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SENSITIVITY OF PIG LIVER ESTERASE IN DETECTING TWELVE CARBAMATE PESTICIDES ON THIN-LAYER CHROMATOGRAMS

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SUMMARY

A comparison of pig and beef liver extracts for the detection of twelve carbamates on thin-layer plates is discussed. The esterase in frozen extracts of pig liver was found sensitive to inhibition by the carbamates studied at the nanogram to picogram levels. Bromine or ultraviolet light was shown to destroy the inhibitory property of the pesticides. Microslides were useful for rapid detection of the carbamates. Silica Gel G and G-HR were superior among the different types of gel layers studied. The hR_F values of the standards in different solvent systems were given. The advantages of using the freeze-dried extracts were discussed.

INTRODUCTION

The thin-layer chromatographic–enzyme inhibition (TLC–EI) technique developed in our laboratory has been used to detect carbamate and organophosphorus pesticides[1,2]. WINTERLIN *et al.*[3] demonstrated by TLC that bee brain cholinesterase was generally more sensitive for detection of these pesticides. Bee brain cholinesterase, in particular, was more sensitive to inhibition by carbaryl (Sevin) than human plasma cholinesterase while the opposite held for aldicarb (Temik). Likewise, we observed that pig liver esterase was more sensitive in detecting carbaryl than beef, sheep, monkey, or chicken liver esterase[4]. Therefore, pig liver esterase was further studied for TLC detection of twelve carbamate pesticides. In addition, the effects of UV light or bromine on these compounds were evaluated. The use of microscope slides for rapid detection of the carbamates was also studied.

MATERIALS AND METHODS

Enzyme solution preparation

The pig and beef liver esterases were extracted following the procedure of MENDOZA *et al.*[4]. The 2000 × *g* extracts were either frozen directly or freeze-dried. Before use, the frozen extracts were diluted eight times with 0.05 *M* Tris–HCl buffer

at pH 8.3. The freeze-dried extracts were reconstituted with the same buffer solution to approximate the amounts of solids present in the spray solution made of frozen extracts. Solids obtained after freeze-drying the pig and beef extracts were 5.8 and 5.6 g/100 ml, respectively.

Preparation of TLC plates

Coating materials were applied approximately 450 μ thick on TLC plates by means of a Desaga applicator (obtainable from Desaga, Heidelberg, G.F.R.). The list below includes the sources of the materials and the amounts used to make slurries.

Aluminum Oxide DS-5, Camag (Arthur H. Thomas Co., Philadelphia, Pa.) 75 g/60 ml of distilled water.

Kieselgel D-5 (silica gel), Camag (Arthur H. Thomas Co.) 50 g/100 ml of distilled water.

MN-Kieselgel G-HR (silica gel), (Macherey, Nagel and Co., Düren, G.F.R.) 50 g/100 ml of distilled water.

Silica Gel H (E. Merck AG, Darmstadt, G.F.R.) 50 g/100 ml of distilled water.

Silica Gel G (E. Merck AG) 50 g/100 ml of distilled water.

SilicAR TLC-7 (Mallinckrodt Chemical Works, St. Louis, Mo.) 50 g/100 ml of distilled water.

Polyamide II (E. Merck AG) 50 g/50 ml of distilled water and 50 ml of acetone.

Some TLC plates with agar-agar were prepared according to the procedure of MAINI[5]. Agar-agar, 0.4 g, was dissolved in 120 ml of boiling distilled water and 50 g of MN-Kieselgel G-HR was added while the solution was still hot. The mixture was shaken vigorously for approximately 1 min before applying it on the preheated plates at 450 μ thickness.

All plates were heated at 110° in an oven for 1 h before use.

Preparation of TLC micro-plates

TLC micro-plates were coated with MN Kieselgel G-HR with or without agar-agar by using the apparatus developed by LOVELADY[6]. The space between the shoe and the micro-plates was adjusted to 450 μ before use. A thin plexiglass slide was fitted snugly in the loader closing the space between the shoe guide and the main body to prevent the slurry from flowing out freely. Before moving the loader across the micro-plates, the plexiglass slide was pulled up to let the slurry out. The slurry was made of 5 g of silica gel in 10 ml of distilled water. The coated micro-plates were incubated 1 h at 110°.

