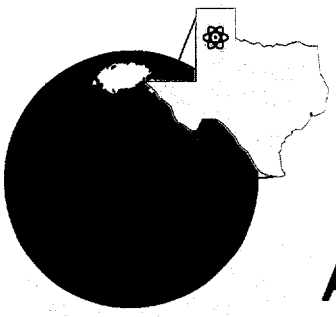


ANRCP-1999-1
January 1999

Amarillo National Resource Center for Plutonium

A Higher Education Consortium of The Texas A&M University System, Texas Tech University, and The University of Texas System

Bioremediation of RDX in the Vadose Zone Beneath the Pantex Plant

Trina L. Shull, Gerald E. Speitel, Jr., and Daene C. McKinney
Department of Civil Engineering
The University of Texas

Edited by

Angela L. Woods
Technical Editor

600 South Tyler • Suite 800 • Amarillo, TX 79101
(806) 376-5533 • Fax: (806) 376-5561
http://www.pu.org

DISCLAIMER

ANRCP-1999-1

AMARILLO NATIONAL RESOURCE CENTER FOR PLUTONIUM/
A HIGHER EDUCATION CONSORTIUM

Final Thesis Report on

Bioremediation of RDX in the Vadose Zone Beneath the Pantex Plant

Trina L. Shull, Graduate Research Assistant
Gerald E. Speitel, Jr., Professor
Daene C. McKinney, Associate Professor

Department of Civil Engineering
Center for Research in Water Resources
University of Texas at Austin
Austin, TX 78712

Submitted for publication to

ANRC Environmental Program

January 1999

EXECUTIVE SUMMARY

Background

The presence of dissolved high explosives (HE), in particular RDX and HMX, is well documented in the perched aquifer beneath the Pantex Plant, but the distribution of HE in the vadose zone has not yet been well defined. Although current remediation activities focus on the contamination in the perched aquifer, eventually regulatory concern is likely to turn to the residual contamination in the vadose zone. Sources of HE include the infiltration of past wastewater discharges from several HE-processing facilities through the ditch drainage system and leachate from former Landfill 3. With limited existing data on the HE distribution in the vadose zone and without preventive action, it must be assumed that residual HE could be leached into infiltrating water, providing a continuing supply of contamination to the perched aquifer. The purpose of this project was to more closely examine the fate and transport of HE in the vadose zone through mathematical modeling and laboratory experimentation. In particular, this report focuses on biodegradation as one possible fate of HE. Biodegradation of RDX in the vadose zone was studied because it is both present in highest concentration and is likely to be of the greatest regulatory concern.

Objectives

This study had several objectives:

- Determine if indigenous soil organisms are capable of RDX biodegradation;
- Determine the impact of electron acceptor availability and nutrient addition on RDX biodegradation;
- Determine the extent of RDX mineralization (i.e., conversion to inorganic carbon) during biodegradation; and
- Estimate the kinetics of RDX biodegradation to provide information for mathematical modeling of fate and transport.

Experimental Approach

Biodegradation studies were conducted on soil obtained from a depth of 55 to 60 feet at location 10, which is near Building 12-43 in Zone 12. The soil had RDX loadings of 10-12 mg/kg. RDX biodegradation was studied in batch experiments using sealed hypovials spiked with ^{14}C-RDX. The use of ^{14}C-RDX permitted measurement of RDX disappearance as well as the formation of water-soluble intermediates and inorganic carbon. Through fractation procedures using gas purging and trapping, and water and ether extraction of the soil, ^{14}C-RDX and its degradation products were measured over time. Three electron acceptor conditions were studied: aerobic, microaerobic, and anoxic. The hypovial headspace contained ambient air for the aerobic experiments, a 3% oxygen in nitrogen gas mixture for the microaerobic experiments, and nitrogen gas for the anoxic experiments. The impact of two nutrient sources, phosphorus and biodegradable organic carbon, alone and in combination also was examined. The fate of ^{14}C-RDX was followed in experiments by sacrificing replicate hypovials for analysis over incubation periods of up to 134 days.

In addition to radiochemical analyses, soil in selected hypovials was extracted and analyzed for total RDX by HPLC. The intent of these measurements was to examine the correspondence between the fate of the radiolabeled RDX and that of the unlabeled RDX originally in the soil. Of particular concern was establishing that the rate of ^{14}C-RDX biodegradation was comparable to the biodegradation rate of all RDX present.

Experimental Results

RDX degraders are indigenous to the contaminated soil located in the unsaturated zone, and they degrade RDX to a significant extent under anoxic and microaerobic conditions. Little biotransformation was observed in the experiments conducted in an aerobic environment. The addition of phosphorus had little effect on the removal rate of RDX, except in the microaerobic experiment and anoxic experiments with biodegradable organic carbon addition. The addition of a biodegradable organic carbon source significantly increased the rate of RDX biodegradation. Mineralization of RDX by the indigenous microorganisms also occurred in the vials. Under microaerobic and anoxic conditions, 40 to 70% of the ^{14}C-RDX was degraded during the experiments. The extent of mineralization varied as a function of the type of nutrient(s) added and the environmental conditions. At the end of the incubation period, mineralization of ^{14}C-RDX ranged from 13 to 59% under microaerobic and anoxic conditions, with most values falling in the range of 30 to 50%. Therefore, a significant fraction of the RDX was completely destroyed, although some unidentified organic intemerdiates were clearly produced during RDX biodegradation.

Loss of original RDX from the soil was measured using HPLC analysis, and in all cases, the removal of unlabeled RDX was somewhat greater than the removal of the ^{14}C-RDX. Therefore, the ^{14}C-RDX data reasonably approximated the fate of all the RDX, and could be used to calculate degradation rate constants. An examination of the data indicated that degradation was a first-order reaction with respect to the RDX loading on the soil; therefore, a first-order equation was fitted to each data set to estimate the rate constant. Another way of representing degradation rate constants for first-order reactions is through calculation of half-lives. The rate constants and half-lives are summarized in Table ES-1. The half-lives for RDX degradation under anoxic and microaerobic conditions ranged from 40 to 70 days, without the addition of a biodegradable organic carbon source. With biodegradable organic carbon addition, the half-lives decreased to less than 40 days.

In comparison, the half-lives for aerobic degradation with and without phosphorus were 690 and 1390 days, respectively. Biodegradation rates were very slow under aerobic conditions, resulting in the model fitting the data poorly (i.e., small R^2) and the 95% confidence intervals for the rate constants being very broad. In one experiment, the confidence interval included zero, indicating that the value of the rate constant was not statistically different from zero. Thus, the estimated values of the half lives under aerobic conditions have a large error associated with them; in particular, the half lives could be much longer than the estimates listed above.

Table ES-1: Summary of Degradation Rate Data

Experiment Name	First Order Degradation Rate Constants (day^{-1})	95% Confidence Interval (Lower, Upper)	R^2	Half-life for RDX Degradation (days)
Aerobic	0.001	0.0002, 0.002	0.19	690
Aerobic w/ Phosphorus	0.0005	-0.003, 0.001	0.06	1390
Microaerobic	0.012	0.010, 0.013	0.91	60
Microaerobic w/ Phosphorus	0.016	0.014, 0.018	0.92	43
Anoxic	0.010	0.007, 0.012	0.68	68
Anoxic w/ Phosphorus	0.011	0.009, 0.013	0.83	64
Anoxic w/ Carbon	0.019	0.015, 0.024	0.83	36
Anoxic w/ Carbon & Phosphorus	0.018	0.014, 0.022	0.78	38

Conclusions and Recommendations

- RDX degraders are indigenous to the Pantex soil tested. Because only one location and depth were tested, tests at additional locations at Pantex should be conducted to confirm that the findings of this research apply across the site.
- Microaerobic or anoxic conditions in the soil pore gas are required to promote significant biodegradation of RDX. Biodegradation of RDX under aerobic conditions is negligible.
- Significant mineralization of RDX is possible, although intermediate organic chemicals also are clearly formed.
- Phosphorus addition appears to be worthwhile only in combination with the addition of a biodegradable organic carbon source.
- Addition of a biodegradable organic carbon source can accelerate RDX biodegradation and can serve as a means of changing the ambient aerobic conditions in the soil to anoxic conditions.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION

1.1 PROBLEM IDENTIFICATION

The U.S. Department of Energy (DOE) Pantex Plant near Amarillo, Texas has identified the high explosives RDX and HMX as soil and groundwater contaminants. Previous remediation activities for the removal of both of these explosives have emphasized pump and treat applications for the perched aquifer groundwater about 270 feet below the Plant. However, the potential for the vadose zone to act as a continuous source of contamination and the possibility that RDX and HMX in the perched aquifer may migrate into the Ogallala aquifer is of particular concern. Thus, research is being sponsored by the Amarillo National Resource Center for Plutonium to determine the feasibility of in situ remediation within the vadose zone above the perched aquifer.

RDX and HMX are common pollutants that arise from the manufacturing, handling, and demilitarization of munitions. These contaminants are released to the environment through the improper disposal of wastewater, the burning of substandard material, or the disarming of out-of-date munitions. Because of the toxicological properties of these compounds, growing concerns have arisen regarding their fate and transport in the environment. Concerns have also arisen because these chemicals are persistent and not easily degraded in the environmental.

The toxic effects of RDX have been investigated through the use of rats, dogs, miniature swine, and fish (Von Oettingen et al., 1949; Schnieder et al., 1977; Bentley et al., 1977). A majority of the studies found that RDX attacks the central nervous system. Limited research is available on the toxic effects of HMX; however, studies investigating exposure limits and tolerances suggest that rodents can handle much higher oral doses of HMX than RDX.

In humans, symptoms and clinical manifestations due to RDX poisoning include convulsions followed by loss of consciousness, muscular cramps, dizziness, headache, nausea, and vomiting (Yinon, 1993). In addition, explosives are readily adsorbed by the skin and can lead to methemoglobinemia and liver damage. As a result of these toxicological effects on humans, the U.S. Environmental Protection Agency has set drinking water health advisories for both RDX and HMX. Based on a lifetime exposure concentration from a drinking water source, the maximum exposure advisories for RDX and HMX are 0.1 and 2.0 mg/L, respectively (U.S. EPA, 1994a).

1.2 PANTEX PLANT - SITE BACKGROUND AND DESCRIPTION

This section gives a brief description of Pantex, its history, and the history of high explosives used at the site. A more extensive description of the facility and site contamination can be found in other publications (Battelle Pantex, 1994; Battelle Pantex, 1996).

1.2.1 Plant Description and History

The Pantex Plant is located on the high plains of the Texas Panhandle approximately 17 miles northeast of Amarillo, Texas (Figure 1.1). The facility consists of roughly 10,177 acres within Carson county. Land use around the plant is primarily agricultural with the remaining portion being industrial.

The Pantex Plant is a government-owned, contractor-operated facility (Batelle Pantex, 1996). The plant's industrial operations are conducted for the Department of Energy (DOE) by Mason & Hanger, the U.S. Army Corps of Engineers (COE), and Sandia National Laboratory. Pantex was

originally constructed as a conventional weapons plant at the beginning of World War II. In the early 1950's, the Pantex Plant was converted into a nuclear weapons assembly plant. Since the early 1990's, the mission of the Pantex Plant has consisted of nuclear weapons disassembly, evaluation of weapons, high explosives research and development, and interim storage of plutonium pits (Battelle Pantex, 1994). Today, Pantex is America's only nuclear weapons assembly and disassembly facility.

The Pantex facility is divided into several functional areas referred to as numbered zones (Figure 1-2). Zone 12 is the zone for weapons assembly-disassembly. Zone 4 is the weapons staging area. Zone 11 is the experimental explosive development facilities. Zones 15 and 13 are the drinking water treatment plant and sanitary wastewater treatment facility (WWTF), respectively. Administrative buildings and vehicle maintenance are located at Zone 16.

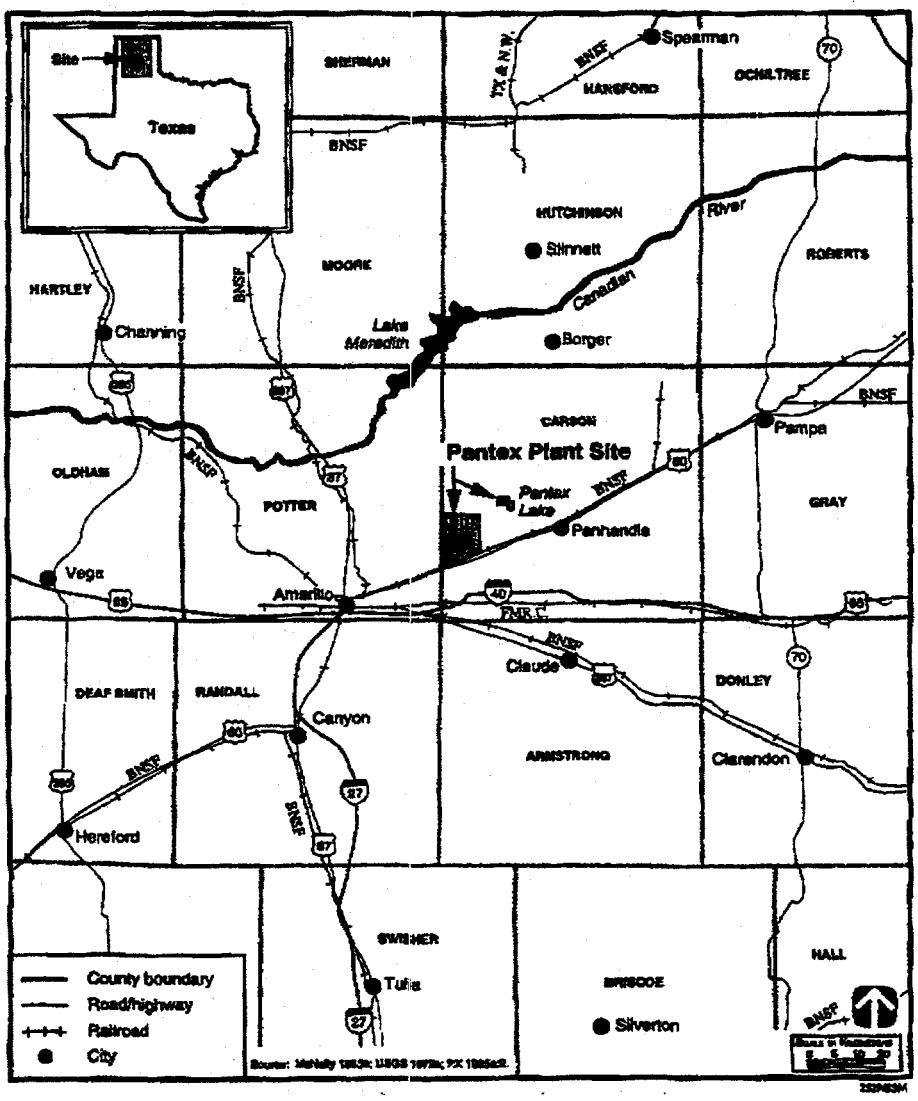


Figure 1.1: Pantex Plant Region Location (USDOE, 1996)

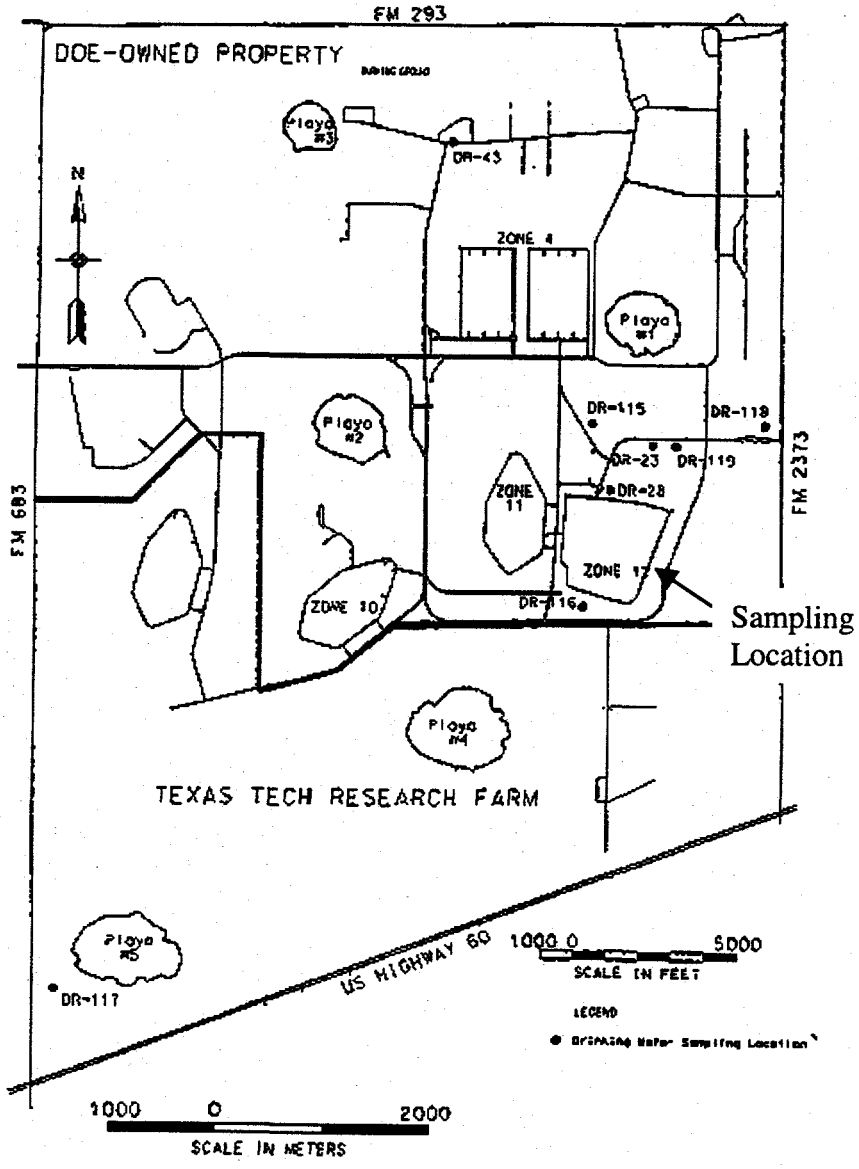


Figure 1.2: Pantex Plant Facility (Battelle Pantex, 1996)

1.2.2 High Explosives Contamination

Site information was obtained from the 1995 and 1996 Environmental Report for Pantex Plant. Both reports give a detailed layout of the site and show the extent of RDX and HMX contamination in the soil and groundwater. RDX has been detected in several groundwater-monitoring wells. Concentrations in 59 samples from 17 wells exceeded the risk reduction level for RDX (Battelle Pantex, 1996). Risk reduction levels are based on the likelihood of injury, disease, or death resulting from human exposure (real or potential) to chemical(s) and are specific for each site. In addition, the risk reduction levels represent the minimum clean-up levels that must be obtained during remediation. The current risk reduction level for RDX at Pantex is 0.026 mg/L for groundwater on site and 0.0077 mg/L for groundwater off site (Battelle Pantex, 1996; USACE, 1995). Samples from 237 perched aquifer wells were used to determine the lateral extent and concentration of RDX in the perched aquifer (Figure 1.3). The RDX levels in the perched aquifer wells were as high as 5 mg/L, and the perched aquifer was estimated to extend beyond the plant's boundary; however, the actual extent of RDX migration beneath the Pantex Plant is still unknown.

Data regarding soil contamination of high explosives at the Pantex site is limited. Some soil analyses are available from the Burning grounds, Playa #3, and Bushland control location (Battelle Pantex, 1997). At these locations, HMX was the only measurable high explosive in the soil during 1996. These results differ slightly from those for 1995, in that several measurable concentrations of 2,4,6-trinitrotoluene

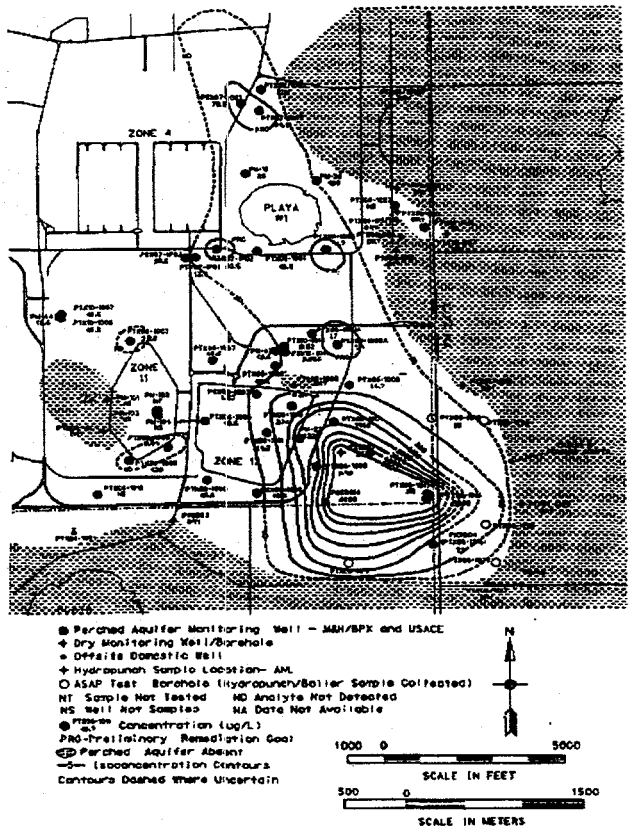


Figure 1.3: RDX Migration at Pantex Plant (Battle Pantex, 1996)

(TNT) was found in 1995 (Battelle Pantex, 1997). The only known data showing RDX contamination in Pantex soil was obtained from the Stoller Corporation via Texas Tech University. These data represent soil taken from location 10, which is near Building 12-43 in Zone 12 (Figure 1.2). The RDX contamination on the soil analyzed ranged from 6 mg/kg at a depth of 124 feet to as much as 27 mg/kg at a depth 4 feet below the surface. These concentrations were confirmed by soil analysis conducted during this study. These data are important because the location overlies the perched aquifer previously mentioned.

1.3 RESEARCH APPROACH

This research focused on a preliminary study of the biodegradation of RDX in the vadose zone at the Pantex site. RDX was chosen as the principal compound in this investigation for a number of reasons. First, RDX is readily available in ^{14}C-radiolabeled form, whereas HMX is not. This is significant because radiolabeled chemicals were to be used for the study. Second, RDX is present in substantially higher quantities at the Pantex site. Third, studies indicate that the human toxicity thresholds are lower for RDX. Finally, RDX has a greater solubility in water and is more mobile through soil than HMX.

1.4 OBJECTIVES

The objectives of the study were the following:

(1) Develop a batch technique for measuring biodegradation of RDX in unsaturated soil using ^{14}C-radiolabeled chemicals.
(2) Investigate the biodegradation of ^{14}C-RDX under different environmental conditions.
(3) Determine the effects of nutrient additions on biodegradation rates in soil.
(4) Determine the extent of mineralization of ^{14}C-RDX and quantify RDX losses from original soil samples.
(5) Estimate the kinetics of RDX biodegradation

2. LITERATURE REVIEW

This chapter discusses background information and reviews literature that deals with the bioremediation of RDX. The first section gives general information about the manufacturing and use of the contaminant. The second section provides chemical and physical properties of RDX. The third section discusses toxicity issues, and the fourth section summarizes pertinent articles regarding the biodegradation of the contaminant.

2.1 GENERAL INFORMATION

One of the most prominent explosives in the United States is hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). RDX was developed in the early 1900's but was not widely used until World War II. During World War II, the use of RDX increased, because of the need for a more powerful explosive than TNT. Since its development, RDX has been used in bombs, boosters, detonators, primers, plastic explosives, and torpedoes (Yinon, 1990).

Two different processes can be used to manufacture RDX, the Woolwich and the Bachmann. In the Woolwich process, hexamine is reacted with nitric acid to produce RDX (Yinon and Zitrin, 1993). This process produces RDX with only traces of a by-product, HMX. In the Bachmann process, hexamine is reacted at 75°C with an ammonium nitrate-nitric acid mixture in the presence of acetic acid and acetic anhydride (Yinon and Zitrin, 1993). This process produces RDX with about 6% of the HMX by-product. Both processes are used; however, the Bachmann process is better for large-scale production.

2.2 CHEMICAL AND PHYSICAL PROPERTIES

RDX is a colorless polycrystalline high explosive with a chemical formula of $C_3H_6N_6O_6$. It has several names such as Research Department Explosive, Royal Demolition Explosive, cyclonite, cyclotrimethylenetrinitramine, hexogen, and hexahydro-1,3,5-trinitro-1,3,5-triazine. It forms a ring that is made of alternating carbon and nitrogen atoms with nitro substituent groups on the ring nitrogens and hydrogen substituent groups on the ring carbons. The structure can be seen in Figure 2.1. RDX is as a white crystalline solid and has a high degree of stability in storage. It is considered the most powerful and brisant of the military high

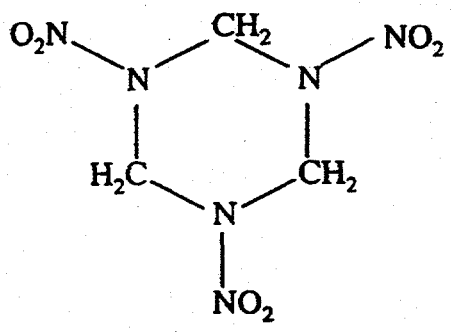


Figure 2.1: Chemical Structure of RDX

explosives, because it has 150% more power than TNT and is much easier to detonate. RDX is less sensitive than mercury fulminate or nitroglycerine. However, it should not be used alone, since it can be set off by a relatively light shock. Therefore, it is classified as a Class A explosive and must be shipped with no less than 10% water (Gibbs, 1980).

Table 2.1 summarizes the physical and chemical properties of RDX. RDX is fairly insoluble in water when compared, for example, to benzene, which has a solubility of approximately 1750 mg/L in water. This chemical does have an affinity for organic matter. RDX's K_{oc} is reported to be approximately 100 L/kg, which is similar to that of benzene at 83 L/kg. However, the magnitude of attraction is relatively low when

compared to pyrene, which has a K_{oc} of 38,000 L/kg. RDX also has a low Henry's constant of about 1.96×10^{-11} atm-m^3/mole at 25°C. The low Henry's constant shows that RDX is essentially nonvolatile. For instance, the Henry's constant of benzene, a volatile chemical, is reported to be 5.59×10^{-3} atm-m^3/mole.

2.3 TOXICITY

Several studies have been conducted to determine the effect of RDX and its threshold limit values. These studies have looked at the effects on rats, dogs, green algae, fathead minnows, and humans. Based on the findings, RDX is toxic to all life forms at high enough doses.

Table 2.1: Chemical and Physical Properties of RDX (Rosenblatt et al. 1991)

Empirical Formula	$C_3H_6N_6O_6$
Molecular Weight (g/mole)	222.15
Melting Point (°C)	205
Density (g/cm^3)	1.83
Water Solubility (mg/L, 25°C)	60
Vapor Pressure (torr, 25°C)	4.03×10^{-9}
K_H (atm-m^3/mole, 25°C)	1.96×10^{-11}
Diffusion Coefficient (air) (cm^2/s)	0.074
Diffusion Coefficient (water) (cm^2/s)	7.15×10^{-6}
Log K_{ow} (L/kg)	0.87, 0.81, 0.86
Log K_{oc} (L/kg)	2.00

In mammals, RDX has adverse affects on the central nervous system. Symptoms include loss of consciousness, convulsions, vomiting, confusion, skin lesions, and renal failure. In the case of human intoxication, recoveries were completed within days to several months after exposure (Rosenblatt et al., 1991). RDX can enter the body by inhalation, ingestion, or skin adsorption. Medical evaluations of workers chronically exposed to RDX at low levels have shown no abnormalities of hemotalogic, hepatic, or renal systems (Hathaway, 1977; Rosenblatt, 1991).

Most data available on the human toxicity of RDX has come from studies done on munitions plant workers and Vietnam soldiers. In 1954, Vogel described RDX poisoning in German munitions workers who had handled finely pulverized RDX powder (Yinon, 1990). Sunderman reported that fumes created during the Bachmann process produced skin lesions (transient erythema and edema about the eye) in munitions workers (1944; Yinon, 1990). Tsa and Lee reported RDX ingestion at a dinner party after having eaten from cooking bowls which had been used 3 years earlier to mix RDX with other chemicals (1982; Yinon 1990). During the Vietnam War, a compound called C-4 was used as a field cooking fuel. This compound was made of 91% RDX, 2.1% polyisobutylene, 1.6% motor oil, and 5.3% inert plasticizers. Several cases of intoxication form C-4 have been reported (Ketel and Hughes, 1972; Merill, 1968; Hollander and Colbach 1969).

The ultimate goal of human toxicological evaluations and epidemiology of RDX is to determine threshold limits. The Environmental Protection Agency-Office of Water has developed health advisories for RDX. Longer-term advisories are based on an exposure duration of approximately 7 years or 10% of an individuals lifetime RDX (U.S. EPA, 1994a). Table 2.2 gives the health advisories.

Toxicity effects in animals included hyperirritability, convulsions, and increase mortality. Von Oettingen et al. (1949) reported that toxic effects were evident

Table 2.2: Health Advisories for RDX (EPA, 1994a)

Exposure Duration	Exposure Concentration of RDX (mg/L)
One day	0.1
Ten day	0.1
Longer-term Child	0.1
Longer-term Adult	0.35
Lifetime	0.002

in rats when daily exposure levels were 50 to 100 mg of RDX per kg of body weight; dogs showed effects at 25 to 50 mg per kg of weight. Kaczorowshi and Syrowatka (1960) found the LD_{50} in rats to be 152.6 mg/kg of RDX. The metabolism of RDX in rats and miniature swine was traced using radiolabeled ^{14}C-RDX by Schnieder et al. (1977). This study found that RDX metabolism primarily takes place in the liver.

The effects of RDX on aquatic plants and animals are less researched. In 1977, Bentley et al. (1977) evaluated the aquatic toxicity of RDX using blue gill, channel catfish, and fathead minnow. RDX exerted an acute, toxic effect on all freshwater fish species tested between the ranges of 3.6 to 6.4 mg/L (Bentley, 1977; Yinon 1990). A study by Sullivan et al. (1979) recommended a 24-hour average concentration of no more than 0.3 mg/L of RDX to protect aquatic life. Two separate studies by Burton et al. (1994a, 1994b) examined the acute and chronic toxicity of RDX on fathead minnows and the toxicity of RDX on freshwater green algae. For fathead minnows, an acute 96-hour LD_{50} was predicted to occur at 12.7 mg/L at 25° C. For the same study, the no observable effect concentration (NOEC) was 1.4 mg/L and the lowest observable effect concentration (LOEC) was 2.4 mg/L. For green algae, the NOEC and LOEC was based on reductions in algal cell density. The NOCE was determined to be 0.5 mg/L and the LOCE was determined to be 4.8 mg/L. Acute toxicity was not observed during this study; Burton et al. believed solubility limits for RDX were the reason.

A final factor to consider when dealing with RDX is that the breakdown products formed by biological degradation may be more toxic than RDX itself. McCormick et al. (1981) proposed a biological breakdown pathway under anaerobic conditions. Several of the chemicals formed, such as dimethylnitrosamine, azoxymethane, and hydrazine, are known mutagens, carcinogens, or both.

2.4 TRANSPORT OF RDX

Potential environmental exposure to RDX exists in manufacturing plants where RDX dust is generated, in nearby waterways into which RDX might have been discharged, and near RDX demilitarization sites where RDX could have reached portable groundwater (Yinon, 1990). Understanding the transport of RDX provides valuable information for its remediation. In soil matrixes, adsorption and desorption properties significantly affect the transport of RDX.

2.4.1 Sorption/Desorption

The transport of RDX through a soil matrix is a function of several properties. From soil partitioning equations, the concentration of chemical on the soil at equilibrium is given by:

$$S = K_d C$$

where: S = Concentration on the soil (mg/kg)

C = Concentration in aqueous solution (mg/L)
K_d = Distribution Coefficient (L/kg)

K_d is equal to the fraction of organic carbon in the soil (f_{oc}) times the organic carbon-partitioning coefficient (K_{oc}). From Table 2.1, the K_{oc} for RDX is 100 L/kg. Therefore, if Pantex soil has about 1% organic matter, the K_d would be 1 L/kg and the concentration of RDX on the soil would be 1 times the concentration of RDX in the pore water at equilibrium. This K_d is small relative to that of pyrene, which would be about 380 L/kg and therefore a highly absorbable chemical.

A study by Spanggord et al. (1990) characterized sorption properties of RDX onto sediments and biomass. This study found an order of magnitude difference between K_{oc} values; however, the magnitudes were relatively small (42 to 167 L/kg) when compared to K_{oc}'s of other chemicals that adsorbed readily to particles. Thus, Spanggord indicated that RDX displays very little tendency to absorb onto any particle. A later study by Haderlein et al. (1996) concluded that RDX has a low affinity for soil and tends to migrate through soil into groundwater.

The rate of sorption onto the soil particles may also play an important role in the transport of RDX. Brannon et al. (1992) tested the mobility of TNT, RDX, and PCB in various soils. The results noted that the pore water concentration of RDX was higher than that of TNT or PCB in all soil types. This was believed to be caused by a slow adsorption and possibly desorption of RDX. Slow adsorption was confirmed by the fact that concentration of RDX in the pore water decreased over time. A more recent article by Selim et al. (1995) showed similar results. In this study, tests were run on three different soils in packed soil columns under steady state flow. For RDX, only limited retention was observed in all columns.

A third consideration in the transport of RDX is competitive sorption. Because most contaminated soil usually has a mixture of compounds, competitive sorption could play a significant role in RDX's phase distribution. No articles were found regarding competitive sorption onto soil particles; however, a study by Burrows (1982) found that TNT displaces sorbed RDX from activated carbon.

2.5 FATE OF RDX

Several degradation processes affect the environmental fate of RDX. The two main abiotic processes are photolysis and hydrolysis. For this research, photolysis is important since Pantex soil was contaminated by wastewater ditches and materials mishandling on the ground surface. Beneath the ground, the biological degradation behavior of the RDX is an important parameter that governs fate of the compound and the design of a treatment system. The biological degradation of RDX may occur under anaerobic or aerobic conditions.

2.5.1 Photolysis of RDX

Photolysis is the degradation of a contaminant by sunlight. Because sunlight is required, photolysis occurs in surface waters and on exposed soil surfaces that have been contaminated with RDX. Aqueous solutions of both RDX and HMX were photolyzed slowly by Spanggord et al. (1990); the half-lives ranged from 1.1 to 12.5 days. The variations in the half-lives were due to geographic latitude and season. Ultra-violet (UV) radiation has also been used to degraded RDX. Burrows and Brueggemann (1986) showed that the degradation by UV follows first-order kinetics. The degradation constants were reported to be 0.212 and 0.197 min^{-1}. The ultimate fate of RDX in both

studies was compounds such as nitrite, ammonia, formaldehyde, and nitroso derivatives.

