to persistently high levels of prolactin during the cycle and the control of mechanisms which lowers prolactin could restore fertility as has been observed in hyperprolactinemic women[10-11].

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DIFFERENTIATION AND ESTIMATION OF *p*-PHENYL ORGANOTHIOPHOSPHATE INSECTICIDES FROM OTHER ORGANOPHOSPHATE INSECTICIDES BY MERCUROUS NITRATE REAGENT

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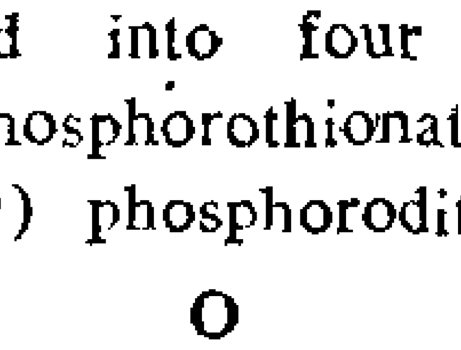
ABSTRACT

Mercurous nitrate with few drops of nitric acid is commonly used for the detection of organophosphate insecticides which can further be used for differentiation and estimation of *p*-phenyl organothiophosphate insecticides from other organophosphate insecticides.

INTRODUCTION

THE use of organophosphate insecticides in agriculture has become very popular and new types of organophosphate insecticides are coming into the market every year. Consequently, in Forensic Toxicology, the characterization of individual organophosphorus insecticides becomes necessary. The organophosphorus insecticides are classified into four classes as (*i*) phosphates, (*ii*) phosphorothionates, (*iii*) phosphorothiolates and (*iv*) phosphorodithioates.



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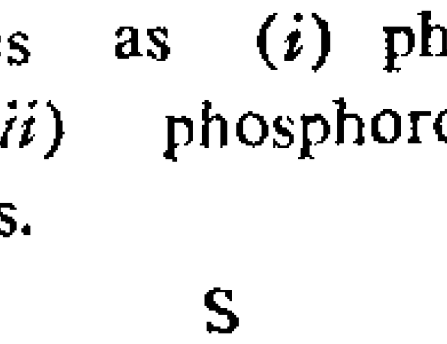

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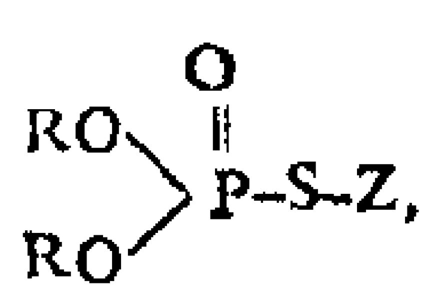

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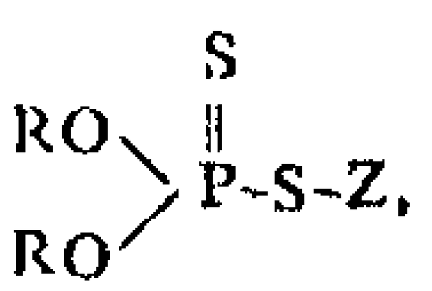

(IV)

In forensic toxicology, for the detection and characterization of individual organophosphate, insecticides can be classified in another way depending upon the nature of –Z. This classification will be of much help. The present paper describes the differentiation based on this classification of *p*-phenyl organophosphate insecticides from other organophosphate insecticides.

Mercuric nitrate gives a black colour on heating with organothiophosphates such as New Tik-20, Dalf, Dasanit, Hinosan, etc., whereas mercurous nitrate gives a black colour instantaneously with these organothiophosphates. Mercurous nitrate can also be used for the detection of organophosphate insecticides with some modification[1]. In our work it was observed that when chromatoplates are sprayed with mercurous nitrate and kept for 2–3 days, a pink colour appeared at the periphery of the black spot. In these cases pink colour was obtained immediately when heated. Among the common organothiophosphate insecticides, it was observed that only *p*-phenyl organothiophosphate insecticides gave a black colour turning to pink with mercurous nitrate reagent. Similar results

