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DETECTION AND DETERMINATION OF ORGANOPHOSPHORUS INSECTICIDES IN TISSUES BY THIN-LAYER CHROMATOGRAPHY

S. N. TEWARI and S. P. HARPALANI
Toxicology Division, Chemical Examiner's Laboratory, Agra (India)
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SUMMARY

The toxicological analysis of 12 common organophosphorus insecticides is described. Suitable methods for the extraction of organophosphorus insecticides from tissues are proposed. The detection, identification and estimation of these insecticides by thin-layer chromatography is described for 25 solvent systems and a series of chromogenic reagents. The distribution of insecticides in human body tissues in five cases of poisoning by ethyl parathion, malathion, dimethoate, sumithion and phosphamidon has also been studied.

INTRODUCTION

In India, poisoning by insecticides has increased at an alarming rate, 50–75% of the cases of poisoning referred to the toxicological laboratories in recent years being due to insecticides. The ease of availability and quick action of organophosphorus insecticides are the main reasons for the use of these compounds in suicides and homicides. The severe toxicity of these insecticides has been reported by several workers[1–5]. It, therefore, became necessary to develop reliable methods for the analysis of organophosphorus insecticides present in autopsy tissues and other biological materials.

Earlier workers[6–15] based their analyses of organophosphorus insecticides on colorimetry and paper chromatography. Methods for the isolation and determination of organophosphorus insecticides by thin-layer chromatography (TLC) have also been mentioned by some workers[16–29]. Abbott and Egan[30] reviewed methods for the detection and determination of phosphorus insecticide residues in food products. Earlier workers confined their work mainly to the detection of insecticides in plants, vegetables and other food products. Little work has been carried out on the TLC analysis of organophosphorus insecticides in tissues. Some chromogenic reagents[31–38] have been used to locate insecticides on TLC plates. However, no systematic work is available on the identification and estimation of common organophosphorus insecticides present in autopsy tissues.

In the present paper, methods are suggested for the extraction of common organophosphorus insecticides from autopsy tissues. The detection and determina-

tion of the extracted insecticides by TLC using 25 solvent systems and 15 different chromogenic reagents have been worked out and are described in detail.

EXPERIMENTAL

The following organophosphorus insecticides have been studied:

(1) Ethyl parathion (Folidol E-605, Bayer)	O,O-Diethyl O-*p*-nitrophenyl thiophosphate
(2) Methyl parathion (Metacid, Bayer)	O,O-Dimethyl O-*p*-nitrophenyl thiophosphate
(3) Malathion (Jyothi oil)	O,O-Dimethyl dithiophosphate of diethyl mercaptosuccinate
(4) Dimethoate (Rogor-Rallis)	O,O-Dimethyl S-(N-methylcarbamoylmethyl) dithiophosphate
(5) Diazinon (Tik-20-Rallis)	O,O-Diethyl O-[2-isopropyl-6-methyl-4-pyrimidinyl]thiophosphate
(6) Sumithion (Fenitrothion, Bayer)	O,O-Dimethyl O-(4-nitro-*m*-tolyl)thiophosphate
(7) Metasystox R (Ciba)	S-[2-(Ethylsulphinyl)ethyl]O,O-dimethyl thiophosphate
(8) Baytex (Fenthion, Bayer)	O,O-Dimethyl O-[(4-methylthio)-*m*-tolyl]thiophosphate
(9) Disyston (Thiodemetone, Bayer)	O,O-Diethyl S-[2-(ethylthioethyl)ethyl] dithiophosphate
(10) Desanit (Bayer)	O,O-Diethyl O-[(4-methylsulphinyl)phenyl] thiophosphate
(11) Phosphamidon (Dimecron, Ciba)	O-2-Chloro-2-(diethylcarbamoyl)-1-methylvinyl O,O-dimethyl phosphate
(12) DDVP (Dichlorovos, Ciba)	O,O-Dimethyl 2,2-dichlorovinylphosphate