2.5.2 Aerobic Biodegradation of RDX

The biodegradation of RDX in the presence of oxygen has had varying success. Osmon and Klausmeier (1973) evaluated the feasibility of biological treatment of wastewater contaminated with high explosives. The focus was to isolate organisms that could use TNT, RDX, or ammonium picrate as a sole carbon source. Microorganisms were found to grow in the presence of these compounds, but no proof of RDX degradation by microorganisms was found. Some years later, Fernando and Aust (1991) found that *Phanerochaete chroysosporium*, a white rot fungus, could aerobically degrade RDX and TNT. This study was conducted with radiolabeled ^{14}C-RDX in both aqueous solution and soil; liquid scintillation counting and high performance liquid chromatograph (HPLC) were used to identify degradation products. In liquid culture, only 4% of the added ^{14}C-RDX was present and 66.6 ± 4.1% was released as $^{14}CO_2$. The experiments performed in soil showed slightly better degradation (76.0 ± 3.9%). The degradation of the RDX was through a lignin-degrading enzyme produced by the fungus. RDX and TNT were both mineralized; however, no metabolites were found in RDX degraded samples.

A composting study by Williams et al. (1992) investigated the degradation of RDX in aerated static piles. This study noted that RDX was significantly reduced; the half lives for RDX degradation were estimated to be 17.3 and 30.1 days for thermophilic and mesophilic condition, respectively. Microorganisms from the compost were not identified.

Griest et al. (1995) studied the chemical characterization and toxicological testing of windrow compost contaminated with explosives. Griest et al. looked at aerated and nonaerated windrow compost. They found that the nonaerated windrow method of composting was slightly more efficient than the aerated windrow method for reducing explosives concentrations in the composts (TNT, 99.9%; RDX, >99.7%; HMX, 98.5%) and in their leachates (>99.9%,>98.8%, and >97.5%, respectively).

Binks et al. (1995) isolated a bacterium, *Stenotrophomonas maltophilia* PB1, from contaminated soil that could use RDX as a sole source of nitrogen under aerobic conditions. The bacterium was isolated by using RDX as the sole source of nitrogen and supplying a carbon source. The carbon source was a mixture of three high biodegradable carbon compounds: glucose, succinate, and glycerol. Once isolated, Binks et al. measured the kinetics of RDX degradation by PB1. RDX degradation was found to correspond to cell growth. A comparison between the growth yields of PB1 grown on RDX versus NH_4Cl suggested that only half of the nitrogen from RDX was available to the bacterium. An analysis, by thin-layer chromatography, of metabolites from the cultures also suggested that only half of total nitrogen was used. Because RDX can provide three nitrogen atoms before the ring is cleaved, it is questionable as to whether the RDX ring was cleaved during this experiment.

2.5.3 Anaerobic/Anoxic Biodegradation of RDX

Several studies have illustrated that RDX can be degraded under anaerobic or anoxic conditions. One of the most referenced articles in the literature is by McCormick et al. (1981). They investigated the anaerobic and aerobic degradation of RDX in sewage sludge and proposed a pathway for RDX degradation. Concentration levels of RDX did not change when cultures were

inoculated with aerobic activated sewage sludge. However, a significant reduction of RDX concentrations was seen in experiments conducted with anaerobic sewage sludge. Under anaerobic conditions, RDX concentration levels of 50 or 100 mg/L disappeared completely with in 4 days. Some experiments were conducted with radiolabeled ^{14}C-RDX, and degradation products were separated using an elaborate process. Liquid scintillation counting was used to quantify ^{14}C-compounds in the gas, ether-soluble, and aqueous phases. Sample mixtures analyzed by GC/MS revealed several intermediates were formed during degradation. Figure 2.2 shows the proposed pathway for the anaerobic degradation of RDX. A list of identified products are as follows: hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX), hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine (DNX), hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX), hydrazine, 1,1-dimethylhydrazine, 1,2-dimethylhydrazine, formaldehyde, and methanol. Funk et al. (1993) studied the bioremediation of soils contaminated with TNT, RDX, and HMX under strict anaerobic conditions. The main focus of the research was on the degradation of TNT; however, the degradation of RDX was reported. Approximately 30 ppm of RDX was initially present in the contaminated soil. After an incubation of 24 days, RDX concentrations dropped below detectable levels, and no identifiable intermediates were detected. This result occurred only under an optimum pH of 6.5 and a temperature of 30° C.

An enteric bacterium *Morganella morganii*, isolated from contaminated soil by Kitts et al. (1994a), cometabolized both RDX and HMX. The metabolism of both explosives required a microaerobic environment, and the degradation occurred by reduction of the nitroso derivatives. The study also founded that nitrate and nitrite decreased degradation rates. In a second study, Kitts et al. (1994b) isolated three strains of enteric bacteria (*Providencia rettgeri* B1, *Morganella morganii* B2, *and*

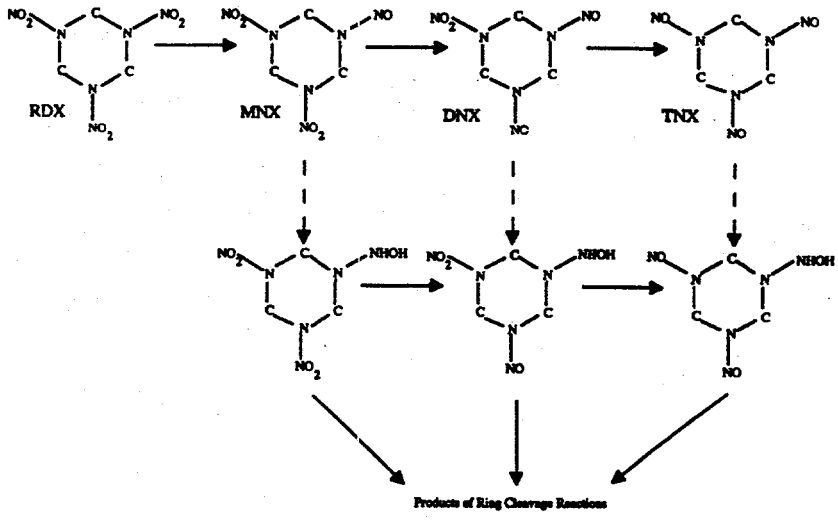


Figure 2.2: Proposed Pathway for the Anaerobic Biodegradation of RDX
(Source: McCormick et al., 1981)

Citrobacter freudii NS2) that could degrade RDX in pure culture. All strains needed to be under oxygen depleting conditions before degradation would occur. For the same study, experiments with radiolabeled ^{14}C-RDX were also conducted. These experiments separated RDX and nitroso deriviates from the aqueous solution by extracting them into ethyl ether. Compounds were confirmed by HPLC analysis.

The *M. morganii* culture had the lowest percentage ^{14}C-compounds and the lowest concentration of nitroso groups in the ether-soluble phase, which means this culture performed the best at degrading RDX. None of the isolated bacteria produced significant amounts of volatile compounds, and breakdown products produced during RDX degradation did not appear to adhere to cell material.

A study by Young et al. (1997a) examined the biotransformation of RDX by a consortium of bacteria found in horse manure. The experiments were performed in liquid cultures, and five bacteria were found to predominate in the consortium. The biotransformation of RDX by all of these bacteria occurred only in the anoxic stationary phase (Young et al., 1997a). Metabolites were identified from solutions degraded by the consortium as well as from solutions degraded with only the most effective bacterium, *Serratia marcescens*. Samples were extracted with ethyl acetate and analyzed by HPLC. In the consortium solution, DNX and TNX were not observed. However, these compounds were observed in the solution degraded by *S. marcescens*. Mass balances indicated that the first step of RDX biotransformation is the conversion to MNX by the isolate, *S. marcescens*, and the consortium (Young et al., 1997a). The ultimate goal of the research was to use the laboratory results to develop a model of the metabolism of explosive contaminants.

A follow up study by Young et al. (1997b) investigated the importance of amendment of known biodegraders and the type of nutrient source required in slurry reactors. The slurry reactors were reaction rate limited and biotransformation occurred on a time scale of days. Both a nutrient broth and corn steep liquor (50% protein, 20% fat) were tested. The corn steep liquor provided the best results and is an inexpensive byproduct of high fructose corn syrup manufacturing. This study focused heavily on identifying and separating kinetic rates for each breakdown path during biotransformation of RDX by different types of amended bacteria.

Light et al. (1997) studied the biological treatability of RDX-contaminated soil. This study used soil obtained from the Pantex Plant to make soil slurries and water extracts of contaminated soil. Four different electron acceptor conditions were tested: aerobic, nitrate-reducing, sulfate-reducing and methanogenic. RDX did not degrade under aerobic conditions; however, its presence did not inhibit microbial growth. Significant reduction in the RDX concentration was seen under nitrate-reducing conditions. In addition, there appeared to be an order of preference under the nitrate-reducing condition in the presence of many nitroaromatic compounds. TNT needed to be degraded before RDX would start to degrade, and RDX needed to be degraded before HMX would start to degrade. RDX was degraded under sulfate-reducing conditions; however, significant losses were only seen when RDX was the only contaminant present. Methanogenesis of RDX was unsuccessful except in low concentration aqueous solution (10 µg/L). Both sulfate-reducing and methanogenic bacteria appeared to be inhibited by the complex mixture of high explosives in Pantex soil.

3. MATERIALS AND METHODS

The biodegradation of RDX was measured in batch experiments using Pantex soil that has been contaminated for over 20 years. Analytical methods were developed to quantify degradation rates. Soil analyses were run to determine optimum depth intervals to be used for the experiment. Finally, biodegradation experiments were conducted.

An overview of all RDX biodegradation experiments is described below. The experimental procedures for the batch study are broken down into several sections. The first section discusses set up procedures for the biodegradation experiments. The second section describes recovery and sampling procedures. The subsequent sections explain soil extraction and headspace analysis techniques.

3.1 PANTEX SOIL SAMPLES

Biodegradation studies were conducted with Pantex soil that was obtained from air rotary drill cuttings. These cuttings were taken from location 10, which is located near Building 12-43 in Zone 12 (Figure 3.1). R. W. Stoller Corporation was the environmental contractor that took samples on March 2, 1997 under the direction of Dr. Kenneth A. Rainwater of Texas Tech University (TTU). The samples were then placed in one-gallon zip lock bags and taken to TTU. On March 23,1997, soil samples were shipped to The University of Texas at Austin (UT) in coolers. Once at UT, soil samples were stored in the dark at 4°C until they were used.

The soil cuttings from location 10 were divided into several depth intervals. The total depth of the samples ranged from 4 to 128 feet with each interval varying from 5 to 8 feet. The Stoller Corporation analyzed the Pantex soil from depths 4 to 124 feet for RDX and HMX contamination. The results of the analyses are presented in Table 3.1. Because soil samples from Pantex were split between TTU, UT, and Stoller Corporation, soil samples received at UT only ranged from 55 to 128 feet for location 10.

3.2 CHEMICALS AND GASES

3.2.1 Contaminant Chemical

RDX is the primary contaminant of interest for the biodegradation studies. Radiolabeled RDX was used in this study because recovery and analysis of radiolabeled compounds are well established. In addition, radiolabeled compounds allow for quantification of breakdown products throughout the biodegradation process. Biological degradation of ^{14}C-RDX for these experiments was monitored by measuring ^{14}C-RDX plus ringed derivatives, ^{14}C-intermediates (cleaved ring compounds), and $^{14}CO_2$ over time.

Radiolabeled RDX was purchased from Chemsyn Science Laboratories in Lenexa, KS. UT received 100 μCi of ^{14}C-RDX in a flame-sealed amber ampule.

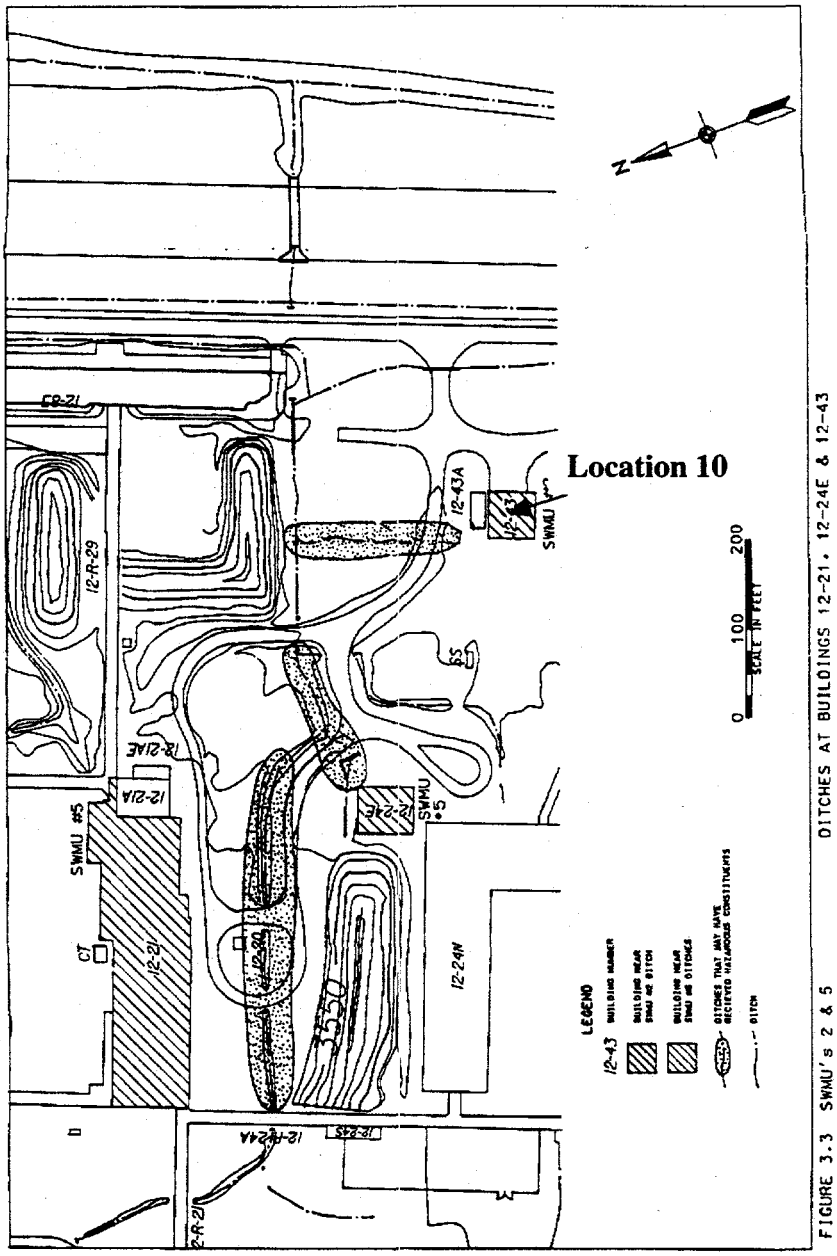


Figure 3.1: Soil Sample Location

Table 3.1: Soil Analyses at Location 10[1]

Depth (ft)	RDX (mg/kg)	HMX(mg/kg)
4	27	4
9	23	2
14	18	1
19	17	1
24	17	1
29	22	2
34	17	2
39	11	2
44	14	0.4
49	11	0.2
54	12	0.1
59	10	0.2
64	9	0.6
69	11	0.8
74	9	0.9
79	6	0.9
84	6	0.7
89	7	0.6
94	8	0.4
99	9	0.2
104	11	0.1
109	7	0.1
114	5	Not run
119	6	0.1
124	6	Not run

[1]Preliminary Data provided by Battelle ER Group from R.W. Stoller Corp.

The radiolabeled compound had a specific activity of 7.5 mCi/mmol and a chemical purity of >99% by radio-HPLC. The ^{14}C-RDX was in the form of a solid dampened with water in excess of 10%. A highly concentrated liquid stock solution was made by dissolving the solid into 5 mL of ethanol. The ethanol-^{14}C-RDX stock solution was placed into a thick walled glass vial with a Teflon lined screw cap. The vial was placed into a secondary container, which was used to prevent accidental contamination and light

exposure. This container was then stored in a refrigerator at 7° C until it was used for experiments.

3.2.2 Other Radiolabeled Chemicals

Carbon dioxide recovery tests were run with radiolabeled sodium bicarbonate ($NaH^{14}CO_3$). The radiolabeled bicarbonate was obtained from Sigma Chemical Company. The $NaH^{14}CO_3$ solution was supplied in several flame-sealed amber ampules. Each ampule contained 1 mL of aqueous solution and had an activity of 1 µCi per vial.

3.2.3 Nutrients

Nutrients were added to determine if the biodegradation of RDX was limited by nutrient availability in the soil. Organic carbon and phosphorus were the two nutrients added since RDX is thought to degrade by the reduction of nitrogen. These nutrients were applied to the soil sample on an individual basis. However, steps were taken to minimize an increase in moisture content, while allowing for flexibility in experimental setup. These steps included adding very small quantities of solution and combining the soluble phosphorus with the ^{14}C-RDX to make a separate phosphorus-^{14}C-RDX spiking solution. In order to allow for flexibility, biodegradable organic carbon was applied separately in a different aqueous solution.

The phosphorus solution used to make the phosphorus-^{14}C-RDX spiking solution was prepared by dissolving 15.13 mg of KH_2PO_4 and 6.86 mg of K_2HPO_4 into 1 L of deionized water. This combination of KH_2PO_4 and K_2HPO_4 was used to minimize pH shifts in the solution. The KH_2PO_4 was purchased from Fisher Chemical Company, and the K_2HPO_4 was purchased from E. M. Science Industries. The total amount of phosphate added was approximately 22 mg/L as P. This value was determined by calculating the required phosphorus needed for the RDX contamination on the soil and supplying 25% in excess. The required phosphorus was determined by using a value of 9.5 mg/kg of RDX, converting this value to mg/kg of theoretical nitrogen available, multiplying that number by an element ratio of N:P for a cell (12%), and finally multiplying by 1.25 to supply excess.

The organic carbon solution was prepared with three highly biodegradable organic chemicals: glucose, succinate, and glycerol. The first chemical was purchased from Fisher Chemical Co. The other two chemicals were obtained from Sigma Chemical Co. The organic carbon solution contained 0.921 g of glucose, 0.72 g of succinate, and 1.35 g of glycerol. The solution was made by dissolving the chemicals into 1 L of deionized water to obtain an organic carbon concentration of approximately 2.9 g/L. The chemical proportions were taken from similar research conducted by Binks et al. (1995). A typical organic carbon to nitrogen mass ratio for the degradation of an organic chemical is 10:1 (Noris, 1994). However, because the main objective was to determine the effect on the degradation rate and not to obtain a residual endpoint, a ratio of 5:1 was added to the soil.

3.2.4 Chemicals

Ethyl ether was used as a partitioning solution during recovery of radiolabeled compounds. The ethyl ether was the spectranalyzed grade available from Fisher Chemical Co. Hydrochloric acid (6N) was used in the experiments to lower the pH prior to purging off inorganic carbon. The hydrochloric acid solution was made from certified ACS liquid HCl and deionized water. The volatile $^{14}CO_2$ was trapped using Carbosorb®II. Carbosorb®II is an organic base compatible with liquid scintillation cocktail (CS-II, United Technologies). Liquid

scintillation cocktail is a specially designed mixture of chemicals used in liquid scintillation counting to transfer radioactivity energy into light photons. ScintiVerse II, purchased from Fisher Chemical Co., was the cocktail solution used throughout the experiments. HPLC grade acetonitrile was used for the soil extraction procedure. This chemical was purchased from Fisher Chemical Co. Calcium chloride was also used for the soil extraction procedure. The calcium chloride was purchased from Spectrum Chemical Manufacturing Corp. and was a dihydrate certified ASC reagent in a powder form.

3.2.5 Gases

Two standard gases were used to create anoxic and microaerobic conditions. Both gases were purchased from Air Liquide located in Austin, TX. Nitrogen gas was used to purge the headspace of vials during the carbon dioxide recovery procedure. This gas was also used during the initial set up of the biodegradation hypovials to create an anoxic condition in the headspace of the hypovials. The nitrogen gas was industrial grade with a purity of >99.99%. Oxygen was used during the initial set up of the microaerobic vials to obtain a 3% oxygen concentration. The oxygen gas was medical grade with a purity of 99.75 %.

3.3 ANALYTICAL METHODS

3.3.1 Moisture Content

Moisture content was determined to calculate soil weights on a dry weight basis. To compute moisture content of Pantex soil, several aluminum pans were weighed and numbered. Next, soil was added to the aluminum pans, and the weights of the pans with the soil were recorded. The aluminum pans were then placed into a drying oven at 105° C for 24 hours. The weights of the pans and dried soil were measured. From this data, moisture content was calculated by subtracting the weight of the wet from the dry soil and dividing this difference by the dry weight of the soil.

3.3.2 Headspace Composition

Headspace analyses were used to determine if any methane, nitrogen, oxygen, carbon monoxide, and carbon dioxide were present in the vials and glove bags. The analytical techniques used were similar to those described by Closmann (1989) and Morley (1996). During initial set up of the biodegradation hypovials, the gases in the glove bags were tested frequently to ensure anoxic or microaerobic conditions. At the end of the biodegradation experiment, headspace analyses were done on selected vials to determine what gases may have been given off during degradation of the RDX. A Fisher Model 1200 Gas Partitioner was used for this analysis. The results were output to a Hewlett Packard HP3396A Integrator. The same operating parameters for the gas partitioner used by Morley (1996) was used for this experiment (Table 3.2). Morley selected these parameters because they gave the best peak separation and resolution after numerous initial tests. A container of standard gas was prepared by Scott Specialty Gas and used to identify the six different gases. The composition of the gas is in Table 3.3.

A typical chromatograph of the standard gas is shown in Figure 3.2. The initial peaks represent the sample injection and the mixture of all gases except carbon dioxide. Thereafter, each gas is represented by a separate peak and detention time. The areas under the peaks correspond to the molar concentration of the gas. By comparing the standard to the experimental samples, headspace gases were identified and quantified.

3.3.3 *Radioactivity Measurements*

All ^{14}C- compounds were measured on a Beckman Model LSC –5000TD liquid scintillation counter using H-number for quench correction. The instrument measures a counting efficiency and the number of counts per minute. These values are used to calculate the disintegrations per minute (dpm) of each sample, which is a measure of radioactivity.

Table 3.2: Gas Partitioner Operating Parameters

Parameters	Description or Value
Instrument	Fisher Scientific Gas Partitioner Model 1200
Detector Type	Thermal Conductivity Detector (TCD)
Bridge Current	225 mA
Column 1	6½ foot long x 0.125-inch aluminum column Packed with 80-100 mesh Columnpak PQ
Column 2	11 foot long x 3/16-inch aluminum packed with 60-80 mesh, Molecular sieve 13X
Carrier Gas	Helium
Carrier Gas Flow Rate	15 mL/min
Injector Temperature	Off
Column Temperature	50°C
Sample Size	1 mL

Table 3.3: Standard Gas Components and Concentrations

Constituent	Concentration (Mole %)	Retention Time (min)
Carbon Dioxide	5.00	0.58
Carbon Monoxide	5.00	1.71
Hydrogen	4.18	--
Methane	4.00	1.55
Nitrogen	5.00	1.07
Oxygen	4.95	0.88
Helium	71.87	--

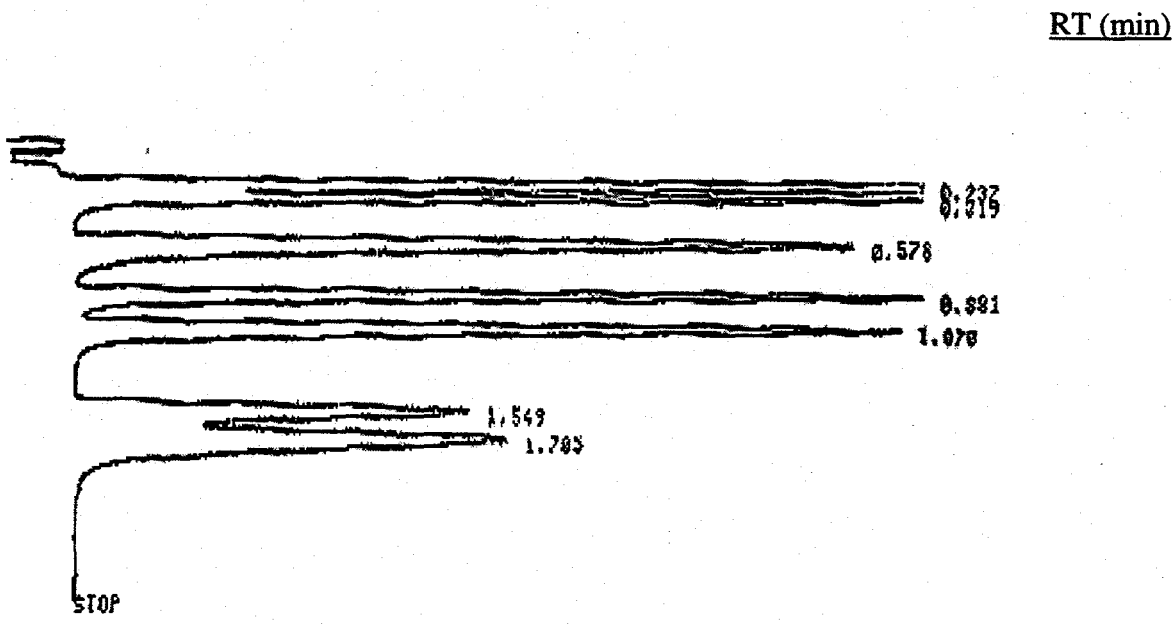


Figure 3.2: Sample Gas Partitioner Chromatogram Analysis of Standard Gas

3.4 SOIL ANALYSIS

Several soil depths were tested to determine the level of RDX contamination. The main goal was to determine which soil depth or depths to use for the study. The samples with the highest level of RDX were expected to contain the greatest number of RDX degraders. Thus, soil extractions were performed on selected samples using EPA Method 8330 (USEPA, 1994b) to determine RDX concentration levels. The extracted solution was then analyzed by HPLC. The results from location 10, Building 12-43 showed RDX contamination as high as 10.3

mg/kg, on a dry weight basis, with an average of about 9.5 mg/kg (dry weight) over a 15-foot section. These data were consistent with RDX levels obtained by the Stoller Corporation on the same soil. The Stoller Corporation obtained an average of 10.5 mg/kg over the same 15-foot section (Table 3.1). Data obtained from other depths showed smaller amounts of RDX; most samples were under 1 mg/kg. Therefore, the soil sample from Building 12-43 (depth 55-65 feet) was selected for this study.

3.5 BIOLOGICAL DEGRADATION EXPERIMENTAL OVERVIEW

The biodegradation experiments were performed in two phases. The first phase was aimed at determining the environmental conditions needed for RDX to be degraded by the indigenous microorganisms present in Pantex soil. Phosphorus addition was investigated to determine if it was a limiting nutrient under the various conditions. The second phase was designed to determine if the rate of degradation could be altered by the addition of a biodegradable organic carbon source or the combination of a biodegradable organic carbon and phosphorus source. Carbon dioxide diffusive losses from the test hypovials were also investigated to determine if a correction should be applied. Nitrogen was not considered a limiting nutrient because RDX degrades by the reduction of the nitrogen from its ring structure.

3.5.1 Phase One

Phase one of the experiments consisted of three different electron acceptor conditions in which oxygen was the electron acceptor that was varied. The first set of hypovials contained air in the headspace. The second set of hypovials contained a reduced oxygen level, roughly 3% oxygen and 97% nitrogen. This set helped to determine if microaerobic conditions were advantageous for RDX metabolism. The final set of hypovials had only nitrogen gas in the headspace to create an anoxic environment. Duplicates of each environmental condition were prepared with the addition of phosphorus (~0.89 mg P/kg of soil). During this phase, autoclaved soil hypovials were also set up to serve as abiotic controls.

3.5.2 Phase Two

Phase two of the experiments was initiated two months after phase one. By this time, initial results from the first phase showed biological degradation of RDX occurred under anoxic conditions. Therefore, a series of hypovials were set up under these conditions. An organic carbon source was prepared using three highly biodegradable compounds (glucose, glycerol, and succinate). Each hypovial was spiked with a 100 µL of the organic carbon solution and ^{14}C-radiolabeled RDX. A duplicate series was made with the addition of phosphorus. Autoclaved soil samples were also set up as an abiotic control with biodegradable organic carbon. All set up procedures were identical to those performed in phase one.

Results from phase one also showed that ^{14}C was being lost from the hypovials. Losses were observed when significant biotransformation of the RDX was occurring or after long periods of time. For instance, because no biological activity occurred in the autoclaved samples, recoveries of ^{14}C were 90% or better for the first 112 days. However, recoveries less than 90% occurred within 36 days under anoxic conditions when significant biodegradation was occurring. Because method development experiments showed that almost all $^{14}CO_2$ could be recovered with the trapping system shortly after being spiked, the ^{14}C losses suggested that $^{14}CO_2$ was leaking out of the vials during incubation. To test this theory and quantify any losses, ^{14}C-radiolabeled bicarbonate

samples were prepared in a similar manner as all other experiments. Table 3.4 shows a summary of all the experiments conducted.

3.6 BIODEGRADATION SET UP PROCEDURES

The biological degradation experiments were prepared individually. Each experiment tested a different environmental condition with or without the addition of nutrients. Thirty, 10-mL, amber, glass hypovials, purchased from Pierce Co., were used for each experiment. Two grams (wet weight) of Pantex soil was placed into each 10-mL hypovial and 100 µL of a ^{14}C-RDX spiking solution was injected onto the soil. Immediately after injection of the ^{14}C-RDX, hypovials were sealed with Teflon-lined septa and aluminum crimp tops. The hypovials were incubated at 20°C, in the dark

Table 3.4: Matrix of Experimental Conditions

Phase	^{14}C-Chemical	Headspace Gases	Nutrients Added	Autoclaved	Vial Set Label
1	RDX	Air	Phosphorus	Yes	Aerobic Autoclaved w/ Phosphorus
1	RDX	Air	Phosphorus	No	Aerobic w/ Phosphorus
1	RDX	Air	None	No	Aerobic
1	RDX	Nitrogen	Phosphorus	No	Anoxic w/ Phosphorus
1	RDX	Nitrogen	None	No	Anoxic
1	RDX	Nitrogen (95%) Oxygen (5%)	Phosphorus	No	Microaerobic w/ Phosphorus
1	RDX	Nitrogen (95%) Oxygen (5%)	None	No	Microaerobic
2	RDX	Nitrogen	Biodegradable Organic Carbon	Yes	Anoxic Autoclaved w/ Carbon
2	RDX	Nitrogen	Biodegradable Organic Carbon & Phosphorus	No	Anoxic w/ Phosphorus & Carbon
2	RDX	Nitrogen	Biodegradable Organic Carbon	No	Anoxic w/ Carbon
2	$NaHCO_3$	Air	None	No	Bicarbonate Control

to eliminate the possibility of photochemical degradation, and under water to minimize losses of $^{14}CO_2$ through the vial septum. A more detailed description of the procedures follows.

3.6.1 Spiking Solutions

Two aqueous ^{14}C-RDX spiking solutions were prepared and used for all experiments described below. One solution contained ^{14}C-RDX in deionized water, while the other solution contained ^{14}C-RDX and phosphate (~22 mg/L P) in deionized water. The first solution was prepared by dissolving 220 µL of the ethanol-^{14}C-RDX stock solution into 20 mL of deionized water. The phosphorus-^{14}C-RDX solution was prepared by adding 220 µL of the ethanol-^{14}C-RDX solution to 20 mL of the phosphate solution (see Nutrients). The amount of ethanol added to the soil was calculated to be 0.55 µL/g. Because the amount was so small, the ethanol should not play a role in the degradation of the RDX and was, therefore, not tested. The radioactivity concentrations of both solutions were determined by directly injecting 100 µL of spiking solution into scintillation vials and measuring them on the Beckman LS-5000TD instrument during hypovial preparation. At least three spiked vials were used, and an average was taken to determine the radioactivity of the dosing solutions. The radioactivity in both solutions was approximately 50,000 dpm.

A third spiking solution was prepared with radiolabeled bicarbonate and added to a set of hypovials. A $H^{14}CO_3^-$ spiking solution was prepared by adding 1 mL of radiolabeled bicarbonate to 8 mL of deionized water. Volatilization was minimized by the use of two glass syringes to mix and store the solution. The smaller syringe (100-µL Hamilton Microliter #810) was used to inject the radiolabeled chemical into the second syringe through the needle opening. Then, the second syringe (10-mL Hamilton 1000 series w/ removable needle) was compressed to create no headspace, and the syringe was sealed with a Teflon lined screw closure. Samples were removed from the second syringe by simultaneously applying pressure to the syringe plunger and removing sample with a third syringe (100-µL Hamilton Microliter #810). This withdraw technique maintained a relatively headspace free environment, thus minimizing volatilization losses. The radioactivity for the solution was approximately 30,000 dpm.

3.6.2 Radiochemical Spiking Procedure

The majority of the techniques for the spiking procedures came from previous work done by Closmann (1989) and Morley (1996). However, some modifications were made to the procedures because RDX is nonvolatile compound and Pantex soil has a high calcium carbonate content.

For each experiment, two grams of Pantex soil was put into 30 hypovials, which were arranged in holding rack. The rack was set up so that after every four biodegradation vials a direct inject vial was present. Vials were spiked in sets of 10 to reduce any likelihood of radioactivity losses. Each vial was spiked with 100 µL of spiking solution via an Eppendorf pipette with a disposable autoclaved tip. This spiking quantity was chosen to minimize the change in moisture content. The moisture content before spiking was approximately 8%, and the moisture content after spiking with 100 µL of water was approximately 11%, based on the dry weight of soil. Direct inject vials were sealed with a polypropylene foil-lined screw cap until the spiking procedure was complete. Biodegradation hypovials were sealed with a Teflon/silicone septum and aluminum crimptop closure. Both closure pieces were purchased from Pierce Co. The Teflon/silicone septa had a thickness of 125

mil (thousandths of an inch) and a diameter of 20 mm. This size was selected based on work done by Closmann (1989). The 125-mil thick septa provided the best reliable seal around the vial lip.

Spiking procedures for the $H^{14}CO_3^-$ solution were similar to the above procedure. In this case, a 100-µL Hamilton gas tight syringe was used to inject the solution into the vials, and vials were spiked in sets of three to minimize volatilization of any $^{14}CO_2$.

