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Analysis of the explosive 2,4,6-trinitrophenylmethylnitramine (tetryl) in bush bean plants

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ABSTRACT

Previous attempts to delineate the metabolism of the explosive 2,4,6-trinitrophenylmethylnitramine (tetryl) in plants have been unsuccessful. Development of an appropriate analytical methodology has been thwarted due to the extreme thermal and base lability of tetryl as well as its propensity to undergo photodecomposition. This study presents a methodology based on solvent extraction of plant tissue followed by fractionation of the organic extract on silica gel with subsequent determination of tetryl by HPLC. This methodology allowed 82.70 ± 5.54% recovery of tetryl from fortified bush bean leaves. The developed methodology was applied to study tetryl uptake and metabolism in bush bean plants exposed to tetryl-amended hydroponic cultures.

INTRODUCTION

Munitions manufacturing, packing, and decommissioning activities result in large quantities of wastewaters. In the past, before the environmental impact of this practice was fully realized, the wastewaters were directed to holding lagoons for primary settling of solid material before being released to rivers and streams. Presently, munitions residues are removed from the wastewater streams by adsorption on carbon columns prior to discharge. However, the pollution legacy of lagooning practices remains because the holding lagoons have since evaporated, leaving heavily contaminated localized areas. Although 2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) are the principal pollutants of concern, significant quantities of tetryl were also released. It has been estimated that approximately 16 kg of tetryl was released from the daily operation of a single manufacturing plant [1]. The contaminated land is presently becoming revegetated, causing further concerns based on the potential for plant uptake of munitions residues and their soil transformation products. The possibility exists that plant metabolism of these compounds may result in the formation of highly toxic metabolites that may propagate through the food chain.

The toxicity of tetryl is well documented. Early munitions workers often suffered dermatitis from exposure to this explosive [2,3]. Tetryl has also been shown to be mutagenic in several different bacterial assay systems [4].

Very little is known about the environmental fate of tetryl. It has recently been demonstrated that tetryl undergoes rapid transformation in the soil environment [5]. Two independent transformation pathways were implicated. The primary transformation pathway involved cleavage of the aniline nitro group resulting in the formation of N-methyl-2,4,6-trinitroaniline. The second, less prominent, pathway involved direct ring nitro reduction of tetryl, resulting in the formation of an aminodinitrophenylmethylnitramine isomer. Other, more polar transformation products were observed in Soxhlet extracts of tetryl-amended soil and were hypothesized to be nitro reduction products of N-meth-

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yl-2,4,6-trinitroaniline and aminodinitrophenylmethylnitramine. Additionally, an unidentified tetryl transformation product was observed in room temperature methanol extracts of tetryl-amended soil that was absent from Soxhlet extracts. This thermally labile transformation product was quantitatively destroyed by conditions utilized for Soxhlet extraction (48 h at 65°C) [5].

Our past studies have shown extreme differences in the plant metabolism of the explosives TNT and RDX [6,7]. The hexahydrotriazine explosive RDX was found to be bioaccumulated in the aerial tissues of plants, whereas the nitroaromatic explosive TNT was rapidly metabolized to polar products which were localized primarily in the root tissue. Virtually nothing is known about the plant metabolism of tetryl. Slow progress in this area is primarily due to the lack of an appropriate analytical methodology to analyze for this explosive in plants. Undoubtedly these problems result from the extreme lability of tetryl. This explosive is photosensitive and known to undergo decomposition in the presence of base [8]. Additionally, tetryl is thermally labile. Decomposition of tetryl to N-methyl-2,4,6-trinitroaniline is known to occur upon gas chromatographic analysis, even when cold on-column injection is utilized [5,9]. Therefore, analysis by milder techniques, such as high-performance liquid chromatography (HPLC), is mandated.

The primary goal of this study was to develop an analytical procedure for the analysis of tetryl in plant tissues that gives both acceptable recoveries and precision. A secondary objective was to apply the developed methodology to study plant metabolism of tetryl in bush bean plants exposed hydroponically to this explosive for either 1 or 7 days. Central to our research approach was the use of uniformly ring-labeled ^{14}C-tetryl. Radiotracer studies allow for both unambiguous identification of metabolic products and a mass balance assessment.

EXPERIMENTAL

Radiolabeled and bulk tetryl

Uniformly ring-labeled ^{14}C-tetryl (specific activity of 14.64 mCi/mmol) was obtained from New England Nuclear (E.I. du Pont de Nemours, Boston, MA, USA). The purity of ^{14}C-tetryl was determined to be 98.70% by HPLC radiochromatography. This purity was judged adequate for plant uptake and metabolism studies and was used without purification. Bulk tetryl was obtained from US Army Biomedical Research and Development Laboratory (Fort Detrick, MD, USA). HPLC analysis of the bulk material indicated a purity in excess of 95%. The identities of both radiolabeled and bulk tetryl were verified by comparison of retention times with an authentic tetryl standard provided by the US Army Toxic and Hazardous Materials Agency (Aberdeen Proving Ground. MD. USA).

