DETECTION AND IDENTIFICATION OF MEXACARBATE AND SOME OF ITS METABOLITES FROM FORESTRY SUBSTRATES BY THIN LAYER CHROMATOGRAPHY

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ABSTRACT

A simple and effective thin layer chromatographic (TLC) method has been developed and reported for the separation and identification of mexacarbate (4-dimethylamino-3,5-xylyl N-methylcarbamate) and five of its metabolites from different forestry substrates. State-of-the-art of solvent system selection development, detection and visual evaluation, relevant to the study, have been discussed. Quantitation *via* autoradiography using C-14 mexacarbate has been attempted. All spots and the corresponding R_f values were identified through comparison to standards. The applicability of the technique to separate and identify the insecticide and its different metabolites from spiked and field samples has been demonstrated.

INTRODUCTION

Mexacarbate (4-dimethylamino-3,5-xylyl N-methylcarbamate), a broad spectrum insecticide, was introduced by the Dow Chemical Company under the trade name Zectran® in 1961.[1] The chemical was field tested for control of spruce budworm (*Choristoneura fumiferana*, Clemens) larvae in different eastern provinces of Canada during the 1972-73 spray seasons.[2] The chemical is currently being re-examined for large scale forestry use in Canada because of its desirable properties such as pest selectivity[3], low mammalian toxicity (LD_{50} 20 mg/kg)[4] and low persistence in the environment.[5,3] One of the primary requirements, with such extensive operational use patterns, is to have sensitive and reliable residue methods to study the distribution, persistence and metabolic fate of the sprayed material found in various forestry substrates. Union Carbide claims[3] that mexacarbate may be highly labile in the environment but that some of its degradation products, especially the 4-methylamino and the 4-amino-3,5-xylyl N-methylcarbamates, were found to be more toxic than the parent material.[6] The formation of such compounds due to the breakdown of the released active ingredient (AI) in the forestry compartments would be on the increasing scale and could persist longer. It has therefore become necessary to monitor simultaneously the parent material and its likely breakdown products. To date, relatively few residue methods have been reported for the derivatives of mexacarbate.

In this study, a simple, inexpensive and effective thin layer chromatographic (TLC) method using a variety of developing solvents

was employed to separate mexacarbate (Zectran®) and its major metabolites on TLC plates coated with Linear K Silica Gel and High Performance (HP) Silica Gel. In addition to viewing the developed plates by UV light, visualization of the spots was facilitated by using a ninhydrin spray and a ferric chloride-potassium ferricyanide spray. Further research combining this TLC technique with autoradiographic detection showed promising results and the work on these lines is being continued in this laboratory for eventual quantification of the separated spots in the chromatogram.

MATERIALS AND METHODS

Chemicals used in the TLC Study

Analytical standards of mexacarbate (Zectran®) and the five derivatives used in the TLC study were supplied by Union Carbide. The structural formulae of the chemicals, their chemical names according to the International Union of Pure and Applied Chemistry (IUPAC), their abbreviations and the corresponding numbers used to identify them in this report are given in Table 1. All solvents used were of pesticide grade and dry. Where necessary and hygroscopic, they were passed through a column of Na_2SO_4 and stored in desiccators.

TLC Plates

Two types of TLC plates were used:

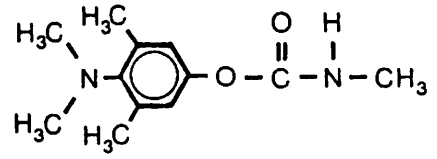
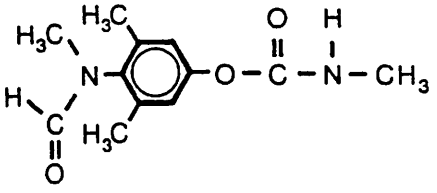
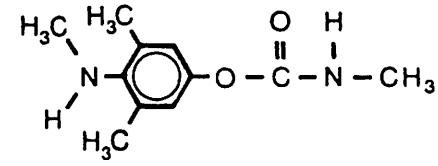
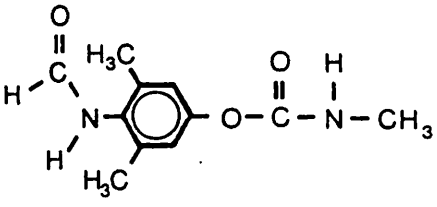
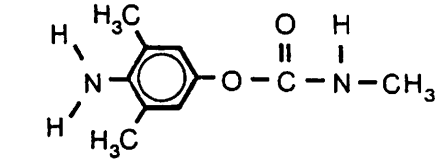
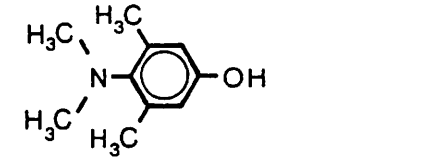
1) LK5F Linear K Silica Gel by Whatman (Cat. #4856-820)

   Layer thickness = 250 µm

   Size = 20 x 20 cm

TABLE 1

Some Common Metabolites of Mexacarbate Used in the Study

No.	CHEMICAL STRUCTURE	NAME (IUPAC USAGE)	ABBR.
1		4-Dimethylamino-3,5-xylyl N-methylcarbamate	M
2		4-Methylformamido-3,5-xylyl N-methylcarbamate	MFM
3		4-Methylamino-3,5-xylyl N-methylcarbamate	MAM
4		4-Formamido-3,5-xylyl N-methylcarbamate	FAM
5		4-Amino-3,5-xylyl N-methylcarbamate	AM
6		4-Dimethylamino-3,5-xylenol	DMAX

2) Si-HPF High Performance (HP) Silica Gel 7011-4 by Baker (Cat. #C5451-162)

Layer thickness = 200 μm (Hard surfaced)

Size = 10 x 10 cm

All TLC plates were heated in an oven at 120°C for at least one hour prior to spot application to activate the sorbent.

Preparation of Standard Solutions and Application

Standard solutions of mexacarbate and its metabolites were prepared in EtOAc at concentrations ranging from 0.8688 mg/mL to 2.389 mg/mL and spotted on both types of thin-layer plates 1.0 cm above the lower edge. The technique used in this study is fully discussed by Touchstone and Dobbins.[7] A 1.0 cm margin was allowed on the 10 x 10 cm plates and a 2.0 cm margin for the 20 x 20 cm plates. The diameters of the spots were maintained within the 0.50 to 0.75 cm range. The plates were allowed to air dry (*ca.* one min) completely before developing.

