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INTRODUCTION

Among the substituted ureas, TH-6040 is becoming increasingly important in the field of insect control, primarily due to its specificity and low mammalian toxicity. Prior to its use in any large scale forest pest control spray programs, it has become necessary to develop suitable analytical methods to estimate the residues of TH-6040 present in nano gram levels in various forest components and to study its persistence and fate in a simulated forest spray operation. Therefore the primary objectives of this program are threefold:

1. Explorative research to gas chromatograph the parent compound and/or its derivatives.
2. Sensitive analytical methodology development for the TH-6040 residues in coniferous foliage, forest soil and natural waters.
3. Study of the degradation, persistence and fate of the

insecticide in forest components after simulated spray application.

The following is a summary of the present progress of the study:

1. GLC Analysis of TH-6040 and its Dimethyl Derivative

The initial observation, after some trial experiments, was that TH-6040 and its derivatives were not readily "GC-able" under ordinary conditions and required an unusually high degree of art and skill and inexhaustible patience on the part of the residue chemist.

(a) Detection of underivatized TH-6040

A standard solution of the compound in benzene was prepared and aliquots (2-6 ul) were injected separately on gas chromatographs equipped with Ni 63 E.C. or FID detectors and OV1 or SE30 columns. Either the peaks obtained were broad or absent indicating the decomposition of the molecule. Variation of the GC parameters were not successful so far to chromatograph the compound intact.

(b) Derivatization of TH-6040 - Methylation

An obvious alternative to detection of the intact TH-6040 is derivatization to a "GC-able" form. We tried methylation using methyl iodide and sodium hydride in presence of DMSO as the solvent medium according to the following procedure:

Two hundred micrograms of TH-6040 was dissolved in 1 ml of DMSO in a centrifuge tube, and after adding ca 50 mg of NaH and 0.30 ml of CH_3I, the solution was shaken for 3 min and allowed to stand for 15 min. Hexane (3 ml) was added followed by water (distilled) in drops until the effervescence (H_2 evolution) stopped. The sample was partitioned twice

with 10 ml of water and the aqueous phase was discarded. The hexane phase was air-evaporated to dryness and the residue (dimethyl derivative of TH-6040) was taken up in hexane for GC analysis using a Coulson conductivity detector in nitrogen mode.

Gas Chromatographic Conditions

G.C.	:	Tracer Model 220
Detector	:	Coulson conductivity in nitrogen mode
Column	:	4' x 0.25" (O.D.) U shaped, 3% OV-1 on Chromosorb W (H.P.) 80/100 mesh
Temperature (^{o}C)	:	Inlet 240 Oven 235 Transfer line 240 Pyrolysis furnace 800
Carrier gas (ml/min)	:	He - 80
Hydrogen (ml/min)	:	50
Resistence bridge	:	Attenuation 1 or 2 Voltage 30
Chart speed (in/hr)	:	7.5
Solvent vent time (min)	:	1 or 1.5
Retention time	:	2 min.35 sec.

The chromatographic profile for the dimethyl derivative is given in Fig. 1. The peak is narrow and symmetrical with good reproductivity and absence of any additional peaks. The derivatization method outlined above is manageable and sensitive enough to detect 0.20 ppm of TH-6040 and could be used to analyse effectively environmental samples, if suitable extraction and cleanup methods are worked out.

2. Methodology Development for coniferous foliage, soil and water

The next phase in the study was to find agreeable extraction and cleanup methods for TH-6040 from forest environmental samples.

Coniferous foliage (white spruce)

Samples (20 g) of Hobart chopped foliage in triplicate were homogenized in a Sorvall for 5 min at speed 5 with 2 x 100 ml of acetonitrile after spiking with 10 ppm (10 ug/g) of TH-6040. The homogenate was filtered under suction through a Whatman No. 1 filter paper. After washing the residue with 20 ml of acetonitrile, the extracts were pooled and passed through a column of Na_2SO_4 (50 g). The column was rinsed with 50 ml of solvent, the filtrate flashed to ca 100 ml and equilibrated thrice with 30 ml of hexane. The nonpolar phase was discarded after partitioning with 30 ml of CH_3CN. The polar phases were pooled and flash evaporated to near dryness. The cleanup was achieved by dissolving the residue in 4 x 1 ml acetone-hexane (1:4) mixture and passing it through a column (35cm x 14 mm) containing 10 g of partly deactivated Florisil (5.5% H_2O) sandwiched between Na_2SO_4 (5 g) and prewashed with 50 ml of petroleum ether. The column was eluted in succession with hexane (45 ml), acetone-hexane (1:9, 30 ml) and acetone-hexane (1:4, 10 ml) discarding them all, and finally with acetone-hexane (1:4, 50 ml). The final eluate containing TH-6040 was flash evaporated to dryness for derivatization according to the precedure outlined in Section 1.