Solvent systems and development of the plates

The standards were applied 2.5 cm from the edge of the gel and were resolved in the solvent systems listed on Table I. The enzyme extracts were sprayed on the plates. As soon as the plates were dry, they were sprayed with the 5-bromoindoxyl acetate solution[7]. Areas of inhibition appeared as white spots on a blue background.

The carbamates spotted on micro-plates were resolved in a 200-ml beaker with 10 ml of solvent and a petri dish cover.

Carbamate standards

Common or proprietary names, chemical nomenclatures, and manufacturers

TABLE I

SOLVENT SYSTEMS USED TO RESOLVE THE TWELVE CARBAMATES AND THE ROOM TEMPERATURE DURING THE TEST

Solvent systems	*Composition*	*Temp. (°C)*
1	20% acetone in cyclohexane	25
2	20% acetone + 10% benzene in hexane	28
3	20% acetone in pentane	28
3[a]	20% acetone in pentane	25
4	30% acetone in hexane	27
4[a]	30% acetone in hexane	25
5	20% acetone + 10% benzene in pentane	28
6	20% acetone in hexane saturated with methanol	28
7	20% acetone in hexane	25
8	20% acetone in 1 part of hexane: 1 part of cyclohexane	26
9	30% acetone in cyclohexane	25
9[a]	30% acetone in cyclohexane	25
10	30% acetone in heptane	25
10[a]	30% acetone in heptane	25
11	20% acetone in 1 part of pentane: 1 part of hexane	25
12	20% acetone in 1 part of hexane: 1 part of heptane	25
17	cyclohexane	28
18	ethanol	28
22	10% ethanol + 10% benzene + 20% acetone in heptane	28
23	chloroform	25
24	20% methanol in benzene	25

[a] Solvent systems were used on plates made with agar-agar.

are listed below. The purity determined by the suppliers is expressed in percent. Carbofuran 3-OH analogue and Mobam did not have purity assay; however, each gave only one spot under the TLC–EI conditions used.

Aldicarb = 2-methyl-2-(methylthio) propionaldehyde O-(methylcarbamoyl)-oxime (Union Carbide, Olefins Division, New York, N.Y.), 99.7%.

Banol = 2-chloro-4,5-xylenol methylcarbamate (Tuco Products Division, Upjohn, Kalamazoo, Mich.), 100%.

Baygon = *o*-isopropoxyphenyl methylcarbamate (Chemagro Corporation, Kansas City, Mo.), 99.2%.

Carbaryl = 1-naphthyl methylcarbamate (Union Carbide), 99.7%.

Carbofuran = 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate Niagara Chemical Division, (FMC Corporation, Middleport, N.Y.), 99.2%.

Carbofuran 3-OH analogue = 2,3-dihydro-2,2-dimethyl-3-hydroxy-7-benzolfuranyl methylcarbamate (FMC Corporation).

Matacil = 4-dimethylamino-*m*-tolyl methylcarbamate (Chemagro Corporation), 99.4%.

Mesurol = 4-(methylthio)-3,5-xylyl methylcarbamate (Chemagro Corporation), 100%.

Mobam = 4-benzothienyl methylcarbamate (Mobil Chemical Company, Industrial Chemical Division, Richmond, Va.).

Ortho 5353 = *m*-(1-ethylpropyl) phenyl methylcarbamate and *m*-(1-methylbutyl) phenylmethylcarbamate mixture is approximately 1:4 proportions (Chevron Chemical Company, Ortho Division, Richmond, Calif.), 99.2%.

TABLE II

DETECTION LIMITS IN NANOGRAMS OF TWELVE CARBAMATE PESTICIDES ON MN KIESELGEL G-HR LAYERS BY TLC–EI PROCEDURE USING PIG AND STEER LIVER EXTRACTS

(+) = TLC spots corresponding to the areas where enzymes were inhibited lasted 5 min; (++) = lasted more than 5 min but less than 30 min; (+++) 30 min or more. (±) = spots lasted 1–2 min, and (—) = no enzyme inhibition detected. TLC plates were coated with 450 μ thick layer of Kieselgel G-HR. The solvent system was 20% acetone in cyclohexane.