3.6.3 Direct Inject Vials

Direct inject vials were used to quantify the amount of radioactivity initially injected into each set of vials. A direct inject vial was present after every fourth degradation vial during the vial set up. Each direct inject vial was a 20-mL scintillation vial with a polypropylene foil-lined screw cap, purchased from Fisher Chemical Co. Each vial received 100 µL of ^{14}C-RDX solution and 10 mL of ScintiVerse II scintillation cocktail. An average radioactivity was calculated from these vials and was considered to be the initial radioactivity that was spiked into each individual biodegradation vial.

3.6.4 Headspace Conditions

Different headspace compositions were prepared to test the biodegradation rates for RDX degraders under aerobic, anoxic, and microaerobic conditions. The three headspace compositions created were air, nitrogen gas, and a mixed gas of approximately 3% oxygen and 97% nitrogen. The biodegradation hypovials that were set up with air in the headspace were sealed under atmospheric conditions in a standard laboratory hood. The biodegradation hypovials that were set up with nitrogen and mix gases in the headspace were prepared in a glove bag (27"x 37"x 18") purchased from Cole-Parmer.

Spiking procedures for the hypovials set up within the glove bag were the same as above except all materials were placed into the glove bag and sealed at least two hours before spiking. During the two hours, the desired gas or gases was injected into the glove bag. The volume of the bag was purged periodically to allow for several gas volume turnovers. After approximately two hours, gas samples were taken from the glove bag with a 1-mL Hamilton Samplelock™ syringe. The contents of the syringe were injected into the gas partitioner to determine the percentage of oxygen present. Several samples were taken in 15-minute intervals until the desired oxygen percentage was reached. Once the desired headspace composition was reached, the spiking procedures were started. When the procedures were halfway done, a second gas sample was taken. Soil spiking procedures were then finished, and a final gas samples was taken. The headspace composition was taken as an average of the three samples collected during dosing.

3.6.5 Organic Carbon Addition

Some experiments required the injection of a biodegradable organic carbon, to determine if the availability of biodegradable organic carbon affected the degradation rate of RDX. An Eppendorf pipette with disposable autoclaved tips was used to inject the organic carbon solution (3.2-Nutrients). Fifty µL of the organic carbon solution was injected into each hypovial just before the ^{14}C-RDX spiking solution was added. Therefore, a total of 150 µL was added to the two experiments where organic carbon was supplied. This addition changed the moisture content in the soil from roughly 8% to 12.5% (dry weight).

3.7 BIODEGRADATION SAMPLING AND RECOVERY

The biodegradation experiments were conducted in several series of hypovials that were analyzed sacrificially over time.

Sampling consisted of a three-step recovery procedure that measured the radioactivity present in each step. The first step used a purge and trap system that recovered $^{14}CO_2$ from within the hypovial. The second step partitioned ^{14}C- ringed compounds into ether. The third step separated the aqueous solution from the soil and tested for ring cleaved ^{14}C-compounds. All biodegradation hypovials were tested exactly the same way throughout the entire experiment.

3.7.1 Incubation and Testing

Once sealed, all hypovials were incubated in the dark to eliminate the possibility of photochemical degradation. The hypovials were also placed under water to minimize diffusion of $^{14}CO_2$ through the seal between the vial and septum. Incubation was conducted in a temperature-controlled refrigerator at 20°C. Sampling consisted of sacrificing hypovials over time to measure ^{14}C-RDX, ^{14}C-intermediates, and $^{14}CO_2$. Testing of incubated hypovials were performed every two to three weeks. Three hypovials were usually analyzed each time for reliability and redundancy.

The radiolabeled bicarbonate experiment was analyzed slightly differently. The $H^{14}CO_3^-$ hypovials were tested once a week for the first five weeks and once every other week for the remainder of the study. Sixteen hypovials were set up for this experiment, so only one or two hypovials were analyzed on each sampling day.

3.7.2 Carbon Dioxide Recovery

The carbon dioxide recovery procedure was taken from previous work conducted by Closmann (1989) and Morley (1996). However, recovery procedures were modified because of the large amount of caliche (weathered calcium carbonate) present in Pantex soil. Modification consisted of lowing the purge gas flow rate, injecting the hydrochloric acid more slowly, increasing the concentration of Carbosorb®II in the trapping solution, and splitting the Carbosorb®II solution from the first trapping vial into several scintillation vials before analyzing. A detailed description of the procedures is provided below.

The carbon dioxide recovery procedure began by preparing two solutions. The first solution was hydrochloric acid. This solution was prepared from a stock solution at 6N (see 3.2-General Chemicals). To make the solution, equal volumes of deionized water and hydrochloric acid were mixed. Five mL of hydrochloric acid was added to the soil via a 5-mL Hamilton syringe. The second solution was the carbon dioxide trapping solution. Carbosorb®II was added to deionized water in a 30/70 ratio by volume.

Two trapping vials were prepared. Each vial was a 20-mL scintillation vial with screw top cap and teflon/silicone septa. The first vial received 10 mL of the CarboSorb solution while the second vial received 5 mL.

Gas purging lines were also constructed. Each line consisted of approximately 5 inches of Teflon 1/32-inch (I.D.) tubing, two flangeless 1/16-inch tubing connectors, two Kel –F ferrules, one 2-inch, 22 gauge removable screw hub needle, and one, 3-inch, 22 gauge removable screw hub needle. All connection materials were purchased from Spectrum Medical Co. A nitrogen gas line was constructed with a 1/16-inch (I.D.) Teflon tube and aluminum screw fitting, which connected to the nitrogen gas cylinder.

Carbon dioxide recovery consisted of connecting a biodegradation hypovial with the two trapping vials in a daisy chain fashion (Figure 3.3). Sampling began by simultaneously purging the headspace with nitrogen gas and injecting 5 mL of hydrochloric acid solution (pH<2) into the biodegradation hypovial. The nitrogen gas

was injected at a rate of 3 mL/min. To avoid sending acid through the gas purge line, the acid was injected slowly over a time period of 3 minutes. The low pH water caused the release of inorganic carbon in the form of CO_2 from the soil and any pore water. The CO_2, which included any $^{14}CO_2$, was then sent through the gas purge line into the first trapping vial. The gas coming from the biodegradation hypovial was introduced at the bottom of the trapping vial, thus allowing the gas to be trapped in the Carbosorb®II solution. All gases from the first vial were then sent to the second trapping vial, which allowed a second opportunity to trap the carbon dioxide. From the second trapping vial, gases were released into a standard laboratory hood. The total nitrogen gas purging time was 7 minutes; then, the nitrogen gas line was pulled from the hypovial. Because Pantex soil has an abundance of $CaCO_3$ that produced large amounts of CO_2, the hypovials were allowed to continue purging on their own for an additional 2 minutes. At approximately 9 minutes, all lines were pulled from the vials. Trapping vials were prepared for analysis in the following fashion. The second trapping vial received 10 mL of ScintiVerse II scintillation cocktail solution and was capped with a polypropylene foil-lined screw cap. The solution from the first trapping vial was split equally into five 20-mL scintillation vials and each received 10 mL of scintillation cocktail. This method was developed because of two-phasing problems between the two solutions. All vials were shaken by hand for several seconds and placed into the Beckman LS 5000TD liquid scintillation counter for analysis. The total $^{14}CO_2$ radioactivity was taken as the sum of the radioactivity in the six vials.

The second trapping vials did not need to be split because a very small amount of $^{14}CO_2$ and CO_2 were trapped in the vials. For instance, the radioactivity in the second trapping vials was always less than 500 dpm with the majority of the vials being below 100 dpm. The radioactivity in the first trapping vial was normally much greater (500-15000 dpm) and a large quantity of unlabeled carbon dioxide was being trapped. The two-phasing problems were believed to be due to the large amount of unlabeled carbon dioxide released from the Pantex soil.

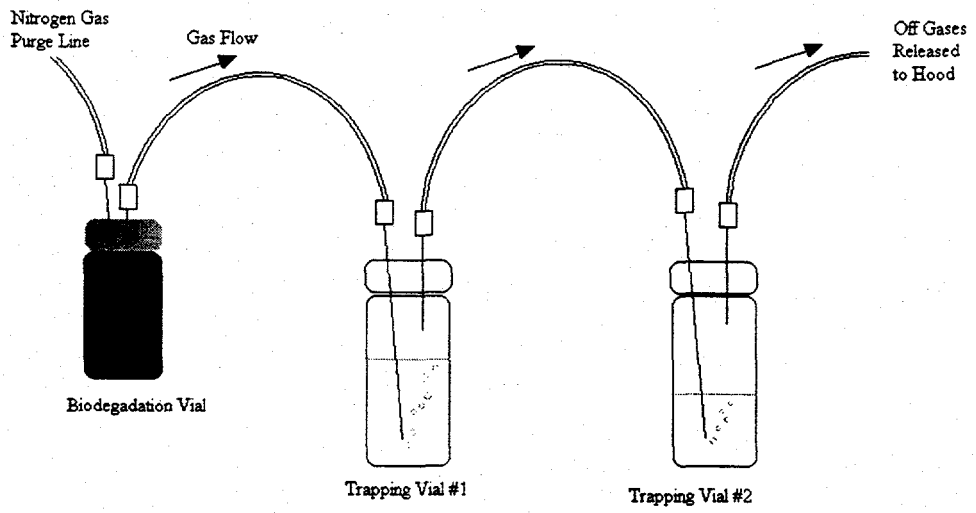


Figure 3.3: Carbon Dioxide Recovery System

3.7.3 Ether Phase Partitioning

At the end of the carbon dioxide recovery procedures, ethyl ether was added to the biodegradation hypovial. The reason for the addition of ethyl ether is to allow chemicals in the aqueous solution (acid water) to partition from the water into the ether. RDX and all derivatives before ring cleavage should partition preferentially into the ether phase, while all compounds created after ring cleavage should largely remain in the aqueous phase (Kitts, et al., 1994). Kitts et al. made this conclusion because they found that the distribution of labeled RDX in the ether phase corresponded well to the amount of RDX and nitroso-RDX intermediates in the unlabeled experiments. Because this partitioning occurs, quantifying the amount of ^{14}C in each phase can be used to monitor the biodegradation of ^{14}C –RDX.

Ether partitioning was achieved by injecting 5 mL of ethyl ether into the biodegradation hypovial using a 5-mL Hamilton syringe. The hypovial was then placed on a Brunswick Scientific shaker table for 7 to 8 minutes at 200 RPM. After shaking, the hypovials were allowed to stand for approximately 45 minutes. The aluminum crimptop and seal were then removed, and ether was taken from the top of the liquid surface. A B-D luer lock disposable syringe was use to remove 1 mL of ether. The ether was placed into a 20-mL scintillation cocktail vial. Ten mL of scintillation cocktail was added to the ether sample, and the vial was sealed with a polypropylene foil-lined screw cap. A second sample of ether was taken the same way for reliability and redundancy purposes. All vials were shaken by hand for several seconds and place into the Beckman LS 5000TD liquid scintillation counter for analysis. The ether phase radioactivity was taken as an average of the two vials.

3.7.4 Water Phase Partitioning

At the end of the ether phase partitioning procedures, water was removed from the biodegradation hypovial. Using a 1000-µL Eppendorf pipette, the water sample was drawn from the bottom of the hypovial. The sample was a mixture of soil and aqueous solution. Approximately 2 to 3 mL of mixture was taken out of the hypovial. This mixture was place into a glass centrifuge tube and centrifuged with an IEC Clinical tabletop centrifuge. The sample was centrifuged for 5 minutes at a setting of two. After centrifuging, the sample was allowed to stand for at least one minute. The supernatant was then poured into a 5-mL glass vial. An Eppendorf pipette was used to place 0.5 mL of supernatant into a 20-mL scintillation vial. Ten mL of scintillation cocktail was then added and the vial was sealed. A second sample of supernatant was taken the same way for reliability and redundancy purposes. All vials were shaken by hand for several seconds and place into the Beckman LS 5000TD liquid scintillation counter for analysis. The aqueous phase radioactivity was taken as the average of the two vials.

3.8 SOIL EXTRACTIONS

Soil extractions were performed at the end of the biodegradation experiments. Extractions were performed to determine if aged, unlabeled RDX present in Pantex soil was also degraded by the organisms during the experiments. Experiments were run on the degraded Pantex soil in the hypovials that was spiked with ^{14}C-RDX and on undegraded, unspiked samples of Pantex soil to provide a comparison for background levels. Soil extraction procedures followed EPA Method 8330 (EPA-Section 7.1.2). Method 8330 is described briefly below. A Waters (2690- 996 P.A.D.) high performance liquid chromatograph using a photo array diode

detector set at a wavelength of 254 nm analyzed the extracted solutions.

Soil extractions started by air drying all soil samples to a constant weight. Approximately two grams of soil was weighed out and placed into a 15-mL glass vial with a Teflon lined screw cap. Ten mL of HPLC grade acetonitrile was then placed into the vial, and the vial was sealed. Sealed vials were vortex swirled for one minute and then placed into a Branson 5200 Sonicater. Ice was periodically placed into the sonicater to keep the water cool. The soil was allowed to sonicate for 18 hours. After sonication, the soil was allowed to settle for 30 minutes. Five mL of supernatant was then removed from the vials and place into a 20-mL scintillation vial. Five mL of a sodium chloride solution was then added. The sodium chloride solution was made by dissolving 5 g of reagent grade sodium chloride into 1 L of deionized water. Upon sealing, the contents were shaken and allowed to set for 15 minutes. Approximately, five mL of supernatant was removed from the vial by a B-D luer lock disposable syringe. The contents of the syringe were filtered through a 0.45-μm Teflon syringe filter. The first 3 mL were discarded and the remainder of the filtered solution was placed into a 5-mL glass vial. The vial was sealed with a Teflon lined screw cap, placed in a box, and stored in a 4°C refrigerator until analyzed by HPLC.

3.9 HEADSPACE ANALYSIS

Headspace analyses on select vials were performed at the end of the biodegradation experiments. These analyses were performed to help confirm electron acceptor conditions in the hypovials and to look for gaseous metabolic products (i.e., carbon dioxide and methane). In addition, two microaerobic vials were analyzed after the first 24 days of incubation to determine the amount of oxygen in the vials. A Hamilton Sample Lock™ syringe was used to extract 1mL of gas from a sealed biodegradation hypovial. The gas sample was then injected into the Fisher Model 1200 Gas Partitioner. A detailed explanation of headspace analysis is in Section 3.3.

4. RESULTS AND DISCUSSION

Results of the carbon dioxide control, biodegradation experiments, soil extractions, and headspace analyses are presented in this chapter. The chapter is divided into four sections. The first section shows results from the carbon dioxide control and explains a correction factor for $^{14}CO_2$ recovery. The second section presents the results from the biodegradation experiments, which is subdivided by experiment type. The third section presents results from the soil extraction experiments and discusses the degradation rate constants for RDX in Pantex soil. The final section discusses headspace analyses that were performed on the biodegradation vials.

4.1 CARBON DIOXIDE CONTROL

The overall recovery of ^{14}C-compounds were calculated by summing the radioactivity of the carbon dioxide that was trapped in the Carbosorb® solution, the nonvolatile products that partitioned into the ether, and the nonvolatile products that remained in the aqueous solution, and dividing this total by the initial amount of radioactivity for each series of vials. Decreasing recoveries over time and between vial series indicated a problem with the experimental system.

Preliminary experiments conducted on the carbon dioxide trapping system showed that on average 96% of the $^{14}CO_2$ could be recovered from the vials. These studies were conducted with and without soil using a $H^{14}CO_3^-$ spiking solution, and vials were tested occurring shortly after set up. Therefore, the losses that were seen in the biodegradation experiments were assumed to have occurred during incubation. To test this assumption, a bicarbonate control experiment was designed to test for any carbon dioxide losses during incubation.

The control experiment was prepared, with soil, in the same manner as all other experiments except ^{14}C-radiolabeled bicarbonate was used for the spiking solution. The experiment was setup by preparing multiple hypovials that were spiked with $H^{14}CO_3^-$. Vials were sacrificially sampled over time to determine losses of $^{14}CO_2$. A total of 21 vials were used for this experiment: five direct inject vials and 16 hypovials. The direct inject vials were used to determine the initial amount of radioactivity in the vial series by taking an average over the five vials. The initial amount of radioactivity for this series was calculated to be 29,830 dpm with a standard deviation of 863 dpm.

Sampling consisted of testing one to two vials each week for the first five weeks and then every two weeks thereafter. The three separation techniques (CO_2, ether, and water) were performed on each vial. Because bicarbonate is not an organic chemical, no radioactivity was expected in the ether. Likewise, the presence of radioactivity was not expected in the water because the pH was lowered during the analysis.

The trapping system was also tested to ensure recovery efficiency. A vial was sacrificing on Day 0 to test the amount of radiolabeled bicarbonate that could be recovered. The recovery efficiency for Day 0 was approximately 105%. The extra 5% in the recovery was within the acceptable experimental error limits. Because complete recovery of $^{14}CO_2$ was obtained on the first day and in preliminary experiments, any difference between recovered $^{14}CO_2$ and initial $^{14}CO_2$ was assumed to be due to the carbon dioxide diffusing through the Teflon septum or between the seal of the Teflon septum and vial rim.

Raw data for all of the vials sampled can be seen in Appendix A, and Figure 4.1 summarizes the results from the control experiment. The radioactivity data in the

figure is represented as percent ^{14}C. The percent ^{14}C was calculated by dividing the measured radioactivity (dpm) for each sample by the initial amount of ^{14}C –RDX radioactivity for the individual experiment. Each point on the figure represents a daily average for the vials sampled on a given day.

As expected, only traces of ^{14}C-chemicals were present in the water and ether phases, demonstrating that no residual carbon dioxide was left in the vial. The radioactivity trapped in the CO_2 recovery system declined over time, which suggested that carbon dioxide was diffusing from the vial. The linear regression applied to the $H^{14}CO_3^-$ experiment is also shown in Figure 4.1. The R^2 for the regression line was 0.58 and the 95% confidence interval for the rate constant ranged from 0.07 to 0.39 day^{-1}.

The resulting equation from the linear regression was used to estimate a correction factor for recovery efficiency. For example, if 15 percent of the ^{14}C recovered from a biodegradation vial after 24 days of incubation was in the form of $^{14}CO_2$, the $^{14}CO_2$ correction was obtained by dividing 15 percent by 0.88, the recovery efficiency of

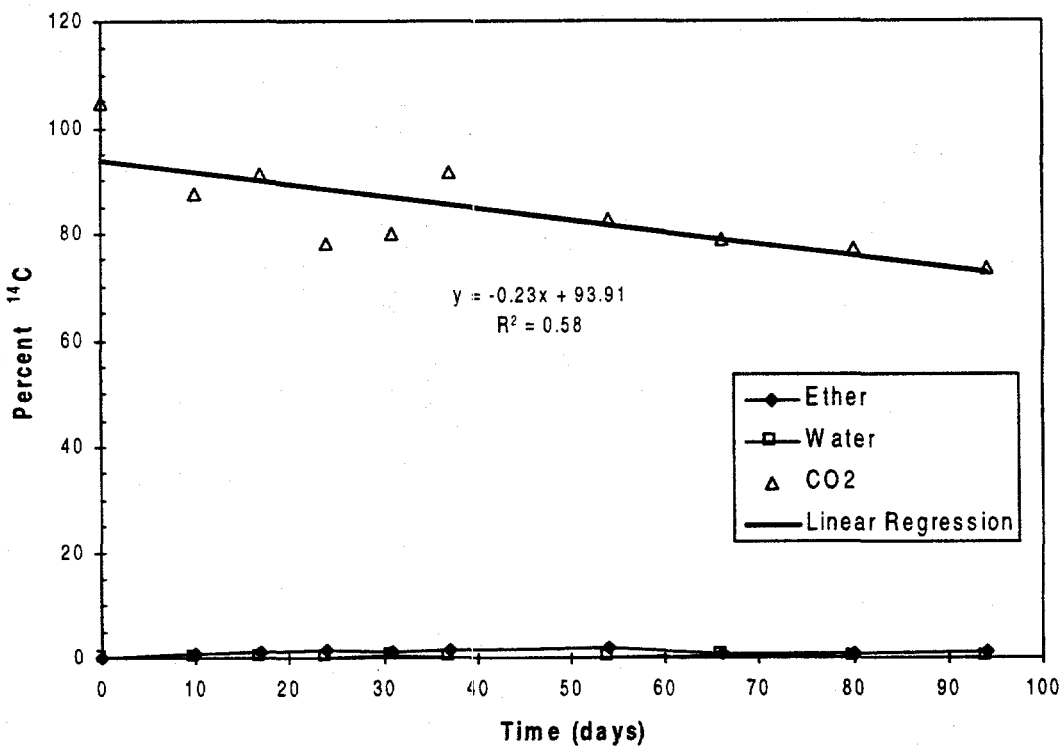


Figure 4.1: ^{14}C-Radioactivity for Aerobic Soil Vials Spiked with Radiolabeled Bicarbonate.

$^{14}CO_2$ after 24 days in the control experiment. This correction gives a carbon dioxide value that is called "CO_2 (meas)" in all remaining figures.

4.2 BIODEGRADATION EXPERIMENTS

The biodegradation experiments were setup by preparing multiple hypovials that were spiked with ^{14}C-RDX. Vials were sacrificially sampled over time to determine losses of ^{14}C-RDX. Vials were also sampled for ^{14}C-breakdown products by partitioning ringed and non-ringed radiolabeled chemicals into ether and water, respectively. A total of ten vial experiments were prepared with spiked Pantex soil.

In Phase One, the effects of environmental conditions were studied. This phase consisted of seven experiments: Aerobic with Phosphorus, Aerobic, Anoxic with Phosphorus, Anoxic, Microaerobic with Phosphorus, Microaerobic, and Aerobic Autoclaved Soil with Phosphorus. The autoclaved experiment provided a baseline for no biological activity.

In Phase Two, the effect of a biodegradable organic carbon source was studied under anoxic conditions. All vials had nitrogen gas in the headspace. The first experiment received phosphorus in addition to the biodegradable organic carbon (Anoxic with Carbon and Phosphorus). The second experiment only received the biodegradable organic carbon (Anoxic with Carbon). The third experiment also received organic carbon; however, the soil was autoclaved so that the experiment could serve as an abiotic control (Anoxic Autoclaved Soil with Carbon). Tables 4.1 presents the vial experiments that were prepared, the number of vials used to calculate the total radioactivity, the total amount of radioactivity, and the standard deviation for each experiment. Raw data used to calculate the total amount of radioactivity in each experiment can be seen in Appendix B.

(a) Phase One – Aerobic Autoclaved Soil with Phosphorus Experiment

Figure 4.2 illustrates the results from the autoclaved experiment where phosphorus was added to the soil, and the vials were sealed with air in the headspace. The data presented in the figure were obtained from the three separation techniques discussed in Chapter 3. The "ether" data represents the percent of original ^{14}C-RDX and ringed derivatives present in the ether phase. Once again, the ether data signifies all radiolabeled chemicals with the ring structure still intact. The "water" data denotes all chemicals, other than $^{14}CO_2$, formed after cleavage of the RDX carbon-nitrogen ring. The "CO_2 (meas)" data stands for the percent of original ^{14}C-RDX that was trapped in the Carbosorb® solution and corrected for $^{14}CO_2$ recovery efficiency (Section 4.1).

Table 4.1: ^{14}C-RDX Spiking Solution Radioactivity for Each Experiment

Experiment	Initial Average Radioactivity (dpm)	Standard Deviation (dpm)	Number of direct inject vials
Aerobic w/ Phosphorus	49,770	309	7
Aerobic	50,070	219	7
Anoxic w/ Phosphorus	49,970	99	7
Anoxic	50,300	224	7
Microaerobic w/ Phosphorus	49,890	542	7
Microaerobic	50,150	211	7
Aerobic Autoclaved Soil w/ Phosphorus	48,970	1582	7
Anoxic w/ Phosphorus and Carbon	46,400	220	7
Anoxic w/ Carbon	47,650	307	7
Anoxic Autoclaved Soil w/ Carbon	47,650	229	7

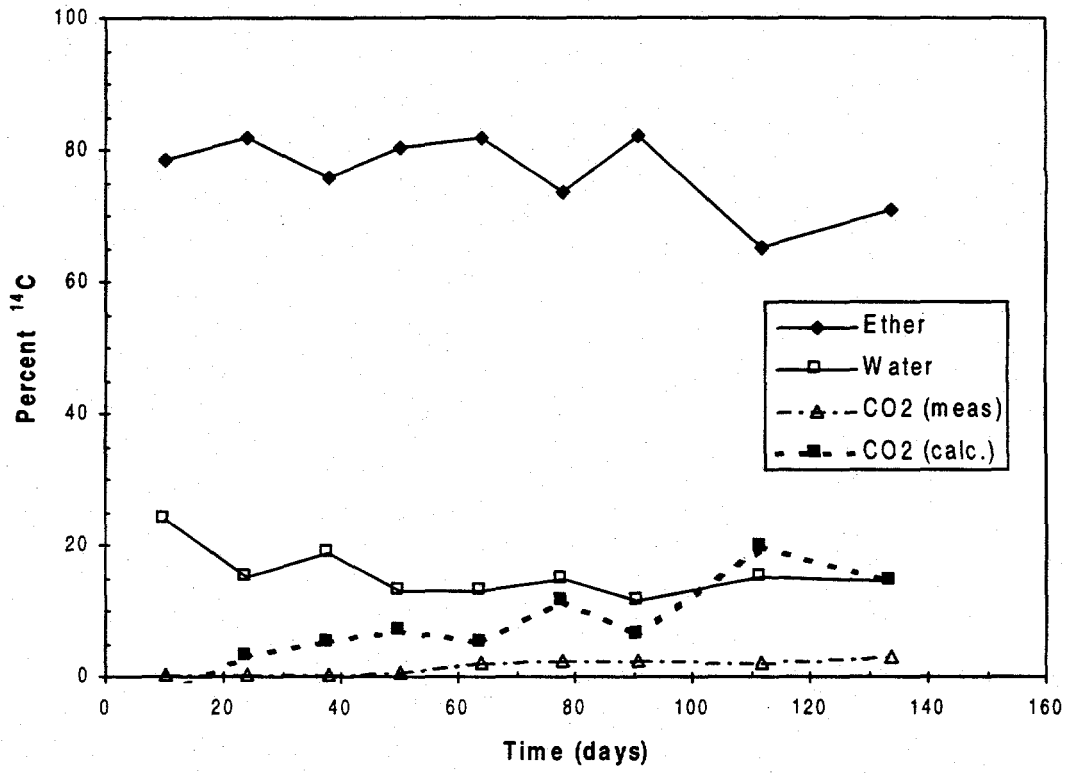


Figure 4.2: ^{14}C-Radioactivity for Aerobic Autoclaved Soil Vials Amended with Phosphorus

The "CO_2 (calc)" data represent the maximum amount of $^{14}CO_2$ that may have been produced. These data were calculated by subtracting the radioactivity in the ether and water from the amount of radioactivity initially added. In essence, this approach assumes 100% mass balance closure and that both measured $^{14}CO_2$ and unaccounted for ^{14}C comprises the $^{14}CO_2$ fraction.

Under aerobic conditions with autoclaved soil, the measured $^{14}CO_2$ production (CO_2 (meas)) was negligible over the entire length of the study (134 days), which suggests that very little or no biological activity occurred during incubation. As expected, the radioactivity in the water and ether showed little variation over the 134 days, which confirms that ^{14}C-chemicals were not being biodegraded. However, Figure 4.2 does show that the radioactivity in the last two ether samples was somewhat less than the rest of the data. At this time, it is unclear whether experimental error or a changing environment (aerobic to anoxic) within the vials was responsible.

The ^{14}C recovery efficiency declined slightly over the length of the study. For example, the average recovery was at 103% on Day 10 and decreased to 88% by Day 134. The unaccounted for ^{14}C is illustrated in Figure 4.2 and the similar figures that follow as the vertical distance between the CO_2 (calc) and CO_2 (meas) curves. The CO_2 (calc) curve lies above the CO_2 (meas) curve when recovery was less than 100%, and below the CO_2 (meas) curve when recovery was greater than 100%. The loss of ^{14}C may be a result of inaccurate carbon dioxide recovery correction, other gas(es) exiting the vial over time, or gas(es) not been trapped in the sampling procedure. Also as time progressed, the recovery of ^{14}C-RDX by the ether extraction

may have been incomplete because of stronger sorption of ^{14}C-RDX into the soil matrix.

Reproducibility of data in the Aerobic Autoclaved vials was fairly high with differences among individual samples, for a given day, fairly small. For instance, the standard deviation values ranged for 0.05% to 8.3%, with the median being 2.5 based on percent ^{14}C. The average standard deviation was calculated to be 3.3%. Therefore, the experimental error was interpreted to be ± 3.3% of the values shown in Figure 4.2.

A limitation in the sampling technique can also be seen in Figure 4.2. Looking at the first day of sampling, the "ether" data shows that only 78 percent of the original radiolabeled chemicals were present. This implies that 22% of the ^{14}C-RDX has been biotransformed within the first 10 days. However, if this was true the radioactivity should steadily decrease over time, which did not occur. Figure 4.2 also shows that about 24 percent of the original radiolabeled chemicals were present in the water; this quantity should have increased if biodegradation occurred. From the above observations and the fact that only 3% of the radiolabeled chemicals were trapped as $^{14}CO_2$, it was determined that very little biodegradation was taking place in this experiment.

The initial distribution of radioactivity between ether and water demonstrated that some of the ^{14}C-RDX partitioned into the water, rather than being completely extracted into the ether. This partitioning was caused by the solubility of RDX in water and may have been enhanced by a co-solvent effect caused by ether dissolution into water. The Merck Index (1989) shows that a saturated water solution contains 8.43% (w/w) of ether at 15°C and 6.05% (w/w) at 25°C. In addition, the solubility of ether in water increases as the concentration of an acid in water increases (i.e., pH decreases). In a concentrated acid, ether is completely soluble (Merck, 1989). Therefore, because an HCl solution was added to the vials during sampling to give a pH<2, considerable dissolution of ether into the water phase may have occurred, thereby enhancing the solubility of RDX in the water phase. Thus, using the radioactivity in the ether phase to represent RDX and the ring intermediates may somewhat underestimate the amount of these chemicals present. Kitts et al. (1994), who used a similar extraction technique, did not report partitioning or ether solubility problems; however, the pH of the aqueous solution in their experiments was not reported, so a comparison cannot be made.

(b) Phase One - Aerobic Experiments

Figures 4.3 and 4.4 represent the vials that were set up with air in the headspace. Under these conditions, the figures look similar to the autoclaved experiment, indicating that little degradation took place. For instance, the radioactivity in the ether only decreased slightly over time, indicating that very little ^{14}C from the ^{14}C-RDX and the nitroso derivatives was moving to the aqueous solution. As expected, the radioactivity in the water stayed fairly constant, because intermediate chemicals were not produced in significant quantities. However, one difference between the autoclaved and aerobic experiments is that the $^{14}CO_2$ production (CO_2 meas) was not at zero on the first day of sampling. This implies some initial degradation took place within the first 10

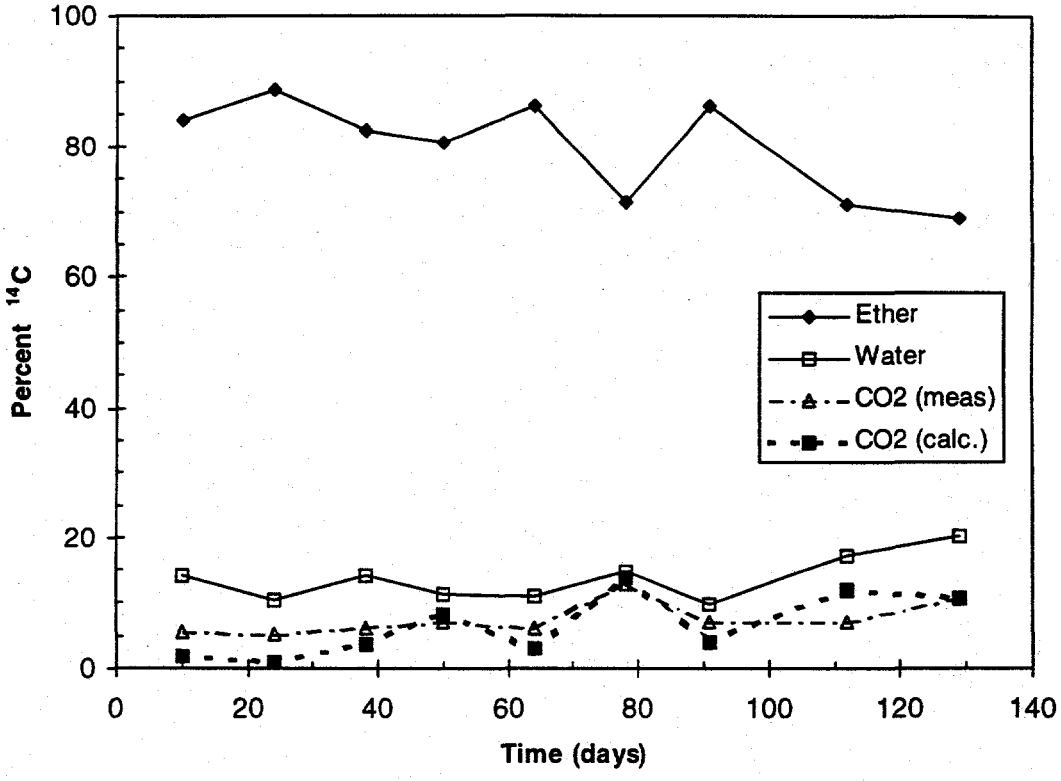


Figure 4.3: ^{14}C-Radioactivity for Aerobic Soil Vials Amended with Phosphorus

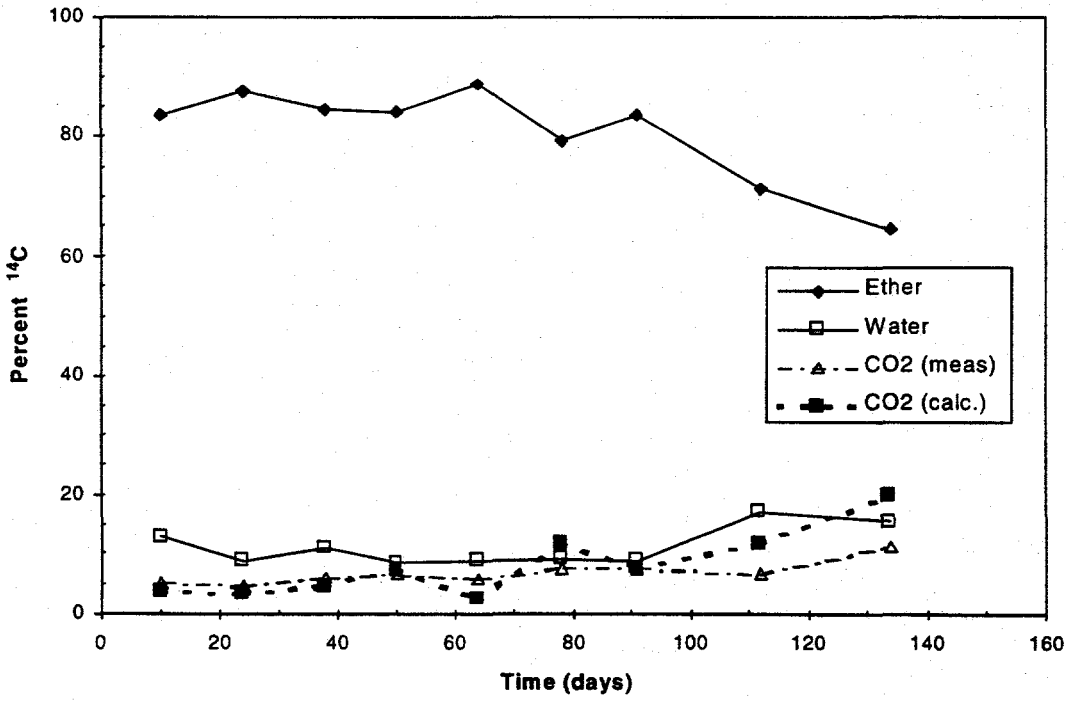


Figure 4.4: ^{14}C-Radioactivity for Aerobic Soil Vials

days that was not present in the autoclaved experiment. In addition, the radioactivity in the water rose slightly and that in the ether declined slightly after the first 91 days, suggesting some partial degradation of ^{14}C-RDX occurred. However, in general the overall degradation of ^{14}C-RDX in both aerobic experiments was minor with approximately 10% of the RDX mineralized.

