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Chromatographic analysis of *cis*- and *trans*-mevinphos in poisoned wildlife

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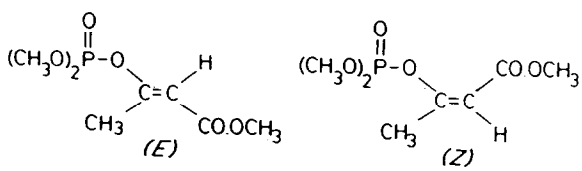
ABSTRACT

A method is described for the analysis of the *cis*- and *trans*-isomers of mevinphos in baits and avian tissues. The procedure involves extraction of the mevinphos isomers with acetone–dichloromethane (1:1) followed by clean-up on alumina. Bait samples were initially screened by thin-layer chromatography and identity of extracts confirmed by infrared spectrophotometry and gas chromatography–mass spectrometry.

INTRODUCTION

Mevinphos, methyl 3-(dimethoxyphosphinyloxy) but-2-enoate, is the active component of the insecticide Phosdrin. The technical product contains approximately 60% (w/w) of the *E* or (*cis*) isomer and the balance of the *Z* or (*trans*) isomer (or α and β isomers, respectively, Fig. 1) [1,2]. The *cis* isomer is much more biologically active than the corresponding *trans* isomer [3,4].



(E) or *cis*-mevinphos (Z) or *trans*-mevinphos

Fig. 1. Chemical structures for the isomers of mevinphos.

Mevinphos is a systemic insecticide and acaricide with contact, respiratory and stomach action. It is formulated as an emulsifiable spray concentrate for the control of chewing and sucking insects and spider mites over a wide range of crops. Generally, mevinphos has little effect on wildlife in practice as it is rapidly broken down to less toxic decomposition products [5]. However, over the years, misuse of agricultural chemicals, pesticides in particular, have resulted in poisoning of native wildlife [6–11]. Mevinphos is also quite toxic to birds with an acute oral LD_{50} of 0.75–7 mg/kg [2].

Native birds are protected by law, however, some cases of deliberate poisonings occur as they may be still considered pests when they compete with man for food and attack his livestock.

A number of methods have been developed to determine mevinphos (and other organophosphorus pesticides) such as thin-layer chromatography (TLC), cholinesterase inhibition and gas chromatography (GC) [12–15]. Of these, GC procedures are more specific and less time-consuming.

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The present paper describes a specific and robust GC and GC–mass spectrometric (MS) procedure for the determination of the *cis* and *trans* isomers (or α and β isomers) of mevinphos in the tissues of poisoned native wedge-tailed eagles (*Aquila audax*).

EXPERIMENTAL

Reagents

Mevinphos (99.0% purity) was obtained from Ajax Chemicals (Sydney, Australia). Standard solutions of mevinphos contained the *cis* (α) and *trans* (β) isomers in the proportions of 60:40. A standard stock solution (10 μg/ml) was prepared in *n*-hexane and refrigerated (*ca.* 4°C). This solution was used to prepare a working standard (1.0 μg/ml) in hexane, in addition to serving as a spiking media for placing mevinphos into the bird tissues at known concentrations. Clean-up was performed using alumina (Brockman activity II, BDH, Poole, UK) previously heated at 600°C, cooled overnight and then deactivated with water (5%, w/w) before use.

All other chemicals used such as acetone, hexane dichloromethane, etc. were either nanograde or chromatographic grade.

Gas chromatography

The chromatographic analyses were carried out on a Varian Aerograph Model 3700 gas chromatograph equipped with a thermionic specific (ceramic-alkali) detector, and a 1.8 m × 2.0 mm I.D. glass column containing 10% Carbowax 20M on Gas-Chrom II (80–100 mesh) was used. The column, injector and detector temperatures were 155, 200 and 300°C, respectively. Nitrogen (high purity) was the carrier gas at a flow-rate of 30 ml/min. Mevinphos concentrations (*cis* and *trans* isomers) were determined by external quantitation with standard solutions of mevinphos using a Varian Model 4400 integrator interfaced to the gas chromatograph and attenuated at 8 mV full-scale deflection (attenuation 2↑3).

