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AN EVALUATION OF THE ENVIRONMENTAL FATE AND BEHAVIOR OF MUNITIONS MATERIEL (TETRYL AND POLAR METABOLITES OF TNT) IN SOIL AND PLANT SYSTEMS

Environmental Fate and Behavior of Tetryl

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Dominic A. Cataldo

March 1992
Project Order No. 88PP8853

Prepared for
U.S. Army Medical Research and Development Command
under a Related Services Agreement
with the U.S. Department of Energy
Contract DE-AC06-76RLO 1830

Pacific Northwest Laboratory
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The objective of the present studies was to elucidate the environmental behavior and fate of 2,4,6-trinitrophenylmethylnitramine (tetryl) in the soil/plant system in three different types of soils incubated for 60 days, no tetryl was detectable after 11 days, most of the radiolabel was associated with non-extractable soil components (43 to 58%), and four transformation products appeared rapidly, of which two were identified as N-methyl-2,4,6-trinitroaniline and *N*-methyl-aminodinitroaniline isomer. Short-term hydroponic studies indicated no significant difference in uptake rates for the three plant species employed. Kinetic studies indicated that plants have a high affinity and capacity for absorbing tetryl. Partitioning patterns indicated that the root is the major accumulation site for tetryl. Chemical fractionation and analyses of tissues showed rapid metabolism of tetryl in tissues of all species, which proceeded toward more polar metabolic products.

Plant maturity studies indicated a significant ($P \leq 0.01$) differences in the total relative uptake of tetryl by all three plant species based on soil type. Fractionation and analysis showed no activity in the F1 fraction of the mature plant. The absence of metabolites in fraction F1 indicates that tetryl has undergone extensive metabolism to compounds that differ dramatically in their polarity.

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EXECUTIVE SUMMARY

The objective of the present studies was to elucidate the environmental behavior and fate of 2,4,6-trinitrophenylmethylnitramine (tetryl) in the soil/plant system. The studies employed three plant species and three soil types as surrogates for understanding the plant root absorption process, soil constraints on plant uptake, plant partitioning following uptake, and the role of tetryl in soils and plants. The study of tetryl behavior required development of chemical extraction and analytical procedures suitable for soil and plant matrices. Using these procedures, the physical and chemical fate of tetryl was assessed for both soils and plants.

The chemical behavior and transformations of tetryl in soil were more complex than those previously reported for either TNT or RDX. Mass balance analyses of the three soil systems, employing Burbank, Palouse, and Cinebar soils, was very good 2 h after amendment, with over 97% of the amended radiolabel present in either the methanol extract or the extracted soils. The mass balance deficit was found to rise with time after amendment. At 60 days after amendment, the mass balance deficit was as high as 21%. Within 11 days after amendment, less than 8% of the extracted radiolabel found speciated as tetryl; by 30 days after amendment, soil concentrations of tetryl were below the detection limit of 2.5 ppm. Immediately after amendment, the bound or non-extractable fractions of radiolabel in Burbank, Palouse, and Cinebar soils were 1.8, 2.8, and 17%, respectively. At the end of the 60-day study, the non-extractable radiolabel content of the soils ranged from 43 to 58% indicating either a strong binding to soil exchange sites or mineral associations.

Soil chemical transformations are rapid, with tetryl concentrations declining rapidly, and the appearance of four principal tetryl transformation products. Two major products identified were *N*-methyl-2,4,6-trinitroaniline and *N*-methyl-aminodinitroaniline isomer, both arising from nitroreduction of *N*-methyl-2,4,6-trinitroaniline. Based on these tetryl transformation products, two independent tetryl soil transformation pathways are proposed.

Hydroponic studies were performed to address several basic needs. These included establishing the physiological capacity of plants to absorb and transport tetryl in the absence of soils and their sorptive components; elucidating the extent of partitioning of tetryl, particularly to the root, which is not amenable to analysis in soil systems; and establishing the short-term chemical fate of tetryl in plant tissues. Short-term studies indicated no significant difference in uptake rates for the three plant species Kinetic studies indicated that plants have a high affinity and capacity to absorb tetryl.

Partitioning patterns for the short-term exposures indicated that the root is the major accumulation site for tetryl. Mass balance studies indicated that mineralization rates for roots were approximately 11 µg equivalents of tetryl per day or 0.4% of supplied tetryl per day. From the tissue analyses performed after 7 days, it is clear that the plants had accumulated the majority of the available label supplied.

Chemical analyses of hydroponically treated bush bean leaf tissues indicated that acid hydrolysis, as compared with water hydrolysis, caused both the release of more radiolabel from the plant matrix and the cleavage of some polar conjugates. Chemical fractionation and analyses of tissues showed rapid metabolism of tetryl in tissues of all species; metabolism was so rapid that in all cases less than 3.1% of the radiolabel was isolated in fraction F1 (the tetryl fraction). Metabolism of tetryl proceeded toward more polar metabolic products, as evidenced by the appearance of a large percentage of radiolabel in the highly polar non-extractable aqueous base fraction. Over all analyses, 27% of the radiolabel was contained in the aqueous base fraction. A significant amount of radiolabel, averaging 14%, was contained in the extractable, yet polar, fraction F3. The pellets were found to sequester an average 54% of the radiolabel over all analyses. This indicates a rapid metabolism, sequestering, or conjugation of tetryl and its transformation products. Root tissues were found to contain N-methyl-2,4,6-trinitroaniline (the photodecomposition product), and *N*-methyl-dinitroaminoaniline. Several other major metabolites were also seen, but remain unidentified.

A major objective of the soil/plant maturity studies was to assess to what extent and in what form tetryl and/or its principal residues are accumulated, stored, and/or metabolized in soil-grown plants (bush bean, blando brome, and wheat) at physiological maturity. Results for plants grown in 25-ppm ^{14}C-tetryl-amended soil indicate significant ($P \leq 0.01$) differences in the total relative uptake of tetryl by all three plant species based on soil type. Bush bean plants grown in the Burbank soil accumulated twice as much tetryl/g fr. wt. as those grown in the Palouse soil (147 vs. 74 µg/g fr. wt.) and four to five times that of the plants grown in the cinnebar soil (32 µg/g fr. wt.). Similar uptake differences are evident in the blando brome, but this trend was not as pronounced in wheat.

Analysis of the tissue distribution patterns indicates that tissue partitioning for mature plants grown on soil differs from that observed in short-term hydroponic studies. While the root remains the primary repository of accumulated tetryl residues, the shoots accumulate substantially more of the residues. For bush bean, the roots contain ~80% of the label, the leaves ~10%, the stems ~7%, and the seed and pod ~3%. In wheat and blando brome, 16 to 20% of the accumulated label is found in the shoot tissues.

Fractionation and analysis of mature plant tissues indicate an overall mass balance of 85.6 ± 22.4%. Radiolabel was not found in fraction F1 of the mature plant tissues analyzed. The absence of metabolites in fraction F1 indicates that tetryl has undergone extensive metabolism to compounds that differ dramatically in their polarity. This precluded detailed chemical analysis of tetryl and its immediate transformation products. Over all analyses, an average of 23.8% of the radiolabel, was found to be associated with the non-extractable aqueous base fraction. This indicates that tetryl metabolism within the plant proceeds with the formation of extremely polar metabolites. The tissue pellets were also found to contain an average of 44.7% of the radiolabel. Incorporation of radiolabel within the insoluble material that composes the tissue pellet is evidently a prominent mechanism employed by plants for the detoxification of tetryl.

It is important to note that while the F1 fraction, which classically contains the early metabolites of tetryl and other munitions materiels, contains no activity, this absence is indicative of either a total degradation and incorporation of residues or a conversion to much more polar metabolites including conjugates. This pattern may represent an important factor in understanding the potential fate and impacts of tetryl present in the environment.

Correlation of cation exchange capacity and organic matter content with plant tetryl concentrations shows a linear response for both total plant (including root) concentration and shoot concentrations. Regression analyses indicate that bushbean is less sensitive to these factors than the monocots, based on whole-plant concentrations However, on a shoot concentration basis, response slopes for the soil parameters are similar. This disparity may indicate that differences in root absorption potential may exist between dicots and monocots, and those differences may be based not so much on tetryl uptake as on the uptake of tetryl soil transformation products.

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1.0 INTRODUCTION

Munitions materiels currently used as propellants or as explosive charges include trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), and 2,4,6-trinitrophenylmethylnitramine (tetryl). These materiels and their decomposition or combustion products may enter the environment as a result of production or manufacturing activities and field usage and disposal (Small and Rosenblatt 1974; Kitchens et al. 1978; Spanggord et al. 1983; Ryon et al. 1984).

The presence of these specific munitions-related components in the environment is, however, not indicative of the existence, or the severity of an environmental impact. Depending on sorptive processes of the soil and on their relative water solubility, organic contaminants such as these may undergo chemical partitioning as they enter terrestrial and aquatic environments, thus affecting their short-term accumulation and mobility. Persistence, and therefore accumulation, of the contaminant can be further influenced by its relative chemical stability and/or the presence biologically mediated degradative processes.

Biotic processes have been clearly indicated as being effective in the degradation/detoxification of a variety of organic xenobiotic compound classes and may interact in a similar fashion with munitions. With this in mind, an understanding of three biotic factors is important in assessing the relative long-term importance of munitions-related materiel released to the environment. These factors are 1) the extent to which soil microbes can degrade and/or modify the contaminant, 2) the extent to which the parent compounds and their major decomposition products are accumulated by food-chain plants, and 3) the extent to which plant-accumulated contaminants are metabolized and degraded.

1.1 REVIEW OF RELATED LITERATURE

Several studies have been conducted on munitions-based chemicals. Most of these studies deal with the development of analytical methods and subsequent characterization of parent compounds and decomposition products associated with waste streams, impoundments, and/or releases from production sites. Far fewer deal with the environmental persistence, bioavailability, and metabolic detoxification of these materiels. Furthermore, even fewer address chemical fate and behavior at environmental concentrations within the solubility constraints of the munitions components.

1.1.1 Chemistry and Analytic Methods

Effluent waste streams associated with TNT production have been carefully characterized and shown to contain over 30 isomeric nitroaromatics and associated by-products (Spanggord et al. 1982). These ranged in concentration from 0.001 to 48 mg/L of ether-extracted effluent. Substantially less work has been performed with respect to RDX, HMX, and tetryl decomposition products. McCormick et al. (1981) have determined that RDX is subject to limited biodegradation with the resulting production of dimethylhydrazines and other compounds. No evidence of significant ring opening is available for TNT or the cyclonites, and the fate of the explosive tetryl in the environment is unknown.

A number of gas chromatrography (GC), gas chromatography/mass spectroscopy (GC/MS), and high-performance liquid chromatography (HPLC) methods permit identification and quantification of parent compounds, by-products, and decomposition products (Spanggord et al. 1980, 1982; Belkin et al. 1985; Jenkins et al. 1986; Bauer et al. 1986). Differing methodologies are often required to establish both chemical and biologically based transformations. Gas-chromatographic analysis of water extracts for tetryl with electron-capture detection can give detection limits ranging from 1000 to 20 parts per trillion (Belkin et al. 1985; Hoffsommer and Rosen 1972). However, in studies designed to examine the thermal decomposition of tetryl at temperatures above 120°C, methyl picramide, a thermal decomposition product, was inevitably observed (Farey and Wilson 1975; Dubovitskii et al. 1961; Yusada 1970). Given the thermal lability of tetryl, use of condensed-phase separation techniques should be considered distinctly advantageous when compared to GC analysis. A number of researchers have described HPLC methods that use detection of ultraviolet (UV) absorption for the analysis of tetryl (Farey and Wilson 1975; Jenkins and Walsh 1987; Bongiovanni et al. 1984). Several of these methods have been applied to the quantitation of explosives in soil extracts (Jenkins and Grant 1987; Jenkins and Walsh 1987; Bongiovanni et al. 1984; Cataldo et al. 1989, 1990; Harvey et al. 1990, 1991)

Palazzo and Leggett (1986) were the first to address the analysis of explosive residues in plant tissues. Since their initial report, only those methods described by Cataldo and co-workers (Cataldo et al. 1989, 1990; Harvey et al. 1990, 1991) have significantly advanced the analytical methodology for determining explosives residues in plant tissues.

1.1.2 Soil Fate and Microbial Decomposition

Although substantial research into soil fate has been performed for nitroguanidine, relatively few data are available for TNT, RDX, and HMX. We are aware of no data relating to the relationship between soil characteristics (i.e., CEC, pH, organic matter content) and the extent of sorption/solubility. An understanding of this relationship is necessary to define limits of environmental mobility and plant availability.

Soil microbial studies, both *in vivo* and *in vitro*, indicate that these munitions compounds are subject to metabolic modification. Kearney et al. (1983), using ^{14}C-TNT, pre-UV irradiated and amended to soil, found respiratory losses of $^{14}CO_2$ to increase with time. The addition of microorganisms that were able to metabolize nitroaromatics resulted in a sharp increase in respiratory losses. Yang et al. (1983), using soil-isolated organisms, demonstrated a 90% reduction in extractable RDX over 3 days, with indications that the organisms were able to use the nitro groups as an alternate N source. Elsewhere, RDX has been shown to be degraded with a half-life of 7 days in a water/sediment system, but only after a lag period of several weeks (Sikka et al. 1980). The latter results indicated that ring opening occurred. *In vitro* anaerobic transformations have been reported for RDX and HMX and their acylated by-products 1-acetyloctahydro-3,5,7-trinitro-1,3,5,7-tetrazocine and 1-acetylhexahydro-3,5-dinitro-1,3,5-triazine (Spanggord et al. 1982, 1983), but rates of decomposition were substantially lower than in sediment systems. The transformations that predominated were nitroreduction and ring opening with further reduction to methanol, formaldehyde, and hydrazines. Although few soil studies have been performed, indications are that both anaerobic and aerobic biotransformations may occur in soils.

Recent studies (Cataldo et al. 1989, 1990; Harvey et al. 1990, 1991) have evaluated the behavior and chemical fate of TNT and RDX in soils. Extensive transformation of TNT to 2-amino-4,6-dinitrotoluene and 4-amino-2,6-dinitrotoluene was found to occur rapidly within three different soils. In addition, a unique volatile transformation product was isolated from one of the soils and partially characterized. This transformation product was most likely a transient intermediate in the derivation of further non-volatile transformation products. The amount of irreversibly bound TNT was found to increase in all soil types throughout the 2-month study, with the highest percentage of bound residue occurring in the soil with the highest organic carbon content. Volatile organics were not evolved by the TNT-amended soils; however, evolution of small amounts of $^{14}CO_2$ from the soils was verified. The mass balance of TNT in three soils was

acceptable throughout the 2-month study. In sharp contrast, no soil transformation products were observed for RDX. Over 68% of the initially amended RDX was recovered as the parent munition in extracts performed 60 days after amendment. Also, in contrast with TNT, only a small proportion (<2.1%) of the RDX-derived radiolabel was irreversibly bound to the soils at the end of the 2-month study.

1.1.3 Plant Uptake and Metabolism

The literature contains very few data related to soil/plant fate and bioavailability of TNT, RDX, HMX, and tetryl. In the 1970s, a substantial amount of research was performed on aquatic organisms, including algae and water plants. In those studies, TNT was found to be toxic to duckweed at levels in excess of 1 µg/g (Schott and Worthley 1974), to inhibit growth of freshwater algae at 2 to 15 µg/g (Smock et al. 1976), and to inhibit the growth and metabolism of microorganisms generally (Klausmeier et al. 1973; Nay et al. 1974). With the exception of the studies by Smock et al. (1976) and Schott and Worthley (1974), no chemical analysis was performed on either culture solutions or materials accumulated.

One study of higher plants was performed using hydroponically grown yellow nutsedge to assess the uptake, toxicity, and metabolic transformations of TNT (Palazzo and Leggett 1986). This study showed shoot and particularly root growth to be inhibited at 5 µg/g. Although this point was not noted by the authors, these toxicity symptoms are characteristic of dinitroaniline herbicide damage, which might be expected based on the chemical structures of TNT and the aminodinitrotoluenes. Chemical analysis showed >90% of all tissue-extractable material to be present as 2-amino-4,6-dinitrotoluene and 4-amino-4,6-dinitrotoluene T, with only a small amount of TNT being recovered. Because these species were not observed in the nutrient solutions, it is assumed that they are metabolic detoxification products.

Detailed plant fate studies related to TNT have recently been conducted (Cataldo et al. 1989, 1990; Harvey et al. 1990, 1991). In the plants, TNT-derived ^{14}C was found to be localized primarily in the roots, with a small amount of radiolabel being transported to the shoot and leaves. Studies examining the xylem exudate of bush beans grown in TNT-containing hydroponic solutions allowed identification of the primary transport forms of TNT. Acid-hydrolyzed xylem exudate was found to contain 2-amino-4,6-dinitrotoluene and 4-amino-2,6-dinitrotoluene, as well as an unidentified TNT metabolite. As these polar conjugates, TNT is transported to the aerial portions of the plant, where it undergoes further metabolic alteration. Studies indicated that the

polar TNT metabolites are sequestered within the plant and are not transpired as volatile organics or $^{14}CO_2$. Chemical fractionation of plant tissues grown in TNT-containing hydroponic solution indicated that less than 12% of the incorporated radiolabel was due to TNT, 2-amino-4,6-dinitrotoluene, or 4-amino-2,6-dinitrotoluene. The majority of the radiolabel was found in either a polar ether-extractable fraction or a more polar non-ether-extractable fraction. The preponderance of these previously unknown but highly polar TNT transformation products was a major finding of these studies. The explosive RDX, on the other hand, was found to be bioaccumulated in the aerial tissues of the plants studied. The levels of RDX in the shoot tissues from mature plants grown in soils containing 10 µg/g RDX reached concentrations as high as 550 µg/g.

A search of the literature failed to reveal any pertinent data on the fate of tetryl in plants. The absence of data addressing the plant metabolism of tetryl is due largely to the lack of an appropriate analytical methodology to analyze for this explosive in plant tissues.

1.1.4 Toxicity of Tetryl

In reports as early as the 1940s, it was shown that workers in munitions production facilities suffered a high incidence of tetryl-induced dermatitis (Whitowski et al. 1942; Schwartz 1944). As more stringent hygiene precautions were implemented, the incidence of dermatitis among workers diminished. Immunological sensitization to tetryl has also been observed in experimental animals. These observations may be related to the ability of tetryl to form covalent adducts with proteins (Brownlie and Cumming 1946) and act as a direct mutagen, as shown in several microbial assay systems (Whong et al. 1980).

1.2 STUDY OBJECTIVES

The objectives of the studies described in this report were to develop the necessary analytical methodology and to assess the chemical fate and behavior of tetryl in soil and plant systems. Particular emphasis was placed on elucidating 1) the chemical transformations that occur in soils and 2) the extent of plant uptake and further metabolism of tetryl.

Specific research objectives include

- understanding the relationship between soil characteristics [pH, organic matter (OM), cation exchange capacity (CEC), mineralogy] that control soil sorption/solubility and plant availability;
- comparative rates of soil decomposition, particularly by microorganisms colonizing the near-surface rhizosphere;
- characterization of principal transformation products produced in the rhizosphere, and their respective bioavailabilities and fates;
- evaluation of the rates of plant uptake, the distribution in edible tissues, and the chemical fate and persistence of accumulated xenobiotics.

1.3 TECHNICAL APPROACH

The major route of entry to the environment for nitro-substituted munitions materiels such as TNT, RDX, HMX, and tetryl is through discharges from production facilities and decommissioning activities. Based on the limited data available, it is certain that these parent materials and many of their by-products are mobile in the environment. However, few systematic data are available with regard to important terrestrial processes that influence mobility, bioavailability, and, more important, chemical fate.

Ongoing research for U.S. Army Medical Research and Development Command with TNT (Cataldo et al. 1989), RDX (Cataldo et al. 1990), and other organic xenobiotics has clearly shown the need to understand the critical aspects controlling the behavior and chemical fate of environmental contaminants. This study will address the environmental fate and behavior of tetryl in soils and plants. In this study the methods and approaches developed for TNT and RDX are applied to understanding the behavior of tetryl. These methods and approaches are essential to developing an understanding of the fate and potential effects of tetryl in the environment.

Although the analytical methodology for the determination of tetryl in process streams, soils, and natural waters is established, present methods are likely to be inadequate for soil/plant systems. Thus, as with TNT and RDX, methods must be adapted or developed to address the question of metabolites. Use of radiotraced tetryl will permit mass balances to be performed.

2.0 MATERIALS AND METHODS

2.1 PURITY AND ANALYSIS OF TETRYL SOURCES

2.1.1 Tetryl Chemical Characterization

A Standard Analytical Reference Material (SARM) sample of tetryl, obtained from the U.S. Army Toxic and Hazardous Materials Agency (Aberdeen Proving Ground, Maryland), was used to verify the identity of both the bulk and radiolabeled tetryl used in this project. Initial efforts attempted to verify product identity and purity by GC with either Fourier transform infrared spectroscopy (FTIR) or mass spectroscopy (MS) detection. Results from these studies were perplexing, because the gas-phase FTIR spectrum of tetryl eluting from the GC column contained a distinctive N-H stretch in the neighborhood of 3400 cm^{-1} (Figure 2.1). Given that the accepted chemical structure of tetryl does not contain a N-H bond, it was assumed that tetryl underwent thermal degradation during chromatography to form a compound that did contain a N-H bond. To further investigate this phenomenon, a tetryl standard was analyzed by GC/MS. The GC/MS studies were conducted with a Hewlett-Packard 5890 gas chromatograph (Palo Alto, California) equipped with an on column injector. Separations were effected by programming the DB-5 (20 m x 250 μm ID, D_f = 0.25 μm) column from 110 to 300°C at a rate of 10°C/min. Components were detected by a Hewlett-Packard 5970 mass selective detector operated in the scanning mode. The total ion current chromatogram for this analysis is presented in Figure 2.2. As can be seen, the chromatogram contains one peak with a retention time of 13.25 min. The mass spectrum of this peak is shown above the total ion chromatogram (Figure 2.2, top). The small peak with a retention time of 17.41 min was a phthalate contaminant that was unrelated to the tetryl standard. Comparison of the spectrum in Figure 2.2 with the standard reference direct-insertion probe spectrum of tetryl (Eight Peak Index, 1983) made it immediately obvious that the compound eluting from the GC column was not tetryl. From the results obtained by FTIR and MS detection, it is hypothesized that tetryl decomposes during GC to a compound having a molecular weight of 242. This change probably involves the substitution of a hydrogen for the nitro substituent attached to the aniline nitrogen, as summarized in Figure 2.3. Such degradation would account for both the mass spectrum and the N-H stretch observed in the FTIR spectrum.

These initial studies caused concern about the stability of the tetryl standard. Because subsequent studies would depend on starting with an uncompromised standard reference material, considerable effort was spent verifying that the tetryl standard had the correct structure.

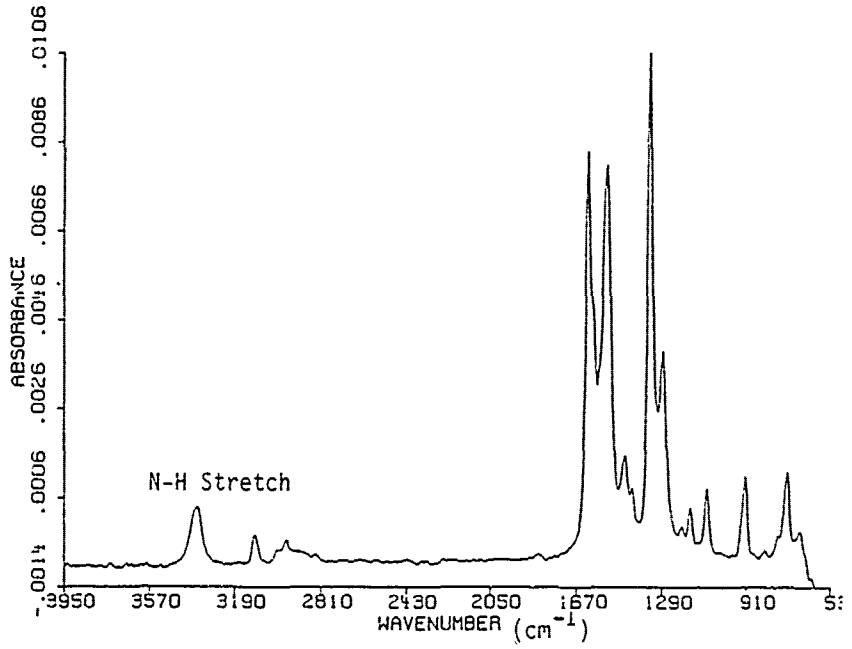

FIGURE 2.1. Fourier Transform Infrared Spectroscopy Spectrum of Tetryl Eluting from a Capillary GC Column

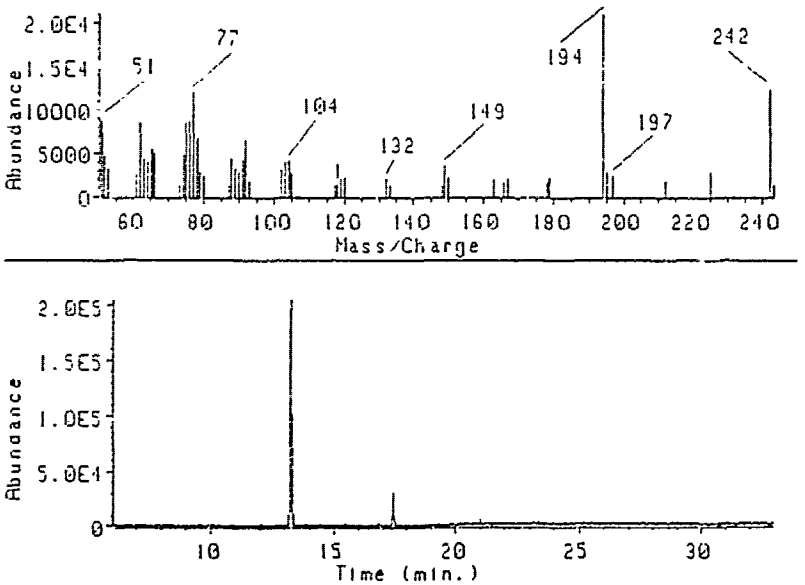

FIGURE 2.2. Total Ion Current Chromatogram (bottom) and Mass Spectrum (top) of a Tetryl Standard

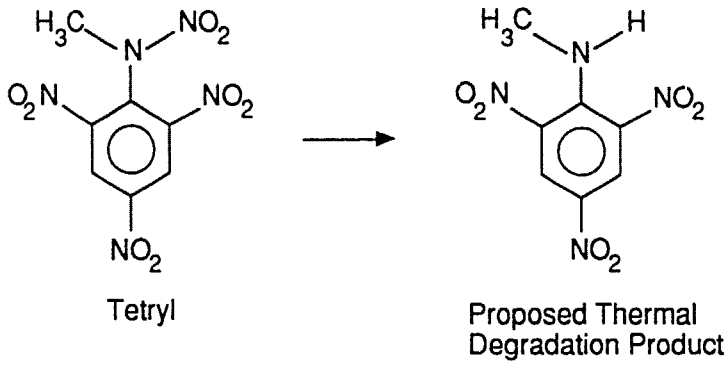

FIGURE 2.3. Proposed Thermal Degradation of Tetryl During Gas-Chromatographic Analysis

A mass spectrum of the tetryl standard was obtained by direct insertion probe analysis with a Hewlett-Packard Model 5985 mass spectrometer. The resulting mass spectrum, shown in the top of Figure 2.4, matches the Eight Peak Index reference spectrum of tetryl. The spectrum shows both the molecular ion (m/e of 287) and the base fragment (m/e of 241) resulting from the loss of NO_2 from the aniline nitrogen. Furthermore, a chemical ionization mass spectrum of tetryl was obtained with isobutane reagent gas. This spectrum is presented in the bottom of Figure 2.4. The protonated molecular ion at 288 a.m.u. and the base fragment at 243 a.m.u. are the principal features of this spectrum. Additional studies (of both ^{1}H and ^{15}N) conducted with a 300-MHz FT-NMR served to further verify that the structure of our tetryl standard indeed corresponded to the accepted structure of tetryl.