Developing Technique

Development (ascending) took place in a glass tank (8.5 x 21 x 21.5 cm) containing an approximate height of 2 mm of developing solvent. A filter paper placed within the tank ensured that the tank was saturated with the solvent vapor.

Mobile Phase Selection

In selecting the final solvent system for TLC, sorbent-mobile phase, sorbent-solute and mobile phase solvent interactions were considered and eventually with patience and using trial-and-error method, a good solvent system was found to separate the mexacarbate and five of

its metabolites. Table 2 outlines the mobile solvent systems which were tested on trial and error basis in the study.

Table 2. Solvent systems tested in the TLC for the separation and identification of Zectran® and its Metabolites

Solvent System	Ratio of Components (v/v)
n-Butanol : Acetic Acid : Water	12:3:5
Hexane : Acetone	1:1
Diethyl Ether : Hexane : Ethanol	77:20:3*
Acetone : Toluene : Pentane	10:10:30
Hexane : Acetone	5:1
Diethyl Ether : Hexane : Ethanol	65:30:5

*Solvent system preferred in the study.

The developed plates were removed from the tank when the solvent front had travelled the desired distance. They were subsequently air-dried (*ca.* one min) and viewed under UV light. Plates spotted with cold material were sprayed with chromogenic reagents for spot visualization while those spotted with hot material were sprayed with EN^{3}HANCE Spray (Cat. #NEF-970, New England Nuclear, Boston, Mass.), a surface autoradiography enhancer for autoradiographic detection.

Two Dimensional Development

Using Hexane: Acetone (1:1/v/v) as the solvent system for both phases, complete resolution of Zectran® and its metabolites can be achieved using normal two-dimensional TLC techniques. A mixture of mexacarbate and its metabolites spotted on a HP precoated silica TLC plate did not show complete separation after development in one-dimension only. Compounds 2 and 5 as well as 6 and mexacarbate had overlapping spots. However, by rotating the plate by 90° and developing it in the second dimension, complete resolution of the parent compound and its

derivatives was obtained (Fig. 1). The spots of solutes on the chromatogram had optimum distribution because of their varying adsorption/desorption interactions with the sorbent and the mobile phase.

Spot Visualization:

Using different visualization reagents, all of them destructive in their action (chemical change of the substance), significant contrast between the spots and the background was obtained to identify the active material and its metabolites.

The reagents used in the visualization studies are:

1. Ninhydrin (1,2,3-Indantrione)

Air-dried plates were sprayed with 10% aqueous sodium hydroxide solution (w/v) in the fume hood and heated for 2-3 min at 60°C in an oven. They were subsequently sprayed with 2% Ninhydrin solution in ethanol (w/v) and heated at 60°C for 30 min.

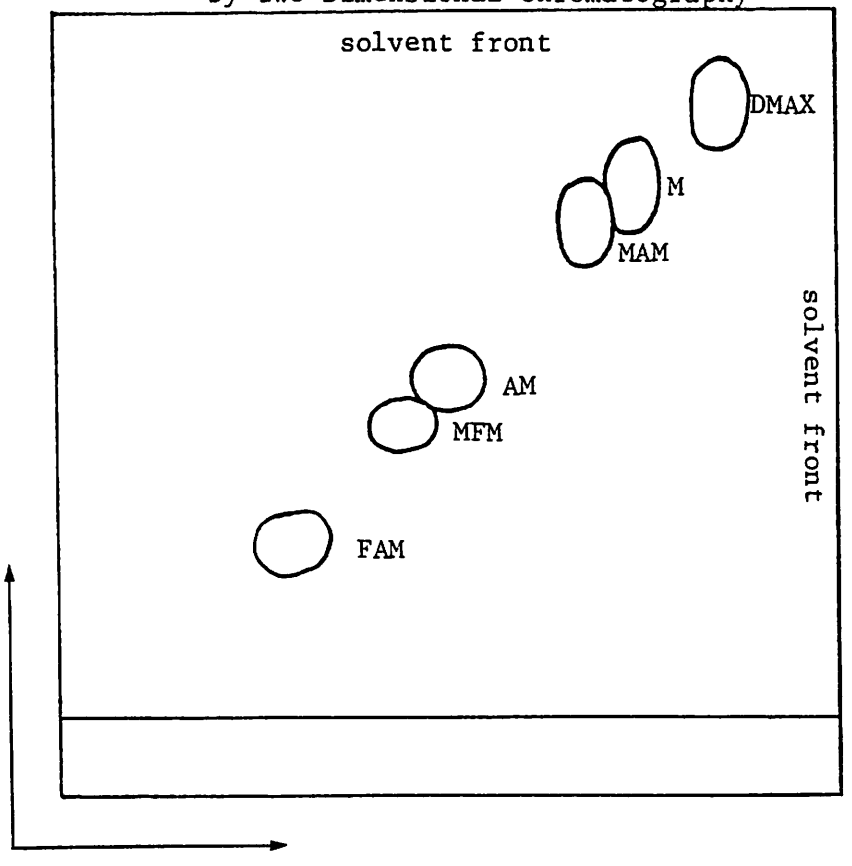
Zectran® and its metabolites appeared as pinkish-purple spots on a white background after spraying.

2. Ferric Chloride-Potassium Ferricyanide

Visualization of aromatic amines was also facilitated by a ferric chloride-potassium ferricyanide spray. A 0.1 M ferric chloride solution and a 0.1 M potassium ferricyanide solution were prepared and mixed 1:1 immediately prior to spraying. Plates were sprayed and then heated at 110°C for 60 min.

Zectran® and its metabolites appeared as blue spots on a light blue background. The spots turned darker with time.

Fig. 1. Separation of Mexacarbate and its
by Two-Dimensional Chromatography



3. Other Reagents

Other visualization methods were also tested but no positive results were obtained with some exceptions.

A Fluorescamine (Fluram, Roche) solution (25 mg in 100 mL of dimethylformamide, DMF) was sprayed on air-dried TLC plates. Plates were observed by UV while they were still wet, however, as expected, no white spots due to fluorescence were observed.

Ehrlich Reagent - a 10% solution of *P*-dimethylaminobenzaldehyde in concentrated HCl - was prepared and mixed immediately in the ratio of 1:4 with acetone before spraying. After approximately 20 seconds, metabolites 3 and 5 produced a bright yellow color signifying the presence of methylamino and amino groups respectively, while mexacarbate produced a faint yellow color. However, metabolites 2, 4 and 6 did not give satisfactory contrast between the visualized area and the background. Very likely, the formamido and phenolic groups have inhibitory effects in color production.