Gas chromatography–mass spectrometry

The GC–MS analyses were carried out on a Hewlett-Packard HP5890 gas chromatograph interfaced to a HP 5970 MSD mass spectrometer system. A 25 m × 0.22 mm I.D. BP20 (a bonded-phase Carbowax 20M) column was used to separate the mevinphos isomers and obtain electron-impact spectra of each. The injector was cool on column, with the oven temperature programmed from 50°C (1 min) to 250°C at 25°C/min. The helium (high purity) carrier gas pressure was set at 100 kPa. The pressure in the ion source (electron-impact mode) was between 10^{-5} and $5 \cdot 10^{-6}$ Torr, the ionising potential 70 eV, and the accelerating voltage 5kV. The mass range examined in the extracts was between 40 to 300 amu.

Sample preparation

Two dead wedge-tail eagles *Aquila audax* were seized from a rural property by a National Parks and Wildlife Service ranger, along with a quantity of Phosdrin found at the site. The liver, crop and gizzard tissues were dissected from each of the poisoned birds, weighed and then cut into small pieces. All tissue samples were stored at −20°C prior to analysis. The tissues were then accurately weighed (5–15 g) into Sorvall cups, and 50 ml of acetone–dichloromethane (1:1) solvent mixture were added to each. The mixtures were then blended at medium speed (No. 5) using a Sorvall omni-mixer for about 2 min and then filtered through a Whatman No. 1 filter into a Kuderna–Danish flask. The samples were re-extracted with a further 25 ml of solvent mixture and the residue was washed several times with the same solvent and passed through the filter into the flask. The combined solvent extracts were then concentrated to *ca.* 2 ml. A 1-ml volume of *n*-decane was added as a "keeper" and the remainder of the solvent removed under a gentle stream of nitrogen to a final volume of 1 ml.

Column clean-up

A slurry of 10 g of prepared alumina in hexane was poured into a 300 mm × 15 mm I.D. glass column plugged with glass wool, and 5 g of anhydrous sodium sulphate were added on top of the adsorbent. The columns were covered with aluminium foil to prevent ultraviolet irradiation conversion of *trans* isomer to *cis* isomer [4]. The residue extracts were transferred with two 1-ml portions of hexane and placed on top of the prepared columns. The columns were then washed with a 50-ml portion of hexane to remove any lipohilic co-extractives in the extracts. After discarding the eluate, the mevinphos (*cis* and *trans* isomers) was eluted from

the column with 100 ml of 20% (v/v) acetone in hexane into a 100-ml volumetric flask and diluted to volume with hexane. This fraction was subsequently diluted further with hexane depending upon the initial screening of the eluate.

Recoveries

A standard additions procedure was followed as no control eagle tissue was available. Recoveries of mevinphos were determined from representative, previously analysed minced eagle liver spiked at 5–10 μg/g mevinphos.

RESULTS AND DISCUSSION

Analysis by GC and GC–MS

Several columns were investigated for separating and detecting the two isomers of mevinphos. Only polar columns such as Carbowax 20M and diethylene glycol succinate (DEGS) gave satisfactory separation with sharp, symmetrical peak responses.

Typical GC–MS total ion current chromatograms of the reference standard mevinphos (*cis* and *trans* isomers) and a liver extract from a poisoned eagle *Aquila audax* are shown in Fig. 2. The mevinphos isomers gave the following retention times: *cis* 9.56 min and *trans* 9.78 min under the GC conditions described. The peaks of *cis*- and *trans*-mevinphos were analysed by GC–MS, the mass spectra of which are shown in Fig. 3. For screening and identification purposes, the ions *m/z* 127 and 192 were monitored.

