Journal of Chromatography, 508 (1990) 199–202
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 22 361

Note

Gas–liquid chromatographic analysis of ethyl fenthion in tissues of native birds

W. J. ALLENDER
Chemistry Branch, Biological and Chemical Research Institute, P.M.B. 10, Rydalmere, 2116 N.S.W. (Australia)
(First received November 22nd, 1989; revised manuscript received February 2nd, 1990)

Ethyl fenthion (O,O-diethyl-O-4-methylthio-*m*-tolyl phosphorothioate) is the active component of the pesticide Luci-jet. It is a contact and stomach insecticide and possesses a useful penetrant and persistent action similar to that of the methyl ester (fenthion)[1]. Luci-jet is formulated as a dip and drench and has been used to control blowfly strike (*Lucilia cuprina*) in sheep[2–4]. It is significantly more toxic than the methyl ester with an acute oral LD_{50} for male rats of 45 mg/kg compared to 190–315 mg/kg for fenthion[1,4].

In recent years, deliberate ethyl fenthion poisoning of wildlife has occurred in New South Wales. Determination by semi-quantiative thin-layer chromatography, based on acetylcholinesterase inhibition[5,6] did not allow sufficiently low detection limits for ethyl fenthion in the liver and kidney tissues, but did provide a useful screening procedure for stomach contents.

The purpose of the present work was to develop a specific, sensitive and robust gas chromatographic method for determining ethyl fenthion poisoning of native wildlife.

EXPERIMENTAL

Extraction and clean-up procedure

The liver, stomach plus contents and kidney tissues dissected from the poisoned birds were cut into small pieces. The tissues were then accurately weighed (5–15 g) into a Sorvall cup and 25 ml of an acetone–dichloromethane (1:1) solvent mixture were added. The mixtures were then blended at medium speed 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 and the residue was washed several times with the same solvent and passed through the filter in the flask. The combined solvent extracts were then concentrated to a low volume. A 1-ml volume of 1% (v/v) acetic acid in hexane was added and the remainder of the solvent removed under a gentle stream of nitrogen to a final volume of *ca.* 1 ml. The residues were transferred with two 1-ml volumes of 1% (v/v) acetic acid in hexane onto silica gel 60 (particle size 0.063–0.200 mm, E. Merck) clean-up columns. The columns were pre-

0021-9673/90/$03.50

pared by adding 3.5 h of deactivated silica gel [deactivated by addition of 20% (w/w) distilled water], followed by 2 g anhydrous sodium sulphate (granular) to a 200 mm × 6 mm I.D. glass column plugged with glass wool. The columns were washed with 10 ml of 1% (v/v) acetic acid in hexane. After the addition of the sample residues the columns were eluted with 10 ml of 1% (v/v) acetic acid in hexane and the eluent was discarded. The ethyl fenthion was eluted with 30 ml of 5% (v/v) diethyl ether in hexane into a 50-ml volumetric flask. This fraction was subsequently concentrated or diluted depending on initial screening of eluate.

Chromatography

The eluate (2 μl) was injected into a Varian 3700 gas chromatography equipped with a thermionic specific (ceramic-alkali) detector and a 1.5 m × 2.0 mm I.D. glass column containing 7% OV-210 and 3.5% OV-101 on Chromsorb Q HP (100–120 mesh) operating at 185°C. Detector and inlet oven temperatures were 300 and 220°C, respectively. Gas flow-rates were as follows: nitrogen (carrier), 50 ml/min; hydrogen, 5.0 ml/min; air, 170–175 ml/min. Ethyl fenthion concentrations were determined by external quantitation with standard solutions of ethyl fenthion using a Hewlett-Packard Model 3390A integrator interfaced to the gas chromatograph and attenuated at 16 mV full scale deflection.

RESULTS AND DISCUSSION

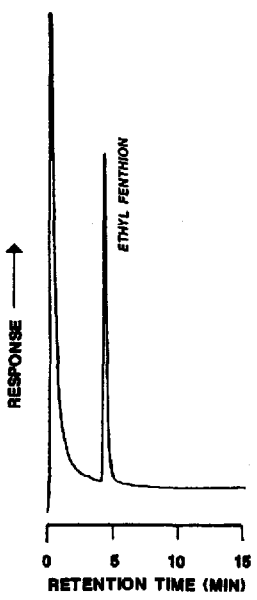
The method described is sensitive and efficient for extraction and clean-up of ethyl fenthion residues in tissues of poisoned birds of prey. The method detection limit for ethyl fenthion in liver and kidney was 0.02 μg/g. Average recoveries from liver and kidney fortified with ethyl fenthion were greater than 91%.