A 1% solution of *P*-dimelthylaminobenzaldehyde in 5% HCl did not give any positive results.

TLC Studies with Spiked Forestry Samples:

To determine the suitability of this TLC methodology in conifer foliage and forest soil extractions and also to study the fate of mexacarbate in these two substrates, foliage and soil samples were spiked with a known concentraton of Zectran® prior to extraction. They were extracted using suitable organic solvents, concentrated under vacuum, then varying amounts of the concentrated extract were spotted on the TLC plate. The plates were developed as described earlier and the presence

of mexacarbate and its metabolites was confirmed by comparing the R_f values with the standards (Table 3) (Figs. 2 and 3).

Autoradiographic Studies

Carbon-14 ring-labelled mexacarbate is useful in metabolic studies because of its inherent sensitivity in detecting and quantifying the intact and breakdown products. In autoradiography, the TLC chromatogram is resolved by exposing it to x-ray film. The radioactivity level will determine the exposure time. The two-fold purpose of this study was to discover:

1. The optimum concentration of mexacarbate and its metabolites that could be detected by autoradiographic technique and
2. The minimum concentration of C-14 (ring) mexacarbate required to spike the soil columns to understand the mobility and fate of the chemical.

A series of TLC plates were spotted with radioactive C-14 mexacarbate, and with soil and foliage extract which had previously been spiked with C-14 (ring) labelled Zectran®. A variety of development parameters (time, temperature, activity) were tested to determine the optimum concentration of C-14 labelled mexacarbate detectable visibly on the x-ray film for a given set of conditions. The film was developed according to the standard photographic procedures outlined in Table 4.

Table 3. R_f values of Zectran and its metabolites with a variety of solvent systems on Baker Si-HPF TLC plates.

Compound	No.	R_f Values on Baker Si-HPF			
		Butanol:Acetic Acid:H_2O 12:3:5	Hexane:Acetone 1:1	Diethyl Ether:Hexane:EtOH 77:20:3	Acetone:Toluene:Pentane 10:10:30
M	1	0.74	0.61	0.69	0.58
MFM	2	0.74	0.34	0.28	0.17
MAM	3	0.44	0.41	0.40	0.26
FAM	4	0.67	0.22	0.13	0.06
AM	5	0.67	0.37	0.34	0.18
DMAX	6	0.44	0.65	0.81	0.66

Table 4. Procedure for Autoradiographic Technique

1) Place completely dry spotted chromatogram in a large, deep box.

2) In a fume hood, evenly saturate the chromatogram with EN^{3}HANCE SPRAY (Surface Autoradiography Enhancer).

3) Allow to dry for several hours.

4) Wrap cardboard sheets of Kodak X-Omatic Cassette in Saran Wrap as protection from radioactive spillings, etc.

5) Tape chromatogram in place in cassette.

6) In the dark room, remove film from individually-wrapped package of Kodak Ready-Pack film. Discard yellow sheet surrounding the film itself.

7) Place film directly on top of chromatogram. Place cardboard sheet on top. Close cassette.

8) Place cassette in freezer for desired length of time.

9) Remove from freezer and allow cassette to warm up while preparing solutions below.

10)

a) X-ray Developer	5 minutes
b) Water + 1% Glacial Acetic Acid	5-10 seconds
c) Stock X-ray Rapid Fixer	10 minutes

11) Remove film from cassette and immerse it in each of the above solutions for the specified time. Gently shake the film approximately every 20 seconds.

*Note: Place a bent corner in the upper right hand corner of the film and use this to transfer the film from one solution to another.

12) White lights may be turned on after 5 minutes in Fixer.

13) Wash developed film in water bath for 15-30 minutes.

14) Rinse with deionized water from wash bottle.

RESULTS AND DISCUSSION

Of all the solvent systems used in this study, the Diethyl Ether:Hexane:Ethanol system (77:20:3) produced the best resolution of Zectran® and its metabolites, as can be seen in the R_f values recorded in Table 3. When n-Butanol:Acetic Acid:Water was used, mexacarbate and 2, 3 and 6, and 4 and 5 were not resolvable. With the Hexane:Acetone solvent system in either a 1:1 ratio or a 5:1 ratio, separation of mexacarbate and 6, and 2 and 5 was not achieved. The Acetone:Toluene:Pentane system was somewhat better as only one pair of metabolites, 2 and 5 was not resolved. Complete resolution, i.e., 6 distinct R_f values, was obtained when Diethyl Ether:Hexane:Ethanol was used as the solvent system. Similar results were obtained when LK5F Linear K Silica Gel was used as the matrix.

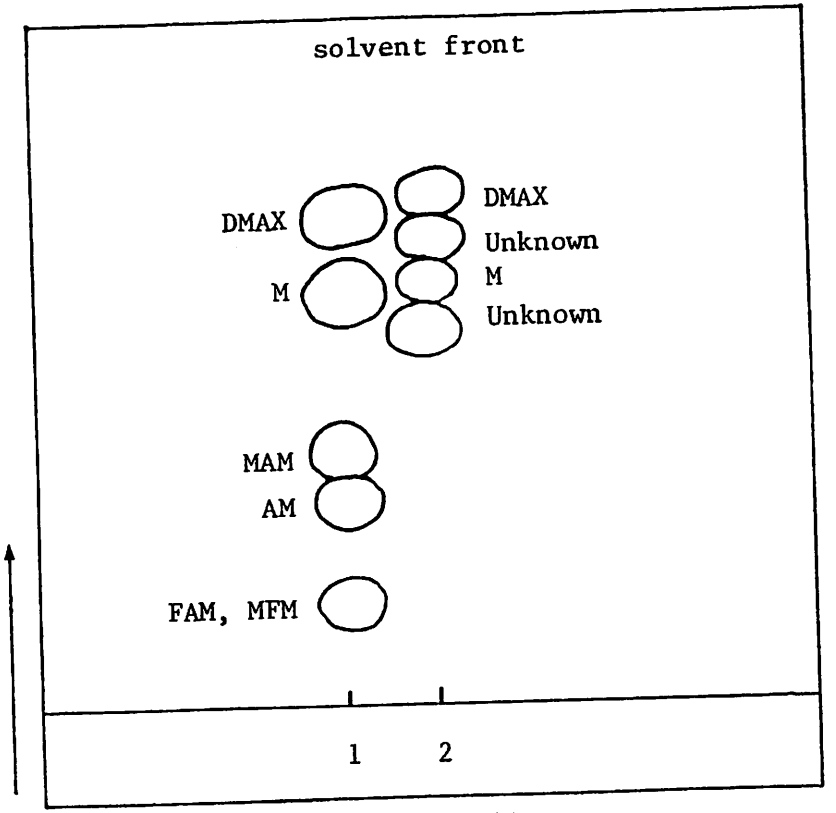
The minimum detection level of mexacarbate and its metabolites was approximately 5 µg. Concentrations below this level were not visible under UV light nor were they detected by a Ninhydrin spray or ferric chloride-potassium ferricyanide spray. When equal concentrations of mexacarbate were applied to both types of plates, the intensity of the spots was considerably lower on the large (20 x 20 cm) Linear K Silica Gel plate compared to the smaller High Performance Silica Gel Plate (10 x 10 cm). The HP Silica Gel plate has been the most appropriate one in this study. The optimum mexacarbate concentration detectable in the larger plate is *ca.* 10 µg.