The recovery efficiency again only slightly declined throughout the two experiments. The average recovery was roughly 101% at the beginning and 97% at the end of the Aerobic with Phosphorus experiment. Thus, complete mass balance closure was achieved within ±5% as indicated by the little difference between the CO_2 (calc) line and the CO_2 (meas) line on Figures 4.3. Recovery efficiency for the Aerobic experiment was lower with recovery starting at 100% and ending at 88%. The exact cause for the lower recovery efficiency is not known; however, soil heterogeneity and previously mentioned reasons are the likely cause.

When comparing the two aerobic experiments to each other, little difference can be seen between the samples with phosphorus and without; thus, it was concluded that phosphorus was not a limiting nutrient under this condition. Likewise, both data sets showed little variation between individual samples tested on a given day, suggesting reproducibility was high. The average standard deviation based on percent ^{14}C, for the Aerobic with Phosphorus experiment was 4.8% with the median being 2.0%. The average standard deviation for the Aerobic experiment was 2.6% with the median being 1.7%. Thus, the average experimental errors for both experiments were within ± 5%.

(c) Phase One-Anoxic Experiments.

Figures 4.5 and 4.6 represent the vials with nitrogen gas in the headspace. Under these conditions, the radioactivity in the ether decreased significantly over time, indicating that RDX and the three nitroso derivatives were biodegraded. As would be expected, the radioactivity in the water increased somewhat as the ring cleavaged products formed. The radioactivity in the water did not increase proportionally to the ether throughout the experiment because some of these intermediate products were further metabolized to inorganic carbon, as indicated by the measured $^{14}CO_2$ production (CO_2 (meas)) in Figures 4.5 and 4.6.

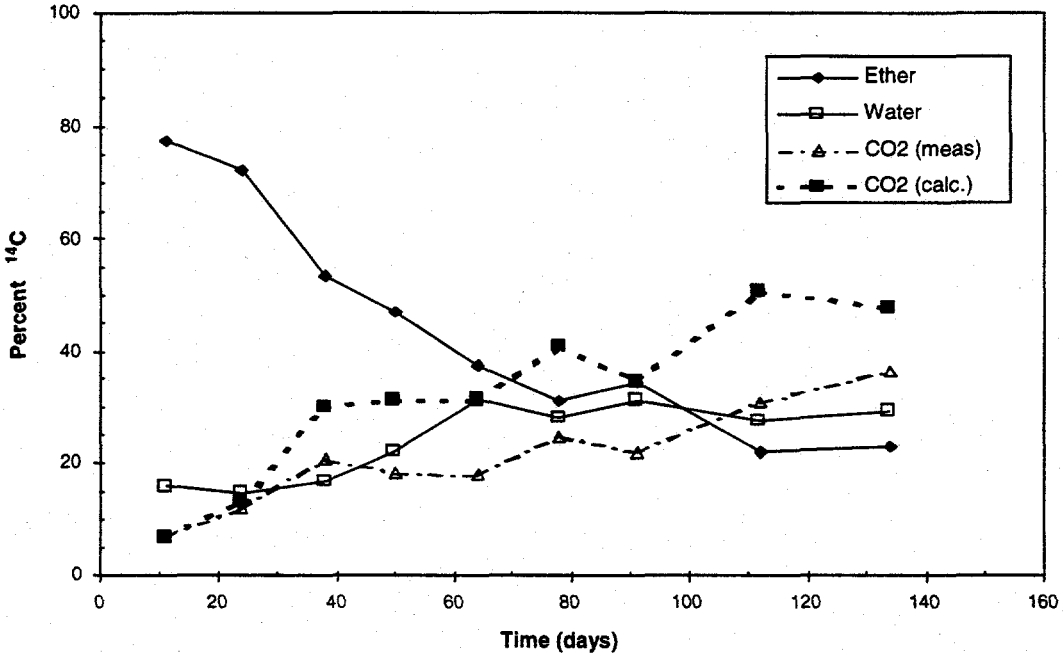


Figure 4.5: ^{14}C-Radioactivity for Anoxic Soil Vials Amended with Phosphorus

The extent of mineralization based on the "CO2 (meas)" data was roughly 36% by the end of the experiment.

The ^{14}C recovery efficiency declined throughout the two anoxic experiments from initial values of approximately 101% to final values of approximately 88%. Because notable amounts of ^{14}C-RDX disappeared and significant amounts of $^{14}CO_2$ were produced, the most likely explanation for the lack of mass balance closure is additional losses of inorganic carbon not accounted for by the recovery efficiency correction. The maximum possible mineralization is shown in Figure 4.5 and 4.6(CO_2 calc) and reached approximately 47% by the end of the experiments. Once again, the difference between the CO_2 (calc) and the CO_2 (meas) lines illustrates losses caused by an inaccurate $^{14}CO_2$-recovery factor or by the loss of the ^{14}C-compounds other than $^{14}CO_2$ in the analytical procedure.

Figures 4.5 and 4.6 also showed small differences between samples with and without phosphorus. In samples with phosphorus, approximately 10% more ^{14}C-RDX was biotransformed than samples without phosphorus. This observation was made by comparing the amount of radioactivity in the ether at the end of both experiments. From these data, it was apparent that phosphorus availability could be limiting RDX biodegradation. A more detailed analysis to determine if phosphorus was limiting is performed in the next section. The differences among replicated samples were small for both anoxic experiments, once again suggesting good reproducibility between vials. The average standard deviations for the Anoxic with Phosphorus and Anoxic experiments were both 4.7 %. The medians for the experiments were 4.0 % and 3.9 %, respectively. These standard deviations were higher than those found in other experiments, and the higher values may be due to significant biodegradation occurring within the vials. Even so, the average standard deviation still fits within an acceptable experimental error of ± 5%.

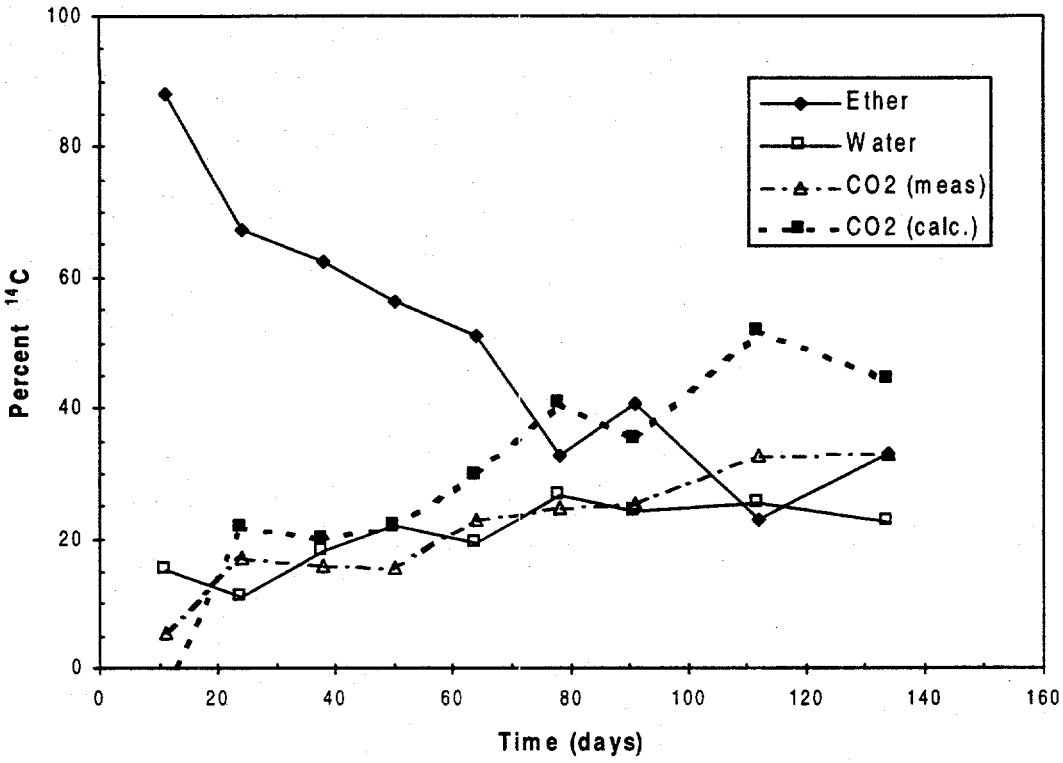


Figure 4.6: ^{14}C-Radioactivity for Anoxic Soil Vials

(d) Phase One - Microaerobic Experiments

Figures 4.7 and 4.8 represent samples with 3% oxygen and 97% nitrogen gas in the headspace initially. Under these conditions, only a small change in the radioactivity measurements occurred over the first 24 days for both experiments. However, after 24 days the radioactivity in the ether fraction declined sharply; this sharp decline was not seen in the Anoxic experiments. Likewise, the radioactivity in the water rose sharply inversely mimicking the ether. However, the increase of ^{14}C radioactivity in the water was not directly proportional to the ether loss, because the aqueous dissolved chemicals were further metabolized to inorganic carbon. This metabolism was indicated by the steady rise in $^{14}CO_2$ (CO2 (meas)) shown in Figures 4.7 and 4.8. The extents of mineralization for the Microaerobic with Phosphorus and Microaerobic experiments were approximately 38% and 46%, receptively.

Based on the significant change in the slopes of the ether data over time, it was hypothesized that the environmental condition in the vials may have changed. To test this hypothesis, vials were sacrificed after day 24, and a headspace analysis was performed on the gases in the vial. The analysis on both vial types showed only

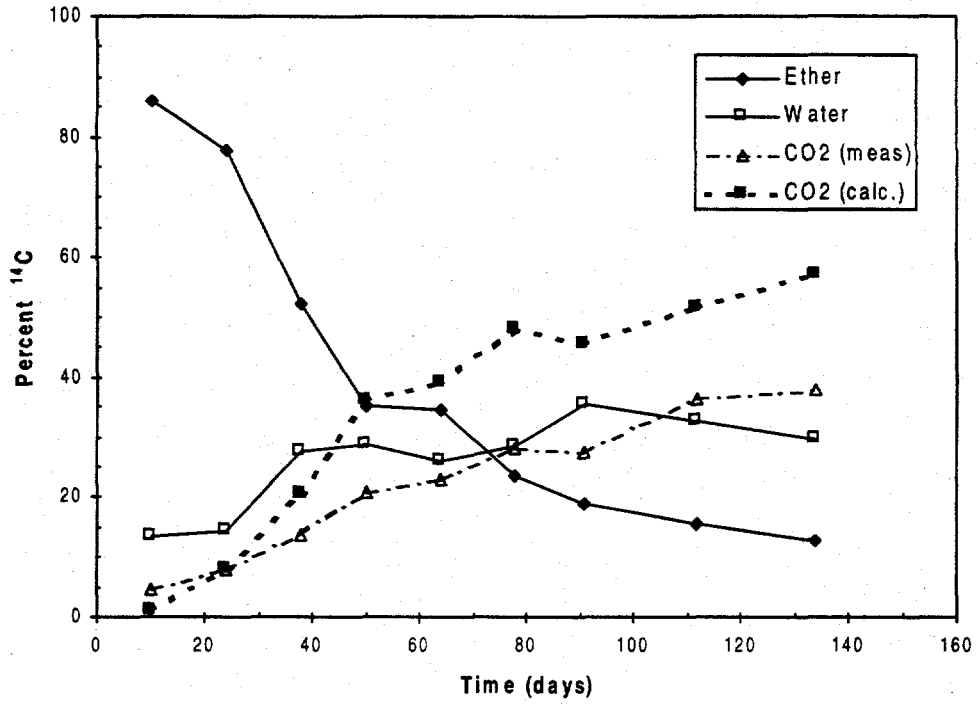


Figure 4.7: ^{14}C-Radioactivity for Microaerobic Soil Vials Amended with Phosphorus

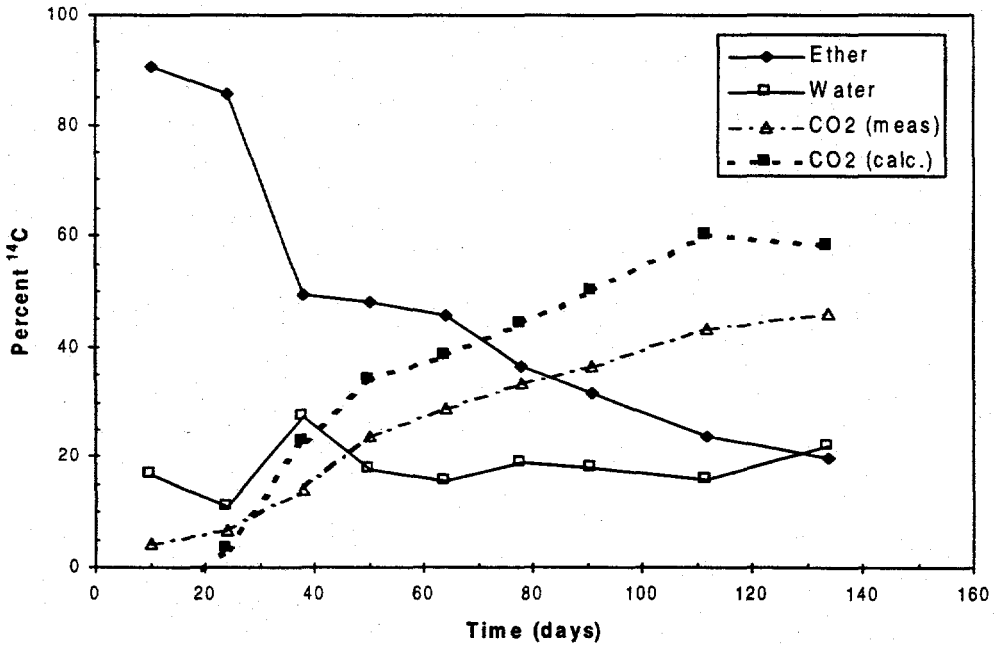


Figure 4.8: ^{14}C-Radioactivity for Microaerobic Soil Vials

traces of oxygen present in the headspace; thus, the vials had shifted to anoxic conditions. Therefore, the data suggest that the onset of significant biodegradation coincided with the establishment of anoxic conditions in the vials. After several weeks of anoxic conditions, a more gradual reduction of RDX in the ether was apparent. The exact reason for the slope change is not known. However, the availability of RDX, water, or nutrients are the most likely causes.

The recovery efficiencies of all ^{14}C-chemicals declined significantly over the duration of the study for both sets of vials. The ^{14}C losses were larger for these experiments than in all previously mentioned experiments. For instance, the initial average recovery was approximately 104% at the beginning and 80% at the end for the Microaerobic with Phosphorus Experiment. Thus, 10% more ^{14}C-compounds were lost under these conditions when significant biodegradation occurred than under anoxic conditions. Assuming that the missing radioactivity was all $^{14}CO_2$, the maximum possible mineralization (CO_2 calc) that may have occurred in these experiments was notably greater than the actual carbon dioxide production (CO_2 meas). The maximum possible mineralization could have reached approximately 57% for both experiments. The significance of this mineralization is that almost 60% of the original RDX may have been converted to a nontoxic, inert gas. As a comparison, the maximum possible extent of mineralization of RDX in the Microaerobic experiments was 21% more than was calculated in the anoxic experiments (57% vs. 47%).

The effect of phosphorus addition was also more significant than in the anoxic experiments. By the end of the experiment, roughly 13% of the initial ^{14}C remained in the ether phase of the vials amended with phosphorus (Figure 4.7), while approximately 33 % of the ^{14}C remained in the vials that received no phosphorus (Figure 4.8). These data strongly suggest that the availability of phosphorus limited ^{14}C-RDX degradation. A more detailed analysis to determine if phosphorus was limiting is presented in Section 4.4.

The differences among replicated samples were small for both Microaerobic experiments. Thus, the standard deviations are similar to the standard deviation seen in other experiments, illustrating reproducibility in the sampling procedures. The average standard deviations for the Microaerobic with phosphorus and Microaerobic experiment were 3.6 % and 3.2 %, respectively. The medians for the experiments were 3.0 % for the Microaerobic with phosphorus and 2.4% for the Microaerobic. Once again, these data fit within the acceptable experimental error of ± 5%.

(e) Phase Two – Anoxic Autoclaved Soil with Organic Carbon Experiment

Figure 4.9 shows the results from the autoclaved soil vials that were amended with biodegradable organic carbon. Under these conditions, the radioactivity in the water and ether changed somewhat over the duration of the study (107 days). As would be expected, $^{14}CO_2$ production (CO_2 (meas)) was negligible, except for one outlining point at Day 64. The extent of mineralization was less than 3% for this experiment. Thus, the data indicate that little biodegradation occurred in the vials.

The ^{14}C recovery efficiency declined similarly to other vials with little biodegradation (Figures 4.2, 4.3, and 4.4). The average ^{14}C recoveries ranged from approximately 91% on Day 9 to approximately 81% on Day 107. Thus, the total decreased in recovery efficiency was roughly 10% over the entire experiment, which is similar to the other autoclaved and

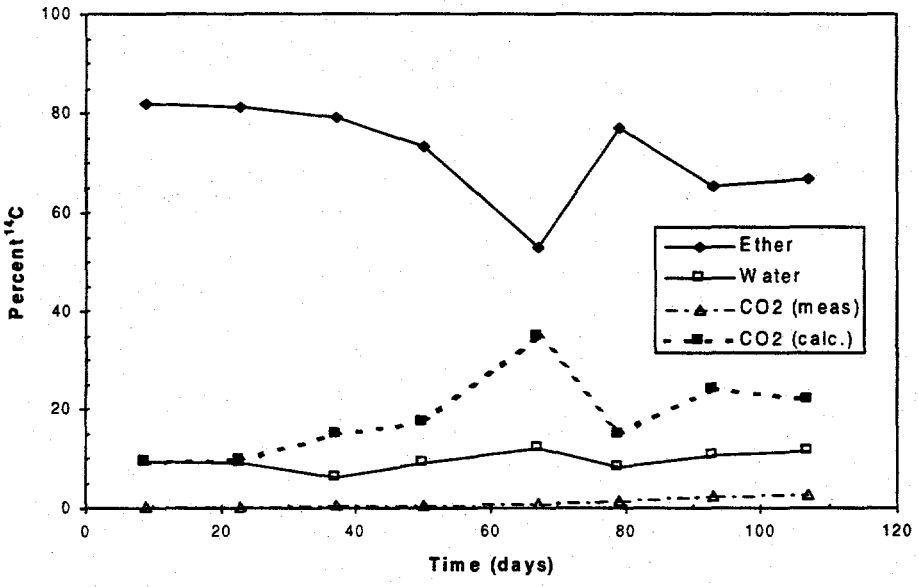


Figure 4.9: ^{14}C-Radioactivity for Anoxic Autoclaved Soil Vials Amended with Organic Carbon

aerobic experiments. Because very little $^{14}CO_2$ was produced, incomplete capture of inorganic carbon does not fully explain the loss of ^{14}C from the vial; this is illustrated in Figure 4.9 with the CO_2 (calc.) line. The CO_2 (calc.) line represents a 100% mass balance closure where an assumption was made that all unrecovered ^{14}C was assigned as $^{14}CO_2$. Therefore, the difference between the CO2 (calc) and the CO2 (meas) lines represents the amount of unrecovered ^{14}C. Lastly, it is unclear why the initial recovery efficiency was lower than 100% for this experiment.

The Anoxic Autoclaved with Organic Carbon experiment consisted of a total of 15 vials; therefore, only one to two vials were tested each sampling day. Thus, reproducibility of the data was only checked if two vials were sampled on the same day. Calculations showed the average standard deviation for days when two vials were sampled was 1.8, and the median was 0.78 based on percent ^{14}C. Thus, the average standard deviation fits within an acceptable experimental error of ± 5%.

(f) Phase Two – Anoxic Organic Carbon Experiments

Figures 4.10 and 4.11 present data for vials which contained nitrogen gas in the headspace. The soil in the vials was amended with readily biodegradable organic carbon. One set of vials was also amended with phosphorus. Under these conditions, the amount of radioactivity in the ether decreased significantly over time. As would be expected, the radioactivity in the water increased notably as the ringed products were cleaved. However as noted previously, a one-to-one relationship did not exist between the decrease of ^{14}C in ether and increase in ^{14}C in the water because some of the aqueous intermediate products were further metabolized to inorganic carbon. The increase of $^{14}CO_2$ (CO_2 meas) as seen on Figures 4.10 and Figure 4.11 demonstrates the further metabolism of the intermediate products. For these experiments, there was also a difference in the extent of mineralization between amended and unamended phosphorus vials. The extents of mineralization for the experiments were

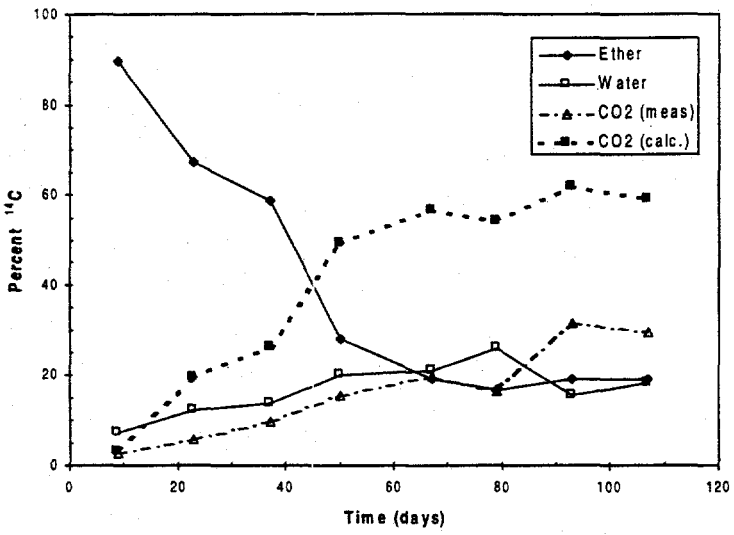


Figure 4.10: ^{14}C-Radioactivity for Anoxic Soil Vials Amended with Carbon and Phosphorus

approximately 30% and 13% for phosphorus amended and unamended vials, respectively. Another difference between the Anoxic Organic Carbon vials with and without phosphorus can be seen in Figure 4.11. The figure shows the ether data for the unamended phosphorus amended soil leveled off at about 40% of the initial ^{14}C by day 37, whereas the ether data for the amended soil leveled off at about 19% by day 67 in Figure 4.10. All other experiments where biodegradation occurred removed at least 7% more ^{14}C from the ether phase by the end of the experiment than the Anoxic with Organic Carbon experiment. Thus, it appears that something was limiting the degradation of ^{14}C-RDX in this experiment. The exact cause of the limitation is not known. However, some possibilities may be that available phosphorus limited the degradation, some RDX was not available due to sorption onto the soil, or a better nitrogen source than RDX was available to the organisms (e.g., recycled nitrogen from endogenous metabolism). A comparison of the organic carbon amended vials to unamended vials shows that the loss of radioactivity from the ether occurred almost two times faster in the vials receiving biodegradable organic carbon. For example, Figure 4.10 shows approximately 19% of the initial ^{14}C was present in the ether at day 67; this percentage is less than almost every experiment from phase one after 134 days. However, the ether data stayed relatively constant at 19% thereafter, whereas values as low as 13% were observed in the Microaerobic with Phosphorus experiment. Once again, the larger quantity of RDX left in the soil may have been due to the lack of available phosphorus, the lack of available RDX, or the availability of an alternative nitrogen source.

^{14}C recovery efficiencies declined rapidly in these experiments. In fact, total recovery efficiency was about 12% lower than other vials where significant biodegradation occurred. The initial average recoveries were approximately 100% on Day 9 and declined to as low as 68% by Day 107. Assuming, as in other experiments, that unaccounted for radioactivity is in the form of $^{14}CO_2$, the maximum possible mineralization is shown in Figure 4.10 and 4.11 (CO_2 calc).

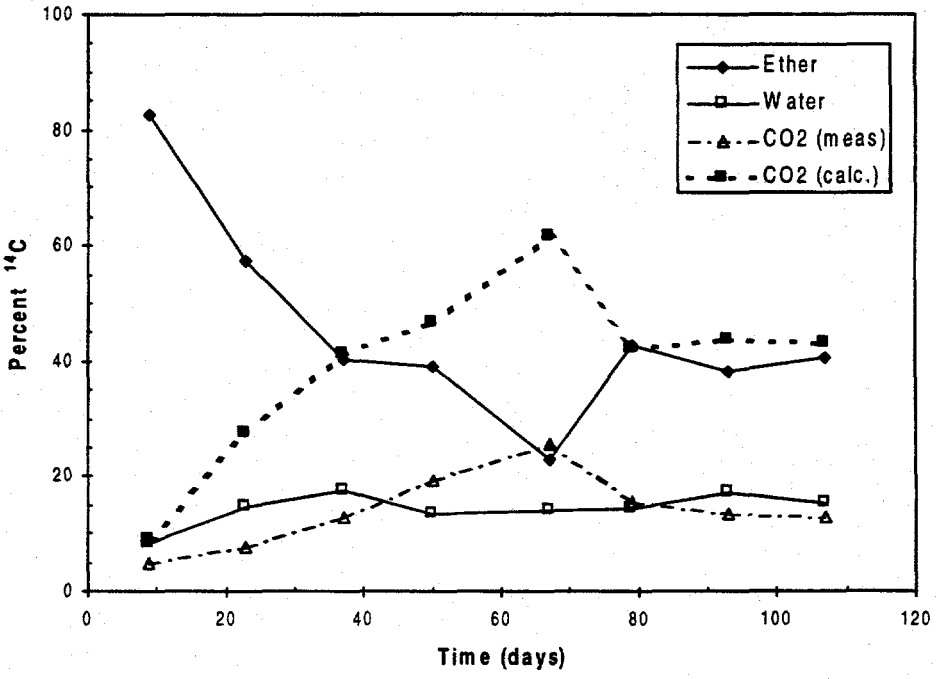


Figure 4.11: ^{14}C-Radioactivity for Anoxic Soil Vials Amended with Carbon

Table 4.2: Summary of Radiochemical Data from the Biodegradation Experiments

Experiment (Length in days)	**Loss of ^{14}C from Ether** (ether %)	**Extent of Mineralization** (CO2 meas, %)	**Maximum Extent of Mineralization** (CO2 calc, %)	**Recovery Efficiency** (Start, Finish %)
Aerobic Autoclaved Soil w/ Phosphorus (134)	10	3	14	103, 88
Anoxic Autoclaved Soil w/ Carbon (107)	15	3	22	91, 81
Aerobic (134)	13	11	14	101, 97
Aerobic w/ Phosphorus (129)	12	10	11	104, 95
Microaerobic (134)	71	46	58	111, 88
Microaerobic w/ Phosphorus (134)	73	38	57	104, 80
Anoxic (134)	55	33	44	108, 88
Anoxic w/ Phosphorus (134)	50	36	47	101, 88
Anoxic w/ Carbon (107)	42	13	43	95, 68
Anoxic w/ Phosphorus and Carbon (107)	70	30	59	99, 67

The maximum possible CO_2 production reached approximately 59% for vials amended with phosphorus, and 43% for unamended vials. Thus, roughly 60% of the RDX may have been mineralized.

The data from the Anoxic Organic Carbon experiments fit within the experimental error of ± 5%. The average standard deviation for the Anoxic with carbon and phosphorus was 4.8% and the median for this experiment was 4.0%. The average standard deviation and median for the Anoxic with Organic Carbon experiment was 2.9% and 3.9%, respectively.

4.2.1 Summary of Biodegradation Data

Table 4.2 shows the loss of ^{14}C for ether, the extent of mineralization, the maximum possible extent of mineralization (assuming all unaccounted for ^{14}C was $^{14}CO_2$), and recovery efficiencies. The loss of ^{14}C from the ether was calculated by subtracting the average amount of ^{14}C present in the ether on the first day of sampling from the last day of sampling. The extent of mineralization is the data called "CO_2 (meas)" from the previous figures, and the maximum extent of mineralization is the data "CO_2 (calc)" from the previous figures. Lastly, average recovery efficiencies are presented with the first number representing the average efficiency on the first day of sampling and the second number representing the average efficiency on the last day of sampling. Raw data for all of the biodegradation experiments can be seen in Appendix C.

A number of important patterns are evident in the table. First, significant loss of ^{14}C from the ether phase only occurred under microaerobic and anoxic conditions. This means that degradation of RDX and ring intermediate products only occurred under these conditions. Second, little loss of ^{14}C from the ether or production of $^{14}CO_2$ occurred under aerobic conditions, suggesting no RDX was degraded. Third, in addition to RDX loss under anoxic and microaerobic conditions, significant CO_2 production was observed indicating that the ring was cleaved and some were resulting products completely mineralized. Fourth, the addition of phosphorus did not have a significant effect on the removal of RDX from most of the vials; however, differences between phosphorus and nonphosphorus vials were evident in the microaerobic experiment and anoxic experiment with organic carbon addition. Finally, the addition of organic carbon affected the rate at which RDX was removed but did not affect the overall removal of RDX from the vials, except for in the Anoxic with Organic Carbon experiment.

4.3 DEGRADATION RATE CONSTANTS

This section is divided into three parts. The first part explains a co-solvent correction factor for incomplete recovery of RDX and the ring intermediates in the ether phase. The second part compares HPLC analyses to radiochemical analyses to show mass losses of unlabeled and labeled RDX. The third part discusses degradation rate constants for each batch biodegradation experiment.

4.3.1 Ether Recovery Correction Factor

The recovery of ^{14}C-RDX and nitroso derivatives was calculated by taking the radioactivity of the nonvolatile products that partitioned into the ether and dividing this total by the initial amount of radioactivity for each series. The initial ether radioactivity for all of the experiments was fairly consistent and less than expected. The initial radioactivity recoveries for the ether phase were expected to be 95% to 100%; however, the actual initial recoveries were around 80%. The low initial ether recovery resulted from a larger than expected fraction of radioactivity partitioning into the water.

A recovery correction factor was, therefore, applied to the ether data to adjust for the incomplete recovery of ^{14}C-RDX. The Aerobic Autoclaved with Phosphorus experiment, excluding the last two sampling days, was used to calculate the correction factor. These data were used because very little degradation occurred and mass balance closures were 90% or better. The last two sampling days were excluded because the radioactivity in the ether decreased and mass balance closure was not within 5% of the other data. The average recovery in the 21 vials considered was 79.17% with a standard deviation of 1.13%.

Autoclaved with Organic Carbon data were not used because triplicate analyses were not performed throughout the experiment. In addition, latter data points in the Autoclaved with Organic Carbon experiment showed some drop in radioactivity in the ether, and in general, the mass balances did not close as well as in the Autoclaved with Phosphorus experiment. However, the Autoclaved with Organic Carbon data generally agreed with the Autoclaved with Phosphorus experiment.

To apply the ether correction factor to all of the experiments, two assumptions were made. First, RDX and ring products were assumed to partition identically between ether and water. Second, it was assumed that non-ringed intermediates did not partition into the ether. With these assumptions, the correction factor was applied by dividing the ^{14}C-ether data by the correction factor to get the corrected recovery for the RDX and nitroso derivatives remaining. For example, if the original ether data for a sample was 76% than the corrected value was 75% / 0.7916 or 94.7%.

4.3.2 RDX Mass Loss Comparison

The radiochemical data report the fate of the ^{14}C-RDX added to the soil. However, of greater importance is the fate of the unlabeled RDX originally present in the soil. Thus, HPLC analyses of RDX soil concentrations were performed on selected vials to compare ^{14}C-RDX removal to total RDX removal. If the comparison shows that the ^{14}C-RDX removal was representative of total RDX removal, then radiochemical data can be used to calculate degradation rate constants.

The following assumptions were made to compare these data. First, the three nitroso derivatives (ring products) were assumed to partition between ether and water identically to RDX. Second, the ring cleaved products were assumed not to partition back into the ether phase. This assumption is conservative, because if they do partition into ether less RDX degradation would be calculated. Table 4.3 shows a summary of all the data for the two analyses and provides some statistical information. The data for the Aerobic Autoclaved with Phosphorus and Autoclaved with Organic Carbon experiments are not reported, because autoclaving destroyed the initial RDX in the soil. The data for the radiolabeled analyses represents the ether data obtained on the last sampling day for each. At least two samples were tested from each experiment by both analyses. Raw data for the HPLC analyses can be seen Appendix D.