Analytical separations

The chromatographic system was manufactured by Waters Assoc. (Milford, MA, USA) and consisted of a Model 600E gradient controller and pump, a WISP Model 710 automatic injector, and a Model 490E variable wavelength detector. A Beckman Ultrasphere (San Ramon, CA, USA) octadecyl silica column (24 cm × 4.6 mm I.D., d_p 5 μm) was utilized for separations. Injection volumes were 20 μl for all chromatographic runs. The column was developed with a water-acetonitrile mobile phase programmed from 35 to 100% acetonitrile over 30 min and held at the final composition for an additional 10 min. HPLC-grade solvents, obtained from J. T. Baker (Phillipsburgh, NJ, USA), were used throughout these studies. Detection was accomplished at 264 nm at a sensitivity of 0.008 AUFS. Integrated peak areas, provided by a Hewlett-Packard 3390A integrator, formed the basis for quantification.

Radiochromatographic detection was performed by collecting successive 0.5-ml fractions of the HPLC column eluate. After addition of 15.0 ml of Ready Solv EP scintillation cocktail (Beckman), the individual fractions were assayed for radiocarbon by liquid scintillation spectrometry.

In several instances, plant-produced tetryl metabolites were characterized by their alkylphenone retention indices [10,11]. These indices were determined by co-injection of a mixture of alkylphenones (Aldrich, Milwaukee, WI, USA) with the plant extract of interest. An authentic N-methyl-2,4,6-trinitroaniline standard was utilized during characterization of tetryl plant metabolites and was synthesized as described previously [5].

Plant cultivation and hydroponic exposures

Bush beans were chosen for these studies as a representative dicotyledon having a wide geographical distribution and agricultural significance. Bush bean (*Phaseolus vulgaris*, var., tendergreen) plants were grown from seed and maintained for 21 to 26 days in hydroponic nutrient solutions [12] prior to tetryl uptake exposures. Plant growth and hydroponic exposures were conducted in a growth chamber environment that simulated the luminous intensity and spectral dispersion of sunlight (180 W m^{-2} of photosynthetically active radiation) during the 16-h daily light cycle. The chamber temperature was regulated at a day/night temperature of 26/22°C and a relative humidity of 50%.

Tetryl-amended exposure solutions were prepared by adding 1 .O ml of methanol containing appropriate quantities of non-radiolabeled and radiolabeled tetryl to 500 ml of nutrient solution to give a final tetryl concentration of 10 ppm with a total of 5 μCi ^{14}C-tetryl/500 ml. The solutions were filter-sterilized and placed in autoclaved 600-ml beakers to minimize microbial contamination that could promote tetryl transformation. Plant exposure beakers were equipped with aeration capillaries and sheathed with opaque covers to protect the roots from the growth chamber lights. Exposures were conducted in triplicate for either 1, 4, or 7 days. Controls were run concurrently with plant exposures and allowed assessment of tetryl loss due to volatilization and/or photodecomposition. Control solutions consisted of three hydroponic cultures; one of these solutions was aerated and exposed to the full intensity of the growth chamber lights, another was exposed to the lights and not aerated, and a third was aerated and maintained in the dark. Hydroponic exposure and control solutions were sampled for liquid scintillation spectrometry and HPLC analyses after amendment and at the conclusion of each exposure period. At harvest, plants were removed from the hydroponic cultures and the roots sequentially rinsed with 0.1 *M* calcium chloride and methanol-water (80:20, v/v). Both rinse solutions were assayed for radiocarbon content by liquid scintillation spectrometry. The plant was then segregated into leaf, stem, and root tissues; the individual tissues were minced and thoroughly mixed. Tissues were stored at − 80°C until sampled for chemical analysis or oxidation.

Tissue fractionation and analysis of' plant tissues

The quantity of radiolabel contained in the plant tissues was determined by total combustion on a Packard Model 306 oxidizer (Packard, Downers Grove, IL, USA). The $^{14}CO_2$ generated by oxidation of the tissues was subsequently assayed for radiocarbon by liquid scintillation spectrometry.

The tissue fractionation and extraction procedure is outlined in Fig. 1. Tissue samples (1 .O g fresh