2.1.2 Chemical Analysis of Tetryl by High Performance Liquid Chromatography

In view of the obvious thermal lability of tetryl, as indicated by the GC studies described above, an analytical procedure based on HPLC was mandated. A UV spectrum of tetryl in acetonitrile was obtained (Figure 2.5) to determine the most sensitive detection wavelength for this explosive. Maximal absorption for tetryl occurred at 264 nm. This wavelength was utilized for detection of tetryl in all subsequent chromatographic separations. The ideal gradient system for tetryl should allow for reasonable retention windows both before and after tetryl in order to observe the full range of possible polar and hydrophobic transformation products. A primary concern was providing a gradient shallow enough to allow resolution of transformation products more polar than tetryl, since these types of products predominated in previous studies of the structurally related 2,4,6-trinitrotoluene (TNT). The HPLC system consisted of a Waters Model 600E system controller and pump and a Waters 490E detector (Waters Associates, Milford,

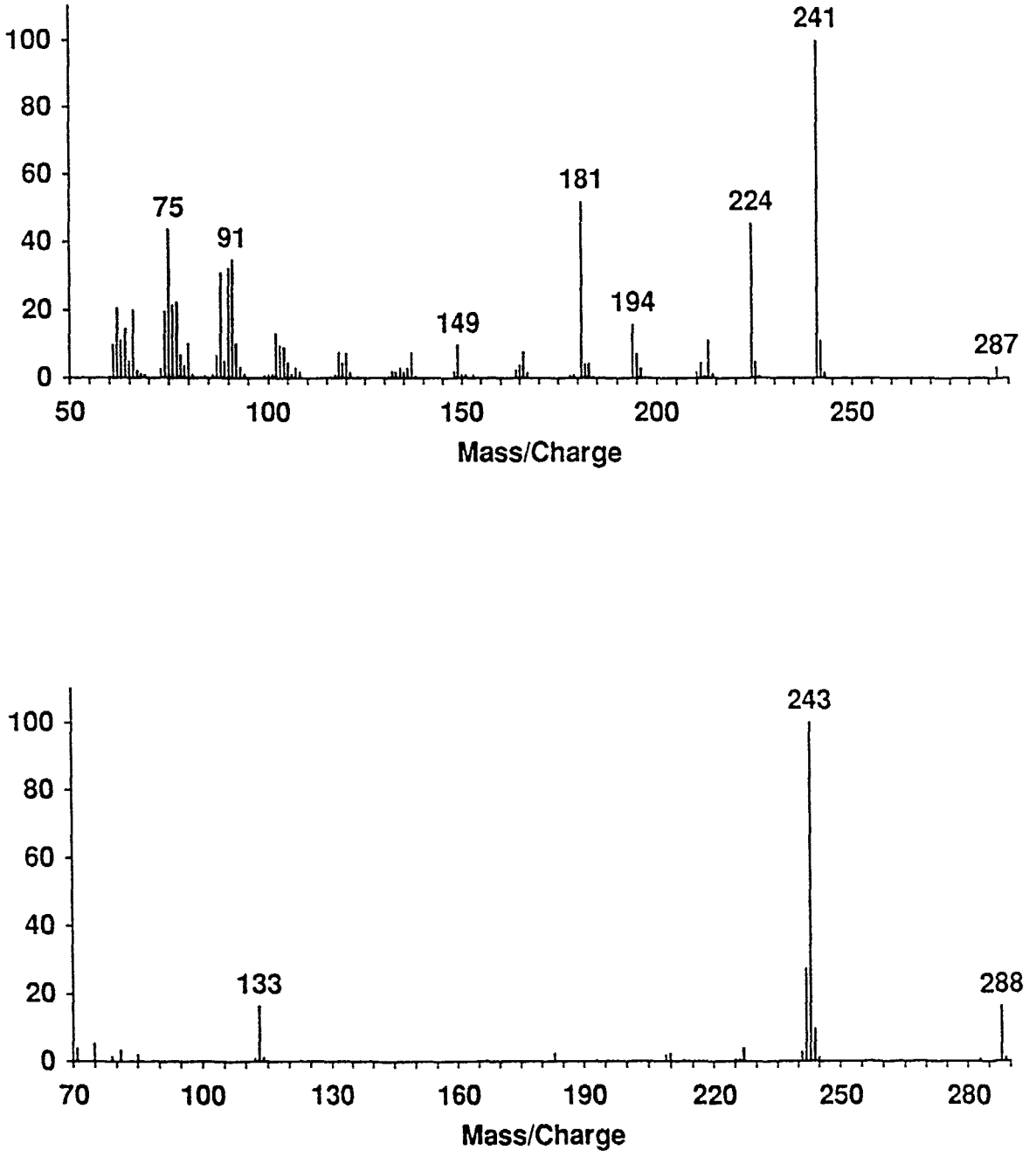

FIGURE 2.4. Direct-Insertion Probe Electron Impact (top) and Isobutane Chemical Ionization (bottom) Mass Spectra of Tetryl

Massachusetts). The detector was operated at 264 nm with a sensitivity of 0.008 AUFS. Samples (20 µL) were injected by a Waters WISP 710A automatic injector onto an Ultrasphere 5-µm ODS (25 cm x 4.6 mm ID) column (Beckman Instruments, San Ramon, California). The column was developed at a flow rate of 1.0 mL/min by a linear gradient from 35 to 100% acetonitrile in 35 min, with a 10-min hold at the final mobile phase composition. The retention time of tetryl under these chromatographic conditions was 16.2 min. Integrated peak areas, provided by a Hewlett-Packard 3390A integrator, formed the basis of quantification. The purity of bulk tetryl determined by HPLC analysis was in excess of 97%.

Analysis of tetryl standards dissolved in methanol and ranging in concentrations from 1.22 to 61.17 µg/g allowed construction of a standard curve. A representative standard curve is presented in Figure 2.6. From the peak height of the 1.22-µg/g standard (Figure 2.7), it is apparent that the chromatographic method has an approximate detection limit of 0.1 µg/g.

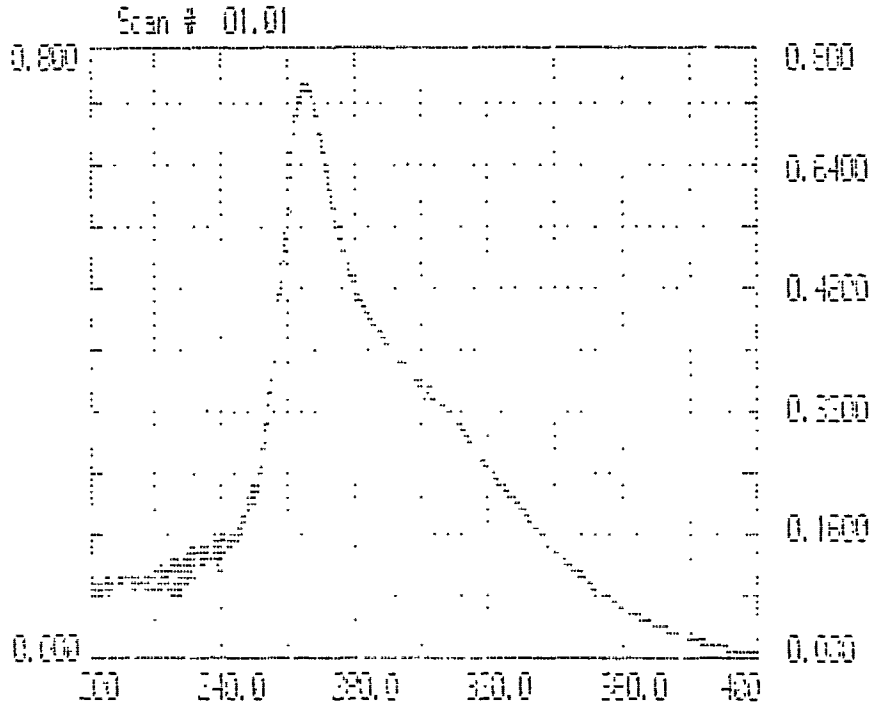

FIGURE 2.5. Ultraviolet Spectrum of Tetryl in Acetonitrile

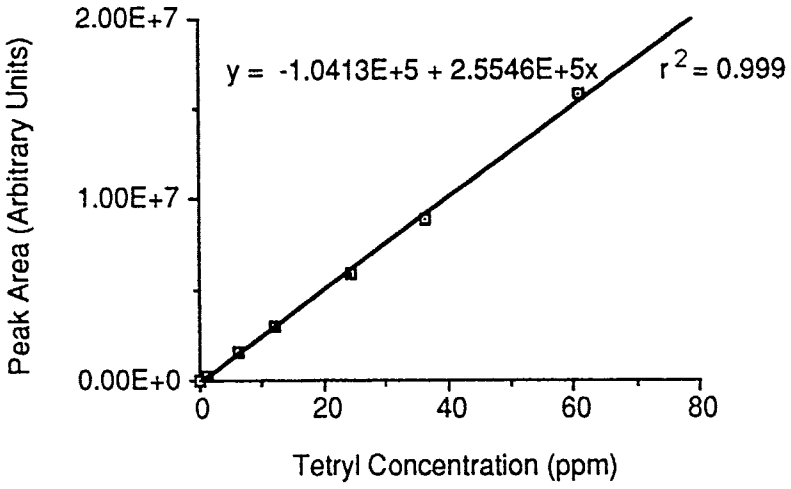

FIGURE 2.6. Standard Curve for Tetryl

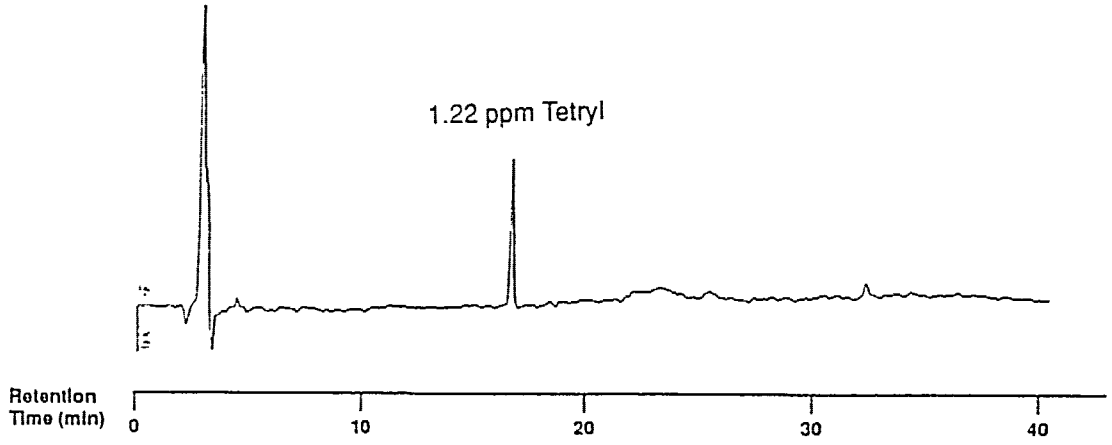

FIGURE 2.7. HPLC Chromatogram of a 1.22-µg/g Tetryl Standard

To verify that the bulk tetryl was the same material as the SARM, equal quantities of each sample were examined for co-elution under high-resolution HPLC conditions. For this experiment, a microcolumn HPLC system was utilized. Microcolumn HPLC has the advantage over conventional HPLC of providing 5 to 10 times higher resolution. The fused silica column used for this work was 1 m x 250 µm ID and slurry-packed with 5-µm ODS. As indicated by anaiysis of test solutes, the microcolumn system produced efficiencies of over 75,000 theoretical plates. Figure 2.8 illustrates the co-elution of the SARM with the bulk tetryl. Because co-elution of different compounds under such high-resolution conditions would be very unlikely, this test serves to demonstrate that the bulk material has the same chemical composition as the SARM.

The stability of tetryl in various organic solvents is important for the present studies. Although acetone has been recommended as a good solvent for tetryl (Meyer 1987). However, we were advised against the use of acetone because dissolution of tetryl in this solvent results in a pink reaction product (S. Summer, personal communication).

To investigate the stability of tetryl in methanol, a flask contaning a concentrated stock solution of tetryl was wrapped in foil and stored in a freezer (-20°C). After 1 month, a dilution was made from the stock solution and chromatographically compared to a tetryl standard that had been stored at room temperature for the same length of time and exposed to the laboratory lights. The results showed that, relative to a freshly prepared standard, 16% of the tetryl present in the light-exposed room-temperature sample had degraded. After 1 month of storage at -20°C, chromatographic analysis of a standard prepared from the concentrated tetryl stock solution did not contain tetryl degradation products and the peak area agreed well with that predicted from the standard curve.

In preparation for hydroponic plant exposures, it was necessary to verify that the water solubility of tetryl is sufficient. For this range-finding experiment, several crystals of tetryl were placed in a vial containing distilled water. The vial was subjected to periodic vortex mixing for 36 h, at which time the supernatant was filtered through a 0.2-µm Nylon-66 filter. The HPLC analysis of the supernatant indicated a tetryl concentration of 11.76 µg/g. Since our previous studies of munition compounds utilized hydroponic exposure solutions containing 10 µg/g TNT or RDX, it appears that the water solubility of tetryl is sufficient to permit the exposure of plants by hydroponic techniques.

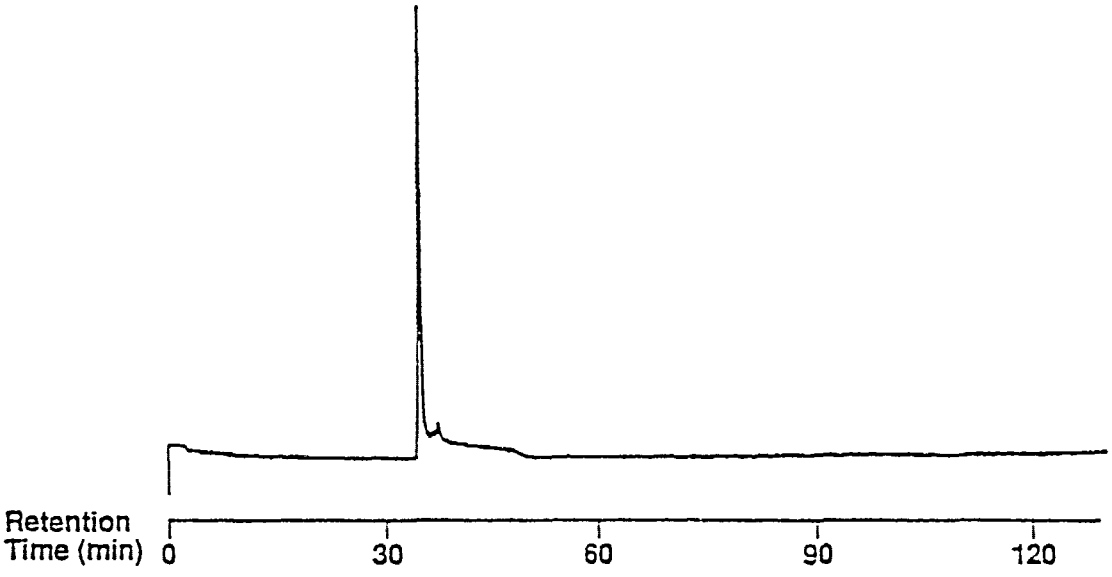

FIGURE 2.8. Microcolumn HPLC Chromatogram Resulting from a Co-Injection of Equal Amounts of SARM and Bulk Tetryl

2.1.3 ^{14}C-Tetryl Radiolabel Purity

Uniformly ring-labeled ^{14}C-tetryl was obtained from New England Nuclear (E.I. du Pont de Nemours & Co., Boston, Massachusetts). The radiolabel was prepared as a slurry in methanol and was shipped on dry ice. Immediately upon receipt, a sufficient quantity of methanol was added to completely dissolve the radiolabel and the purity of the tetryl in the resulting solution was determined by radiochromatography. The resulting radiochromatogram, shown in Figure 2.9, indicates a purity of 98.70%, which was judged adequate for the planned fate studies. Co-elution of the radiolabel with the SARM, under conventional HPLC conditions, served to verify the chemical integrity of the ^{14}C-tetryl. It was determined that a total of 4.83 mCi [containing 92.64 mg of radiolabeled tetryl (specific activity of 14.64 mCi/mmol)] was available for the tetryl fate studies.

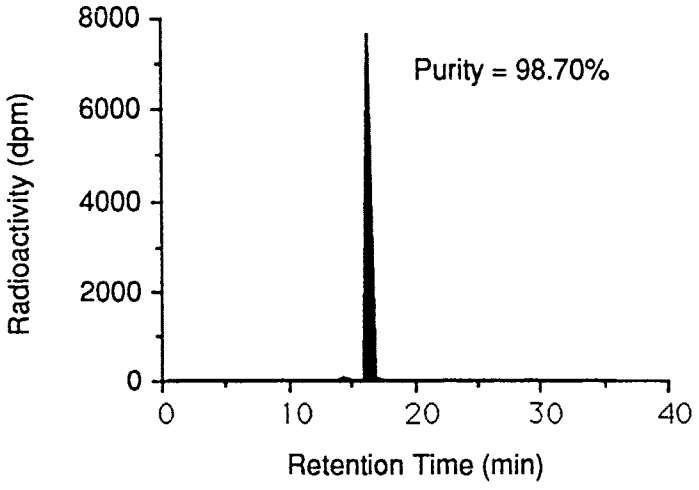

FIGURE 2.9. Radiochromatogram of ^{14}C-Tetryl

2.2 SOIL CHARACTERIZATION AND SAMPLING

The chemical and physical characteristics of soils employed in these studies are tabulated in Table 2.1. The Burbank soil is a sandy loam (sandy, skeletal, mixed xeric Torriorthent) and is representative of the desert areas of Washington, Oregon, and Idaho. The sample was collected on the Hanford Site in southeastern Washington, and consisted of the Ap horizon. Cinebar soil is a clay loam (typic, dystrandepps medial, mesic soil). The Cinebar soil is a Washington forest soil from the Cascade Mountain Range. The sample was collected near Yale, Washington, and consisted of the Ap horizon. Palouse soil represents a typical Washington state agricultural soil. The Palouse soil is a silt loam, mixed mesic Pachic Ultic Haploxeroll. The sample was collected at Pullman, Washington, and consisted of the Ap horizon.

For soil experiments, a solution containing appropriate proportions of labeled and unlabeled tetryl was prepared in 2.0 mL of methanol and amended with quantities of air-dried soil corresponding to oven-dry weights of 400 g, to give final concentrations of 60 µg/g tetryl containing 10 or 20 µCi of labeled tetryl. Soils were brought to 66% of field capacity with water immediately before amendment and they were maintained at this moisture level throughout the experiment. Initial sampling was performed to ensure both mixing efficiency and activity levels. Soils were maintained in a growth chamber that simulated the luminous intensity and spectral dispersion of sunlight (500 µE m^{-2} sec^{-1}) during the 16/8-h daily light/dark cycle, at day/night temperatures of 26/22°C, and 50% relative humidity.

TABLE 2.1. Selected Properties of Test Soils

Soil Property	Burbank Sandy Loam	Palouse Silt Loam	Cinebar Clay Loam
% sand	45.1	1.1	35.2
% silt	51.4	77.5	51.4
% clay	4.0	21.4	13.4
% ash	98.0	93.8	nd[a]
pH (100% field capacity)	7.4	5.4	5.6
Organic carbon (%)	0.5	1.7	7.2
Sulfur (%)	0.053	0.043	nd
Nitrogen (%)	0.061	0.16	0.44
Total P (µg/g)	2400	3770	3400
Phosphate-P (µg/g)	4.8	5.8	26
Carbonate/bicarbonate (%)	<0.1	<0.1	<0.1
Ammonium-N (µg/g)	6.1	18.3	15.0
CEC (meq/100 g)	5.5	23.8	38.2

[a]nd = not determined

2.3 PLANT CULTIVATION AND SAMPLING

The chemical fate of tetryl in plants was evaluated using bush beans (*Phaseolus vulgaris*), wheat (*Triticum aestivum*), and blando brome (*Bromus mollis*). All plants used for either hydroponic studies or soil studies were grown from seed. All plants were maintained in controlled-environment chambers with a 16/8-h light/dark cycle [500 µE m^{-2} sec^{-1}, photosynthetically active radiation (PAR), at leaf surface], at day/night temperatures of 26/22°C, and 50% relative humidity.

2.3.1 Hydroponic Studies

Plants were grown for 18 to 26 days on hydroponic nutrient solutions as described previously (Cataldo et al. 1978). After 18 to 26 days, solutions were amended with 1 to 25 µg/g tetryl, containing 5 µCi of radiolabeled tetryl per 500 mL. These solutions were filter sterilized and placed in autoclaved 500-mL beakers to minimize bacterial contamination, which could promote transformation of tetryl. Plants were placed in these solutions and maintained in a growth chamber until harvested. The beakers were jacketed in an opaque sheath to protect the roots

from light, as well as to minimize the photolysis of tetryl. Solutions were analyzed by HPLC and liquid scintillation spectrometry at intervals. At harvest, plants were removed from the hydroponic solutions and the roots rinsed with 0.1 M $CaCl_2$ followed by a rinse in 80:20 methanol:water. Plants were then separated into component tissues (roots, stems, leaves); the component tissues were minced, thoroughly mixed, analyzed for radiocarbon, and stored at -80°C until chemical analysis could be performed.

2.3.2 Soil/Plant Studies

Soil studies were conducted with both unlabeled and radiolabeled tetryl. Soils were uniformly mixed to the concentration noted, and plants grown to maturity. Shoot tissues were harvested, and roots washed free of adhering soil. All tissues were assayed for radioactivity and analyzed for tetryl and residues.

2.4 CHEMICAL/ANALYTICAL PROCEDURES

2.4.1 Radioanalyses

Soils and plant tissues were oxidized by total combustion in a Packard Model 306 oxidizer (Packard Instrument Co., Downers Grove, Illinois) to determine the amount of radiocarbon associated with each sample. Combusted samples and liquid samples were counted using a Beckman 9800 liquid scintillation spectrometer (Beckman Instruments, Downers Grove, Illinois) with appropriate quench correction.

2.4.2 Residue Analysis

Soil and plant-tissue extracts were analyzed by reversed-phase HPLC with detection at 264 nm, as described above (Section 2.1.2). To facilitate future research on the environmental fate of tetryl, the bracketed retention indices of tetryl soil transformation products and plant metabolic products encountered during this work are reported. Alkylphenone retention indices are independent of temperature, flow rate, and column length. Provided that a similar solvent program utilizing the same mobile phase constituents (acetonitrile/water) and the same general type of column (octadecyl silica) is used, the retention indexes of a component determined by independent laboratories should be identical. Therefore, a compound's retention index is far more useful than its retention time (Smith 1982; Hill et al. 1984).

To determine retention indices, compounds of interest are bracketed between members of a homologous series of alkylphenones during chromatographic co-injection studies. Each alkylphenone retention marker is assigned a retention index, corresponding to the number of carbon atoms in the molecule multiplied by 100. For example, acetophenone contains 8 carbon atoms and is assigned a retention index of 800. A compound eluting between two alkylphenones is given an intermediate retention index that is proportional to those of the bracketing alkylphenones. For example, a compound eluting midway between acetophenone and propiophenone (retention index of 900) would be assigned a retention index of 850 (Smith 1982; Hill et al. 1984).

2.4.3 Soil Extraction

Soil extraction was based on exhaustive Soxhlet extraction as previously described for TNT and RDX (Cataldo et al. 1989, 1990; Harvey et al. 1990, 1991). At predetermined times after amendment, 10-g soil samples were removed from the pots of amended soil and subjected to exhaustive Soxhlet extraction with 200-mL methanol for 48 h. Methanol was chosen over acetonitrile as an extraction solvent because of its lower boiling point and corresponding lower probability of inducing thermal degradation of the explosive during the 48-h reflux period. The Soxhlet apparatus was covered with foil during extraction to minimize photodecomposition of tetryl. After extraction was complete, residual methanol was removed from the extracted soils by vacuum, and an accurate dry weight of soil obtained. The methanol extract was filtered through a 0.45-µm Nylon-66 filter before the volume was reduced to approximately 20 mL by rotary evaporation. The concentrated extract was again filtered through a 0.45-µm Nylon-66 filter and the final volume brought to 25.0 mL. This solution was analyzed by HPLC and, in experiments that utilized ^{14}C-tetryl, by liquid scintillation spectrometry.

During preliminary studies, it was noted that refluxing tetryl in methanol caused the clear solution to acquire a greenish hue. This discoloration may indicate tetryl decomposition. An experiment was conducted to determine the recovery of tetryl from a 15-µg/g methanol solution after refluxing in a Soxhlet extraction apparatus for 48 h. Concentrations of tetryl before and after reflux were determined by HPLC. Comparisons between pre- and post-reflux tetryl concentrations allowed for calculation of tetryl recovery. It was found that the recovery of tetryl in refluxed solutions was 82.46 ± 1.68%. A radiochromatogram of the refluxed tetryl solution is shown in Figure 2.10. The radiocarbon profile shows that 18% of the radiolabel is contained in polar decomposition products (indicated with an asterisk in Figure 2.10) that elute with the column dead volume. The results of this experiment were surprising in light of previous work that addressed the thermal stability of tetryl (Yinon 1990). These prior studies indicated that tetryl was

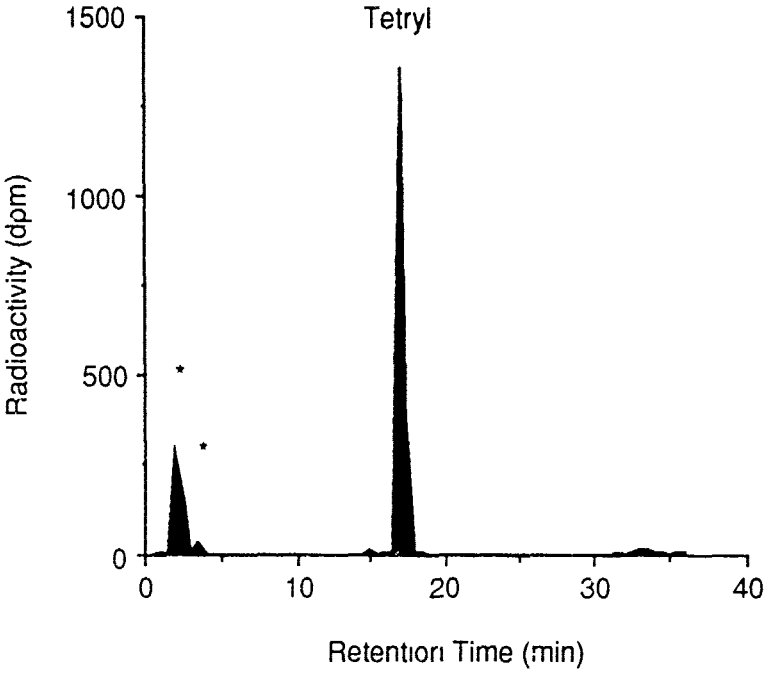

FIGURE 2.10. Radiochromatogram of Refluxed Tetryl Solution

generally stable and not prone to thermal degradation at temperatures below 120°C. Although the Soxhlet procedure induced some tetryl decomposition, the utility of this technique was evident because tetryl transformation products could be determined, provided that elution is not coincident with the column dead volume.

2.4.4 Tissue Extraction and Fractionation

An extraction scheme previously developed for RDX was evaluated for its suitability for the analysis of tetryl in plant tissues (Cataldo et al. 1990; Harvey et al. 1991). The plant extraction procedure for RDX is shown in Figure 2.11. When the RDX procedure was utilized for fractionation of tetryl-spiked leaf tissues, reasonable distributions of radiolabel among the various fractions were obtained; however, chromatographic analysis revealed that tetryl had undergone decomposition to several products, resulting in poor chromatographic recovery. Several steps in the procedure were suspected of contributing to tetryl decomposition. These steps included 1) acid hydrolysis, 2) partitioning between ammonium hydroxide and diethyl ether, and 3) chromatography on Florisil adsorbent. Development of an appropriate analytical fractionation procedure for tetryl involved modifications to eliminate the problem steps.

