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SIMPLIFIED METHOD FOR THE ANALYSIS OF SOME CARBAMATE INSECTICIDES IN FOLIAGE, FOREST SOIL AND FISH TISSUE BY DIRECT GAS–LIQUID CHROMATOGRAPHY

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SUMMARY

A simplified and sensitive method for the analysis of eight carbamate insecticide residues in foliage, forest soil and fish tissue by direct gas–liquid chromatography (GLC) is described. After fortification, the carbamate residues were extracted from the natural substrates by homogenization in ethyl acetate. The interfering co-extractives present in the crude extracts were removed by filtration through Whatman GF/A glass microfibre filters after coagulation. The carbamate residues were re-extracted into dichloromethane and directly analyzed intact by GLC with a Tracor Model 702 nitrogen–phosphorus detector. The rate of recovery for aminocarb, carbofuran, carbaryl, methomyl, mexacarbate, pirimicarb and propoxur was better than 80% at 5.0-ppm and 0.50-ppm levels. Slightly less than 60% was recovered for methiocarb.

INTRODUCTION

Carbamate insecticides have been extensively applied in recent years to the forest to control various insect pests. Aminocarb (4-dimethylamino-*m*-tolyl N-methylcarbamate) is perhaps the most widely used insecticide against the spruce budworm (*Choristoneura fumiferana* Clem.) in eastern Canada[1,2]. Other carbamate insecticides such as carbaryl (1-naphthyl N-methylcarbamate), mexacarbate (4-dimethylamino-3,5-xylyl methylcarbamate), propoxur (*o*-isopropoxyphenyl N-methylcarbamate), methomyl (S-methyl N-((methylcarbamoyl)-oxy)thioacetimidate) and carbofuran (2,3-dihydro-2,2-dimethyl benzofuran-7-yl methylcarbamate) have been used either on an operational or an experimental scale to control various forest pests[3]. As a result, a multiresidue method for the extraction and analysis of these carbamates is required for studying their distribution, persistence and dynamics in the forest ecosystem.

The existing methods for analyzing carbamate insecticide residues generally consist of solvent extraction, coagulation followed by column chromatography clean-up, chemical derivatization of the parent compounds or their alkaline hydrolysis products, further clean-up and then gas–liquid chromatographic (GLC) analysis of

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the derivatives[4–11]. The derivatives prepared by bromination[5], acylation[4–6,8,9,11] or reaction with pentafluorobenzyl bromide[10] or 2,4-dinitrobenzene[7] were analyzed by electron-capture gas chromatography. The advantage of these methods was not only their high sensitivity but also the greater thermal stability of the derivatives. However, these methods are tedious and time consuming. Furthermore, the analysis of mexacarbate and aminocarb is not possible as their phenolic products obtained upon alkaline hydrolysis cannot be extracted from the aqueous media[10].

Recently Lorah and Hemphill[12] reported that carbaryl, Mesurol® (4-methylthio-3,5-xylyl methylcarbamate), promecarb (*m*-cym-5-yl methylcarbamate) and mexacarbate were successfully chromatographed intact on Chromosorb W support, surface-modified with Carbowax 20M, and that the parent compounds were detected by an alkali flame ionization detector (AFID). This paper describes a simplified and sensitive method for the extraction and analysis of eight carbamate insecticide residues in foliage, forest soil and fish tissue by direct GLC–AFID.

MATERIALS AND METHODS

Reagents and natural substrates

The carbamate insecticides used in this study are listed in Table I. Stock solutions of these were prepared at 1000 μg/ml in acetone. The working solutions used for substrate fortification and GLC–AFID analysis were prepared by appropriate dilution with acetone and ethyl acetate respectively. The coagulating solution consisted of 15.0 g ammonium chloride, 6.0 ml 85% phosphoric acid in one liter of aqueous solution. All organic solvents were glass-distilled pesticide-grade.