Isolation of organophosphorus insecticides from tissues

Method 1. 20 g of tissue were macerated into a fine slurry by mixing with an equal amount of anhydrous sodium sulphate, and the slurry was then transferred to a conical flask fitted with an air condenser. 50 ml of *n*-hexane were added and the flask was heated on a boiling water-bath for 1 h. After cooling, the *n*-hexane layer was filtered. The extraction of the remaining slurry was repeated twice with 25-ml portions of *n*-hexane. The filtered extracts were combined, placed in a separating funnel, and vigorously shaken for 5 min with 15-, 10- and 10-ml portions of acetonitrile which had been previously saturated with *n*-hexane. The acetonitrile layers were mixed, placed in another separating funnel and diluted ten times with distilled water. 25 ml of a saturated solution of Na_2SO_4 were than added, and the mixture was extracted three times with 25-ml portions of *n*-hexane which had been previously saturated with distilled water. The final *n*-hexane extracts were combined and then reduced in volume to 2 ml by evaporation on a warm water-bath. The remaining solvent was removed in a current of dry air. The residue was dissolved in 1 ml of acetone.

Method 2. The tissue sample (20 g) was minced thoroughly by mixing it with

an equal amount of anhydrous Na_2SO_4 and then transferred to a conical flask fitted with an air condenser. 10 ml of 0.1 *N* sulphuric acid and 50 ml of acetone were added to the flask and the mixture was heated under reflux over a hot water-bath for 2 h. The acetone layer was then filtered. The remaining slurry was again refluxed with another 50 ml of acetone and then filtered. The acetone layers were combined, reduced in volume to *ca.* 25 ml by evaporation on a warm water-bath and then placed in a separating funnel. The acetone solution was diluted four times with distilled water, 20 ml of a saturated solution of Na_2SO_4 were added and the mixture was extracted three times with 25-ml portions of chloroform. The chloroform extracts were combined, washed with 25 ml of distilled water and then passed through anhydrous Na_2SO_4 (25 g). The resulting chloroform extract was evaporated just to dryness on warm water-bath. The remaining solvent was removed in a current of dry air, and the residue was dissolved in 1 ml of acetone.

Thin-layer chromatography

A suitable TLC apparatus and analytical grade reagents were used.

Glass plates (20 × 20 cm) were coated with a 250-μm thick layer of a slurry prepared by mixing 25 g of silica gel G with 50 ml of distilled water. The plates were dried in air and then activated at 120° for 30 min. An aliquot portion of the extracted residue was spotted on to an activated TLC plate together with acetone solutions containing authentic samples of the organophosphorus insecticides[1–12]. The spotted plate was then developed by the ascending technique at 25° in a saturated glass chamber containing the solvent. After a 10-cm run, the plate was removed from the chamber and dried at room temperature.

Because of the differences in polarity of the different organophosphorus insecticides, and the variations in their solubility in water, the following solvent systems were successfully used in their separation on the TLC plate:

(I)	Cyclohexane–liquid paraffin	(9:1)
(II)	Cyclohexane–chloroform	(7:3)
(III)	Cyclohexane–acetone–acetonitrile	(17:2:1)
(IV)	Cyclohexane–acetone–ethanol	(95:1:4)
(V)	Cyclohexane–acetone–liquid paraffin	(8:1:1)
(VI)	Cyclohexane–diethyl ether	(9:1)
(VII)	*n*-Hexane–ethyl methyl ketone	(39:1)
(VIII)	*n*-Hexane–ethyl acetate	(3:2)
(IX)	*n*-Hexane–acetone	(17:3)
(X)	*n*-Hexane saturated with acetonitrile	
(XI)	*n*-Hexane–Chloroform	(2:3)
(XII)	*n*-Hexane-benzene	(2:3)
(XIII)	Benzene	
(XIV)	Benzene–light petroleum	(3:1)
(XV)	Benzene–methanol	(3:2)
(XVI)	Xylene	
(XVII)	Toluene	
(XVIII)	Light petroleum	
(XIX)	Light petroleum ether–ethyl methyl ketone	(19:1)