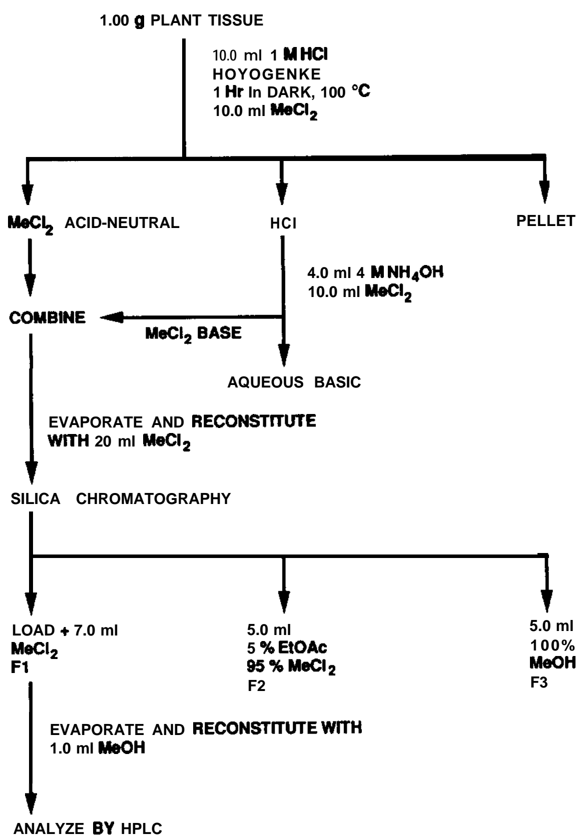

Fig. 1. Flowchart outlining the fractionation of tetryl from plant tissues. After homogenization and acid hydrolysis of tissue, the mixture was partitioned with methylene chloride, giving rise to the methylene chloride acid-neutral (MeCl, AN), aqueous hydrochloric acid (HCl), and pellet fractions. The HCl layer was made basic and again partitioned with methylene chloride. The resulting layers were designated the aqueous basic (Aqueous basic) and methylene chloride base ($MeCl_2$ base) fractions. The pooled methylene chloride layers were evaporated, reconstituted with 2.0 ml of methylene chloride ($MeCl_2$) and subjected to chromatography on silica with various mobile phases. The mobile phase compositions consisted of $MeCl_2$, a mixture of $MeCl_2$ and ethyl acetate (EtOAc), or methanol (MeOH), as described above. Tetryl, which was contained in fraction F1, was further analyzed by reversed-phase HPLC. with detection at 246 nm.

weight) were homogenized for 2.5 min in 10.0 ml of 1 A4 HCI in a Sorval Omni-Mixer (Newton, CT, USA). The homogenized samples were transferred to 25-ml Corex centrifuge tubes and acid hydrolysis was performed by submerging the tubes in a boiling water bath for l h. The Corex tubes were wrapped in aluminum foil to exclude light during acid hydrolysis. After cooling to room temperature, the hydrolysis mixture was extracted with 10.0 ml of methylene chloride. The organic layer ($MeCl_2$ acid-neutral fraction) and pellet were separated from the aqueous hydrochloric acid layer (HCl fraction) after centrifugation for 10 min at 3000 g. Aliquots of each fraction (100 μl) were removed for liquid scintillation spectrometry. The tissue pellet was oxidized to determine the amount of sequestered radiolabel. The acidic aqueous layer was next made basic by the addition of 4.0 ml of 4 A4 NH_4OH and extracted with a second lO.O-ml portion of methylene chloride. After centrifugation the resulting organic ($MeCl_2$ base fraction) and the aqueous (aqueous basic fraction) phases were separated. Aliquots for liquid scintillation spectrometry were then removed, the organic fractions pooled, and the solvent evaporated to dryness with a gentle stream of nitrogen.

The residue remaining after evaporation of the pooled methylene chloride layers was reconstituted with 2.0 ml of methylene chloride prior to fractionation on silica gel. Silica Sep-Pak (Waters Assoc.) cartridges were preconditioned with 10.0 ml methylene chloride immediately before use. Three fractions were collected from silica gel chromatography of the plant extracts. The first fraction (fraction F1) resulted from application of the plant extract followed by 7.0 ml of methylene chloride mobile phase. Tetryl was contained within fraction F1. The second fraction (fraction F2) was eluted with 5.0 ml of methylene chloride-ethyl acetate (95:5, v/v). The final fraction (fraction F3) was eluted with 5.0 ml of methanol in an attempt to strip the silica of the maximal amount of adsorbed material. After removal of 100-μl aliquots from each fraction for scintillation spectrometry, fraction F1 was evaporated to dryness and reconstituted with 1.O-ml methanol in preparation for HPLC analysis. The spent silica sorbent was removed from the Sep-Pak cartridge and adsorbed radiolabel was determined by liquid scintillation spectrometry.

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

Volatile organic and $^{14}CO_2$ emissions were measured by a previously described technique [13]. Briefly, a 28-day-old bush bean plant was placed in a specially designed split-chamber enclosure that isolated the roots from the aerial tissues. The plant was maintained on a hydroponic culture containing 7.5 ppm tetryl with a total of 22 μCi radiolabel. Air was pulled by vacuum through each chamber, then through a pair of sorbent traps, and finally through a series of bubbler traps at a flow-rate of 500 ml/min. Two tandem sorbent columns (10 × 1.0 cm) packed with XAD-II resin were located immediately after the chambers to sorb volatile organic emissions. This was followed by four sequential bubbler traps filled with 3 *M* NaOH designed to remove $^{14}CO_2$. Radiocarbon present in the sampling train was analyzed every 24 h for 3 consecutive days. Radiolabel contained in the NaOH traps and the methanol eluated from the XAD resin was determined by liquid scintillation spectrometry.

RESULTS AND DISCUSSION

Plant hydroponic exposures

Analysis of hydroponic control solutions indicated rapid tetryl photodecomposition. The control solution that was aerated and exposed to the growth chamber lights maintained a constant quantity of radiolabel throughout the 7-day exposure period; however, tetryl progressively decomposed from an initial 4.73 mg/beaker to 3.64 and 0.83 mg/beaker after 1 and 7 days of exposure, respectively. Similar results were obtained for the control solution exposed to the growth chamber lights but not aerated. HPLC analysis of the light-exposed control solutions revealed the formation of a photodecomposition product that increased in concentration throughout the 7-day exposure period. This photodecomposition product (alkylphenone retention index of *920)* was subsequently identified by co-injection experiments as N-methyl-2,4,6-trinitroaniline. Formation of this colored product accounted, at least in part, for the bright yellow hue acquired by tetryl solutions upon exposure to light. The control solution that was maintained in the dark remained colorless and displayed only a small loss of tetryl throughout the exposure period. For this so-

lution, the quantity of radiolabel remained constant at 5.51 μCi/beaker, while the tetryl content decreased from an initial 4.71 mg/beaker to 4.33 and 4.06 mg/beaker after 1 and 7 days of exposure, respectively. HPLC analysis of this control solution did not reveal the presence of transformation products absorbing at 264 nm.