TABLE I

LIST OF CARBAMATE INSECTICIDES USED IN THIS STUDY

Chemical	*Purity*	*Chemical name*
Aminocarb	99.4%	4-Dimethylamino-*m*-tolyl N-methylcarbamate
Carbofuran	99.2%	2,3-Dihydro-2,2-dimethyl benzofuran-7-yl methylcarbamate
Carbaryl	99.0%	1-Naphthyl N-methylcarbamate
Methiocarb	99.0%	4-(Methylthio)-3,5-xylyl N-methylcarbamate
Methomyl	99.0%	S-Methyl N-((methylcarbamoyl)-oxy)thioacetimidate
Mexacarbate	99.0%	4-Dimethylamino-3,5-xylyl methylcarbamate
Pirimicarb	99.4%	2-(Dimethylamino)-5,6-dimethyl-4-pyrimidinyl dimethylcarbamate
Propoxur	98.7%	*o*-Isopropoxyphenyl N-methylcarbamate

Foliage of white spruce (*Picea glauca* (Moench) Voss) and forest soil (sandy loam, 22% moisture content and pH 6.3) were collected from a forest north of Sault Ste. Marie, Canada. Prior to fortification of the test materials with carbamates, foliage samples including the small twigs were pulverized in a Hobart meat grinder and soil samples were sieved (screen mesh No. 4) to remove any small branches and stones. For treatment of rainbow trout (*Salmo gairdneri* Richardson), the heads and internal organs were removed and the remaining body tissue macerated. The prepared samples of foliage, forest soil and fish tissue were then fortified with the appropriate working solution containing all eight carbamate insecticides in acetone to give a concentration of 5.0 ppm or 0.50 ppm.

Extraction and clean-up of carbamate insecticides from foliage, forest soil and fish tissue

Aliquots of fortified foliage (25 g), forest soil (50 g) and fish tissue (10 g) were extracted with ethyl acetate (3 × 100 ml) by homogenization and anhydrous sodium sulfate (10 g) was added in the first extraction. Foliage and fish tissue samples were homogenized for 1.5 min in a Polytron (Model PT-20) and forest soil samples were blended for 5 min in a Sorvall Omni mixer. The homogenates were filtered through a layer of anhydrous sodium sulfate (3 cm) over Whatman No. 1 filter paper in a büchner funnel, followed by rinsing with ethyl acetate (2 × 10 ml). The volume of the crude extracts was measured and recorded.

Aliquots of crude extracts equivalent to 5 g (wet weight) of foliage and fish tissue or 20 g (wet weight) of forest soil were used for clean-up. The crude extracts in a 250-ml round bottom flask were evaporated just to dryness in a flash evaporator at 38°C and the residues were dissolved in acetone (2 ml), followed by the addition of coagulating solution (25 ml). After 30 min, *ca.* 8-ml portions of the aqueous suspensions were filtered through two layers of Whatman GF/A glass microfibre filters (2.4 cm in diameter) in a Millipore filter holder (No. XX 1002500) under aspiration. The filters were replaced until the whole suspension had been filtered. All used filters were then returned to the 250-ml round-bottom flasks and washed with the coagulating solution (3 × 5 ml) and glass-distilled water (3 × 5 ml). The combined filtrates were neutralized with saturated sodium carbonate (aqueous) and quantitatively transferred into a 250-ml separatory funnel. The carbamate residues were extracted by partitioning with dichloromethane (50, 25, 25 and 25 ml). The combined extracts were dried on anhydrous sodium sulfate (10 g) and then evaporated just to dryness in a flash evaporator at 38°C and the carbamate residues were dissolved in ethyl acetate (2 ml) for GLC–AFID analysis.

The effect of pH and solvent selection on extraction efficiency

The effect of pH and solvent selection was investigated using aminocarb and carbaryl as indicators. Phosphate buffers of pH 3.0, 5.0 and 7.5 were prepared by titration with 0.2 *M* solutions of mono-, di- and tribasic sodium phosphate and phosphoric acid. Four 100-ml aliquots of these were fortified with aminocarb and carbaryl to give a concentration of 5.0 ppm or 0.50 ppm. After 30 min, the residues were extracted from the buffers with dichloromethane, ethyl acetate, benzene or diethyl ether (50, 25, 25 and 25 ml).