In general, data from Figure 4.3 show that the removal of RDX from the soil analyzed by HPLC was greater than the removal of spiked RDX from the soil analyzed by liquid scintillation counting. The maximum removal of original RDX was seen in the Microaerobic with Phosphorus experiment (95%). Four other

Table 4.3: Comparison of HPLC and Radiolabeled Analyses

Experiment Name	Radiolabeled Study Removal (%)	Mean (%)	Standard Deviation (%)	HPLC Study Removal (%)	Mean (%)	Standard Deviation (%)
Aerobic	11.05 33.11* 11.84	11.44	0.56	10.90 23.34 18.25	17.50	6.25
Aerobic w/ Phosphorus	44.03* -1.18 -4.75	-2.96	2.53	24.63 21.51 11.01	19.05	7.13
Microaerobic	77.19 72.91 74.85	74.98	2.14	81.55 79.73 89.12	83.46	4.98
Microaerobic w/ Phosphorus	84.05 83.29	83.67	0.54	93.24 95.89	94.57	1.87
Anoxic	82.62 62.17 54.4	66.4	14.58	58.67 87.02 67.98	71.22	14.45
Anoxic w/ Phosphorus	65.83 81.34 65.99	71.05	8.91	87.99 96.95	92.47	6.34
Anoxic w/ Carbon	81.48 87.14	84.31	4.00	83.10 92.29 82.75	86.05	5.41
Anoxic w/ Carbon & Phosphorus	89.76 65.82 72.03	75.87	12.42	95.71 82.12 82.61 76.01	84.13	7.71

* Data not used for mean and standard deviation

experiments removed over 83% of the original RDX: Anoxic with Phosphorus, Microaerobic, Anoxic with Organic Carbon and Phosphorus, and Anoxic with Carbon. Removal of ^{14}C–RDX, however, was approximately 10% less than removal of total RDX in the above experiments.

Vials under aerobic conditions showed considerably different removal efficiencies. For example, the percent removals obtained from the Aerobic with Phosphorus radiolabeled analysis showed negative values, -1.18 and - 4.75 percent. Negative values were possible because the ether recovery correction factor was an average value and imply that no degradation occurred. For the same experiment, the HPLC data showed that roughly 19% of the total RDX was removed from the soil. These data are opposite of what is normally seen in spiked soil studies. Normally, more removal of radiolabeled chemicals would be expected, because spiked chemicals are presumed more readily available to microorganisms. A possible explanation may be that the radiochemical analyses underestimated RDX metabolism, because RDX and ringed intermediates are included in the measurement, whereas the

HPLC analysis only measures RDX. Also, any partitioning of ring cleavage intermediates into the ether would decrease the apparent RDX removal in the radiochemical analyses.

4.3.3 Biodegradation Batch Experiments

Because removal efficiency of ^{14}C-RDX was approximately the same as total RDX removal, radiochemical data were used to calculate removal rate constants. A total of eight rate constants were computed from the eight experiments used in the comparison analyses. Raw data and statistical analyses can be seen in Appendix E.

The conversion from ^{14}C percent to mass loading was made by multiplying the corrected ether data by the total mass RDX loading on the soil. The RDX mass loading on the soil was determined to be 12.65 mg/kg, which includes the ^{14}C-RDX added and the RDX originally present on the soil. The spiked mass of ^{14}C-RDX was calculated from the average of the direct inject vials for each series; this value was roughly 0.32 mg/kg with a standard deviation of 0.0088 mg/kg.

Because a new HPLC was brought on-line to sample the biodegraded soil for the comparison study, original soil samples that had not been spiked or biodegraded were retested. Average RDX loadings greater on the new HPLC than on the old HPLC; therefore, the new average RDX loadings was used in the total mass and degradation rate constants calculations. The initial RDX loading was calculated from an average of triplicate HPLC samples performed on the soil coring. The average mass of RDX present on the soil was 12.33 mg/kg.

Figures 4.12 and 4.13 show the results for the two aerobic experiments. As noted previously, very little degradation of RDX was observed under aerobic conditions. The degradation rate constants were determined by fitting both a zero and first-order kinetic functions to the data. The lines for the zero and first-order were very similar; however statistically, the zero order function fit the data slightly better. The zero-order degradation rate constant for the Aerobic Phosphorus experiment was calculated

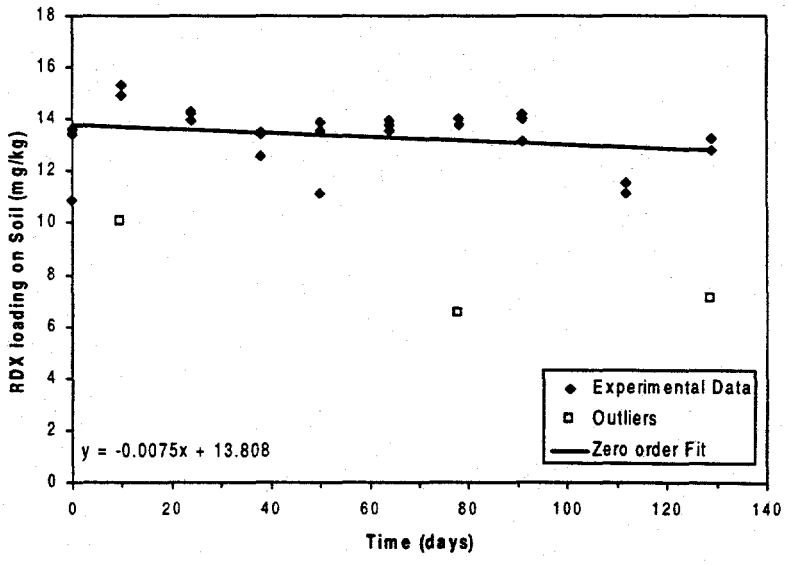


Figure 4.12: Estimated RDX Loading on Soil for Aerobic Vials Amended with Phosphorus

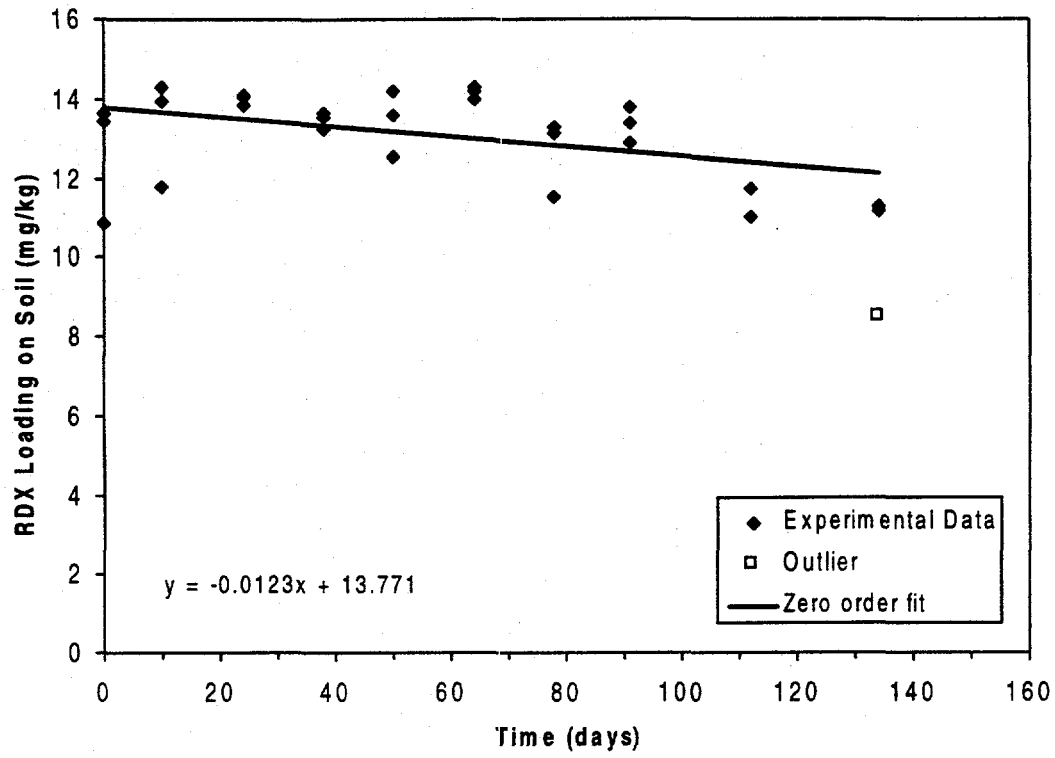


Figure 4.13: Estimated RDX Loading on Soil for Aerobic Vials

to be 0.0075 mg/kg·day. The 95% confidence interval for the Aerobic with Phosphorus data ranged -0.019 and 0.004 mg/kg·day. Thus, the rate constant is not statistically different from zero. The R^2 value for this experiment was 0.07, as might be expected because the RDX loading showed essentially no dependence on time. For comparison with the other experiments, the first-order degradation rate constant of 0.0005 day^{-1} was used to calculate the half-life for the degradation of RDX. The half-life for this experiment was 1390 days.

The zero-order rate constant for the Aerobic experiment was calculated to be 0.0123 mg/kg·day. The 95% confidence interval for these data was between 0.002 and 0.022 mg/kg·day. This rate constant was statistically different from zero; thus a small amount of degradation occurred in this vial series. The R^2 for this experiment was 0.20, which is somewhat better than for the phosphorus-amended experiment but still indicative of very poor fit. To compare with the other experiments, the first-order degradation rate constant of 0.001 day^{-1} was used to calculate the half-life for the degradation of RDX. The half-life for this experiment was 690 days.

When the two aerobic experiments were compared against each other, the 95% confidence intervals were found to overlap. Therefore, they are not statistically different from each other. The overlapping of confidence intervals indicates that the effect of phosphorus addition on the degradation rate was not statistically significant, which was to be expected because no biodegradation occurred.

Figures 4.14 and 4.15 show the data for the Anoxic with Phosphorus and Anoxic experiments. These figures illustrate that substantial biodegradation occurred and that the extent of degradation was similar for both experiments. A total of approximately 9.5 mg/kg was removed over the duration of the study.

Visually, the data from both experiments were curved; therefore, a first-order kinetic function was fit to these data to determine the degradation rate

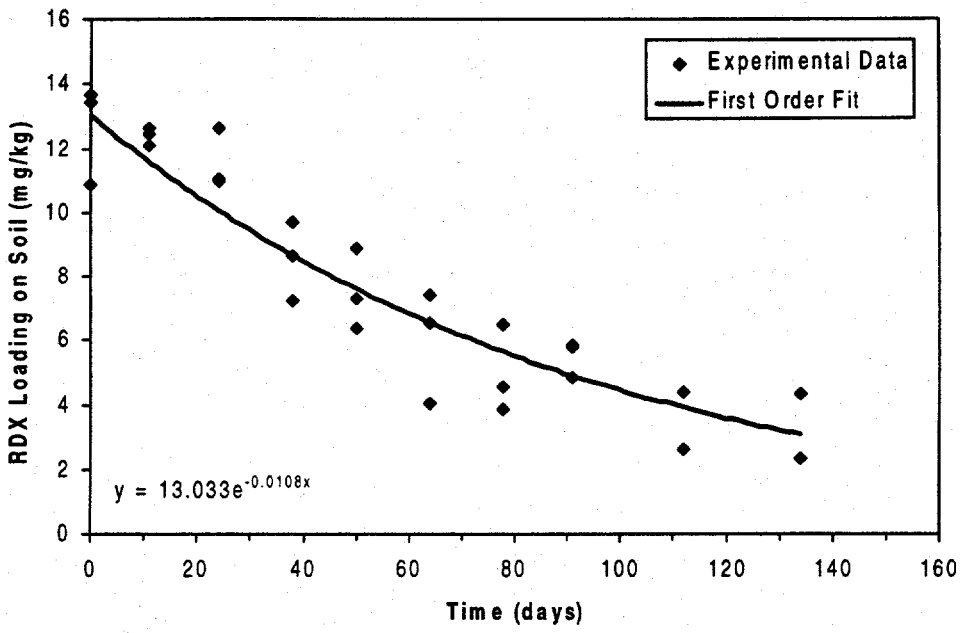


Figure 4.14: Estimated RDX Loading on Soil for Anoxic Vials Amended with Phosphorus

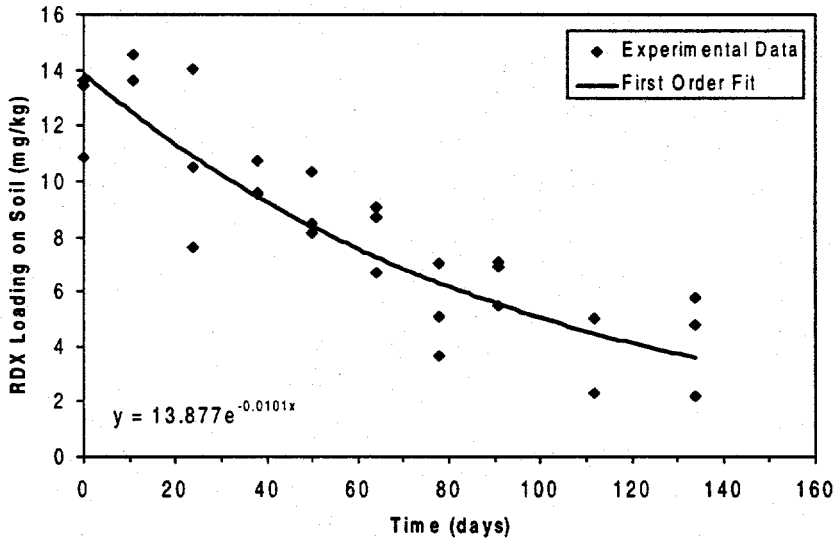


Figure 4.15: Estimated RDX Loading on Soil for Anoxic Vials

constants. The first order rate constant was estimated from a linear regression of the natural log of RDX loading versus time. The first order degradation rate constant for the Anoxic with Phosphorus was determined to be 0.0108 day^{-1}. The 95% confidence interval ranged from 0.009 to 0.013 day^{-1}. R^2 for the linear regression was 0.83, which implies that the data fit the regression line quite well. For this rate constant, the half-life for RDX degradation would be 64 days.

The Anoxic experiment had a slightly lower first-order degradation rate constant of 0.0101 day^{-1}. Using the same statistical method as mentioned above, the 95% confidence interval ranged from 0.007 to 0.012 day^{-1}. The R^2 for this experiment was 0.68, which implies that the data varied more than in the Anoxic with Phosphorus experiment. At this rate constant, the half-life for the degradation of RDX would be 68 days.

A comparison of the 95% confidence intervals for the rate constant of both experiments shows that the intervals overlapped. Therefore, the rate constants were not statistically different from each other, suggesting no discernible difference in the observed degradation rate between the vials with and without phosphorus.

Figures 4.16 and 4.17 show the Microaerobic with Phosphorus and Microaerobic experiments. For these experiments, substantial biodegradation was again seen. The overall mass removal for both experiments was approximately 10.8 mg/kg, which was a slightly greater removal than seen in the anoxic experiments.

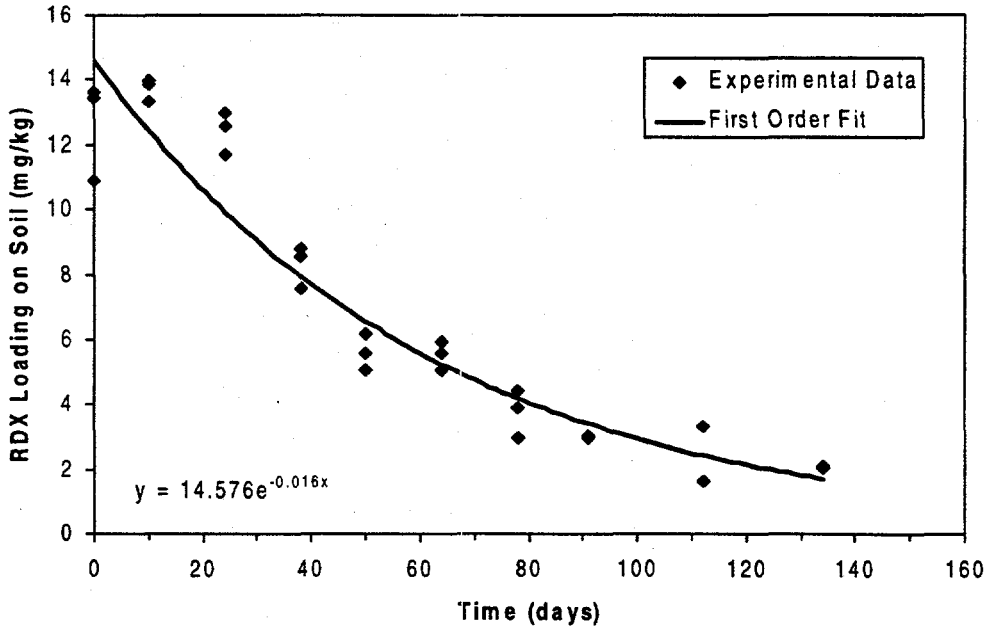


Figure 4.16: Estimated RDX Loading on Soil for Microaerobic Vials amended with Phosphorus

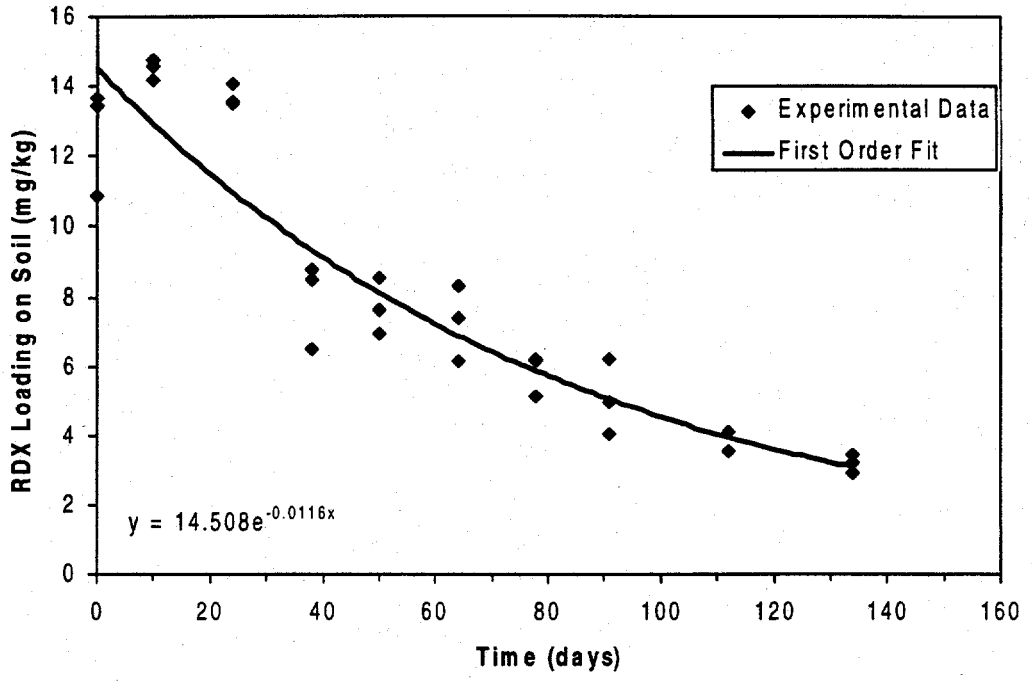


Figure 4.17: Estimated RDX Loading on Soil for Microaerobic Vials

First-order kinetic functions were fit to the data for both experiments to determine the degradation rate constants. Once again, the rate constant was estimated from a linear regression of the natural log of RDX loading versus time. The first order degradation rate constant for the Microaerobic with Phosphorus was 0.016 day^{-1}. The 95% confidence interval ranged from 0.014 and 0.018 day^{-1}. The R^2 for this experiment was 0.92, indicating an excellent fit to the data. At this degradation rate constant, the half-life for RDX degradation would be 43 days.

The Microaerobic experiment had a first-order degradation rate constant of 0.012 day^{-1}. The 95% confidence interval for the degradation rate constant in the Microaerobic experiment ranged from 0.010 to 0.013 day^{-1}. The R^2 value for this experiment was 0.91, again indicating an excellent fit to the data. At this degradation rate constant, the half-life for the degradation of RDX would be 60 days.

A comparison of the confidence intervals for these two experiments revealed that they do not overlap. Thus, they are statistically different from each other. This suggests that phosphorus addition had a discernible effect on the RDX degradation rate.

Figures 4.18 and 4.19 show the Anoxic with Organic Carbon and Phosphorus and Anoxic with Organic Carbon experiments. These data show that significant biodegradation of RDX occurred. In addition, the removal of RDX to levels seen by previously mentioned experiments occurred within the first 67 days. Samples taken after 67 days show a leveling off of removal rates in both experiments. In particular, samples taken on the last three sample days (Days 79, 93, and 107) for the Anoxic with Organic Carbon experiment showed that a larger amount of RDX remained in the soil than on Day 67; the exact cause of the higher residual amount is unknown. However, some possible explanations are that a residual amount of RDX was too tightly bound to the soil to be available for biodegradation, the ringed

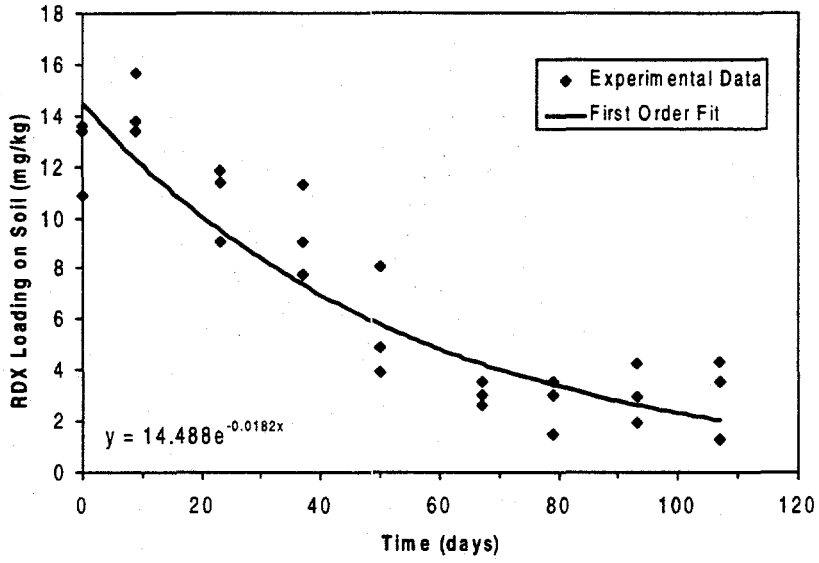


Figure 4.18: Estimated RDX Loading on Soil for Anoxic Vials Amended with Organic Carbon and Phosphorus

products slowly degraded, or organisms used another source of nitrogen. A first-order kinetic model was fit to the data for both experiments in order to determine the degradation rate constants. The first-order degradation rate constant for the Anoxic with Organic Carbon and Phosphorus was determined to be 0.018 day^{-1}. The 95% confidence interval ranged from 0.014 to 0.022 day^{-1}. The R^2 value for this fit was 0.78, which implies that the data varied somewhat from the model fit. The lower R^2 value may have been caused by a possible slope change or leveling of after 67 days. At this degradation rate constant, the half-life for the degradation of RDX would be 38 days. The first-order degradation constant for the Anoxic with Organic Carbon experiment was calculated to

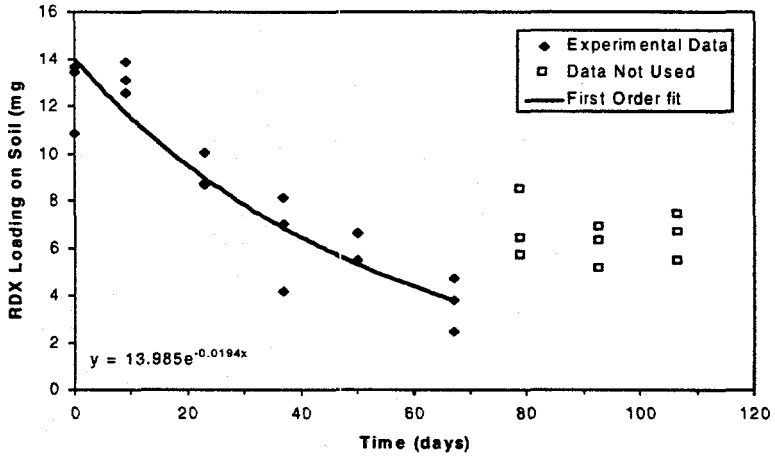


Figure 4.19: Estimated RDX Loading on Soil for Anoxic Vials Amended with Organic Carbon

be 0.019 day^{-1} by disregarding the last three days of data. The 95% confidence interval for the degradation rate constant in this experiment ranged from 0.015 to 0.024 day^{-1}. The R^2 value for this experiment was 0.83. Thus, the data fit the kinetic equation fairly well for the first 67 days. At this degradation rate constant, the half-life for the degradation of RDX would be 36 days.

The confidence intervals for these two experiments, as calculated above, overlap, so they are not statistically different from each other. This suggests that there was no discernible difference in the observed degradation rate between the vials with and without phosphorus addition. However if all of the data were used to calculate the rate constant for the Anoxic with Organic Carbon experiment, there would clearly be a difference in rate constants suggesting that phosphorus plays a role.

4.3.4 Summary of Degradation Rate Data

Table 4.4 displays the first-order degradation rate constants, the 95% confidence interval, the R^2 values, and the half-life for RDX degradation. A comparison of the degradation rate constants shows an order of magnitude difference between the aerobic degradation rate constants and the other experiments. In addition, the amendment of phosphorus significantly affected the degradation rates constants under microaerobic and anoxic conditions with organic carbon addition. Likewise, the degradation rates were influenced by the addition of an organic carbon source. However, a comparison between the Microaerobic with Phosphorus experiment and the experiments where organic carbon was added shows that the 95% confidence intervals for these experiments overlapped. Thus, there was no statistical difference among the rate constants for these three experiments. Finally, the half-lives among environmental conditions differed greatly. The half-lives for aerobic conditions were roughly 20 times greater than the half-lives for microaerobic conditions and anoxic conditions.

Table 4.4: Summary of Degradation Rate Data

Experiment Name	First Order Degradation Rate Constants (day^{-1})	95% Confidence Interval (Lower, Upper)	R^2 values	Half-life for RDX Degradation (days)
Aerobic*	0.001	0.0002, 0.002	0.19	690
Aerobic w/ Phosphorus*	0.0005	-0.003, 0.001	0.06	1390
Microaerobic	0.012	0.010, 0.013	0.91	60
Microaerobic w/ Phosphorus	0.016	0.014, 0.018	0.92	43
Anoxic	0.010	0.007, 0.012	0.68	68
Anoxic w/ Phosphorus	0.011	0.009, 0.013	0.83	64
Anoxic w/ Carbon	0.019	0.015, 0.024	0.83	36
Anoxic w/ Carbon & Phosphorus	0.018	0.014, 0.022	0.78	38

* First order equations were fit to the data in order to compare with other experiments.

4.4 HEADSPACE ANALYSES

Headspace analyses on the biodegradation vials were conducted to determine if gases other than CO_2 were produced. Analyses were conducted for five gases: CH_4, CO, CO_2, N_2, and O_2. One to three samples were collected from each experiment at the end of the degradation batch study. The molar concentration (%) of the five gases were determined by comparing 1 mL of a standard gas mixture (Table 3.3) to 1 mL of the headspace composition. Raw data for this analysis can be see in Appendix F.

Table 4.5 summarizes the data from the headspace analyses. As expected, all the vials showed some amount of carbon dioxide in the headspace, which means carbon dioxide was generated after the vial was sealed. Vials sealed with air in the headspace had some carbon dioxide initially present; however, vials sealed inside the glove bag where contained only nitrogen, or nitrogen and oxygen.

Table 4.5 also shows that the oxygen concentration in the microaerobic and aerobic experiments dropped by the end of the study. The microaerobic experiments was set up with 3% (mole%) oxygen in the vial, and the oxygen level dropped to less than a half of a percent by the end of the experiment. Vials that were sealed with air in the headspace were actually under oxygen depleting conditions by the end of the experiment (2% O_2). This low concentration of oxygen may have created anoxic conditions in the vials and may account for the slight loss of ^{14}C

Table 4.5: Summary of Headspace Analyses

Experiment (# of samples)	**Methane** CH_4 (mole %)	**Carbon Monoxide** CO (mole %)	**Carbon Dioxide** CO_2 (mole %)	**Nitrogen** N_2 (mole %)	Oxygen O_2 (mole %)
Aerobic Autoclaved Soil w/ Phosphorus (1)	0.00	0.00	0.07	10.2	2.43
Anoxic Autoclaved Soil w/ Carbon (1)	0.00	0.00	0.21	19.5	0.43
Aerobic (2)	0.00	0.04	0.39	14.0	2.1
Aerobic w/ Phosphorus (1)	0.00	0.00	0.17	9.0	1.9
Microaerobic (1)	0.00	0.00	0.41	19.0	0.27
Microaerobic w/ Phosphorus (1)	0.00	0.00	0.25	17.0	0.28
Anoxic (1)	0.00	0.00	0.15	16.3	0.11
Anoxic w/ Phosphorus (1)	0.00	0.00	0.14	17.2	0.11
Anoxic w/ Carbon (2)	0.00	0.00	0.17	15.3	0.10
Anoxic w/ Phosphorus and Carbon (3)	0.00	0.01	0.19	18.5	0.12

from the ether in the aerobic experiments during the last few days of sampling.

Table 4.5 also shows that no methane was produced in any of the experiments. However, the presence of carbon monoxide was detected in the Aerobic experiment and Anoxic with Phosphorus and Organic Carbon experiment. The detection of carbon monoxide in the headspace gas suggests that some of the ^{14}C may have escaped as CO during the carbon dioxide trapping procedure. Other unidentifiable peaks were also seen on the gas partitioning printouts; however, the area of the peaks was insignificant, suggesting very low concentrations.

5. CONCLUSIONS AND RECOMMENDATIONS

The main objective of this research was to study the biodegradation of RDX in unsaturated Pantex soil. A batch technique using ^{14}C-chemicals was developed to investigate the degradation of RDX under aerobic, anoxic, and microaerobic conditions. In addition, nutrients (organic carbon and phosphorus) were added to determine their effect on biodegradation rates in the soil. The extent of mineralization was quantified by monitoring the production of $^{14}CO_2$ in the vials. Finally, RDX biodegradation rates were estimated for each environmental condition.

5.1 CONCLUSIONS

RDX degraders are indigenous to Pantex soil located in the vadose zone of Zone 12. In the batch experiments, these bacteria were observed to degrade RDX to a significant extent under anoxic and microaerobic conditions. Little biotransformation was observed in the experiments conducted in an aerobic environment. The addition of phosphorus had little effect on the removal rate of RDX, except in the microaerobic experiment and anoxic experiment with organic carbon addition. In these two experiments, results showed a 20% difference in the final ^{14}C concentrations (ether phase) between vials with and without phosphorus amendment. The amendment of a biodegradable organic carbon source significantly increased the rate at which RDX was degraded. Mineralization of RDX by the indigenous microorganisms was also found to occur in the vials. The extent of mineralization varied depending on the nutrient(s) added and environmental condition applied. For instance, approximately 11% of the ^{14}C-RDX was transformed to $^{14}CO_2$ under aerobic conditions and approximately 46% was transformed under microaerobic conditions. Furthermore, the addition of an organic carbon caused the extent of mineralization to be somewhat less than those in other bioactive vials. Loss of original RDX from the soil was measured using HPLC analysis, and in all cases, the removal of unlabeled RDX was greater than the removal of the ^{14}C-RDX. Therefore, the degradation rates were estimated in terms of a total mass loading and were largest when organic carbon was added.

Overall, experiments conducted with air in the headspace showed minimal degradation of RDX with approximately 15% of the initial ^{14}C being removed from the ether phase. Under these conditions, the addition of phosphorus did not affect the degradation rate. Experiments conducted with nitrogen gas in the headspace had a much better RDX removal percentage of roughly 50%. However, the microaerobic experiment that was amended with phosphorus provided the greatest removal of radiolabeled RDX by the end of the experiment. Under these conditions, approximately 73% of the initial ^{14}C-RDX was biodegraded within 134 days and at least 38% of the ^{14}C-RDX was completely mineralized to $^{14}CO_2$. However, the vials amended with organic carbon and phosphorus also provided a high removal percentage in half the time. By Day 67, the Anoxic with Organic Carbon and Phosphorus experiment had removed roughly 70% of the initial ^{14}C-RDX and converted 26% of that to $^{14}CO_2$. After Day 67, the removal of RDX from these vials tapered off.

Soil extractions using EPA Method 8330 showed that unlabeled RDX present in Pantex soil was also degraded by the indigenous bacteria. In fact, the percentage of total RDX (radiolabeled and unlabeled) removed from soil was in all cases greater than the removal percentage of labeled RDX alone. For example, the Microaerobic with Phosphorus experiment showed that roughly 84% of the ^{14}C-RDX was degraded when

analyzed by liquid scintillation counting whereas roughly 95% of the RDX was degraded when analyzed by HPLC. In general, approximately 10% more RDX was degraded in all bioactive experiments analyzed by HPLC. Better removal of unlabeled RDX may be because the radiochemical analyses underestimated RDX metabolism. The radiochemical analysis measurements include RDX and ringed intermediates, whereas the HPLC analysis only measures RDX. Also, any partitioning of ring cleavage intermediates into the ether would decrease the apparent RDX removal in the radiochemical analyses

The results from the HPLC analyses allowed degradation rate constants to be calculated based on total mass of RDX present in the Pantex soil. For all biologically active vials, the RDX losses were described well by first order kinetics. RDX losses under aerobic conditions were described by zero-order kinetics. The largest rate constant was obtained from the two experiments amended with organic carbon. The first-order rate constants for these experiments were 0.018 and 0.019 day^{-1} for the phosphorus amended and non-amended experiments, respectively. At these rates, the half-lives for RDX degradation with and without phosphorus would be roughly 38 and 36 days, respectively. However, the Microaerobic with Phosphorus experiment with a degradation rate constant of 0.016 day^{-1} should also be noted, because the 95% confidence interval for this experiment overlapped the latter two experiments. Thus, there was no statistical difference among the rate constants for these three experiments. A statistical difference was seen between the two-microaerobic experiments indicating the phosphorus was a limiting nutrient.

Some problems with mass balance closure were present in this study. ^{14}C recovery efficiencies declined as a function of time and biological activity. In non-biologically active vials, roughly 10% of the ^{14}C was lost over time. Lower recovery efficiencies (88-67%) were seen in the biologically active vials. All losses may have been caused by an inaccurate $^{14}CO_2$ recovery correction factor, by other gas(s) exiting the vial, or by incomplete ether extractions due to stronger sorption of ^{14}C-RDX into the soil matrix overtime.

Attempts were made to determine the source of the losses. The carbon dioxide control experiment showed losses of $^{14}CO_2$ during incubation accounted for anywhere from 0 to 17% of the total ^{14}C. Headspace analyses also suggested another source of ^{14}C losses. These analyses showed that carbon monoxide might have been formed during biodegradation. Trace amounts of CO was found in the headspaces of the Anoxic with Organic Carbon and Phosphorus and Aerobic experiments.

In general, these results show that indigenous bacteria found in Pantex soil can degrade RDX. However, anoxic or microaerobic conditions are needed. To create these conditions in the vadose zone, the injection of either an inert gas or highly degradable organic substance would be required. Supplemental nutrients (organic carbon and phosphorus) are not necessary for RDX degradation, but the addition of organic carbon increases the degradation rate significantly.