Hydroponic exposure solutions that supported bush beans (solutions were aerated and shielded from light) displayed loss of radiolabel due to plant uptake and root-catalyzed transformation of tetryl. Initial exposure solutions contained 5.56 ± 0.09 μCi radiolabel with a total tetryl content of 5.03 ± 0.07 mg/beaker. Analysis of solutions after supporting bush beans for 1 day indicated that practically all the tetryl had been transformed. Although these solutions contained 55% of the initial radiolabel, tetryl accounted for only 3% of the mass originally amended. Polar products that eluted coincident with the column dead volume were the principal transformation products observed in the plant exposure solutions. Analysis of solutions from the 7-day exposure period indicated that 1.23 ± 0.07 μCi radiolabel remained in solution and tetryl concentrations were below the detection limit of 0.10 ppm.

The quantity of radiolabel assimilated by the plant was calculated by subtracting the sum of the radiolabel remaining in the post-exposure hydroponic solution, the 0.1 *M* calcium chloride rinse, and the methanol-water (80:20, v/v) rinse solutions from the amount initially amended to the hydroponic solution. Total radiolabel uptake calculated in this manner was 1.89 ± 0.25, 3.04 ± 0.34, and 3.60 ± 0.19 μCi for the l-, 4-, and 7-day exposures, respectively. The actual amount of radiolabel contained within the tissues was determined by oxidation. Plants contained 1.53 ± 0.31, 2.04 ± 0.51, and 2.27 ± 0.20 μCi for the l-, 4-, and 7-day exposures, respectively, as based on oxidation. Values calculated from the oxidation data were consistently lower than values based on analysis of the hydroponic and rinse solutions. This discrepancy between plant uptake and tissue content of radiolabel may reflect tetryl metabolism to volatile organic products or $^{14}CO_2$.

Analytical methodology for analysis of tetryl in plant tissue

Previous studies conducted in this laboratory have developed analytical methods for the analysis of TNT [5] and RDX [6] in plant tissues. Methodologies developed for these explosives were similar to the scheme outlined in Fig. 1, with the exceptions that diethyl ether rather than methylene chloride was utilized for solvent extraction and chromatographic fractionation was performed on Florisil (Sep-Pak cartridges, Waters, Milford, MA, USA) rather than silica adsorbent. The method previously developed for RDX [6] was evaluated for the analysis of tetryl-fortified bush bean leaf tissue as an entry point for studies described here. Application of the RDX method resulted in both low recoveries and decomposition of tetryl. A suitable analytical method for tetryl (shown in Fig. 1) was developed by investigating each step of the entire RDX fractionation scheme (starting from the last step and working backward) and incorporating changes that maximized tetryl recovery. RDX analysis steps that were not appropriate for tetryl analysis were traced to a combination of the following: (1) fractionation on Florisil adsorbent, (2) the use of diethyl ether as an extraction solvent, and (3) the acid hydrolysis procedure. These areas are individually discussed below.

Florisil adsorbent was evaluated by fractionating a methylene chloride extract of acid-hydrolyzed bush bean leaf tissue that was spiked with tetryl. It was found that Florisil chromatography of the tetryl-containing plant extract led to decomposition of this explosive. The composition of Florisil includes 18% magnesium oxide which confers basic properties to this adsorbent. It is likely that Florisil promotes tetryl decomposition due to the lability of this explosive toward basic conditions. This observation is consistent with previous studies describing irreversible alteration of tetryl resulting from chromatography on Florisil [14]. For this reason, silica gel was evaluated as an alternative chromatographic sorbent. Tetryl was not strongly adsorbed by silica and was eluted from silica with methylene chloride mobile phase. Methylene chloride was of sufficient solvent strength to elute some plant carotenoid pigments from silica within the same fraction that contained tetryl; however, these pigments did not interfere with the subsequent HPLC determination of tetryl. Liquid scintillation and HPLC analyses of fraction F 1 revealed nearly quantitative recovery of tetryl from the silica gel. The majority of the plant

pigments were eluted within the second fraction by methylene chloride-ethyl acetate (95:5, v/v) mobile phase. Methanol was used to elute the final fraction, which contained a moderate quantity of plant pigment.

