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Determination of the explosive 2,4,6-trinitrophenylmethylnitramine (tetryl) and its transformation products in soil

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ABSTRACT

Soils amended with uniformly ring-labeled [^{14}C]-2,4,6-trinitrophenylmethylnitramine (tetryl) were subjected to exhaustive Soxhlet extraction followed by high-performance liquid chromatographic analysis immediately after amendment and at 11, 30, and 60 days post-amendment. Transformation of tetryl was found to be extremely rapid. Tetryl was below the detection limit in soil extracts by 30 days post-amendment. Radiochromatographic profiles revealed the presence of a variety of transformation products. The primary transformation product appeared in soil extracts immediately after amendment and was subsequently identified as N-methyl-2,4,6-trinitroaniline. A minor transformation pathway was identified that involved direct ring nitro reduction of tetryl, resulting in the production of an aminodinitrophenylmethylnitramine isomer. The mass balance of the soil system was greater than 79% over the 60-day study, with 43% of the amended radiolabeled found to be non-extractable at the conclusion of the study.

INTRODUCTION

The explosive 2,4,6-trinitrophenylmethylnitramine, commonly referred to as tetryl (structure given in Fig. 7), detonates with more force and less shock or frictional provocation than either 2,4,6-trinitrotoluene (TNT) or hexahydro-1,3,5-trinitrotriazine (RDX). As such, tetryl has found its primary use as either a booster explosive or a base charge in blasting caps and detonators. Substitution of more stable high explosives, such as RDX, for uses traditionally dominated by tetryl has been a recent trend that has culminated in discontinuation of tetryl production within the United States [1].

Munitions manufacturing, packing and decommissioning facilities produce large quantities of waste waters containing explosives residues. In the past, these manufacturing rinse waters were directed to lagoons for primary settling before being discharged to rivers and streams. For environmental reasons, this practice has long been abandoned; however, environmental concerns persist because evaporation of lagoons has resulted in localized areas of severe contamination. Although TNT and RDX were the primary contaminants contained in discharge waste waters, significant amounts of other explosives residues, including tetryl, were released. The potential pollution problem becomes apparent when one considers that tetryl production resulted in daily release of an estimated 16 kg of tetryl from a single manufacturing plant [2]. As the toxic parent munitions and their soil transformation products are available for plant uptake, it becomes imperative to delineate soil transformation pathways, the propensity for plant uptake of these compounds and further plant metabolic alteration of the munitions residues. Such studies will ultimately result in understanding the impact of lagooning practices on food-chain transfer of munitions residues.

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The toxicity of tetryl has been documented. Acute toxic effects of tetryl exposure include skin irritation and dermatitis [3,4]. These symptoms were commonly manifested by munitions workers in early production facilities that lacked vigorous hygiene precautions. Tetryl has also been found to be mutagenic in several different bacterial assays [5].

Although studies elucidating the environmental fate of tetryl are absent from the literature, the toxicity of the possible transformation products is sufficient for concern. The related polynitro aromatic explosive TNT is known to undergo bacterial metabolism to nitroso metabolites [6]. Further reduction of the nitroso group to an amino functionality is a transformation pathway that also is well documented for TNT [6]. Transformation of tetryl could proceed in an analogous manner by reduction at either the aniline nitro group or the aromatic nitro positions resulting in formation of N-nitroso or nitroso transformation products, respectively. The mutagenicity of both these classes of compounds is well established.

A survey of the analytical literature pertaining to tetryl reveals that gas chromatographic analysis of water exracts with electron-capture detection can achieve detection limits ranging from *ca.* 1000 to 20 parts per 10^{12} (ppt) [7,8]. However, the thermal lability of this explosive would seem to indicate an obvious advantage for the use of condensed-phase over gas chromatographic separation techniques. A number of researchers have described high-performance liquid chromatographic (HPLC) methods utilizing ultraviolet absorption detection for the determination of tetryl [9–11]. Several of these methods have been applied to the determination of explosives in soil extracts [10–13].

The aim of this study was to investigate the environmental fate of tetryl in soil with the primary objective of gaining a clear understanding of the products available for plant uptake. Uniformly ring-labeled tetryl was utilized to evaluate the soil system for a mass balance of amended analyte and to allow for unambiguous identification of transformation products.

EXPERIMENTAL

Uniformly ring-labeled [^{14}C]tetryl, having a specific activity of 14.64 mCi/mmol, was obtained from New England Nuclear (DuPont, Boston, MA, USA). Radio-HPLC was utilized to assess the purity of [^{14}C]tetryl. During the chromatographic analysis (conditions described below), successive 0.5-ml increments of the column eluate were collected. After the addition of 15.0 ml of Ready-Solv EP cocktail (Beckman Instruments, San Ramon, CA, USA), the individual fractions were assayed for radiocarbon by liquid scintillation spectrometry. The purity of [^{14}C]tetryl was determined to be 98.70%. This purity was judged adequate for subsequent environmental fate studies and the material was used without further purification. Bulk tetryl was obtained from the US Biomedical Research and Development Laboratory (Fort Detrick, Frederick, MD, USA). A small amount of authentic tetryl was obtained from the US Army Toxic and Hazardous Materials Agency (Aberdeen Proving Ground, MD, USA) and served as a standard analytical reference material (SARM). HPLC co-injection experiments with the SARM provided verification of the identity of radiolabeled and bulk tetryl.