Application of Foliage and Soil Extract

Various concentrations of foliage and soil extracts spiked with Zectran® were applied after solvent extraction to both types of TLC plates. Due to its hard surfaced layer, the extract did not absorb as well into the High Performance Silica Gel Plate as it did into the Linear K Silica Gel Plate. For both soil and foliage extract, resolution was poor for every solvent system with the one exception of Diethyl Ether:Hexane:Ethanol. Resolution of the soil extract was possible using the Diethyl Ether solvent system when extract in concentrations of 1.0 g soil/mL and 2.0 g soil/mL were passed through microcolumn cleanup to remove extraneous, interfering materials. Three distinct spots were visible by UV light indicating mexacarbate degradation. Among the 3 spots observed (Fig. 3) one corresponded to the R_f value for mexacarbate (R_f = 0.71) and the other for metabolite 5 (R_f = 0.37). The third spot with the R_f value of 0.44 did not correspond to any of the known mexacarbates. Resolution was not possible for soil concentrations exceeding 2.0 g soil/mL without further clean-up. Resolution of foliage extract spiked with Zectran® was facilitated by using the following clean-up procedure.

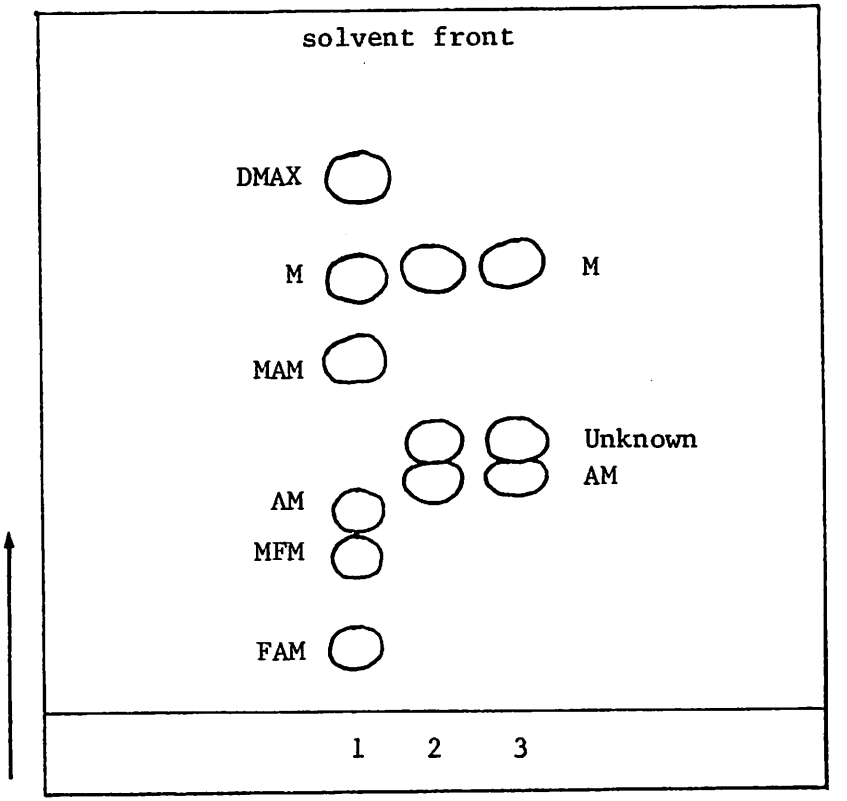
The foliar extract of required concentration was N-evaporated to dryness using a gentle stream of pure nitrogen. The residue was treated with 1 mL of 1.0 M HCl and 1 mL of CH_2Cl_2. The CH_2Cl_2 layer containing chlorophyll was discarded and the remaining aqueous layer containing mexacarbate in the ionic form was neutralized with 10% sodium carbonate to pH 7. The solution was partitioned with 0.5 mL of benzene and the mexacarbate was extracted into the organic phase by shaking.

Fig. 2. TLC Chromatogram of Conifer Foliage



1 Mexacarbate plus metabolites
2 Foliage extract samples

Fig. 3. TLC Chromatogram of Forest Soil



1 Mexacarbate plus metabolites
2,3 Soil extract samples.

The organic layer was removed by micropipet and N-evaporated to a volume of 20 μL for TLC application.

The cleaned-up foliage extract was resolved into 4 different spots (Fig. 2) (Table 5) as seen by UV light and subsequent ninhydrin spray. One spot corresponded exactly to the spot for mexacarbate (R_f = 0.65). One spot was similar to the spot for metabolite 6 under the same development conditions with an R_f value of 0.76. Two other spots with R_f values of 0.59 and 0.80 did not correspond to any of the other four known metabolites.

Table 5. TLC of foliage and soil extract samples

Sample	RF Values for Standards		Sample RF Values		Compound Identified
Foliage Extract	0.17	FAM, MFM	R_{f1}	= 0.59	Unknown
	0.32	AM	R_{f2}	= 0.65	M
	0.40	MAM	R_{f3}	= 0.76	DMAX
	0.65	M	R_{f4}	= 0.80	Unknown
	0.77	DMAX			
Soil Extract	0.10	FAM	R_{f1}	= 0.37	AM
	0.25	MFM	R_{f2}	= 0.44	Unknown
	0.33	AM	R_{f3}	= 0.71	M
	0.56	MAM			
	0.70	M			
	0.85	DMAX			

Pseudoautography, conducted using nonradioactive mexacarbate under similar experimental conditions (Table 4), did not produce any spurious blackening on the x-ray film.