Typical chromatograms of the reference standard ethyl fenthion and a cleaned stomach contents extract from a poisoned magpie (*Gymonorhina tibicen*) obtained with thermionic specific detection are shown in Figs. 1 and 2.

Table I shows the tissue concentrations of ethyl fenthion in two species of birds of prey. The ethyl fenthion had been extensively metabolished in all the bird livers, probably to the corresponding sulphoxide and sulphone, both of which are also toxic[7]. Ethyl fenthion residues in the magpie (*Gymonorhina tibicen*) were greater than those found in the currawong (*Strepera melanoptera*). This may be due to variation in species sensitivity to the pesticide and/or metabolic rates prior to death.

The use of ethyl fenthion appeared to be limited to mainly meat baits. Birds of prey such as crows, magpies and currawongs were probably the primary target, particularly during the lambing season; however, dogs, including sheep dogs, can also be at risk. The misuse of pesticides to poison vertebrates can put at risk both the person laying the bait and the general public.[8]

The method has proved useful for the analysis of samples derived from a number of instances of ethyl fenthion poisoning of wildlife. Several ethyl fenthion intoxications have been confirmed involving native granivorous birds in addition to birds of prey. The silica gel clean-up method has been successfully used on a variety of substrates. It eliminated co-extractives and provided adequate sensitivity. However, as most baits contained large amounts of the toxic chemical, the method is generally suitable without the clean-up procedure.



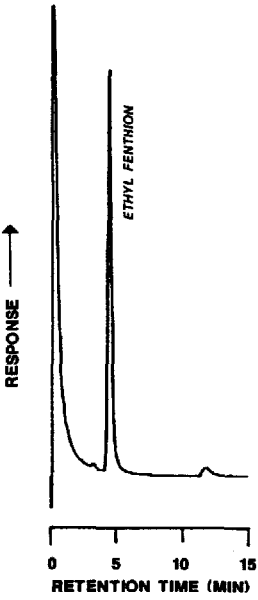


Fig. 1. Chromatogram of 2 μl of 0.5 μg/ml ethyl fenthion references standard (retention time, 4.42 min).

Fig. 2. Chromatogram of a cleaned extract of stomach contents of an ethyl fenthion-intoxicated magpie.

In summary, this method is a useful tool for screening purposes and for quantitation of suspected ethyl fenthion intoxication of wildlife.

TABLE I

ETHYL FENTHION RESIDUES FOUND IN TISSUES OF BIRDS OF PREY

Species	*Tissue*	*Ethyl fenthion content (mg/kg)*
Magpie	Liver	<0.02
(*Gymonorhina tibicen*)	Kidney	0.08
	Stomach contents	199
Magpie	Liver	0.03
(*Gymonorhina tibicen*)	Kidney	0.11
	Stomach contents	99
Currawong	Liver	<0.02
(*Strepera melanoptera*)	Kidney	0.12
	Stomach	<0.02
Currawong	Liver	<0.02
(*Strepera melanoptera*)	Kidney	0.06
	Stomach	0.04

REFERENCES

1 C. R. Worthing (Editor), *The Pesticide Manual — A World Compendium*, British Crop Protection Council, London, 7th ed., 1983, p. 269.
2 G. J. Shanahan and P. B. Hughes, *Vet. Rec.*, 102 (1978) 582.
3 J. H. Viljoen, *Onderstepoort J. Vet. Res.*, 45 (1978) 155.
4 Technical Information Service, *Lu-jet (Bayer S. 1751)*, H. H. York & Co., Sidney, 1962, p. 2.
5 H. Ackermann, *J. Chromatogr.*, 36 (1968) 309.
6 H. Ackermann, *J. Chromatogr.*, 44 (1969) 414.
7 K. D. Rainsford, *Pestic. Biochem. Physiol.*, 8 (1978) 302.
8 W. Bourne and S. Bourne, *Birds*, 15 (1974) 31.