The method detection limit for mevinphos (*cis* and *trans* isomers) in the liver tissues was estimated at 0.04 μg/g). The liver recoveries (n = 4) averaged 76.0%, with a relative standard deviation (R.S.D.) of 4.3%, and were somewhat lower than expected; whilst recoveries of the standard corresponding to

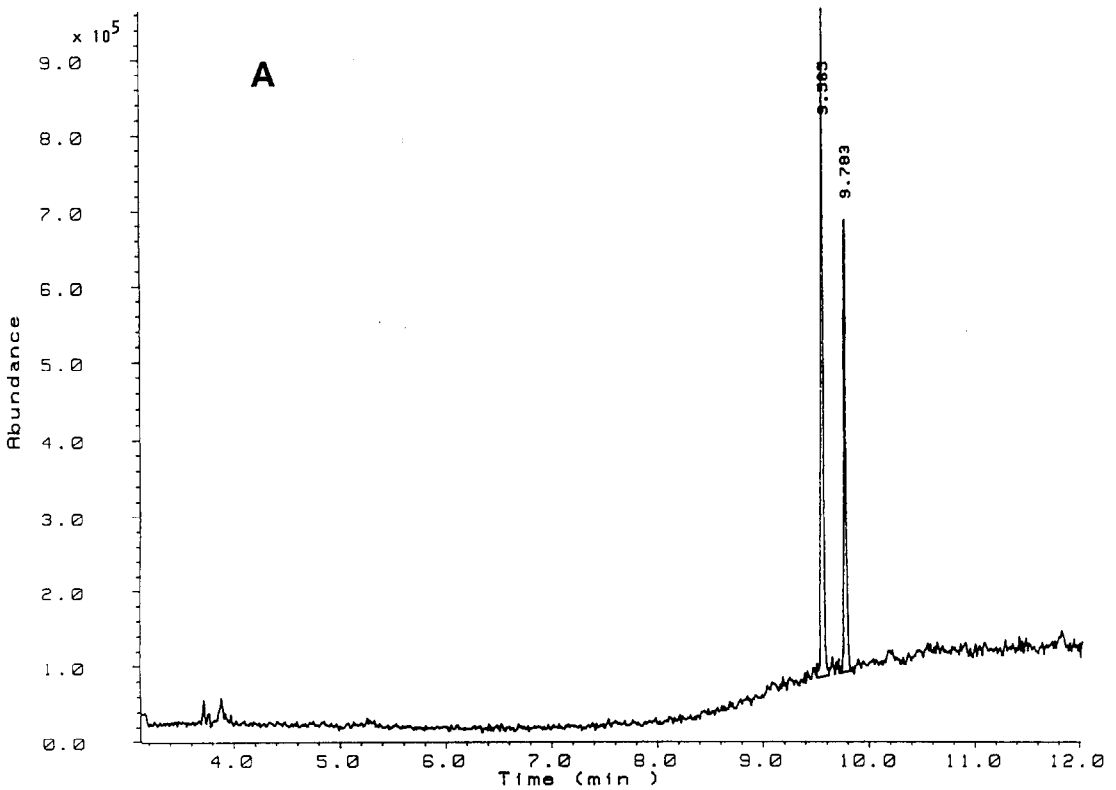

Fig. 2.