During the autoradiographic studies, a TLC plate was spotted with C-14 Zectran® and developed for 72 hours at -21°C (household freezer). Approximately 1000 DPM were detected. However, on development, the spots which appeared as black areas on x-ray films were very

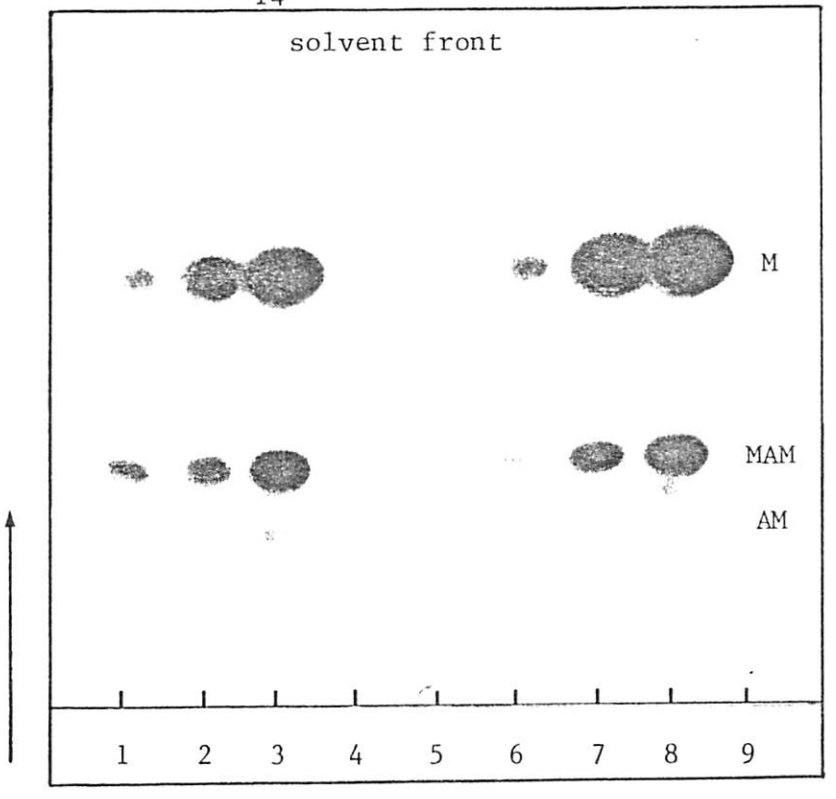
faint. Thus, 1000 DPM was the minimum detection level for Zectran® standard under these conditions.

As the intensity and size of the spots are a function of time spent in freezer, freezer temperature and number of DPM, increasing the development time to 96 hours produces larger and more intense spots, thus making detection easier.

Foliage samples which were spiked with approximately 4.6×10^5 DPM prior to extraction were spotted on TLC plates. The extract concentrations ranged from 0.05 g to 0.5 g, representing approximately 500 DPM to 5,000 DPM based on 100% recovery. The activity on the x-ray film was absent. Further work is necessary with increased concentrations of active material for definite identification of the insecticide and its metabolites from foliage.

Three forest soil extractions were performed using LHF (litter) (sandy loam) samples (2 g soil/mL) spiked with 50.03 µg cold Zectran®. In addition, each sample was spiked with a known quantity of DPM: 4.6×10^5 DPM, 9.2×10^5 DPM and 2.3×10^6 DPM respectively. The purpose of this experiment, as stated earlier, was to determine the optimum concentration of C-14 labelled Zectran® for soil column leaching studies as well as studying the metabolic breakdown products of Zectran® in forest soils. Samples which were passed through an aluminum oxide (neutral) microcolumn cleanup were compared to pre-microcolumn samples on the same TLC plate (Fig. 4a, 4b). Pre-microcolumn samples were more intense than post-microcolumn samples as seen on X-ray film (Fig. 4), but relative intensities as seen by UV light were approximately equal (Fig. 4b).

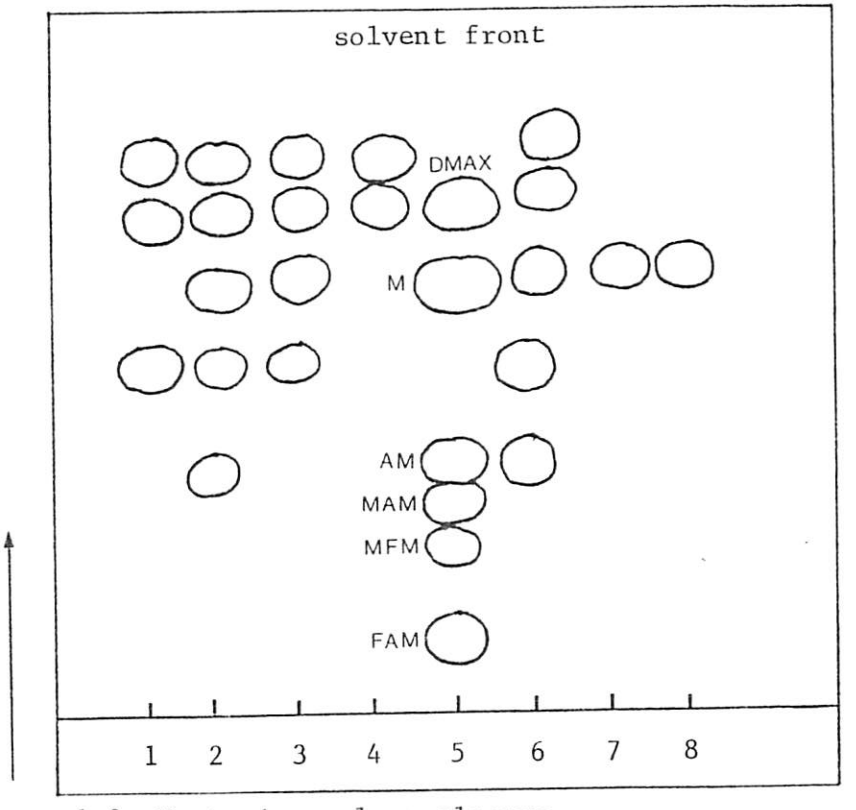
Fig. 4a. Autoradiographic Chromatogram of C_{14} in LHF (litter) Layer



1-3 Post-microcolumn cleanup
5 Mexacarbate plus metabolites
7-9 Pre-microcolumn cleanup

1,6 4.6×10^5 DPM
2,7 9.2×10^5 DPM
3,8 2.3×10^6 DPM

Fig. 4b. TLC Chromatogram of LHF (litter) Layer



1-3 Post-microcolumn cleanup
4 Extract only
5 Mexacarbate plus metabolites
6-8 Pre-microcolumn cleanup

Also, a greater number of spots were visible under UV light for pre-microcolumn samples (Fig. 4b) than for post-microcolumn samples.