(XX)	Light petroleum ether–acetone	(7:3)
(XXI)	Carbon tetrachloride–acetone	(9:1)
(XXII)	Carbon tetrachloride–ethyl methyl ketone	(49:1)
(XXIII)	Diethyl ether–methanol	(3:1)
(XXIV)	Chloroform–liquid paraffin	(9:1)
(XXV)	Carbon tetrachloride–ethyl acetate	(3:2)

Location of the spots of insecticide. The dry developed plate was viewed under UV light (254 nm). Dark spots were observed for insecticides 1, 2 and 6 (*p*-nitrophenyl derivatives). The plate was then sprayed with a 1% solution of silver nitrate [prepared by dissolving 1.0 g of AnalaR $AgNO_3$ in redistilled ethanol containing 5.0 ml of ammonia solution (sp. gr. 0.88), and made up to 100 ml with ethanol]. Insecticides 1, 2 and 6 gave bright yellow spots. The plate was then dried in air and then irradiated with UV light (366 nm) for 10 min. Insecticides 11 and 12, (containing chlorine) gave dark grey to black spots.

The irradiated plate was then sprayed with a 0.5% solution of palladium chloride in 0.1 *N* HCl and, after drying in air, was kept in a case at 80° for 20 min. Yellow spots, which turned brown on heating, were observed for insecticides3, 4, 5, 7, 8, 9 and 10. The spots of insecticide 1, 2 and 6 also changed from yellow to brown on heating in the case. The colours of the spots are given in Table I, and the R_F values of the organophosphorus insecticides in the different solvent systems are recorded in Table II.

Other chromogenic reagents were also used to locate the spots of the organophosphorus insecticides (Table III).

TABLE I

COLOURS OF THE SPOTS LOCATED BY A NUMBER OF METHODS ON THE SAME TLC PLATE

No.	*Insecticide*	*Colour of the spots*			
		(a) Viewed under UV light (254 nm)	*(b) Sprayed with $AgNO_3$ reagent in ammonia*	*(c) Viewed under UV light (366 nm)*	*(d) Sprayed with $PdCl_2$ reagent and warmed*
1	Ethyl parathion	Dark	Yellow		Yellowish brown
2	Methyl parathion	Dark	Yellow		Yellowish brown
3	Malathion				Brown
4	Dimethoate				Brown
5	Diazinon				Brown
6	Sumithion	Dark	Yellow		Yellowish brown
7	Metasystox R				Brown
8	Baytex				Brown
9	Disyston				Brown
10	Desanit				Brown
11	Phosphamidon			Black	Grey
12	DDVP			Black	Grey