5.2 RECOMMENDATIONS

To better assess the in-situ bioremediation of RDX in the vadose zone beneath Pantex, further research should be conducted. Additional research should include:

- Modification of methods to eliminate RDX partitioning problems between ether and water; in particular, the impact of pH

on ether extractions should be evaluated in detail.

- Because significant accumulation of water-soluble intermediates was observed, identification of these intermediates would be desirable to determine if they are of environmental concern.
- Characterization of toxicity effects due to high concentrations of explosives (hot spots) and other contaminants.
- Determination of delivery methods to create microaerobic/anoxic conditions in the vadose zone.
- Optimization of substrate concentrations and application methods to stimulate indigenous RDX degraders.
- Field studies to determine the actual response of indigenous bacteria and the ability to create optimal conditions.
- Study HMX degradation, since HMX is frequently present with RDX.

REFERENCES

1. Battelle Pantex. 1997. *1996 Environmental Report for Pantex Plant.* Environmental Protection Department, Environmental, Safety, and Health Division, Battelle Pantex, Mason & Hanger – Silas Mason Co., Amarillo, TX. DOE/AL/65030-9704.

2. Battelle Pantex. 1996. *1995 Environmental Report for Pantex Plant.* Environmental Protection Department, Environmental, Safety, and Health Division, Battelle Pantex, Mason & Hanger – Silas Mason Co., Amarillo, TX. DOE/AL/65030-9602.

3. Battelle Pantex. 1994. 1993 *Environmental Report for Pantex Plant.* Environmental Protection Department, Environmental, Safety, and Health Division, Battelle Pantex, Mason & Hanger – Silas Mason Co., Amarillo, TX. DOE/AL/65030-9413.

4. Bentley, R. E., Dean, J. W., Ellis, S. J., Hollister, T. A., LeBlanc, G. A., Sauter, S., and Sleight, B. H., III. 1977. *Laboratory Evaluation of the Toxicity of Cyclotrimethylenetrinitramine (RDX) to Aquatic Organisms.* Final Rep. No. DAMD-17-74-C-4104. EG&G Bionomics. Warcham, MA

5. Binks, P.R., Nicklin, S., and Bruce, N.C. 1995. "Degradation of Hexahydro-1,3,5-Trinitro-1,3,5-Triazine (RDX) by *Stenotrophomonas maltophilia* PB1." *Applied and Env. Micr.* 61(4):1318-1322.

6. Brannon, J. R., Adrian, D. D., Pennington, J. C., Hayes, C., and Myers, T. E. 1992. *Slow Release of PCB, TNT, and RDX from Soils and Sediments.* EL-92-38, U.S. Army Waterways Experiment Station, Vicksburg, MS

7. Burrows, W. D. 1982. *Tertiary Treatment of Effluent from Holston AAP Industrial Liquid Waste Treatment Facility. I. Bath carbon adsorption studies: TNT, RDX, HMX, TAX, and SEX.* Technical Report 8207, ADA121244, U.S. Army Armament Research and Development Command, New Jersey.

8. Burrows, W. D. and Brueggemann, E. E. 1986. *Tertiary Treatment of Effluent from Holston AAP Industrial Waste Treatment Facility. V. Degradation of Nitramines in Holston AAP Wastewaters by Ultraviolet Radiation.* Technical Report 8602. ADA 176195, U.S. Army Toxic and Hazardous Materials Agency, Aberdeen Proving Ground, Maryland.

9. Burton, D.T., Turley, S. D. and Peters, G. T. 1994a. "The Toxicity of Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) to the Fathead (*Pimephales promelas*)." *Chemosphere* 29: 567-579.

10. Burton, D.T., Turley, S. D. and Peters, G. T. 1994b. " The Toxicity of Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) to the Freshwater Green Alga *Selenastum capricornutum." Water, Air & Soil Poll.* 79: 449-457.

11. Fernando, T., and S. D. Aust. 1991. "Biodegradation of Munitions Waste, TNT (2,4,6-Trinitrotoluene), and RDX (Hexahydro-1,3,5-Trinitro-1,3,5-Triazine) by *Phanerochaete chysosporium*" in *Emerging Technologies in Hazardous Waste Management II*, D.W. Tedder and F. G. Pholand (eds.). American Chemical Society, Washington DC.

12. Funk, S. B., D. J. Roberts, D. L. Crawford, and R. L. Crawford. 1993. "Initial-Phase Optimization for Bioremediation of Munitions Compound-Contaminated Soils." *Applied and Env. Micr.* 59(7):2171-2177.

13. Gibbs, T. R., and Popolato, A. 1980. *LASL explosive property data. Los Alamos Scientific Laboratory.* University of California Press, Berkeley, California.

14. Gorontzy, T., Drzyzga, M.W. Kahl, D. Bruns-Nagel, J. Breitung, E. van Loew, and K.H. Blotevogel. 1994. " Microbial Degradation of Explosives and Related Compounds." *Critical Reviews in Microbiology* 20(40):265-284.

15. Griest, W. H., R. L. Tyndall, A. J. Stewart, J. E. Caton, A. A. Vass, C. H. Ho, and W. M. Caldwell. 1995. "Chemical Characterization and Toxicological Testing of Windrow Composts from Explosives-Contaminated Sediments." *Environmental Toxicology and Chemistry*, 14(1):51-59.

16. Harderlein, S.B., Weissmahr K.W., and Schwarzenback, R.P. 1996. "Specific Adsorption of Nitroaromatic Explosives and Pesticides to Clay Minerals." *Environmental Science & Technology* 30:612-622.

17. Hathaway, J.A. and Buck, C.R. 1997. "Absence of Health Hazards Associated with RDX Manufacture and Use." *Journal of Occup. Med.* 19: 269-278.

18. Hollander, A. I. and Colbach, E. M. 1969. "Composition C-4 Induced Seizures: A Report of Five Cases". *Military Med.* 134: 1529-1539.

19. Kaczorowshi, T. and Syrowatka, T. 1960. "Toxicity of Hexogen." *Roez. Panst. Zakl. Hig.* II: 373-382.

20. Ketel, W. B. and Huges, J. R. 1972. "Toxic Encephalopathy with Seizures Secondary to Ingestion of Composition C-4." *Neurology* 22: 871-876.

21. Kitts, C.L., E.A. Margiotta, and P.J. Unkefer. 1994a. "*Morganella morganii* Nitramine Reductase Activity is Induced by Nitroaromatic Compounds." [Abstract Q-126] American Society for Microbiology, 94th General Meeting, Las Vegas, NV.

22. Kitts, C.L., D.P. Cunningham, and P.J. Unkefer. 1994b. "Isolation of Three Hexahydro-1,3,5-Trinitro-1,3,5,-Triazine-Degrading Species of the Family *Enterobateriance*ae from Nitramine Explosive-Contaminated Soil." *Applied and Env. Micr.* 60(12):4608-4611.

23. Light, C. L., Wilber, G. G., and Clarkson, W.W. 1997. "Biological Treatment of RDX Contaminated Soils." Proceeding of the 52nd Purdue Industrial Waste Conference. Industrial Waste Conference. Purdue University, Lafayette, IN.

24. Merill, S. L. 1968. "Ingestion of an Explosive Material, Composition C-4." *USARV Med. Bull.* 3: 5-9.

25. McCormick, N.G., J.H. Cornell, and A.M. Kaplan. 1981. "Biodegradation of Hexahydro-1,3,5-Trinitro-1,3,5-Triazine." *Applied and Env. Micr.* 42(5): 817-823.

26. Norris, R. D. 1994. "In-situ Bioremediation of Soils and Ground Water Contaminated with Petroleum Hydrocarbons" in *The Handbook of*

Bioremediation, J. E. Matthews and R. S. Kerr (eds.). Lewis Publishers: Boca Raton, Florida: p. 18.

27. Osmon, J.L., and R. E. Klausmeier. 1973. "The Microbial Degradation of Explosives." *Dev. Ind. Microbiology*, 14:247-252.

28. Rosenblatt, D. H., E.P. Burrows, W. R. Mitchell, and D. L. Parmer. 1991. "Organic Explosives and Related Compounds" in *The Handbook of Environmental Chemistry Volume 3: Anthropogenic Compounds*, ed. By O. Hutzinger. Springer-Verlag, Berlin.

29. Schnieder, N. R., Bradley, S. L., and Anderson, M. E. 1977. "Toxicology of Cyclotrimethylenetrintramine: Distribution and Metabolism in the Rat and the Miniature Swine." *Toxicol. Appl. Pharmacol.* 39: 531-541.

30. Selim, H. Madgi and Amacher, M. C. 1995. *Reactivity and Transport of Heavy Metals in Soils.* CRC/Lewis Publishers: Boca Raton, Fla.

31. Spanggord, R. J., Mill, T. Chou, T., Mabey, W. R. Smith, J. H., and Lee, S. 1990. Environmental Fate Studies on Certain Munitions Wastewater Constituents. Final Rep. Phase II – laboratory studies. LSU- 7934. SRI International: Menol Park, CA.

32. Sullivan, J. H., Jr., Putnam, H. D., Keirn, M. A. Pruitt, B. C., Jr., Nichols, J. C., and McLave, J. T. 1979. *A Summary and Evaluation of Aquatic Environmental Data in Relation to Establishing Water Quality Criteria for Munitions-unique Compounds*. Rep. No. DAMD 17-77-C-7207 (AD-A087683). Water and Air Research, Inc., Gainesville, FL.

33. Sunderman, F. W. 1944. *Hazards to the Health of Individuals Working with RDX.* Rep. OSRD No. 4174. University of Pennsylvania, Philadelphia.

34. Tsa, M. T. and Lee, J. 1982. "Food Poisoning Caused by Hexogen: A Report of 8 Cases." *Chin. Journ. Prevent. Med.* 16: 229-240.

35. U.S. Army Corps. of Engineers. 1995. *Risk Reduction Rule Guidance for Pantex Plant RCRA Facility Investigations.* Tulsa District. Revision 5.

36. U.S. Department of Energy. 1996. *Final Programmatic Environmental Impact Statement for Stockpile Stewardship and Management.* DOE/EIS-0236 vol. 1 of 4.

37. U.S. Environmental Protection Agency. 1994a. *Drinking Water Regulations and Health Advisories.* Report No. 822-R-94-003. Office of Water, Washington D.C.

38. U.S. Environmental Protection Agency. 1994b. *Test Methods for Evaluating Solid Waste.* SW – 846: Revision 0, September 1994. Office of Solid Waste and Emergency Response, Washington D.C.

39. Von Oettinagen, W.F., Donahue, D. D., Yagoda, H., Monaco, A. R., and Harris, M. 1949. "Toxicity and Potential Dangers of Cyclotrimethylenetrinitramine (RDX)." *Ind. Hyg. Toxicol.* 31: 21-31.

40. Williams, R. T., P. S. Ziegenfuss, and W. E. Sisk. 1992. "Composting of Explosives and Propellant Contaminated Soils Under Thermophilic and Mesophilic Conditions." *Journal of Industrial Microbiology* 9: 137-144.

41. Yinon, J. 1990. Toxicity and Metabolism of Explosives. CRC Press: Boca Raton, Florida.

42. Yinon, J. and Zitrin, S. 1993. *Modern Methods and Applications in Analysis of Explosives*. John Wiley & Son: West Sussex, England.

43. Young, D.M., Unkefer, P.J., and Ogden, K.L. 1997a. "Biotransformation of Hexahydro-1,3,5-Trinitro-1,3,5-Triazine (RDX) by a Prospective Consortium and Its Most Effective Isolate *Serratia marcescens*." *Biotechnology and Bioengineering*. 53(5): 515-522.

44. Young, D.M., Kitts, C. L, Unkefer, P.J., and Ogden, K.L. 1997b. "Biological Breakdown of RDX in Slurry Reactors Proceeds with Multiple Kinetically Distinguishable Paths." *Biotechnology and Bioengineering*. 56(3): 256-267.

Appendix A

Bicarbonate Control Study and Recovery Correction Factor

Bicarbonate Controls Experiment

Raw data

		Ether (dpm)		Water (dpm)		CO2 (dpm)	
Date	Run time	Vial 1	Vial 2	Vial 1	Vial 2	Vial 1	Vial 2
11/7/97	0					31241	
11/17/97	10	375		29		26036	
11/23/97	17	306	355	-11	33	27745	26646
12/1/97	24	422		14		23297	
12/8/97	31	409	319	83	154	22197	25467
12/14/97	37	422		26		27282	
12/31/97	54	346	816	18	37	25164	24194
1/12/98	66	320	75	208	56	21761	25276
1/26/98	80	313	217	9	44	24333	21572
2/9/98	94	297	340	15	30	22768	20962

		Averages (dpm)				Standard Deviation dpm		
Date	Run time	Ether	Water	CO2	CO2 corr	Ether	Water	CO2
11/7/97	0			31241	29825			
11/17/97	10	375	29	26036	29420			
11/23/97	17	331	11	27196	29484	34.7	31.2	777.2
12/1/97	24	422	14	23297	29390			
12/8/97	31	364	118	23832	29343	63.9	50.5	2311.7
12/14/97	37	422	26	27282	29377			
12/31/97	54	581	27	24679	29217	332.5	13.4	686.0
1/12/98	66	197	132	23518	29496	173.2	107.3	2485.7
1/26/98	80	265	27	22952	29534	67.6	24.5	1952.5
2/9/98	94	319	23	21865	29484	30.9	10.6	1277.1

		Averages (%)				Standard Deviation %		
Date	Run time	Ether	Water	CO2	CO2 (calc.)	Ether	Water	CO2
11/7/97	0	0.00	0.00	104.75	100.00			
11/17/97	10	0.79	0.06	87.30	61.74			
11/23/97	17	1.11	0.04	91.18	98.85	0.12	0.10	2.61
12/1/97	24	1.41	0.05	78.11	98.54			
12/8/97	31	1.22	0.40	79.91	98.38	0.21	0.17	7.75
12/14/97	37	1.41	0.09	91.47	98.50			
12/31/97	54	1.95	0.09	82.75	97.96	1.11	0.04	2.30
1/12/98	66	0.66	0.44	78.85	98.90	0.58	0.36	8.33
1/26/98	80	0.89	0.09	76.96	99.02	0.23	0.08	6.55
2/9/98	94	1.07	0.08	73.31	98.86	0.10	0.04	4.28

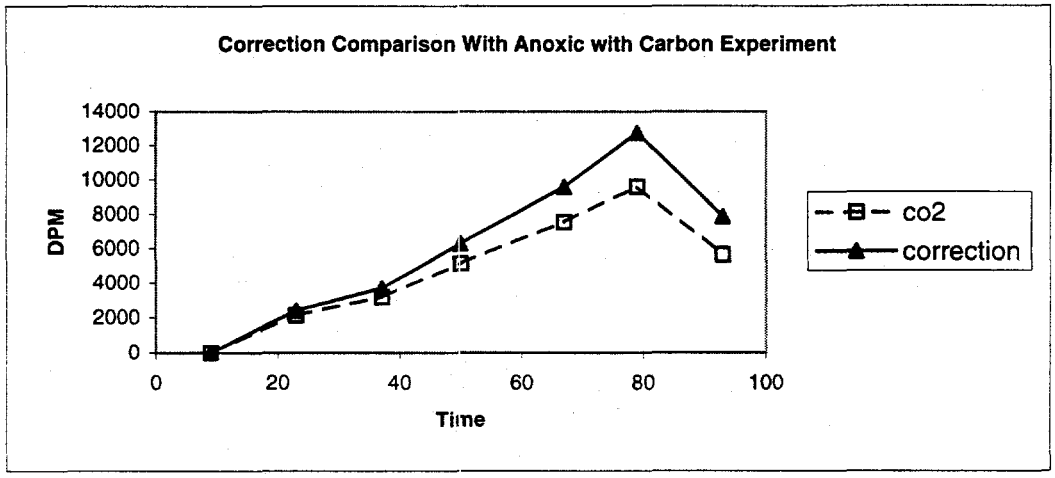
Bicarbonate Recovery Correction Factor

		Recovery (%)			Correction Factor		
Date	Run time	Vial 1	Vial 2	average	CO2dpm	linear Reg	Recovery (L)
11/7/97	0	104.7		104.7	31241	28415	0.95
11/17/97	10*	88.7		88.7	*	*	*
11/23/97	17	94.0	90.6	92.3	27196	27155	0.91
12/1/97	24	79.6		79.6	23297	26636	0.89
12/8/97	31	76.1	87.0	81.5	23832	26117	0.88
12/14/97	37	93.0		93.0	27282	25673	0.86
12/31/97	54	85.6	84.0	84.8	24679	24413	0.82
1/12/98	66	74.7	85.2	80.0	23518	23523	0.79
1/26/98	80	82.7	73.2	77.9	22952	22485	0.75
2/9/98	94	77.4	71.5	74.5	21865	21448	0.72

*Data not used for correction

Comparison of the effect of Carbon Recovery Correction on Anoxic with Carbon Experiment

Run time	CO2	CO2 corr.
9	0	0
23	2150	2408
37	3189	3705
50	5155	6298
67	7559	9584
79	9603	12738
93	5658	7868
107	4600	



Appendix B

Initial Radioactivity Amounts in the Batch Biodegradation Experiments

Initial Radioactivity in Each Experiment

VIAL NUMBERS	AEROBIC W/ PHOS. (dpm)	AEROBIC (dpm)	ANOXIC W/ PHOS. (dpm)
1	49835	50464	49863
2	49922	49947	50048
3	49566	49827	49912
4	49513	50199	50054
5	50161	50286	50035
6	49456	50035	50151
7	50213	50004	49947
AVERAGE	49809	50109	50001
Average - background	49773	50073	49965
Standard Dev	309	219	99
PERCENT DIFF	0.62	0.44	0.20

VIAL NUMBERS	ANOXIC (dpm)	MICROAEROBIC W/ PHOS. (dpm)	MICROAEROBIC (dpm)
1	50140	51094	49990
2	34309*	49840	50354
3	50218	49584	49848
4	50329	49608	50106
5	50204	50030	50393
6	50759	49776	50299
7	50386	49551	50341
AVERAGE	50339	49926	50190
Average - background	50303	49890	50154
Standard Dev	224	542	211
PERCENT DIFF	0.45	1.09	0.42

* Some of the sample missed the vials: vial was not used in calculating average

VIAL NUMBERS	AUTO-AEROBIC W/ PHOS. (dmp)	ANOXIC W/ PHOS & ORG. CARBON (dpm)	ANOCIX W/ ORG. CARBON (dpm)
1	49440	46204	47720
2	49704	46219	48035
3	49782	46702	47487
4	49786	46482	47633
5	49595	46453	47941
6	45444	46725	47138
7	49323	46256	47876
AVERAGE	49010	46434	47690
Average - background	48974	46398	47654
Standard Dev	1582	220	307
PERCENT DIFF	3.23	0.47	0.64

VIAL NUMBERS	AUTO-ANOXIC W/ ORG. CARBON (dpm)	BICARBONATE CONTROL (dpm)
1	47946	30560
2	47773	29493
3	47612	30556
4	47763	30169
5	47337	28528
6		
7		
AVERAGE	47686	29861
Average - background	47650	29825
Standard Dev	228	863
PERCENT DIFF	0.48	2.89

Appendix C

Batch Biodegradation Experiments

Aerobic with Phosphorus Experiment - Data

		Ether (dpm)			Water (dpm)			CO2 (dpm)		
Date	Run time	Vial 1	Vial 2	Vial 3	Vial 1	Vial 2	Vial 3	Vial 1	Vial 2	Vial 3
9/1/97	10	31195	47810	46421	11230	5240	4544	2882	2497	2338
9/15/97	24	44396	44547	43420	5273	5492	4929	2522	2306	2157
9/29/97	38	39188	41996	41894	7979	6598	6351	2971	2433	2512
10/13/97	50	42281	43294	34809	4523	4303	7913	2242	2275	4284
10/27/97	64	43493	42227	42850	5630	5310	5419	2308	2256	2583
11/10/97	78	42967	43611	20181	4737	5299	11761	2987	2504	9218
11/23/97	91	43632	40898	44217	4563	4988	4924	2285	3072	2265
12/14/97	112	34804	35933		9175	7787		2387	2491	
12/31/97	129	22056	39869	41278	11316	8658	10045	3445	2105	4878

		Averages (dpm)				Standard Deviation dpm		
Date	Run time	Ether	Water	CO2 (act.)	CO2 (corr)	Ether	Water	CO2
9/1/97	10	41809	7005	2572	960	9218	3676	280
9/15/97	24	44121	5231	2328	421	612	283	184
9/29/97	38	41026	6976	2639	1771	1592	877	290
10/13/97	50	40128	5580	2934	4066	4634	2023	1170
10/27/97	64	42857	5453	2382	1464	633	162	176
11/10/97	78	35586	7266	4903	6922	13345	3903	3745
11/23/97	91	42916	4825	2541	2033	1771	229	460
12/14/97	112	35368	8481	2439	5924	798	981	74
12/31/97	129	40573	10007	3476	-806	10714	1330	1387

		Averages (%)					Standard Deviation %		
Date	Run time	Ether	Water	CO2 (act)	CO2 (meas)	CO2 (calc)	Ether	Water	CO2
9/1/97	10	84.00	14.07	5.17	5.64	1.93	18.52	7.39	0.56
9/15/97	24	88.64	10.51	4.68	5.29	0.85	1.23	0.57	0.37
9/29/97	38	82.43	14.02	5.30	6.22	3.56	3.20	1.76	0.58
10/13/97	50	80.62	11.21	5.89	7.15	8.17	9.31	4.07	2.35
10/27/97	64	86.10	10.96	4.79	6.04	2.94	1.27	0.33	0.35
11/10/97	78	71.50	14.60	9.85	12.95	13.91	26.81	7.84	7.52
11/23/97	91	86.22	9.69	5.10	6.98	4.08	3.56	0.46	0.92
12/14/97	112	71.06	17.04	4.90	7.18	11.90	1.60	1.97	0.15
12/31/97	129	81.52	20.10	6.98	10.85	-1.62	21.53	2.67	2.79

		Recovery (%)				
Date	Run time	Vial 1	Vial 2	Vial 3	average	Corr. Ave
9/1/97	10	91.03	111.60	107.09	103.24	103.71
9/15/97	24	104.86	105.17	101.47	103.83	104.44
9/29/97	38	100.73	102.52	101.98	101.74	102.66
10/13/97	50	98.54	100.20	94.44	97.73	98.98
10/27/97	64	103.33	100.04	102.17	101.85	103.10
11/10/97	78	101.84	103.29	82.69	95.94	99.04
11/23/97	91	101.42	98.36	103.28	101.02	102.90
12/14/97	112	93.15	92.84		93.00	95.27
12/31/97	129	73.97	101.72	112.91	96.20	112.47

Aerobic Experiment - Data

		Ether (dpm)			Water (dpm)			CO2 (dpm)		
Date	Run time	Vial 1	Vial 2	Vial 3	Vial 1	Vial 2	Vial 3	Vial 1	Vial 2	Vial 3
9/1/97	10	36889	43741	44743	6317	6605	6430	2436	2351	2537
9/15/97	24	44006	44204	43400	5526	5056	2930	2279	2096	1721
9/29/97	38	41507	42445	42779	7064	6377	3147	2682	2554	2602
10/13/97	50	44467	42635	39188	4715	4934	3389	2498	2410	3528
10/27/97	64	43865	44471	44794	5061	5340	2816	2170	2674	2105
11/10/97	78	41198	41556	36176	4994	5528	3138	2396	2481	3730
11/23/97	91	41857	43238	40276	5101	5511	2681	2356	2850	3234
12/14/97	112	34521	36749		8353	8811		2311	2239	
1/5/98	134	35263	26517	34948	8185	10639	4654	2861	5359	2629

		Averages (dpm)				Standard Deviation dpm		
Date	Run time	Ether	Water	CO2 (act.)	CO2 (corr)	Ether	Water	CO2
9/1/97	10	41791	6451	2441	1831	4275	145	93
9/15/97	24	43870	4504	2032	1699	419	1383	284
9/29/97	38	42244	5529	2613	2300	660	2091	64
10/13/97	50	42097	4346	2812	3630	2680	836	622
10/27/97	64	44377	4406	2316	1291	472	1383	312
11/10/97	78	39643	4553	2869	5876	3008	1255	746
11/23/97	91	41790	4431	2813	3852	1482	1529	440
12/14/97	112	35635	8582	2275	5856	1576	324	51
1/5/98	134	35106	7826	3617	7141	4961	3008	1514

		Averages (%)					Standard Deviation %		
Date	Run time	Ether	Water	CO2 (act)	CO2 (meas)	CO2 (calc)	Ether	Water	CO2
9/1/97	10	83.46	12.88	4.88	5.32	3.66	8.54	0.29	0.19
9/15/97	24	87.61	8.99	4.06	4.59	3.39	0.84	2.76	0.57
9/29/97	38	84.36	11.04	5.22	6.12	4.59	1.32	4.18	0.13
10/13/97	50	84.07	8.68	5.62	6.81	7.25	5.35	1.67	1.24
10/27/97	64	88.62	8.80	4.63	5.84	2.58	0.94	2.76	0.62
11/10/97	78	79.17	9.09	5.73	7.53	11.73	6.01	2.51	1.49
11/23/97	91	83.46	8.85	5.62	7.69	7.69	2.96	3.05	0.88
12/14/97	112	71.17	17.14	4.54	6.66	11.69	3.15	0.65	0.10
1/5/98	134	70.11	15.63	7.22	11.42	14.26	9.91	6.01	3.02

		Recovery (%)				
Date	Run time	Vial 1	Vial 2	Vial 3	average	corr. Ave
9/1/97	10	91.15	105.24	107.26	101.22	101.66
9/15/97	24	103.47	102.56	95.96	100.67	101.20
9/29/97	38	102.36	102.60	96.91	100.62	101.53
10/13/97	50	103.21	99.81	92.08	98.37	99.56
10/27/97	64	102.04	104.82	99.29	102.05	103.26
11/10/97	78	97.03	98.99	85.96	93.99	95.80
11/23/97	91	98.48	103.05	92.25	97.93	100.00
12/14/97	112	90.24	95.46		92.85	94.96
1/5/98	134	92.48	84.91	84.34	87.24	97.16

Anoxic with Phosphorus Experiment - Data

		Ether (dpm)			Water (dpm)			CO_2 (dpm)		
Date	Run time	Vial 1	Vial 2	Vial 3	Vial 1	Vial 2	Vial 3	Vial 1	Vial 2	Vial 3
9/2/97	11	37876	38936	39360	7794	7774	8124	3323	2584	3558
9/15/97	24	34291	39403	34428	8406	5853	7714	6181	4170	5311
9/29/97	38	27043	22582	30318	7782	9260	8088	7871	12189	6600
10/13/97	50	19971	22793	27704	13154	9616	10261	8091	8914	5788
10/27/97	64	20396	23259	12588	14545	10920	21450	7126	8414	5697
11/10/97	78	14287	20307	12069	11380	16431	14127	11440	5596	11023
11/23/97	91	18090	15107	18335	13673	19560	13402	7749	7067	9133
12/14/97	112	8244	13616		15398	11990		10152	10922	
1/5/98	134	13518	7381	13454	12408	16178	15490	11422	11730	11265

		Averages (dpm)				Standard Deviation dpm		
Date	Run time	Ether	Water	CO_2 (act.)	CO_2 (corr)	Ether	Water	CO_2
9/2/97	11	38724	7897	3155	3344	765	196	508
9/15/97	24	36041	7324	5221	6600	2912	1320	1009
9/29/97	38	26647	8376	3886	14941	3883	780	2930
10/13/97	50	23489	11010	7598	15466	3913	1884	1620
10/27/97	64	18748	15638	7079	15579	5523	5350	1359
11/10/97	78	15554	13979	9353	20432	4263	2529	3260
11/23/97	91	17177	15545	7983	17243	1797	3479	1052
12/14/97	112	10930	13694	10537	25342	3799	2410	545
1/5/98	134	11451	14692	11472	23822	3525	2008	237

		Averages (%)					Standard Deviation %		
Date	Run time	Ether	Water	CO_2 (act)	CO_2 (meas)	CO_2 (calc)	Ether	Water	CO_2
9/2/97	11	77.50	15.81	6.31	6.91	6.69	1.53	0.39	1.02
9/15/97	24	72.13	14.66	10.45	11.82	13.21	5.83	2.64	2.02
9/29/97	38	53.33	16.76	17.79	20.87	29.90	7.77	1.56	5.86
10/13/97	50	47.01	22.04	15.21	18.44	30.95	7.83	3.77	3.24
10/27/97	64	37.52	31.30	14.17	17.88	31.18	11.05	10.71	2.72
11/10/97	78	31.13	27.98	18.72	24.61	40.89	8.53	5.06	6.52
11/23/97	91	34.38	31.11	15.98	21.86	34.51	3.60	6.96	2.11
12/14/97	112	21.88	27.41	21.09	30.89	50.72	7.60	4.82	1.09
1/5/98	134	22.92	29.41	22.96	36.31	47.68	7.05	4.02	0.47

		Coefficient of Variation			Recovery (%)				
Date	Run time	Ether	Water	CO_2	Vial 1	Vial 2	Vial 3	average	corr. Ave
9/2/97	11	0.02	0.02	0.16	98.05	98.66	102.16	99.62	100.22
9/15/97	24	0.08	0.18	0.19	97.82	98.92	94.97	97.24	98.61
9/29/97	38	0.15	0.09	0.33	85.45	88.12	90.07	87.88	90.97
10/13/97	50	0.17	0.17	0.21	82.49	82.70	87.57	84.25	87.49
10/27/97	64	0.29	0.34	0.19	84.19	85.25	79.53	82.99	86.69
11/10/97	78	0.27	0.18	0.35	74.26	84.73	74.49	77.83	83.72
11/23/97	91	0.10	0.22	0.13	79.08	83.53	81.80	81.47	87.35
12/14/97	112	0.35	0.18	0.05	67.63	73.11		70.37	80.17
1/5/98	134	0.31	0.14	0.02	74.75	70.63	80.47	75.28	88.63

Anoxic Experiment

		Ether (dpm)			Water (dpm)			CO2 (dpm)		
Date	Run time	Vial 1	Vial 2	Vial 3	Vial 1	Vial 2	Vial 3	Vial 1	Vial 2	Vial 3
9/2/97	11	42926.75	45775	89981	7551	7721	11205	2519	2674	5473
9/15/97	24	44208.81	33149	24010	5487	3560	7538	2939	7704	12097
9/29/97	38	30212.91	33783	29946	8098	8443	10483	6980	5626	7918
10/13/97	50	32528.38	26734	25587	10188	11774	11106	5397	6129	7729
10/27/97	64	21174.03	28450	27410	9958	8946	10272	11476	7764	8191
11/10/97	78	22114.56	11380	15941	11008	16178	12791	9213	10093	9200
11/23/97	91	17185.76	22385	21868	12807	10131	13383	10238	10214	7568
12/14/97	112	15738.95	7277		12030	13634		9468	13080	
1/5/98	134	6920.758	15066	18158	28540	12149	10545	32297	10723	10240

		Averages (dpm)				Standard Deviation dpm		
Date	Run time	Ether	Water	CO2 (act.)	CO2 (corr)	Ether	Water	CO2
9/2/97	11	44351	7636	2597	-1684	2014	120	110
9/15/97	24	33789	5528	7580	10986	10115	1989	4580
9/29/97	38	31314	9008	6841	9982	2142	1289	1152
10/13/97	50	28283	11023	6419	10997	3721	796	1193
10/27/97	64	25678	9726	9144	14899	3935	693	2031
11/10/97	78	16478	13326	9502	20499	5387	2626	512
11/23/97	91	20479	12107	9340	17717	2864	1735	1535
12/14/97	112	11508	12832	11274	25963	5983	1134	2554
1/5/98	134	16612	11347	10481	22344	2186	1134	341

		Averages (%)					Standard Deviation %		
Date	Run time	Ether	Water	CO2 (act)	CO2 (meas)	CO2 (calc)	Ether	Water	CO2
9/2/97	11	88.17	15.18	5.16	5.65	-3.35	4.00	0.24	0.22
9/15/97	24	67.17	10.99	15.07	17.04	21.84	20.11	3.95	9.11
9/29/97	38	62.25	17.91	13.60	15.96	19.84	4.26	2.56	2.29
10/13/97	50	56.23	21.91	12.76	15.47	21.86	7.40	1.58	2.37
10/27/97	64	51.05	19.33	18.18	22.93	29.62	7.82	1.38	4.04
11/10/97	78	32.76	26.49	18.89	24.84	40.75	10.71	5.22	1.02
11/23/97	91	40.71	24.07	18.57	25.41	35.22	5.69	3.45	3.05
12/14/97	112	22.88	25.51	22.41	32.83	51.61	11.89	2.25	5.08
1/5/98	134	33.02	22.56	20.84	32.95	44.42	4.35	2.25	0.68

		Recovery (%)				
Date	Run time	Vial 1	Vial 2	Vial 3	average	corr. Ave
9/2/97	11	105.35	111.66	212.03	143.02	108.99
9/15/97	24	104.63	88.29	86.76	93.23	95.20
9/29/97	38	90.03	95.12	96.11	93.76	96.12
10/13/97	50	95.65	88.74	88.31	90.90	93.61
10/27/97	64	84.70	89.78	91.19	88.56	93.31
11/10/97	78	84.16	74.85	75.41	78.14	84.09
11/23/97	91	79.98	84.94	85.12	83.35	90.19
12/14/97	112	74.02	67.57		70.80	81.21
1/5/98	134	134.70	75.42	77.42	95.84	88.53

Microaerobic with Phosphorus Experiment - Data

		Ether (dpm)			Water (dpm)			CO2 (dpm)		
Date	Run time	Vial 1	Vial 2	Vial 3	Vial 1	Vial 2	Vial 3	Vial 1	Vial 2	Vial 3
9/3/97	10	41612	43279	43596	7358	6309	6279	2353	2034	1800
9/17/97	24	40478	36500	39170	6169	8294	7039	3101	3813	3583
10/1/97	38	23694	26726	27470	14610	13346	12906	6187	5878	5602
10/15/97	50	19189	17436	15771	12493	14600	15689	8163	8058	9296
10/29/97	64	18480	17362	15742	14321	10628	14017	7864	9334	9772
11/12/97	78	9279	13736	12132	9479	17334	15443	15154	7854	9063
11/25/97	91	9348	9442		15796	19358		10910	9111	
12/16/97	112	10440	5136		19825	12642		9712	15050	
1/7/98	134	6301	6600		16531	13013		10865	13042	