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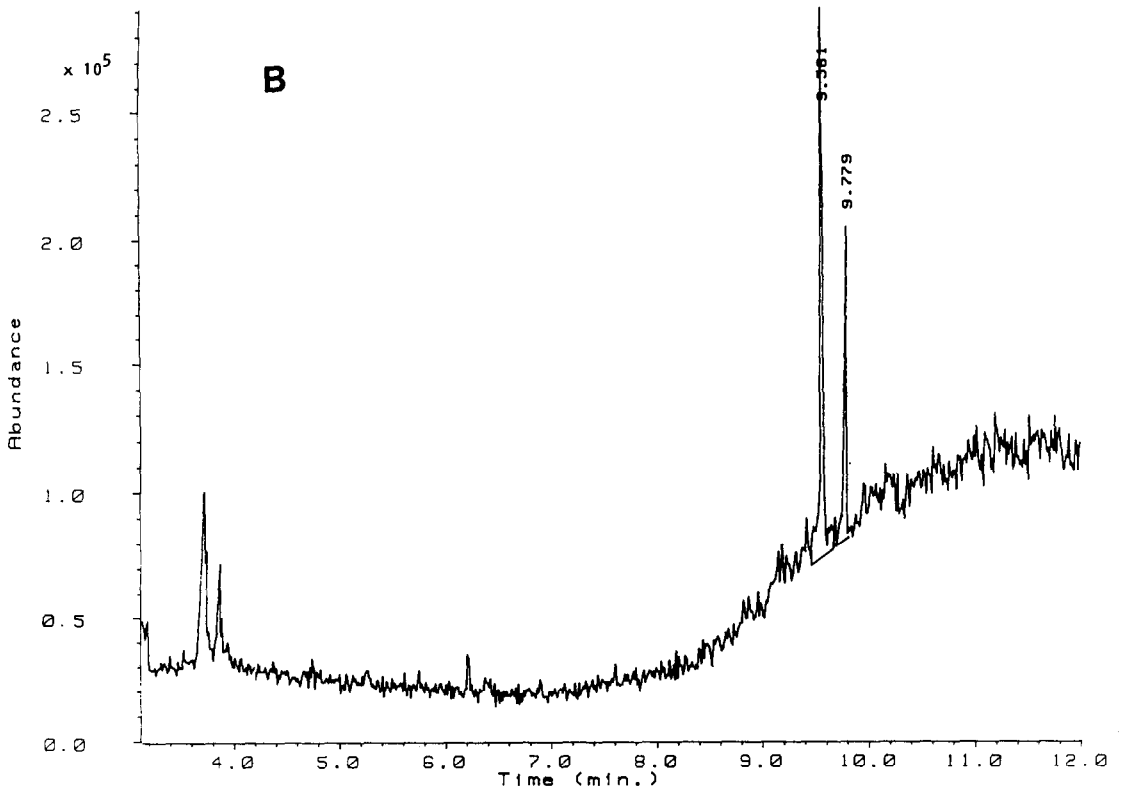

Fig. 2. GC–MS total ion current chromatograms of (A) *cis*- and *trans*-mevinphos reference standard and (B) a liver extract from a poisoned eagle *Aquila audax*.

10 μg/g were 95.5% (R.S.D. 3.5%). This may be due to losses of mevinphos from tissues before extraction and clean-up rather than during the procedure.

Infrared spectrophotometry

Infrared spectrophotometry was used to confirm the identity of the peaks in the bait extract found in one eagle's beak. Fig. 4 shows the infrared spectrum

TABLE I

MEVINPHOS RESIDUES FOUND IN TISSUES OF POISONED WEDGE-TAILED EAGLES *AQUILA AUDAX*

Bird No.	Tissue	Total mevinphos (*cis* + *trans*) found in tissue "as received" (mg/kg)	Ratio of *cis* to *trans* isomer found in tissue
1	Food found in beak	32.0	58:42
1	Crop and gizzard	16.8	58:43
1	Liver	1.7	62:38
2	Crop	4.6	58:42
2	Gizzard	4.5	58:42
2	Liver	0.8	61:39

