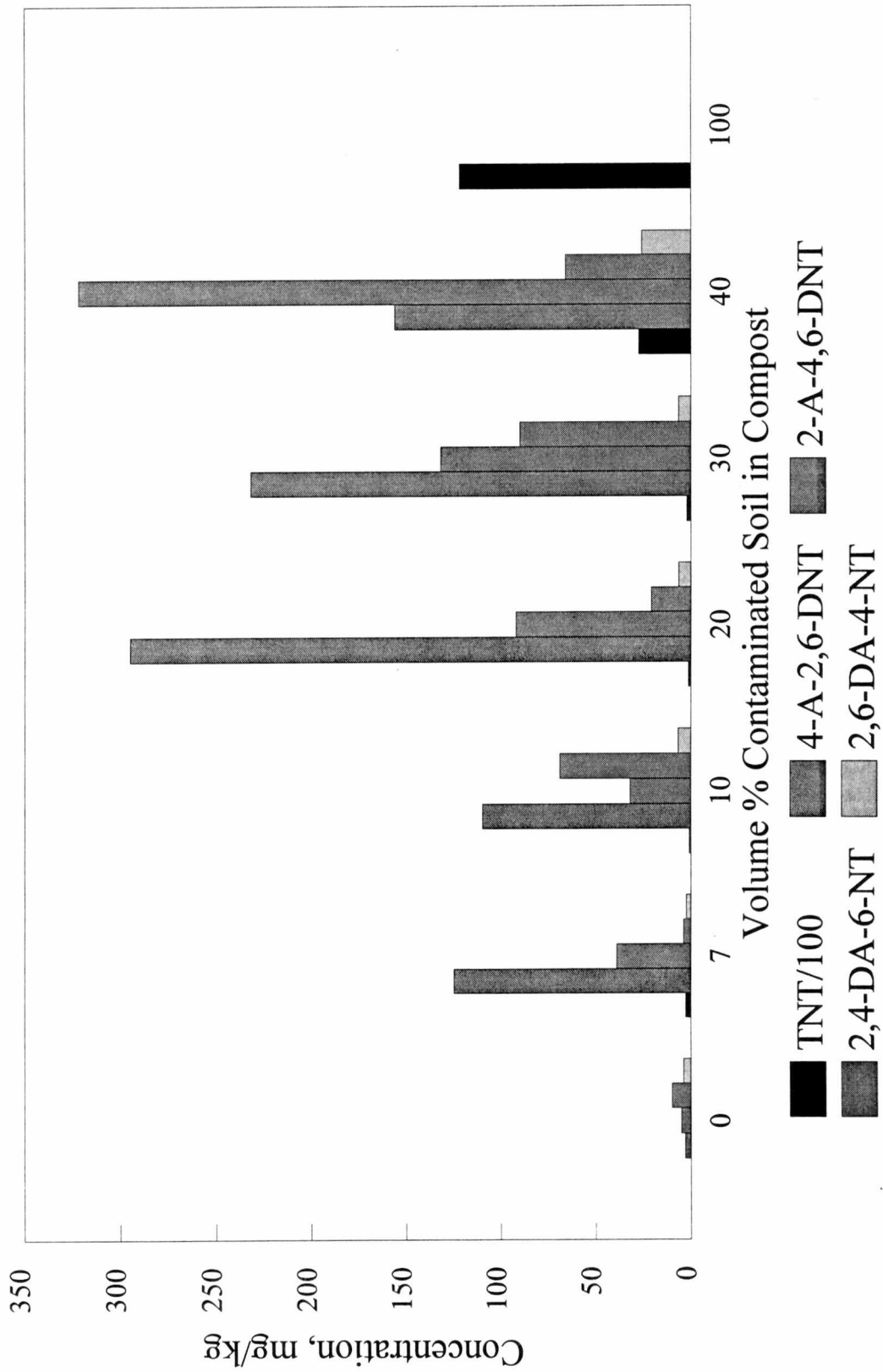
Unlike the results seen in the CCLT leachates, no rise in TNT concentration at the day 10 time point was detected in the acetonitrile extracts. This lends further support to the hypothesis that the increase in TNT concentration noted in the CCLT leachates for this time point was due to a decrease in pH which allowed greater extraction of TNT in the leachate. Almost all transformation of TNT occurred by the day 44 midpoint. 4-A-2,6-DNT initially rose in concentration for the first 44 days, then fell by day 90. At day 0, 2-A-4,6-DNT was present at a concentration of 278 mg/l, then dropped steadily to a final concentration of 32 mg/l. Neither of the diamino metabolites were detected until day 44. 2,6-DA-4-NT, which was not detected at all in the CCLT analysis, was found at concentrations of 8.3 and 6.7 mg/l at days 44 and 90, respectively. 2,4-DA-6-NT increased from a concentration of 35 mg/l at day 44 to a final concentration of 69 mg/l. Again, as with the CCLT analysis, the diaminonitrotoluene metabolites increased as the monoaminodinitrotoluene concentrations decreased. This relationship between the TNT metabolites would be expected because the diaminonitrotoluene metabolites are formed as an additional nitro group is reduced to an amino group on the monoaminodinitrotoluene to form the new metabolite.

Bar graphs comparing the concentrations of TNT and its metabolites in the extracts of the composts at day 90 are shown in Figures 3.4 A and 3.4 B. Figure 3.4 A shows the results of TNT and its metabolites on the same scale, while Figure 3.4 B has TNT at 1/100 scale in order to show greater resolution of the metabolite activity. These results show very little TNT detected in the final composts up to and including the 30% soil compost. The 40% soil compost pile had a considerable drop in composting efficiency. Metabolite concentrations also increased with soil



A. TNT at normal scale

Figure 3.4. Bar graphs of TNT and metabolites concentrations in acetonitrile extracts at Day 90.



B. TNT at 1/100 scale
Figure 3.4 (continued).

loading increases.

HMX did not appear to be transformed; while HMX concentrations increased as soil percentage increased, the concentration of HMX within each compost did not vary appreciably over the 90-day composting period. RDX concentrations also increased with soil percentage, but apparently did undergo some biotransformation in the 7, 10, and 20% soil composts; the 30 and 40% soil composts yielded only minimal decreases in RDX. The results of the RDX and HMX analysis can be seen in Table 3.2.