Soil amendment and incubation conditions

Palouse soil, a typical Washington State agricultural soil, was used for most studies. Palouse is a silt-loam, mixed Pachic Ultic Haploxeroll. The sample was collected at Pullman, WA, USA, and consisted of the Ap horizon [14]. This soil is 77% silt and 21% clay, contains 2% organic matter, has a cation-exchange capacity of 23.8 mequiv. per 100 g and a pH of 5.4. For comparison to Palouse soil, Burbank (a sandy loam having a pH of 7.4) and Cinebar (a silt loam having a pH of 5.6) soils were also studied. Solutions containing appropriate proportions of non-radiolabeled and ^{14}C-labeled tetryl were prepared in 2.0 ml of methanol and amended to 400 g of air-dried soil to give a final concentration of 60 ppm tetryl containing 10 μCi of radiolabeled tetryl. Uniform amendment was facilitated by thoroughly cutting the tetryl solution into the soil for 15 min with a spatula prior to packing the soil into a plastic-lined carton. Amended soils were immediately brought to and maintained at 66% field capacity with water. Soils were incubated in a growth chamber environment that simulated the luminous intensity and spectral dispersion of sunlight during the 16-h light period. The chamber was maintained at a day/night temperature of 26/22°C and a relative humidity of 50%.

Analytical separations

Analytical separations were performed on a system consisting of a Waters Model 600E controller and pump and a Waters Model 490E detector (Waters Assoc., Milford, MA, USA). Injections (20 μl) were provided to a Beckman Ultrasphere ODS (5 μm) (24 cm × 4.6 mm I.D.) column by a Waters WISP Model 710 automatic injector. The absorbance maximum of tetryl in acetonitrile was determined to be 264 nm (Beckman DU-7 spectrophotometer). This wavelength was utilized at a detector sensitivity of 0.008 a.u.f.s. for all chromatographic profiles generated in this study. Separations were effected by solvent programming the acetonitrile–water mobile phase from 35 to 100% acetonitrile over 30 min and maintaining the final mobile phase composition for an additional 10 min. HPLC-grade solvents, obtained from J. T. Baker (Phillipsburgh, NJ, USA), were used throughout these studies. Integrated peak areas, provided by a Hewlett-Packard (Avondale, PA, USA) Model 3390A integrator, formed the basis for quantification.

Representative soil extracts were further analyzed by radiochromatography in order to identify unambiguously tetryl transformation products. Once incorporation of radiolabel had been verified by radiochromatography, soil transformation products were further characterized by determining their alkylphenone retention indices [15,16].

Ancillary techniques

The column eluate corresponding to the elution of tetryl transformation products was collected during repetitive chromatographic runs to collect sufficient material for further chemical characterization. Fourier transform infrared (FT-IR) spectra were obtained on NaCl pellets by use of an IR microscope (IR-Plan advanced analytical microscope, Spectra-Tech, Stamford, CT, USA) interfaced to a Nicolet (Fremont, CA, USA) Model 740 spectrometer. Analysis by gas chromatography–mass spectrometry (GC–MS) of the HPLC-purified transformation products was conducted on a Hewlett-Packard Model 5890 gas chromatograph interfaced to a Hewlett-Packard Model 5970 mass-selective detector. Samples were introduced to a 30 m × 250 μm I.D. DB-5 (d_f = 1.0 μm) column (J & W Scientific, Folsom, CA, USA) by on-column injection, at which time the column was programmed from 110 to 300°C at 10°C/min. Direct insertion probe analyses were performed on a Hewlett-Packard Model 5985 mass spectrometer operated at a source temperature of 200°C in either the 70-eV electron impact or the chemical ionization mode. For chemical ionization experiments, isobutane reagent gas delivery was adjusted to achieve $1.8 \cdot 10^{-4}$ Torr as measured external to the source.

Extraction of soils

Soils were sampled (10.00 g) in triplicate immediately after amendment and at 11, 30 and 60 days post-amendment. Samples were placed in preweighed glass extraction thimbles and subjected to exhaustive Soxhlet extraction with 200 ml of methanol for 48 h. The first soil extractions (t = 0) were initiated within 30 min of amendment. The Soxhlet apparatus was wrapped with aluminum foil during extractions to minimize photodecomposition of tetryl. After extraction, the methanol extract was filtered through a 0.45-μm nylon 66 filter (Alltech, Deerfield, IL, USA) and reduced in volume to *ca.* 20 ml by rotary evaporation. The concentrated extract was then filtered through a 0.45-μm nylon 66 filter and the final volume adjusted to 25.0 ml. Samples of the final extract were removed for liquid scintillation spectrometry and HPLC analysis. The methanol distillates resulting from rotary evaporation and spent filters were assayed for radioactivity by liquid scintillation spectrometry.

Residual methanol contained in the soils after extraction was removed under vacuum (*ca.* 10^{-4} Torr) for 14 h. Accurate soil dry weights were then obtained and sub-samples removed for oxidation. Oxidation of the extracted soils, performed on a Packard (Downers Grove, IL, USA) Model 306 oxidizer, allowed the determination of the non-extractable radiolabel that was associated with the soil matrix.

Room-temperature methanol extraction was performed on tetryl-amended Palouse soil for comparison with extracts generated by the Soxhlet extraction procedure. Extraction was effected by agitating 6.0 g of soil with 10 ml of methanol on a vortex mixer for 15 min. After centrifugation, the supernatant was decanted and the extraction repeated with two additional portions of methanol. The methanol extracts were then pooled, filtered and reduced to dryness with a stream of dry nitrogen. The

residue was reconstituted with 15.0 ml of methanol prior to analysis by HPLC.

Determination of $^{14}CO_2$ and volatile organic emissions

The emission of volatile organics and $^{14}CO_2$ from tetryl-amended soil was monitored by a previously described technique [17]. Briefly, soil pots were enclosed within a sealed canister through which air was drawn by vacuum at a rate of 500 ml/min. Air passed successively over the soil, through a column containing XAD-2 resin which sorbed volatile organics and finally through three consecutive bubbler traps containing 3 *M* NaOH to trap $^{14}CO_2$. After collection, the organics were eluted from the XAD column with methanol. Liquid scintillation spectrometry was utilized to determine radiocarbon present in the NaOH and the methanol eluate from the XAD column.

