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### Thin layer chromatography of some degradation products of organophosphorus insecticides

This note describes a thin layer chromatographic system, and two modifications of spray reagents for separating and visualising degradation products of certain organophosphorus insecticides.

#### Chromatography

Acetonitrile-water mixtures provide a simple system for the separation of degradation products of dialkyl aryl phosphorothionates on Silica Gel G layers. The  $R_F$  values are very dependent upon the water content. Good separations are obtainable with technical acetonitrile and water in the ratio of 88:12, v/v. A 10 cm development is achieved in 10 min, an advantage when speed is of importance. The silica gel layers

TABLE I

$R_F$  VALUES OF SOME COMPOUNDS IN THE SYSTEM ACETONITRILE-WATER (88:12)

Compound	$R_F$
Inorganic phosphate	0.0
Inorganic phosphorothionate	0.01
Methyl phosphate	0.02
Methyl phosphorothionate	0.15
Dimethyl phosphate	0.05
Dimethyl phosphorothionate	0.33
Ethyl phosphorothionate	0.16
Diethyl phosphorothionate	0.40
Ethyl ethylphosphonate	0.10
Ethyl ethylphosphonothionate	0.30
Phenyl phosphate	0.00
Phenyl phosphorothionate	0.70
Methyl phenyl phosphorothionate	0.30
Diphenyl phosphate	0.50
4-Bromo-2,5-dichlorophenyl phosphorothionate	0.75
Methyl-4-bromo-2,5-dichlorophenyl phosphate	0.39
Methyl-4-bromo-2,5-dichlorophenyl phosphorothionate	0.59
Ethyl-4-bromo-2,5-dichlorophenyl phosphorothionate	0.46
4-Nitrophenyl phosphate	0.0
4-Nitrophenyl phosphorothionate	0.75
Methyl-4-nitrophenyl phosphate	0.28
Methyl-4-nitrophenyl phosphorothionate	0.37
4-Nitro-3-methylphenyl phosphorothionate	0.70
Methyl-4-nitro-3-methylphenyl phosphate	0.27
Methyl-4-nitro-3-methylphenyl phosphorothionate	0.50
2,4,5-Trichlorophenyl ethylphosphonate	0.27
2,4,5-Trichlorophenyl ethylphosphonothionate	0.70

should be activated at 100° for 30 min when complete resolution of the compounds with high  $R_F$  values is required. The  $R_F$  values for some substances are listed in Table I. The system is especially suitable for the separation of O-methyl-O-4-bromo-2,5-dichlorophenyl phosphorothionate (desmethylbromophos) and the corresponding

monoaryl phosphorothionate (bisdsmethyl bromophos). These substances have nearly identical  $R_F$  values in the butanol-acetic acid-water systems commonly used for separation of mono- and diesters of phosphoric and phosphorothionic acid.

#### *Spray reagents*

BRAITHWAITE<sup>1</sup> reported that the use of DQC, (2,6-dibromobenzoquinone-4-chloroimide) in cyclohexane as a spray reagent for thin layer detection of phosphorothionates, as described by MENN *et al.*<sup>2</sup>, works better on layers when HCl is included in the gel. This was also the experience in our laboratory. Use of acetic acid instead of cyclohexane as a solvent for DQC, had the same effect as that described by BRAITHWAITE. 1% DQC in acetic acid was therefore used as spray reagent. This modification is especially favourable with monoalkyl phosphorothionates. They (*e.g.* monomethyl and monoethyl phosphorothionate, and alkyl aryl phosphorothionates) appear as yellow spots which develop instantly. The limit of detection is about 0.2–0.5  $\mu\text{g}$ . Dialkyl phosphorothionates and ethyl, ethylphosphonothionate and aryl, ethylphosphonothionates as well as monoaryl phosphorothionates yield red colours, with full colour development after heating at 100° for 5 min. The limits of detection are about 0.1  $\mu\text{g}$ .

JUNGNICKEL<sup>3</sup> developed a very sensitive method for the detection of phosphorus substances on paper chromatograms. With a modification which provides for the destruction of the compounds, his method is useful for thin layer detection of degradation products of insecticides. A suitable destruction method consists in the combination of heating and U.V.-irradiation. The layers are sprayed very lightly with 1% w/v ammonium molybdate in 0.1 *M* HCl, with 5% v/v of 60% w/v HClO<sub>4</sub> added, and then heated directly with the flame of a Thuringer or Bunsen burner, the flame being directed downward upon the gel side of the plate for 2 min. If the plates tend to shatter, because of the quality of the glass, they can be placed in an oven at 200° for an hour, instead. The layers are then exposed to strong U.V.-light for 30 min. Finally, they are sprayed with JUNGNICKEL's reagent No. 2. Crystal violet and Brilliant green provide the highest sensitivity, while Iodine green provides low sensitivity.

Compared to the HANES AND ISHERWOOD method<sup>4</sup>, this modification of JUNGNICKEL's method gives a higher sensitivity and is more easily accomplished.

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