Problems associated with the solvent extraction portion of the analytical scheme were traced to the use of diethyl ether. Diethyl ether, a relatively high-polarity solvent, was used in the past studies of TNT and RDX due to its ability to extract fairly polar plant metabolites. However, for tetryl, use of this solvent led to poor recoveries. For example, diethyl ether extraction combined with silica gel fractionation of bush bean leaves spiked with tetryl gave a 73.85 ± 1.3 1% recovery based on radiocarbon analysis and a 68.26 ± 6.26% chromatographic recovery of tetryl within fraction F 1. Higher recoveries of tetryl were obtained from tetryl-spiked acid-hydrolyzed tissues when methylene chloride was substituted for diethyl ether. An explanation of this result again emphasizes the lability of tetryl toward base. Diethyl ether allows a relatively large quantity of base to partition into the organic phase during extraction of the ammonium hydroxide-containing aqueous phase. Residual base is then concentrated during evaporation of the pooled organic extract which, in turn, leads to the decomposition of tetryl. By virtue of the low solubility of water in methylene chloride, extraction with this solvent minimizes transfer of base into the pooled organic extract and, consequently, tetryl decomposition is minimized.

The acid hydrolysis procedure was also found to contribute to tetryl decomposition. Tetryl decomposition was not felt to be due to the acidic conditions, but rather to sensitization of tetryl to photodecomposition at the elevated hydrolysis temperature. This concern was readily addressed by wrapping the hydrolysis tube in aluminum foil to exclude illumination from the laboratory fluorescent lights. Further alterations of the hydrolysis variables (i.e., temperature and duration) were not pursued, as exclusion of light during this step resulted in reasonable tetryl recoveries. As a precaution against photodecomposition of tetryl, all extraction and fractionation steps were performed either under subdued light or, when possible, in the dark *(i.e.,* during evaporation of solvents).

Consolidation of the RDX methodology modifications summarized above resulted in the analysis scheme for tetryl outlined in Fig. 1. Triplicate samples of bush bean leaf tissue were spiked with 4.58 ppm tetryl containing a total of 63 953 dpm radiolabeled tetryl and analyzed in accordance with Fig. 1 to evaluate the recovery and precision of this method. The distribution of radiolabel among the various chemical fractions is summarized in Table 1. Radiolabel recovery of tetryl in fraction F1 was 80.49 ± 0.59%. HPLC analysis of fraction F1 gave an 82.70 ± 5.54% chromatographic recovery of tetryl. A representative chromatogram of the F1 fraction resulting from this triplicate spike experiment is presented in the top of Fig. 2. The peak eluting with a retention time of 17.00 min in this chromatogram is due to tetryl. For comparison, a chromatogram of the F1 fraction of a bush bean leaf blank is shown in the bottom of Fig. 2. Several peaks that elute well after tetryl (retention times from 25 to 32 min) are due to the presence of carotenoid pigments within fraction F1. Examination of the tissue blank indicates that these pigments do not interfere with tetryl quantification. Application of the analytical scheme summarized in Fig. 1 allows both high recovery and precision for the analysis of tetryl in plant tissues.

TABLE I

RADIOLABEL DISTRIBUTION AMONG VARIOUS CHEMICAL FRACTIONS OF BUSH BEAN LEAVES SPIKED IN TRIPLICATE WITH 4.58 PPM TETRYL

Chemical fraction	Percentage of total radiolabel ± S.D. (n = 3)
HCl	2.60 ± 0.14
$MeCl_2$ acid-neutral	84.33 ± 0.73
Aqueous basic	0.36 ± 0.34
$MeCl_2$ base	0.04 ± 0.06
F1[a]	80.49 ± 0.59
F2	3.87 ± 0.89
F3	0.68 ± 0.18
Silica sorbent	0.68 ± 0.18
Pellet	1.49 ± 0.45

[a] Chromatographic recovery of tetryl in fraction F1 was 82.70 ± 5.54%.

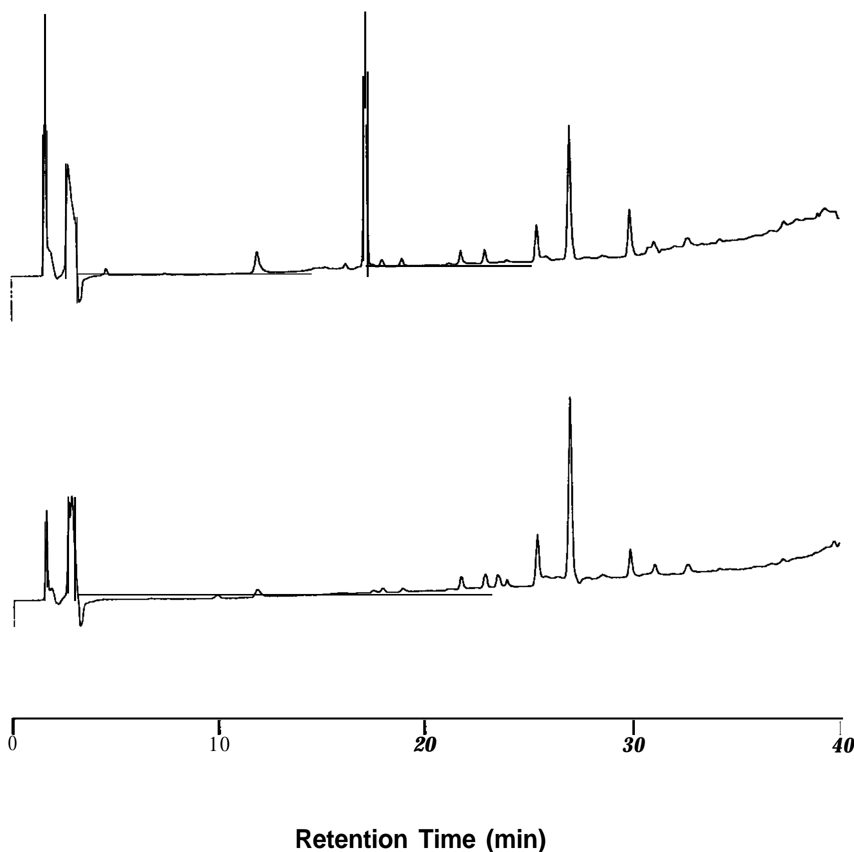

Fig. 2. Chromatograms of fraction F1 from leaf tissue spiked with tetryl (top) and a leaf blank tissue (bottom).