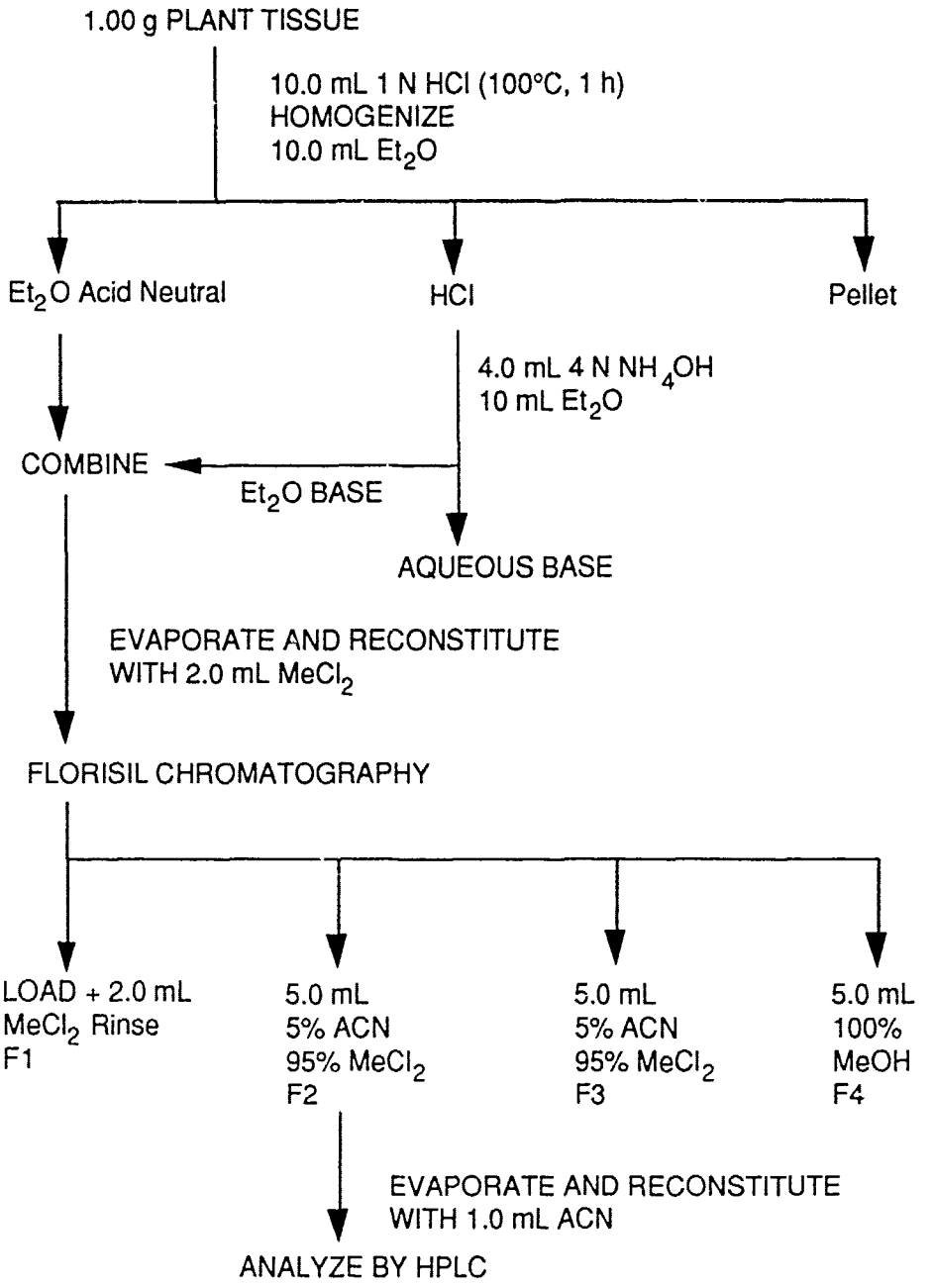

FIGURE 2.11. Flow Chart for the Analysis of RDX in Plant Tissues

Acid hydrolysis involved homogenizing the tissue in 1 M HCl, then heating the mixture at 100°C for 1 h. This step is necessary to cleave the polar conjugates resulting from tetryl metabolism. It was suspected that this harsh treatment may have caused tetryl decomposition, not because of the acidic conditions, but rather because of the accelerated photodecomposition of tetryl at elevated temperatures. This concern was readily addressed by wrapping the Corex tube that contained the acidic homogenate in aluminum foil during the acid hydrolysis process. After hydrolysis, the tube was cooled in an ice bath to bring it to room temperature before it was exposed to laboratory illumination. Extractions of tetryl-spiked solutions that were hydrolyzed in the dark gave no indication of decomposition.

Triplicate spike experiments continued to show both poor recovery and poor reproducibility of tetryl from plant tissues hydrolyzed in the dark and fractionated according to the procedure shown in Figure 2.11. The data indicated that problems remained at other steps of the procedure. The next concern addressed was the instability of tetryl under alkaline conditions. When the HCl layer was made alkaline and extracted with diethyl ether, some ammonia may have remained dissolved in the water-saturated ether layer. In addition, it was difficult to remove the ether layer in the presence of plant material without transferring a quantity of the aqueous base solution, however minuscule In the fractionation step that followed, the ether layers were pooled and evaporated to dryness. Any ammonia present during evaporation could cause decomposition of tetryl. Another concern was the use of Florisil adsorbent, which contains 16% magnesium oxide. Adsorption chromatography on this strongly alkaline adsorbent could also cause decomposition of tetryl.

The first of these concerns to be experimentally evaluated was the Florisil chromatography. Initial experiments performed in the absence of plant material did not indicate decomposition of tetryl on Florisil. However, in the presence of plant extracts, tetryl inevitably displayed a degree of decomposition that was attributable to the Florisil adsorbent. For this reason, silica was substituted for Florisil as the chromatographic adsorbent. Studies indicated that tetryl is not strongly adsorbed by silica and that elution could be accomplished with a reasonable volume (7.0 mL) of methylene chloride. The intense yellow coloration of this fraction indicated that the plant carotene pigments also eluted with the tetryl in fraction F1. Fraction F2 was eluted with 5.0 mL of methylene chloride: acetonitrile (95:5). This fraction contained the majority of the plant pigments. The last fraction was eluted from the silica Sep-Pak with 5.0 mL of methanol. Although fraction F1 contained carotene pigments, these components did not interfere in the chromatographic determination of tetryl, as will be further discussed below.

At this point, several triplicate spike experiments were conducted to evaluate the effect of altered hydrolysis conditions and silica chromatography on the recovery of tetryl. Bush bean leaf tissue was spiked with 4.58 µg/g tetryl containing 63,953 dpm and fractionated by the modified procedure. The distribution of radiolabel is presented in Table 2.2. The percentage recovery of radiolabel in fraction F1 was 73.85 ± 1.31, which was in reasonable agreement with the chromatographic recovery of 68.97 ± 6.26%. Although these results represent a dramatic improvement over results obtained by other methods, higher recoveries of tetryl are certainly desirable.

One final modification was made in an attempt to increase the recovery of tetryl: extractions were performed with methylene chloride rather than diethyl ether. The decision to make this substitution was based on the low solubility of water in methylene chloride, and hence, the lower probability of transferring base with the organic extract. The separation of aqueous and methylene chloride phases in the presence of plant material is also easier than extractions performed with diethyl ether. A flow chart for the final procedure for extracting tetryl from plant tissues is shown in Figure 2.12. Bush bean leaf tissues were spiked in triplicate and fractionated according to the procedure outlined in Figure 2.12. The distribution of radiolabel among the chemical fractions is summarized in Table 2.3.

TABLE 2.2. Distribution of Radiolabel among the Chemical Fractions Generated by a Modified Procedure Previously Developed for RDX

Fraction	% Total Radiolabel
HCl	4.75 ± 1.66
Et_2O acid-neutral	87.81 ± 5.47
Aqueous base	0.93 ± 0.85
Et_2O base	7.22 ± 0.54
F1	73.85 ± 1.31
F2	1.77 ± 0.63
F3	3.98 ± 0.62
Sorbent	0.15 ± 0.11
Pellet	2.51 ± 0.19

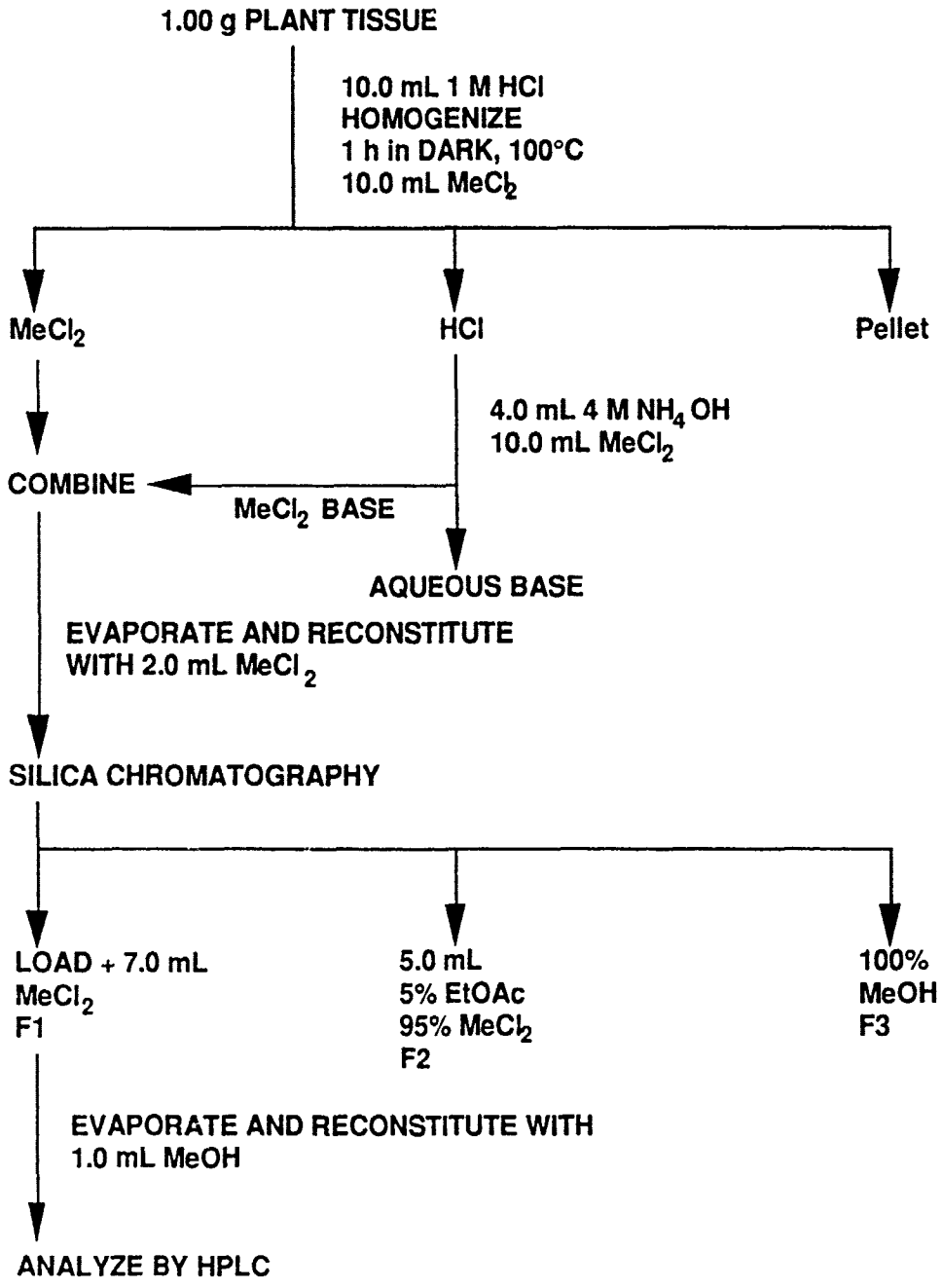

FIGURE 2.12. Flow Chart for the Analysis of Tetryl in Plant Tissues

TABLE 2.3. Distribution of Radiolabel among the Chemical Fractions Generated During the Tetryl Fractionation

Fraction	% Total Radiolabel
HCl	2.60 ± 0.14
$MeCl_2$ acid-neutral	84.33 ± 0.73
Aqueous base	0.36 ± 0.34
$MeCl_2$ base	0.04 ± 0.06
F1	80.49 ± 0.59
F2	0.66 ± 0.07
F3	3.87 ± 0.89
Sorbent	0.68 ± 0.18
Pellet	1.49 ± 0.45

The radiolabel recovery of tetryl in fraction F1 was 80.49 ± 0.59%, whereas chromatographic recovery was 82.70 ± 5.54%. Figure 2.13 illustrates chromatograms for the fraction F1 blank (top) and the F1 fraction of a bush bean leaf spiked with tetryl (bottom). Although fraction F1 contains carotene pigments, examination of the blank chromatogram indicates that these components do not interfere in the analysis of tetryl. In fact, the carotene pigments contained in fraction F1 cause very little interference at 264 nm throughout the entire chromatographic elution range. Figure 2.14 illustrates the triplicate spike chromatograms obtained from this study. The high degree of reproducibility obtainable from this procedure is evident by examination of these chromatograms.

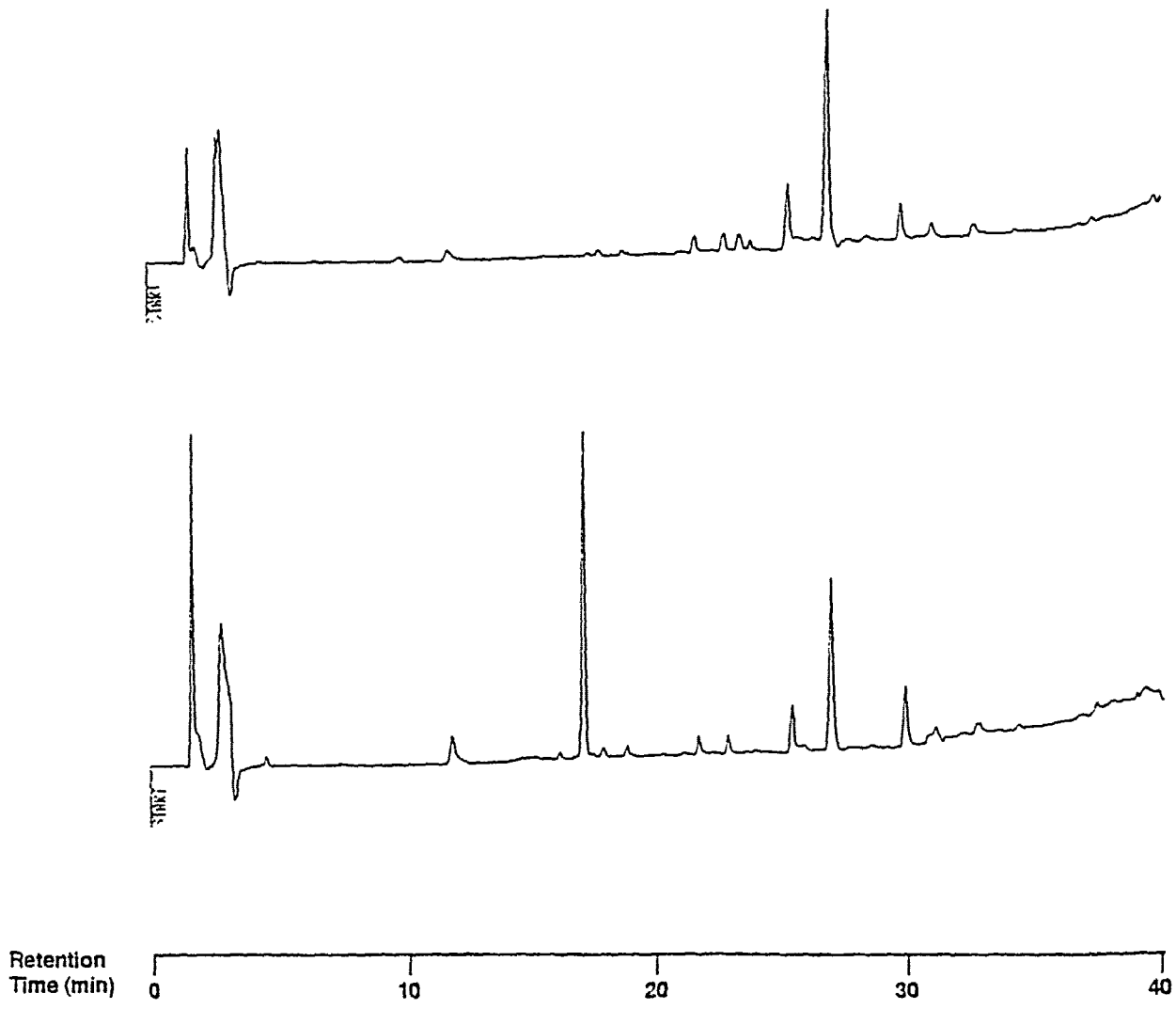

FIGURE 2.13. Chromatograms of Fraction F1 from Leaf Blank Tissue (top) and Leaf Tissue Spiked with Tetryl (bottom)

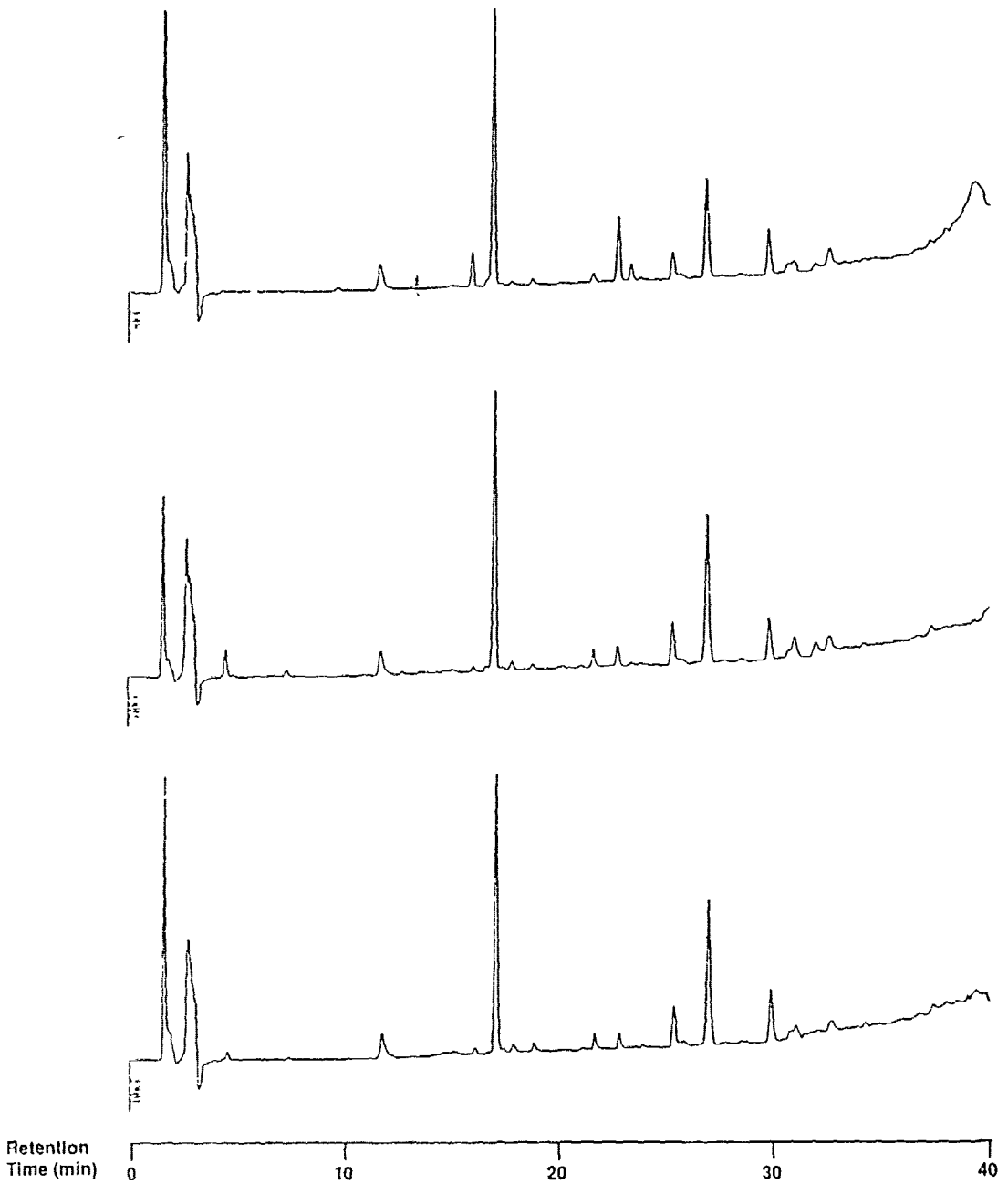

FIGURE 2.14. Chromatograms of F1 Fractions from the Triplicate Spike Experiment

3.0 RESULTS AND DISCUSSION

The purpose of this study was to provide an understanding of the environmental fate and behavior of 2,4,6-trinitrophenylmethylnitramine (tetryl). The methods and approaches employed were similar to those employed to study the environmental fates of TNT and RDX (Cataldo et al. 1989, 1990; Harvey et al. 1990, 1991). The important parameters investigated include 1) the extent of sorption in soils, 2) the chemical transformations of tetryl in soils, and 3) the relative availability of tetryl to plants and the chemical forms of tetryl-related residues in plant tissues. In support of these activities, a principal goal was the development of suitable analytical methodology to separate and characterize transformation products in soils and plant tissues.

3.1 FATE AND BEHAVIOR OF TETRYL IN SOIL

3.1.1 Extractability and Mass Balance of Tetryl in Soil

Soils were amended with 60 µg/g tetryl containing 10 µCi ^{14}C-tetryl and brought up to and maintained at 67% of field capacity with water. Once amended, the soil pots were maintained in a growth chamber. Soils were sampled immediately after amendment (0 days) and 11, 30, and 60 days after amendment. Results for the 60-day study are presented in Table 3.1. The second column is the percentage of radiolabel that was recovered in the methanol extracts, as determined by liquid scintillation spectrometry. The third column lists the amount of the parent explosive that was recovered, as determined by HPLC analysis. The amount of radiolabel that remained irreversibly bound to the soils after extraction is listed in the fourth column. These values were determined by oxidation of the extracted soils. Finally, the mass balance deficit [100 -(column 2 + column 4)] is listed in the last column. The mass balance deficit does not account for any radiolabel that may have been volatilized from the soils during the course of the study.

Several trends are immediately identifiable in Table 3.1. Initially, most of the radiolabel was extracted and present in the methanol extract. It is noteworthy that 50% or less of the radiolabel was speciated as tetryl in extracts processed immediately after amendment. This indicates that transformation of tetryl in soil was an extremely facile process. Transformations continued at a rapid rate, with less than 8% of the extracted radiolabel found speciated as tetryl in the 11-day extracts. By 30 days after amendment, soil concentrations of tetryl were below 2.5 µg/g, a value representing the approximate analytical detection limit. The amount of radiolabel that was initially irreversibly bound to the soils was commensurate with the amount of organic carbon contained in the soils. Immediately after amendment, Burbank and Palouse soils

TABLE 3.1. Mass Balance of Soils Containing 60 µg/g Tetryl

Soil/Time (days)	% Radiolabel in Methanol Extract	% Unaltered Tetryl	% Radiolabel in Soil After Extraction	% Mass Balance Deficit
Burbank				
0	95 ± 5	52 ± 2	1.8 ± 0.4	3
11	45 ± 4	4 ± 1	42.7 ± 4.3	12
30	29 ± 1	-------	54.7 ± 3.8	16
60	21 ± 2	-------	58.2 ± 5.4	21
Palouse				
0	95 ± 21	46 ± 9	2.8 ± 0.2	2
11	67 ± 5	8 ± 4	32.4 ± 0.8	1
31	45 ± 4	-------	40.2 ± 1.8	15
60	36 ± 3	-------	43.3 ± 2.3	21
Cinebar				
0	82 ± 3	43 ± 7	17.1 ± 0.4	1
11	43 ± 1	4 ± 0.2	47.9 ± 0.1	9
31	37 ± 1	-------	50.3 ± 3.1	13
60	32 ± 2	-------	52.8 ± 1.5	15

contained 1.8% and 2.8% non-extractable radiolabel, respectively. Significantly, Cinebar soil was found to immediately bind more than 17% of the radiolabel in non-extractable forms. For each soil type, sequestration of radiolabel was initially rapid but continued at a slower rate by the 30- and 60-day sampling periods. At the end of the 60-day study, the non-extractable radiolabel content of the soils ranged from 58 to 43%.

The mass balance of the soil systems was initially very good, with more than 97% of the amended radiolabel present in either the methanol extract or the extracted soils. As the study progressed, the mass balance deficit rose continuously. At the end of the study, the mass balance deficit was as high as 21% for Palouse soil. This trend was a good indication that $^{14}CO_2$ and/or radiolabeled volatile organics were being evolved from the tetryl-amended soils. Results from studies specifically designed to detect and quantitate the emission of volatile compounds from the soils are described in Section 3.1.2.

The behavior of tetryl in sterile soils was examined to see whether the facile transformations observed in nonsterile soils were due to catalytic or microbial mechanisms. Soils were exposed to 1 MRad of gamma radiation from a ^{60}Co source for sterilization purposes. This treatment was expected to eliminate all bacterial populations; however, fungal spores are

particularly difficult to obliterate by this treatment. Nonetheless, fungal populations can be expected to be severely attenuated at the very least by the radiation treatment. With this type of sterilization, functional free enzymes may be present in the sterilized soil matrix.

The mass balance of tetryl in sterile soils amended with 60 µg/g of the parent munition is shown in Table 3.2. The large mass balance deficits observed for several of the samples (Palouse soil sampled immediately after amendment, Burbank soil at 6 days after amendment, and Cinebar soil at 9 days after amendment) were most likely due to inhomogeneity in the amendment, nonrepresentative sampling, or incomplete sterility. Of particular interest, however, were the discrepancies between the percentage of radiolabel recovered in the methanol extracts and the percentage recovered as tetryl. These large discrepancies indicate the quantity of tetryl that has been transformed. The HPLC analysis of the methanol extracts revealed the presence of the same transformation products that were observed during studies of nonsterile soils.

TABLE 3.2. Mass Balance of Tetryl in Sterile Soils Containing 60 µg/g Tetryl

Soil/Time (days)	% Radiolabel in Methanol Extract	% Unaltered Tetryl	% Radiolabel in Soil After Extraction	% Mass Balance Deficit
Burbank				
0	96.3	55.5	0.8	2.9
3	96.1	47.6	5.5	-1.6
6	57.6	35.8	8.9	33.5
9	84.4	30.8	11.3	4.3
Palouse				
0	72.8	42.7	0.8	26.4
3	78.8	34.5	6.8	14.4
6	77.7	30.4	9.4	12.9
9	83.1	18.5	12.3	4.6
Cinebar				
0	93.6	62.3	1.1	5.3
3	70.1	27.5	12.6	17.3
6	86.4	15.7	14.5	-0.9
9	57.5	0.8	17.0	25.5

3.1.2 Rates of Tetryl Mineralization and Volatilization in Soils

Gas-exchange experiments using ^{14}C-tetryl-amended soils were conducted to determine the release of volatile organics and $^{14}CO_2$ from the three soil types and thus assess the mass balances observed in the soils. Soils were amended with 10 μg/g (μg/g) ^{14}C-tetryl and allowed to equilibrate for 28 days after which organic volatiles and respiratory CO_2 were trapped at daily intervals over three consecutive days and counted.

All three soil types were subdivided and samples from each group were irradiated with gamma radiation from a ^{60}Co source to produce a sterile sample along with the nonsterile original soil. All irradiated soils recieved at least 20,000 rad over a 24-h period. All soils were then aseptically amended with 5 μCi (185 KBq) of ^{14}C tetryl at a 10-μg/g concentration. The amended soils were then incubated in the growth chamber for 28 days to permit the materiel to equilibriate with the soil matrices. During this period, soils were maintained at approximately one-half field capacity with the periodic (twice weekly) addition of sterile distilled water. All soils were then sequentially placed in the gas- trapping apparatus described in previous reports (Cataldo et al. 1989, 1990) and the rate of $^{14}CO_2$ and potential organic constituents emissions were determined. The data are given in Table 3.3.

TABLE 3.3 Volatility and Mineralization of Soil-Amended ^{14}C-Tetryl. Data are averages ± SD (N=3)

Soil	Sterility	Organic Volatiles[a] (dpm/day)	% Total Amended Label/Day	Daily Respiration[b] (dpm/day)	% Total Amended Label/Day
Burbank	Sterile	0.0 ± 0.0	0.0 ± 0.0	8902 ± 2040	0.080
	Nonsterile	0.0 ± 0.0	0.0 ± 0.0	14195 ± 290	0.127
Palouse	Sterile	0.0 ± 0.0	0.0 ± 0.0	14456 ± 670	0.130
	Nonsterile	0.0 ± 0.0	0.0 ± 0.0	19840 ± 3472	0.178
Cinebar	Sterile	0.0 ± 0.0	0.0 ± 0.0	2885 ± 504	0.025
	Nonsterile	0.0 ± 0.0	0.0 ± 0.0	8096 ± 2040	0.072

[a] Trapped with two tandem 1 x 15 cm XAD resin columns and eluted with 100% MeOH

[b] Trapped using 3 N NaOH (10 mL each in 4 traps in series)

Two immediate points were evident in the results of this experiment. First, either the "sterile" soils were not sterile even after receiving 20,000 rad of gamma radiation, or there was significant nonorganic mineralization of the tetryl occurring in all three soil types. A lack of sterility may have been a result of either a higher self-absorbency by the soils, which prevented complete sterilization, or inadvertent contamination during the subsequent experiment. Given the potential resistance of both bacterial and fungal spores within the soil,incomplete sterilization may have been the more likely.

