Journal of Chromatography, 133 (1977) 245–251

CHROM. 9688

Note

Comparison of drosophila, rat-liver and bee-head esterases in detecting residues of organophosphorus and carbamate pesticides in vegetables and fruits

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(Received September 20th, 1976)

The combination of thin-layer chromatography with an enzyme-inhibition technique has been used to detect organophosphorus and carbamate pesticides[1–5]; we have found that bee-head esterase is generally the most sensitive enzyme for use in detection of a great number of these pesticides[4]. As alternative enzyme sources, we have compared drosophila and rat-liver esterases[6–7] with this bee-head esterase, and we now report the application of these enzymes in the detection of many organophosphorus and some carbamate pesticides in vegetables and fruits, without the need for a clean-up procedure.

EXPERIMENTAL

Preparation of enzymes

Drosophila extract. The flies were cultivated according to the method described by Haskell[8].

Mix 300 mg of flies with 75 ml of ice–water for 3 min with an Ultra-Turrax mixer at moderate speed, and filter through a G1 glass filter; prepare a fresh solution daily. Note that live flies can be frozen at —20° after being anaesthetized with diethyl ether and stored at this temperature for at least 3 months.

Rat liver extract. The rat-liver esterase was extracted according to the procedure of Mendoza and Wales[1].

Homogenize 10 g of fresh rat liver and 90 ml of water in the Ultra-Turrax; keep the livers and glassware in a bath of ice while preparing the homogenate. Transfer 1.5-ml portions of the homogenate into plastic tubes, and freeze them at —20°; before use, dilute each of these portions with 150 ml of water. Note that the frozen rat-liver extracts can be kept for *ca.* 6 months.

Bee-head extract. Mix 25 bee heads with 75 ml of ice–water for 3 min in the Ultra-Turrax at moderate speed, and filter through a G1 glass filter; prepare a fresh solution daily. Note that live bees can be frozen with solid carbon dioxide and stored at —20° for at least 1 year (see ref. 4).

Preparation of substrate

Dissolve 20 mg of 2-naphthyl acetate in 8 ml of ethanol, and separately dissolve 50 mg of Fast Blue B in 32 ml of water; mix these two solutions immediately before spraying the chromatogram.

Sample extraction

Cut the samples into small pieces with a food cutter, and homogenize 50 g of chopped sample with 100 ml of dichloromethane in a 500-ml jar with use of the Ultra-Turrax at moderate speed; dry the homogenate with anhydrous sodium sulphate and filter.

Thin-layer chromatography

Evaporate 10 ml of the dichloromethane extract to 1 ml, and apply two 5-μl portions, and two 5-μl portions of a standard solution (1 ng/1 μl in ethyl acetate) to a silica gel G plate. Develop the chromatogram of pesticides or vegetables or fruit extracts according to any known method[1–5].

After development, dry the plate in air, and oxidise the pesticides by exposure to bromine vapour as follows. Place a porcelain dish containing 0.1 ml of bromine on the bottom of a desiccator, close the desiccator, and wait until bromine colour is evenly distributed. Expose the plate for 30 sec to the bromine vapour, and wait until all the smell of bromine has disappeared. Then spray the plate lightly, but evenly, with 20 ml of enzyme solution, and place it for 30 min in a moist atmosphere at 37°.

TABLE I

DETECTION LIMITS FOR PESTICIDES

The developing solvent used was chloroform–diethyl ether (24:1), and R_F values are relative to dichlorvos (R_F = 1.00). In this Table, — indicates not detectable.