RESULTS AND DISCUSSION

The chemical integrity of the radiolabeled and bulk tetryl was verified by chromatographic co-elution experiments with the SARM standard under

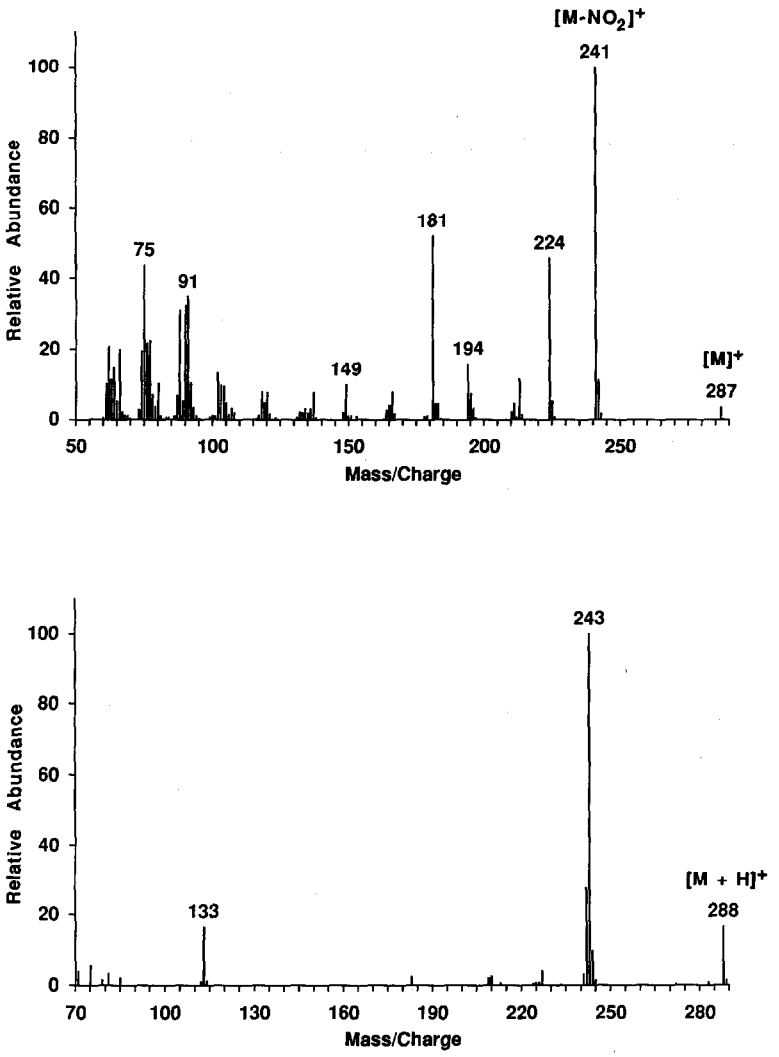

Fig. 1. Direct insertion probe electron impact (top) and isobutane chemical ionization (bottom) mass spectra of tetryl.

the HPLC conditions described above. Analysis of the SARM tetryl (molecular weight 287) by GC–MS yielded a single peak with a retention time of 19.9 min. The corresponding mass spectrum gave an apparent molecular ion at m/z 242 with a base fragment of 194, indicating that tetryl had undergone thermal decomposition on GC analysis. A possible thermal degradation mechanism would involve the cleavage of the aniline nitro group, resulting in the formation of N-methyl-2,4,6-trinitroaniline (molecular weight 242). Artifactitious formation of N-methyl-2,4,6-trinitroaniline, resulting from thermal decomposition of tetryl on GC analysis, has been described previously [18]. Further efforts to verify the identity of the SARM standard centered on direct insertion probe mass spectrometry due to the more thermally mild analytical conditions. Direct introduction of the standard material into the mass spectrometer source gave the electron impact and isobutane chemical ionization mass spectra shown in Fig. 1. These spectra match those previously reported for tetryl [19,20]. The spectra in Fig. 1 are presented here for comparison with subsequent mass spectral studies of tetryl transformation products that were conducted under identical source pressure and temperature conditions.

Tetryl transformation products in soil extracts

The stability of tetryl under the extraction conditions was examined by refluxing triplicate 200-ml methanol solutions containing 15 ppm of tetryl for 48 h. After this reflux period, the solutions had a distinct greenish hue, indicating that decomposition of the parent explosive had occurred. Chromatographic recovery of tetryl in the heated solutions

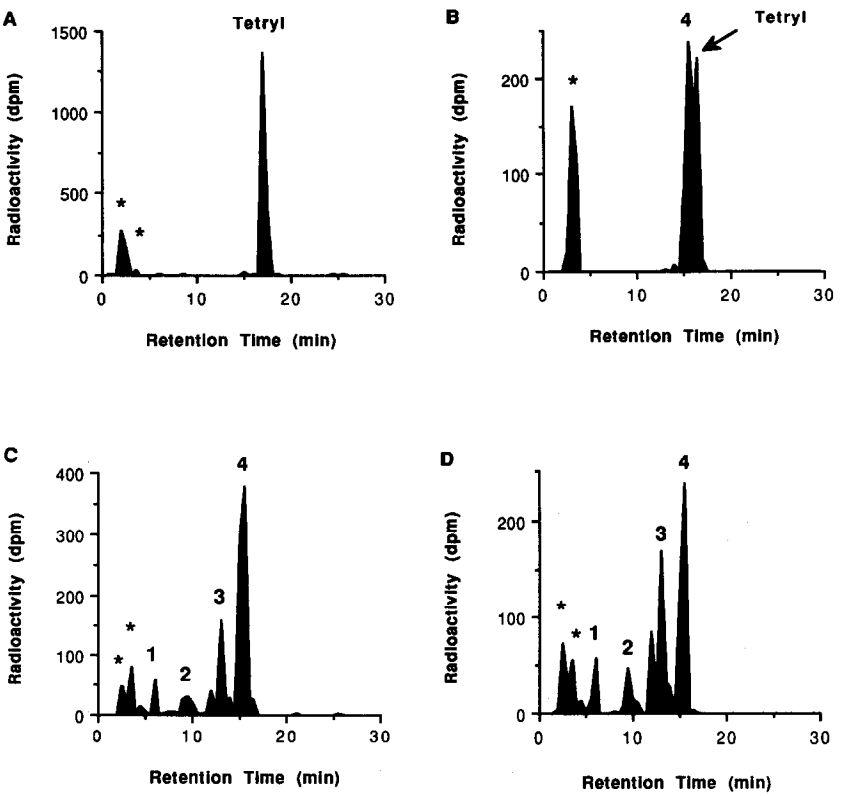

Fig. 2. Representative radio-HPLC profiles of (A) a methanolic solution of tetryl after 48 h of reflux, and extracts of tetryl-amended soil performed (B) immediately after amendment, (C) 11 days post-amendment and (D) 30 days post-amendment. Peaks labeled 1–4 correspond to tetryl soil transformation products. Peaks marked with asterisks elute in a retention window that contains tetryl decomposition products that arise from the Soxhlet extraction procedure.