GLC–AFID analysis

Analysis of carbamate residues present in the extracts of foliage, forest soil and fish tissue samples was performed on a Tracor Model 550 gas chromatograph, equipped with a Model 702 nitrogen–phosphorus detector. Five Pyrex glass columns (90 cm × 3.0 mm I.D.) were used with the following packings: (1) 3% OV-101, (2) 3% OV-25, (3) 1.5% OV-17 + 1.95% OV-210, (4) 2% OV-101 + 6% OV-210; all on Chromosorb W HP, 80–100 mesh; and (5) 1% OV-17 + 1% OV-210 on Ultra-Bond 20M, 80–100 mesh (as suggested by Lorah and Hemphill[12]). The operating parameters were as follows: detector temperature 240°C; inlet and outlet temperature 210°C; column temperature 150°C; carrier gas (helium) flow-rate 80 ml/min; plasma gas flow-rate hydrogen 1.5 ml/min, air 120 ml/min.

Calibration curves were prepared daily before and after sample analysis to confirm the detector stability.

Quantification of carbamates was done by external standardization based on peak height.

RESULTS AND DISCUSSION

Performance of various GLC columns

Prior to sample analysis, repeated injections of sample extracts significantly enhanced and stabilized the detector response. Table II summarizes the retention times of the eight carbamates relative to carbaryl at 150°C. The 2% OV-101 + 6% OV-210 column gave the best over-all resolution of the eight carbamates although baseline separation of mexacarbate, pirimicarb and methomyl could not be achieved. However, good separation of these three carbamates could be obtained with the 1.5% OV-17 + 1.95% OV-210 column. Methomyl was successfully chromatographed intact on only two columns, namely, (1)1.5% OV-17 + 1.95% OV-210 (column 3) and (2) 2% OV-101 + 6% OV-210 (column 4). Considerable peak tailing occurred on column 4, but only slightly tailing on column 3.

TABLE II

RETENTION TIMES OF CARBAMATES RELATIVE TO CARBARYL AT 150°C

Compound	*Column (90 cm × 3.0 mm I.D.)*				
	3% OV-101	*3% OV-25*	*1.5% OV-17 + 1.95% OV-210*	*2% OV-101 + 6% OV-210*	*1% OV-17 + 1% OV-210 on Ultra-Bond 20M*
Aminocarb	0.75	0.45	0.46*	0.44	0.36
Carbofuran	0.53	0.52*	0.47*	0.40	0.38*
Carbaryl	1.00	1.00**	1.00**	1.00	1.00
Methiocarb	1.21	1.02**	0.98**	0.81	0.80
Methomyl	—	—	0.40	0.64	—
Mexacarbate	0.78*	0.51*	0.53	0.51	0.38*
Pirimicarb	0.79*	0.69	0.65	0.56	0.32
Propoxur	0.33	0.23	0.26	0.28	0.20

*,** Peaks superimposed with each other.

Except methomyl, the test carbamates were successfully chromatographed intact on the GLC column suggested by Lorah and Hemphill[12] (1% OV-17 + 1% OV-120 on Ultra-Bond 20M [a commercial Chromosorb W support, surface-modified with Carbowax 20M]). However, the improvement in column performance, if any, compared with the other four GLC columns tested was negligible and did not justify the high cost of this material.

Influence of pH and solvent selection on extraction efficiency

At pH 3.0, the recovery rate of aminocarb from phosphate buffers was slightly less than 60%. However, at pH 7.5, the recovery was quantitative regardless of the organic solvent used excepting diethyl ether. The poor recovery of aminocarb at low

TABLE III

RECOVERY OF CARBAMATE RESIDUES FROM FOLIAGE, FOREST SOIL AND FISH TISSUE
Values indicate mean recovery ± standard deviation (%) ($n = 4$).