Secondly however, it must be remembered that decomposition of the tetryl was very rapid once the tetryl was added to all three soil types (see Section 3.1.1) and that there was a significant mass balance difference over a 60-day incubation period. While the percentage of daily label evolution by the pots following a 21-day incubation period was much less than the 1.3% ^{14}C-contaminant in the tetryl (Section 2.1.3), summation of the counts from the daily rate over the full 21 day period and beyond would exceed this contaminant level. Although there is no evidence from this experiment that the rate of $^{14}CO_2$ evolution would be linear, mass balance studies indicate definite a microbial and/or inorganic-driven mineralization of the tetryl over time

3.1.3 Chemical Speciation of Tetryl Residues in Soil

To investigate the potential for transformation of tetryl in soil, a preliminary experiment was conducted with unlabeled explosive. A 400-g pot of Palouse soil was amended with 60 µg/g of tetryl, allowed to age for 8 days, and extracted for chemical analysis. The chromatographic profile of the extract from Palouse soil incubated with tetryl for 8 days is presented at the top of Figure 3.1. Tetryl in this extract was identified by co-injecting a tetryl standard with the soil extract. The co-injection is presented at the bottom of Figure 3.1. As is indicated in Figure 3.1, tetryl is a minor constituent of the extract. Clearly, the transformation of tetryl in this soil is a very labile process, with only small amounts of the parent munition remaining after 8 days.

The remainder of the experiments utilized soils containing 60 µg/g tetryl containing 10 µCi ^{14}C-tetryl per 400 g of air-dried soil. Radiochromatograms were performed on extracts of Palouse soil extracted immediately after amendment and after 11 and 30 days to verify the incorporation of radiolabel into these possible tetryl transformation products. The resulting radiochromatograms are presented in Figures 3.2 through 3.4. Radiolabel appearing coincident with the column dead

volume is labeled with asterisks in Figures 3.2 through 3.4 and corresponds to the retention time of artifacts generated by the Soxhlet extraction procedure, as previously discussed in Section 2.4.3. The radiocarbon profiles identify the presence of at least 4 tetryl-transformation products The transformation products labeled 1-4 in Figures 3.2 through 3.4 correspond to the transformation products observed during the preliminary study that utilized non-radiolabeled tetryl.

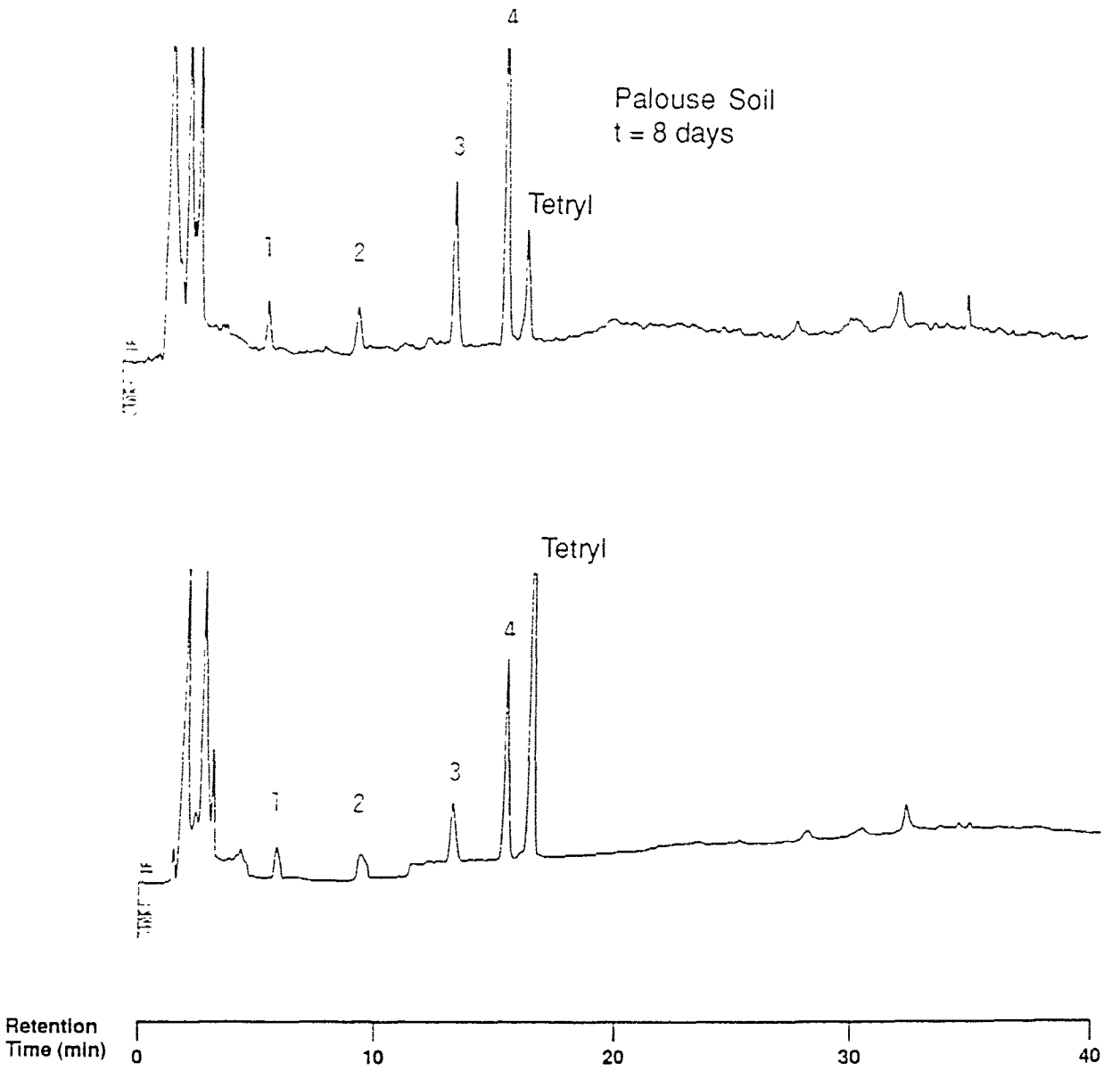

FIGURE 3.1. Chromatographic Profile of an Extract of Tetryl-Amended Soil Performed 8 Days After Amendment (top) and a Co-Injection of the Same Extract with Tetryl (bottom)

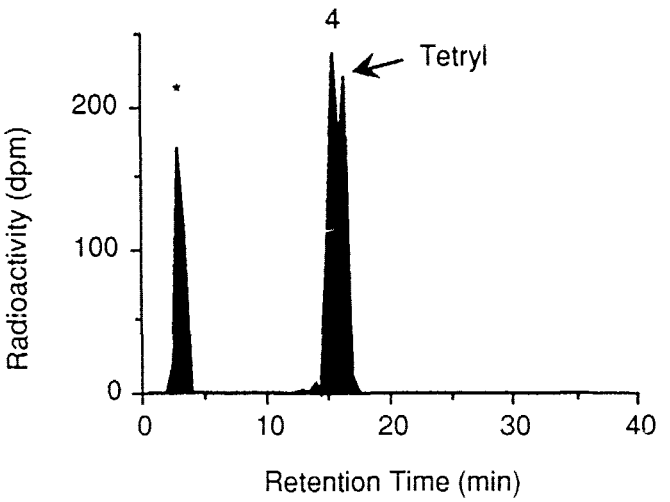

FIGURE 3.2. Radiochromatogram of Tetryl-Amended Palouse Soil Extracte Immediately After Amendment.

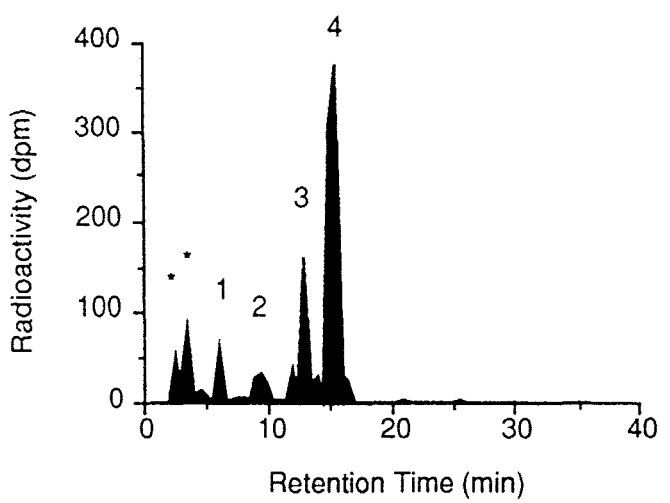

FIGURE 3.3 Radiochromatogram of Tetryl-Amended Palouse Soil Extracted 11 Days After Amendment

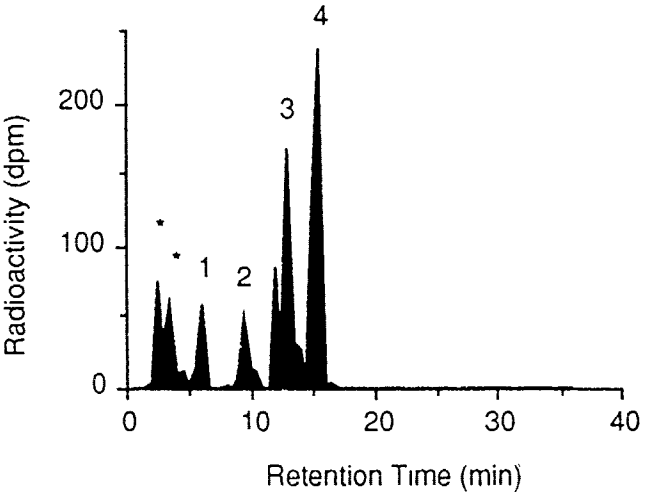

FIGURE 3.4. Radiochromatogram of Tetryl-Amended Palouse Soil Extracted 30 Days After Amendment

The peak areas of transformation products 1 through 4 as a function of soil incubation time for the three different soils are presented graphically in Figures 3.5 through 3.7. The primary purpose of these graphs is to illustrate that transformation product 4 was clearly the first transformation product to appear in all three soils. Formation of this transformation product occurred most rapidly in Cinebar soil (Figure 3.7). In Burbank and Palouse soils, the highest concentration of transformation product 4 was observed in the 11-day extracts. Further trends are difficult to interpret because of complex and competitive processes including product formation and irreversible binding, and because of the propensity for further transformation in the soils. The potential for these processes can be expected to differ for each compound in a given soil, and to differ for each soil type for a given compound. Despite these complexities, a logical start into identifying tetryl transformation products would be to target transformation product 4, because this compound is the principal transformation product and is likely to be a parent of further transformation products.

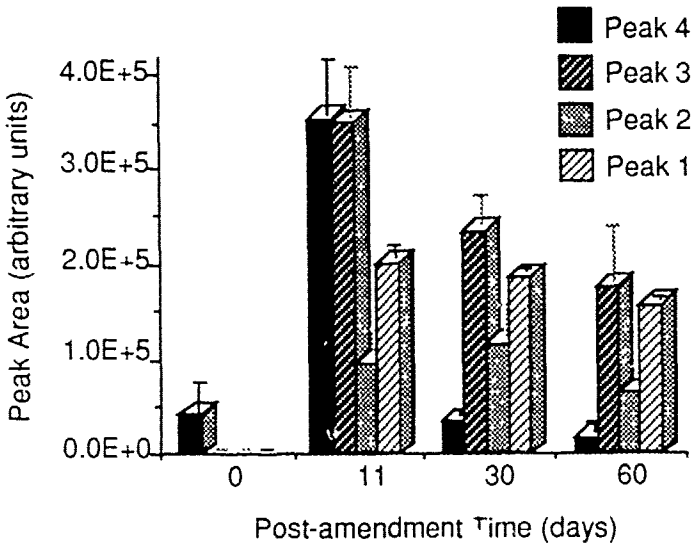

FIGURE 3.5. Peak Areas of Tetryl Transformation Products in Burbank Soil During the 60-Day Study

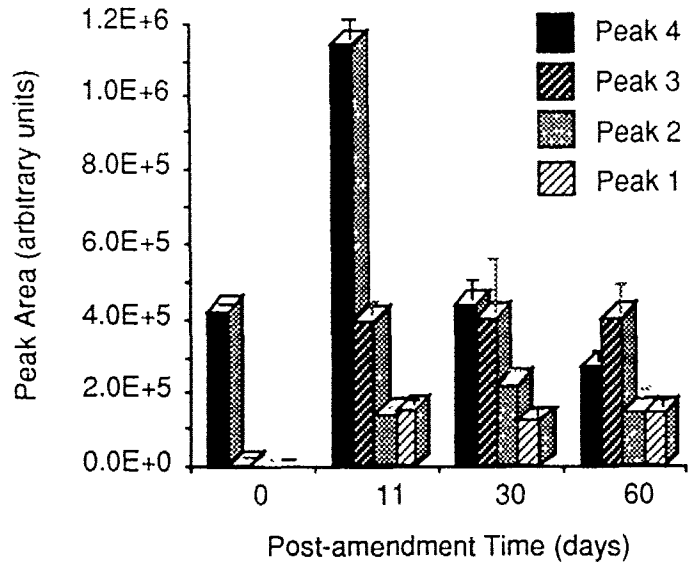

FIGURE 3.6. Peak Areas of Tetryl Tranformation Products in Palouse Soil During the 60-Day Study

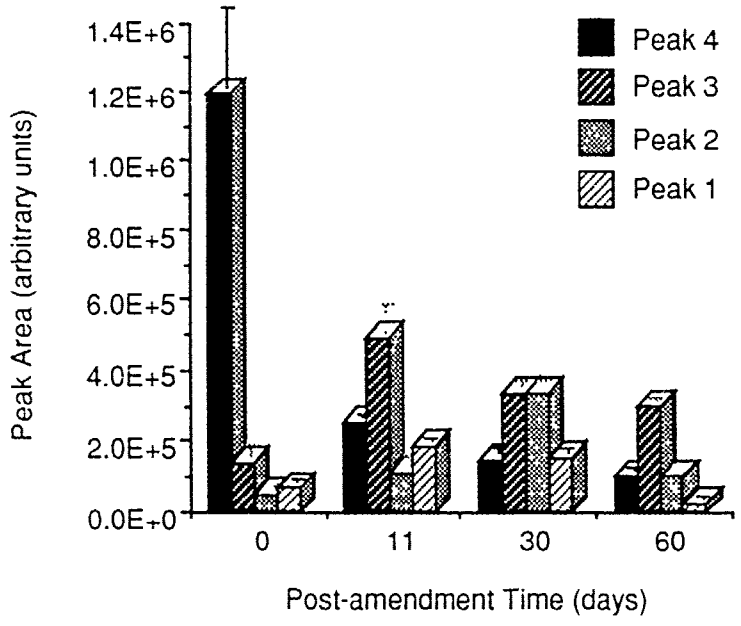

FIGURE 3.7. Peak Areas of Tetryl Tranformation Products in Cinebar Soil During the 60-Day Study

For structural elucidation of peak 4, sufficient material was purified by repetitive HPLC collection of the column eluant corresponding to peak 4. The acetonitrile in the mobile phase was removed by a stream of nitrogen prior to extraction of the eluant with several portions of toluene. The toluene was reduced in volume by a stream of dry nitrogen and the concentrated extract analyzed by GC/MS and by a direct-insertion probe MS. The results of the GC/MS analysis are presented in Figure 3.8. The total ion current chromatogram (shown at the bottom of Figure 3.8) indicates that the material collected by HPLC contains only one component. The mass spectrum of the transformation product is presented in the top of Figure 3.8. This mass spectrum is identical to those obtained during the GC/MS studies of tetryl (see Figure 2.2). Furthermore, the gas-chromatographic retention time of the transformation product is identical to that of tetryl analyzed under the same conditions. The most plausible explanation for this result is that, on injection to the gas chromatograph, both tetryl and the tetryl-derived transformation product undergo thermal degradation to the same product. Alternatively, tetryl transformation product 4 may be the same compound as results from thermal decomposition of tetryl.

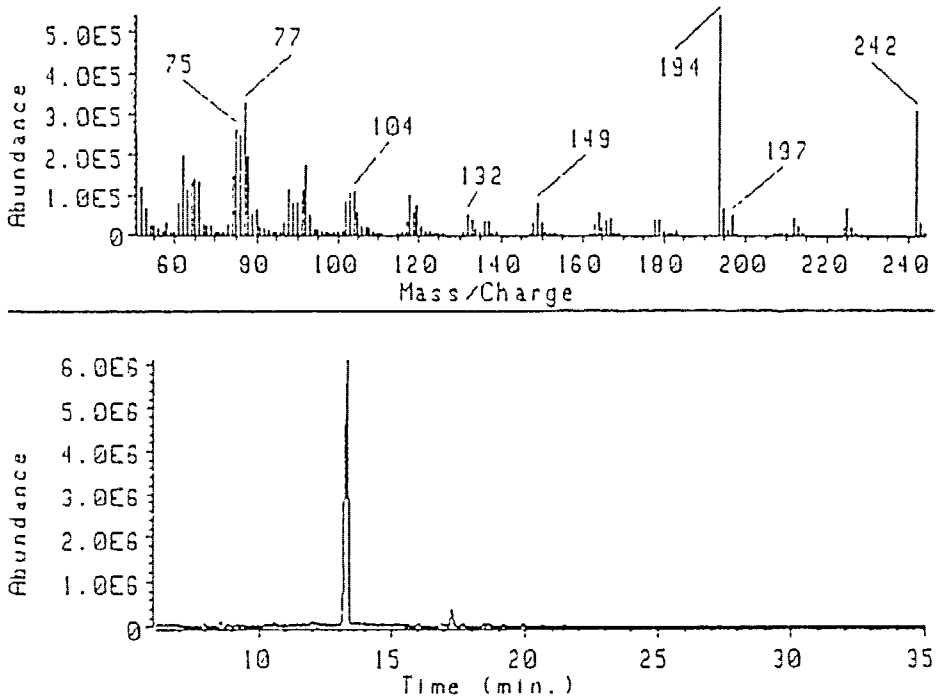

FIGURE 3.8. Total Ion Current Chromatogram (bottom) and Mass Spectrum (top) Resulting from GC/MS Analysis of Transformation Product 4

Additional experiments centered on derivatization of transformation product 4 with either *N,O*-bis-trimethylsilyltrifluoroacetamide (BSTFA) or trifluoroacetic acid. Derivatization of transformation product 4 did not occur, as evidenced by the persistence of the 242 a.m.u. product upon GC/MS analysis. Although these data seem to indicate the absence of an active hydrogen in the transformation product, they should be interpreted with care, because the presence of electron-withdrawing nitro groups may diminish reactivity of functional groups that would normally react with the derivatization reagents.

The next information on transformation product 4 was obtained from the FTIR spectrum. Initial attempts, based on incorporation of transformation product 4 in a KBr pellet, resulted in poor spectra with broad absorption bands and weak features. Further experiments were conducted with an infrared microscope interfaced to an FTIR spectrophotometer. With this experimental arrangement, a few microliters of the transformation product dissolved in methylene chloride were applied to the surface of a NaCl window. After evaporation of the solvent, the microscope was focused on the residue remaining on the NaCl window surface. The resulting

spectrum is shown in Figure 3.9. For comparison, a FTIR spectrum of tetryl obtained under identical experimental conditions is shown in Figure 3.10. The most compelling difference between these spectra is the presence of an absorption at 3331 cm^{-1}. This absorption is suggestive of an aniline N-H stretch.

An UV spectrum provided the next evidence for the identity of transformation product 4. This spectrum is presented in Figure 3.11 and features absorption maxima at 344 and 415 nm. These absorption maxima, as well as their relative intensities, are in good agreement with a spectrum of *N*-methyl-2,4,6-trinitroaniline reported by Dubovitskii et al. (1961).

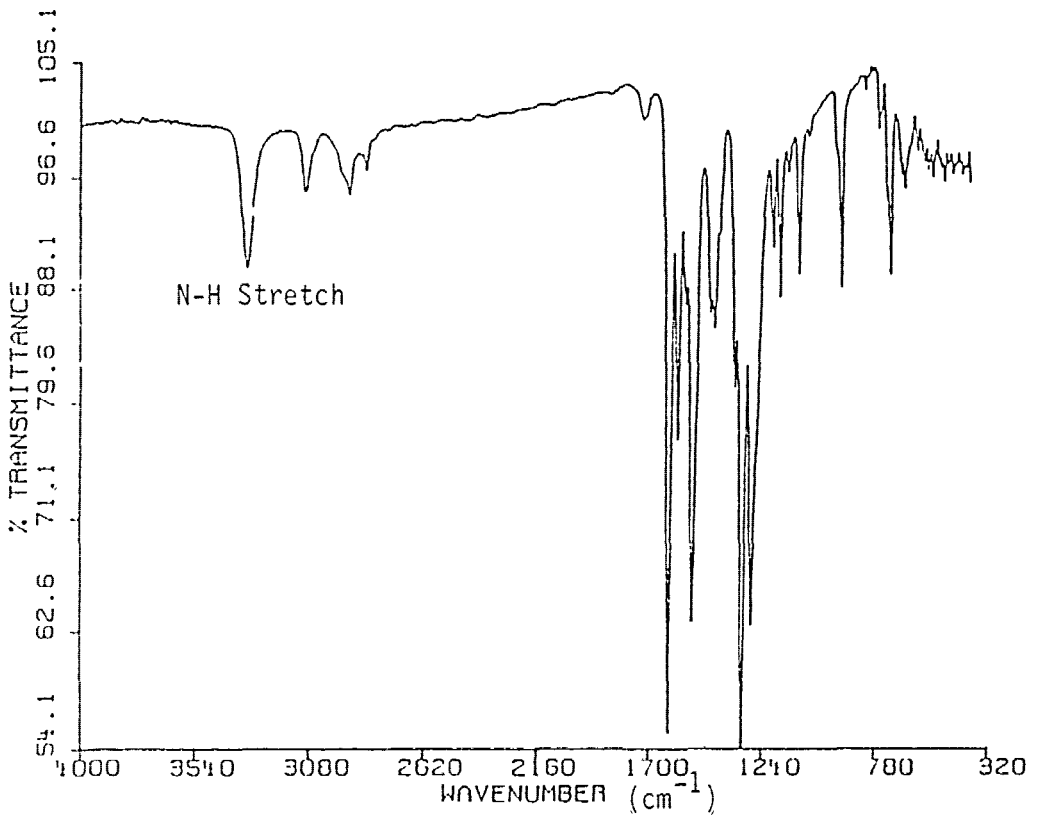

FIGURE 3.9. Fourier Transform Infrared Spectroscopy Spectrum of Transformation Product 4

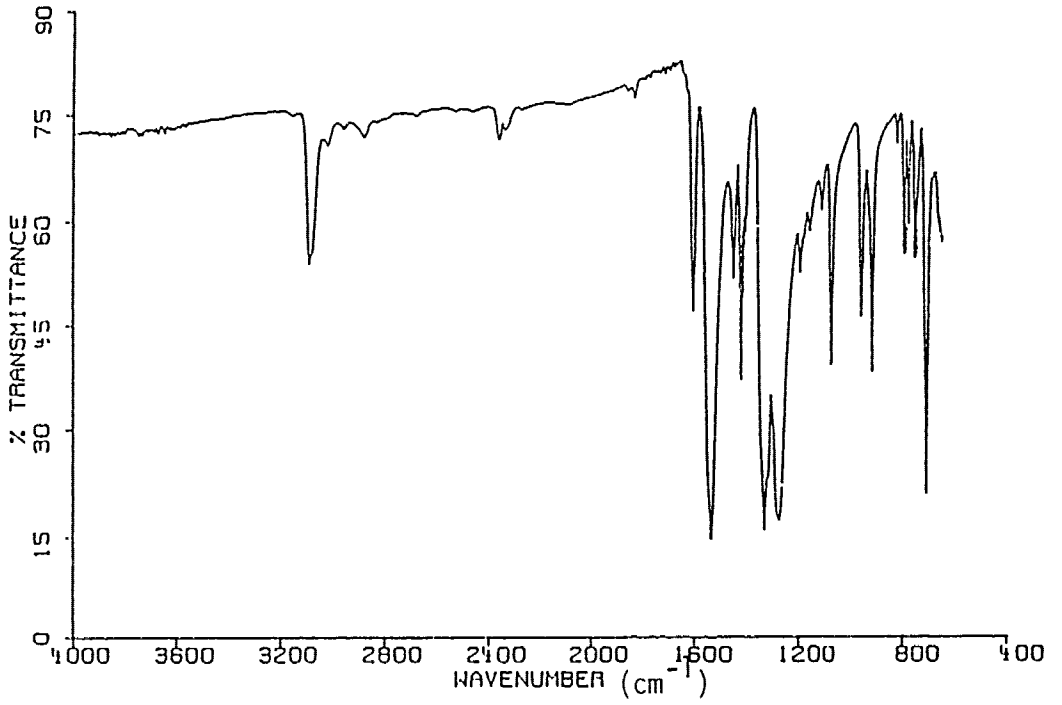

FIGURE 3.10. Fourier Transform Infrared Spectroscopy Spectrum of Tetryl

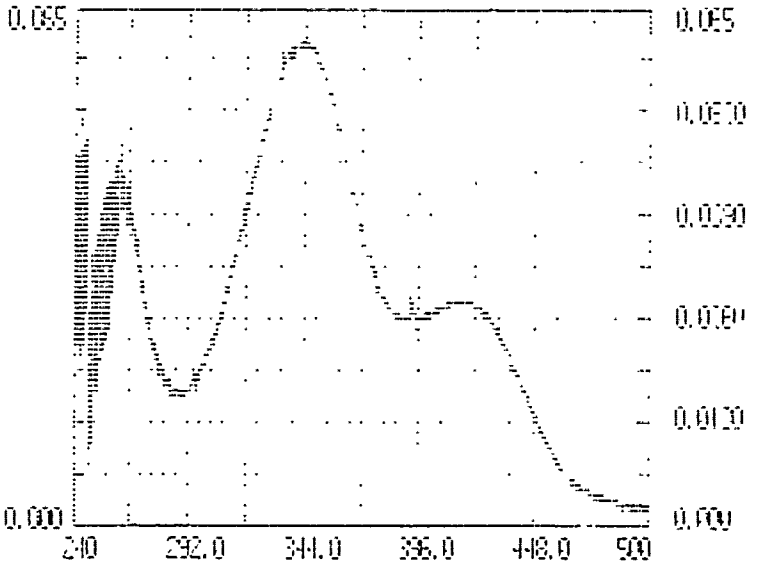

FIGURE 3.11. Ultraviolet Spectrum of Transformation Product 4

A direct-insertion probe mass spectrum was obtained for comparison with the spectra obtained during GC/MS studies. If the spectra obtained by these two methods differ, thermal decomposition may be occurring during GC analysis. Direct-insertion probe spectra were obtained in both the 70-ev electron impact and the isobutane chemical ionization modes. The mass spectrum resulting from the direct-insertion probe analysis of transformation product 4 is presented at the top of Figure 3.12. This spectrum is identical to those collected during the GC/MS analysis of transformation product 4 (see Figure 3.8). It appears therefore that transformation product 4 remains intact during GC analysis. Chemical ionization mass spectrometry was used to determine the molecular weight of transformation product 4. To verify the tuning of the mass spectrometer, a spectrum of benzophenone was obtained before analysis of the tetryl transformation sample. The chemical ionization spectrum of benzophenone gave the expected protonated molecular ion at m/e of 183 a.m.u., thereby indicating proper source conditions. The chemical ionization spectrum of transformation product 4 is presented at the bottom of Figure 3.12. Appearance of the $(M + 1)^{+1}$ ion at 243 a.m.u. provided clear evidence that the molecular weight of transformation product 4 is 242 a.m.u. Together these studies allowed a high degree of certainty in the identification of transformation product 4 as *N*-methyl-2,4,6-trinitroaniline.

Positive identification of transformation product 4 required synthesis of *N*-methyl-2,4,6-trinitroaniline. Synthesis proceeded by the aromatic nucleophilic substitution reaction of 2,4,6-trinitrochlorobenzene (picryl chloride) with methylamine. Methylamine was generated from the hydrochloride salt by treatment with aqueous sodium hydroxide. The free base was then extracted into methylene chloride during generation. After the methylene chloride solution was dried over anhydrous sodium sulfate, excess methylamine was added to 1.0 g of picryl chloride (also dissolved in methylene chloride) over a period of 4 h at room temperature. The reaction was allowed to proceed overnight, at which time the methylene chloride was removed under a stream of dry nitrogen. Analysis of the reaction product by GC/MS indicated quantitative conversion to the *N*-methyl-2,4,6-trinitroaniline product. The mass spectrum obtained was identical to that previously observed for transformation product 4. Additional chromatographic co-elution experiments illustrated that the synthetic product was identical to transformation product 4. The top chromatogram in Figure 3.13 is a profile for Palouse soil at 11 days after amendment, the bottom chromatogram is a co-injection of the same extract with synthetic *N*-methyl-2,4,6-trinitroaniline. The relative increase in the peak area of transformation product 4 verifies the structural assignment of tetryl transformation product 4 as *N*-methyl-2,4,6-trinitroaniline.