Pesticide [common and alternative name(s)]	*Chemical name*	*Limit of detection (ng)*			R_F *value*
		Drosophila extract	*Rat-liver extract*	*Bee-head extract*	
Abate	O,O,O′,O′-Tetramethyl-O,O′-(thiodi-*p*-phenylene) diphosphorothioate	100.0	100.0	2.0	2.56
Aldicarb	2-Methyl-2-(methylthio) propionaldehyde O-(methylcarbamoyl)oxime	100.0	—	5.0	0.70
Amidithion	O,O-Dimethyl S-(2-methoxyethylcarbamoyl methyl) phosphorodithioate	25.0	200.0	25.0	0.30
Aminocarb	4-Dimethylamino-3-tolyl methylcarbamate	200.0	150.0	10.0	0.7
Azinfos-ethyl, Gusathion A	O,O-diethyl S-(4-oxobenzo-1,2,3-triazino-3-ylmethyl) phosphorodithioate	0.1	1.0	0.1	1.82
Azinfos-methyl, Guthion, Gusathion	O,O-dimethyl S-(4-oxobenzo-1,2,3-triazino-3-ylmethyl) phosphorodithioate	0.1	1.0	0.1	1.75
Azodrin, Monocrotophos	Dimethyl (1-methyl-2-methylcarbamoyl)vinyl phosphate	500.0	—	5.0	0.00

TABLE I *(continued)*

Pesticide [common and alternative name(s)]	*Chemical name*	*Limit of detection (ng)*			R_F *value*
		Drosophila extract	*Rat-liver extract*	*Bee-head extract*	
Bidrin, Dicrotophos	Dimethyl *cis*-(1-methyl-2-dimethylcarbamoylvinyl) phosphate	1000.0	—	5.0	0.00
Bromophos-ethyl, Nexagan	O,O-Diethyl O-(2,5-dichloro-4-bromophenyl) phosphorothioate	0.1	1.0	0.5	2.85
Bromophos-methyl, Nexion	Dimethyl (2,5-dichloro-4-bromophenyl) phosphorothioate	0.1	1.0	0.5	2.94
Butonate	Dimethyl 2,2,2-trichloro-1-butyryloxy-ethyl phosphonate	—	100.0	500.0	0.00
Carbaryl, Sevin	1-Naphthyl methylcarbamate	5.0	5.0	0.1	1.06
Chlorfenvinfos, Birlane	Diethyl 2-chloro-1-(2,4-dichlorophenyl)vinyl phosphate	1.0	1.0	5.0	1.44
Cidial, Phenthoate, Dimephenthoate	O,O-dimethyl S-α-ethoxycarbonyl benzylphosphorothioate	0.1	10.0	0.1	2.78
Coumaphos, Co-Ral, Asuntol	O,O-diethyl O-(3-chloro-4-methyl-2-oxo-2H-1-benzopyranyl-7) phosphorothioate	1.0	5.0	2.0	2.21
Demeton-O	O,O-diethyl O-2-ethylthioethyl phosphorothioate	10.0	5.0	5.0	0.66
Demeton-O-methyl	O,O-dimethyl O-2-ethylthioethyl phosphorothioate	10.0	100.0	5.0	0.54
Demeton-O-methyl-sulphoxide	O,O-dimethyl S-2-ethylsulphinylethylphosphorothioate	20.0	100.0	5.0	0.51
Di-allate, Avadex	S-(2,3-Dichloroallyl) di-isopropylthiolcarbamate	—	—	5.0	1.86
Diazinon, Basudin	Diethyl (2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate	1.0	1.0	0.5	1.66
Dichlorvos	Dimethyl 2,2-dichlorovinyl phosphate	0.5	1.0	0.2	1.00
Dimethoate, Rogor	O,O-Dimethyl S-methylcarbamoylmethyl phosphorodithioate	20.0	—	500.0	0.17
Dimetilan	1-Dimethylcarbamoyl-5-methylpyrazol-3-yl dimethylcarbamate	25.0	50.0	50.0	0.22
Dioxathion, Delnav	1,4-Dioxan-2,3-ylidene S,S-bis-(O,O-diethyl phosphorodithioate)	50.0	1.0	5.0	2.63
Dithion, Coumithoate	Diethyl 7,8,9,10-tetrahydro-6-oxobenzo[c]chroman-3-yl phosphorothioate	5.0	2.0	100.0	2.21
Dowco 199, Plondrel	O,O-Diethyl phthalimido phosphorothioate	100.0	50.0	200.0	2.08

(Continued on p. 248)

