Gas-Liquid Chromatographic Determination of Ethion, Ethion Monooxon, and Ethion Dioxon in Tissues of Turkeys and Cattle

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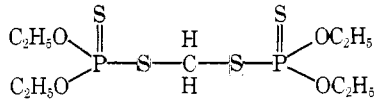
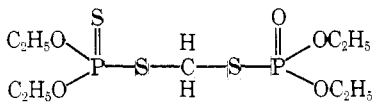
A gas chromatograph equipped with a flame photometric detector provided a highly sensitive method of determining residues of ethion (*S,S'*-methylene *O,O,O',O'*-tetraethyl phosphorodithioate), ethion monooxon (*O,O*-diethyl *S*-(mercaptomethyl) phosphorothioate *S*-ester with *O,O*-diethyl phosphorodithioate), and ethion dioxon (*S,S'*-methylene *O,O,O',O'*-tetraethyl phosphorothioate) in avian and animal tissues. With extraction and cleanup, 0.002 ppm of ethion, 0.002 ppm of ethion monooxon, and 0.005 ppm of ethion dioxon could be detected. Recoveries in tissues of turkeys and cattle were 79–107% for ethion and ethion monooxon and 76–98% for ethion dioxon.

Ethion (*S,S'*-methylene *O,O,O',O'*-tetraethyl phosphorodithioate), an insecticide and acaricide used extensively on crops and citrus, was tested by Graham and Drummond (1964) for control of female ticks (by preventing reproduction) of *Boophilus annulatus* (*Say*) and *B. microplus* (Canestrini) on cattle. More recently Price and Kunz (1970) and Kunz *et al.* (1971) demonstrated ethion to be effective for control of *Neoschongastia americana* (Hirst), the turkey chigger.

Graham and Orwall (1963), Archer *et al.* (1963), Forman and Gilbert (1961), and Gunther *et al.* (1962) described methods of detecting ethion. However, a gas chromatographic method was needed for determining the residues of ethion and its two most likely metabolites, ethion monooxon (*O,O*-diethyl *S*-(mercaptomethyl) phosphorothioate *S*-ester with *O,O*-diethyl phosphorodithioate) and ethion dioxon (*S,S'*-methylene *O,O,O',O'*-tetraethyl phosphorothioate) in tissues of turkeys and cattle. The present paper describes a method of the required sensitivity that was developed to determine all three compounds from the same sample of tissue from turkeys or cattle. This method makes use of a gas chromatograph equipped with a Melpar flame photometric detector operating in the phosphorus mode. The structural formulas for ethion (I), ethion monooxon (II), and ethion dioxon (III) are as shown.



I



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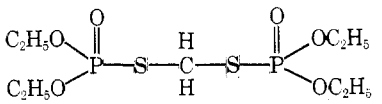

III

EXPERIMENTAL SECTION

Reagents and Equipment. All solvents were redistilled in glass. The silicic acid was Mallinckrodt's 100-mesh powder, analytical reagent grade. It was heated 3 hr at 200° and cooled, and 9% water was added and allowed to equilibrate. The glass wool was dichloromethane–hexane extracted and dried. The chromatographic columns were Kontes technical glassware drawing No. 11416-B with 24/40 joints. The gas chromatograph was a Micro-Tek Model 160, or equivalent, equipped with a Melpar flame photometric detector operating in the phosphorus mode.

Gas Chromatography. A borosilicate glass column 4 mm i.d. × 1.22 m filled with 5% DC-200 coated Gas-Chrom Q (80–100 mesh) was used. A column filled with 10% OV-17 coated Gas-Chrom Q was tried, but it did not give the separation (recorder pen returning to the base line) between peaks as desired. Carrier gas was prepurified nitrogen adjusted to a flow rate of 260 ml/min (exhaust). The column was heated isothermally to 205°, the injector to 245°, and the detector heating block to 165°. Hydrogen and oxygen flowing to the detector were adjusted to 200 and 40 ml/min, respectively. At these conditions, the retention times for ethion, ethion monooxon, and ethion dioxon were about 2.72, 2.16, and 1.68 min, respectively.

A series of standard solutions for ethion and for ethion monooxon in hexane ranging from 0.05 to 0.25 μg/ml and for ethion dioxon ranging from 0.1 to 0.5 μg/ml was prepared. Ten microliters of each concentration was injected into the gas chromatograph, and a standard curve was prepared by plotting peak heights against nanograms of the insecticides. These curves were used to estimate the residues in test samples. A standard solution of about the same concentration as the test sample was then injected to determine the true value more accurately. Peak heights were proportional to the amounts of solute if they were injected in the same volume of solvent. Five-tenths nanogram of ethion in 10 μl of hexane gave a response of about 10% full-scale deflection (FSD), the same amount of ethion monooxon gave a response of about 6%, and 1 ng of ethion dioxon in 10 μl of hexane gave a response of about 5% FSD. At the described conditions for the gas chromatograph, ethion, ethion monooxon, and ethion dioxon would not completely separate, but complete separation was not necessary since they were completely separated on the cleanup column.