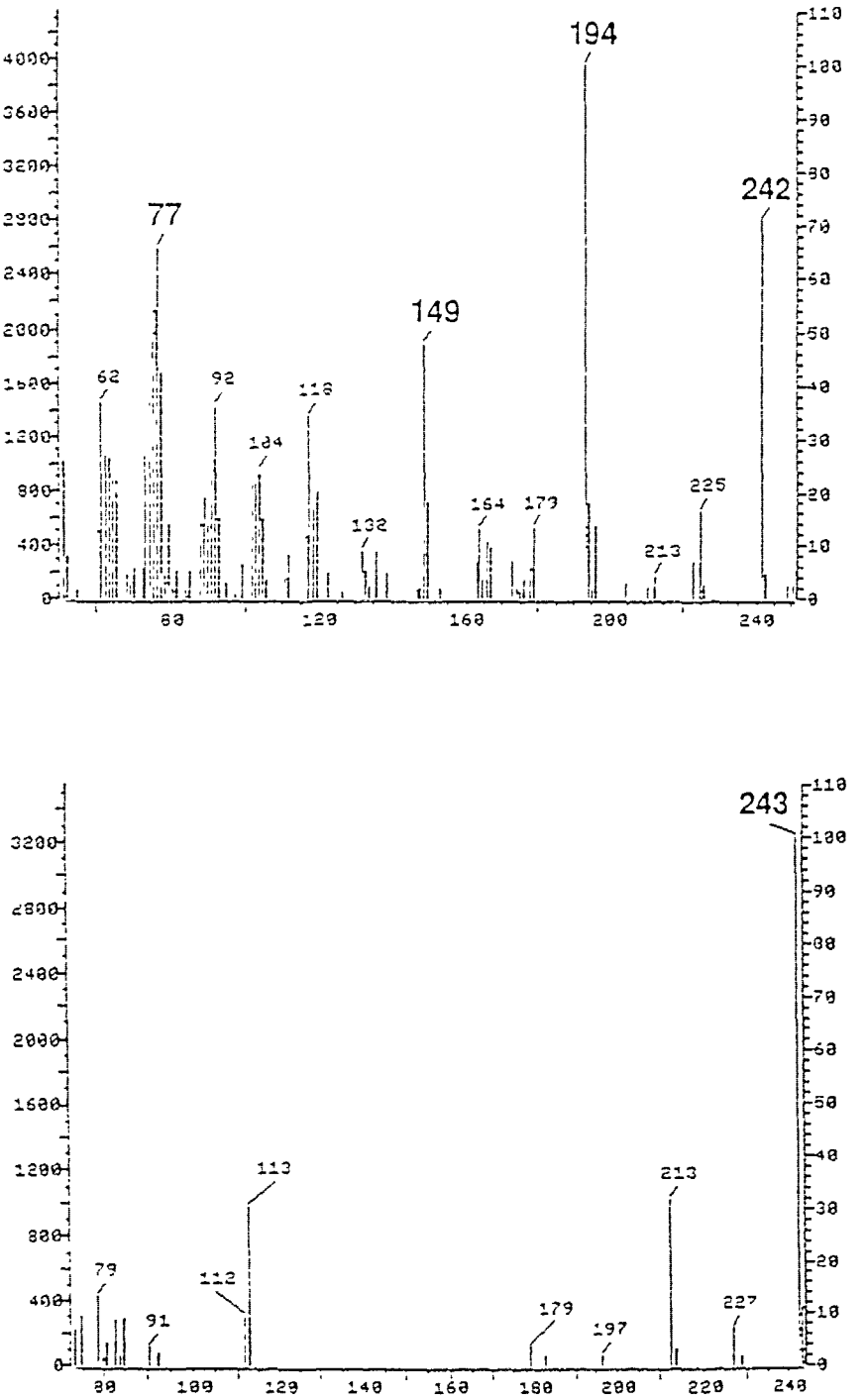

FIGURE 3.12. Direct-Insertion Probe Electron Impact (top) and Isobutane Chemical Ionization Mass Spectra of Transformation Product 4 (bottom)

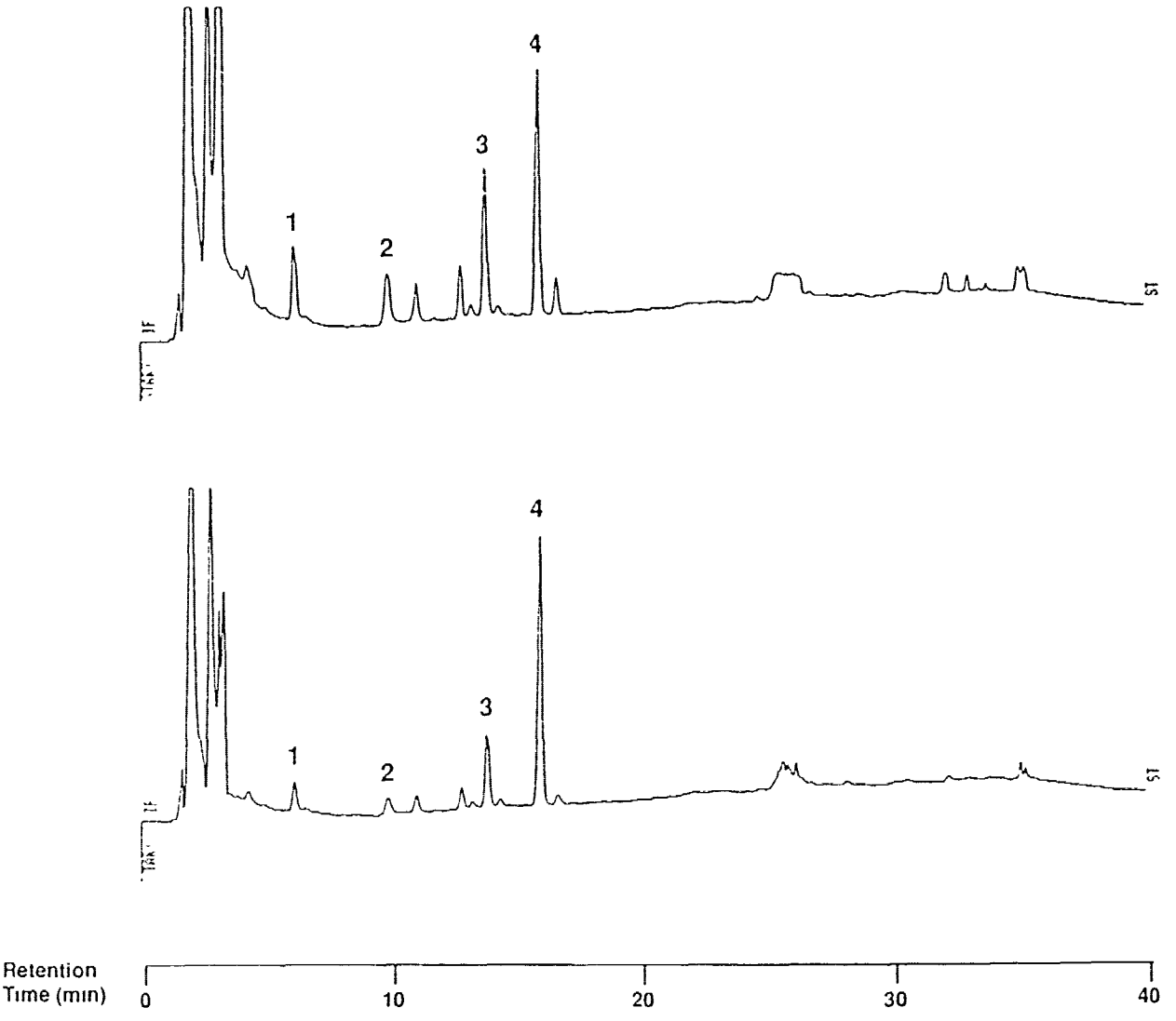

FIGURE 3.13. Chromatographic Profile of an Extract from Palouse Soil Aged 11 Days with Tetryl (top) and a Co-Injection of the Same Extract with Synthetic *N*-methyl-2,4,6-trinitroaniline (bottom)

Transformation product 3 was purified by semi-preparative reversed-phase HPLC of an extract of Palouse soil that had been incubated with tetryl for 11 days. The proportion of acetonitrile in the acetonitrile/water mobile phase was reduced by a stream of dry nitrogen, after which transformation product 3 was extracted into methylene chloride. After drying over anhydrous sodium sulfate, the purity of the preparation was assessed by GC/MS. The HPLC-purified material exhibited one peak on GC/MS analysis, as illustrated at the bottom of Figure 3.14. The mass spectrum of this material (Figure 3.14, top) featured an apparent molecular ion at 212 a.m.u. On the basis of this molecular weight, this compound was hypothesized to be a dinitroaminophenylmethylnitramine isomer arising from nitroreduction of *N*-methyl-2,4,6-trinitroaniline.

Both electron impact and isobutane chemical ionization mass spectra of transformation product 3 were obtained by direct-insertion probe analysis for comparison to the mass spectra of tetryl. The electron impact and isobutane chemical ionization mass spectra of the parent munition are presented in the top and bottom of Figure 2.4, respectively. Figure 3.15 presents the corresponding mass spectra for transformation product 3. The electron impact spectrum of transformation product 3 (Figure 3.15, top) is different from that obtained under GC/MS conditions. The difference between these spectra can be attributed to the thermal decomposition of transformation product 3 that occurs under the stringent thermal conditions necessary for GC analysis. The thermally more gentle analysis conditions normally utilized for direct-insertion probe mass spectrometry allow analysis of the intact transformation product. The electron impact mass spectrum features a base fragment at m/e of 211. Interestingly, tetryl exhibits a base fragment at 241 a.m.u. resulting from the loss of the aniline nitro group. The difference in mass between the base fragment of tetryl and transformation product 4 is 30 a.m.u.

Isobutane chemical ionization mass spectrometry of transformation product 3 gave the spectrum presented at the bottom of Figure 3.15. This spectrum features the protonated molecular ion at m/e of 258, indicating a molecular weight of 257 a.m.u. The molecular weight is 30 less than that of the parent munition, which suggests that transformation product 3 is a direct nitroreduction product of tetryl. The base fragments seen in the chemical ionization spectra of both tetryl and transformation product 3 result from the protonated thermal decomposition products. It is likely that both compounds undergo thermal decomposition by cleavage of the aniline nitro group. This hypothesis is further supported by examination of the thermal decomposition compounds produced under GC conditions. The molecular ion at 212 a.m.u. observed during GC/MS experiments with transformation product 3 (Figure 3.14) serves to further localize the site of nitroreduction to a ring position.

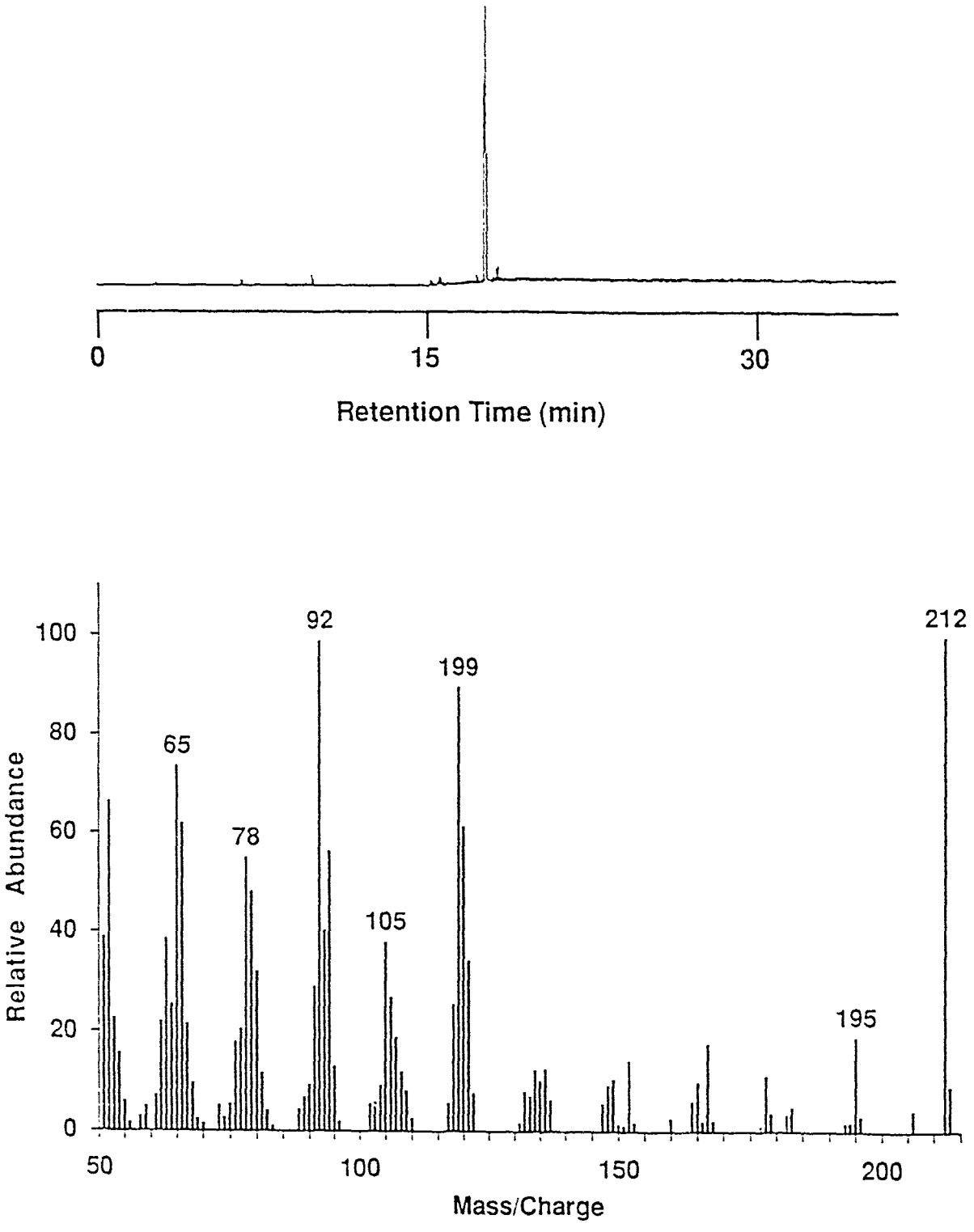

FIGURE 3.14. Total Ion Current Chromatogram (top) of Transformation Product 3 and the Mass Spectrum of the Major Component (bottom)

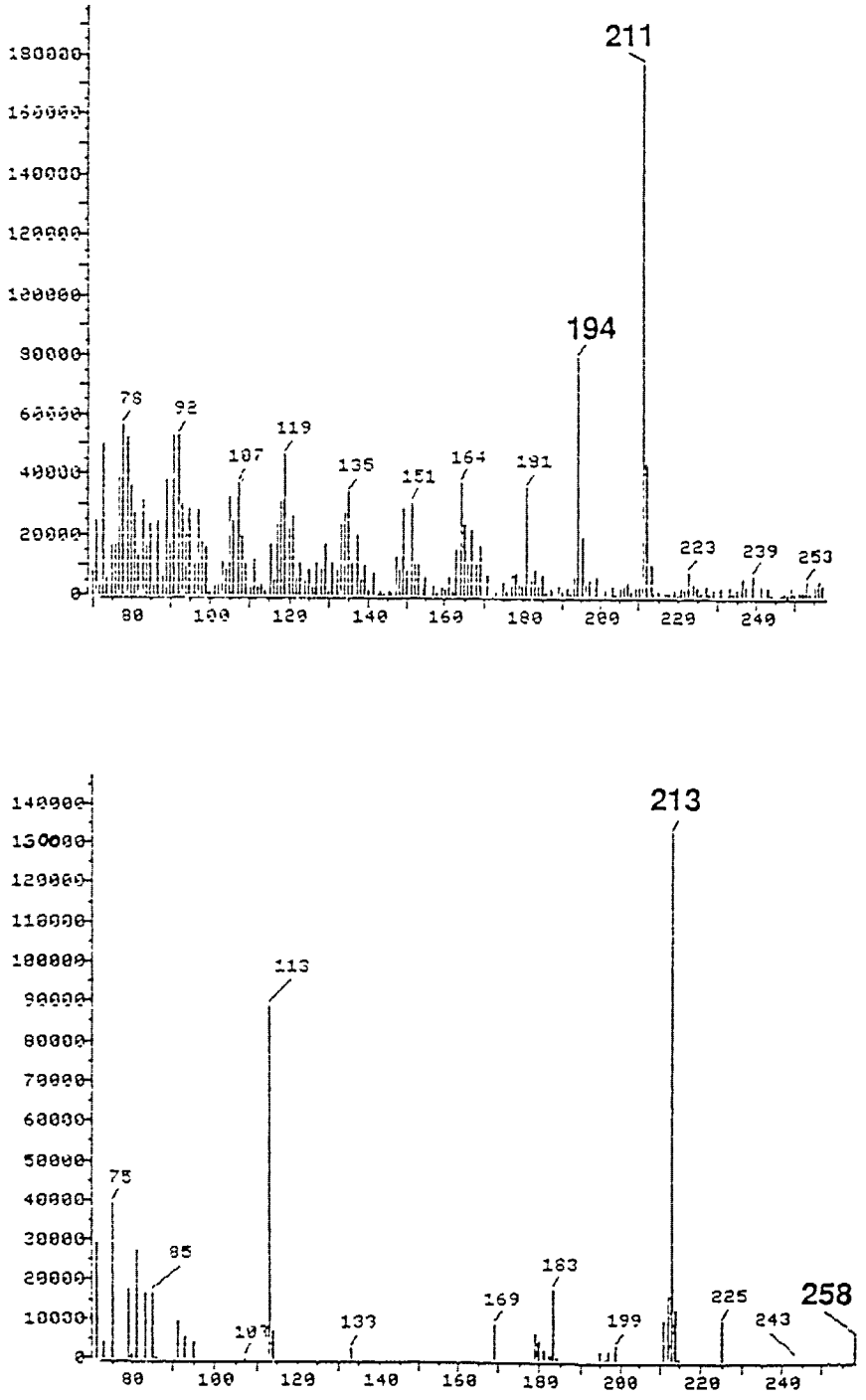

FIGURE 3.15. Direct-Insertion Probe Electron Impact (top) and Isobutane Chemical Ionization Mass Spectra of Transformation Product 3 (bottom)

Figure 3.16 summarizes the two independent tetryl soil transformation pathways identified by the above studies. The principal transformation pathway involves the formation of *N*-methyl-2,4,6-trinitroaniline (transformation product 4). The less prominent pathway involves direct nitroreduction of the parent munition to form a dinitroaminophenylmethylnitramine isomer (transformation product 3). Other compounds observed in radiochromatographic profiles of soil aged with ^{14}C-tetryl are most likely further reduction products of the initial transformation products.

An attempt was made to synthesize the thermal decomposition product of transformation product 3. The method chosen for the synthesis of *N*-methyl-dinitroamino-aniline was stoichiometric reduction of *N*-methyl-2,4,6-trinitroaniline with sulfide ion. The balanced oxidation reduction reaction is

$$3S^{-2} + 4H_2O + NO_2\text{——} \dashrightarrow NH_2\text{——} + 6OH^- + 3S^0$$

N-methyl-2,4,6-trinitroaniline (0.16 g, 0.66 mmol) was dissolved in 50 mL of 50:50 methanol:water. A 0.40 M solution of $Na_2S\ 9H_2O$ was prepared in distilled water. The sulfide solution (5.0 mL, 2.0 mmol) was added to the *N*-methyl-2,4,6-trinitroaniline over 50 min, and an additional 4 h at ambient temperature was allowed for the reaction to reach completion. The initial step in product isolation was removal of elemental sulfur by filtration. Next, the methanol was removed under a stream of dry nitrogen and the reaction mixture extracted with methylene chloride. After drying with anhydrous sodium sulfate, the methylene chloride was removed by rotary evaporation. Analysis of the isolated reaction product by HPLC and GC/MS revealed approximately equal quantities of methypicramide starting material and a product that was assumed to be *N*-methyl-dinitroaminoaniline product, based on its apparent molecular weight of 212 a.m.u.

The total ion current chromatogram of the *N*-methyl-dinitroaminoaniline reaction product is shown at the bottom of Figure 3.17. The first peak is due to *N*-methyl-2,4,6-trinitroaniline starting material. The mass spectrum corresponding to the second peak is shown at the top of Figure 3.17. Although extremely similar, the mass spectrum of synthetic material contains a fragment at m/e of 130 that was not observed in the mass spectrum of transformation product 3 obtained by GC/MS (see Figure 3.14). It is difficult to rationalize this discrepancy in the mass spectra. It is possible that the sulfide reduction and environmental processes cause reduction at different ring positions and that the different isomers give rise to slightly different mass spectra.

To facilitate the future identification of the tetryl soil transformation products that were observed during our studies, the tetryl transformation products were characterized by their alklyphenone retention indices. These retention indices are tabulated in Table 3.4. Table 3.4 also includes the retention indices for tetryl and the products synthesized during the experiments.

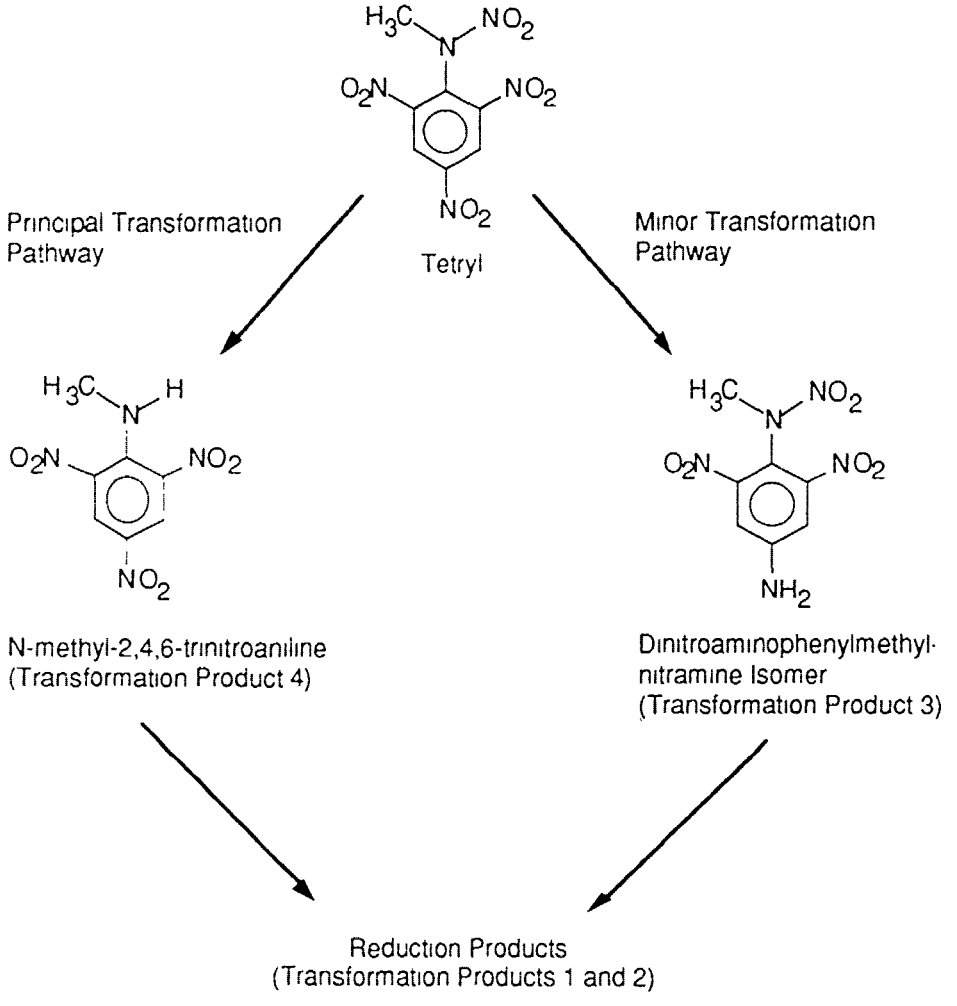

FIGURE 3.16. Transformation Pathway for Tetryl in Soil

TABLE 3.4. Retention Indices of Tetryl Soil Transformation Products

Compound	Retention Index
Soil transformation product #1	714
Soil transformation product #2	813
Soil transformation product #3	872
Soil transformation product #4	922
Tetryl	946
N-methyl-2,4,6-trinitroaniline	922 ± 2
N-methyl-dintroaminoaniline	851 ± 1

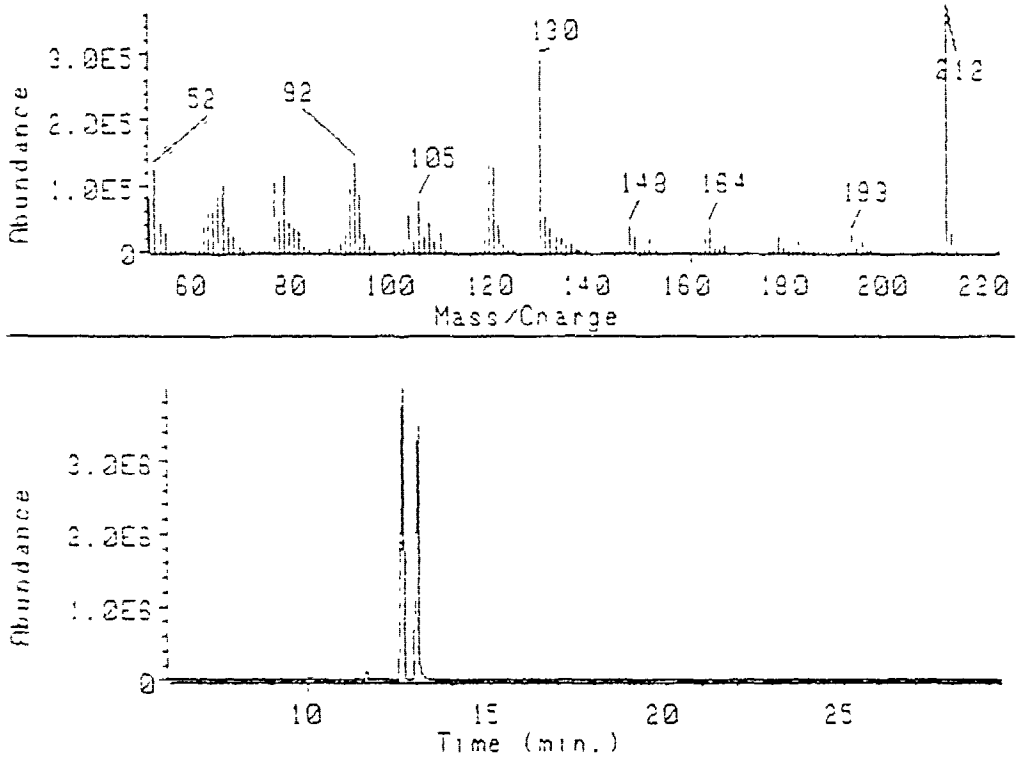

FIGURE 3.17. Total Ion Current Chromatogram (bottom) and Mass Spectrum (top) of the *N*-methyl-dinitroaminoalinine Synthetic Product

3 2 HYDROPONIC STUDIES OF SHORT-TERM PLANT AVAILABILITY AND CHEMICAL FATE OF TETRYL

Hydroponic studies were performed to address several basic needs. The first was to establish the physiological capacity of plants to absorb and transport tetryl in the absence of soils and their sorptive components. In other words, the studies addressed the question, "how does the soluble concentration of tetryl affect its biological availability?" The second need was to elucidate the extent of partitioning of tetryl, particularly to the root, which is not amenable to analysis in soil systems. And finally, the studies addressed the need to establish the short-term chemical fate of tetryl in plant tissues, including roots.

3.2.1 Plant Availability of Tetryl from Solution Culture

To determine the rate of uptake and relative bioavailability of tetryl, a series of experiments were undertaken with three plant species (bush bean, wheat, and blando brome). In these experiments, plants were grown in solution culture and roots were exposed to increasing concentrations of ^{14}C-tetryl. The solution concentrations were limited to 1 to 10 mg/L because of the relative low solubility of tetryl in water. The average rates of uptake (in micrograms tetryl per gram fresh weight root per hour, ± 1 standard deviation based on ^{14}C equivalents) by the plants are shown in Figure 3.18.