Compound	*Fortification (ppm)*					
	5.0			*0.50*		
	Foliage	*Soil*	*Fish*	*Foliage*	*Soil*	*Fish*
Aminocarb	100 ± 0.34	96.4 ± 1.11	92.2 ± 2.82	99.6 ± 1.43	90.0 ± 1.76	97.9 ± 1.93
Carbofuran	81.1 ± 4.15	93.8 ± 2.92	94.5 ± 5.00	89.4 ± 1.97	97.0 ± 1.87	88.4 ± 2.86
Carbaryl	81.5 ± 1.42	89.6 ± 1.44	83.5 ± 1.22	86.3 ± 4.80	92.2 ± 1.35	90.1 ± 3.68
Methiocarb	12.5 ± 5.60	59.9 ± 10.2	58.6 ± 1.03	60.0 ± 9.83	55.4 ± 18.0	50.3 ± 8.77
Methomyl	97.0 ± 2.04	96.5 ± 0.97	98.0 ± 1.84	97.3 ± 3.92	98.0 ± 2.15	96.4 ± 1.80
Mexacarbate	102 ± 1.26	91.5 ± 2.07	89.0 ± 0.58	95.0 ± 0.00	96.3 ± 1.59	103 ± 3.30
Pirimicarb	96.8 ± 1.19	98.9 ± 3.00	97.4 ± 1.80	93.4 ± 3.04	99.6 ± 1.56	97.2 ± 1.15
Propoxur	91.3 ± 1.94	97.4 ± 1.81	101 ± 1.15	88.5 ± 0.55	94.1 ± 3.68	105 ± 5.51

pH was probably due to the formation of quaternary ammonium ion ($R_2R'NH^+$) of the carbamate molecule by protonation as suggested by Sundaram *et al.*[14]. This product cannot be extracted with organic solvent. The effect of low pH was much less pronounced for carbaryl probably because it does not form the quarternary ion. The recoveries of carbaryl from phosphate buffers were always better than 90%.

The extraction efficiency of dichloromethane, ethyl acetate and benzene were about the same. Dichloromethane was selected as the extracting solvent for convenience because of its higher specific gravity than water which enabled easy removal from the separatory funnel during extraction.

Recovery of carbamates from fortified foliage, forest soil and fish tissue

Except for methiocarb, the recoveries of the test carbamates were better than 80% at 5.0-ppm and 0.50-ppm levels in all three substrates (Table III) when analyzed intact by GLC–AFID. The highest average recovery of methiocarb was only about 60% obtained in foliage samples fortified at 0.50-ppm level. Because methiocarb is practically insoluble in water[13] and does not dissolve in the coagulating solution, its poor recovery may well have been due to the loss of residues at the filtration step after coagulation. The water solubilities of the other carbamates (Table IV) according to Martin and Worthington[13] are much greater. Therefore, even carbaryl which has

TABLE IV

SOLUBILITY OF CARBAMATES IN WATER ADOPTED FROM PESTICIDE MANUAL[13]

Compound	*Solubility (ppm)*
Aminocarb	Slightly soluble
Carbofuran	700 at 25°C
Carbaryl	40 at 30°C
Methiocarb	Practically insoluble
Methomyl	58,000 at 25°C
Mexacarbate	100 at 25°C
Pirimicarb	2700 at 25°C
Propoxur	2000 at 20°C

a water solubility as low as 40 ppm at 30°C gives a recovery rate of better than 80% for the described method.

Celite 545, an adsorbent commonly used as filtering aid was compaired with the Whatman GF/A glass microfibre filter. It was evident that significant loss (10–15%) of carbamate residues occurred at the filtration step after coagulation apparently due to residue adsorption on Celite 545. However, no appreciable adsorption had been observed with the glass microfibre filter. As a result, the over-all recovery of carbamate residue was greatly improved.

CONCLUSIONS

The method described in this paper for the extraction and direct analysis of carbamates in foliage, forest soil and fish tissue gives good recovery (>80%) for seven of the carbamates tested. The recovery of methiocarb was somewhat lower (<60%) and inconsistant which was attributed to its insolubility in water. Because of the high sensitivity of the modern alkali fiame ionization detector, our method provides a direct, time and labor saving approach to the extraction and analysis of carbamates from environmental samples at residue level.

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