Compounds	*Liver extracts*			
	Pig		*Steer*	
	Frozen	*Freeze-dried*	*Frozen*	*Freeze-dried*
Aldicarb	50 (±)	100 (±)	100 (±)	100 (±)
	30 (—)	50 (—)	50 (—)	50 (—)
Banol	0.5 (+)	5 (±)	5 (±)	10 (±)
	0.1 (—)	1 (—)	1 (—)	5 (—)
Baygon	10 (±)	50 (±)	100 (±)	100 (±)
	5 (—)	10 (—)	50 (—)	50 (—)
Carbaryl	0.1 (+)	0.5 (±)	0.5 (±)	5 (+)
	0.05 (—)	0.1 (—)	0.1 (—)	1 (—)
Carbofuran	1 (+)	50 (+)	100 (±)	100 (+)
	0.5 (—)	10 (—)	50 (—)	50 (—)
Carbofuran 3-OH	10 (±)	100 (±)	100 (±)	400 (±)
	5 (—)	50 (—)	50 (—)	300 (—)
Matacil	10 (±)	50 (±)	50 (±)	50 (+)
	5 (—)	10 (—)	10 (—)	10 (—)
Mesurol	0.1 (±)	10 (±)	10 (±)	10 (±)
	0.05 (—)	5 (—)	5 (—)	5 (—)
Mobam	0.5 (+)	1 (±)	5 (++)	5 (+)
	0.1 (—)	0.5 (—)	1 (—)	1 (—)
Ortho 5353	1 (+)	5 (±)	5 (+)	5 (+)
	0.1 (—)	0.5 (—)	1 (—)	1 (—)
Tranid	100 (±)	500 (±)	300 (++)	100 (±)
	50 (—)	400 (—)	200 (—)	50 (—)
Zectran	50 (+++)	50 (++)	50 (±)	50 (±)
	10 (—)	10 (—)	10 (—)	10 (—)

Tranid = 5-chloro-6-oxo-2-norbornanecarbonitrile O-(methylcarbamoyl)oxime (Union Carbide), 99.9%.

Zectran = 4-dimethylamino-3,5-xylyl methylcarbamate (Dow Chemical Company, The Agricultural Products Department, Midland, Mich.), 99%.

All the standards were dissolved in methanol and kept in a refrigerator.

RESULTS AND DISCUSSION

Sensitivity tests

Table II shows the detection limits in nanograms (ng) of different carbamate pesticides on MN Kieselgel G-HR layer by TLC–EI procedure using pig and steer liver extracts. In general, the pig liver extracts and frozen extracts were more sensitive than the steer liver extracts and freeze-dried extracts, respectively, in detecting the pesticides tested. Carbaryl was detected at 0.1 ng by the frozen extract of pig livers and at 0.5 ng by the freeze-dried extract of pig livers or frozen extract of steer livers. With the frozen extract of pig livers, Banol and Mobam were both detectable at the

TABLE III

DETECTION LIMITS IN NANOGRAMS OF TWELVE CARBAMATE PESTICIDES ON SILICA GEL G AND ALUMINUM OXIDE DS-5 LAYERS USING FROZEN PIG LIVER EXTRACTS

(+) = TLC spots corresponding to the area where enzymes were inhibited lasted 5 min, (++) = lasted more than 5 min but less than 30 min, (+++) = 30 min or more.

Compounds	*Types of layer*	
	Silica Gel G	*Aluminum Oxide DS-5*
Aldicarb	30 (±)	10 (±)
	10 (−)	5 (−)
Banol	0.5 (++)	0.5 (++)
	0.1 (−)	0.1 (−)
Baygon	10 (±)	10 (+)
	5 (−)	5 (−)
Carbaryl	0.1 (+)	0.1 (±)
	0.05 (−)	0.05 (−)
Carbofuran	1 (±)	1 (±)
	0.50 (−)	0.5 (−)
Carbofuran 3-OH	10 (+)	5 (+)
	5 (−)	1 (−)
Matacil	5 (++)	1 (±)
	1 (−)	0.5 (−)
Mesurol	0.1 (±)	0.1 (±)
	0.05 (−)	0.05 (−)
Mobam	0.1 (+)	0.5 (+++)
	0.05 (−)	0.1 (−)
Ortho 5353	1 (±)	1 (±)
	0.5 (−)	0.5 (−)
Tranid	50 (+)	5 (++)
	10 (−)	1 (−)
Zectran	0.5 (±)	5 (+++)
	0.1 (−)	1 (−)

0.5-ng level and carbofuran and Ortho 5353 at the 1-ng level. The carbofuran 3-OH analogue was less sensitive than carbofuran. Tranid, the least inhibitory among the pesticides tested, was detected at 100–500 ng levels.