The metabolic breakdown products which were observed for LHF (litter layer) extractions 1, 2 and 3 are summarized below (Table 6).

Table 6. TLC of LHF Layer - Soil Extract

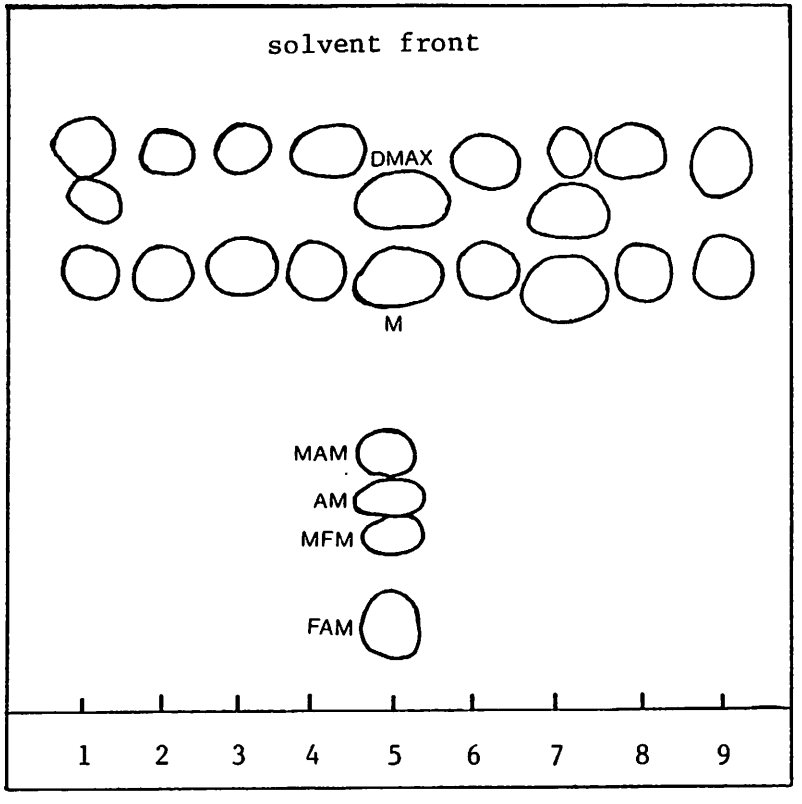
Plate #	Extraction #	Sample R_f Value	Compound Identified
53	LHF #1	R_{f1} = 0.41	MAM
		R_{f2} = 0.69	M
	LHF #2	R_{f1} = 0.41	MAM
		R_{f2} = 0.69	M
	LHF #3	R_{f1} = 0.32	AM
		R_{f2} = 0.41	MAM
		R_{f3} = 0.69	M

TLC of Forest Substrates

The TLC methodology developed with foliage and soil extract samples was applied to a variety of forest substrates including aluminum coils and glass plates (used as collectors of spray droplets), air, and litter as well as foliage and soil. The results of the experiments are outlined in Table 7 and in Figs. 5-8. Some revised R_f values obtained for spiked forest soils are given in Table 8.

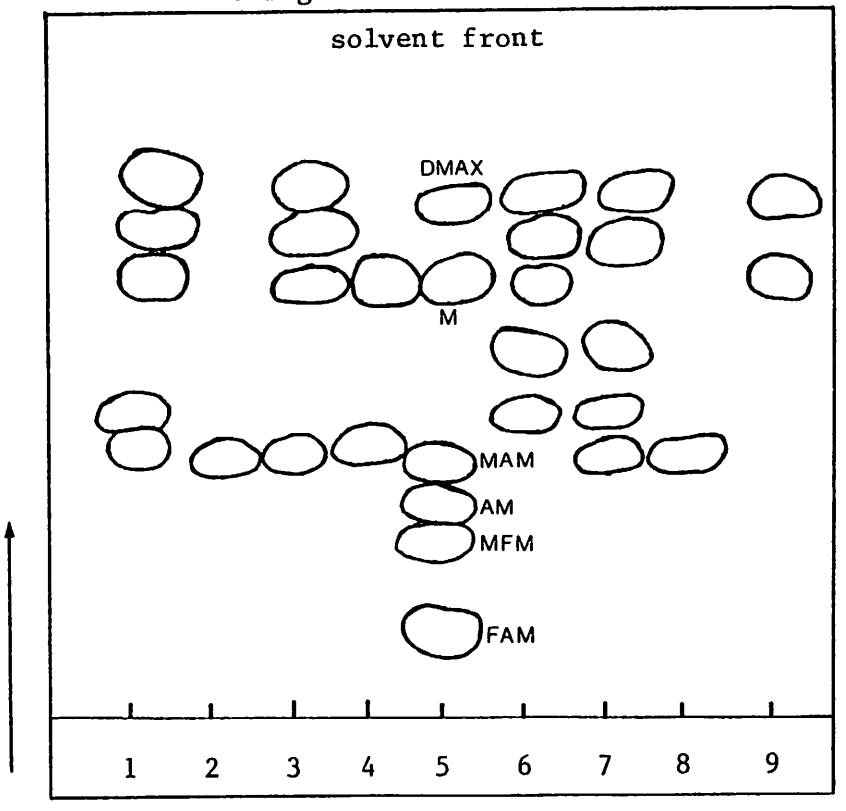
The variation of R_f values among the chemicals studied due to adsorption, desorption, partition, etc., their mode of metabolic breakdown in different forestry components, and the rate of loss of some of these compounds from the diverse substrates studied are beyond the scope of this preliminary report. It is interesting to note that, in spite of the differences in substrate studies, mobile phases used and other