Fate of Biotransformed Explosives

The ultimate fate of the TNT biotransformed in these composts is not clear at present. Previous studies (Kaplan and Kaplan 1982) suggest that only a small percentage of the TNT is actually mineralized, and that a significant percentage can be covalently attached to macromolecular constituents in the compost, forming an inaccessible, bound fraction. These final products may represent mineralization of the TNT; the formation of unextractable bound products; or the formation of products that are extractable, but that cannot be detected by HPLC at the three wavelengths monitored (280, 254, and 230 nm). The first two possibilities seem more likely (Griest, 1991).

COMPARISON OF CHEMICAL ANALYSIS AND MUTAGENICITY

The results of the HPLC analyses and the Ames tests demonstrated similar trends in evaluating the effectiveness of composting as a means of remediating explosives-contaminated soil. A comparison of the specific mutagenicity and

chemical concentrations of TNT and major metabolites of the acetonitrile extracts from the 10% contaminated soil compost as a function of composting time can be made by viewing Figures 3.1 and 2.3, respectively. During the first 20 days of composting, the mutagenicity of the extracts varied widely and then diminished rapidly. HPLC analysis showed that over the course of the composting period the TNT concentration dropped steadily and rapidly; while the monoaminonitrotoluene metabolite concentrations rose then fell; and the diaminonitrotoluenes rose slowly. TNT has a much higher specific mutagenicity than any of the metabolites observed by HPLC (Tan, 1992) and should have been the principal mutagen; however, a one-to-one relationship between TNT concentration and mutagenicity was not found. No TNT metabolites were detected in the uncomposted 100% contaminated soil control.

The measured mutagenicity was compared with the mutagenicity predicted from the concentrations of TNT and metabolites determined by HPLC. Predicted mutagenicity was calculated by multiplying the known concentrations of TNT and its metabolites by the mutagenic rate for each. TNT is the most mutagenic of the compounds detected by HPLC; the specific mutagenic activity of its amino-metabolites decreases as the number of nitro groups reduced to amino groups increases (Tan, 1992). HMX and RDX do not have measurable mutagenic activity (Griest, 1990) with strains TA-98 and TA-100, hence, were not considered in these calculations.

Table 3.3 lists the calculated percentage of mutagenic activity in strains TA-98 and TA-100 attributable to TNT and its detectable metabolites, (based on their concentrations in the acetonitrile extracts as determined by HPLC). The predicted

Table 3.3. Accounting of Mutagenic Activity in Composts by TNT and Metabolites Determined by HPLC.

Compost	Mutagenicity Accounted for Strains TA-98, TA-100, %*				
	Day 0	Day 10	Day 20	Day 44	Day 90
7%	5, 3				5, 26
10%	18, 19	5, 12	3, 3	3, 5	3, 4
20%	7, 5				3, 6
30%	14, 10				2, 4
40%	23, 16				14, 23
100%	23, 31				

* Format is: % accounting of mutagenicity measured with strain TA-98 (w/o S9), % accounting of mutagenicity measured with strain TA-100 (w/o S9).

activity was typically much lower than the measured activity; moreover, the longer the composting time, the smaller the percentage of observed mutagenicity could be predicted by calculation. The 10% uncontaminated soil control did produce detectable mutagenicity at the day 10 time point, but did not produce any detectable mutagenicity at any of the other time points, hence, the amendments and soil most likely did not contribute to the activity observed. Therefore, the unaccounted-for mutagenicity may have been due to a combination of factors including the presence of undetected compound(s) in the contaminated soil, compounds created by biotransformation in the composting process that were not detected by HPLC, and incomplete recovery of TNT and its metabolites in the acetonitrile extraction process. Synergism among mutagens may have affected the activity.

CHAPTER 4

COMPARISON OF AMES TEST RESULTS WITH *CERIODAPHNIA DUBIA* TOXICITY TESTS

INTRODUCTION

Seven-day *Ceriodaphnia dubia* bioassays were conducted on CCLT leachates from each of the static compost piles. The 10% contaminated and uncontaminated soil composts were tested at days 0, 10, 22, 44, and 90; while the 7, 10, 20, 30, 40 and 100% soil piles were tested at days 0 and 90. Survival and fecundity endpoints for each of the compost leachates were calculated.

Survival as LC_{50}

Within each temporal block of tests, a leachate's toxicity was determined by comparing survival and reproduction of *Ceriodaphnia* among the concentrations tested. The concentration of leachate at which survival was reduced by 50% with respect to the controls (LC_{50}) was determined graphically by interpolation.

Fecundity as EC_{50} and SR_{15}

The concentration of leachate at which reproduction was reduced 50% with respect to the controls (EC_{50}) was calculated. A leachate concentration was considered to reduce fecundity when it decreased reproduction of *Ceriodaphnia* to 15 offspring per female. This concentration, the SR_{15} , was expressed in terms of

toxicity units (TUs) and computed by taking the reciprocal of the leachate concentration. The criterion of 15 offspring per female was selected because it was consistently lower than the number of offspring in the controls, was well above zero, and is the minimum level of fecundity acceptable for valid controls according to EPA protocol (Weber, 1989).

RESULTS

Leachate toxicity to *Ceriodaphnia* is summarized in Table 4.1 as LC_{50} for survival and EC_{50} and SR_{15} for fecundity. The full set of data are included in the Appendix. In most instances, the survival and reproductive responses of *Ceriodaphnia* differed markedly [or, at least, significantly] among leachate concentrations and generated conspicuous dose-response curves. Additionally, in almost every leachate tested, *Ceriodaphnia* fecundity was reduced at lower leachate concentrations than those affecting survival.

An examination of the time course fate of leachate toxicity to *Ceriodaphnia* of the 10% contaminated soil compost in Figure 4.1 shows that $1/LC_{50}$ dropped rapidly by day 10 and continued to slowly decline through day 90. The $1/EC_{50}$ plot shows a decrease at day 10, but is followed by an increase at day 20. From day 20 until the end of the 90 day period a clear decrease in toxicity is observed. The data demonstrate that the longer the composting time, the lower the toxicity of the leachates, regardless of the starting percentage of soil in a particular compost. Note, however, that the initial percentage of soil in a compost has an effect on the toxicity of the leachate from it at the end of the 90-day composting period (Figure 4.2). However, the 10 and 20% compost piles did exhibit greater toxicity than the

Table 4.1. *Ceriodaphnia dubia* Endpoint Data for Leachates.