The sensitivity of detection was found to be affected by the types of gel layers used, Table III. Silica Gel G was much more sensitive than MN Kieselgel G-HR (*cf.* Table II) in detecting aldicarb, Matacil, Tranid and Zectran. The detectability of Banol, Baygon, carbaryl, carbofuran, carbofuran 3-OH analogue, and Ortho 5353 on Silica Gel G was comparable to that on MN Kieselgel G-HR. The aluminum oxide TLC plates did not give discrete spots corresponding to the carbamates tested. The spots, though larger than those on Silica Gel G or G-HR, were diffused. Nevertheless, the detectable levels of aldicarb, carbofuran 3-OH analogue, and Tranid, were lower on this layer than on Silica Gel G.

The TLC spots, corresponding to the areas where the enzymes were inhibited, were clearly defined on Silica Gel G and MN Kieselgel G-HR layers. The development of the background and the spots were consistently rapid and intense on Silica Gel G. Therefore, considering the low limit of detection, Silica Gel G is as useful as MN Kieselgel G-HR in the TLC–EI procedure for these carbamates.

Silica Gel D-5 was satisfactory for use in carbamate detection but less superior

than Silica Gel G and G-HR. It did not give a uniform background as did the Silica Gel G and G-HR.

The use of Aluminum Oxide DS-5 layer may be limited for the detection of Aldicarb, Carbofuran 3-OH analogue, and Tranid.

Polyamide 11 did not form a uniform suspension in water unless acetone was added. The carbamates spotted on the polyamide TLC plates streaked. The aqueous spray droplets were not readily adsorbed on the layer, thus the blue background obtained was uneven.

Silica TLC-7 and Silica Gel H layers gave poor TLC background which obliterated spots where the pesticides were located.

TABLE IV

EFFECT OF BROMINE AND UV ON TWELVE CARBAMATE INSECTICIDES RESOLVED ON MN KIESELGEL G-HR AND SPRAYED WITH THE PIG LIVER EXTRACTS

Compounds	*Levels evaluated (ng)*	*Br_2*	*UV*
Aldicarb	1000	slight decrease	decrease
Banol	10	decrease	decrease
Baygon	100	decrease	decrease
Carbaryl	1	increase	decrease
Carbofuran	10	decrease	decrease
Carbofuran 3-OH	10	decrease	increase
Matacil	1000	slight increase	decrease
Mesurol	10	slight decrease	decrease
Mobam	10	no change	no change
Ortho 5353	100	no change	no change
Tranid	100	no change	no change
Zectran	100	decrease	decrease

Effect of bromide and UV on twelve carbamate pesticides

Table IV shows that bromine and UV generally reduced the ability of the compounds to inhibit the pig liver esterase. However, carbaryl exposed to bromine vapor and carbofuran 3-OH analogue exposed to UV showed an appreciable increase in their ability to inhibit the enzymes used. Also, a slight increase in enzyme inhibition was observed with Matacil exposed to bromine. The decrease in enzyme inhibition due to 10 ng of Banol that was exposed to bromine contradicted the observation of WALES *et al.*[2]. Both bromine and UV had negligible effect on Mobam, Ortho 5353, and Tranid.

Solvent system on different TLC plates

Table V shows the hR_F values of the eleven carbamates resolved with different solvent systems on MN Kieselgel G-HR. The best separation was obtained with solvent system 5; only 4 pairs of carbamates had overlapping hR_F values (in parenthesis): (a) Matacil (70)–Mobam (69); (b) Baygon (77)–carbaryl (77); (c) carbofuran (80)–Mesurol (80); (d) Banol (89)–Ortho 5353 (89). Carbamates with the same or a difference of two hR_F values were considered overlapping. With the other solvent systems, three to six carbamate hR_F values overlapped at one area.