Extraction of Fat. A 20-g sample was blended in a Waring Blendor with 50–60 g of anhydrous sodium sulfate and 150 ml of 5% acetone in hexane. (The acetone was used to obtain a quantitative extraction of ethion dioxon.) The mixture was transferred to a 600-ml beaker and stirred with 2 g of Celite. The mass was heated on a hot plate or steam bath to near boiling, transferred onto a folded filter paper, and filtered into a 500-ml erlenmeyer flask. The blender, beaker, and filter were washed with another 150 ml of hot acetone–hexane. The solvent was concentrated to 75 ml by distillation through a Snyder column and cooled, and the extract was transferred to a

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Table I. Recovery of Ethion (0.025 ppm), Ethion Monooxon (0.025 ppm), and Ethion Dioxon (0.05 ppm) from Turkey and Cattle Tissues[a,b]

Tissue	500 ng of ethion		500 ng of ethion monooxon		1000 ng of ethion dioxon	
	Found	% recovery	Found	% recovery	Found	% recovery
			Turkey			
Fat	465	93	410	82	840	84
Skin	475	95	435	87	860	86
Muscle	465	93	440	88	760	76
Liver	465	93	480	96	760	76[b]
Kidney	470	94	470	94	860	86[b]
Gizzard	500	100	535	107	930	93
			Cattle			
Fat	500	100	505	101	860	86
Muscle	495	99	450	90	990	99
Liver	490	98	395	79	630	63[b]
Kidney	450	90	400	80	840	84[b]
Heart	430	86	465	93	980	98

[a] Control values were <0.002, <0.002, and <0.005 ppm, respectively, for ethion, ethion monooxon, and ethion dioxon. [b] Percentage recovery shown is for immediate extraction.

500-ml separatory funnel by using 75 ml of hexane to make the transfer. One hundred milliliters of hexane was added to a second separatory funnel, and the extract was partitioned four times with 50-ml portions of acetonitrile. Each portion of acetonitrile was drained into the second separatory funnel and shaken with the hexane. The acetonitrile extracts were combined in a 300-ml erlenmeyer flask and concentrated to a volume of 5–10 ml by distillation through a Snyder column. The last traces of acetonitrile were removed by the addition and evaporation of three 25-ml portions of hexane; the last 2–3 ml of solvent was removed by use of an aspirator or jet of dry, filtered air at room temperature. The residue was dissolved in 5 ml of a mixture of dichloromethane–hexane (3:7), stoppered, and held for the chromatographic cleanup column. (If it is desired to base the analysis on extracted fat, transfer the hexane solutions remaining after the acetonitrile extraction into a tared flask, evaporate the solvent, weigh the fatty residue, and record it as the weight of sample.)

Extraction of Skin, Muscle, Liver, Kidney, Heart, and Gizzard. A 20-g sample of a tissue was blended with 125 ml of acetone. The mixture was transferred to a 600-ml beaker, stirred with 2 g of Celite, and filtered into a 500-ml erlenmeyer flask. The blender, beaker, and filter were washed with another 125 ml of acetone. The filter paper and mass were returned to the blender, and the extraction and filtration (into a different flask) steps were repeated with hexane. A Snyder column was attached to the acetone flask, and the extract was concentrated by distillation to about 50 ml and cooled to room temperature. The Snyder column was removed, and the corresponding hexane extract was transferred to the acetone flask and again concentrated to about 50 ml or until the solvent vapors reached 63–64° (this temperature indicates removal of acetone). The extract was transferred to a 500-ml separatory funnel with 150 ml of hexane. One hundred milliliters of hexane was added to a second separatory funnel, and if any water was present in the first funnel, it was drained through the second funnel and then discarded. The extract was partitioned with acetonitrile, and the acetonitrile was removed as described for fat.

Cleanup of Extracts. For the cleanup of ethion, ethion monooxon, and ethion dioxon, a chromatographic column was prepared by adding, in order, a plug of glass wool, 1.5 cm of anhydrous sodium sulfate, 8 g of silicic acid, 1.5 cm of sodium sulfate, and a plug of glass wool. The silicic acid was packed by attaching the column to an aspirator and tapping it gently on a table top until no more settling occurred. The column was prewashed with 50 ml of the dichloromethane–hexane (3:7) solvent. Three 10-ml portions of the solvent were used to transfer the sample extract to the column, and the column was then washed with 40 ml more solvent. The receiver was changed, and the ethion was eluted with 140 ml more of the same solvent. The receiver was changed, the column was washed with 75 ml of dichloromethane, and the solvent discarded. The receiver was changed again, and the ethion monooxon was eluted with 115 ml of acetone–dichloromethane (1:99). The receiver was changed again, and the ethion dioxon was eluted with 140 ml of acetone–dichloromethane (1:9). (Note: Each lot of silicic acid must be calibrated to determine the correct volume of eluting solvents for the different compounds.) Snyder columns were attached to the receivers, and the solvent was condensed by distillation to 5–10 ml and cooled; then the contents were quantitatively transferred to glass-stoppered 50-ml flasks with hexane. The solvents were condensed to about 5 ml and evaporated just to dryness with a jet of dry, filtered air at room temperature. The residues were dissolved in 2