TABLE I

TLC of p-phenyl organothiophosphate insecticides

Insecticides	Chemical Name	Hexane: Acetone 4:1 Rf	Chloroform; Hexane 4:6 Rf	Carbon-tetrachloride: Benzene 2:1 Rf
1. Ekatox, Follidol, Paramar E-50 (Ethyl parathion)	(0, 0-Diethyl-0-*p*-nitrophenyl phosphorothionate)	0·51	0·44	0·66
2. Paramar M-50 Metacid-50 (Methyl parathion)	[0, 0-Dimethyl-0-(*p*-nitrophenyl) phosphorothionate]	0·48	0·40	0·63
3. New Tik-20 (Sumithion)	[0, 0-Dimethyl-0-(3-methyl-*p*-nitrophenyl) phosphorothionate]	0·53	0·30	0·50
4. Dalf, Baytex, Lebaycid-50 (Fenthion)	[0, 0-Dimethyl-0-(4-methyl mercapto-3-methyl phenyl)-phosphorothionate]	0·59	0·50	0·70
5. Dasanit (Fensulfothion)	[0, 0-Diethyl-0-(4-methyl sulfinylphenyl) phosphorothionate]	0·80	0·52	0·72
6. Hinosan	(0-Ethyl-S, S-Diphenyl dithiophosphate)	0·41	0·15	0·26

were obtained with Millon's[2] reagent which contained mercuric nitrate in addition to mercurous nitrate. Organophosphate insecticides which are not *p*-phenyl organothiophosphates (Malathion, Rogor, etc.), do not give this pink colour reaction. Millon's reagent is used for the estimation of *p*-phenyl thiophosphate insecticide as it quickly develops the colour.

EXPERIMENTAL

(1) *Mercurous nitrate.*—A few drops of concentrated nitric acid are added to a saturated solution of mercurous nitrate in water.

(2) *Millon's reagent*[3].—3 ml of mercury is dissolved in 27 ml of concentrated nitric acid and then diluted by an equal volume of distilled water.

PROCEDURE

I. *Thin Layer Chromatography*

A number of *p*-phenyl organothiophosphate insecticides are chromatographed using silica gel, as adsorbent (layer thickness—0·25 mm) and *n*-Hexane : Acetone (4 : 1) as solvent. After a run of 10 cm, the plate is taken out from the chamber, dried at room temperature and sprayed with mercurous nitrate reagent. The black spots appear. The plate is then heated at 110° C for 10 min, the black spots turn pink. TLC is repeated with two other solvent systems (1) chloroform ; Hexane (4 : 6), (2) carbon tetrachloride : benzene (2 : 1). The results are shown in Table I.

II. *Colorimetric Estimation*

1 ml of the test solution is mixed with 2 ml of fresh Millon's reagent and 4 ml of distilled water in a test-tube and kept in boiling water bath for 5 minutes, allowed to cool and the optical density of the colour formed is determined at 525 nm.

Calibration curve.—Standard stock solution of ethyl parathion in ethyl alcohol (5 gm/100 ml) and a series of working standards from the standard stock solution to give concentrations of 10, 25, 50, 100, 150, 250, 300, 350, and 400 μg/ml are prepared. The optical density of the colour produced after following the above procedure is recorded. The calibration is repeated with other *p*-phenyl organothiosphates. The graphs of optical density *versus* concentration of *p*-phenyl organothiophosphates μg/ml is shown in Fig. 1.

Recovery experiment—1 mg of parathion was added to 100 g of finely minced viscera and mixed well and kept for 1 day. The *p*-phenyl organothiophosphate is extracted with ether and the ether evaporated at room temperature. The residue is dissolved in *n*-hexane and transferred to celite column for clean up. The loaded material was eluted by hexane, the solvent evaporated and the residue dissolved in 10 ml of ethyl alcohol,