CCLT		Leachate Concentration (%)		
Leachate of Compost	Composting Days	LC ₅₀	EC ₅₀	SR ₁₅
Blank	-	> 90	> 90	> 90
Non Cont.	0	> 20	17	17.9
	10	16.7	6.31	5.7
	20	> 20	3.0	2.2
	44	> 50	> 50	> 50
	90	> 50	43	> 50
7% Soil	0	10	< 5	< 5
	90	> 50	31	34.5
10% Soil	0	1.3	0.5	< 0.5
	10	5.8	0.7	0.6
	20	6.4	< 0.5	< 0.5
	44	8.3	2.0	2.3
	90	18	7.2	7.2
20% Soil	0	4	< 1	< 1
	90	> 20	8.4	8.1
30% Soil	0	4	1	1.1
	9	> 50	21	19.5
40% Soil	0	4	1	1.3
	90	15	4.6	4.6
100% Soil	-	> 5	2.5	2.4

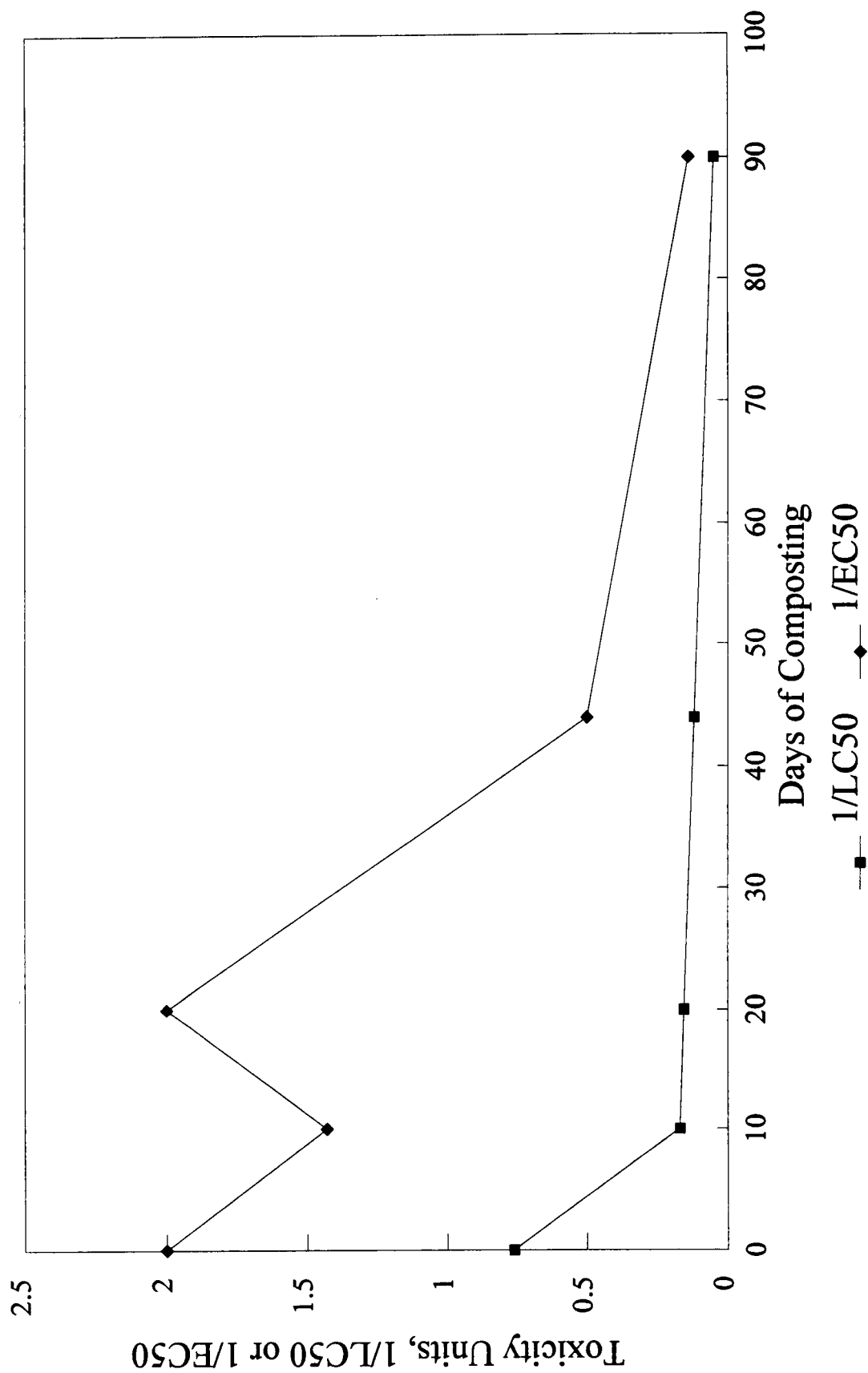


Figure 4.1 Time course plot of toxicity to *C. dubia* from 10% soil compost leachates