TABLE II

R_F VALUES OF ORGANOPHOSPHORUS INSECTICIDES ON SILICA GEL G TLC PLATES

No.	*Insecticide*	$R_F \cdot 100$ *in various solvent systems*																								
		I	*II*	*III*	*IV*	*V*	*VI*	*VII*	*VIII*	*IX*	*X*	*XI*	*XII*	*XIII*	*XIV*	*XV*	*XVI*	*XVII*	*XVIII*	*XIX*	*XX*	*XXI*	*XXII*	*XXIII*	*XXIV*	*XXV*
1	Ethyl parathion	50	69	80	86	56	68	58	95	80	48	90	48	86	90	23	64	45	44	72	80	90	95	23	90	100
2	Methyl parathion	40	61	51	75	42	51	53	90	54	28	84	35	74	94	22	53	35	30	52	68	83	88	32	85	100
3	Malathion	32	31	45	58	38	22	25	84	48	30	38	8	83	32	31	22	9	15	25	63	35	64	46	75	84
4	Dimethoate	35	35	55	24	12	3	31	52	9	7	63	11	54	47	33	30	0	19	8	39	20	10	43	15	60
5	Diazinon	23	30	43	60	37	20	34	78	44	12	35	7	78	67	20	20	7	16	27	71	30	60	40	78	89
6	Sumithion	45	64	56	72	46	48	38	93	60	35	88	32	82	98	14	51	38	27	55	74	88	82	29	87	100
7	Metasystox R	58	91	95	31	20	4	96	66	14	9	28	6	98	97	39	3	5	70	13	43	95	18	27	50	85
8	Baytex	30	75	88	80	62	64	64	92	85	50	92	51	92	94	18	60	40	40	70	82	75	91	35	88	100
9	Disyston	32	85	90	86	72	79	70	97	97	55	96	60	95	96	24	64	53	54	86	91	93	97	35	92	100
10	Desanit	4	20	24	4	3	0	28	73	10	0	7	2	12	20	41	3	2	10	11	25	40	7	34	35	83
11	Phosphamidon	5	5	22	6	–	6	4	6	5	–	5	–	14	25	25	–	–	–	23	24	27	6	49	55	22
12	DDVP	0	10	42	0	–	3	0	5	0	–	0	–	35	23	28	–	–	–	0	5	–	–	36	43	34

TABLE III

OTHER CHROMOGENIC REAGENTS USED

Chromogenic reagent	*Treatment of the plate*	*Spot colours*	*Sensitivity (μg)*
Rhodamine B (0.05% ethanolic solution)	Sprayed, dried in air and exposed to Br_2 vapour for 30 sec	purple against a white background (for sulphur-containing insecticides)	0.2–1.0
Light green (0.5% solution in acetone containing 5 ml of water)	sprayed when still damp, exposed to Br_2 vapour for 30 sec	dark green against a white background (for sulphur-containing insecticides)	0.5–1.0
Tartrazine yellow [0.5% solution in ethanol–water (1:1)]	sprayed, dried in air and exposed to Br_2 vapours for 30 sec	yellow against a white background (all insecticides)	1.0
Congo red (0.4% solution in 50% aqueous ethanol)	sprayed, dried in air and exposed to Br_2 vapour	blue against a white background	0.5–1.0
Fluoresceine–sodium (0.25% solution in ethanol)	sprayed, dried in air and exposed to Br_2 vapour for 30 sec	yellow against a red background	0.1–0.5
Dimethyl yellow (0.1% solution in isopropanol)	exposed to Br_2 vapour for 1 min, kept in air for 2 h and then sprayed	pink against a yellow background	0.5–1.0
Bromophenol blue [0.05% solution in 50% ethanol and 1% aqueous silver nitrate solution (1:1)]	sprayed, dried in air and then sprayed with 2% aqueous citric acid solution	blue against a white background	1.0
Thymol blue (0.25% solution in ethanol)	sprayed, dried in air and then exposed to Br_2 vapour	violet against a yellow background	1.0
Palladium chloride (0.25% solution in 0.1 *N* HCl)		yellow against a white background	0.2–0.5
Mercurous nitrate (1% solution in 0.1 *N* HNO_3)	(a) no treatment	(a) black for phosphamidon	2.0–5.0
	(b) sprayed with 10% aqueous sodium bicarbonate, dried in air and then sprayed with the reagent	(b) black for DDVP	

Estimation of the insecticides. The extracted residue was dissolved in 0.5 ml of acetone and an aliquot portion (10 μl) of this solution was spotted on to the TLC plate together with known amounts (1, 2.5, 5 and 10 μg) of the control insecticide, the presence of which in the extracted residue had already been established by qualitative TLC. (The diameter of the applied spots was restricted to 2 mm). The spotted plate was developed (10-cm run) in the appropriate solvent system. For the estimation of thiophosphoric acid esters (1–10) the developed plate was sprayed with a 0.25% solution of palladium chloride in 0.1 *N* HCl and dried in air. After exactly 10 min, the area of the yellow spot which had developed was measured and compared with that of the spot of the known amount of insecticide run alongside. The measurement of the spot area was made: (a) by visual observation, which gave only a rough estimation; (b) with the help of transparent graph paper fixed between two thin glass sheets, which gave a result having a fair accuracy; and (c) by scanning the developed spot on the TLC plate with the help of a photoelectric densitometer, which gave the most