Fate of tetryl in plants

Tissue fractionation. Acid hydrolysis was included as an initial step in the analysis scheme to cleave polar conjugates formed during plant metabolism of tetryl. Insight regarding the formation of acid-hydrolyzable conjugates during tetryl metabolism was provided by comparing tissue hydrolyses performed in acid or water. This experiment was conducted with bush bean leaf tissue from a plant exposed to a tetryl-amended hydroponic culture for 7 days. Triplicate samples of tissue were homogenized either in water or 1 *M* HCl. Hydrolyses proceeded in the dark at 100°C for 1 h, at which time the preparations were extracted with methylene chloride. For 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. The acid hydrolysis treatment resulted in a 62.1 ± 1.2, 7.1 ± 0.7, and 31.7 ± 8.5% distribution of radiolabel in the HCl layer, the methylene chloride phase, and the pellet, respectively. 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) as well as the cleavage of some polar conjugates (reflected in the higher percentage of solubilized radio: label that partitions into the methylene chloride layer).

The quantity of radiolabel contained in the bush bean tissues from plants exposed to tetryl hydroponic solutions for either 1 or 7 days is summarized in the top row of Table II. Assimilation of radiolabel occurred throughout the study, as evidenced by

TABLE II

TOTAL RADIOACTIVITY (BASED ON OXIDATION), PERCENTAGES OF TOTAL RADIOACTIVITY IN CHEMICAL FRACTIONS, AND MATERIAL BALANCE FOR THE ANALYSIS OF BUSH BEAN TISSUES

Values are the average ± standard deviation from the analysis of 3 plants.

	Day 1			Day 7		
	Leaves	Stem	Roots	Leaves	Stem	Roots
Activity (dpm/g)	$(5 \pm 2)10^3$	$(21 \pm 5)10^3$	$(7 \pm 2)10^5$	$(17 \pm 4)10^3$	$(3 \pm 1)10^4$	$(37 \pm 6)10^4$
Fraction (% total activity)						
HCl	54 ± 16	24 ± 5	37 ± 9	62 ± 1	36 ± 6	19 ± 1
Aqueous basic	41 ± 14	16 ± 3	27 ± 6	45 ± 5	29 ± 8	14 ± 1
$MeCl_2$ acid-neutral	6 ± 5	11 ± 6	14 ± 3	7 ± 1	12 ± 2	7 ± 2
$MeCl_2$ base	1 ± 1	4 ± 1	4 ± 1	5 ± 1	6 ± 1	2.8 ± 0.2
F1	0.3 ± 0.6	0.6 ± 0.7	3.1 ± 0.8	0.8 ± 0.7	0.3 ± 0.5	0.9 ± 0.5
F2	0 ± 0	1.4 ± 1.4	1.5 ± 0.2	1.2 ± 1.2	1.6 ± 1.0	1.0 ± 0.2
F3	7 ± 3	9 ± 3	11 ± 3	12 ± 7	10 ± 1	6 ± 1
Silica sorbent	2.1 ± 0.6	1.0 ± 0.3	1.4 ± 0.3	1.1 ± 0.1	0.8 ± 0.3	0.7 ± 0.1
Pellet	37 ± 5	37 ± 15	46 ± 11	32 ± 8	62 ± 10	64 ± 19
Tetryl equivalents in fraction F1						
ng equivalents [a]	9 ± 15	60 ± 78	$(8.3 \pm 2.6)10^3$	53 ± 46	25 ± 44	$(1.3 \pm 0.9)10^3$
Material balance						
HCl + $MeCl_2$ acid-neutral + pellet	97	72	97	101	110	90

[a] Calculated from specific acitivity of hydroponic solutions.

the larger quantities of radiolabel in stem and leaf tissues from 7-day as compared to 1-day exposures. For both exposure periods the majority of radiolabel was localized within the root tissue. Stem tissue contained intermediate amounts of radiolabel, whereas the smallest quantity of radiocarbon was localized in the leaf tissue.