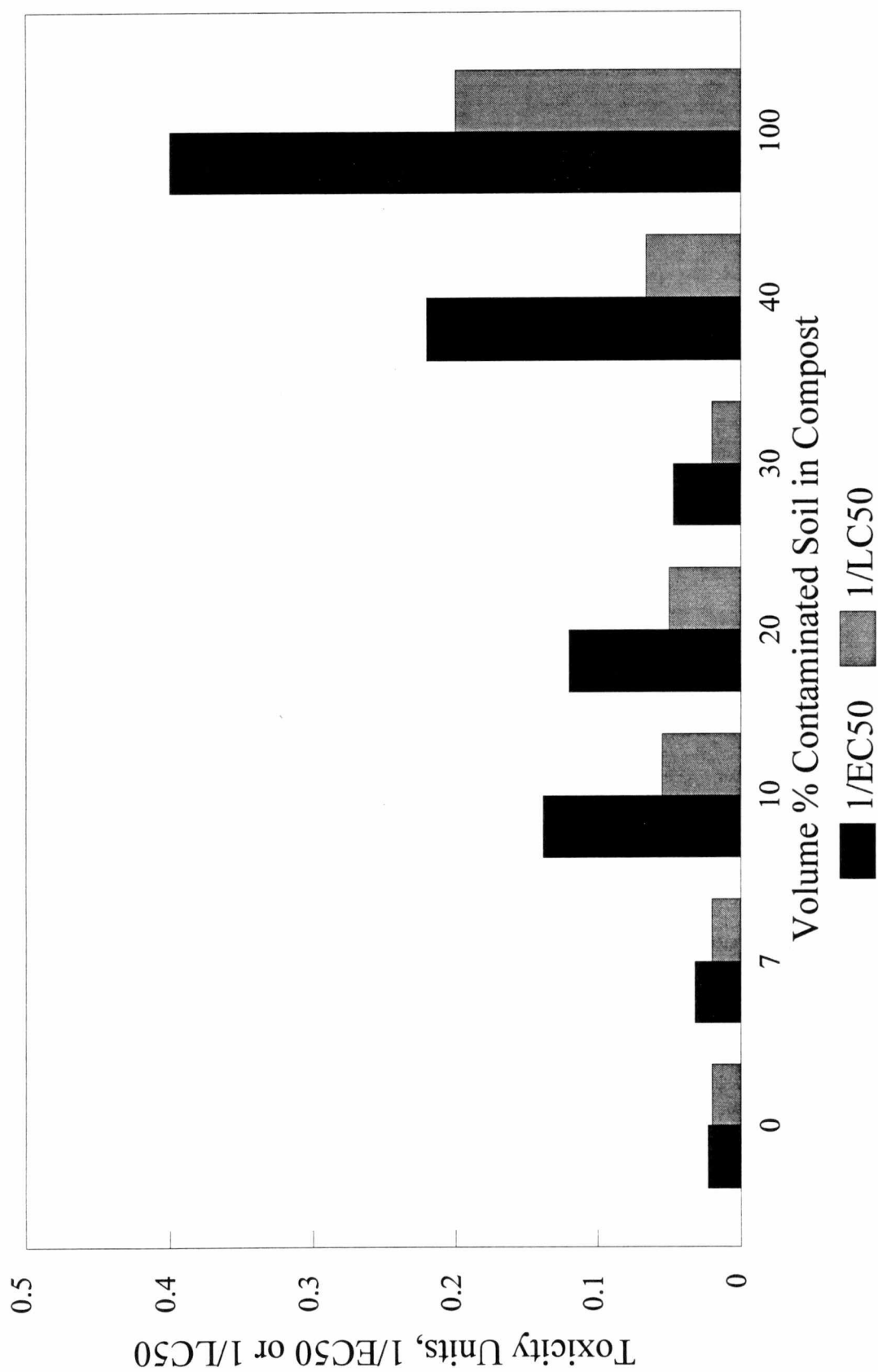


Figure 4.2 Leachate toxicity to *C. dubia* at Day 90

30% compost pile. Both lower amounts of soil (and correspondingly higher percentage of amendments) and longer composting times were important determinants in decreasing the toxicity of the leachates in the composting experiments.

COMPARISON OF MUTAGENICITY AND TOXICITY TO *CERIODAPHNIA DUBIA*

By comparing Figure 2.1 with Figure 4.1, a correlation can be seen in the Ames mutagenicity and *Ceriodaphnia* toxicity for the time course study of the 10% contaminated soil compost. Both the TA 100 and $1/EC_{50}$ plots show a decrease at day 10 which is followed by an increase at day 20. The two plots then decline significantly by day 44 and continue to drop off through day 90. The relationship between the two tests is not as striking for the day 90 bar graphs (Figures 2.2 and 4.2), but a similar trend is evident. For the mutagenicity test, increasing soil percentage always correlated with increasing mutagenicity. The *Ceriodaphnia* toxicity graph shows that the 10 and 20% soil composts had greater toxicity than the 30% soil compost. However, the trend toward greater starting soil percentage corresponding to greater toxicity was visible. For both tests, a drop in composting efficiency was noted for the 40% soil compost.

CHAPTER 5

RISK ASSESSMENT AND CALCULATION OF PRELIMINARY REMEDIATION GOALS

INTRODUCTION

One of the objectives of this study was to evaluate the loading capacity of the selected compost mixture and optimize the process to bioremediate the greatest quantity of soil possible per unit time. The Army's goal is to return the remediated soil to the environment. In order to evaluate the feasibility of this goal, a risk assessment needs to be conducted on the residual contamination in the composted material to determine the potential adverse health effects associated with exposure.

In 1989, EPA published the Risk Assessment Guidance for Superfund, Volume I, Human Health Evaluation Manual, Part A (EPA, 1989). This guidance document provides a methodology for evaluating adverse human health effects associated with exposure to chemicals at Superfund sites. Specific objectives of the risk assessment process are to (1) provide an analysis of baseline risks and help determine the need for action at sites; and (2) provide a basis for determining levels of chemicals that can remain onsite and still be adequately protective of public health. The risk assessment process employs four steps: data collection and evaluation; exposure assessment; toxicity assessment; and risk characterization.

Data collection and evaluation involves gathering and analyzing chemical data from the various media at the site and identifying the substances which will be

the chemicals of concern.

The exposure assessment is conducted to estimate the magnitude of actual and/or potential human exposures, the frequency and duration of these exposures, and the pathways by which humans are potentially exposed. The exposure assessment is based on reasonable maximum estimates of exposure for current and future land-use assumptions.

The toxicity assessment is performed to weigh available evidence regarding the potential for particular contaminants to cause adverse effects in exposed individuals and to provide, where possible, an estimate of the relationship between the extent of exposure to a contaminant and the increased likelihood and/or severity of adverse effects.