The recovery of TH-6040 from spruce foliage after fortification with 10 ug/g is given below.

Sample No.	Percent Recovery
I	83.5
II	74.7
III	81.0
Average	79.7

Lower fortification levels (1 and 5 ppm levels) yielded lower recovery values showing that this method is suitable if the substrate matrix contains ca an optimum value 10 ug/g of TH-6040. The average recovery is ca 80% which is acceptable for forest environmental samples especially for coniferous foliage which are loaded with plant waxes and terpenes. The low value may be due to poor extraction efficiency, losses in partition and cleanup and probably in derivatization steps.

The GLC response is shown in Fig. 2. The background interference in the chromatogram is negligible showing that the extraction, separation, cleanup and derivatization operations were, though not excellent, just adequate. With minor modifications to increase the percent residue recovery, this procedure could be adopted for residue analysis of TH-6040 from coniferous foliage.

Different solvents for extraction (acetone, chloroform, dichloromethane), column adsorbents (Florisil 0% H_2O, 10% H_2O, Al_2O_3, charcoal, SiO_2 etc) and column eluants [1,2] (CH_2Cl_2 hexane-CH_2Cl_2) were tried but none were satisfactory due to low recoveries and inconsistent results.

Ethyl acetate being less polar than CH_3CN will minimize the coextractive impurities and appears to be a suitable solvent for extracting TH-6040 from coniferous foliage. This aspect of the study will be continued on a later date.

Forest Soil

The extraction, partition, cleanup and derivatization procedures adopted were very similar to that for foliage. The average recovery values obtained were low (28 ± 11%). Although the column cleanup was satisfactory with minimum GLC interferences, the method was found to be unreliable and needed modifications. The low values obtained could be attributed to the strong adsorptive interactions between the soil matrix and the polar insecticide molecule as well as to its possible metabolic breakdown in the soil substrate and incomplete derivatization due to the possible presence of phenolic moieties in the final extract.

Water Samples

Fifty millilitre volumes of distilled water samples were taken in 250 ml separatory funnels and spiked with 0.5, 1.0, 2.0 and 3.0 ug/g of TH-6040 to give concentrations ranging from 0.003 to 0.02 ppm. The samples were extracted by shaking vigorously for 3 min. with 3 x 50 ml of dichloromethane. The organic layer was collected after equilibration, dried by passing through columns of Na_2SO_4, flash evaporated to dryness, derivatized and analysed by GLC as outlined earlier under foliage.

Recovery values were low and ranged from 17 to 39%. Dichloromethane, although easy to handle and work with (no emulsion problems, distinct and rapid phase separations, etc.) gave low recoveries. Other solvents like chloroform and toluene are being tried at present and encouraging results are anticipated.

3. Persistence of TH-6040 in the Forest Environment

Since the methodology of TH-6040 is still not yet perfected, this aspect of the study will be examined more thoroughly on a later

date. At present foliage, soil and water samples were sampled at frequent intervals from Larose Forest after spraying (5 oz AI/acre) a few trees with TH-6040 in July 1975, using Mr Hopewell's device for simulated aerial spray. The insecticide residues are extracted and stored in freezers for future analysis. Random studies in quantifying the TH-6040 residues in the foliar extracts showed that the compound appeared to degrade rapidly in the forest environment. A detailed and thorough investigation on the fate and persistence of TH-6040 is required for evaluating properly the potentialities of the insecticide in a forest ecosystem.

Conclusions

The data presented in this report indicate that although it is not possible to assay TH-6040 directly by GLC, derivatization (methylation) techniques developed at this laboratory show considerable promise. The parent compound present in standard solutions could be quantified in nano gram levels through methylation of the parent molecule; the experimental conditions for derivatization and the necessary GLC parameters for the final analysis using the Coulson Conductivity detector (in nitrogen mode) were worked out.

A reasonably simple and sensitive GLC method involving solvent extraction, partitioning, Florisil column clenaup, derivatization by methylation and final determination of TH-6040 in coniferous foliage is developed at this Institute and described. Recovery values for spiked samples were ca 80% with a sensitivity of 0.2 ppm. Extension of the procedure to soil samples gave low recovery values and needed modifications in the experimental method. Recovery studies of TH-6040 using fortified water samples were low when CH_2Cl_2 was used as an

extractant. In future, alternative solvents should be used for extraction to improve the recovery of the insecticide.

Attempts are being made to modify and extend the procedure to forest environmental samples. Preliminary studies show that the insecticide is appreciably unstable in some of the forest components. A detailed and thorough investigation on the fate and persistence of TH-6040 is planned for the future.