The distribution of radiolabel among the various chemical fractions for plants exposed to tetryl hydroponic solutions for either 1 or 7 days is also summarized in Table II. The resulting distributions are indicative of very rapid metabolism of tetryl toward more polar products. Metabolism was so extensive that less than 3.1% of the radiolabel contained in the tissues was associated with fraction F1. A significant quantity of radiolabel, an average of 9% over all analyses, was associated with fraction F3. This fraction contains methylene chloride extractable metabolites that are considerably more polar than tetryl. Large quantities of radiolabel were contained in the aqueous basic fraction. This fraction contains highly polar compounds resulting from extensive metabolism of the parent munition. Over all analyses, the aqueous basic fraction was found to contain an average of 29% of the radiolabel. The largest percentage of radiolabel was found to be sequestered within the tissue pellets. This fraction contained an overall average of 46% of the radiolabel. Incorporation of radiolabel within the non-soluble biopolymeric pellet matrix is believed to represent an end point for metabolism of xenobiotics. Once the plant has compartmentalized xenobiotic metabolites within the lignin and cellulose pools, these metabolites are effectively removed from interfering with normal plant metabolic processes. A variety of aromatic xenobiotic residues, including chlorinated anilines, pentachlorophenol, 2,4-dichlorophenoxyacetic acid, and benzo[*a*]pyrene quinones, have been demonstrated to be incorporated within the lignin fraction [15]. It is interesting that all pellets from the 1-day-exposure tissues contained approximately the same proportion of sequestered radiola-

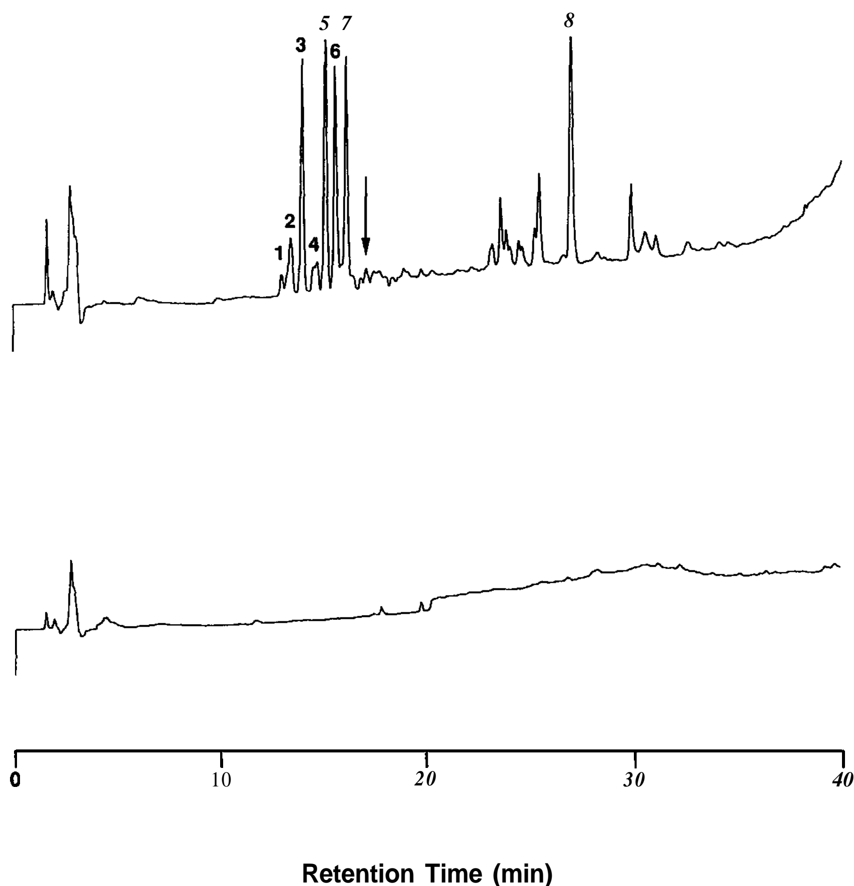

Fig. 3. Chromatographic profile of the F1 fraction of tetryl-exposed bush bean root tissue (top) compared to a control root extract (bottom). Metabolites of tetryl are numbered in the top chromatogram.

bel; whereas stem and root pellets from 7-day-exposure plants contained higher percentages of radiolabel than the corresponding leaf pellets. This result suggests that initial detoxification of tetryl involved sequestration of tetryl-derived radiolabel within the root and stem tissues. The mass balance for the fractionation scheme is included in the bottom row of Table III. These values represent the sum of the hydrochloric acid, the methylene chloride acid-neutral, and the pellet fractions. The material balance ranged from 72 to 110% with an overall average of 95%.

HPLC analysis. HPLC analysis was conducted on the F1 fractions; however, only the root tissues contained a sufficient amount of radiolabel to allow detection of tetryl metabolites. The top of Fig. 3 shows a representative chromatogram of fraction F1 from a bush bean root tissue that had been exposed to a tetryl-amended hydroponic culture for 4 days. The bottom of Fig. 3 shows a chromatogram of the corresponding fraction from a control bush bean root. The arrow in Fig. 3 indicates the retention time of tetryl. The parent explosive is present in this tissue at approximately the detection limit of 0.10 ppm. The most striking feature of this chromatogram is the series of tetryl metabolites appearing within the retention window of 12.85-15.93 min (compounds labeled 1 through 7 in Fig. 3). These compounds are slightly more polar than the parent munition and likely represent the very initial metabolic alterations of tetryl. Additionally, a compound eluting with a retention time of 26.79 min (labeled 8

TABLE III

ALKYLPHENONE RETENTION INDICES OF TETRYL AND PLANT METABOLITES OF TETRYL

Compound	Retention index
Aminodinitrophenylmethylnitramine[a]	872
N-methyl-2,4,6-trinitroaniline standard	922
Tetryl standard	946
Metabolite 1	852
Metabolite 2	861
Metabolite 3	873
Metabolite 4	889
Metabolite 5	coelutes with propiophenone
Metabolite 6	908
Metabolite 7	923