TABLE I *(continued)*

Pesticide [common and alternative name(s)]	*Chemical name*	*Limit of detection (ng)*			R_F *value*
		Drosophila extract	*Rat-liver extract*	*Bee-head extract*	
Dursban, Dowco 179, chlorpyrifos	O,O-Diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate	1.0	1.0	0.1	2.57
Dyfonate, Fonofos	O-Ethyl S-phenyl ethylphosphonodithioate	2.0	2.0	0.5	2.56
Endothion, Endocide	O,O-Dimethyl S-(5-methoxy-4-oxo-4H-pyran-2-ylmethyl) phosphorothioate	100.0	100.0	25.0	0.00
EPN	O-Ethyl O-4-nitrophenyl phenyl phosphonothioate	2.0	2.0	0.1	2.51
Eptam, EPTC	S-Ethyldipropylthiocarbamate	1000.0	—	1000.0	2.31
Ethion, Nialate, Diethion	Tetraethyl S,S′-methylene-bis-(phosphorodithioate)	10.0	2.0	10.0	3.00
Fenthion, Tiguvon	O,O-Dimethyl O-3-methyl-4-(methylthio)phenyl phosphorothioate	25.0	—	40.0	2.81
Folimat, Omethoate	Dimethyl S-methylcarbamoylmethyl phosphorothioate	50.0	—	25.0	0.00
Folithion, Sumithion, Fenitrothion	O,O-Dimethyl O-(3-methyl-di-4-nitrophenyl) phosphorothioate	5.0	1.0	0.5	2.44
Formothion	O,O-Dimethyl S-(N-formyl-N-methylcarbamoyl) phosphorodithioate	10.0	—	10.0	1.12
HOE 2873, Pyrazophos	O,O-Diethyl O-(6-ethoxycarbonyl-5-methylpyrazolo [1,5-*a*]pyrimidin-2-yl) phosphorothioate	0.5	1.0	0.1	1.90
H 14503, Torak, Dialifor	O,O-Diethyl S-(2-chloro-1-phthalimidoethyl) phosphorodithioate	0.5	1.0	0.1	2.44
Imidan, Phosmet, Prolate	O,O-Dimethyl S-phthalimidomethyl phosphorodithioate	1.0	—	0.2	2.21
Malaoxon	O,O-Dimethyl S-[1,2-di-(ethoxycarbonyl)ethyl] phosphorothioate	1.0	—	0.2	0.40
Malathion	O,O-Dimethyl S-[1,2-di-(ethoxycarbonyl)ethyl] phosphorodithioate	1.0	—	0.2	2.38
Menazon	O,O-Dimethyl S-(4,6-diamino-1,3,5-triazin-2-ylmethyl) phosphorodithioate	1.0	—	0.5	0.00
Mevinphos, Phosdrin	Dimethyl 2-methoxycarbonyl-1-methylvinyl phosphate	1.0	2.0	0.1	0.44
Nemafos, Zinophos, Thionazin	O,O-Diethyl O-2-pyrazinyl phosphorothioate	1.0	1.0	0.1	1.41