The risk characterization is the final step of the risk assessment process. In this step, the toxicity and exposure assessments are summarized and integrated into quantitative and qualitative expressions of risk. The risk characterization endpoint is expressed as either (1) risk, which is an estimate of increased lifetime cancer risks (with an acceptable range of 10^{-6} to 10^{-4}) or (2) hazard quotient, which is an estimate of increased noncarcinogenic (systemic) effects (with an acceptable hazard of 1).

METHODOLOGY

Risks and hazard quotients are calculated for a site by determining the chemical concentration of the contaminated media, selecting an exposure pathway, selecting the appropriate EPA assigned toxicity values, and characterizing the risk. The standard equations are shown as follows:

$$\text{Risk} = \frac{C \times IR \times EF \times ED \times SF}{BW \times AT}$$

$$\text{Hazard} = \frac{C \times IR \times EF \times ED}{RfD \times BW \times AT}$$

Where:

Risk	= Increased cancer risk
Hazard	= Ratio to reference dose (non-carcinogenic)
C	= Concentration of contaminant in medium of concern
IR	= Ingestion Rate
EF	= Exposure Frequency in days per year
ED	= Exposure Duration
SF	= Slope Factor of contaminant of concern
RfD	= Reference Dose of contaminant of concern
BW	= Body Weight
AT	= Averaging Time (period over which exposure is averaged)

In these equations, IR, EF, ED, BW, and AT have assigned standard default values (EPA, 1991a) which are based on the exposure scenario selected. The only variables in the equations are the chemical concentration of the contaminated medium, and the slope factor or reference dose of the contaminant of concern. Because the slope factor or reference dose has an EPA assigned value through the Integrated Risk Information System (IRIS, 1994), the only remaining variable is the concentration of the chemical contaminant. Therefore, the risk or hazard quotient is solved as a function of the contaminant concentration.

As a supplement to Part A of the Human Health Evaluation Manual, Part B - Development of Risk-based Preliminary Remediation Goals, was published (EPA, 1991). This guidance document outlines the procedure used to develop risk-based preliminary remediation goals (PRGs) at Superfund sites.

The PRGs are calculated by algebraic manipulation of the risk assessment

equation. Because the risk and hazard index are determined as a function of chemical concentration, the chemical concentration can be solved as a function of risk or hazard. By establishing a predetermined acceptable risk or hazard level (10^{-6} for Group A and B carcinogens, 10^{-5} for Group C carcinogens, and a hazard index of 1), the equation can be solved as a "back-calculation" for the concentration in the medium of concern. Although the acceptable risk range is 10^{-6} to 10^{-4} , the target risk level is set at 10^{-6} for Group A and B carcinogens and at 10^{-5} for Group C carcinogens to compensate for multiple pathways and multiple contaminant exposures. The calculation is based on a "reasonable worse case" and therefore, even if multiple contaminants existed, the PRG should be adequately protective.

For human exposure, the primary contributor to risk is direct ingestion of the contaminated medium. In the development of PRGs, the primary pathway is selected because other pathways generally contribute only a small portion of the risk or hazard (usually an order of magnitude difference). For the explosives-contaminated soil at UMDA, inadvertent ingestion of the soil is the primary contributor to risk. The equation for calculating the ingestion rate for inadvertent soil ingestion during residential exposure is made up of two parts. The first part is exposure during childhood, which assumes 200 mg/day soil intake for a six-year period. The second part of the equation assumes 100 mg/day exposure for a 24-year period. This difference in intake for the child and adult makes it necessary to use an age-adjusted ingestion factor (IF), which is calculated to be 114 mg-yr/kg-day for a 30 year duration. The ingestion rate used in a commercial/industrial scenario is 50 mg/day for a 25-year exposure duration.

The original risk equations and the "back-calculated" equations for residential

and industrial exposure scenarios are shown in the following.

Residential Risk Equation:

$$\text{Risk} = \frac{\text{SF} \times \text{C} \times 10^{-6} \text{ kg/mg} \times \text{EF} \times \text{IF}}{\text{AT} \times 365 \text{ days/yr}}$$

$$\text{C} = \frac{\text{TR} \times \text{AT} \times 365 \text{ days/yr}}{\text{SF} \times 10^{-6} \text{ kg/mg} \times \text{EF} \times \text{IF}}$$

Where:

<u>Parameter</u>	<u>Default Value (units)</u>
C =	unknown in (mg/kg)
TR =	10^{-6} or 10^{-5} target risk (unitless)
SF =	chemical specific (mg/kg-day) ⁻¹
AT =	70 (yr)
EF =	350 (days/yr)
IF =	114 (mg-yr/kg-day)

Industrial Risk Equation:

$$\text{Risk} = \frac{\text{SF} \times \text{C} \times 10^{-6} \text{ kg/mg} \times \text{EF} \times \text{ED} \times \text{IR}}{\text{BW} \times \text{AT} \times 365 \text{ days/yr}}$$

$$\text{C} = \frac{\text{TR} \times \text{BW} \times \text{AT} \times 365 \text{ days/yr}}{\text{SF} \times 10^{-6} \text{ kg/mg} \times \text{EF} \times \text{ED} \times \text{IR}}$$

Where:

<u>Parameter</u>	<u>Default Value (units)</u>
C =	unknown (mg/kg)
TR =	10^{-6} target risk (unitless)
SF =	chemical specific (mg/kg-day) ⁻¹
AT =	70 (yr)
BW =	70 (kg)

EF	=	250 (days/yr)
ED	=	25 (yr)
IR	=	50 (mg/day)

A hazard-based PRG also needs to be calculated for residential and industrial scenarios. The more stringent of the hazard-based or risk-based value is used as the PRG. The original hazard equations and the "back-calculated" equations for residential and industrial exposure scenarios are shown in the following.