No statistically significant differences in uptake rates (P=0.05) were observed among those plants used in this study. However, there was a slight tendency for the bean plants to have a higher rate at the lowest concentration used (1.0 mg/L) and a lower rate at the highest concentration (10 mg/L). The uptake of tetryl differed from those reported for either TNT (Cataldo et al. 1989) or RDX (Cataldo et al. 1990), for which uptake rates for bush beans were substantially greater for all concentrations employed.

Data from these experiments were subsequently analyzed by the use of Lineweaver-Burke reciprocal plots. Over the concentrations used (1 to 10 mg/L), it was apparent that either there was a saturation of the absorption process, or a second isotherm appeared between the 5- and 10-mg/L concentrations. The final plots used for the calculations were therefore limited to the 1-, 2.5-, and 5-mg/L uptake rates. From these, a value for K_s, which is analogous to the Michaelis-Menten K_m, and a root absorption rate (V_{max}) were calculated for each species. These are given in Table 3.5.

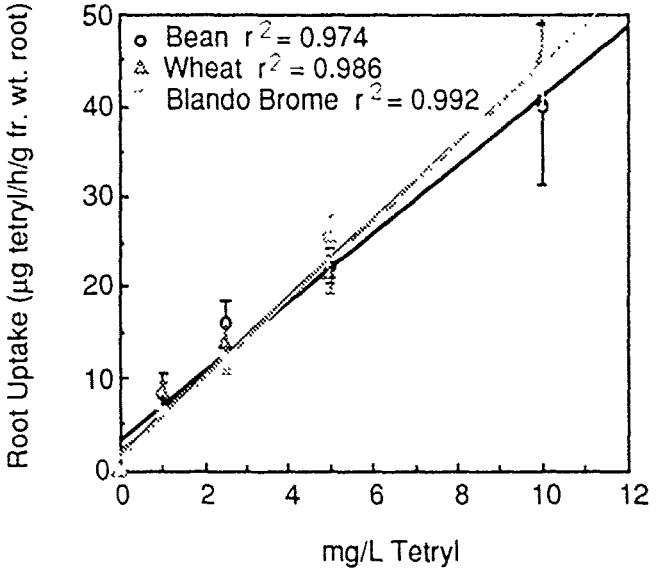

FIGURE 3.18. Uptake Rates of Radiolabeled Tetryl by Roots of Bush Bean, Wheat, and Blando Brome from 1-, 2.5-, 5-, and 10-mg/L Tetryl-Amended Nutrient Solutions. Data are averages ± standard deviation (N=3).

TABLE 3.5. Calculated K_s and V_{max} Values from Apparent Isotherms Observed from Double Reciprocal Plots of Tetryl Uptake from Nutrient Solution by Bush Bean, Wheat, and Blando Brome Plants.

Species	K_s Isotherm (mmol)	V_{max} Isotherm (mmol tetryl/g/fr. wt. root/h)
Bush Bean	12.8	0.172
Wheat	10.7	0.101
Blando Brome	38.3	0.199

The K_s values for all three plant species are much lower than those previously observed for either TNT or RDX (Cataldo et al. 1989, 1990). This may indicate that the plants have a greater affinity for tetryl as opposed to the other munitions. The bush bean K_S value was only 12.8 µM for the tetryl, while similar studies for the TNT and RDX yielded values of 280 and 389 µM, respectively (Cataldo et al. 1989, 1990). Correspondingly, for wheat the tetryl K_S of 10.7 µM was also lower than the respective TNT and RDX values of 133 and 270 µM, while for blando brome,

the tetryl K_S of 38.3 μM was not quite as low relative to the respective values of 138 and 96 μM for the TNT and RDX, but it was still significantly less.

However, while the plants exhibited a greater affinity for tetryl, the apparent capacity of the roots to absorb tetryl [i.e., the V_{max} (Table 3.5)] was much lower than that reported for either TNT or RDX. The V_{max} values observed for TNT ranged from 5.6 to 15.4 μM per hour per gram fresh weight root among the three species, while those for RDX ranged from 0.24 to 0.98 μM per hour per gram fresh weight root. The tetryl values were thus 2 to 150 times less.

These results suggest that tetryl is effectively absorbed by the roots of plants. The absorption affinity for tetryl is tempered by the comparatively low absorption capacity of the roots, at least for short-term exposures. However, even with a reduced root uptake capability, the plants did appear to accumulate a significant amount of the materiel over a short-term, exposure as seen in Table 3.6. Based on ^{14}C equivalents, within the 1-h exposure time, the roots of the bean accumulated approximately 40 μg of tetryl/g fr. wt. This is more than that observed for the TNT (29 μg/g fr wt. in 2 h) (Cataldo et al. 1989) and much more than that of the RDX (6 μg/g fr. wt. in 2 h) (Cataldo et al. 1990) at an exposure concentration of 10 mg/L. The other species also showed similar uptake increases across all exposure concentrations tested. Furthermore, unlike the other munitions materials tested, no significant variations between plant species were evident for tetryl.

TABLE 3.6. Distribution of Tetryl in Plants Following 1-h Exposure in Solution Culture at Various Tetryl Concentrations. Data given in specific tissue cencentration (μg tetryl/g fr. wt. tissue)[a] and percentage of ^{14}C.

Species/Tissue	1 mg/L	2.5 mg/L	5 mg/L	10 mg/L	% Plant ^{14}C
	Average μg Tetryl/g fr. wt. Tissue ± SD (n=3)				
Bush Bean					
Root	9.46 ± 2.26	15.64 ± 3.39	22.12 ± 2.37	39.48 ± 4.25	99.6
Shoot[b]	0.04 ± 0.02	0.04 ± 0.03	0.16 ± 0.12	0.09 ± 0.05	0.4
Wheat					
Root	8.66 ± 2.13	14.18 ± 2.34	21.44 ± 3.81	44.10 ± 6.85	99.5
Shoot[b]	0.03 ± 0.01	0.07 ± 0.02	0.08 ± 0.04	0.29 ± 0.23	0.5
Blando Brome					
Root	6.06 ± 1.08	11.30 ± 1.89	24.75 ± 4.69	43.28 ± 9.22	99.3
Shoot[b]	0.02 ± 0.01	0.08 ± 0.04	0.09 ± 0.03	0.40 ± 0.30	0.7

[a] Based on ^{14}C equivalents

[b] Includes stem plus leaves

Partitioning patterns for the short-term exposures (1 h uptake, Table 3.6) more closely resembled those for TNT, where the overwhelming majority of the label was retained by the root (>99%) (Cataldo et al. 1989). This trend continued for up to 7 days after amendment. The chemical structures of tetryl and TNT are similar and their particular partitioning pattern resembles those of the dinitroanaline herbicides, which they both also resemble structurally. This pattern was unlike that of the RDX, which has a substantially different chemical structure and for which distribution between the root and the shoot was more uniform (Cataldo et al. 1990).

3.2.2 Short-Term Partitioning of Tetryl Within the Plant

Species differences, including the rate at which tetryl may be absorbed by a plant's roots, could also affect partitioning of the absorbed tetryl between tissues and organs, and the subsequent chemical fate or metabolism of the partitioned tetryl. Description of the metabolic fate of plant-absorbed tetryl involved analysis of tissues derived from the short-term hydroponic studies and is reported in Section 3.2.4. The partitioning patterns up to 7 days after amendment are given in Table 3.7. Even up to one week after amendment, the partitioning patterns do not differ and the majority of the tetryl (or ^{14}C equivalents) is retained by the roots following uptake.

TABLE 3.7. Percentage of Radiolabel Contained in Different Tissues (Average ± SD, N=3) of Bush Bean, Wheat, and Blando Brome Following Incubation in Hydroponic Solutions Containing 10 µg/g ^{14}C-Tetryl (0.185 MBq/500 mL)

Species	Tissue	1 day	4 day	7 day
		Avg. % Total Label in Plant ± SD		
Bush Bean	Leaves	1.19 ± 0.25	1.78 ± 1.14	2.00 ± 0.66
	Stem	8.38 ± 1.85	9.67 ± 4.42	6.12 ± 0.94
	Roots	90.44 ± 2.00	88.55 ± 5.05	91.88 ± 0.27
Wheat	Shoot	1.42 ± 0.92	5.74 ± 5.27	7.25 ± 5.08
	Root	98.58 ± 0.42	94.26 ± 5.27	92.75 ± 5.08
Blando Brome	Shoot	6.68 ± 1.55	7.39 ± 0.27	9.71 ± 1.60
	Root	93.32 ± 1.55	92.61 ± 0.27	90.29 ± 1.60

3.2.3 Respiration and Volatility of Tetryl by Plants Grown in Solution Culture

Tetryl and other related residues that can be absorbed by plant roots and exported to the stem and leaves 1) may be further metabolized to a form that would be innocuous to the plant, 2) may be sequestered and/or stored, 3) may undergo further metabolic conversions to achieve a final form as CO_2, and/or 4) may pass through the plant *via* the transpiration stream to be released to the atmosphere essentially unchanged. To assess the potential for the last two processes, a 28-day-old hydroponic-grown soybean plant was exposed to a nutrient solution containing 7.5 µg/g ^{14}C-tetryl. The plant and its rooting solution were placed into the CO_2/volatiles-trapping chamber described by Cataldo et al. (1989). Over a period of 72 h, volatile organic compounds and CO_2 emissions from the shoot and root portions of the plant were sampled and assayed. From the collected data, a ^{14}C mass balance was constructed between the initial and remaining amounts of radiolabel in the nutrient solution, trapped volatiles, and plant tissues.

The results, given in Table 3.8, show a mass balance recovery of approximately 91%. Over a 72-h period, ~42% or 1100 µg tetryl was accumulated within the plant. This was much higher than the 15%, or 692 µg, reported for RDX but was much less than that seen for TNT (~68%) (Cataldo et al. 1989, 1990). Respiratory losses from the root accounted for 11-µg equivalents of tetryl. This was less than that observed with the RDX, where 21-µg equivalents were reported. The assumed root respiration of tetryl from the plant was shown to increase linearly in time. This trend is shown in Figure 3.19. In addition, observable quantities of organic volatiles were detected from the root but not from the shoot (Table 3.8, 0.56 µg). This disparity may be due to aerosolization of the material from the solution culture and indicates that the material is not transpired directly from the plant.

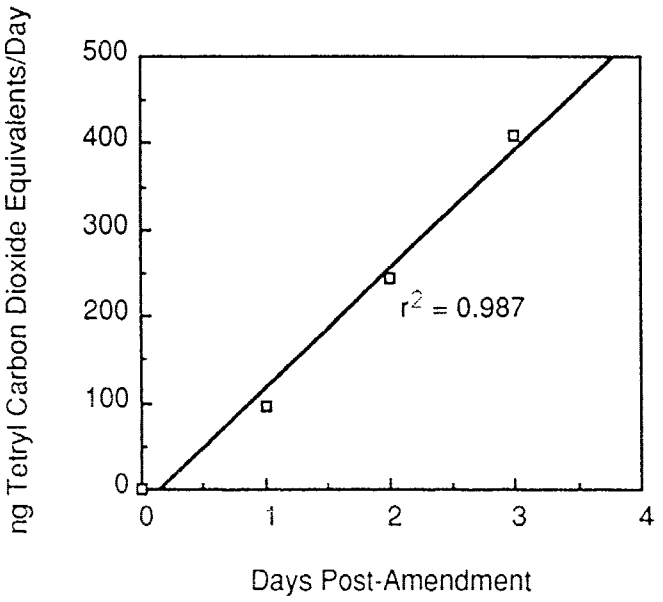

FIGURE 3.19. Tetryl Evolution from Shoot of Bean Plant Following Amendment of $^{14}CO_2$-Tetryl (ng Calculated from $^{14}CO_2$ Equivalents)

TABLE 3.8 Distribution of Radio-Tetryl in a Hydroponically Grown Bush Bean Following 72-h Exposure to a 7.5-µg/g Solution. Plant was maintained under normal growth conditions and in a split chamber.

Tissue/Fraction	TET (µg)	% Total Initial ^{14}C
Original [^{14}C]Tetryl Solution	2647.82[a]	100.00
Plant Segment	1103.30[b]	41.69
Shoot CO_2[c]	0.75[d]	0.03
Shoot XAD[e]	0.00	0.00
Root CO_2[c]	11.16	0.42
Root XAD[e]	0.56	0.02
Transpired Water[f]	0.40	0.02
Total	1116.25	42.18
$CaCl_2$ Root Wash	70.21	2.65
Methanol Root Wash	247.25	9.34
Final [^{14}C]Tetryl Solution	985.84[f]	37.23
Total	1303.30	91.40

[a] Based on HPLC analysis of the 350-mL starting solution.

[b] Determined by tissue oxidation and based on [^{14}C] tetryl equivalents from solution's specific activity

[c] Based on [^{14}C] tetryl starting solution equivalents from NaOH traps

[d] Not detected

[e] Based on [^{14}C] tetryl starting solution equivalents from XAD resin columns.

[f] Based on [^{14}C] tetryl starting solution equivalents in condensed transpired water in upper (shoot) chamber

3.2.4 Chemical Fate of Tetryl in Hydroponically Grown Plants

Hydroponic solutions were prepared from filter-sterilized nutrient solutions and methanol solutions of tetryl. Each 500-mL beaker was amended to approximately 10 µg/g tetryl containing 5 µCi radiolabeled tetryl. For each species, nine plants were transferred to the amended hydroponic solutions. Plants were harvested in triplicate after 1, 4, and 7 days of exposure to tetryl-amended hydroponic solutions. Three control solutions were maintained in the growth chamber along with the hydroponic plants. The controls consisted of two beakers that were wrapped with an opaque sheath to exclude light. One of these solutions was aerated, whereas the other was not. The third solution was aerated and exposed to the full intensity of the growth chamber lights.

Hydroponic solutions were analyzed by liquid scintillation spectrometry and HPLC immediately after amendment and at each harvest. The results of these analyses are presented in Table 3.9. Control solutions that were exposed to the growth chamber lights immediately acquired a bright yellow coloration, indicating that tetryl had undergone photodecomposition Analysis of the colored solutions by HPLC indicated the initial presence of methyl picramide. After 7 days of exposure to light, the yellow solutions were devoid of methyl picramide (as shown by HPLC). Comparison to controls maintained in the dark indicated rapid photodecomposition of tetryl. This decomposition is reflected in the discrepancy between radiolabel contained in the solutions and the HPLC determinations of tetryl. After 1 day, there was a significant decrease in the quantity of tetryl in the light-exposed beakers, but the amount of tetryl in the dark controls remained approximately the same as the initial concentration. The difference in tetryl concentration between controls maintained in the dark and those exposed to the light was even more exaggerated by the end of the 7-day experimental period.

Analysis of solutions containing plants revealed evidence for both plant uptake and root-catalyzed transformations (Table 3.9). The amount of tetryl was near or below the detection limit of 0.1 µg/g in all plant hydroponic solutions by 4 days. However, even after all the tetryl had been transformed, radiolabel continued to be assimilated by the plants.

At harvest, the plants were removed from the hydroponic solutions and the roots sequentially rinsed in 0 1 M $CaCl_2$ followed by 80:20 methanol:water. The rinse solutions were analyzed by liquid scintillation spectrometry. The quantity of radiolabel (tetryl and tetryl transformation products) that was taken up by the plant was estimated by subtracting the amount of radiolabel contained in the remaining hydroponic and rinse solutions from the amount initially in the solution These values are presented in Table 3.10. From these data, it is clear that wheat and blando brome have a higher affinity than bush bean for tetryl and its transformation products After 1 day of exposure, the monocotyledons had assimilated approximately 3.3 µCi of radiolabel, as compared to the 1 9 µCi assimilated by the bush bean. This trend continues throughout the study, with the monocotyledons adsorbing approximately 4 8 µCi and the dicotyledons 3.6 µCi of radiolabel by the end of the 7-day exposure.

TABLE 3.9. Quantities of Tetryl and Radiolabel in Hydroponic Solutions at the Beginning of the Study and at Two Harvest Times

Species/ Treatmen	0 day		1 day		7 day	
	µCi	mg tetryl	µCi	mg tetryl	µCi	mg tetryl
BUSH BEANS						
Control, light, bubbled	5.37	4.73	5.56	3 64	5.86	0.83
Control, light, not bubbled	5.39	4 69	5.81	3.54	5.06	0.60
Control, dark, bubbled	5 51	4.71	5.41	4 33	5 46	4.06
Plants 1-3[a]	5.12 ± 0.06	4.76 ± 0 29	3.00 ± 0.09	BD[b]	----	----
Plants 7-9[a]	5 44 ± 0 12	4 42 ± 0 05	----	----	1.24 ± 0.43	BD
WHEAT						
Control, light, bubbled	5 36	4 90	5.20	2.30	5.05	0.23
Control, light, not bubbled	5.34	4 76	5.34	1.89	5.10	0.14
Control, dark, bubbled	5 22	4 81	5 26	4.72	5 12	2.28
Plants 1-3	5 06 ± 0 31	4 67 ± 0.16	2 45 ± 0 50	BD	----	----
Plants 7-9	5 14 ± 0 27	4.84 ± 0 01	----	----	0.77 ± 0.39	BD
BLANDO BROME						
Control, light, bubbled	5 53	4 40	5.56	4 07	5.35	1.46
Control, light, not bubbled	5 54	4 26	5.38	3.95	5.44	2.84
Control, dark bubbled	5 54	4 37	5 38	4 90	5.38	5.03
Plants 1-3	5 59 ± 0 04	4 63 ± 0 13	1 94 ± 0 18	BD	----	----
Plants 7-9	5 81 ± 0 18	4.91 ± 0 07	----	----	0 95 ± 0.09	BD

[a]Of the nine amended plants, three (1-3) were sampled at day 1, three (4-6) at day 4 (data not shown), and three at day 7 (7-9)

[b]BD=below detection limits

TABLE 3.10. Total Radiolabel Uptake (in Microcuries) by Plants from Original 5-μCi Hydroponic Solution. Values are the average ± SD (N=3 for each sampling period).

Species	1 day	4 day	7 day
		μCi/Plant	
Bush Bean	1.89 ± 0.25	3.60 ± 0.34	3.60 ± 0.23
Wheat	3.24 ± 0.38	4.47 ± 0.08	4.79 ± 0.10
Blando Brome	3.34 ± 0.15	4.50 ± 0.02	4.76 ± 0.17

To investigate the proportion of tetryl metabolites that exist as acid-hydrolyzable conjugates, two identical samples of bush bean leaf tissue from the 7-day exposure group were homogenized and hydrolyzed for 1 h at 100°C in either water or 1 M HCl prior to extraction with methylene chloride. In the water hydrolysis treatment, the aqueous layer contained 47.4 ± 0.4%, the methylene chloride layer 2.9 ± 2.2%, and the pellet 42.0 ± 0.7% of the total radiolabel. For the acid hydrolysis treatment, the HCl layer contained 62.1 ± 1.2%, the methylene chloride 7.1 ± 0.7%, and the pellet 31.7 ± 8.5% of the total radiolabel. This experiment demonstrates that the acid hydrolysis treatment caused both the release of more radiolabel from the plant matrix (reflected in the higher percentage of extractable radiolabel) and the cleavage of some polar conjugates (reflected in the higher percentage of solubilized radiolabel that partitioned into the methylene chloride layer).

Tissues generated during the hydroponic exposures were analyzed in accordance with the analytical methodology summarized in Section 2.4.4. The resulting distribution of radiolabel among the various chemical fractions is summarized for bush bean (Tables 3.11 and 3.12), wheat (Table 3.13), and blando brome (Table 3.14) tissues. The chemical analyses show rapid metabolism of tetryl in tissues of all species, even in plants exposed for only 1 day. In fact, metabolism was so rapid that in all cases less than 3.1% of the radiolabel was isolated in fraction F1. Metabolism of tetryl proceeded toward the yield of more polar metabolic products, as evidenced by the appearance of a large percentage of radiolabel in the highly polar non-extractable aqueous base fraction. Over all analyses, 27% of the radiolabel was contained in the aqueous base fraction. A significant amount of radiolabel, averaging 14%, was contained in the extractable, yet polar, fraction F3. The pellets were found to sequester an average of 54% of the radiolabel over all analyses.

TABLE 3.11. Percentage of Total Radioactivity in the Various Chemical Fractions of Bush Bean Plants Exposed to Tetryl-Amended Hydroponic Cultures for 1 Day

Fraction	Plant Segment		
	Root	Stem	Leaves
HCl	37 ± 9	24 ± 5	54 ± 16
$MeCl_2$ acid-neutral	14 ± 3	11 ± 6	6 ± 5
Aqueous base	27 ± 6	16 ± 3	41 ± 14
$MeCl_2$ base	4 ± 1	4 ± 1	1 ± 1
F1	3.1 ± 0.8	0.6 ± 0.7	0.3 ± 0.6
F2	1.5 ± 0.2	1.4 ± 1.4	0 ± 0
F3	11 ± 3	9 ± 3	7 ± 3
Sorbent	1.4 ± 0.3	1.0 ± 0.3	2.1 ± 0.6
Pellet	46 ± 11	37 ± 15	37 ± 5

TABLE 3.12. Percentage of Total Radioactivity in the Various Chemical Fractions of Bush Bean Plants Exposed to Tetryl-Amended Hydroponic Cultures for 7 Days

Fraction	Plant Segment		
	Root	Stem	Leaves
HCl	15 ± 8	36 ± 6	62 ± 1
$MeCl_2$ acid-neutral	5 ± 2	12 ± 2	7 ± 1
Aqueous base	11 ± 6	29 ± 8	45 ± 5
$MeCl_2$ base	2 ± 1	6 ± 1	5 ± 1
F1	0.6 ± 0.2	0.3 ± 0.5	0.8 ± 0.7
F2	0.8 ± 0.4	1.6 ± 1.0	1.2 ± 1.2
F3	4 ± 2	10 ± 1	12 ± 7
Sorbent	0.5 ± 0.2	0.8 ± 0.3	1.1 ± 0.1
Pellet	54 ± 37	62 ± 10	32 ± 8

TABLE 3.13. Percentage of Total Radioactivity in the Various Chemical Fractions of Wheat Plants Exposed to Tetryl-Amended Hydroponic Cultures

Fraction	Day 1		Day 7	
	Root	Shoots	Roots	Shoots
HCl	45 ± 13	153 ± 85	22 ± 5	44 ± 3
$MeCl_2$ acid-neutral	23 ± 14	45 ± 74	5 ± 2	5.1 ± 0.3
Aqueous base	26 ± 9	35 ± 43	13 ± 2	32 ± 4
$MeCl_2$ base	5 ± 3	16 ± 28	1 ± 1	1 ± 1
F1	0.3 ± 0.3	0 ± 0	0.03 ± 0.02	0.03 ± 0.06
F2	2.6 ± 1.8	4 ± 7	0.5 ± 0.2	0.01 ± 0.01
F3	14 ± 8	64 ± 95	2.9 ± 0.7	4.0 ± 0.3
Sorbent	2.3 ± 1.2	7 ± 7	0.5 ± 0.2	0.5 ± 0.2
Pellet	36 ± 10	165 ± 118	31 ± 13	31 ± 1

TABLE 3.14 Percentage of Total Radioactivity in the Various Chemical Fractions of Blando Brome Plants Exposed to Tetryl-Amended Hydroponic Cultures

Fraction	Day 1		Day 7	
	Root	Shoots	Roots	Shoots
HCl	43 ± 13	36 ± 4	31 ± 10	55 ± 11
$MeCl_2$ acid-neutral	17 ± 2	13 ± 3	9 ± 2	9 ± 3
Aqueous base	27 ± 9	17 ± 3	18 ± 6	35 ± 5
$MeCl_2$ base	5 ± 1	5 ± 1	4 ± 1	3 ± 1
F1	3.0 ± 0.1	0.2 ± 0.3	0.7 ± 0.3	0.4 ± 0.3
F2	2.5 ± 0.4	1.3 ± 0.7	1.3 ± 0.3	0.6 ± 0.5
F3	14 ± 3	17 ± 8	14 ± 10	14 ± 12
Sorbent	1.6 ± 0.2	0.9 ± 0.3	1.0 ± 0.2	0.82 ± 0.03
Pellet	59 ± 13	48 ±10	63 ± 21	60 ± 16

The wheat shoot tissue data from plants exposed for 1 day exhibited a large amount of variability (Table 3.13). For this reason, these tissues were analyzed a second time. Results from the second analysis were almost identical to the data shown in Table 3.13. It appears, therefore, that variability in the tissues, rather than any deficiencies in the analytical procedure, was responsible for the large standard deviations observed. It should also be noted that the

shoots of the plants were diced and the tissue randomized prior to analysis. If the labelled material had in fact been restricted to only certain portions of the stem (e.g., through reduced capability for translocation), this would have added to the variability.

Only the root tissues contained metabolites in quantities above the chromatographic detection limit. Figure 3.20 (top) is a chromatogram of the F1 fraction of bush bean root tissue that contains a number of tetryl metabolites. For comparison, a corresponding chromatogram of a bush bean control root is presented at the bottom of Figure 3.20. The metabolites indicated in Figure 3.20 are representative of those appearing in the chromatographic profiles for root tissue of all three species. The chromatogram in Figure 3.20 represents an extraction of approximately 5.0 g of tissue; the profiles performed on 1.0-g aliquots of tissue contain correspondingly lower quantities of metabolites. *N*-methyl-2,4,6-trinitroaniline was present in the chromatographic profiles of root tissue; however, the parent explosive was below the detection limit. The observation that the chromatographic behavior of *N*-methyl-2,4,6-trinitroaniline on silica is similar to that of tetryl is in agreement with previous studies performed by Yusada (1970). It may be possible to quantitate *N*-methyl-2,4,6-trinitroaniline along with tetryl within the fraction F1 eluate. It is interesting that the metabolites observed in fraction F1 were of higher polarity (as evidenced by a shorter retention time) than the parent explosive. It is likely that these compounds represent the initial steps of tetryl metabolism in plants. Table 3.15 compiles the retention indices of the observed tetryl plant metabolites Results of the retention study suggest that the identity of plant metabolite 7 is *N*-methyl-2,4,6-trinitroaniline. In addition, it is probable that the identity of plant metabolite 1 is *N*-methyl-dinitroaminoaniline.