		Averages (dpm)				Standard Deviation dpm		
Date	Run time	Ether	Water	CO2 (act.)	CO2 (corr)	Ether	Water	CO2
9/3/97	10	42829	6648	2062	413	1066	615	277
9/17/97	24	38716	7167	3499	4007	2027	1068	363
10/1/97	38	25963	13621	5889	10306	2000	885	293
10/15/97	50	17465	14261	8506	18164	1709	1625	686
10/29/97	64	17195	12989	8990	19707	1377	2050	999
11/12/97	78	11716	14085	10690	24090	2258	4100	3912
11/25/97	91	9395	17577	10011	22918	66	2519	1272
12/16/97	112	7788	16233	12381	25869	3750	5079	3775
1/7/98	134	6450	14772	11953	28668	212	2488	1539

		Averages (%)					Standard Deviation %		
Date	Run time	Ether	Water	CO2 (act)	CO2 (meas)	CO2 (calc)	Ether	Water	CO2
9/3/97	10	85.85	13.33	4.13	4.51	0.82	2.14	1.23	0.56
9/17/97	24	77.60	14.37	7.01	7.93	7.97	4.06	2.14	0.73
10/1/97	38	52.04	27.30	11.80	13.85	20.49	4.01	1.77	0.59
10/15/97	50	35.01	28.58	17.05	20.67	36.11	3.43	3.26	1.38
10/29/97	64	34.47	26.03	18.02	22.74	39.18	2.76	4.11	2.00
11/12/97	78	23.48	28.23	21.43	28.17	47.89	4.53	8.22	7.84
11/25/97	91	18.83	35.23	20.07	27.46	45.56	0.13	5.05	2.55
12/16/97	112	15.61	32.54	24.82	36.35	51.43	7.52	10.18	7.57
1/7/98	134	12.93	29.61	23.96	37.89	56.99	0.42	4.99	3.09

		Recovery (%)				
Date	Run time	Vial 1	Vial 2	Vial 3	average	corr. Ave
9/3/97	10	102.87	103.47	103.58	103.31	103.68
9/17/97	24	99.71	97.43	99.80	98.98	99.90
10/1/97	38	89.18	92.10	92.16	91.15	93.19
10/15/97	50	79.87	80.37	81.69	80.64	84.27
10/29/97	64	81.51	74.81	79.24	78.52	83.23
11/12/97	78	67.97	78.02	73.44	73.14	79.89
11/25/97	91	72.27	75.99		74.13	81.52
12/16/97	112	80.13	65.80		72.96	84.50
1/7/98	134	67.54	65.45		66.50	80.43

Microaerobic Experiment - Data

		Ether (dpm)			Water (dpm)			CO2 (dpm)		
Date	Run time	Vial 1	Vial 2	Vial 3	Vial 1	Vial 2	Vial 3	Vial 1	Vial 2	Vial 3
9/3/97	10	44523	45662	46248	6027	10188	8919	2034	1493	2389
9/17/97	24	44078	42289	42448	5040	6069	5421	2968	3251	2850
10/1/97	38	27508	20399	26634	11834	16905	12697	5486	6749	5895
10/15/97	50	23907	21755	26742	9507	8307	8905	9994	11165	8199
10/29/97	64	23190	19361	26143	8309	7760	7554	11775	12536	10206
11/12/97	78	19331	16125	19477	10108	9056	9491	11800	13850	12601
11/25/97	91	19487	12749	15476	8758	8612	9917	13279	15162	11861
12/16/97	112	12821	11100		8947	7089		13049	16523	
1/7/98	134	9056	10756	9987	11721	6648	14584	13825	17686	12353

		Averages (dpm)				Standard Deviation dpm		
Date	Run time	Ether	Water	CO2 (act.)	CO2 (corr)	Ether	Water	CO2
9/3/97	10	45478	8378	1972	-3701	877	2132	451
9/17/97	24	42938	5510	3023	1706	990	520	206
10/1/97	38	24847	13812	6043	11495	3877	2713	644
10/15/97	50	24135	8906	9786	17113	2502	600	1494
10/29/97	64	22898	7874	11506	19382	3401	390	1188
11/12/97	78	18311	9552	12751	22291	1895	529	1033
11/25/97	91	15904	9096	13434	25154	3389	715	1656
12/16/97	112	11960	8018	14786	30176	1217	1313	2456
1/7/98	134	9933	10984	14622	29237	851	4019	2754

		Averages (%)					Standard Deviation %		
Date	Run time	Ether	Water	CO2 (act)	CO2 (meas)	CO2 (calc)	Ether	Water	CO2
9/3/97	10	90.68	16.70	3.93	4.29	-7.36	1.75	4.25	0.90
9/17/97	24	85.61	10.99	6.03	6.82	3.39	1.97	1.04	0.41
10/1/97	38	49.54	27.54	12.05	14.14	22.85	7.73	5.41	1.28
10/15/97	50	48.12	17.76	19.51	23.66	34.02	4.99	1.20	2.98
10/29/97	64	45.66	15.70	22.94	28.94	38.53	6.78	0.78	2.37
11/12/97	78	36.51	19.05	25.42	33.43	44.31	3.78	1.05	2.06
11/25/97	91	31.71	18.14	26.79	36.65	50.01	6.76	1.42	3.30
12/16/97	112	23.85	15.99	29.48	43.18	59.99	2.43	2.62	4.90
1/7/98	134	19.80	21.90	29.15	46.10	58.12	1.70	8.01	5.49

		Recovery (%)				
Date	Run time	Vial 1	Vial 2	Vial 3	average	corr. Ave
9/3/97	10	104.84	114.33	114.76	111.31	111.67
9/17/97	24	103.85	102.90	101.13	102.63	103.42
10/1/97	38	89.38	87.83	90.17	89.13	91.22
10/15/97	50	86.55	82.20	87.42	85.39	89.54
10/29/97	64	86.28	79.07	87.54	84.30	90.30
11/12/97	78	82.22	77.82	82.88	80.98	88.98
11/25/97	91	82.79	72.82	74.28	76.63	86.50
12/16/97	112	69.42	69.21	0.00	46.21	83.01
1/7/98	134	68.99	69.96	73.62	70.86	87.81

Aerobic Autoclaved Soil with Phosphorus Experiment - Data

		Ether (dpm)			Water (dpm)			CO2 (dpm)		
Date	Run time	Vial 1	Vial 2	Vial 3	Vial 1	Vial 2	Vial 3	Vial 1	Vial 2	Vial 3
9/1/97	10	39063	37462	38875	10721	13124	11473	110	80	64
9/15/97	24	38939	38006	43255	8449	5220	8792	150	110	102
9/29/97	38	34830	36206	40402	13578	5467	8757	93	203	45
10/13/97	50	37633	37153	42989	8686	5115	5228	322	39	213
10/27/97	64	40259	42336	37717	7061	3407	8748	502	1214	648
11/10/97	78	35782	35364	37228	8308	4887	8591	1421	1055	145
11/23/97	91	41046	42459	37228	6321	2025	8591	785	1345	463
12/14/97	112	31874	32044		10078	4669		938	475	
1/5/98	134	35531	33690	35093	9113	4683	7610	240	1305	1288

		Averages (dpm)				Standard Deviation dpm		
Date	Run time	Ether	Water	CO2 (act.)	CO2 (corr)	Ether	Water	CO2
9/1/97	10	38467	11773	85	-1265	875	1229	23
9/15/97	24	40067	7487	120	1421	2800	1971	26
9/29/97	38	37146	9267	114	2561	2903	4080	81
10/13/97	50	39258	6343	191	3373	3240	2030	142
10/27/97	64	40104	6405	788	2465	2313	2730	376
11/10/97	78	36125	7262	874	5587	978	2062	657
11/23/97	91	40244	5645	865	3085	2706	3335	446
12/14/97	112	31959	7374	707	9642	121	3825	327
1/5/98	134	34772	7135	944	7068	962	2253	610

		Averages (%)					Standard Deviation %		
Date	Run time	Ether	Water	CO2 (act)	CO2 (meas)	CO2 (calc)	Ether	Water	CO2
9/1/97	10	78.54	24.04	0.17	0.19	-2.58	1.79	2.51	0.05
9/15/97	24	81.81	15.29	0.25	0.28	2.90	5.72	4.02	0.05
9/29/97	38	75.85	18.92	0.23	0.27	5.23	5.93	8.33	0.16
10/13/97	50	80.16	12.95	0.39	0.47	6.89	6.62	4.14	0.29
10/27/97	64	81.89	13.08	1.61	2.03	5.03	4.72	5.57	0.77
11/10/97	78	73.76	14.83	1.78	2.35	11.41	2.00	4.21	1.34
11/23/97	91	82.17	11.53	1.77	2.42	6.30	5.53	6.81	0.91
12/14/97	112	65.26	15.06	1.44	2.11	19.69	0.25	7.81	0.67
1/5/98	134	71.00	14.57	1.93	3.05	14.43	1.96	4.60	1.25

		Recovery (%)				
Date	Run time	Vial 1	Vial 2	Vial 3	average	corr. Ave
9/1/97	10	101.88	103.45	102.93	102.76	102.77
9/15/97	24	97.07	88.49	105.48	97.34	97.38
9/29/97	38	99.03	85.50	100.47	95.00	95.04
10/13/97	50	95.23	86.39	98.89	93.50	93.59
10/27/97	64	97.65	95.88	95.20	96.58	97.00
11/10/97	78	92.93	84.34	93.85	90.38	90.94
11/23/97	91	98.32	93.58	94.50	95.47	96.12
12/14/97	112	87.58	75.94		81.76	82.43
1/5/98	134	91.65	81.02	89.82	87.50	88.62

Anoxic with Organic Carbon and Phosphorus Experiment - Data

		Ether (dpm)			Water (dpm)			CO2 (dpm)		
Date	Run time	Vial 1	Vial 2	Vial 3	Vial 1	Vial 2	Vial 3	Vial 1	Vial 2	Vial 3
11/3/97	9	40061	45573	38925	3538	2715	3939	1102	638	1670
11/17/97	23	33115	34448	26349	4841	4709	7280	2189	2118	2907
12/1/97	37	22592	32939	26425	6685	5736	6508	5774	2309	3439
12/14/97	50	14371	23504	11576	9859	5609	8705	4282	6501	7573
12/31/97	67	10310	8799	7724	8579	9472	11191	7054	8450	6326
1/12/98	79	8902	10279	4342	11817	9193	14973	6860	6224	4176
1/26/98	93	12420	5737	8656	9630	7035	4943	5819	11948	14264
2/9/98	107	3763	12557	10275	9191	9263	7052	11144	6716	10804

		Averages (dpm)				Standard Deviation dpm		
Date	Run time	Ether	Water	CO2 (act.)	CO2 (corr)	Ether	Water	CO2
11/3/97	9	41519	3397	1136	1482	3556	624	517
11/17/97	23	31304	5610	2405	9484	4343	1448	437
12/1/97	37	27318	6310	3841	12770	5231	504	1767
12/14/97	50	12974	9282	5928	24143	1976	817	2327
12/31/97	67	8945	9747	7277	27707	1299	1327	1080
1/12/98	79	7841	11995	5753	26563	3108	2894	1402
1/26/98	93	8938	7203	10677	30258	3350	2348	4364
2/9/98	107	8865	8502	9554	29032	4563	1256	2464

		Averages (%)					Standard Deviation %		
Date	Run time	Ether	Water	CO2 (act)	CO2 (meas)	CO2 (calc)	Ether	Water	CO2
11/3/97	9	89.48	7.32	2.45	2.67	3.03	7.66	1.34	1.11
11/17/97	23	67.47	12.09	5.18	5.85	19.37	9.36	3.12	0.94
12/1/97	37	58.88	13.60	8.28	9.69	26.08	11.27	1.09	3.81
12/14/97	50	27.96	20.01	12.78	15.49	49.30	4.26	1.76	5.02
12/31/97	67	19.28	21.01	15.68	19.96	56.57	2.80	2.86	2.33
1/12/98	79	16.90	25.85	12.40	16.35	54.24	6.70	6.24	3.02
1/26/98	93	19.26	15.52	23.01	31.69	61.78	7.22	5.06	9.41
2/9/98	107	19.11	18.32	20.59	29.66	59.28	9.83	2.71	5.31

		Recovery (%)				
Date	Run time	Vial 1	Vial 2	Vial 3	average	corr. Ave
11/3/97	9	96.34	105.45	95.98	99.26	99.47
11/17/97	23	86.52	88.96	78.74	84.74	85.41
12/1/97	37	75.54	88.33	78.39	80.75	82.17
12/14/97	50	61.45	76.76	60.03	66.08	63.46
12/31/97	67	55.91	57.59	54.40	55.97	60.24
1/12/98	79	59.44	55.38	50.63	55.15	59.10
1/26/98	93	60.06	53.28	60.05	57.80	66.47
2/9/98	107	51.94	61.50	60.63	58.02	67.09

Anoxic with Organic Carbon Experiment - Data

		Ether (dpm)			Water (dpm)			CO2 (dpm)		
Date	Run time	Vial 1	Vial 2	Vial 3	Vial 1	Vial 2	Vial 3	Vial 1	Vial 2	Vial 3
11/3/97	9	39102	37487	41454	3904	4555	3478	2546	2492	1413
11/17/97	23	25991	25853	29892	7416	7181	6117	3645	3074	2848
12/1/97	37	24226	20966	12468	7948	7500	9508	4421	4532	6513
12/14/97	50	19583	16365	19721	6288	6411	6262	5814	9721	7141
12/31/97	67	7291	11217	14001	6084	8106	5842	10833	8636	9340
1/12/98	79	18990	25143	16821	7838	7456	5117	3434	3189	10352
1/26/98	93	20522	18612	15274	7956	8496	7904	3239	4005	6557
2/9/98	107	22089	16179	19869	7033	8662	6161	3456	4807	4491

		Averages (dpm)				Standard Deviation dpm		
Date	Run time	Ether	Water	CO2 (act.)	CO2 (corr)	Ether	Water	CO2
11/3/97	9	39348	3979	2150	4328	1995	542	639
11/17/97	23	27245	6905	3189	13504	2293	692	411
12/1/97	37	19220	8319	5155	20116	6070	1054	1177
12/14/97	50	18557	6320	7559	22777	1899	80	1986
12/31/97	67	10836	6677	9603	30140	3371	1243	1122
1/12/98	79	20318	6804	5658	20532	4317	1473	4067
1/26/98	93	18136	8119	4600	21399	2656	328	1737
2/9/98	107	19379	7286	4251	20990	2985	1270	707

		Averages (%)					Standard Deviation %		
Date	Run time	Ether	Water	CO2 (act)	CO2 (meas)	CO2 (calc)	Ether	Water	CO2
11/3/97	9	82.57	8.35	4.51	4.91	8.84	4.19	1.14	1.34
11/17/97	23	57.17	14.49	5.69	7.55	27.57	4.81	1.45	0.86
12/1/97	37	40.33	17.46	10.82	12.66	41.07	12.74	2.21	2.47
12/14/97	50	38.94	13.26	15.86	19.23	46.51	3.99	0.17	4.17
12/31/97	67	22.74	14.01	20.15	25.65	61.54	7.07	2.61	2.35
1/12/98	79	42.64	14.28	11.87	15.66	41.92	9.06	3.09	8.53
1/26/98	93	38.06	17.04	9.65	13.29	43.69	5.57	0.69	3.64
2/9/98	107	40.67	15.29	8.92	12.85	42.86	6.26	2.66	1.48

		Recovery (%)				
Date	Run time	Vial 1	Vial 2	Vial 3	average	corr. Ave
11/3/97	9	95.59	93.45	97.25	95.43	95.83
11/17/97	23	77.75	75.77	81.54	78.35	79.21
12/1/97	37	76.79	69.25	59.78	68.61	70.45
12/14/97	50	66.49	68.19	69.51	68.06	71.44
12/31/97	67	50.80	58.67	61.24	56.90	62.40
1/12/98	79	63.50	75.10	67.76	68.79	72.57
1/26/98	93	66.56	65.29	62.40	64.75	68.39
2/9/98	107	68.36	62.22	64.05	64.88	68.81

Anoxic Autoclaved Soil with Organic Carbon Experiment - Data

		Ether (dpm)		Water (dpm)		CO2 (dpm)	
Date	Run time	Vial 1	Vial 2	Vial 1	Vial 2	Vial 1	Vial 2
11/3/97	9	38943.81		4445		98	
11/17/97	23	38735.87		4319		129	
12/1/97	37	37726.33		2836		246	
12/14/97	50	34739.85	35221	4146	4662	150	250
12/31/97	67	25299.69		5755		294	
1/12/98	79	35515.08	37915	4671	3145	268	809
1/26/98	93	31362.02	30796	5566	4652	729	928
2/9/98	107	30736.93	32875	5472	5459	1017	822

		Averages (dpm)				Standard Deviation dpm		
Date	Run time	Ether	Water	CO2 (act.)	CO2 (corr)	Ether	Water	CO2
11/3/97	9	38944	4445	98	4261			
11/17/97	23	38736	4319	129	4596			
12/1/97	37	37726	2836	246	7087			
12/14/97	50	34980	4404	200	8266	340	365	70
12/31/97	67	25300	5755	294	16595			
1/12/98	79	36715	3908	538	7028	1697	1079	382
1/26/98	93	31079	5109	829	11462	400	647	141
2/9/98	107	31806	5466	920	10379	1512	9	138

		Averages (%)					Standard Deviation %		
Date	Run time	Ether	Water	CO2 (act)	CO2 (meas)	CO2 (calc)	Ether	Water	CO2
11/3/97	9	81.73	9.33	0.21	0.22	8.94			
11/17/97	23	81.29	9.06	0.27	0.31	9.64			
12/1/97	37	79.17	5.95	0.52	0.60	14.87			
12/14/97	50	73.41	9.24	0.42	0.51	17.35	0.71	0.76	0.15
12/31/97	67	53.09	12.08	0.62	0.78	34.83			
1/12/98	79	77.05	8.20	1.13	1.49	14.75	3.56	2.26	0.80
1/26/98	93	65.22	10.72	1.74	2.40	24.05	0.84	1.36	0.30
2/9/98	107	66.75	11.47	1.93	2.78	21.78	3.17	0.02	0.29

		Recovery (%)			
Date	Run time	Vial 1	Vial 2	average	cor. Ave
11/3/97	9	91.26		91.26	91.28
11/17/97	23	90.63		90.63	90.66
12/1/97	37	85.64		85.64	85.73
12/14/97	50	81.92	84.22	83.07	83.16
12/31/97	67	65.79		65.79	65.96
1/12/98	79	84.90	87.87	86.38	86.74
1/26/98	93	79.03	76.34	77.69	78.34
2/9/98	107	78.12	82.17	80.15	81.00

Appendix D

Soil Extraction Data Analyses by HPLC
&
Summary of Radiolabeled Corrected Data for Comparison

HPLC Analysis Data

RDX 1/8/98

	Area	Conc (µg/L)	RDX (mg/kg)	dry weight (mg/kg)
Control1	178013	1281	12.77	13.11
Control2	180295	1297	12.97	13.32
Control3	143256	1030	10.27	10.55
Aerobic w/P	120837	869	9.05	9.29
Aerobic	143505	1032	10.70	10.98
Anoxic w/ P	18280	131	1.44	1.48
Anoxic	64523	464	4.96	5.09
Micro w/ P	10508	75	0.81	0.83
Micro	29179	210	2.22	2.27
Auto w/P	7295	52	0.51	0.53

RDX 2/9/97

	Conc (µg/L)	RDX (mg/kg)	dry weight (mg/kg)
Carbon &P1	54	0.52	0.53
Carbon & P2	229	2.15	2.20
Carbon	212	2.03	2.08
Anoxic	164	1.56	1.60
Micro	250	2.43	2.50
Aerobic w/P	990	9.42	9.68

RDX 2/24/97

	Conc (µg/L)	RDX (mg/kg)	dry weight (mg/kg)
Anoxic w/ P	34	0.37	0.38
Anoxic	359	3.84	3.95
Micro w/ P	45	0.49	0.51
Micro	120	1.31	1.34
auto w/ C	43	0.43	0.44
auto w/ P	45	0.45	0.46
Aerobic w/P	1031	10.68	10.97
Carbon w/P1	193	2.09	2.14
Carbon w/P2	267	2.87	2.95
Carbon 1	87	0.93	0.95
Carbon 2	198	2.07	2.13
Aerobic	851	9.20	9.45
Aerobic	923	9.81	10.08

HPLC- Summary of Data

Soil extractions

Sample	Date	Concentration (mg/L)	dry weight (mg/kg)	average (mg/kg)	Stand. Dev	Run Times
Aerobic w/ P	1/8/98	3.04	24.63			137
Aerobic w/ P	2/9/98	2.65	21.51	19.05	7.13	
Aerobic w/ P	2/24/98	1.36	11.01			
Aerobic	1/8/98	1.34	10.90			137
Aerobic	2/24/98	2.88	23.34	17.50	6.25	184
Aerobic	2/24/98	2.25	18.25			184
Anoxic w/ P	1/8/98	10.85	87.99	92.47	6.34	137
Anoxic w/ P	2/24/98	11.95	96.95			184
Anoxic	1/8/98	7.23	58.67			137
Anoxic	2/9/98	10.73	87.02	71.22	14.45	169
Anoxic	2/24/98	8.38	67.98			184
Micro w/ P	1/8/98	11.49	93.24	94.57	1.87	135
Micro w/ P	2/24/98	11.82	95.89			182
Micro	1/8/98	10.05	81.55			135
Micro	2/9/98	9.83	79.73	83.46	4.98	167
Micro	2/24/98	10.99	89.12			182
Anoxic-C&P	2/9/98	11.80	95.71			107
Anoxic-C&P	2/9/98	10.12	82.12	84.13	7.71	107
Anoxic-C&P	2/24/98	10.18	82.61			122
Anoxic-C&P	2/24/98	9.38	76.07			122
Anoxic w/ C	2/9/98	10.24	83.10			107
Anoxic w/ C	2/24/98	11.38	92.29	86.05	5.41	122
Anoxic w/ C	2/24/98	10.20	82.75			122
Auto w/ P	1/8/98	11.80	95.71*			137
Auto w/ P	2/24/98	11.86	96.23*	95.98		184
Auto w/ C	2/24/98	11.89	96.45*			122

* High removal efficiencies in the autoclaved vials is because autoclaving destroys RDX.

Summary of Radiolabeled Data

The removal % data represent the ether data corrected by the ether recovery factor

Sample	Date	Removal%	average	Standard Dev.	Run time
Aerobic w/ P	12/31/97	44.03*			129
Aerobic w/ P	12/31/97		-2.96	2.53	129
Aerobic w/ P	12/31/97	-4.75			129
Aerobic	1/5/98	11.05			134
Aerobic	1/5/98	33.11*	11.44	0.56	134
Aerobic	1/5/98	11.84			134
Anoxic w/ P	1/5/98	65.83			134
Anoxic w/ P	1/5/98	81.34	71.05	8.91	134
Anoxic w/ P	1/5/98	65.99			134
Anoxic	1/5/98	82.62			134
Anoxic	1/5/98	62.17	66.40	14.58	134
Anoxic	1/5/98	54.40			134
Micro w/ P	1/7/98	84.05	83.67	0.54	134
Micro w/ P	1/7/98	83.29			134
Micro	1/7/98	77.19			134
Micro	1/7/98	72.91	74.98	2.14	134
Micro	1/7/98	74.85			134
Anoxic-C&P	2/9/98	89.76			107
Anoxic-C&P	2/9/98	65.82	75.87	12.42	107
Anoxic-C&P	2/9/98	72.03			107
Anoxic w/ C	2/9/98	84.70			107
Anoxic w/ C	2/9/98	76.46	77.26	7.07	107
Anoxic w/ C	2/9/98	70.62			107
Auto w/ P	1/5/98	8.36			134
Auto w/ P	1/5/98	13.11	10.32	2.48	134
Auto w/ P	1/5/98	9.49			134
Auto w/ C	2/9/98	18.52	12.47	4.01	107
Auto w/ C	2/9/98	12.86			107

* Data not used for average

Appendix E

Degradation Rate Contants for Biodegradation Experiments

Aerobic with Phosphorus Experiment

Total RDX Mass on Soil= 12.65 mg/kg

Run Time	Ether (dpm)	Corrected Ether (norm)	RDX Loading (mg/kg)	Outliers (mg/kg)	ln (RDX)
0	--	1.00	13.44		2.60
0	--	1.00	13.64		2.61
0	--	1.00	10.87		2.39
10	31195	0.79		10.02	
10	47810	1.21	15.35		2.73
10	46421	1.18	14.90		2.70
24	44396	1.13	14.25		2.66
24	44547	1.13	14.30		2.66
24	43420	1.10	13.94		2.63
38	39188	0.99	12.58		2.53
38	41996	1.07	13.48		2.60
38	41894	1.06	13.45		2.60
50	42281	1.07	13.57		2.61
50	43294	1.10	13.90		2.63
50	34809	0.88	11.18		2.41
64	43493	1.10	13.96		2.64
64	42227	1.07	13.56		2.61
64	42850	1.09	13.76		2.62
78	42967	1.09	13.79		2.62
78	43611	1.11	14.00		2.64
78	20181	0.51		6.48	
91	43632	1.11	14.01		2.64
91	40898	1.04	13.13		2.57
91	44217	1.12	14.20		2.65
112	34804	0.88	11.17		2.41
112	35933	0.91	11.54		2.45
129	22056	0.56		7.08	
129	39869	1.01	12.80		2.55
129	41278	1.05	13.25		2.58

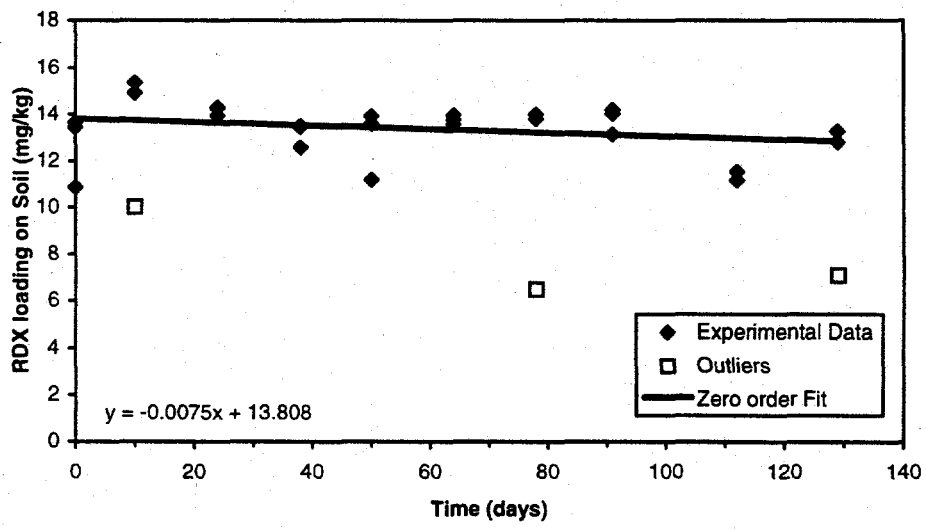
Zero Order
SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.268205651
R Square	0.071934271
Adjusted R Sq	0.033264866
Standard Error	1.097241156
Observations	26

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	2.239610693	2.239610693	1.860237326	0.185253454
Residual	24	28.8945157	1.203938154		
Total	25	31.13412639			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	13.80814014	0.377022137	36.62421589	1.41428E-22	13.03000486	14.58627543
X Variable 1	-0.00752448	0.005516864	-1.363905175	0.185253454	-0.018910726	0.003861766



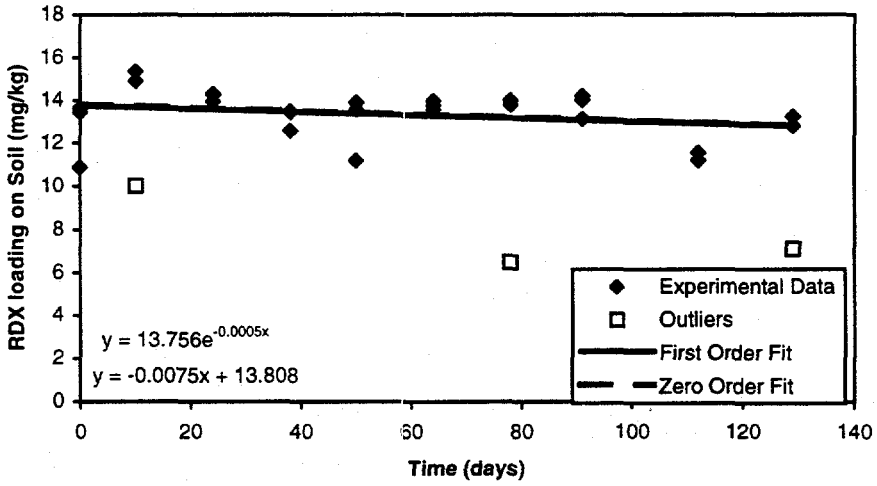
First Order
SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.250638873
R Square	0.062819845
Adjusted R Sq	0.023770671
Standard Error	0.08615654
Observations	26

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.011941572	0.011941572	1.608736869	0.216835172
Residual	24	0.178150785	0.007422949		
Total	25	0.190092357			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	2.621488876	0.029604178	88.55131357	1.0516E-31	2.560388867	2.682588884
X Variable 1	-0.000549441	0.00043319	-1.268359913	0.216835172	-0.001443501	0.000344619



Aerobic Experiment

Total RDX Mass on Soil= 12.65 mg/kg

Run Time	Ether (dpm)	Corrected Ether (norm)	RDX Loading (mg/kg)	Outliers (mg/kg)	ln (RDX)
0	--	1.00	13.44		2.39
0	--	1.00	13.64		2.60
0	--	1.00	10.87		2.61
10	36889	0.93	11.77		2.47
10	43741	1.10	13.96		2.64
10	44743	1.13	14.28		2.66
24	44006	1.11	14.05		2.64
24	44204	1.12	14.11		2.65
24	43400	1.09	13.85		2.63
38	41507	1.05	13.25		2.58
38	42445	1.07	13.55		2.61
38	42779	1.08	13.65		2.61
50	44467	1.12	14.19		2.65
50	42635	1.08	13.61		2.61
50	39188	0.99	12.51		2.53
64	43865	1.11	14.00		2.64
64	44471	1.12	14.19		2.65
64	44794	1.13	14.30		2.66
78	41198	1.04	13.15		2.58
78	41556	1.05	13.26		2.59
78	36176	0.91	11.55		2.45
91	41857	1.06	13.36		2.59
91	43238	1.09	13.80		2.62
91	40276	1.02	12.86		2.55
112	34521	0.87	11.02		2.40
112	36749	0.93	11.73		2.46
134	35263	0.89	11.26		2.42
134	26517	0.67		8.464003658	
134	34948	0.88	11.16		2.41

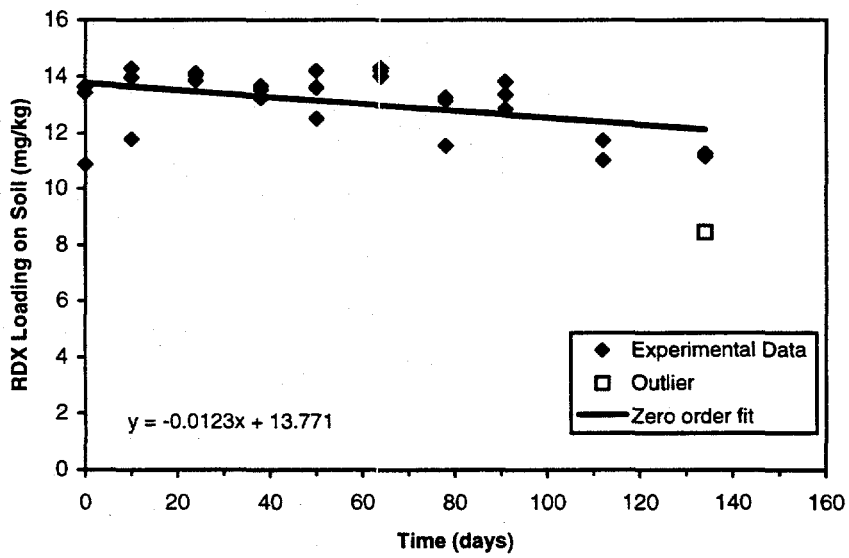
Zero Order
SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.442317943
R Square	0.195645162
Adjusted R Sq	0.164708438
Standard Error	1.025221509
Observations	28

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	6.647069217	6.647069217	6.324042553	0.018430449
Residual	26	27.32805768	1.051079142		
Total	27	33.9751269			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	13.77069417	0.334626785	41.15239655	3.37062E-25	13.08285851	14.45852983
X Variable 1	-0.01233843	0.004906395	-2.51476491	0.018430449	-0.022423677	-0.002253184



First Order

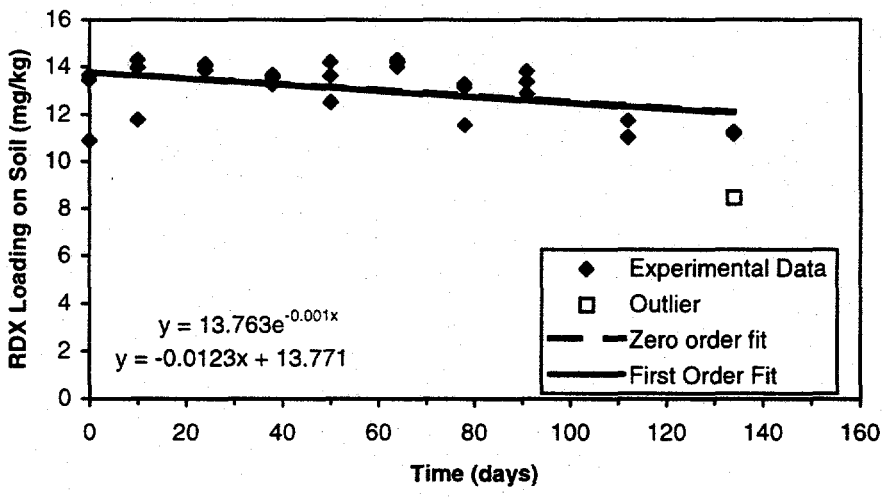
SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.440068663
R Square	0.193660429
Adjusted R Sq	0.162647368
Standard Error	0.081623661
Observations	28

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.04160336	0.04160336	6.244479771	0.019105153
Residual	26	0.173222974	0.006662422		
Total	27	0.214826334			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	2.621979199	0.026641524	98.41701266	5.63006E-35	2.567216726	2.676741672
X Variable 1	-0.000976133	0.000390626	-2.49889571	0.019105153	-0.001779076	-0.00017319