The only advantage obtained with MN Kieselgel G-HR and agar-agar com-

TABLE V

hR_F VALUES OF ELEVEN CARBAMATES RESOLVED WITH DIFFERENT SOLVENT SYSTEMS ON MN KIESELGEL G–HR

Compounds	*1*	*2*	*3*	*3*[a]	*4*	*4*[a]	*5*	*6*	*7*	*8*	*9*	*9*[a]	*10*	*10*[a]	*11*	*12*	*17*	*18*	*22*	*23*
Aldicarb (500)[b]	20	33	66	60	45	50	62	23	21	27	33	35	25	32	29	30	0	75	33	11
Banol (50)	33	48	77	72	65	57	89	40	35	39	46	47	39	40	40	32	3	79	45	33
Baygon (500)	27	40	76	63	50	53	77	28	30	32	35	33	38	35	27	25	1	76	35	11
Carbaryl (5)	24	38	75	63	43	57	77	28	29	28	35	27	25	37	32	24	1	75	35	25
Carbofuran (500)	26	39	71	61	49	43	80	29	20	27	37	38	31	33	31	24	2	70	33	8
Carbofuran 3-OH (500)	6	13	23	22	32	35	28	7	7	7	33	20	15	19	7	5	0	55	39	0
Matacil (500)	27	37	75	67	54	55	70	28	28	33	39	40	30	37	33	25	2	70	33	7
	P 0[c]	P 0	P 0	P52	P31	P47	P 0	P15		P20		P27	P 0		P 0	P 0		P 0		
	P 6	P 9	P48		P39		P10						P10			P 3				
		P24			P67		P27						P18			P12				
							P53						P25			P20				
Mesurol (50)	32	48	77	72	64	61	80	40	35	39	45	51	39	43	37	31	0	77	37	30
													P 7					P 0		
Mobam (5)	22	37	71	61	50	52	69	26	25	28	35	37	27	33	32	21	3	76	33	23
Ortho 5353 (50)	37	53	80	77	68	65	89	44	39	41	51	53	40	47	40	33	5	78	43	33
										P35										
Zectran (500)	35	48	79	77	66	61	86	50	37	40	46	47	40	47	39	33	5	77	43	27
		P26	P 0	P53	P25	P47	P 0	P17		P 7		P27	P 0		P10	P 6	Po			
			P57		P40		P 5			P20			P 7		P20	P13				
							P25			P30			P23							
							P53						P27							

[a] The TLC plates were prepared with agar-agar.
[b] Figures in parenthesis indicate the pesticide amounts (in ng) spotted.
[c] P = inhibitor detected in the standard solution.

bination was the improvement of resistance to mechanical breakage[5]. The carbamate hR_F values on these plates were comparable to those on TLC plates without agar-agar.

It is interesting to note that the hR_F values of some pesticides increased while those for the others decreased or remained the same in another system. The position of carbofuran 3-OH relative to other carbamates shifted most often (*cf.* solvent system 22 *versus* solvent systems 1, 2, 5, 6, 7, 8, 11, or 12; *cf.* 3 *versus* 4; *cf.* 5 *versus* 9).

The carbamates on MN Kieselgel G-HR migrated to the solvent front when developed with 30% chloroform and 10% ethanol in petroleum ether or with acetonitrile; the exceptions were carbofuran 3-OH analogue and Tranid, with hR_F values of approximately 90. No migration of the carbamates was observed with pentane, hexane, heptane, benzene, or petroleum ether. These solvents may be important in certain TLC clean-up procedures for carbamates.

With ethanol, solvent system 18, the hR_F values for the carbamates were approximately 90 on Silica Gel H and SilicAR TLC-7 and approximately 80 for MN Kieselgel G-HR. As previously observed, carbofuran and Tranid migrated slower than the other carbamates studied.

Two-dimensional TLC in one solvent system was carried out to determine whether additional spots for Matacil, Mesurol, Ortho 5353, and Zectran standards were in the standard solution or breakdown products during chromatography (see Table V). For Matacil, Mesurol, and Ortho 5353, additional spots were obtained after the first resolution only indicating that the standards had some impurities. Zectran gave several spots after the first and one spot, besides that of Zectran, after the second resolution. Therefore, Zectran standard had impurities and was breaking down during chromatography.