TABLE 3.15. Retention Indices of Plant Metabolites of Tetryl

Compound	Retention Index
Plant metabolite #1	852
Plant metabolite #2	861
Plant metabolite #3	873
Plant metabolite #4	889
Plant metabolite #5	(coelutes with propiophenone)
Plant metabolite #6	908
Plant metabolite #7	923
Tetryl	946
N-methyl-2,4,6-trinitroaniline	922 ± 2
N-methyl-dintroaminoaniline	851 ± 1

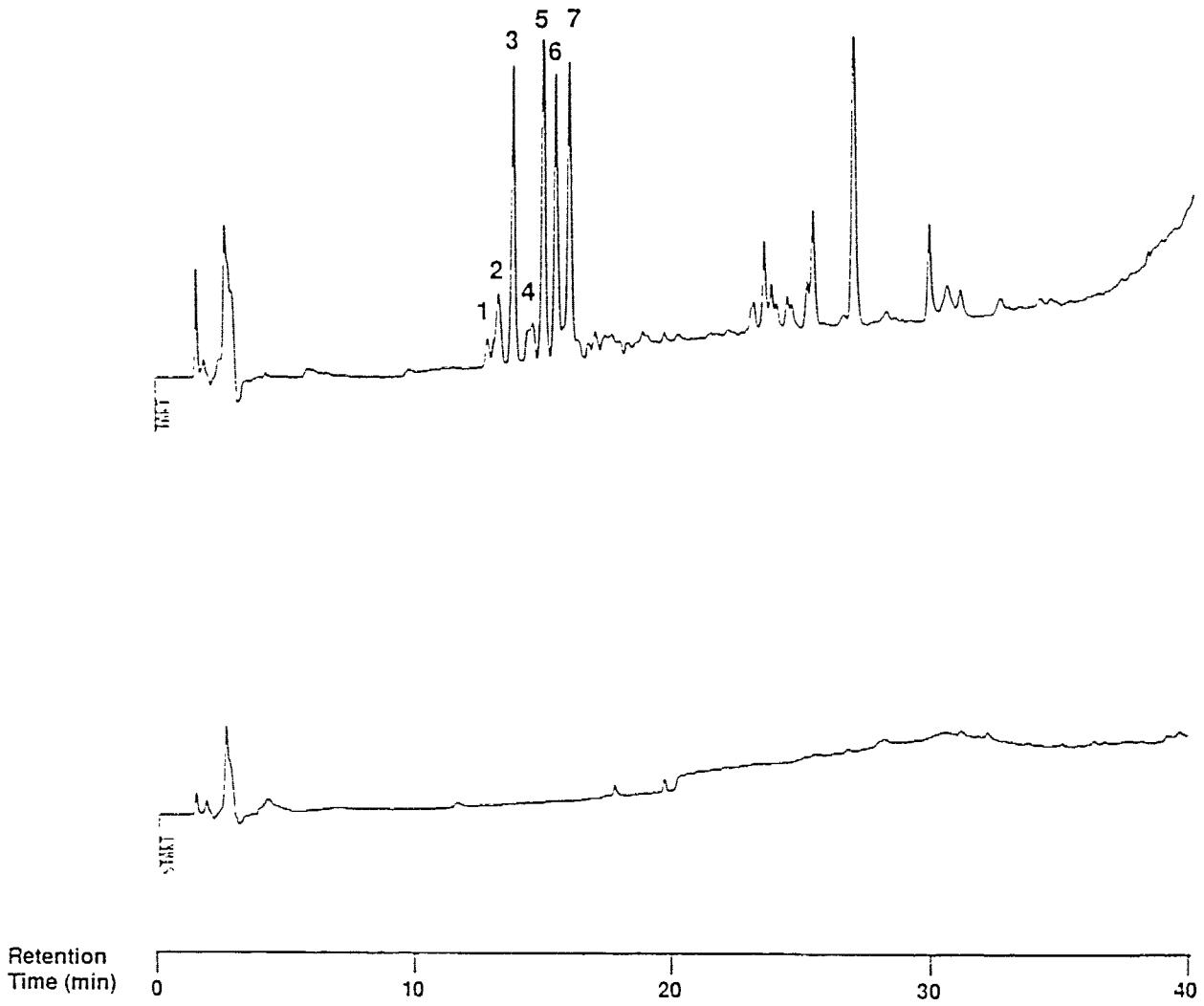

FIGURE 3.20. Chromatographic Profile of the F1 Fraction of Tetryl-Exposed Bush Bean Root Tissue (top) Compared to a Control Root Extract (bottom). Metabolites are numbered in the top chromatogram

To verify the incorporation of radiolabel in these suspected tetryl metabolites, radiochromatography was performed on the F1 fraction of bush bean root tissue from a plant exposed to tetryl-amended hydroponic solution for 4 days. The resulting radiochromatogram is presented in Figure 3.21. A variety of tetryl metabolites are observed within the 10- to 15-min retention window Unfortunately, the loss of chromatographic resolution inherent with the use of 0.5-mL fractions for radioassay precludes separation of the closely eluting individual tetryl metabolites; however, the radiochromatogram does verify elution of tetryl metabolites in the same retention window as was previously observed by UV detection. It appears that the metabolites numbered 1-7 in Figure 3.20 do contain radiolabel. Additionally, the radiochromatogram indicates that several of the later-eluting peaks (retention times of 24-27 min) are also tetryl metabolites.

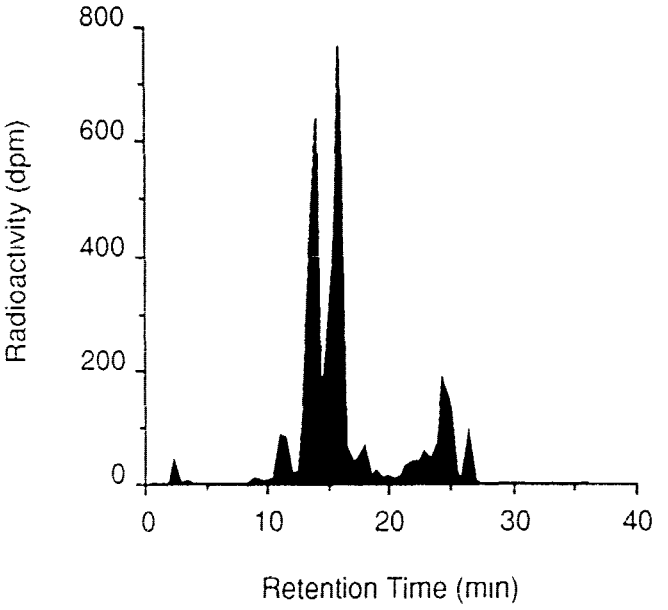

FIGURE 3.21 Radiochromatogram of the F1 Fraction of a Bush Bean Root Exposed to a Tetryl-Amended Hydroponic Solution for 4 Days

3.2.5 Transport Form of Tetryl in Xylem Exudates

Several attempts were made to colect and analyze the plant transport forms for tetryl in xylem exudates. However, in each case the plants failed to produce enough material for chemical analysis.

3.3 ABSORPTION AND CHEMICAL FATE OF TETRYL IN MATURE PLANTS GROWN IN SOIL

A major objective of the soil/plant maturity studies was to assess to what extent and in what form tetryl and/or its principal residues are accumulated, stored, and/or metabolized in soil-grown plants (bush bean, blando brome, and wheat) at physiological maturity. Preliminary studies were conducted to determine the maximum concentration of soil tetryl that could be employed in both hydroponic and long-term soil studies without inducing adverse plant effects or toxicity; this information was needed to ensure that toxicity artifacts were not encountered. In addition, the plant maturity studies were structured to elucidate accumulation and tissue partitioning of ^{14}C derived from tetryl-amended soil. Plants were grown to physiological maturity, subsampled, analyzed for total ^{14}C content, and fractionated for detailed chemical analyses. The data sets discussed below include treatments referred to as "chamber controls;" these are plants in non-amended soil that were maintained in the same growth chambers as the treatment plants. This sort of control is a standard procedure when dealing with ^{14}C-labeled organics that have the potential for either volatilization or oxidative decomposition. Actual "treatment controls" were maintained in a separate growth chamber.

3.3.1 Soil/Plant Toxicity Studies

Preliminary tetryl soil-dosing studies were performed to evaluate the potential phytotoxicity of tetryl for subsequent soil and hydroponic experiments. Pots containing 400 g of Palouse, Burbank, or Cinebar soil were amended with 0, 10, 25, 50, or 75 µg/g tetryl. For each soil type and for each concentration, the pots were seeded with either bush bean, wheat, or blando brome and the plants allowed to grow for 70 days, during which time visual phytotoxic assessments were conducted. Observations at 70 days after seeding indicated no apparent toxicity or growth effects of the tetryl in the case of the wheat and blando brome grown in any of the three soil types at concentrations up to 50 µg/g. There appeared to be a slight growth reduction in wheat and blando brome grown in Burbank soil, but these effects have not proven significant (Table 3.16). Furthermore, while the bush bean initially demonstrated an apparent visible toxicity response at concentrations over 10 µg/g in all three soil types, that also was not significant. These studies were conducted as a preliminary screening test only and so only two pots were planted for each

species. The effects of a single concentration at 60 days (the results of the partitioning studies described in Section 3.3.2) are given in detail in Tables 3.17 (bean) and 3.18 (wheat and grass).

TABLE 3.16. Plant Dry Weights Following Growth for 70 Days in Soil Amended with Tetryl at Concentrations of 0, 10, 25, 50, and 75 µg/g. Data are averages of plant above-ground dry weight (g) per plant ± variance (N=2).

Soil/Species	Tetryl Concentration (µg/g) 0	10	25	50	75
Cinebar					
Bush Bean	5.52 ± 0.93	4 88 ± 0.62	4.18 ± 1 30	5.75 ± 0.55	4.24 ± 1.32
Blando Brome	1 99 ± 0 56	1 85 ± 0.36	1 83 ± 0.30	1 81 ± 0 25	1.68 ± 0.16
Wheat	2.50 ± 0.79	2 40 ± 0.63	2.61 ± 1.01	2 34 ± 0.44	2 31 ± 0.63
Palouse					
Bush Bean	2 36 ± 0 83	2 33 ± 0 58	2.31 ± 0.41	2 48 ± 0 25	2.21 ± 0.31
Blando Brome	1 08 ± 0.08	0 90 ± 0.41	1 00 ± 0.012	0 99 ± 0.32	0 95 ± 0.09
Wheat	1 35 ± 0 25	1 34 ± 0.13	1 02 ± 0.22	1 16 ± 0.41	1 15 ± 0 19
Burbank					
Bush Bean	1.85 ± 0 33	2.84 ± 1 03	2.07 ± 0.57	1.23 ± 0 28	1.61 ± 0 11
Blando Brome	0.42 ± 0 09	0 41 ± 0.11	0.40 ± 0 05	0.37 ± 0.01	0.33 ± 0 0012
Wheat	0 38 ± 0.02	0 51 ± 0 08	0 40 ± 0 06	0 51 ± 0 06	0.62 ± 0 18

TABLE 3.17. Average Fresh Weight (g) of Bean Plants Grown for 60 Days in either Soils Amended with 25 µg/g ^{14}C-Tetryl (0.74 MBq/400 g pot) or Non-Amended Control Soils. Data are expressed as average fresh weight (g) per plant segment ± standard deviation. Corrections for fresh weight to dry weight are as follows: leaves, 0.104; stems, 0.145; pods, 0.126; seed, 0.203; and roots, 0.063.

Condition/ Soil	Plant Segment Leaf	Stem	Pod	Seed	Root	Total
Tetryl-Amended						
Cinebar	7 67 ± 2 01[a]	3 70 ± 0 47[a]	5 28 ± 3 09[a]	3 38 ± 2 38	3.41 ± 0.79	23 44 ± 7.31[a]
Palouse	4.80 ± 0 89[a]	2 50 ± 0.32	2.20 ± 0 68	0.02 ± 0 01	2.50 ± 0.32[a]	15.30 ± 2.15
Burbank	4.06 ± 1 20	2.87 ± 1.01	5.02 ± 1 12	0.79 ± 0.49	2.55 ± 0 46[a]	15.30 ± 2 15
Chamber Control						
Cinebar	10 69 ± 1 78	5.45 ± 0 57	6 29 ± 2.61	0.64 ± 0.43	3.12 ± 0.35	27 09 ± 4.07
Palouse	5.62 ± 0.53	2.72 ± 0 22	2.47 ± 0.01	0.02 ± 0.01	1.81 ± 0.32	13.14 ± 2 46
Burbank	3.35 ± 1 17	2.54 ± 0 28	4.84 ± 1.50	0 33 ± 0 30	2.08 ± 0 32	13.14 ± 2.46

[a] Differences between control and exposed plants is significant (P≤0.05) according to one-tailed t-test.

TABLE 3.18. Average Fresh Weights of Wheat and Blando Brome Plants Grown for 60 Days in either Soils Amended with 25 µg/g ^{14}C-Tetryl (0.74 MBq/ 400 g pot) or Non-Amended Control Soils Maintained in the Same Growth Chamber. Data are expressed as average fresh weight (g) per plant segment ± standard deviation (N=5 for tetryl-amended plants and N=3 for controls).

Species/ Condition	Soil	Plant Segment			
		Shoot	Root	Seed	Total
WHEAT					
Tetryl-Amended					
	Cinebar	7 02 ± 1 01[a]	8.37 ± 1 23	1 01 ± 0 871	5.99 ± 1 91[b]
	Palouse	3 78 ± 0.17	5 83 ± 0.57[a]	.[c]	9 66 ± 0 44[b]
	Burbank	2 12 ± 0 27[b]	5 24 ± 0.37[b]	0 30 ± 0.42	7 43 ± 0.37[a]
Chamber Control					
	Cinebar	10 06 ± 1 43	10 37 ± 2 87	.[c]	21.34 ± 1.87
	Palouse	3 27 ± 0 66	3 68 ± 0.68	.[c]	6 96 ± 1 07
	Burbank	3 87 ± 0 63	6 42 ± 0.43	.[c]	10 30 ± 0 19
BLANDO BROME					
Tetryl-Amended					
	Cinebar	6 66 ± 0 19	11.78 ± 0 91	1.19 ± 0.24	9 63 ± 0.82
	Palouse	2 86 ± 0 14[b]	5 29 ± 0 20[b]	0.47 ± 0 11[b]	8 61 ± 0.18[b]
	Burbank	2 11 ± 0 44	4 40 ± 1 23	0 46 ± 0 17	6 98 ± 1 70[b]
Chamber Control					
	Cinebar	8.04 ± 1 02	10 37 ± 1 86	0 43 ± 0 50	18 69 ± 1 30
	Palouse	3 58 ± 0 23	3 81 ± 0 27	0 21 ± 0 20	7 61 ± 0 26
	Burbank	3 00 ± 0 71	8 31 ± 0 91	0 22 ± 0 32	11 54 ± 0 22

[a]Differences between tetryl and control weights significant (P≤0 01) according to one-tailed t-test

[b]Differences between tetryl and control weights significant (P≤0.05) according to one-tailed t-test

[c]No tissue present

3.3.2 Long-Term Partitioning of Tetryl within the Plant

To determine effects of long-term incubation of plants in tetryl-amended soil, all three species of plants were grown in each of the test soils. As a control, plants were also grown in non-amended soil both in the same chamber as the test plants to act as a "chamber control" and in another chamber.

The specific activities of plant segments of bush beans grown in 25 µg/g ^{14}C-tetryl-amended soil are shown in Table 3.19; those of blando brome and wheat are given in Table 3.20 Based on soil type, the data show significant ($P=\leq 0.01$) differences as to the relative capabilities of tetryl uptake from the soil by all three plant species. Bush bean plants grown in the Burbank soil accumulated twice as much tetryl per gram of fresh weight as those grown in the Palouse soil (65 vs. 126 µg/g fr. wt. for the root) and four to five times that of the plants grown in the Cinebar soil (26 vs. 126 µg/g fr. wt. for the root). Similar effects are evident in the blando brome (31 vs. 78 µg/g fr. wt), but the trend was less pronounced in wheat (47 vs. 59 µg/g fr. wt.). This observation is similar to those for TNT and RDX (Cataldo et al. 1989, 1990), for which such differences were attributed to the soil organic matter content and cation exchange capacity.

The test soils show an organic carbon content of only 0.5% for the Burbank soil, as compared to 1 7% for the Palouse and 7.2% for the Cinebar (Table 2.1). Furthermore, the total CEC (meq/100 g) for the soils ranges from 5.5 for Burbank to 23.8 and 38.2 for the Palouse and Cinebar soils, respectively. These are the only soil properties to show a positive correlation with these observations of specific activity. Therefore it is possibile that a soil with the lower CEC and organic matter content is less able to bind the tetryl, rendering it more available to the plant for uptake

The total amount of specific uptake (microgram per gram fresh weight) by the plants for all three species was higher for tetryl than for either TNT or RDX. For the bush bean, tetryl specific activity was 126 µg/g fr wt. of root in plants grown in Burbank soil, as opposed to 104 for TNT and only 0.75 for the RDX. Similar differences were apparent for the other soils as well. This pattern may be a factor of the lower K_s for tetryl than for the other munitions tested to date. See Section 3.2.1. for discussion of the significance of K_s

There is an apparent accumulation, although much lower, of the tetryl in the control plants that were grown within the same chamber as the plants in amended soil (Tables 3 19 and 3.20). Thus, it appears that absorption and fixation of radiocarbon from plants within the growth chamber will have minimal effects on the results. No apparent accumulations occurred within those plants grown in other chambers (treatment controls) The tetryl calculations are based on ^{14}C equivalents from the original specific activities amended to the pots and given the observable rate

TABLE 3.19 Tetryl Uptake (µg Tetryl Equivalents[a]/g fr. wt.) for Plant Tissues of Bush Bean Grown for 60 Days in either Soils Amended with 25 µg/g ^{14}C Tetryl (0.74 MBq/400 g pot) or Non-Amended Control Soils Maintained in the Same or Separate Growth Chambers. Data are averages ± standard deviation (N=15 for tetryl-amended plants, N=6 for controls grown in another chamber, and N=3 for controls grown in the same tetryl chamber).[b]

Condition/ Soil	Plant Segment				
	Leaf	Stem	Pod	Seed	Root
Tetryl-Amended					
Cinebar	1.41 ± 0.19	2.37 ± 0.41	0.43 ± 0.13	0.54 ± 0.11	26.23 ± 5.78
Palouse	3.04 ± 0.76	4.51 ± 1.62	0.39 ± 0.13	0 51 ± 0.28	65.33 ± 14.28
Burbank	9.46 ± 2.68	9.13 ± 2.28	0.70 ± 0.24	1.02 ± 0.32	126.65 ± 25.78
Chamber Control					
Cinebar	0.18 ± 0.01	0.24 ± 0.02	0.07 ± 0.01	0.09 ± 0.01	0.23 ± 0.01
Palouse	0.15 ± 0 02	0 18 ± 0 04	0.00 ± 0.00	0.00 ± 0.00	0.22 ± 0.00
Burbank	0.24 ± 0.01	0.23 ± 0.05	0.11 ± 0.05	0.16 ± 0.01	0.23 ± 0.04
Treatment Control					
Cinebar	0.00	0.00	0.00	0.00	0.00
Palouse	0 00	0.00	0.00	0.00	0.00
Burbank	0 00	0 00	0.00	0.00	0.00

[a]Derived from specific activity

[b]Corrections from fresh weight to dry weight are as follows: leaves, 0 104, stems, 0 145, pods, 0 125, seed, 0 203, and roots 0 063

TABLE 3 20 Tetryl Uptake [µg Tetryl Equivalents[a]/g fr. wt.] for Tissues of Wheat and Blando Brome Plants Grown for 60 Days in either Soils Amended with 25 µg/g ^{14}C-Tetryl (0.74 MBq/ 400 g pot) or Non-Amended Control Soils Maintained in the Same Growth Chamber. Data are averages ± standard deviation (N=5 for tetryl-amended plants and N=3 for controls)[d].

Species/ Condition	Soil	Plant Segment		
		Shoot	Seed	Root
WHEAT				
Tetryl-Amended	Cinebar	2.40 ± 0.92	0.26 ± 0.10	8.86 ± 1.46
	Palouse	10.69 ± 3.14	-[d]	47.31 ± 14.19
	Burbank	29.04 ± 9.01	0.26 ± 0.10	59.24 ± 9.77
Chamber Control[b]	Cinebar	0.31 ± 0.02	-[d]	0.21 ± 0.01
	Palouse	0 30 ± 0.01	-[d]	0.21 ± 0.01
	Burbank	0.33 ± 0.05	-[d]	0.25 ± 0.01
Treatment Control[c]	Cinebar	0.00	-[d]	0.00
	Palouse	0.00	-[d]	0.00
	Burbank	0.00	-[d]	0.00
BLANDO BROME				
Tetryl-Amended[b]	Cinebar	2.64 ± 0.69	0.45 ± 0 05	5.25 ± 1.42
	Palouse	14.61 ± 3.72	1.83 ± 0.56	31.18 ± 8.19
	Burbank	34.41 ± 7.30	2.96 ± 0.40	78.21 ± 15.68
Chamber Control[b]	Cinebar	0.38 ± 0.05	0.31 ± 0.02	0.22 ± 0.02
	Palouse	0.26 ± 0.04	0.16 ± 0.04	0.20 ± 0.01
	Burbank	0.33 ± 0.01	0.11 ± 0.01	0.08 ± 0.04
Treatment Control[c]	Cinebar	0.00	-[d]	0.00
	Palouse	0.00	-[d]	0 00
	Burbank	0.00	-[d]	0 00

[a] Derived from specific activity

[b] Control plants grown in same chamber as amended plants

[c] Control plants grown in different chamber from amended plants

[d] Seeds not present on plant

[e] Corrections from fresh weight to dry weight are as follows: shoot, 0 188, seed 0 203, and roots, 0 127

of $^{14}CO_2$ evolution from tetryl-amended pots and soils. The tetryl accumulations were apparently caused by photosynthetic assimilation of evolved CO_2. This hypothesis is also supported by the ^{14}C-distribution patterns (shown for the control plants in Tables 3.21 and 3.22), in which there is an even distribution of the material between the leaves, stem, and root of the plants in all three soil types

Also evident from Tables 3.21 and 3.22 is a similar pattern of label distribution within the plants from all three soil types when they were amended with the tetryl. In each case, more than 75% of the total label recovered from the plants was contained within the roots. This pattern is very similar to that for TNT (a structurally similar compound), where 65 to 75% was retained within the roots, and different from that for RDX, where the label was distributed evenly throughout the plant.

TABLE 3.21 Distribution of ^{14}C in Bean Plants Grown for 60 Days in either Soils Amended with 25 µg/g ^{14}C-TETRYL (0.74 MBq/400 g pot) or Non-Amended Control Soils Maintained in the Same Growth Chamber (chamber controls). Data are expressed as average percent total ^{14}C per plant ± SD (N=5 for tetryl amended plants and N=3 for controls).

Condition/ Soil	Plant Segment				
	Leaf	Stem	Pod	Seed	Root
Tetryl-Amended					
Cinebar	11.80 ± 2.32	8.34 ± 1.21	2.02 ± 0.73	2.18 ± 0.99	75.66 ± 3.30
Palouse	9.31 ± 4.89	6.44 ± 2.31	0.57 ± 0.36	0.01 ± 0.00	83.67 ± 7.02
Burbank	7.91 ± 2.48	6.74 ± 1.86	0.93 ± 0.51	0.22 ± 0.17	84.20 ± 3.63
Chamber Control					
Cinebar	39.62 ± 1.66	34.24 ± 2.35	8.45 ± 1.01	0.36 ± 0.01	17.69 ± 0.56
Palouse	45.59 ± 4.58	25.51 ± 5.69	0.20 ± 0.16	-(a)	28.70 ± 0.45
Burbank	23.01 ± 1.21	27.27 ± 4.42	25.43 ± 10.63	5.20 ± 0.21	24.29 ± 4.08

[a] No seeds on plants

TABLE 3.22. Percentage of Total ^{14}C-Distribution for Segments of Wheat and Blando Brome Plants Grown for 60 Days in either Soils Amended with 25 µg/g ^{14}C-Tetryl (0.74 MBq/ 400 g pot) or Non-Amended Control Soils Maintained in the Same Growth Chamber. Data are average percent total label ± standard deviation (N=5 for tetryl amended plants and N=3 for controls).

Species/ Condition	Soil	Plant Segment Shoot	Seed	Root
WHEAT				
Tetryl-Amended	Cinebar	18.18 ± 4.70	0.33 ± 0.25	81.57 ± 4.67
	Palouse	15.72 ± 5.31	-(b)	84.28 ± 8.55
	Burbank	16.41 ± 4.43	0.13 ± 0.15	83.46 ± 4.43
Chamber Control	Cinebar	50.79 ± 2.79	-(b)	49.21 ± 3.18
	Palouse	49.41 ± 2.67	-(b)	50.59 ± 5.35
	Burbank	56.50 ± 8.58	-(b)	43.50 ± 9.35
BLANDO BROME				
Tetryl-Amended	Cinebar	22.24 ± 8.89	0.65 ± 0.29	77.11 ± 9.10
	Palouse	20.39 ± 3.70	0.22 ± 0.25	79.39 ± 3.78
	Burbank	17.78 ± 3.16	0.17 ± 0.20	82.05 ± 3.70
Chamber Control	Cinebar	49.79 ± 2.89	3.17 ± 1.23	47.04 ± 3.68
	Palouse	50.37 ± 6.16	3.84 ± 1.27	45.79 ± 4.84
	Burbank	52.61 ± 20.57	7.59 ± 3.04	39.80 ± 15.36

(a)Derived from specific activity

(b)Seeds not present on plant

3.3.3 Chemistry of Tetryl in Mature Plants

Tables 3.23 through 3.28 summarize the data obtained from chemical fractionation of mature soil-grown plants. These tables include the microgram equivalents of tetryl contained in each tissue. Microgram equivalents were calculated from the specific activity of the soil spike solution and the amount of radiocarbon contained in each tissue. The amount of radioactivity in the tissue samples was determined by oxidation (Section 2.4.1). Overall, 17 tissue samples were fractionated by the tetryl analysis methodology described Section 2.4.4. A mass balance can be calculated by summing the percentage of radiolabel in the HCl, the $MeCl_2$ acid-neutral, and the pellet fractions. The average mass balance calculated in this manner was 85.58 ± 22.41% overall analyses. The spent silica sorbent was found to contain an insignificant quantity of radiolabel. In all cases, the amount of radiolabel retained on the silica adsorbent after elution of fraction F3 with

methanol was less than 0.69% of the total activity contained in the tissue.

Radiolabel was not found in fraction F1 of the mature plant tissues. This is true for all tissues except shoots from wheat plants grown in Palouse soil, in which, however, only 0.01% of the total radiolabel was contained within fraction F1. The absence of radiolabel within this fraction precluded observation of the tetryl metabolites with HPLC analysis of the F1 fractions. The absence of metabolites in fraction F1 indicates that tetryl has undergone extensive metabolism to compounds whose polarity differs dramatically from that of tetryl. An average of 23.79 ± 13.84% of the radiolabel over all analyses was found to be associated with the non-extractable aqueous base fraction. This indicates that tetryl metabolism within the plant proceeds with the formation of extremely polar metabolites. The tissue pellets were also found to contain a large percentage of radiolabel. The radiolabel sequestered within the tissue pellets averaged 44.67 ± 15.09% over all analyses. Incorporation of radiolabel within the insoluble material that composes the tissue pellet is evidently an important mechanism for the detoxification of tetryl. There is also an indication of small amounts of radiolabel contained in the extractable, yet polar, F3 fraction. The amount of radiolabel contained in this fraction averages 1.79 ± 2.16% over all analyses.

TABLE 3.23 Microgram Tetryl Equivalents[a] and Percentage of Total Radioactivity, Based on Oxidations, in the Various Chemical Fractions of Mature Bush Bean Plants Grown in Burbank Soil[b]

Fraction	Plant Segment		
	Leaves	Stem	Pod
Tetryl equivalents (µg)	10.99	7.06	0.90
HCl	34	52	39
$MeCl_2$ acid-neutral	5	6	0
Aqueous base	23	38	4
$MeCl_2$ base	0	4	0
F1	0	0	0
F2	0	0	0
F3	8	4	0
Pellet	29	75	18
Sorbent	0.24	0.42	0.36

[a]Derived from specific activity

[b]Values are based on the fractionation of 1.00 g of tissue.

TABLE 3.24. Microgram Tetryl Equivalents[a] and Percentage of Total Radioactivity, Based on Oxidations, in the Various Chemical Fractions of Mature Bush Bean Plants Grown in Palouse Soil[b]

Fraction	Plant Segment		
	Leaves	Stem	Pod
Tetryl equivalents (µg)	1.84	2.35	0.42
HCl	48	68	42
$MeCl_2$ acid-neutral	0	0	0
Aqueous base	35	57	0
$MeCl_2$ base	0	1	0
F1	0	0	0
F2	0	0	0
F3	0	8	0
Pellet	34	47	29
Sorbent	0.53	0.44	0.69

[a]Derived from specific activity

[b]Values are based on the fractionation of 1.00 g of tissue

TABLE 3.25. Microgram Tetryl Equivalents[a] and Percentage of Total Radioactivity, Based on Oxidations, in the Various Chemical Fractions of Mature Bush Bean Plants Grown in Cinebar Soil[b]

Fraction	Plant Segment		
	Leaves	Stem	Pod
Tetryl equivalents (µg)	1.06	1.93	0.35
HCl	30	41	52
$MeCl_2$ acid-neutral	0	0	0
Aqueous base	13	26	9
$MeCl_2$ base	0	0	0
F1	0	0	0
F2	0	0	0
F3	0	0	0
Pellet	18	43	63
Sorbent	0.66	0.39	0.46

[a]Derived from specific activity

[b]Values are based on the fractionation of 1.00 g of tissue.

TABLE 3.26. Microgram Tetryl Equivalents[a] and Percentage of Total Radioactivity, Based on Oxidations, in the Various Chemical Fractions of Mature Wheat and Blando Brome Plants Grown in Burbank Soil[b]

Fraction	Plant Segment	
	Wheat Shoot	Blando Brome Shoot
Tetryl equivalents (µg)	29.14	28.70
HCl	36	29
$MeCl_2$ acid-neutral	6	6
Aqueous base	31	22
$MeCl_2$ base	1	1
F1	0	0
F2	1	0
F3	4	5
Pellet	55	58
Sorbent	0.37	0.38

[a]Derived from specific activity

[b]Values are based on the fractionation of 1.00 g of tissue

TABLE 3.27. Microgram Tetryl Equivalents[a] and Percentage of Total Radioactivity, Based on Oxidations, in the Various Chemical Fractions of Mature Wheat and Blando Brome Plants Grown in Palouse Soil[b]

Fraction	Plant Segment	
	Wheat Shoot[c]	Blando Brome Shoot
Tetryl equivalents (µg)	11.06 ± 2.68	12.66
HCl	35 ± 10	27
$MeCl_2$ acid-neutral	3 ± 1	3
Aqueous base	28 ± 8	19
$MeCl_2$ base	0.09 ± 0.10	0
F1	0.01 ± 0.01	0
F2	0.27 ± 0.46	0
F3	2.2 ± 0.5	3
Pellet	44 ± 10	60
Sorbent	0.25 ± 0.05	0.33

[a]Derived from specific activity.