was determined to be 82.46 ± 1.68%. A radiochromatogram of the refluxed tetryl solution is shown in Fig. 2A. This radiocarbon profile indicates that all tetryl decomposition products (18% of the radiolabel) are contained in two peaks that eluted immediately after the column dead volume. These extraction artifacts are labeled with asterisks in Fig. 2A. Although the extraction conditions induced a limited amount of tetryl decomposition, the utility of this technique was evident as tetryl transformation products could be determined, provided that elution is not coincident with the column void volume.

A variety of tetryl transformation products were observed in the chromatograms of the soil extracts. Radiochromatographic analysis of extracts from tetryl-amended soils aged for 0, 11 and 30 days are shown in Fig. 2B, C and D, respectively. Fig. 2B shows the presence of a primary tetryl transformation product (peak 4) that appeared in extracts initiated immediately after amendment. Tetryl and transformation product 4 are not fully resolved in Fig. 2 owing to the loss of chromatographic resolution that occurs when assaying individual 0.5-ml fractions for radiocarbon. These compounds were, however, baseline resolved in chromatograms utilizing UV absorption detection. Chromatograms of extracts from the 11- and 30-day periods show the persistence of peak 4 and the appearance of several additional transformation products of higher polarity (peaks 1–3). The same transformation products were observed in extracts from tetryl-amended Burbank and Cinebar soils. The first-eluting peaks in these chromatograms (labeled with asterisks) appear at the same retention time as the artifacts formed from tetryl during the reflux period.

The soil transformation products observed in this study were further characterized by their alkylphenone retention indices [15,16]. Calculations were based on a co-injection of alkylphenone standards with an extract of Palouse soil that had been incubated with tetryl for 11 days. The retention indices were determined to be 714, 813, 872 and 922 for transformation products 1, 2, 3 and 4, respectively. An alkylphenone retention index of 946 was calculated for tetryl.

Identification of the primary tetryl transformation product (transformation product 4)

Interestingly, GC–MS analysis of the HPLC-purified transformation product 4 gave a single peak with a retention time and mass spectrum that were identical with those previously observed during GC–MS studies of tetryl. The most plausible explanation of this result is that soil transformation product 4 is actually the same product formed by the thermal decomposition of tetryl during GC analysis. Alternatively, the transformation product could contain an alteration of the aniline nitro group that decomposes in a manner similar to that hypothesized for tetryl when subjected to the temperatures necessary for GC.

Additional information about the identity of transformation product 4 was provided by FT-IR and UV–VIS spectrometry. The FT-IR spectrum of transformation product 4 featured an absorbance at 3332 cm^{-1} that was absent from the spectrum of tetryl. This spectral feature suggests the presence of an N–H bond in transformation product 4. The UV–VIS spectrum of transformation product 4 contained maxima at 344 and 415 nm. These absorbance maxima, and their relative absorption intensities, are in good agreement with a UV–VIS spectrum of N-methyl-2,4,6-trinitroaniline reported by Dubovitskii *et al.* [21].

A direct insertion probe electron impact mass spectrum of the HPLC-purified transformation product 4 was acquired to evaluate whether the spectrum obtained during GC–MS studies was due to a thermal decomposition product. The resulting mass spectrum is presented at the top of Fig. 3. This spectrum is identical with those previously obtained during GC–MS analysis of both transformation product 4 and tetryl. It appears, therefore, that transformation product 4 remains intact during GC analysis. Further mass spectral studies centered on isobutane chemical ionization to verify the molecular weight of transformation product 4. The chemical ionization mass spectrum of HPLC-purified transformation product 4 is presented at the bottom of Fig. 3. The prominent $[M + H]^+$ ion at m/z 243 indicates a molecular weight of 242. The studies described above strongly implicate N-methyl-2,4,6-trinitroaniline as the identity of transformation product 4.

Final structural verification of the identity of transformation product 4 involved the synthesis of N-methyl-2,4,6-trinitroaniline by the nucleophilic aromatic substitution reaction of picryl chloride