Anoxic with Phosphorus Experiment

Total RDX Mass on Soil= 12.65 mg/kg

Run Time	Ether (dpm)	Corrected Ether (norm)	RDX Loading (mg/kg)	ln(RDX)
0	--	1.00	13.44	2.60
0	--	1.00	13.64	2.61
0	--	1.00	10.87	2.39
11	37876	0.96	12.11	2.49
11	38936	0.98	12.45	2.52
11	39360	1.00	12.59	2.53
24	34291	0.87	10.97	2.40
24	39403	1.00	12.60	2.53
24	34428	0.87	11.01	2.40
38	27043	0.68	8.65	2.16
38	22582	0.57	7.22	1.98
38	30318	0.77	9.70	2.27
50	19971	0.50	6.39	1.85
50	22793	0.58	7.29	1.99
50	27704	0.70	8.86	2.18
64	20396	0.52	6.52	1.88
64	23259	0.59	7.44	2.01
64	12588	0.32	4.03	1.39
78	14287	0.36	4.57	1.52
78	20307	0.51	6.50	1.87
78	12069	0.31	3.86	1.35
91	18090	0.46	5.79	1.76
91	15107	0.38	4.83	1.58
91	18335	0.46	5.86	1.77
112	8244	0.21	2.64	0.97
112	13616	0.34	4.36	1.47
134	13518	0.34	4.32	1.46
134	7381	0.19	2.36	0.86
134	13454	0.34	4.30	1.46

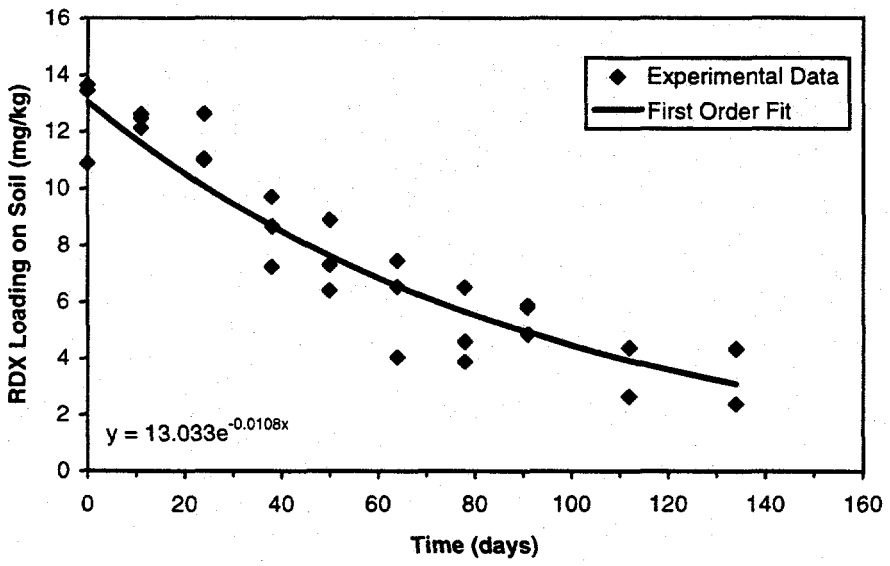
First Order
SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.908852392
R Square	0.826012671
Adjusted R Sq	0.819568695
Standard Error	0.210865481
Observations	29

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	5.699592847	5.699592847	128.1837142	9.28174E-12
Residual	27	1.200534778	0.044464251		
Total	28	6.900127625			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	2.567448474	0.067898186	37.81321178	6.35876E-25	2.428132998	2.70676395
X Variable 1	-0.010751243	0.000949603	-11.32182469	9.28174E-12	-0.012699667	-0.008802819



Anoxic Experiment

Total RDX Mass on Soil= 12.65 mg/kg

Run Time	Ether (dpm)	Corrected Ether (norm)	RDX Loading (mg/kg)	ln(RDX)
0		1.00	13.44	2.60
0		1.00	13.64	2.61
0		1.00	10.87	2.39
11	42927	1.08	13.64	2.61
11	45775	1.15	14.55	2.68
11	89981	2.26	*	
24	44209	1.11	14.05	2.64
24	33149	0.83	10.53	2.35
24	24010	0.60	7.63	2.03
38	30213	0.76	9.60	2.26
38	33783	0.85	10.73	2.37
38	29946	0.75	9.52	2 25
50	32528	0.82	10.34	2.34
50	26734	0.67	8.50	2.14
50	25587	0.64	8.13	2.10
64	21174	0.53	6.73	1.91
64	28450	0.71	9.04	2.20
64	27410	0.69	8.71	2.16
78	22115	0.56	7.03	1.95
78	11380	0.29	3.62	1.29
78	15941	0.40	5.07	1.62
91	17186	0.43	5.46	1.70
91	22385	0.56	7.11	1.96
91	21868	0.55	6.95	1.94
112	15739	0.40	5.00	1.61
112	7277	0.18	2.31	0.84
134	6921	0.17	2.20	
134	15066	0.38	4.79	1.57
134	18158	0.46	5.77	1.75

*Data not used

First Order

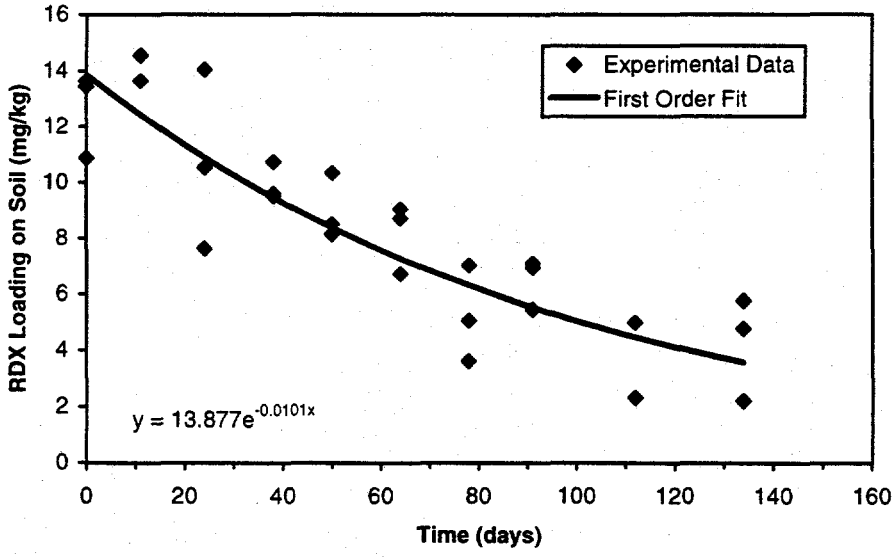
SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.825046878
R Square	0.680702351
Adjusted R Sq	0.667930445
Standard Error	0.255744045
Observations	27

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	3.485881314	3.485881314	53.29684954	1.1949E-07
Residual	25	1.635125408	0.065405016		
Total	26	5.121006722			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	2.596251064	0.087364082	29.71760251	5.03037E-21	2.416321495	2.776180633
X Variable 1	-0.009185126	0.001258156	-7.300469131	1.1949E-07	-0.011776343	-0.006593908



Microaerobic with Phosphorus Experiment

Total RDX Mass on Soil= 12.65 mg/kg

Run Time	Ether (dpm)	Corrected Ether (norm)	RDX Loading (mg/kg)	ln (RDX)
0		1.00	13.44	2.60
0		1.00	13.64	2.61
0		1.00	10.87	2.39
10	41612	1.05	13.33	2.59
10	43279	1.10	13.86	2.63
10	43596	1.10	13.97	2.64
24	40478	1.02	12.97	2.56
24	36500	0.92	11.69	2.46
24	39170	0.99	12.55	2.53
38	23694	0.60	7.59	2.03
38	26726	0.68	8.56	2.15
38	27470	0.70	8.80	2.17
50	19189	0.49	6.15	1.82
50	17436	0.44	5.59	1.72
50	15771	0.40	5.05	1.62
64	18480	0.47	5.92	1.78
64	17362	0.44	5.56	1.72
64	15742	0.40	5.04	1.62
78	9279	0.23	2.97	1.09
78	13736	0.35	4.40	1.48
78	12132	0.31	3.89	1.36
91	9348	0.24	2.99	1.10
91	9442	0.24	3.02	1.11
112	10440	0.26	3.34	1.21
112	5136	0.13	1.65	0.50
134	6301	0.16	2.02	0.70
134	6600	0.17	2.11	0.75

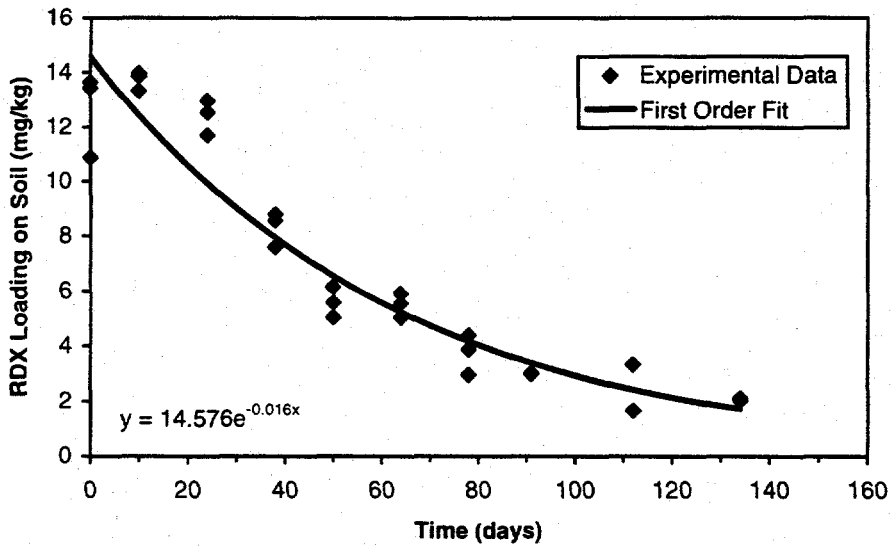
First Order

Regression Statistics	
Multiple R	0.960938232
R Square	0.923402286
Adjusted R Sq	0.920338377
Standard Error	0.189525975
Observations	27

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	10.82561606	10.82561606	301.3804961	1.85057E-15
Residual	25	0.898002376	0.035920095		
Total	26	11.72361844			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	2.679366465	0.061887444	43.29418549	4.93031E-25	2.551906978	2.806825952
X Variable 1	-0.015985629	0.000920815	-17.36031383	1.85057E-15	-0.017882081	-0.014089177



Microaerobic Experiment

Total RDX Mass on Soil= 12.65 mg/kg

Run Time	Ether (dpm)	Corrected Ether (norm)	RDX Loading (mg/kg)	ln (RDX)
0		1.00	13.44	2.60
0		1.00	13.64	2.61
0		1.00	10.87	2.39
10	44523	1.12	14.19	2.65
10	45662	1.15	14.55	2.68
10	46248	1.16	14.74	2.69
24	44078	1.11	14.05	2.64
24	42289	1.07	13.48	2.60
24	42448	1.07	13.53	2.60
38	27508	0.69	8.77	2.17
38	20399	0.51	6.50	1.87
38	26634	0.67	8.49	2.14
50	23907	0.60	7.62	2.03
50	21755	0.55	6.93	1.94
50	26742	0.67	8.52	2.14
64	23190	0.58	7.39	2.00
64	19361	0.49	6.17	1.82
64	26143	0.66	8.33	2.12
78	19331	0.49	6.16	1.82
78	16125	0.41	5.14	1.64
78	19477	0.49	6.21	1.83
91	19487	0.49	6.21	1.83
91	12749	0.32	4.06	1.40
91	15476	0.39	4.93	1.60
112	12821	0.32	4.09	1.41
112	11100	0.28	3.54	1.26
134	9056	0.23	2.89	1.06
134	10756	0.27	3.43	1.23
134	9987	0.25	3.18	1.16

First Order

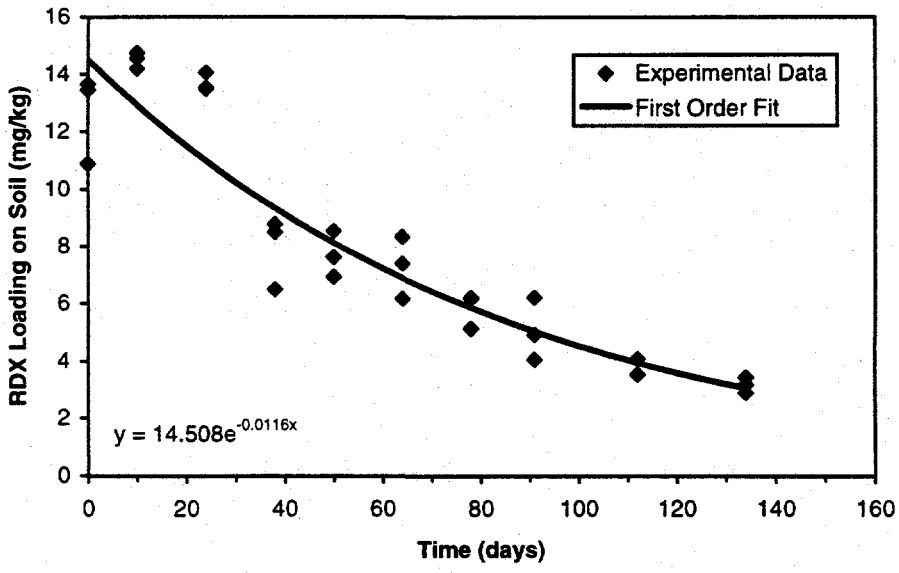
SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.954581476
R Square	0.911225795
Adjusted R Sq	0.907937861
Standard Error	0.155399098
Observations	29

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	6.692678517	6.692678517	277.1424016	1.00658E-15
Residual	27	0.652019752	0.02414888		
Total	28	7.344698269			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	2.67467041	0.049882464	53.61945222	5.76807E-29	2.572320116	2.777020704
X Variable 1	-0.011616512	0.000697789	-16.64759447	1.00658E-15	-0.013048256	-0.010184768



Aerobic Autoclaved Soil with Phosphorus Experiment

Total RDX Mass on Soil= 12.65 mg/kg

Run Time	Ether (dpm)	Corrected Ether (norm)	RDX Loading (mg/kg)	ln (RDX)
0		1.00	13.44	2.60
0		1.00	13.64	2.61
0		1.00	10.87	2.39
10	39063	1.01	12.74	2.54
10	37462	0.97	12.22	2.50
10	38875	1.00	12.68	2.54
24	38939	1.00	12.70	2.54
24	38006	0.98	12.40	2.52
24	43255	1.12	14.11	2.65
38	34830	0.90	11.36	2.43
38	36206	0.93	11.81	2.47
38	40402	1.04	13.18	2.58
50	37633	0.97	12.27	2.51
50	37153	0.96	12.12	2.49
50	42989	1.11	14.02	2.64
64	40259	1.04	13.13	2.57
64	42336	1.09	13.81	2.63
64	37717	0.97	12.30	2.51
78	35782	0.92	11.67	2.46
78	35364	0.91	11.53	2.45
78	37228	0.96	12.14	2.50
91	41046	1.06	13.39	2.59
91	42459	1.10	13.85	2.63
91	37228	0.96	12.14	2.50
112	31874	0.82	10.40	2.34
112	32044	0.83	10.45	2.35
134	35531	0.92	11.59	2.45
134	33690	0.87	10.99	2.40
134	35093	0.91	11.45	2.44

Zero Order

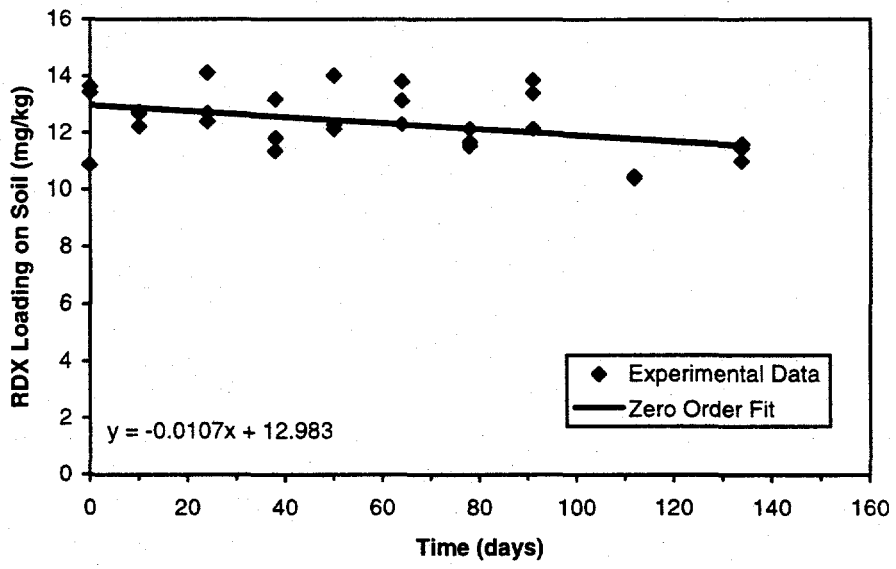
SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.426793806
R Square	0.182152953
Adjusted R Sq	0.151862322
Standard Error	0.973269867
Observations	29

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	5.696320876	5.696320876	6.013507965	0.020946373
Residual	27	25.57586429	0.947254233		
Total	28	31.27218517			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	12.98261804	0.31241558	41.55560374	5.18591E-26	12.34159465	13.62364143
X Variable 1	-0.010716994	0.004370278	-2.452245494	0.020946373	-0.019684057	-0.001749931



First Order

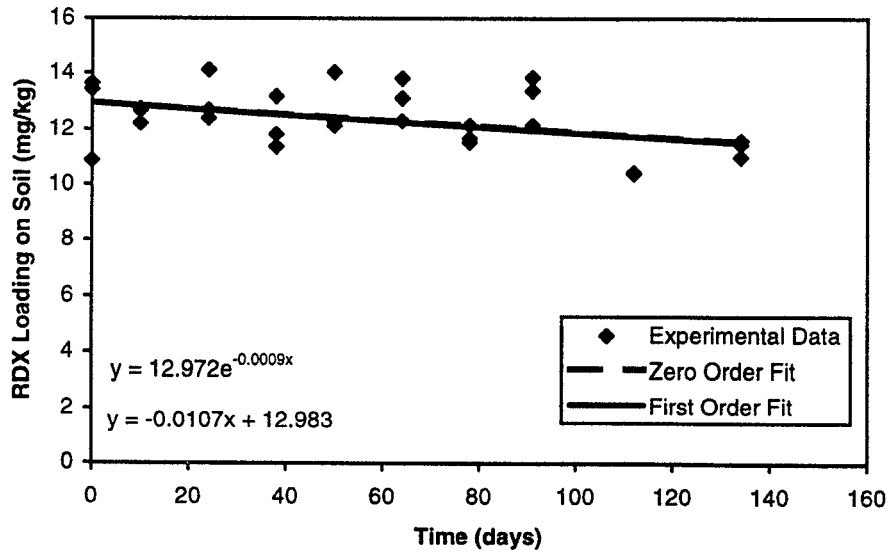
SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.435662695
R Square	0.189801984
Adjusted R Sq	0.15979465
Standard Error	0.079053805
Observations	29

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.039529279	0.039529279	6.325186516	0.018161899
Residual	27	0.16873661	0.006249504		
Total	28	0.208265889			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	2.562768333	0.025375943	100.9920446	2.37099E-36	2.510701235	2.614835432
X Variable 1	-0.000892761	0.000354976	-2.514992349	0.018161899	-0.00162111	-0.000164412



Anoxic with Organic Carbon and Phosphorus Experiment

Total RDX Mass on Soil= 12.63 mg/kg

Run Time	Ether (dpm)	Corrected Ether (norm)	RDX Loading (mg/kg)	ln (RDX)
0		1.00	13.42	2.60
0		1.00	13.62	2.61
0		1.00	10.85	2.38
9	40061	1.09	13.77	2.62
9	45573	1.24	15.67	2.75
9	38925	1.06	13.38	2.59
23	33115	0.90	11.39	2.43
23	34448	0.94	11.84	2.47
23	26349	0.72	9.06	2.20
37	22592	0.62	7.77	2.05
37	32939	0.90	11.32	2.43
37	26425	0.72	9.09	2.21
50	14371	0.39	4.94	1.60
50	23504	0.64	8.08	2.09
50	11576	0.32	3.98	1.38
67	10310	0.28	3.54	1.27
67	8799	0.24	3.03	1.11
67	7724	0.21	2.66	0.98
79	8902	0.24	3.06	1.12
79	10279	0.28	3.53	1.26
79	4342	0.12	1.49	0.40
93	12420	0.34	4.27	1.45
93	5737	0.16	1.97	0.68
93	8656	0.24	2.98	1.09
107	3763	0.10	1.29	0.26
107	12557	0.34	4.32	1.46
107	10275	0.28	3.53	1.26

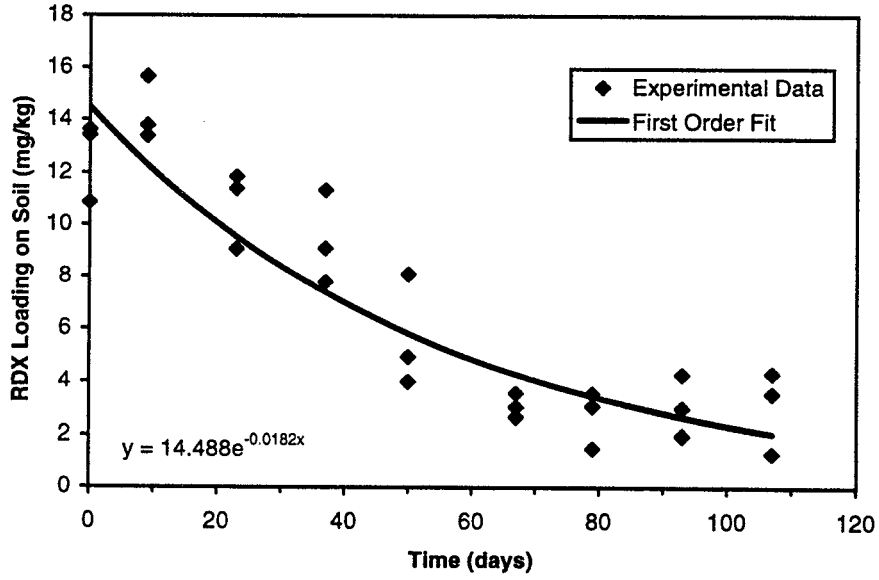
First Order
SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.881389231
R Square	0.776846977
Adjusted R Sq	0.767920856
Standard Error	0.35942917
Observations	27

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	11.24344381	11.24344381	87.0307475	1.27786E-09
Residual	25	3.229733208	0.129189328		
Total	26	14.47317702			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	2.673351994	0.122368264	21.8467755	8.34228E-18	2.421330013	2.925373975
X Variable 1	-0.018226195	0.001953708	-9.329027147	1.27786E-09	-0.022249929	-0.014202461



Anoxic with Organic Carbon Experiment

Total RDX Mass on Soil= 12.64 mg/kg

Run Time	Ether (dpm)	Corrected Ether (norm)	RDX Loading (mg/kg)	Outliers (mg/kg)	ln (RDX)
0		1.00	13.43		2.60
0		1.00	13.63		2.61
0		1.00	10.86		2.39
9	39102	1.04	13.10		2.572462591
9	37487	0.99	12.56		2.530284396
9	41454	1.10	13.89		2.630887973
23	25991	0.69	8.71		2.16406412
23	25853	0.69	8.66		2.158729119
23	29892	0.79	10.01		2.303877688
37	24226	0.64	8.11		2.09370558
37	20966	0.56	7.02		1.949215028
37	12468	0.33	4.18		1.429432408
50	19583	0.52	6.56		1.880968705
50	16365	0.43	5.48		1.70144375
50	19721	0.52	6.61		1.88800252
67	7291	0.19	2.44		0.892961952
67	11217	0.30	3.76		1.323724621
67	14001	0.37	4.69		1.545420074
79	18990	0.50		6.36	
79	25143	0.67		8.42	
79	16821	0.45		5.63	
93	20522	0.54		6.87	
93	18612	0.49		6.23	
93	15274	0.40		5.12	
107	22089	0.59		7.40	
107	16179	0.43		5.42	
107	19869	0.53		6.66	

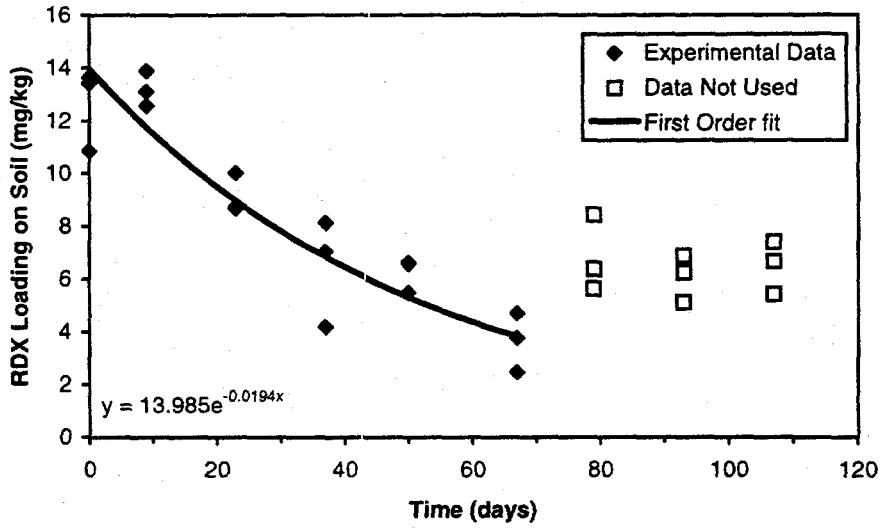
First Order
SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.911773704
R Square	0.831331288
Adjusted R Sq	0.820789493
Standard Error	0.21410076
Observations	18

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	3.614897589	3.614897589	78.8605095	1.39543E-07
Residual	16	0.73342617	0.045839136		
Total	17	4.348323759			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	2.637961704	0.08445378	31.23556696	9.08429E-16	2.458927729	2.81699568
X Variable 1	-0.019398882	0.002184474	-8.880343997	1.39543E-07	-0.02402976	-0.014768005



Anoxic Autoclaved Soil with Organic Carbo Experiment

Total RDX Mass on Soil= 12.65 mg/kg

Run Time	Ether (dpm)	Corrected Ether (norm)	RDX Loading (mg/kg)	ln (RDX)
0		1.00	13.43	2.60
0		1.00	13.63	2.61
0		1.00	10.86	2.38
9	38944	1.03	13.05	2.57
23	38736	1.03	12.98	2.56
37	37726	1.00	12.64	2.54
50	34740	0.92	11.64	2.45
50	35221	0.93	11.80	2.47
67	25300	0.67	8.48	2.14
79	35515	0.94	11.90	2.48
79	37915	1.01	12.70	2.54
93	31362	0.83	10.51	2.35
93	30796	0.82	10.32	2.33
107	30737	0.81	10.30	2.33
107	32875	0.87	11.01	2.40

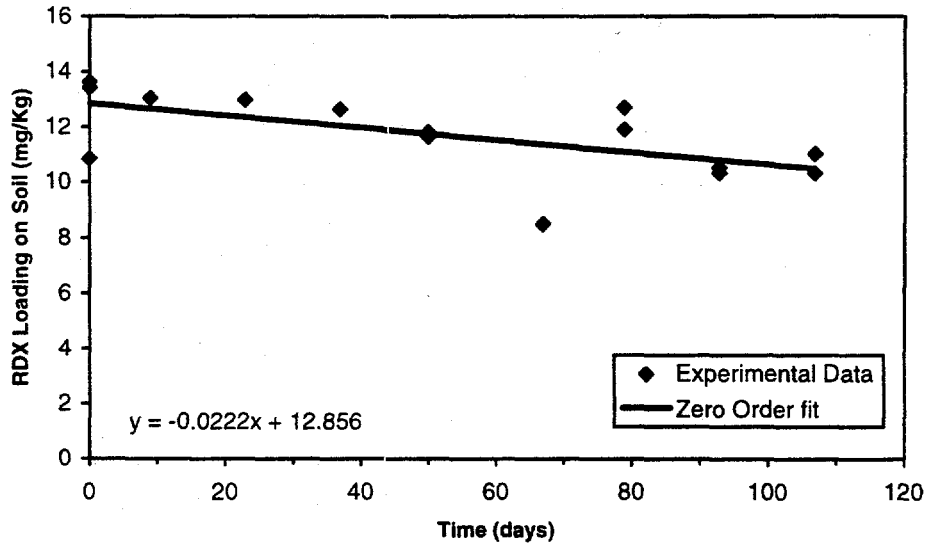
Zero Order
SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.613988817
R Square	0.376982267
Adjusted R Sq	0.329057826
Standard Error	1.177557204
Observations	15

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	10.9075671	10.9075671	7.866179748	0.014895932
Residual	13	18.02633259	1.386640969		
Total	14	28.9338997			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	12.85611075	0.517796034	24.82852302	2.4429E-12	11.73748064	13.97474086
X Variable 1	-0.022207593	0.007918074	-2.804671059	0.014895932	-0.039313549	-0.005101637



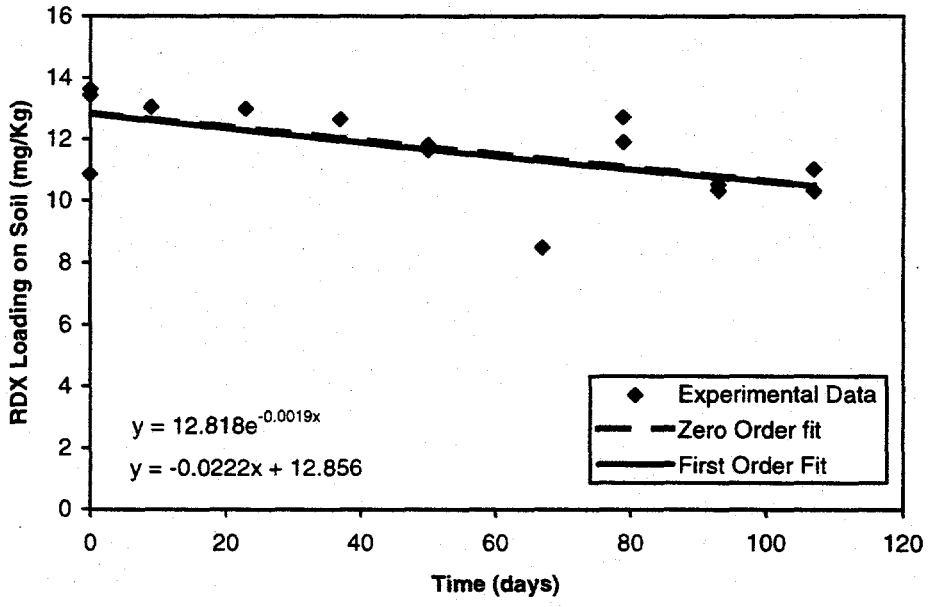
First Order
SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.584294161
R Square	0.341399667
Adjusted R Sq	0.290738103
Standard Error	0.108742562
Observations	15

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.079686292	0.079686292	6.738829986	0.022176743
Residual	13	0.153724281	0.011824945		
Total	14	0.233410574			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	2.550881432	0.047816333	53.34749199	1.29648E-16	2.447580545	2.654182319
X Variable 1	-0.001898145	0.000731202	-2.595925651	0.022176743	-0.003477809	-0.00031848



Appendix F

Headspace Analysis Data

Headspace Analysis Data

Standard Gas Mixture 2/3/98

	Sample 1 (area)	Sample 2 (area)	Sample 3 (area)	Average area	Conc. (mole %)
CO2	911820	789499	790007	830442	5
O2	706317	604898	607725	639647	4.95
N2	878165	711782	707592	765846	5
Methane	534301	457472	452232	481335	4
CO	1013879	824823	709895	849532	5

Anoxic with Carbon and Phosphorus Experiment

	Area	Conc. (mole %)
CO2	48661	0.29
O2	21066	0.16
N2	2796894	18.26
Methane	21717	0.18
CO	29166	0.17

Standard Gas Mixture 2/24/98

	Sample 1 (area)	Sample 2 (area)	Sample 3 (area)	Sample 4 (area)	Average area
CO2	660497	597930	774912	920452	738448
O2	508977	460352	596194	708913	568609
N2	596147	544405	695449	820923	664231
Methane	394090	361429	458205	533465	436797
CO	651494	613947	763605	920140	737297

Aerobic with Phosphorus Experiment

	Area	Conc. (mole %)
CO2	28002	0.17
O2	249676	1.93
N2	1390929	9.08
Methane		0.00
CO		0.00

Aerobic Experiment- vial #1

	Area	Conc. (mole %)
CO2	40149	0.24
O2	240804	1.86
N2	1958460	12.79
Methane	11313	0.09
CO	14028	0.08

Aerobic Experiment- vial #2

	Area	Conc. (mole %)
CO2	56486	0.34
O2	298794	2.31
N2	2318200	15.13
Methane		0.00
CO		0.00

Anoxic with Phosphorus Experiment

	Area	Conc. (mole %)
CO2	23244	0.14
O2	14649	0.11
N2	2626765	17.15
Methane		0.00
CO		0.00

Anoxic Experiment

	Area	Conc. (mole %)
CO2	24896	0.15
O2	13781	0.11
N2	2505046	16.35
Methane		0.00
CO		0.00

Microaerobic w/ Phosphorus Experiment

	Area	Conc. (mole %)
CO2	41356	0.25
O2	36049	0.28
N2	2599662	16.97
Methane		0.00
CO		0.00

Microaerobic Experiment

	Area	Conc. (mole %)
CO2	68672	0.41
O2	35330	0.27
N2	2920858	19.07
Methane		0.00
CO		0.00

Anoxic with Organic Carbon and Phosphorus Eperiment - vial #1

	Area	Conc. (mole %)
CO2	16998	0.10
O2	10342	0.08
N2	2846566	18.58
Methane		0.00
CO		0.00

Anoxic with Organic Carbon and Phosphorus Experiment - vial #2

	Area	Conc. (mole %)
CO2	29222	0.18
O2	14679	0.11
N2	2878378	18.79
Methane		0.00
CO		0.00

Anoxic with Organic Carbon - vial #1 (injection problems)

Anoxic with Organic Carbon Experiment- vial #2

	Area	Conc. (mole %)
CO2	24460	0.15
O2	10455	0.08
N2	1714304	11.19
Methane		0.00
CO		0.00

Anoxic Autoclaved with Organic Carbon Experiment

	Area	Conc. (mole %)
CO2	34385	0.21
O2	55155	0.43
N2	2983989	19.48
Methane		0.00
CO		0.00

Aerobic Autoclaved with Phosphorus Experiment

	Area	Conc. (mole %)
CO2	12087	0.07
O2	314393	2.43
N2	1565559	10.22
Methane		0.00
CO		0.00