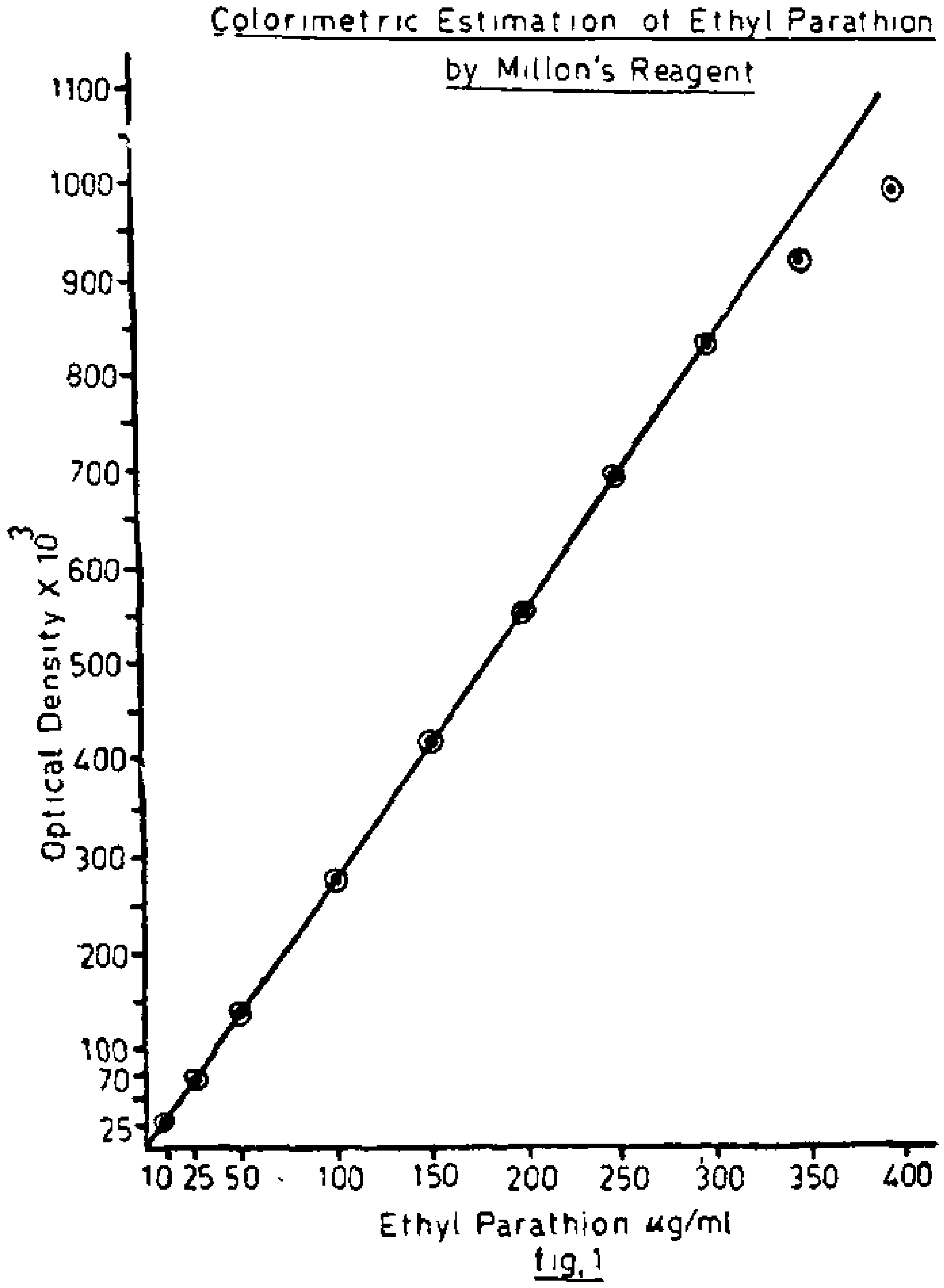

FIG. 1

One ml of this is used for the estimation. The experiment is repeated with other *p*-phenyl organothiophosphate insecticides in triplicate. The results are shown in Table II.

TABLE II

Percentage recoveries of insecticides from viscera

Insecticides (1 mg/100 g of viscera)	Quantity recovered (average of 3 readings (mg)	Percentage recovery %
Ethyl parathion	0·92	92
Methyl parathion	0·90	90
Sumithion	0·94	94
Baytex	0·80	80
Dasanit	0·82	82
Hinosan	0·87	87

RESULTS AND DISCUSSION

As organophosphate insecticides easily hydrolise and form phenolic compounds, which further react with the reagent to give pink colour ; this colour reaction is similar to the reaction of Millon's[4] reagent with *p*-hydroxy substituted compounds. Mercurous nitrate reagent, thus, in addition to detecting organothiophosphate insecticides, also serves the purpose of differentiating *p*-phenyl organothiophosphate insecticides from other organophosphate insecticides. The calibration curve obtained for each *p*-phenyl organothiophosphate insecticide shows linearity upto 300 µg. The sensitivity of estimation is ~ 15 µg/ml. The recoveries of these insecticides from viscera are good. This method for differentiating and estimating *p*-phenyl organothiophosphate insecticides is very useful in the toxicological analysis.

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S.L. HORA MEMORIAL GOLD MEDAL FOR 1977

The S.L. Hora Memorial Gold Medal, given annually for the most outstanding contribution in the field of Ichthyology, has been awarded to Dr. A. G. K. Menon, Deputy Director, Zoological Survey of India, Madras.

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