[a] Tentative identification based on mass spectral data [5]

in Figure 3) is present in the tetryl-exposed root and absent from the corresponding control tissue. HPLC radiochromatography served to verify incorporation of radiolabel in each of these metabolites. Of the metabolites implicated in Fig. 3, one compound was identified by co-injection experiments. An authentic N-methyl-2,4,6-trinitroaniline standard was found to co-elute with metabolite number 7. Additionally, the alkylphenone retention index of metabolite number 3 matched that of an aminodinitrophenylmethylnitramine isomer that was tentatively idenified during previous studies of tetryl in soils [5]. To aid future investigations focusing on the identification of tetryl plant metabolites, compounds 1 through 7 were characterized by their alkylphenone retention indices. These indices are presented Table III.

Monitoring of $^{14}CO_2$ and volatile organic emissions. Experiments designed to monitor $^{14}CO_2$ and volatile organic emissions indicated that a small amount of the radiolabel was oxidized to $^{14}CO_2$. No volatile organics evolved from the shoot during the 72-h exposure. Reliable data for emission of volatiles organics from plant roots could not be obtained due to aerosolization of small amounts of ^{14}C-tetryl from the hydroponic solution. The $^{14}CO_2$ respiration amounted to 0.07 and 1.0% of the plant-accumulated radiolabel for the shoot and root, respectively. The amount of respired $^{14}CO_2$ was not sufficient to account for the discrepancy noted earlier between the amount of radiolabel assimilated from hydroponic cultures and the amount actually contained within the plant tissues. Possible explanations for this discrepancy include inefficient trapping of small volatile organic compounds on the XAD resin due to the large volumes and high flow-rates required to maintain plant turgor or stress-related metabolic differences induced by maintenance of the plant in the volatile emissions experimental chamber.

Comparison to plant metabolism of TNT and RDX. The plant metabolism of tetryl described in this study closely parallels results previously obtained for TNT [6]. Radiolabel from both nitroaromatic explosives was found to be localized primarily in the root tissue. Interestingly, plants exposed to dinitroaniline herbicides also display localization of radiolabel within the root tissue [16]. The presence of the nitroaromatic nucleus of these compounds seems to be associated with the tendency for root tissue accumulation. Chemical fractionation of TNT-exposed plants indicated extensive metabolism toward more polar metabolites; a result that again parallels the present studies with tetryl. Additionally, exposures of plants to either TNT or tetryl resulted in significant quantities of radiolabel being sequestered within the tissue pellets. Results from plant metabolism studies performed with the hexahydrotriazine explosive RDX [7] contrast sharply with those for TNT and tetryl. RDX-exposed plants were found to bioaccumulate the parent munition within the aerial tissues. Metabolism toward

polar products was also observed for RDX; however, the quantity of parent munition far exceeded the level of polar metabolites in leaf tissues. Additionally, the tissue pellets from RDX-exposed plants contained lower percentages of radiolabel than pellets from either TNT- or tetryl-exposed plants.

CONCLUSIONS

Studies described here allow, for the first time, analysis of the explosive tetryl in plant tissues with high recovery and precision. Analytical methodology is based on acid hydrolysis of the tissue to free polar conjugates followed by solvent extraction. The organic extract is then fractionated by silica gel chromatography to remove interfering indigenous plant components prior to HPLC determination of tetryl. Chromatographic recovery of tetryl from fortified bush bean leaves was 82.70 ±5.54%, whereas radiolabel recovery was 80.49 ± 0.59%. The methodology was applied to study plant uptake and metabolism of tetryl in bush bean plants maintained on tetryl-amended hydroponic cultures for either 1 or 7 days. Importantly, the use of ^{14}C-tetryl combined with HPLC analysis of fraction F1 allowed assessment of the proportion of plant-sequestered radiolabel that was present as the parent munition. Analysis of plants exposed to tetryl-amended hydroponic solutions indicated very rapid metabolism of this explosive to polar metabolites, with less than 3.1% of the radiolabel found associated with the tetryl-containing silica gel fraction. Tetryl was at or below the chromatographic detection limit in the root tissues. As with previous studies on TNT plant metabolism [6], the majority of tetryl-derived radiolabel was found associated with the root tissue. Sequestration of radiolabel in the tissue pellet fraction was found to represent a major route for tetryl detoxification in the bush bean plants.

HPLC analysis of fraction F1 from root tissue indicated the presence of a variety of a tetryl metabolites that likely represent the initial metabolic transformation products of the parent munition. These tetryl metabolites were characterized by their alkylphenone retention indices. One of these components was identified by chromatographic co-elution experiments as N-methyl-2,4,6-trinitroaniline. This compound was also present in light-exposed hydroponic control solutions and was previously implicated as being a principal soil transformation product of tetryl [5]. A match in the retention indices between metabolite 3 and an aminodinitrophenylmethylnitramine isomer, that was characterized during previous studies [5], suggests that one metabolic route involves ring nitro reduction of the parent munition. Chemical identification of these tetryl-derived plant metabolites should be the focus of further studies.

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