[b]Values are based on the fractionation of 1.00 g of tissue

[c]Values are the average ± standard deviation from the analysis of 3 plants

TABLE 3.28. Microgram Tetryl Equivalents[a] and Percentage of Total Radioactivity, Based on Oxidations, in Various Chemical Fractions of Mature Wheat and Blando Brome Plants Grown in Cinebar Soil[b]

Fraction	Plant Segment	
	Wheat Shoot	Blando Brome Shoot
Tetryl equivalents (µg)	3.39	2.41
HCl	37	26
$MeCl_2$ acid-neutral	2	0
Aqueous base	27	15
$MeCl_2$ base	0	0
F1	0	0
F2	0	0
F3	0.2	0
Pellet	52	42
Sorbent	0.28	0.21

[a]Derived from specific activity

[b]Values are based on the fractionation of 1.00 g of tissue

The amount of radiolabel found in each tissue was clearly dependent on the soil in which the plants were grown. The greatest quantities of tissue radiolabel (i.e., leaves, stems, pods, or shoots) were found in plants grown in Burbank soil. For plants grown in any given soil, shoots from wheat and blando brome contained higher amounts of radiolabel than did bush bean leaf or stem tissues. For any given soil, heat shoots contained the highest amount of radiolabel of all the plant tissues. Wheat shoots from plants grown in Burbank, Palouse, and Cinebar soils contained 29.14, 11.06, and 3.39 µg tetryl equivalents per gram fresh tissue, respectively. Bush bean pod tissue contained the lowest quantity of tetryl-derived radiolabel. Pod tissue from bush bean plants grown in Burbank, Palouse, and Cinebar soils contained 0.90, 0.42, and 0.35 µg tetryl equivalents per gram fresh weight tissue, respectively. The same radiolabel availability (Burbank > Palouse > Cinebar) was observed in previous studies of both TNT and RDX (Cataldo 1989, 1990).

3.4 TETRYL BEHAVIOR IN SOIL AND RELATIVE PLANT AVAILABILITY

Whatever the phenomenon controlling tetryl solubility in soil, it has a direct influence on the availability and tissue concentrations of tetryl in plants. The tissue accumulation and tissue concentration patterns shown in Tables 3.19 and 3.20 clearly demonstrate that tetryl is not highly mobile within the plant. Furthermore, these patterns show that the extent of uptake is characteristic of soil type when the specific activity of the tissue is considered. However, it should

also be noted that, as shown in Tables 3.17 and 3.18, significant differences in fresh weight are evident between plants grown in the differing soils. These differences may contribute to the differences observed in specific activities when there is less tissue in the plant to begin with. As a confirmation of the potential for soil differences to affect tetryl uptake, Table 3.29 shows uptake per plant This data still show significant differences between the soil types.

Two soil properties correlate with these observations, CEC and soil organic matter. In Figures 3.22 through 3 25, concentrations of tetryl in whole plants and shoots are plotted against CEC and soil organic matter. Shoot concentrations of tetryl are based on all shoot tissues following harvest of mature plants grown on Burbank, Palouse, and Cinebar soils. Overall, correlations of plant concentrations with either CEC or soil organic matter show a distinct inverse relationship that is consistently significant ($r^2 > 0.7$). Slope variations for the whole plant and the shoot alone concentrations are not statistically different, although overall the bush bean whole-plant values are somewhat less than those of the two monocots.

TABLE 3.29. Average Total Plant Uptake (microgram tetryl equivalents[a] per plant, N=5) for Bush Bean, Wheat, and Blando Brome Grown for 60 Days in Soils Amended with 25 µg/g ^{14}C-Tetryl (0.74 MBq/400 g pot). Data are averages ± standard deviation.

Soil	Species		
	Bush Bean	Wheat	Blando Brome
Cinebar	113.25 ± 11.24	90.11 ± 6.19	77.14 ± 5.13
Palouse	195 04 ± 33.77	302.88 ± 49.48	195.04 ± 33.77
Burbank	376.69 ± 44 32	371.30 ± 41 49	405.92 ± 57.95

[a]Derived from specific activity

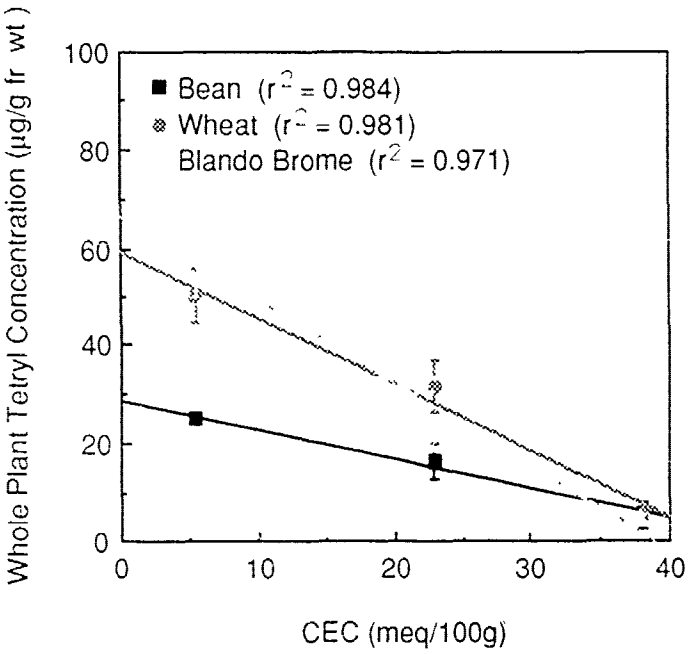

FIGURE 3.22. Relationship of Cation Exchange Capacity to Whole-Plant Tetryl Concentrations in Bush Bean, Wheat, and Blando Brome

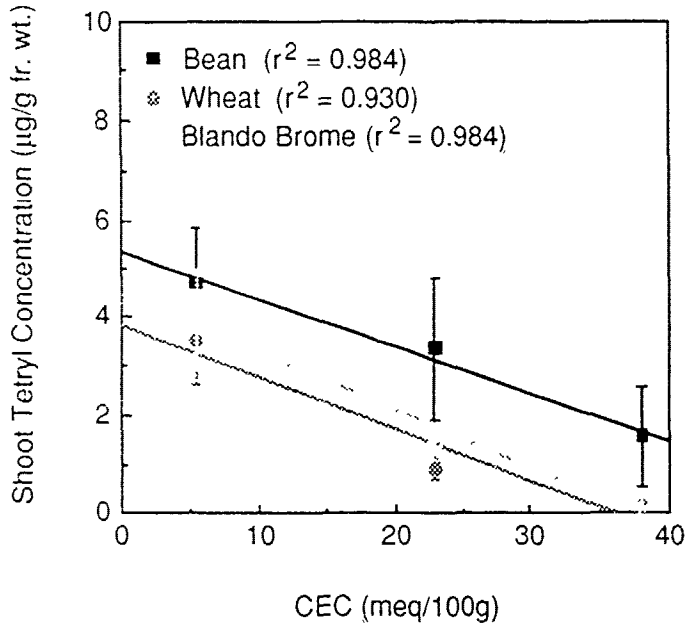

FIGURE 3.23. Relationship of Cation Exchange Capacity to Shoot Tetryl Concentrations in Bush Bean, Wheat, and Blando Brome

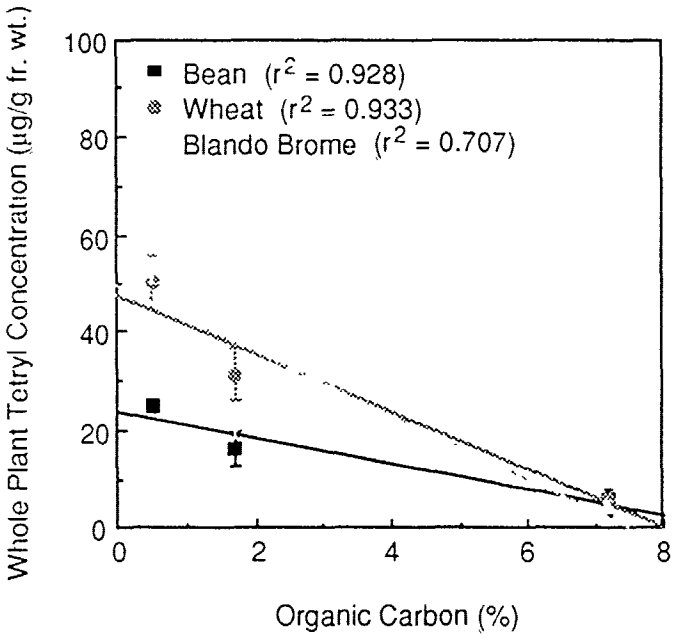

FIGURE 3.24. Relationship of Soil Organic Carbon Content to Whole-Plant Tetryl Concentrations in Bush Bean, Wheat, and Blando Brome

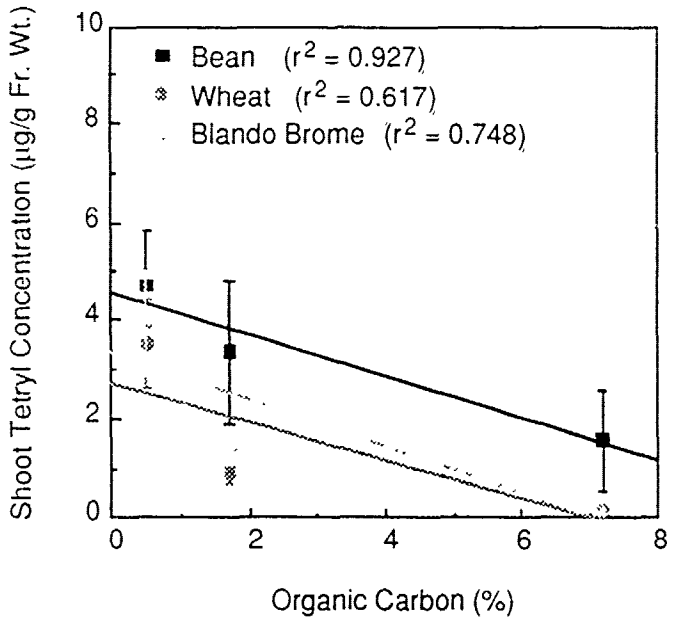

FIGURE 3.25. Relationship of Soil Organic Carbon Content to Shoot Tetryl Contentrations in Bush Bean, Wheat, and Blando Brome

4.0 SUMMARY AND CONCLUSIONS

The purpose of the present studies was to determine the environmental behavior and fate of 2,4,6-trinitrophenylmethylnitramine (tetryl) in the soil/plant system, with particular emphasis on chemical transformations that may impact transport in the food chain. Studies employed three plant species and three soil types as surrogates for understanding the plant root absorption process, soil constraints on plant uptake, plant partitioning following uptake, and the role of tetryl in soils and plants. The study of tetryl behavior required that suitable chemical extraction and analytical procedures be developed for soil and plant matrices. Using these methodologies, the physical and chemical fate of tetryl was assessed for both soils and plants.

4.1 TETRYL BEHAVIOR IN SOILS AND PLANTS

4.1.1 Tetryl Fate in Soils

Soil Partitioning and Fate

The chemical behavior and transformations of tetryl in soil were more complex than those reported for either TNT (Cataldo et al. 1989) or RDX (Cataldo et al. 1990). Mass-balance analyses of the three soil systems, employing Burbank, Palouse, and Cinebar soils, was very good at 2 h after amendment, with over 97% of the amended radiolabel present in either the methanol extract or the extracted soils. The mass-balance deficit was found to rise with time after amendment. At 60 days after amendment, the mass-balance deficit was as high as 21%, indicating losses due to either volatilization of transformation products or mineralization.

Immediately following amendment (in soils amended with radiolabeled-tetryl at 60 µg/g), most of the radiolabel was extracted and present in the methanol extract. However, at most 50% of the radiolabel was speciated as tetryl in these extracts, indicating that transformation of tetryl in soil is extremely facile. Transformations continued at a rapid rate, with less than 8% of the extracted radiolabel found speciated as tetryl in the 11-day extracts; by 30 days after amendment, soil concentrations of tetryl were below the detection limit of 2.5 µg/g. The amount of radiolabel that was irreversibly bound to the soils was commensurate with the amount of organic carbon contained in the soils. Immediately after amendment, the bound or non-extractable radiolabel in Burbank, Palouse, and Cinebar soils was 1.8, 2.8 and 17%, respectively. At the end of the 60-day study, the non-extractable radiolabel content of the soils ranged from 43 to 58%, indicating a strong binding to soil exchange sites or mineral associations. Soil sterilization increased the

recovery of unaltered tetryl in Burbank and Palouse soils, but not in Cinebar soil.

Mineralization

Gas-exchange experiments were conducted to assess any release of volatile organics or mineralization for the three soils, and thus to refine the mass-balance deficits observed in these soils. No volatilization of organic residues was indicated at 28 days after amendment. Mineralization rates ($^{14}CO_2$ evolution) for soils amended with 10 µg/g ^{14}C-tetryl (5 µCi, 185 KBq) at 21 to 24 days after amendment ranged from about 3000 to 20,000 dpm per day. While the tetryl was only 98.7% pure, the total activity released over the 21 days would indicate that approximately 3 to 7 µg of tetryl equivalents per day might have been converted to CO_2. These mineralization rates are greater than those observed for RDX-amended soils (Cataldo et al. 1990) and those reported for TNT (Cataldo et al. 1989).

Chemical Transformations

The extent of tetryl chemical transformation was investigated in soils (60 µg/g) incubated over a 60-day period. Transformations were rapid, with tetryl concentrations rapidly declining (50% of extractables at 2 h, < 2% at 11 days) and the appearance of four principal tetryl transformation products Stringent analytical methodologies were employed to identify these unknown transformation products. Peak 4, which is the first and also the major transformation product to appear, was shown to be *N*-methyl-2,4,6-trinitroaniline, which was previously reported by Dubovitskii et al. (1961). Tetryl transformation product 3 was purified by semi-preparative reversed-phase HPLC from an extract of Palouse soil that had been incubated with tetryl for 11 days. This compound was shown to be *N*-methyl-aminodinitroaniline isomer arising from nitroreduction of *N*-methyl-2,4,6-trinitroaniline. Given these tetryl transformation products, two independent tetryl soil transformation pathways are proposed. The principal transformation pathway involves the formation of *N*-methyl-2,4,6-trinitroaniline (transformation product 4). The less prominent pathway involves direct nitroreduction of the parent munition to form a dinitroaminophenylmethylnitramine isomer (transformation product 3). Other compounds observed in radiochromatographic profiles of soil aged with ^{14}C-tetryl are probably further reduction products of these initial transformation products.

4.1.2 Plant Availability and Fate - Short-Term Hydroponic Studies

Hydroponic studies were performed to address several basic needs. The first was to establish the physiological capacity of plants to absorb and transport tetryl in the absence of soils

and their sorptive components. The second need was to elucidate the extent of partitioning of tetryl, particularly to the root, because that is not amenable to analysis in soil systems. And the third need was to establish the short-term chemical fate of tetryl in plant tissues, including roots.

Plant Availability of Tetryl

To determine the rate of uptake and relative bioavailability of tetryl, a series of hydroponic experiments were undertaken with three plant species: bush bean, wheat, and blando brome. Plants exposed to solutions containing 1 to 10 μg/g tetryl showed significant differences (P=0.05) in uptake rates in the absence of soil mediating factors (i.e., soil adsorption). The uptake rates determined for tetryl differ from those reported for either TNT (Cataldo et al. 1989) or RDX (Cataldo et al. 1990), in that uptake rates for bush bean were substantially greater for TNT and RDX at all concentrations employed.

Calculated K_s values (analogous to the Michaelis-Menten K_m) for all three plant species are much lower than those previously observed for either TNT or RDX (Cataldo et al. 1989, 1990). This difference may indicate that the plants have a greater affinity for tetryl than for the other munitions. The bush bean K_S value was only 12.8 μM for the tetryl, but similar studies yielded values of 280 and 389 μM for the TNT and RDX, respectively (Cataldo et al. 1989, 1990). Correspondingly, the wheat K_S of 10.7 μM was also lower than the respective TNT and RDX values of 133 and 270 μM, and the K_S of the blando brome, 38.3 μM, was not quite as low in comparison to the 138- and 96-μM values for the TNT and RDX, respectively, but it was still significantly less. Although the plants exhibited a greater affinity for tetryl, the apparent capacity of the roots to absorb tetryl (V_{max}) was much lower than those reported previously for either TNT or RDX. The V_{max} values observed for TNT ranged from 5.6 to 15.4 μM per hour per gram fresh weight root among the three species, and those for RDX ranged from 0.24 to 0.98 μM per hour per gram fresh weight root. The tetryl values were therefore 2 to 150 times less.

These results suggest that tetryl is effectively absorbed by the roots of plants. The absorption affinity for tetryl is tempered by the comparatively low root absorption capacity, at least for short-term exposures. After 1 h of uptake from 10-μg/g solutions, each plant had accumulated approximately 40 μg of tetryl per gram fresh weight. This accumulation is more than that observed for TNT (29 μg/g fr. wt. in 2 h) (Cataldo et al. 1989) and much more than that for RDX (6 μg/g fr. wt. in 2 h) (Cataldo et al. 1990) at an exposure concentration of 10 μg/g. Furthermore, in contrast to TNT and RDX, no significant variations in tetryl uptake rates were noted between plant species.

Plant Partitioning of Tetryl

Partitioning patterns for the short-term exposures (1 to 7 day uptake) more closely resembled those for TNT, where the overwhelming majority of the label was retained by the root (>90%) (Cataldo et al. 1989). In bush bean, after 7 days of uptake, 92% of the label was associated with the root, 6% with the stem, and only 2% with the leaves, indicating that the plant mobile species is not readily transported and is probably sequestered. The chemical structures of TNT and tetryl are similar and their partitioning pattern resembles those of the dinitroanaline herbicides, which are structurally similar to the munitions. The overall partitioning pattern for tetryl was unlike that for RDX, where a more uniform distribution between the root and the shoot was observed (Cataldo et al. 1990).

Mineralization

Tetryl and related residues that are absorbed by plant roots and exported to the stem and leaves may 1) be further metabolized to a form that would be innocuous to the plant, 2) be sequestered and/or stored, 3) undergo further metabolic conversions to achieve a final form as CO_2, and/or 4) pass through the plant *via* the transpiration stream to be released to the atmosphere essentially unchanged. To determine the potential for these last two processes, hydroponic-grown bush bean plants were exposed to a nutrient solution containing 7.5 µg/g ^{14}C-tetryl and mass balances determined, including losses due to volatilization of organic residues and mineralization.

Mass balance for the 72-h study was 91%. Approximately 42%, or 1100 µg tetryl, was accumulated within the plant. This was much higher than the 15%, or 692 µg, reported for RDX but less than that for TNT (~68%) (Cataldo et al. 1989, 1990). Respiratory losses from the root accounted for 0.4% or 11-µg tetryl equivalents. This loss was less than that observed with the RDX, for which 21-µg equivalents were reported. The assumed root respiration of tetryl from the plant was shown to increase in a linear manner with time. No significant quantities of organic volatiles were detected from the roots or shoots of plants.

Plant Chemical Transformations

Hydroponically grown plants, exposed to 10 µg/g tetryl containing 5 µCi radiolabeled tetryl, were employed to investigate plant-related chemical transformations. Control solutions, without plants, were employed to determine tetryl stability during the study. Solutions exposed to growth chamber lights immediately acquired a bright yellow coloration, indicating that tetryl had

undergone photodecomposition; this was evident in tetryl concentrations after 1 day; after 7 days, 50 to 90% of the tetryl had undergone transformation. Decomposition was not as evident in controls kept in the dark. Analysis of the colored solutions by HPLC indicated the initial presence of methyl picramide. After 7 days of exposure to light, HPLC profiles showed that the yellow solutions solutions were devoid of methyl picramide.

Analysis of plant-containing solutions revealed both plant uptake (based on tracer depletion) and root-catalyzed transformations (based on the disappearance of tetryl). The amount of tetryl was near or below the detection limit of 0.1 µg/g in all plant hydroponic solutions after 4 days. However, radiolabel continued to be assimilated by the plants even after all of the tetryl had been transformed, indicating that tetryl transformation products as well as tetryl were being absorbed by the plant root.

From the tissue analyses performed after 7 days, it is clear that the plants had accumulated the majority of the available label. A significant quantity (28%) of the bush bean root-associated label was removed by washing, indicating that, at least in bush bean, tetryl residues are sorbed to the exterior of the root. This phenomenon was less pronounced in wheat and blando brome.

Plant Chemistry of Tetryl

Preliminary analyses of hydroponically treated bush bean leaf tissues were undertaken to investigate the proportion of tetryl metabolites that exist as acid-hydrolyzable conjugates. This study indicated that acid hydrolysis, as compared with water hydrolysis, caused both the release of more radiolabel from the plant matrix (reflected in the higher percentage of extractable radiolabel) and the cleavage of some polar conjugates (reflected in the higher percentage of solubilized radiolabel that partitions into the methylene chloride layer).

Analysis of tissues generated during the hydroponic exposures followed the chemical analyses by acid hydrolysis. Chemical fractionation and analyses show rapid metabolism of tetryl in tissues of all species, even in plants exposed for only 1 day. In fact, metabolism was so rapid that in all cases less than 3.1% of the radiolabel was isolated in fraction F1 (the tetryl fraction). Metabolism of tetryl proceeded toward more polar metabolic products, as evidenced by the appearance of a large percentage of radiolabel in the highly polar non-extractable aqueous base fraction. Over all analyses, 27% of the radiolabel was contained in the aqueous base fraction. A significant amount of radiolabel, averaging 14%, was contained in the extractable, yet polar, fraction F3. The pellets were found to sequester an average 54% of the radiolabel over all

analyses. This indicates a rapid metabolism, sequestering, or conjugation of tetryl and its transformation products.

Only the root tissues contained extractable metabolites in quantities above the chromatographic detection limit for the methods employed. Analysis of the F1 fraction of bush bean root tissue, which contains a number of tetryl metabolites, indicates that although tetryl is below detection limits, *N*-methyl-2,4,6-trinitroaniline (the photodecomposition product that had disappeared at 7 days in the hydroponic study) is present in high concentration, and that *N*-methyl-dinitroaminoaniline is present. Several other major metabolites are also seen. Given that the metabolites observed in fraction F1 were of higher polarity (as evidenced by a lower retention time) than the parent explosive, it is likely that these compounds represent the initial steps of tetryl metabolism in plants.

4.1.3 Tetryl Behavior and Chemical Form in Mature Plants

A major objective of the soil/plant maturity studies was to assess to what extent and in what form tetryl and its principal residues are accumulated, stored, and/or metabolized in soil-grown plants (bush bean, blando brome, and wheat) at physiological maturity. Preliminary studies were conducted to determine the maximum concentration of soil tetryl that could be employed in both hydroponic and long-term soil studies without inducing adverse plant effects or toxicity; this test ensured that toxicity artifacts were not encountered. In addition, the plant maturity studies were structured to elucidate accumulation and tissue partitioning of ^{14}C derived from soil amended with tetryl.

Tetryl Toxicity

Preliminary tetryl soil-amendment studies were performed to evaluate the potential phytotoxicity of tetryl for subsequent soil and hydroponic experiments; plants were seeded to pots containing tetryl concentrations of 0, 10, 25, 50, or 75 µg/g, and growth was monitored for 50 days. Observations 50 days after seeding indicated no apparent toxicity or significant growth effects of the tetryl on the wheat and blando brome grown in any of the three soil types at concentrations up to 50 µg/g. To preclude toxicity problems, the maturity studies were conducted at 25 µg/g tetryl.

Plant Uptake and Partitioning

Results for plants grown in 25-μg/g ^{14}C-tetryl-amended soil indicate significant ($P \leq 0.01$) differences in the total relative uptake of tetryl by all three plant species based on soil type. Bush bean plants grown in the Burbank soil accumulated twice as much tetryl per gram fresh weight as those grown in the Palouse soil (74 vs. 147 μg/g fr. wt.) and four to five times as much as plants grown in the Cinebar soil (32 vs. 147 μg/g fr. wt.). Similar uptake differences are evident for the blando brome (48 and 115 μg/g fr. wt. for Burbank and Palouse), but this trend was not as pronounced in wheat (58 vs. 89 μg/g fr. wt.). These observations are similar to those reported for TNT and RDX (Cataldo et al. 1989, 1990), for which such differences were attributed to the soil organic matter content and cation exchange capacity.

Analysis of the tissue distribution patterns indicates that tissue partitioning for mature plants grown on soil differs from that observed in short-term hydroponic studies. Although the root remains the primary repository of accumulated tetryl residues, the shoots accumulate substantially more of the residues. For bush bean, the roots contain ~80% of the label, the leaves ~10%, the stems ~7%, and the seed and pod ~3%. In wheat and blando brome, 16 to 20% of the accumulated label is found in the shoot tissues. This distribution pattern indicates that significant transport of tetryl residues to plant shoots can occur over their life cycle. This pattern is very similar to that for TNT (a structurally similar compound), where 65 to 75% was retained within the roots, and different from that for RDX, where the label was distributed evenly throughout the plant.

Chemical Fate of Tetryl in Mature Plants

Fractionation and analysis of mature plant tissues indicate an overall mass balance of 83.6 ± 22.4%, by summing the percentage of radiolabel in the HCl, the $MeCl_2$ acid-neutral, and the pellet fractions.

Radiolabel was not found in fraction F1 of the mature plant tissues analyzed. The absence of metabolites in fraction F1 indicates that tetryl has undergone extensive metabolism to compounds that differ dramatically in their polarity. This absence precluded detailed chemical analysis of tetryl and its immediate transformation products. An average of 23.8 ± 13.8% of the radiolabel over all analyses was found to be associated with the non-extractable aqueous base fraction. This indicates that tetryl metabolism within the plant proceeds with the formation of extremely polar metabolites. The tissue pellets were also found to contain an average of 44.7 ± 15.1% over all analyses. Incorporation of radiolabel within the insoluble material that composes the tissue pellet is evidently an important mechanism for the detoxification of tetryl.

It is important to note that although the F1 fraction, which classically contains the early metabolites of tetryl and other munitions, contains no activity, this pattern may be indicative of either total degradation and incorporation of residues or conversion to much more polar metabolites, including conjugates. These alternatives may represent an important factor in understanding the potential fate and impacts of tetryl in the environment.

4.1.4 Influence of Soil Type on Plant Availability of Tetryl

Soil acts as a mediator in controlling the availability of plants and the absorption of both xenobiotics and nutrients contained in soil. Studies with tetryl clearly indicate that soil characteristics play a role in both tetryl availability and tetryl residues available to the plant through the reported chemical transformations. Prior studies with TNT and RDX (Cataldo et al. 1989, 1990) indicated that soil CEC and organic matter content were important in plant uptake of these munitions. In the present study with tetryl, these soil properties were again found to be important and to influence plant uptake in a predictable manner.

Correlation of CEC and organic matter content with plant tetryl concentrations shows a linear response for both total plant (including root) concentration and shoot concentrations. Regression analyses indicate that bush bean is less sensitive to these factors than the monocots are based on whole plant concentrations. On a shoot concentration basis, response slopes for the soil parameters are similar. These correlations may indicate that there are differences in root absorption potential between dicots and monocots, and the difference may be based not so much on teryl uptake as on the uptake of tetryl transformation products in soil.

4.2 RESEARCH NEEDS

Tetryl, like TNT and RDX, is readily accumulated from soils by plants. The ability of plants to accumulate both the parent compound and tetryl soil transformation products is important to understanding potential environmental and health impacts associated with manufacturing and disposal. Although these studies indicate that the uptake, plant distribution, and chemical fate of tetryl may vary with plant species and soil parameters, a number of critical questions require resolution.

The first is, what is the relative availability to plants of munitions residues for those plant species currently used in site reclamation or in agricultural use? Will the plant tissue distributions and concentrations vary for plants with typical growing seasons of 120 days or perennials be different from those seen with representative species? Second, and possibly more critical, what

is the actual fate of accumulated residues? The absence of tetryl or known tetryl residues does not indicate that there is no potential impact. The formation of polar residues is a normal biological approach to detoxification of xenobiotics in both plants and animals. Do these polar residues and/or conjugates represent a hidden risk to the environment and humans? These questions must be addressed to allow unimpeded remediation of currently contaminated sites.

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