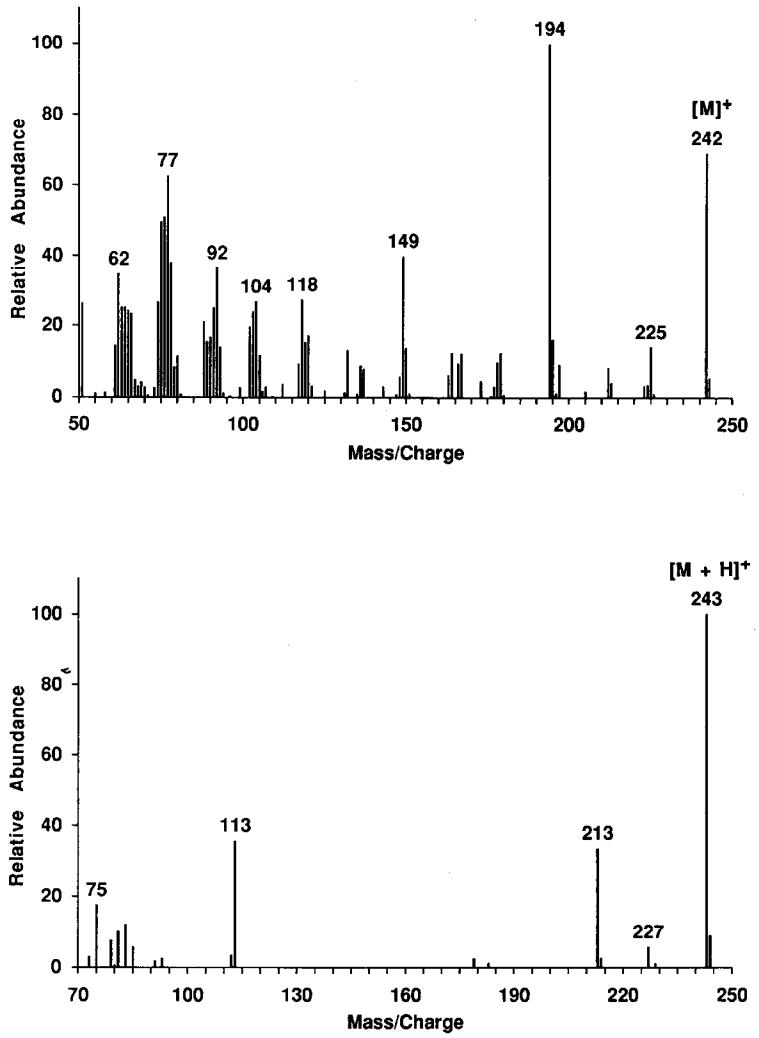

Fig. 3. Direct insertion probe electron impact (top) and isobutane chemical ionization (bottom) mass spectra of tetryl transformation product 4.

with excess of methylamine. The reaction proceeded quantitatively to the expected product at room temperature in methylene chloride solvent. HPLC co-elution of synthetic N-methyl-2,4,6-trinitroaniline with transformation product 4 is illustrated in Fig. 4. The top chromatogram is a profile of an extract from soil incubated with tetryl for 30 days; the bottom chromatogram resulted from co-injection of the same extract with synthetic N-methyl-2,4,6-trinitroaniline. The bottom chromatogram illustrates an increased relative area of peak 4 due to co-elution of synthetic N-methyl-2,4,6-trinitroaniline and transformation product 4. The synthetic product also exhibited mass spectral and chromatographic properties identical to the primary tetryl transformation product when analyzed by GC–MS, thereby verifying the structural assignment of transformation product 4 as N-methyl-2,4,6-trinitroaniline.

At room temperature, tetryl is stable and may be stored for years without indications of decomposition [9]. However, at temperatures above 120°C,

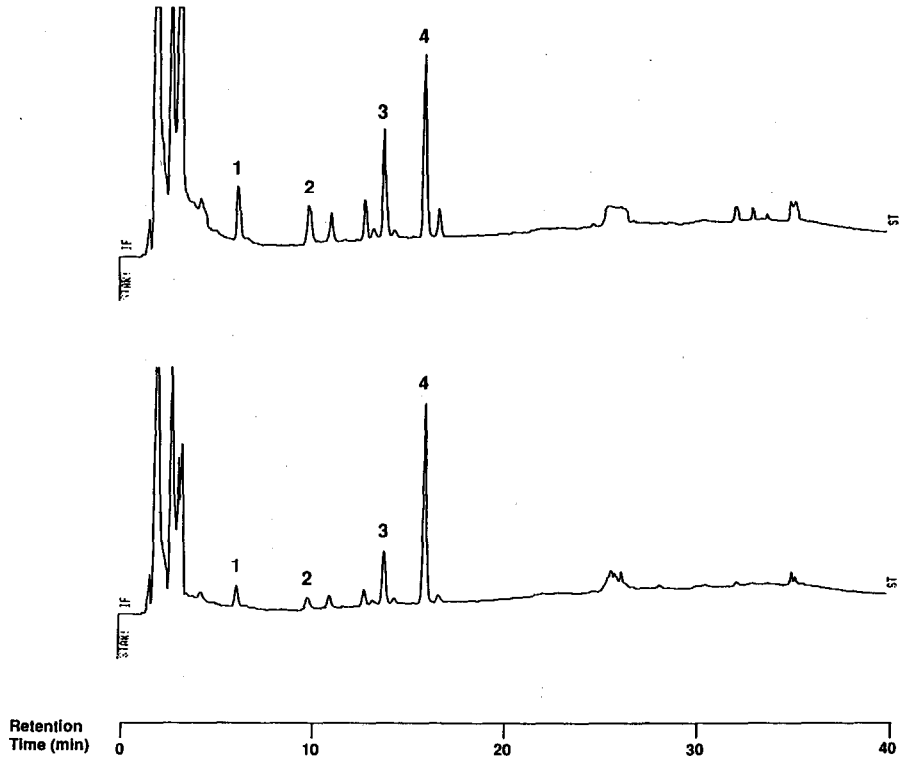

Fig. 4. HPLC profile of an extract of tetryl-amended soil performed 11 days after amendment (top) and a co-injection of the same extract with synthetic N-methyl-2,4,6-trinitroaniline (bottom). Peaks labeled 1–4 correspond to tetryl soil transformation products identified by radiochromatography (Fig. 2). This co-elution experiment establishes N-methyl-2,4,6-trinitroaniline as the identitity of tetryl transformation product 4.

thermal decomposition of tetryl readily occurs. Several studies have focused on the identification of residual components remaining after heating tetryl. Inevitably, N-methyl-2,4,6-trinitroaniline was observed in tetryl samples subjected to prolonged heating [9,21,22]. It should be emphasized that the N-methyl-2,4,6-trinitroaniline observed in this study was a true soil transformation product and did not arise as an extraction or analysis artifact.