Residential Hazard Equation:

$$\text{Hazard} = \frac{C \times 10^{-6} \text{ mg/kg} \times \text{EF} \times \text{IF}}{\text{RfD} \times \text{AT} \times 365 \text{ days/yr}}$$

$$C = \frac{\text{HI} \times \text{RfD} \times \text{AT} \times 365 \text{ days/yr}}{10^{-6} \text{ mg/kg} \times \text{EF} \times \text{IF}}$$

Where:

<u>Parameter</u>	<u>Default Value (units)</u>
C	unknown (mg/kg)
HI	1 (unitless)
RfD	chemical specific (mg/kg-day) ⁻¹
AT	30 (yr)
EF	350 (days/yr)
IF	114 (mg-yr/kg-day)

Industrial Hazard Equation:

$$\text{Hazard} = \frac{C \times 10^{-6} \text{ kg/mg} \times \text{EF} \times \text{ED} \times \text{IR}}{\text{RfD} \times \text{BW} \times \text{AT} \times 365 \text{ days/yr}}$$

$$C = \frac{\text{HI} \times \text{RfD} \times \text{BW} \times \text{AT} \times 365 \text{ days/yr}}{10^{-6} \text{ kg/mg} \times \text{EF} \times \text{ED} \times \text{IR}}$$

Where:

<u>Parameter</u>	<u>Default Value (units)</u>
C =	unknown (mg/kg)
HI =	1 (unitless)
RfD =	chemical specific (mg/kg-day) ⁻¹
BW =	70 (kg)
AT =	25 (yr)
EF =	250 (days/yr)
ED =	25 (yr)
IR =	50 (mg/day)

RESULTS

PRGs were calculated for TNT, RDX, and HMX based on residential and industrial exposure scenarios. For each exposure scenario, a risk-based and hazard-based PRG was calculated for TNT and RDX. The risk-based PRGs are based on an acceptable risk level of 10^{-5} because TNT and RDX are Group C carcinogens. Only a hazard-based PRG could be calculated for HMX because it has been classified as a Group D non-carcinogen by EPA. All of the hazard-based PRGs are based on a hazard index of 1. TNT has a slope factor of 3×10^{-2} and a reference dose of 5×10^{-4} ; RDX has a slope factor of 1.1×10^{-1} and a reference dose of 3×10^{-3} . The reference dose for HMX is 5×10^{-2} (IRIS, 1994).

Residential and industrial scenarios were evaluated to determine potential uses and/or restrictions for the remediated soil. The residential exposure scenario is the most conservative exposure scenario, and thus residential PRGs are protective of human health under unrestricted use applications. Soil concentrations that do not meet residential PRGs, can be compared to industrial PRGs. Industrial PRGs are protective of human health where certain restrictions are met. Under the industrial scenario, children are not exposed, and exposure is limited to 250 days/yr for a 25-

year maximum. Therefore, the Army would need to maintain control of the property where the remediated soil was disposed in order to keep deed restrictions on the property that would guarantee that industrial use was preserved.

The results of the PRG calculations and the final soil concentrations for the various compost piles are shown in Tables 5.1 and 5.2, respectively. The lower of the risk-based or hazard-based value is used as the PRG for each exposure scenario. The lower value is marked in bold in Table 5.1. The residential PRGs are as follows: TNT is 135 mg/kg; RDX is 58 mg/kg; and HMX is 13,500 mg/kg. Using these residential PRGs, all of the compost piles have final concentrations that exceed the PRG for RDX and would not be protective of human health. For TNT, only the 7 and 10% piles are below the acceptable concentration of 135 mg/kg. The PRG for HMX is very high at 13,500 mg/kg, and the starting material (100% soil) was below the threshold level.

The industrial PRGs are higher than the residential PRGs at 1,005 mg/kg for TNT, 520 mg/kg for RDX, and 100,575 mg/kg for HMX. Even with the higher values, only the 7% and 10% compost piles have final concentrations of RDX that do not exceed the threshold limit of 520. The concentrations of TNT are well below the PRG for the volumes up to and including 30%. All of the compost piles are below the PRG for HMX.

DISCUSSION

Based on the PRG results for all of the explosive contaminants, none of the soils could be returned to the environment for a residential exposure scenario and only the 7 and 10% soil compost could be returned under an industrial exposure

Table 5.1. Residential and Industrial PRGs for TNT, RDX, and HMX.

Explosive	Residential PRG		Industrial PRG	
	Hazard-based	Risk-based	Hazard-based	Risk-based
TNT	135	213	1,005	1,906
RDX	810	58	6,034	520
HMX	13,500	NA	100,575	NA

All values are in mg/kg.

The values in bold are the lower values for the particular exposure scenario and are selected as the PRG.

Table 5.2. Initial and Final Concentrations of TNT, RDX, and HMX for Compost Piles.

Soil Volume	Concentration (mg/kg)		
	TNT	RDX	HMX
7% Day 0	1240	762	220
7% Day 90	104	256	136
10% Day 0	4830	909	203
10% Day 90	97	395	153
20% Day 0	6550	1100	320
20% Day 90	143	647	241
30% Day 0	7950	1030	296
30% Day 90	222	778	319
40% Day 0	9410	1240	340
40% Day 90	2750	1440	376
100% Soil (uncomposted)	12,200	1380	409

scenario. The PRG values for HMX are at a concentration that is high enough to preclude the need for remediation of the soil. The results of the PRGs for TNT show that for an industrial use scenario composting is effective, but for a residential use scenario the effectiveness is less certain.

By focusing on the industrial PRG for TNT for comparative purposes, a pattern similar to that seen in the Ames test results is observed. This pattern is that the 30% soil compost appears to be the maximum soil loading volume before composting efficiency drops off. Based solely on the PRG for TNT, the compost piles containing up to and including 30% soil could be returned to the environment with the only restriction being that the property needs to be maintained under industrial use conditions.

Composting effectively reduced the TNT concentrations to a level where the soil could be returned to the environment (based on TNT only) for all of the compost piles except the 40% soil pile. However, the composting process did not sufficiently reduce the RDX concentrations to make them protective of human health under a residential exposure scenario, and under an industrial scenario, only for the 7 and 10% soils. Thus, most of the soil could not be returned to the environment based on the PRG methodology. Nearly a 3-fold reduction in RDX concentration was observed for the 7 and 10% soil composts, and approximately half of the RDX was removed in the 20% soil compost. Given sufficient time, composting may be able to lower the RDX concentration to a level where the soil could be returned to the environment under an industrial exposure for the 20 and 30% soil volume composts.

Ecological risk was not considered in this PRG evaluation because standard

default values and PRG equations have not been published for ecological receptors.