ACKNOWLEDGEMENTS

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THGOHO STANDARD

Volume Injected 4µl
Concentration 25ng/µl
Attenuation 2
G C. 26/9/75

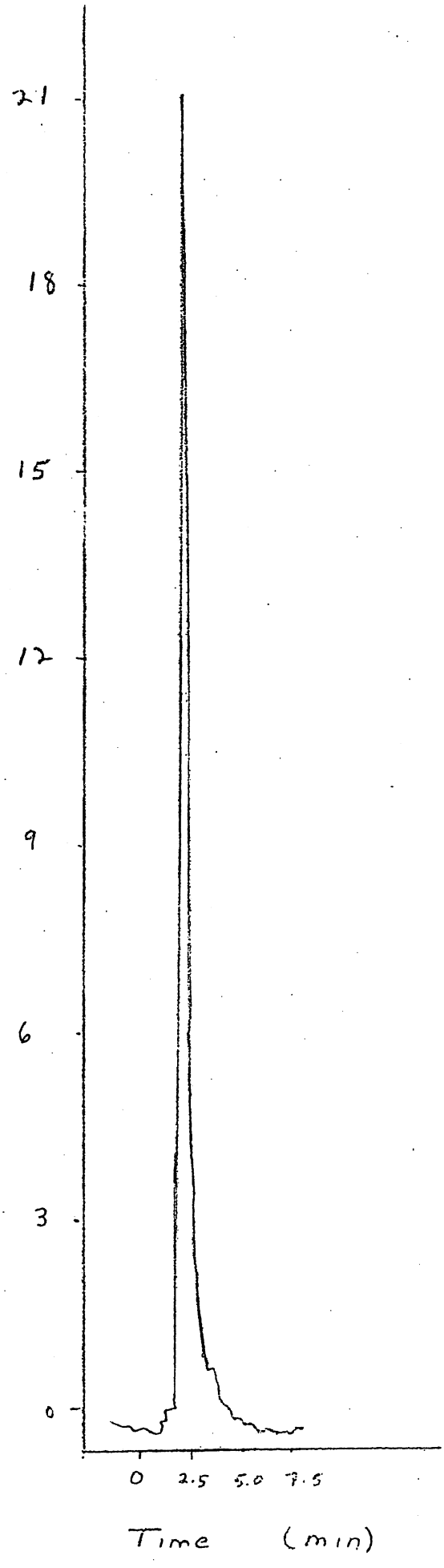
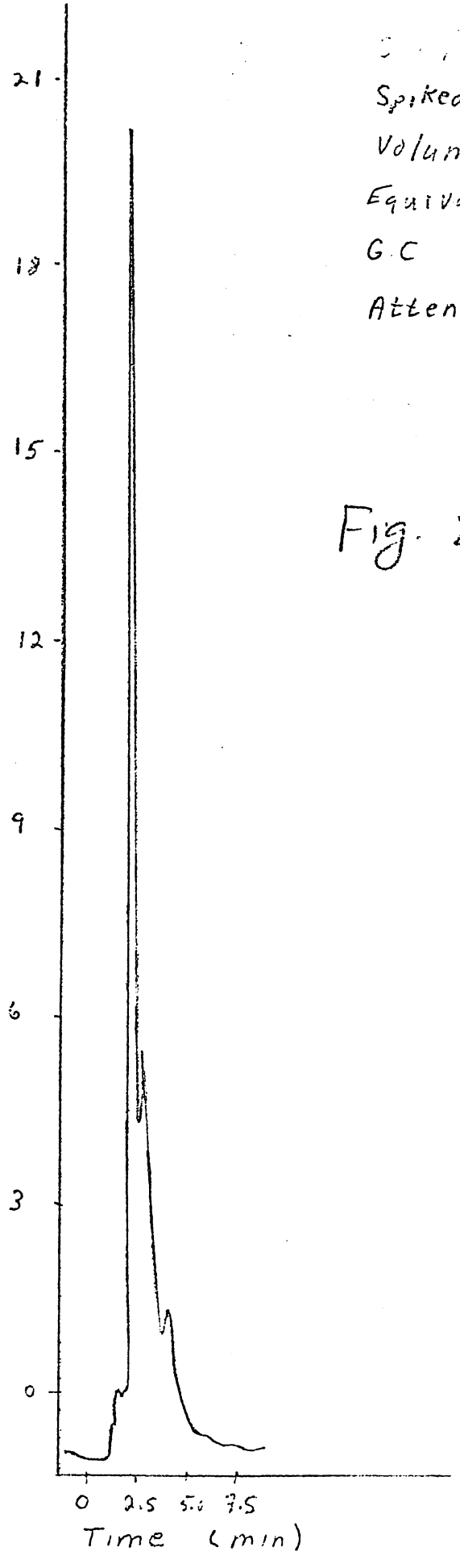


Fig. 1.

Spruce Foliage



Spiked 10 ppm
Volume Injected 4 µl
Equivalent weight 4 mg/µl
G.C 26/9/75
Attenuation 2

Fig. 2.