Tentative identification of transformation product 3

HPLC-purified tetryl transformation product 3 was examined by GC–MS and direct insertion probe mass spectrometry. GC–MS analysis of HPLC peak 3 resulted in the total ion current chromatogram shown at the top of Fig. 5. The mass spectrum corresponding to the peak eluting at 20.2 min is presented at the bottom of Fig. 5. The results from this study suggest that transformation product 3 has a molecular weight of 212; however, direct insertion probe mass spectrometry resulted in an electron impact mass spectrum of transformation product 3 that differed from that obtained during the GC–MS study. The direct insertion probe mass spectrum of transformation product 3 is shown at the top of Fig. 6. The differences between spectra obtained by GC–MS and direct insertion probe analysis are attributable to thermal decomposition of transformation product 3 during GC analysis to yield a product with a molecular weight of 212.

Isobutane chemical ionization mass spectrometry of transformation product 3 resulted in the spectrum shown at the bottom of Fig. 6. The $[M + H]^+$ ion at m/z 258 indicates that transformation product 3 has a molecular weight of 257. This molecular weight (30 less than for tetryl) suggests that trans-

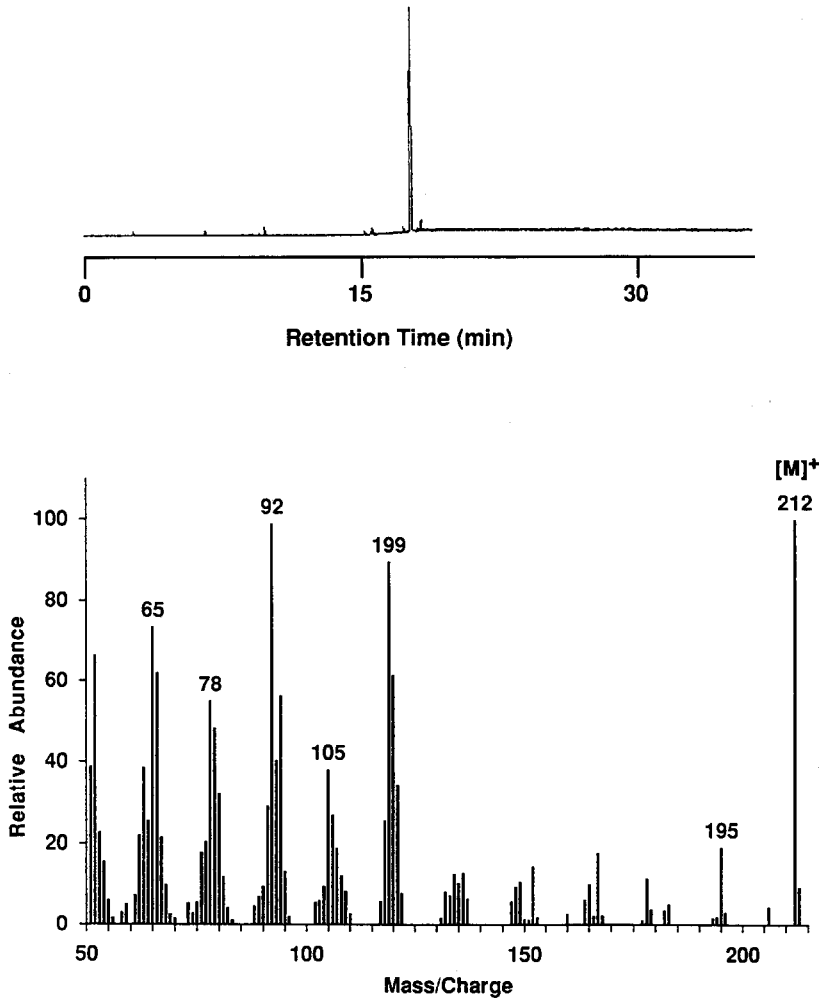

Fig. 5. Total ion current gas chromatogram (top) and corresponding mass spectrum (bottom) of transformation product 3.

formation product 3 is a nitro reduction product of the parent munition. By examination of the electron impact mass spectrum, it is possible to assign the site of nitro reduction. The base ion at m/z 211 results from cleavage of the aniline nitro group in a manner analogous to that previously observed for tetryl (see top of Fig. 1 for the spectrum of tetryl). The site of nitro reduction must therefore be at a ring position. Thermal decomposition of both tetryl and transformation product 3 occurred on GC analysis and proceeded by cleavage of the aniline nitro group. The mass spectrum of the thermal decomposition product of transformation product 3 (Fig. 5) further serves to localize the site of nitro reduction at a ring position. These data strongly suggest that transformation product 3 is a dinitroaminophenylmethylnitramine isomer. Nitro reduction products have been identified as the primary soil transformation products of the chemically related explosive TNT [12].

Thermal stability of transformation products and tentative tetryl transformation pathway

A methanol solution containing 16.8 ppm of N-methyl-2,4,6-trinitroaniline was subjected to reflux conditions for 48 h. The chromatographic recovery