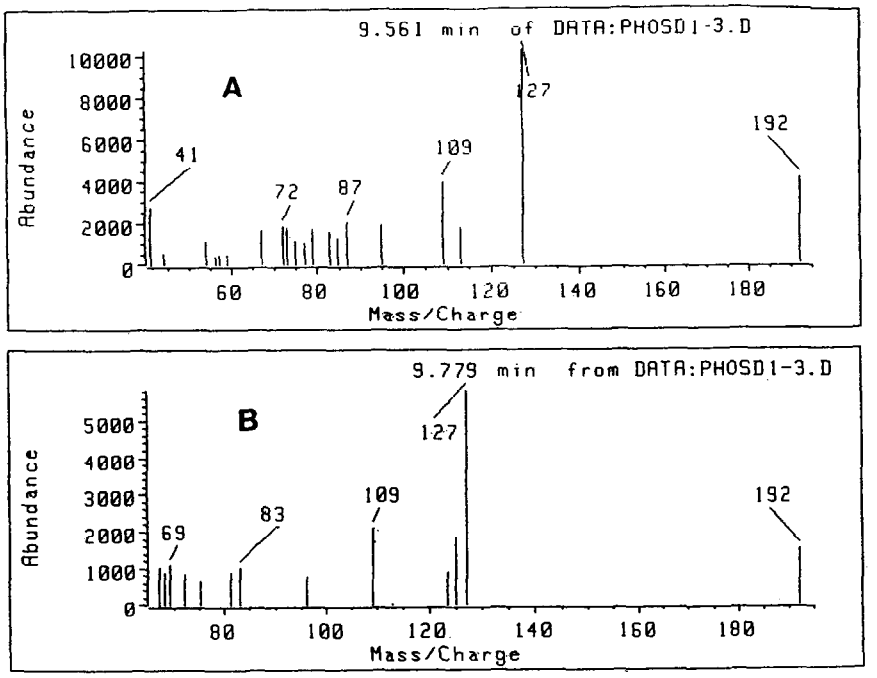

Fig. 3. Electron-impact mass spectrum of (A) *cis*- and (B) *trans*-mevinphos isomers.

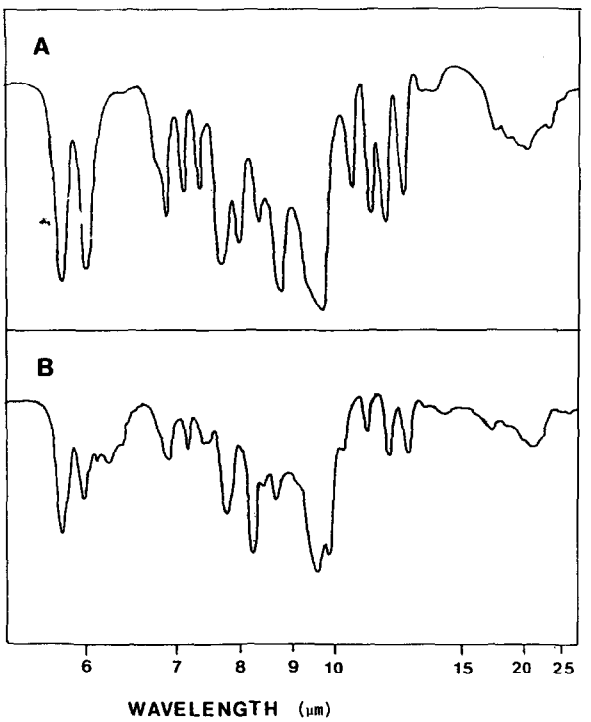

Fig. 4. Infrared spectra of (A) *cis* (or α) and *trans* (or β) isomers of mevinphos.

of both isomers separated and collected by preparative TLC (based on the work of Mendoza *et al.* [16]) and rechromatographed by GC. These data correspond with those reported by Stiles *et al.* [1].

Application to a baiting incident

The Phosdrin pesticide found at the site was found to contain 62% (w/v) of mevinphos with an isomeric *cis*/*trans* ratio of 59:41. Table I shows the tissue concentrations of mevinphos in the dead eagles found at the site.

In one of the birds (No. 1), death had apparently occurred quite rapidly as a portion of the poisoned meat was still present in the bird's beak. The *cis* and *trans* (α/β) isomeric ratios were little different from the Phosdrin found at the site. The *trans* (β) isomer of mevinphos is known to be less active as a cholinesterase inhibitor being some 22 times less toxic than the *cis* isomer [4,17]. Morello *et al.* [18] showed that *trans*-mevinphos (β-mevinphos) was degraded faster than the *cis* (α) isomer by mouse liver homogenates at approximately 3 and 2 μmol, respectively. This may also occur in birds of prey and would account for the slightly increased isomeric ratio of *cis* to *trans* in the livers of the poisoned eagles.

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