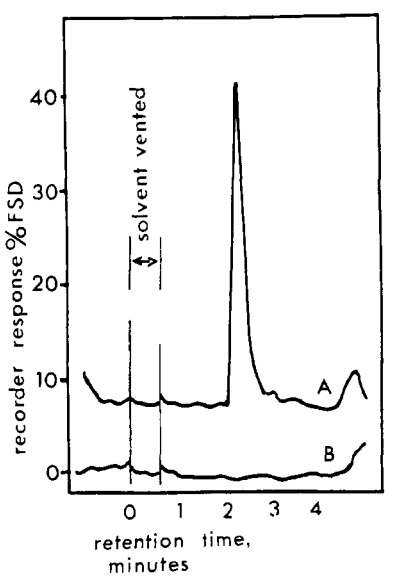

Figure 1. Chromatograms of extracts from turkey fat: (A) control sample containing 2.5 ng of ethion; (B) untreated sample.

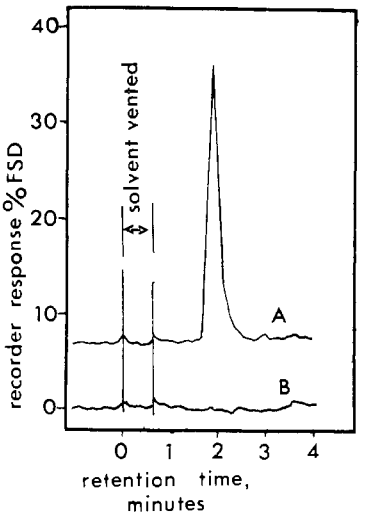

Figure 2. Chromatograms of extracts from turkey fat: (A) control sample containing 2.5 ng of ethion monooxon; (B) untreated sample.

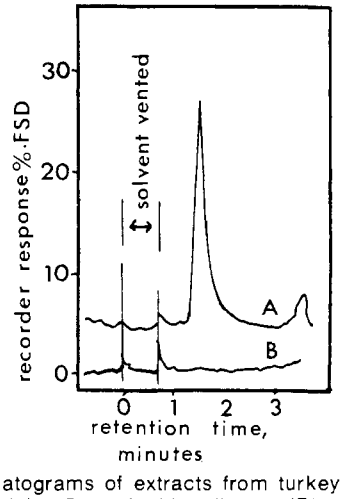

Figure 3. Chromatograms of extracts from turkey fat: (A) control sample containing 5 ng of ethion dioxon; (B) untreated sample.

ml of hexane and stoppered tightly. The residues of ethion, ethion monooxon, and ethion dioxon were determined by injecting a 10-μl aliquot of each into the gas chromatograph and comparing peak heights with those of an injection of a standard solution of about the same concentration.

RESULTS AND DISCUSSION

Recovery Experiments. The efficiency of the overall procedure was tested by adding known amounts of ethion, ethion monooxon, and ethion dioxon to control samples of the various tissues before blending. The recoveries of ethion, ethion monooxon, and ethion dioxon from fortified control turkey and cattle tissues are shown in Table I. The recoveries of ethion dioxon from liver and kidney tissues of turkeys and cattle were 63–84% when extraction was immediate and 0–10% when ethion dioxon was allowed to remain 30 min in contact with the tissue before extraction. This loss is apparently a result of an enzymatic activity or some combining effect. A similar difficulty was encountered with ethion monooxon, but to a lesser extent: recoveries were 40–55% when ethion monooxon was permitted to remain 30 min in contact with the tissue before blending. The same thing did not occur with the other tissues. Therefore, it seems likely that no ethion dioxon *per se* would be present in these tissues of treated turkeys or cattle, and probably no ethion monooxon either. Figures 1–3 are chromatograms showing recoveries of ethion, ethion monooxon, and ethion dioxon from control fat.

Sensitivity. With the input attenuator at 10^3, the output attenuator at 16, and the bucking range at 10^{-8}, 0.2 ng of ethion in 10 μl of hexane gave a response of 4–5% FSD; also, 0.2 ng of ethion monooxon in 10 μl of hexane gave a response of 2–3% FSD; and 0.5 ng of ethion dioxon in 10 μl of hexane gave a response of 2–3% FSD. The control samples showed no peaks at the retention time for ethion, ethion monooxon, or ethion dioxon; however, some kidney samples have a small peak on the trailing edge of the ethion dioxon peak. This peak does not interfere with quantitation since quantitation is based on peak height. (A 4–6 min wait between injections was necessary because two peaks of variable response eluted at this point.) At the conditions described, 0.2 ng of ethion, 0.2 ng of ethion monooxon, and 0.5 ng of ethion dioxon were readily detected, and 0.002, 0.002, and 0.005 ppm, respectively, could be detected in the body tissues.

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