Fig. 5. TLC Chromatogram of Forest Substrates Aluminum Coils and Glass Plates



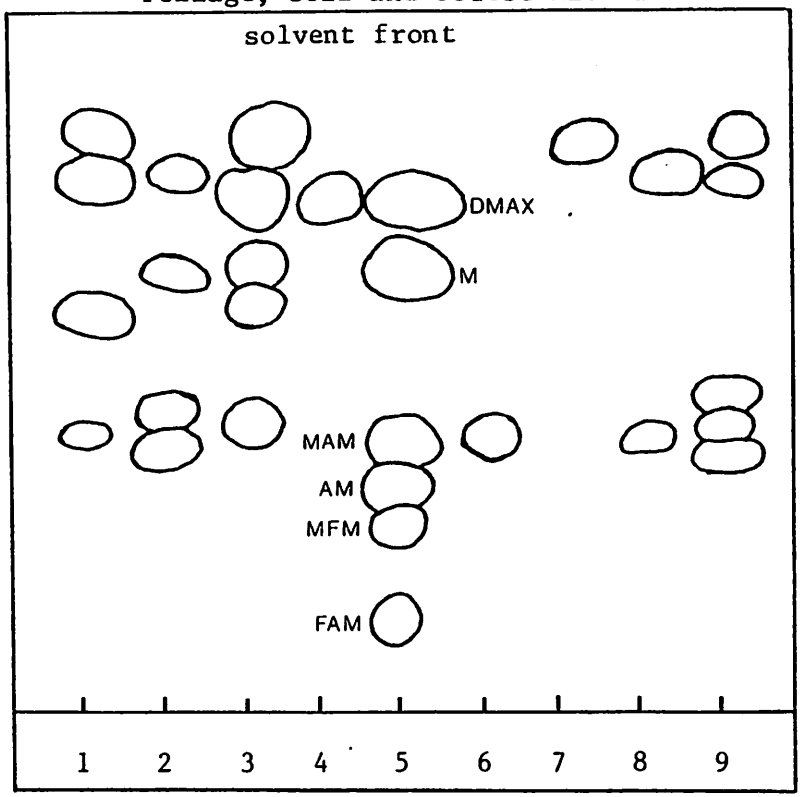
1-4 Aluminum coils
5 Mexacarbate plus metabolites
6-9 Glass plates

Fig. 6. TLC Chromatogram of Forest Substrates Foliage



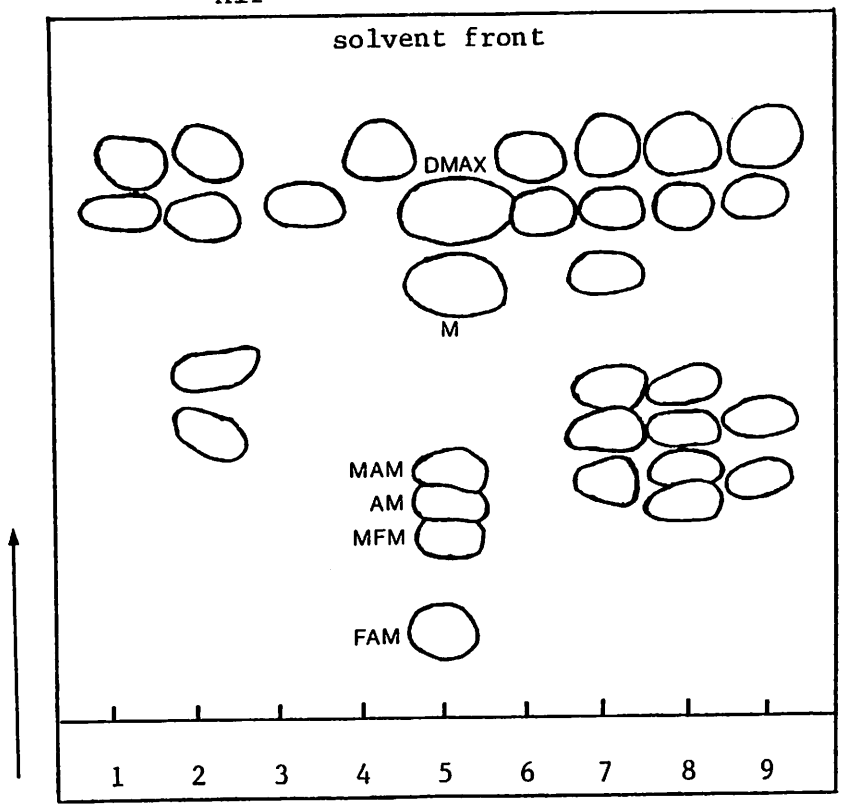
1-4, 6-9 Foliage
5 Mexacarbate plus metabolites

Fig. 7. TLC Chromatogram of Forest Substrates
Foliage, Soil and Forest Litter



1,2 Foliage
3,4,6,7 Soil
5 Mexacarbate plus metabolites
8,9 Litter

Fig. 8. TLC Chromatogram of Forest Substrates
Air



1-4, 6-9 Air
5 Mexacarbate plus metabolites

Table 7. TLC of Forest Substrates

Substrate	RF Value	Compound Identified
Aluminum Coils:		
a) Plot 5 2nd App.	R_{f1} = 0.70	M
	R_{f2} = 0.81	DMAX
	R_{f3} = 0.90	Unknown
b) Plot B70 Top	R_{f1} = 0.70	M
	R_{f2} = 0.90	Unknown
c) Plot B140 Top	R_{f1} = 0.70	M
	R_{f2} = 0.90	Unknown
d) Plot 4 2nd App. Top	R_{f1} = 0.70	M
	R_{f2} = 0.90	Unknown
e) Control	R_{f1} = 0.90	Unknown
Plates #54 & #55		
Glass Plates:		
a) Plot 4 2nd App.	R_{f1} = 0.70	M
	R_{f2} = 0.90	Unknown
b) Plot B140	R_{f1} = 0.70	M
	R_{f2} = 0.81	DMAX
	R_{f3} = 0.90	Unknown
c) Plot B70	R_{f1} = 0.70	M
	R_{f2} = 0.90	Unknown
d) Plot 5 2nd App.	R_{f1} = 0.70	M
	R_{f2} = 0.90	Unknown
e) Control	R_{f1} = 0.38	MAM
	R_{f2} = 0.53	Unknown
	R_{f1} = 0.59	Unknown
	R_{f2} = 0.88	Unknown
Plate #55		
Foliage:		
a) Plot 4		
i) F1 - Prespray	R_{f1} = 0.41	MAM
	R_{f2} = 0.47	Unknown

(cont'd)