accurate results. For the estimation of chlorine-containing insecticides (such as 11 and 12), the developed plate was sprayed with a 1.0% ethanolic silver nitrate solution containing 5 ml of concentrated ammonia solution. After drying in air, the plate was irradiated with UV light (366 nm) for 10 min. After exactly 10 min, the areas of the developed spots were measured and compared with that of a known amount of the control insecticide run on the same plate.

The measurement and comparison of the area of the developed spot gave the amount of the insecticide present in the applied spot. Thus the amount of the insecticide in the extracted residue and then that in 100 g of tissue or other biological material could be estimated.

Application of the TLC method to cases of poisoning

In five different cases of suspected poisoning by insecticides 1, 3, 4, 6 and 11, the insecticides from different autopsy tissues, *viz.*, stomach, intestine, liver, kidney, spleen, lung and heart, were extracted by the suggested method, and the identification was made by qualitative TLC. Estimations were carried out by measurement of the area of the developed spots on the TLC plates by densitometry. The data are given in Table IV.

TABLE IV

ESTIMATED AMOUNTS OF INSECTICIDES IN DIFFERENT AUTOPSY TISSUES IN CASES OF POISONING

Insecticide detected	*Amount in different tissues (mg/100 g)*							
	Stomach	*Intestine*	*Liver*	*Kidney*	*Spleen*	*Lung*	*Heart*	*Brain*
Ethyl parathion	8.75	6.25	4.00	3.75	2.08	—	—	—
Malathion	46.90	—	2.31	2.86	1.67	—	—	—
Dimethoate	3.25	—	4.75	2.50	4.38	2.75	1.06	—
Sumithion	12.50	—	12.50	6.25	4.17	—	—	—
Phosphamidon	0.09	0.11	0.25	0.25	0.13	0.35	0.13	0.31

RESULTS AND DISCUSSION

In Table II are recorded the R_F values of 12 common organophosphorus insecticides obtained by using 25 different solvent systems on silica gel G plates. These solvent systems provided a satisfactory separation of the insecticides. However, a better separation was achieved by use of the solvent systems I–IV, XIII, XX and XXIV. For chlorine-containing insecticides, *e.g.*, 11 and 12, solvent systems III, XIII and XXIII–XXV were most suitable for clear resolution and differentiation. The distinction between ethyl and methyl parathion was best made by using solvent systems III, X, XII, XVIII and XIX which produced a wide difference in the R_F values. The 25 solvents provided satisfactory alternative systems for the separation of the group of insecticides under consideration. Furthermore, the scheme suggested for the location of spots on the TLC plates (see Table I) provided a method for the detection of organophosphorus insecticides which contain sulphur as well as for those which do not contain sulphur, *i.e.*, those containing chlorine, and the procedure could be applied as a screening method. From control experiments, it was demon-

strated that recoveries of 92% of the added insecticides in tissues were obtained by using the suggested methods of extraction and TLC identification and estimation. The chromogenic reagents used for locating the spots of the insecticides on the TLC plates (see Table II) were sensitive down to 0.2 μg of the insecticides.

The proposed technique of extraction and quantitative estimation was successfully applied to the analysis of five different cases of suspected poisoning caused by insecticides 1, 3, 4, 6 and 11. The first four cases were of suspected oral administration of insecticides, while that of phosphamidon (11) was thought to be due to inhalation of the insecticide during spraying in the fields. The amounts of the insecticides found in different autopsy tissues are recorded in Table IV. The distribution of phosphamidon in the tissues also suggests that the insecticide is ingested by the lungs.

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