TABLE I *(continued)*

Pesticide [common and alternative name(s)]	*Chemical name*	*Limit of detection (ng)*			R_F *value*
		Drosophila extract	*Rat-liver extract*	*Bee-head extract*	
Ortho-Dibrom, Naled	Dimethyl 1,2-dibromo-2,2-dichloroethyl phosphate	10.0	25.0	0.1	1.26
Parathion	O,O-Diethyl O-4-nitrophenyl phosphorothioate	2.0	1.0	0.2	2.85
Parathion-methyl	O,O-Dimethyl O-4-nitrophenyl phosphorothioate	1.0	1.0	0.2	2.75
Paraoxon	Diethyl 4-nitrophenyl phosphate	1.0	1.0	0.2	0.88
Phenkapton	O,O-Diethyl S-(2,5-dichlorophenylthiomethyl) phosphorodithioate	5.0	1.0	0.5	2.56
Phosalone	O,O-Diethyl S-(6-chloro-2-oxobenzoxazolin-3-ylmethyl) phosphorodithioate	2.0	1.0	0.5	2.26
Phosphamidon, Dimecron	Dimethyl 2-chloro-2-diethylcarbamoyl-1-methylvinyl phosphate	500.0	—	100.0	0.05
Prothoate, Fac 20	O,O-Diethyl S-(N-isopropylcarbamoylmethyl) phosphorodithioate	100.0	500.0	1000.0	0.79
Ronnel, Fenchlorphos	O,O-Dimethyl O-2,4,5-trichlorophenyl phosphorothioate	2.0	5.0	0.5	2.46
Sulfotep, Dithione	O,O,O′,O′-Tetraethyldithiopyrophosphate	20.0	20.0	25.0	2.71
TEPP	Tetraethyl pyrophosphate	5.0	5.0	1.0	0.06
Tetrachlorvinphos, Rabond, Gardona	Dimethyl 1-(2,4,5-trichlorophenyl) 2-chlorovinyl phosphate	1000.0	—	1.0	1.30
Thimet, Phorate	O,O-Diethyl S-(ethylthiomethyl) phosphorodithioate	5.0	5.0	5.0	2.82
Thiometon, Ekatin	O,O-Dimethyl S-(2-ethylthioethyl) phosphorodithioate	25.0	25.0	5.0	2.76
Trichloronate, Agritox, Phytosol	Ethyl 2,4,5-trichlorophenyl ethylphosphonothioate	500.0	0.5	5.0	2.50
Trichlorphon, Dipterex	Dimethyl 1-hydroxy-2,2,2-trichloroethylphosphonate	10.0	1.0	1.0	0.30
Trithion, Carbophenothion	O,O-Diethyl S-4-chlorophenylthiomethyl phosphorodithioate	10.0	5.0	5.0	2.94
Ultracid, Supracid	O,O-Dimethyl S-5-methoxy-2-oxothiadiazol-3-ylmethyl phosphorodithioate	5.0	25.0	0.1	1.93
Unden, Baygon, Aprocarb, Propoxur	2-Isopropoxyphenyl methylcarbamate	—	—	0.5	0.77
Vamidothion	O,O-Dimethyl S-2-(1-methylcarbamoylethylthio)-ethyl phosphorothioate	50.0	—	10.0	0.00
V.C. 13, Nemacide, Dichlofenthion	O,O-Diethyl O-2,4-dichlorophenyl phosphorothioate	10.0	2.0	100.0	2.56

Spray the plate with freshly prepared substrate solution, and again place it in a moist atmosphere at 37° for 15 min; the pesticides appear as white spots on a violet background.

RESULTS AND DISCUSSION

Table I shows the limits of detection (in nanograms) obtained for many organophosphorus and some carbamate pesticides by using the enzyme-inhibition technique. The results indicate that bee-head esterase is generally more sensitive in detecting these pesticides then are the drosophila and rat-liver esterases, but these last two esterases are useful alternative enzymes in screening for these pesticides.

Rat-liver esterase is particularly sensitive to inhibition by dithion, V.C. 13 and trichloronate.

The sensitivity to inhibition by butonate and trichlorphon was improved by spraying the plate with 5% ammonia solution instead of exposing it to bromine vapour[5].

Table II shows that most of the vegetable or fruit extracts can be applied to the plate without clean-up. For most extracts, a limit of detection of 0.01 ppm can be attained, and those extracts that show interferences on the plate can be cleaned-up according to an accepted method[9].

The pesticides can be determined quantitatively by gas–liquid chromatography[10,11].

TABLE II

TLC OF VEGETABLE AND FRUIT EXTRACTS ON SILICA GEL G WITHOUT ELABORATE CLEAN-UP

The developing solvent used was chloroform–ethyl ether (24:1). − = No interference; + = interference.

Sample	*Interference*	*Sample*	*Interference*
Broccoli	+	Spinach	−
Roots	−	Lettuce	−
Paprika	−	French beans	−
Chicory	−	Strawberries	−
Melon	−	Apples	−
Pineapple	−	Potatoes	−
Cabbage*	+	Plums	−
Red peppers	−	Cauliflowers	+
Garden cress	+	Cucumbers	−
Purslane	−	Grapefruit	−
Bananas	−	Pears	−
Oranges	−	Blackberries	−
Celery	+	Rhubarb	+
Lemons	−	White grapes	−
Endive	−	Red grapes	−
Onions	+	Swedes	+
Leeks	+	Curly kale	+

* Different kinds of cabbages.

ACKNOWLEDGEMENT

We thank W. Visser for his technical assistance.

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