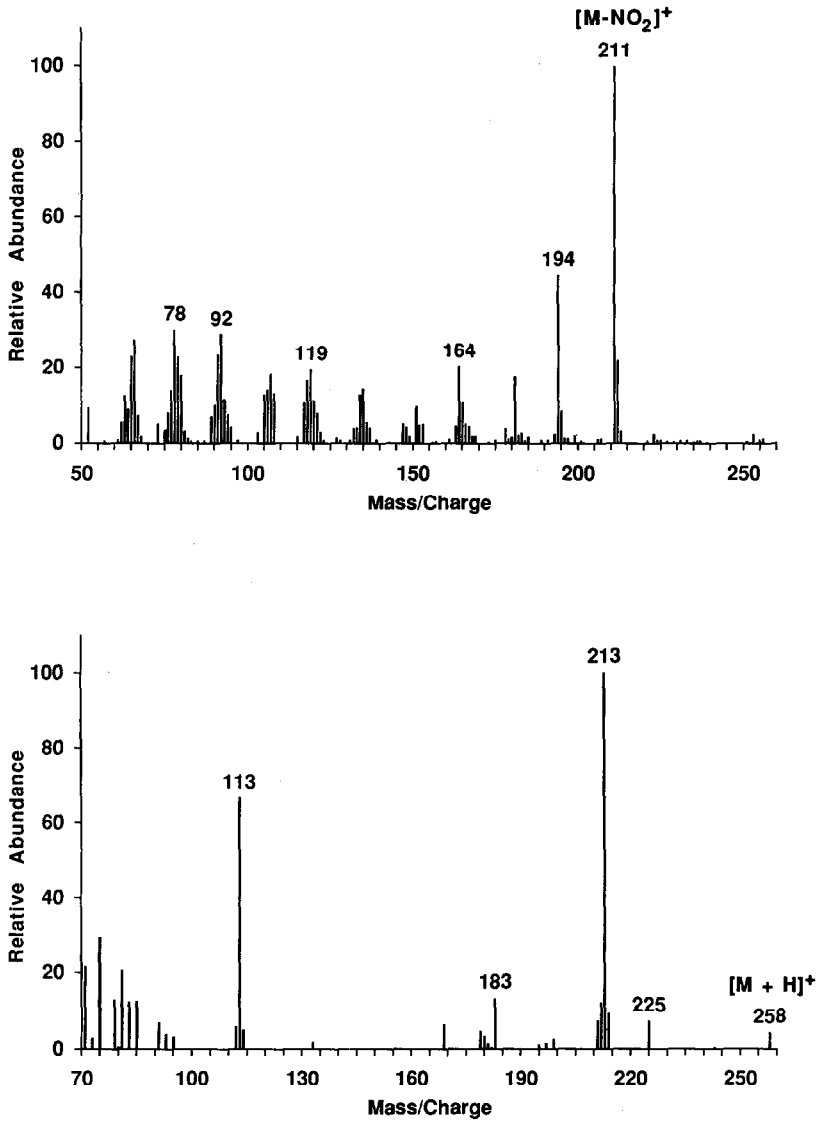

Fig. 6. Direct insertion probe electron impact (top) and isobutane chemical ionization (bottom) mass spectra of tetryl transformation product 3.

in the heated methanol solution was 95.2%. This high recovery indicated that N-methyl-2,4,6-trinitroaniline was sufficiently stable under the conditions employed for Soxhlet extraction.

A room-temperature methanol extraction was performed on Palouse soil that had been incubated with non-radiolabeled tetryl for 11 days to investigate whether Soxhlet extraction promoted decomposition of labile tetryl soil transformation products. HPLC analysis of this soil extract revealed the presence of tetryl and transformation products 1–4. Additionally, a previously unobserved transformation product (retention index of 894) was present in the room-temperature extract. The peak areas of the unknown transformation product and transformation product 3 were approximately equal. Subsequent heating of the room-temperature extract in a sealed vial at 65°C for 48 h resulted in the com-

plete destruction of the unknown tetryl transformation product. In an attempt to delineate products arising from thermal decomposition of this material, a methanol solution of HPLC-purified transformation product was heated at 65°C for 48 h. Although chromatographic studies of the heated solution confirmed rapid thermal decomposition of the unknown transformation product, the appearance of decomposition products absorbing at 246 nm was not observed.

From these studies, it is possible to postulate a tentative tetryl transformation pathway in soils as summarized in Fig. 7. The transformations proceed by two independent pathways. The most prominent tetryl transformation involves conversion of tetryl into N-methyl-2,4,6-trinitroaniline. A minor transformation pathway involves ring nitro reduction of the parent explosive, resulting in the formation of a dinitroaminophenylmethylnitramine isomer. The role of the thermally labile transformation product (retention index 894) in the tetryl transformation pathway remains uncertain. Further studies are necessary to elucidate the structure of this compound. Other more highly polar transformation products were observed in this study by radiochromatography (see peaks 1 and 2 in Fig. 2). These compounds are possible further reduction products of N-methyl-2,4,6-trinitroaniline (transformation product 4) and dinitroaminophenylmethylnitramine (transformation product 3).

Mass balance of tetryl in soil

Table I presents the mass balance of tetryl in Palouse soil that was obtained over the 60-day study. The second column is the percentage of radiolabel that was recovered in the methanol extracts, as de-

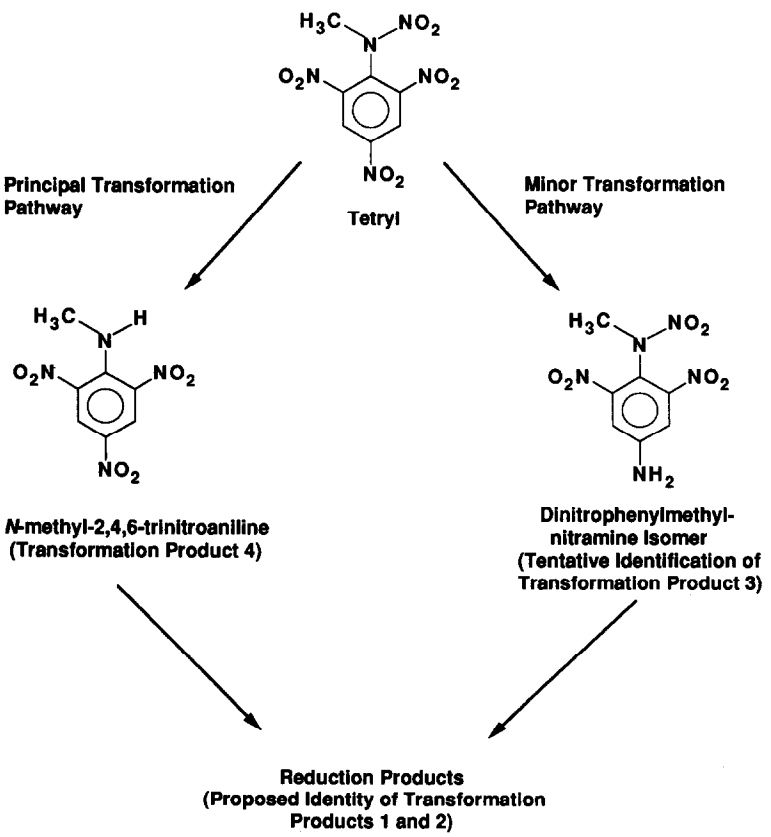

Fig. 7. Transformation pathway of tetryl in soils.