The hR_F values of twelve carbamates on Silica Gel G, D-5, H, and SilicAR

TABLE VI

hR_F VALUES OF TWELVE CARBAMATES ON DIFFERENT TYPES OF GEL LAYERS AND RESOLVED WITH SOLVENT SYSTEM 5 OR 7 AT 25°

Compounds	*Types of layer*									
	Silica Gel G		*Silica Gel D-5*		*Silica Gel H*		*SilicAR TLC-7*		*Aluminum Oxide DS-5*	
	5	*7*	*5*	*7*	*5*	*7*	*5*	*7*	*5*	*7*
Aldicarb	43	14	38	8	43	17	—[b]	—	60	20
Banol	64	26	55	18	63	28	—	27	73	42
Baygon	57	22	42	14	49	22	47	23	63	20
Carbaryl	46	18	33	12	51	22	52	26	57	20
Carbofuran	45	18	33	12	51	21	53	26	57	20
Carbofuran 3-OH	20	5	12	2	10	8	20	13	3	0
Matacil	45	18	33	12	51	21	47	21	57	22
Mesurol	54	22	47	18	60	27	62	33	63	27
Mobam	45	14	33	9	51	20	47	26	52	16
Ortho 5353	65	25	61	19	68	32	60	35	73	30
Tranid	12	3	7	1	16	4	15	5	3	0
Zectran	64	24	61	19	71	30	60	39	73 P	30

[a] P = inhibitor detected in the standard solution.
[b] — = area of the spot, not definite.

TABLE VII

MEAN hR_F VALUES OF TWELVE CARBAMATES RESOLVED ON SILICA GEL G WITH 20% METHANOL IN BENZENE SYSTEM IN GLASS TANKS WITH (A) AND WITHOUT (B) FILTER PAPER LINING

Each mean was based on four observations.

Compounds	*A*	*B*
Aldicarb	52.0 ± 1.4	68.0 ± 5.2
Banol	61.8 ± 1.1	76.0 ± 3.4
Baygon	55.5 ± 1.0	68.2 ± 3.7
Carbaryl	54.8 ± 0.6	65.0 ± 2.9
Carbofuran	56.5 ± 0.6	68.0 ± 2.3
Carbofuran 3-OH	39.8 ± 3.4	54.5 ± 2.7
Matacil	53.8 ± 0.8	65.0 ± 2.8
Mesurol	60.2 ± 1.0	74.2 ± 1.4
Mobam	53.0 ± 0.0	67.5 ± 2.8
Ortho 5353	62.2 ± 1.1	78.8 ± 2.4
Tranid	39.2 ± 3.2	53.5 ± 1.0
Zectran	62.0 ± 1.2	78.2 ± 2.4

TABLE VIII

MEAN hR_F VALUES OF TWELVE CARBAMATES ON MICROSLIDES (75 × 25 mm) COATED WITH MN KIESELGEL G-HR WITH OR WITHOUT AGAR-AGAR

Compounds	*Solvent*[a] *system*	*Number observed*	*Mean hR_F*	
			Without agar-agar	*With agar-agar*
Aldicarb	7	4	24.9 ± 0.6	25.5 ± 1.0
	9	2	47.5	42.5
Banol	7	4	34.8 ± 1.4	34.3 ± 0.7[b]
	9	3	46.7 ± 0.9	47.3 ± 0.7
Baygon	7	3	32.0 ± 1.0	30.3 ± 1.2
	9	3	43.7 ± 1.7	44.7 ± 0.9
Carbaryl	7	4	24.9 ± 1.4	28.5 ± 1.3
	9	3	48.0 ± 1.0	45.0 ± 0.0
Carbofuran	7	4	25.8 ± 1.6	29.5 ± 1.5
	9	5	44.4 ± 1.4	43.6 ± 1.0
Carbofuran 3-OH	7	4	5.5 ± 0.3	6.2 ± 1.2
	9	4	17.0 ± 0.7	17.5 ± 0.5
Matacil	7	3	25.7 ± 0.7	24.7 ± 0.9
	9	3	41.3 ± 0.3	40.7 ± 0.3
Mesurol	7	4	33.0 ± 0.9	32.0 ± 0.0[b]
	9	3	46.7 ± 0.3	47.7 ± 0.3
Mobam	7	3	23.0 ± 1.0	22.3 ± 0.9
	9	3	44.0 ± 1.0	42.7 ± 0.3
Ortho 5353	7	4	37.5 ± 2.0	38.8 ± 1.3
	9	3	50.0 ± 1.0	49.3 ± 0.3
Tranid	7	4	0.0	0.0
	9	3	14.7 ± 0.3	14.3 ± 0.7
Zectran	7	3	34.7 ± 0.3	35.0[c]
	9	3	48.7 ± 1.8	51.0 ± 0.0

[a] 7 = 20% acetone in hexane, 9 = 30% acetone in cyclohexane.
[b] Based on three observations only.
[c] A mean of two observations, both 35.