The literature review and this study have shown the toxic effects of TNT to ecological receptors at concentrations in the low ppm. Aquatic receptors are especially sensitive to TNT (Won, 1976). Before the soil is returned to the environment, an ecological impact study should be performed.

CHAPTER 6

CONCLUSIONS

- 1) Composting can effectively reduce the genotoxic effects of explosives in contaminated soil as demonstrated by the Ames test results.
- 2) Reduction in mutagenicity correlated with lower explosives concentrations as determined by HPLC and with reduced toxicity as determined by the *Ceriodaphnia dubia* assay.
- 3) The increase of the volume of soil and the corresponding decrease in amendment volume limits the effectiveness of the composting process to lower mutagenicity. Thirty percent soil appears to be the maximum volume for effective composting.
- 4) Although a correlation of genotoxic response and chemical concentration was observed, a direct relationship was not. While chemical concentration declined steadily throughout the study, genotoxic response showed an increase during the first 20 days before eventually declining. The final (day 90) results showed the best correlation, as increasing soil percentage in the compost piles resulted in increased toxicological response.

- 5) The majority of the mutagenicity detected in acetonitrile extracts could not be accounted for as determined by the amount of TNT and metabolites observed during HPLC analysis and the corresponding known mutagenic rate of the explosives. The discrepancy between the predicted and observed rate of mutagenicity increased with composting time.
- 6) Because mutagenic response did not correlate with chemical detection during the first 20 days of composting, there is a need for toxicological verification of a measured chemical reduction in a bioremediation project where the transformation products are unknown.
- 7) The success of this and the previous composting study in different geographic locations and using different amendment mixtures, suggests that composting of explosives as a remediation alternative may be feasible on a widespread basis.
- 8) The risk assessment showed that the 30% soil was the maximum volume which would reduce the concentration of TNT in the soil to a level at which it could be safely returned to the environment under an industrial use scenario. However, the RDX concentrations were above industrial use PRGs for all but the 7% and 10% soil composts and would require additional remediation or composting time before being returned to the environment.

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APPENDIX

Summary of Results of *Ceriodaphnia* Toxicity Tests
of Compost Leachates

Test Date	Sample	Leachate Conc. (%)	Survival (%)	Repro. (Mean $\pm$ SD)
Oct 18	Control	100	100	25.0 $\pm$ 4.5
"	CCLT	90	100	22.3 $\pm$ 2.7
"	"	70	90	22.3 $\pm$ 2.2
"	"	50	100	22.3 $\pm$ 3.8
"	"	30	90	22.3 $\pm$ 2.1
"	"	10	100	22.0 $\pm$ 4.4
			100	21.5 $\pm$ 3.9
"	7% cont, 0 d	90	0	-- $\pm$ --
"	"	70	0	-- $\pm$ --
"	"	50	0	-- $\pm$ --
"	"	30	0	-- $\pm$ --
"	"	10	50	0.0 $\pm$ --
"	"	5	100	0.2 $\pm$ 0.4
Nov 1	Control	100	100	30.8 $\pm$ 9.1
"	10% noncon., 0d	20	100	12.6 $\pm$ 2.7
"	"	10	100	24.3 $\pm$ 2.5
"	"	5	100	29.8 $\pm$ 4.0
"	"	2.5	100	32.2 $\pm$ 4.2
"	"	1	90	30.2 $\pm$ 3.8
"	40% cont., 0 d	20	0	-- $\pm$ --
"	"	10	0	-- $\pm$ --
"	"	5	0	-- $\pm$ --
"	"	2.5	100	3.8 $\pm$ 1.9
"	"	1	100	17.9 $\pm$ 3.8
"	"	0.5	0	-- $\pm$ --

Test Date	Sample	Leachate Conc. (%)	Survival (%)	Repro. (Mean $\pm$ SD)
"	30% cont., 0 d	20	0	-- $\pm$ --
"	"	10	0	-- $\pm$ --
"	"	5	10	0.0 $\pm$ --
"	"	2.5	100	0.0 $\pm$ --
"	"	1	100	16.0 $\pm$ 4.9
"	"	0.5	0	-- $\pm$ --
"	20% cont., 0 d	20	0	-- $\pm$ --
"	"	10	0	-- $\pm$ --
"	"	5	20	0.0 $\pm$ --
"	"	2.5	90	0.0 $\pm$ --
"	"	1	100	13.1 $\pm$ 4.4
"	"	0.5	20	12.0 $\pm$ 16.9
Nov 14	Control	100	90	28.9 $\pm$ 3.3
"	Noncon., 10 d	20	30	1.3 $\pm$ 1.5
"	"	10	90	9.3 $\pm$ 3.3
"	"	5	90	15.9 $\pm$ 3.6
"	"	2.5	100	27.5 $\pm$ 4.8
"	"	1	100	39.4 $\pm$ 7.5
"	"	0.5	100	39.5 $\pm$ 9.2
"	10% cont., 10 d	20	0	-- $\pm$ --
"	"	10	0	-- $\pm$ --
"	"	5	80	1.0 $\pm$ 1.9
"	"	2.5	90	3.4 $\pm$ 1.3
"	"	1	90	13.9 $\pm$ 7.5
"	"	0.5	100	36.0 $\pm$ 7.2
"	100% cont.	5	100	0.8 $\pm$ 1.6
"	"	1	100	22.9 $\pm$ 2.6