TABLE I

MASS BALANCE OF PALOUSE SOIL CONTAINING 60 ppm OF TETRYL

Time (days)	Radiolabel in methanol extract (%)	Unaltered tetryl (%)	Radiolabel in soil after extraction (%)	Mass balance deficit (%)
0	95 ± 21[a]	46 ± 9[a]	2.8 ± 0.2[a]	2 ± 21[a]
11	67 ± 5[a,b]	8 ± 4[b]	32.4 ± 0.8[b]	1 ± 5[a]
31	45 ± 4[b]	<0.5	40.2 ± 1.8[c]	15 ± 4[a]
60	36 ± 3[b]	<0.5	43.3 ± 2.3[c]	21 ± 3[a]

[a,b] Significant differences within each column are denoted by different superscripts (one-factor ANOVA for repeated measures with subsequent comparison by Scheffe F-test, $p < 0.05$). Values of <0.5% are below the chromatographic detection limit.

termined by liquid scintillation spectrometry. The third column lists the amount of the parent explosive that was speciated as tetryl, as determined by HPLC analysis. The amount of radiolabel that was not extractable in methanol is listed in column 4. 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 take into account radiolabel that was volatilized from the soils during the course of the study.

The filters used for the preparation of the final extracts and the methanol distillate resulting from rotary evaporation were found to contain negligible amounts of radiolabel. Initially, most of the radiolabel was extracted and present in the methanol extracts. It is noteworthy that only 46% of the radiolabel was speciated as tetryl in extracts initiated immediately after amendment. This indicates that transformation of tetryl was an extremely rapid process. Transformations continued at a rapid rate with less than 8% of the amended radiolabel speciated as tetryl in extracts from soils aged for 11 days. By 30 days post-amendment, the concentration of tetryl was below the chromatographic detection limit of 0.1 ppm. This detection limit corresponds to *ca.* 0.5% recovery of the amended tetryl. Immobilization of radiolabel was initially rapid, with 32% of the amended radiolabel irreversibly bound to the soil by 11 days post-amendment. Binding to the soil matrix continued at a slower rate throughout the remainder of the study. By the end of the 60-day study, 43% of the radiolabel was non-extractable. The mass balance deficits were found to be statistically equivalent throughout the study. At the end of the 60-day study, over 79% of the radiolabel initially amended to the soil was recovered in either the methanol extracts or the extracted soils.

Tetryl-amended soil was allowed to equilibrate for 21 days prior to monitoring the emission of volatile products for three consecutive days. A moderate amount of $^{14}CO_2$ was evolved from the soils. Assuming a constant rate of $^{14}CO_2$ evolution throughout the 60-day study, an amount equal to 9% of the amended radiolabel was mineralized to $^{14}CO_2$. Emission of volatile organic compounds was not observed for tetryl-amended soils.

The fate of tetryl in soil can be compared with results previously obtained for the explosives RDX and TNT [12,13]. Valid comparisons are possible because the same Palouse soil and post-amendment incubation conditions were utilized for all three munitions fate studies. Additionally, the soil extraction and analytical conditions were similar for all three explosives, with the exception that RDX was extracted with acetonitrile rather than methanol. TNT showed rapid soil transformation to 2- and 4-aminodinitrotoluene isomers; however, transformation was less rapid than observed for tetryl. For example, 88 ± 1 and 36 ± 3% of the amended TNT were recovered as the parent compound immediately after amendment and at the end of the 60-day study, respectively. Although TNT displayed substantial irreversible binding to the soil constituents (30 ± 1% after 60 days of incubation), this process was more pronounced for tetryl. The fates of both TNT and tetryl contrast sharply with that of RDX. RDX did not undergo transformations within the soil and only 1.10 ± 0.26% was non-extractable at the end of the 60-day study. Ad-

ditionally, moderate amounts of $^{14}CO_2$ were evolved from both TNT and tetryl-amended soils. Mineralization to $^{14}CO_2$ accounted for 4 and 9% of the amended TNT and tetryl, respectively, over the 60-day study. The amount of RDX that was oxidized to CO_2 (0.31%) was small in comparison with the aromatic explosives.

CONCLUSIONS

A method based on exhaustive Soxhlet extraction and subsequent HPLC analysis of tetryl was developed and applied to study the fate of this explosive in the soil environment. Tetryl was found to undergo extremely rapid transformation. Two independent tetryl transformation pathways were identified. The first and principal pathway resulted in the formation of N-methyl-2,4,6-trinitroaniline. The second, less prominent transformation pathway resulted in the formation of an aminodinitrophenylmethylnitramine isomer. Other, more polar transformation products were identified by radiochromatography and characterized by their alkylphenone retention indices. These products were likely reduction products of N-methyl-2,4,6-trinitroaniline and aminodinitrophenylmethylnitramine. Transformation of tetryl was so extensive that the parent compound was below the detection limit in soil extracts by 30 days post-amendment. Progressive binding of radiolabel to the soil constituents occurred throughout the study and was particularly rapid during the first 10 days of incubation. The mass balance of radiolabel in amended soil was better than 79% throughout the 60-day study.

It is clear that a broad range of compounds are available for uptake by plants grown in tetryl-contaminated soils. A large proportion of tetryl-derived residues will eventually become immobilized by binding to the soils. Although binding with the soil matrix precludes dispersal of contamination by leaching, it must be emphasized that the bound residues may still be available for plant uptake and further metabolism.

This study clearly emphasizes the advantages of HPLC for the analysis of tetryl and tetryl soil transformation products. Had our study drawn exclusively on gas-phase separation techniques, tetryl transformation product 4 could not have been distinguished from the parent munition. Additionally, tetryl transformation product 3 would have been erroneously assigned a molecular weight of 212. The studies described here provide independent verification that previously described GC methods for the determination of tetryl [7,8] have resulted in the separation and detection of the thermal decomposition product of tetryl, N-methyl-2,4,6-trinitroaniline [18].

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