TLC-7, and Aluminum Oxide DS-5 are shown in Table VI. Solvent systems 5 and 7 were used. The carbamates had lower hR_F values on these gels than on MN Kieselgel G-HR when solvent systems 5 and 7 were used. The exception was Banol, which had an hR_F value of 42 on Aluminum Oxide DS-5 and only 35 on MN Kieselgel G-HR with solvent system 7 (*cf.* Table V). With the same solvent system 7, carbofuran had hR_F values of 21, 20 and 20 on Silica Gel H, Aluminum Oxide DS-5 and MN Kieselgel G-HR, respectively; carbofuran 3-OH had hR_F values of 8 on Silica Gel H and 7 on G-HR (*cf.* Table V). Solvent system 24 gave variable hR_F values for the carbamates on Silica Gel G, Table VII. The hR_F values in the same solvent system decreased when the tanks were lined with filter papers. Moreover, the standard deviations of the means also decreased, with the exception of those for carbofuran 3-OH analogue and Tranid.

The migration rates of the twelve carbamates on microslides coated with MN Kieselgel G-HR with or without agar-agar were comparable and consistent in either solvent systems 7 or 9, Table VIII. However, the migration rates were much increased when solvent system 9 was used. As previously stated, agar-agar improved the mechanical resistance of the gel layer.

CONCLUSIONS

The results showed that pig liver esterase was more sensitive in detecting carbamates than was beef liver esterase. Although the esterase lost some sensitivity to inhibition when the extracts were freeze-dried, the amounts detected were still at the ng levels. Pig liver esterase was particularly sensitive to inhibition by carbaryl.

In comparison with the data of WINTERLIN *et al.*[3], the three types of esterases are given in the order of increasing sensitivity for (a) aldicarb: human plasma cholinesterase (H) > bee head cholinesterase (B) > pig liver esterase (P); (b) carbaryl: B > P > H; (c) carbofuran: B > P = H. Comparative study of some published methods using enzymes is in progress.

The results also showed that bromine or UV generally decreased the inhibitory property of the carbamates. The exception was carbaryl whose inhibitory property was markedly enhanced after exposure to bromine. The effect of UV may be comparable to the effect of sunlight on the carbamates.

The sensitivity of the TLC–EI detection of the pesticides studied was affected by the types of gel layers. Nanograms to picograms of standards were consistently detected on Silica Gel G and G-HR; these gels readily produced uniform blue backgrounds. For rapid detection of the carbamates, microslides could be used.

The freeze-dried liver extract has properties that are desirable for storage, transport, and large scale preparation of the extracts. They are as follows: (1) The storage is limited to approximately 6 g of powder for every 100 ml of extracts freeze-dried instead of the usual 10 to 12 test tubes (13 × 100 mm). (2) The freeze-dried extracts can readily be reconstituted with water or the buffer solution. (3) The exact amount of the freeze-dried enzyme needed for spraying can easily be weighed. Thawing out the whole tube of the frozen enzyme, when only a small amount of spray is needed, is wasteful. (4) Like the frozen extracts, the freeze-dried extracts can be used even after a year of storage at below 0°.

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REFERENCES

1 C. E. MENDOZA AND P. J. WALES, *Analyst*, 93 (1968) 34.
2 P. J. WALES, H. A. MCLEOD AND W. P. MCKINLEY, *J. Assoc. Offic. Agr. Chemists*, 51 (1968) 1239.
3 W. WINTERLIN, G. WALKER AND H. FRANK, *J. Agr. Food Chem.*, 16 (1969) 808.
4 C. E. MENDOZA, D. L. GRANT, B. BRACELAND AND K. A. MCCULLY, *Analyst*, 94 (1969) 805.
5 P. MAINI, *J. Chromatog.*, 42 (1969) 266.
6 H. G. LOVELADY, *Microchem. J.*, 14 (1969) 22.
7 C. E. MENDOZA, P. J. WALES, D. L. GRANT AND K. A. MCCULLY, *J. Agr. Food Chem.*, 17 (1969) 1196.