Test Date	Sample	Leachate Conc. (%)	Survival (%)	Repro. (Mean $\pm$ SD)
"	"	0.5	100	24.1 $\pm$ 2.4
"	"	0.1	100	24.2 $\pm$ 6.3
"	"	0.05	100	21.1 $\pm$ 9.8
Dec 6	Control	100	90	29.9 $\pm$ 10.2
"	10% cont.	20	0	-- $\pm$ --
"	"	10	0	-- $\pm$ --
"	"	5	0	-- $\pm$ --
"	"	2.5	0	-- $\pm$ --
"	"	1	70	0.0 $\pm$ 0
"	"	0.5	100	10.9 $\pm$ 3.0
Feb 28	20% cont., 90 d	0.5	100	32.5 $\pm$ 5.4
"	"	1.0	100	31.8 $\pm$ 8.8
"	"	2.5	100	33.1 $\pm$ 7.9
"	"	5.0	90	28.6 $\pm$ 6.7
"	"	10.0	90	6.8 $\pm$ 1.9
"	"	20.0	90	0.4 $\pm$ 0.9
"	Control	--	80	27.3 $\pm$ 4.8
Mar 7	10% cont., 90 d	0.5	100	37.0 $\pm$ 9.2
"	"	1.0	100	34.5 $\pm$ 8.2
"	"	2.5	100	32.8 $\pm$ 10.8
"	"	5.0	100	21.1 $\pm$ 5.8
"	"	10.0	100	7.0 $\pm$ 2.5
"	"	20.0	40	3.0 $\pm$ 2.9
"	Control	--	100	29.9 $\pm$ 8.1
Mar 14	10% cont., 20 d	0.5	100	9.2 $\pm$ 6.6
"	"	1.0	90	2.8 $\pm$ 0.9
"	"	2.5	90	0.2 $\pm$ 0.4

Test Date	Sample	Leachate Conc. (%)	Survival (%)	Repro. (Mean $\pm$ SD)
"	"	5.0	70	0
"	"	10.0	0	-- $\pm$ --
"	"	20.0	0	0
"	Control	--	100	23.8 $\pm$ 3.3
Mar 14	Noncon., 20 d	0.5	90	19.9 $\pm$ 9.9
???	"	1.0	80	19.9 $\pm$ 8.9
"	"	2.5	100	13.8 $\pm$ 6.4
"	"	5.0	100	2.4 $\pm$ 2.1
"	"	10.0	60	0.5 $\pm$ 1.2
"	"	20.0	70	0
"	Control	--	100	23.8 $\pm$ 3.3
Mar 20	10% cont., 44 d	0.5	90	26.4 $\pm$ 12.6
"	"	1.0	80	33.0 $\pm$ 8.6
"	"	2.5	80	12.3 $\pm$ 7.3
"	"	5.0	70	8.9 $\pm$ 5.4
"	"	10.0	40	4.8 $\pm$ 5.1
"	"	20.0	10	0
"	Control	--	100	38.6 $\pm$ 4.0
Apr 4	10% cont., 10 d	0.5	90	18.9 $\pm$ 4.1
"	"	1.0	100	4.2 $\pm$ 1.9
"	"	2.5	90	1.2 $\pm$ 1.6
"	"	5.0	60	0
"	"	10.0	0	0
"	"	20.0	0	0
"	Control	--	100	24.3 $\pm$ 3.0
Apr 11	30% cont., 90 d	0.5	90	34.8 $\pm$ 3.2
"	"	1.0	100	35.1 $\pm$ 5.3

Test Date	Sample	Leachate Conc. (%)	Survival (%)	Repro. (Mean $\pm$ SD)
"	"	2.5	100	37.2 $\pm$ 4.1
"	"	5.0	100	36.7 $\pm$ 4.6
"	"	10.0	100	36.8 $\pm$ 6.8
"	"	20.0	100	24.9 $\pm$ 6.0
Apr 11	40% cont., 90 d	0.5	100	28.0 $\pm$ 8.9
"	"	1.0	100	25.0 $\pm$ 6.2
"	"	2.5	100	24.3 $\pm$ 7.6
"	"	5.0	100	13.4 $\pm$ 2.9
"	"	10.0	100	0.0 $\pm$ --
"	"	20.0	0	-- $\pm$ --
"	Control	--	90	30.1 $\pm$ 7.8
May 2	Noncon., 90 d	10.0	100	34.8 $\pm$ 11.8
"	"	20.0	100	35.6 $\pm$ 4.9
"	"	30.0	90	24.5 $\pm$ 10.7
"	"	40.0	90	21.8 $\pm$ 12.2
"	"	50.0	100	17.6 $\pm$ 12.2
"	Control	--	100	41.1 $\pm$ 5.7 $\pm$
May 9	7% cont., 90 d	10.0	100	28.2 $\pm$ 6.7
"	"	20.0	100	27.0 $\pm$ 4.0
"	"	30.0	100	19.5 $\pm$ 6.6
"	"	40.0	100	9.6 $\pm$ 6.1
"	"	50.0	100	9.3 $\pm$ 5.8
"	Control	--	90	36.0 $\pm$ 8.2
May 30	30% cont., 90 d	10.0	100	23.0 $\pm$ 4.2
"	"	20.0	90	14.6 $\pm$ 9.9
"	"	30.0	100	0.6 $\pm$ 1.1
"	"	40.0	100	0.0 $\pm$ --

Test Date	Sample	Leachate Conc. (%)	Survival (%)	Repro. (Mean $\pm$ SD)
"	"	50.0	70	0.0 $\pm$ --
"	Control	--	100	26.5 $\pm$ 3.5
Jun 6	noncon., 44 d	10.0	100	36.3 $\pm$ 9.5
"	"	20.0	100	39.7 $\pm$ 4.4
"	"	30.0	100	36.3 $\pm$ 3.9
"	"	40.0	90	31.8 $\pm$ 9.7
"	"	50.0	100	28.0 $\pm$ 8.4
"	Control	--	100	34.6 $\pm$ 6.6

VITA

Kevin Stewart Ironside was born in Knoxville, Tennessee, on June 7, 1963, the son of David Stewart Ironside and Barbara Barnes Ironside. Kevin remained in Knoxville where he attended Shannondale Elementary School and Gresham Junior High School and graduated from Central High School in 1981. He entered the University of Tennessee, Knoxville in September 1981 and received a Bachelor of Arts with a major in Microbiology in June 1985. In September 1985 he began work with the Health and Safety Research Division at Oak Ridge National Laboratory (ORNL) as a research assistant. In August 1988, he reentered the University of Tennessee at Knoxville while continuing employment at ORNL. In August 1991 he began work at the Hazardous Waste Remedial Actions Program (HAZWRAP) in the field of risk assessment. He received the Master of Science Degree with a major in Life Sciences, a concentration in Environmental Toxicology, in May 1994. He is currently employed with an environmental consulting firm in Oak Ridge, Tennessee as a Project Manager and toxicologist in support of environmental restoration programs at hazardous waste sites.