Table 7. TLC of Forest Substrates

Substrate	R_f Value	Compound Identified
Foliage: (cont'd)		
a) Plot 4		
i) F1 - Prespray	R_{f3} = 0.63	Unknown
	R_{f4} = 0.76	Unknown
	R_{f5} = 0.85	Unknown
ii) F115	R_{f1} = 0.41	MAM
iii) F124	R_{f1} = 0.41	MAM
	R_{f2} = 0.63	Unknown
	R_{f3} = 0.76	Unknown
	R_{f4} = 0.85	Unknown
iv) F126	R_{f1} = 0.41	MAM
	R_{f2} = 0.63	Unknown
Plate #56		
b) Plot 5	R_{f1} = 0.41	MAM
	R_{f2} = 0.47	Unknown
	R_{f3} = 0.58	Unknown
	R_{f4} = 0.63	M
	R_{f5} = 0.76	DMAX
	R_{f6} = 0.85	Unknown
c) Plot B140 1 hr Top	R_{f1} = 0.41	MAM
	R_{f2} = 0.46	Unknown
	R_{f3} = 0.48	M
	R_{f4} = 0.84	DMAX
Plate #57		
d) F1 - Control	R_{f1} = 0.43	MAM
	R_{f2} = 0.61	Unknown
	R_{f3} = 0.84	DMAX
	R_{f4} = 0.91	Unknown
Soil:		
a) Control	R_{f1} = 0.48	Unknown
	R_{f2} = 0.64	Unknown
	R_{f3} = 0.69	M
	R_{f4} = 0.81	DMAX
	R_{f5} = 0.91	Unknown

Table 7. TLC of Forest Substrates

Substrate	R_f Value	Compound Identified
Soil: (cont'd)		
b) Plot 4 1 hr	R_{f1} = 0.81	DMAX
c) Plot 5 1 hr 1st App.	R_{f1} = 0.43	MAM
d) Plot 5 1 hr 2nd App.	R_{f1} = 0.89	Unknown
Litter:		
a) Plot 5 1 hr 1st App.	R_{f1} = 0.42	MAM
	R_{f2} = 0.84	M
b) Plot 4 1 hr	R_{f1} = 0.39	Unknown
	R_{f2} = 0.44	MAM
	R_{f3} = 0.49	Unknown
	R_{f4} = 0.83	DMAX
	R_{f5} = 0.91	Unknown
** c) Plot 4 - Prespray	R_{f1} = 0.38	MAM
** d) Plot 5 1 hr 2nd App.	R_{f1} = 0.38	MAM
	R_{f2} = 0.48	Unknown
	R_{f3} = 0.60	M
	R_{f4} = 0.76	Unknown
Air:		
a) Prespray	R_{f1} = 0.79	DMAX
	R_{f2} = 0.88	Unknown
Plate #58		
b) Plot B140 0 hr	R_{f1} = 0.44	Unknown
	R_{f2} = 0.54	Unknown
	R_{f3} = 0.79	DMAX
	R_{f4} = 0.89	Unknown
c) Plot B140 1 hr	R_{f1} = 0.80	DMAX
d) Plot B140 6 hr	R_{f1} = 0.89	Unknown
e) Plot 4 0 hr	R_{f1} = 0.81	DMAX
	R_{f2} = 0.89	Unknown

Table 7. TLC of Forest Substrates

Substrate	R_f Value	Compound Identified
Air: (cont'd)		
f) Plot 4 1 hr	R_{f1} = 0.38	MAM
	R_{f2} = 0.45	Unknown
	R_{f3} = 0.52	Unknown
	R_{f4} = 0.70	M
	R_{f5} = 0.81	DMAX
	R_{f6} = 0.90	Unknown
g) Plot B 70 0 hr	R_{f1} = 0.33	AM
	R_{f2} = 0.39	MAM
	R_{f3} = 0.45	Unknown
	R_{f4} = 0.52	Unknown
	R_{f5} = 0.81	DMAX
	R_{f6} = 0.92	Unknown
h) Plot B70 3 hr	R_{f1} = 0.38	MAM
	R_{f2} = 0.47	Unknown
	R_{f3} = 0.81	DMAX
	R_{f4} = 0.92	Unknown

** The low R_f values corresponded to similar low values obtained for mexacarbate and its metabolites on this particular plate. Work is being continued to achieve consistency on all plates.

Table 8. TLC of sandy loam forest soil fortified with 10 μg of mexacarbate/gm of soil: A 12 h kinetic study*

Compound	R_f value+		Comments
	Ether:Hexane: EtOH 77:20:3	Ether:Hexane: EtOH 65:30:5	(Variation in spot intensity with time)
M	0.69	0.65	Spot intensity decreased to half in 45 min, after 3 h, it has become 1/4; diminished considerably after 6 h
MFM	0.28	0.32	None up 20 min; noticeable up to 3 h and decreased afterwards
MAM	0.40	0.50	Noticeable after 30 min; significant from 45 min, to 3 h and then decreased
FAM	0.13	0.23	None up to 20 min; traces afterwards; noticeable after 2 h
AM	0.34	0.36	Traces up to 20 min; increased gradually up to 6 h; then decreased
DMAX	0.81	0.76	Negiligible up to 1 h; gradual increase up to 3 h and rapid loss afterwards
4-methylamino-3, 5-xylenol**	0.82?	0.86?	Traces
4-Amino-3, 5-xylenol**	0.82?	0.95?	Traces

*Time intervals: 0, 10, 20, 30, 45 min, 1, 2, 3, 6 and 12 h.

**Ref. standards unavailable; speculation only.

+R_f values obtained by Abdel-Wahab *et al.* [Jour. Agric. Food Chem. 14(3), 290-297 (1966)] are listed below for comparison.

Compound	R_f Values	
	Ether 4:Hexane 1	CH_3CN 1:Toluene 1
M	0.78	0.80
MFM	0.27	0.51
FAM	0.05	0.38
MAM	0.44	0.60
AM	0.44	0.60

experimental conditions, the R_f values recorded here are not far different from those obtained earlier by Abdel-Wahab *et al.*[8].

In conclusion, the application of TLC techniques to isolate and study the metabolic fate of mexacarbate, at least qualitatively, from the forestry substrates appears to be simple, inexpensive and very effective. Quantitation of the solutes from sorbent following removal and solvent extraction using gas chromatographic and high performance liquid chromatographic methods for nonradioactive materials as well as counting by liquid scintillation of labelled moieties are in progress at this laboratory.

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