Gas–Liquid Chromatographic Determination of Ronnel (*O,O*-Dimethyl *O*-2,4,5-Trichlorophenyl Phosphorothioate) and the Oxygen Analog of Ronnel (Dimethyl 2,4,5-Trichlorophenyl Phosphate) in Tissues of Cattle

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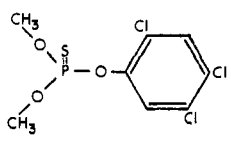
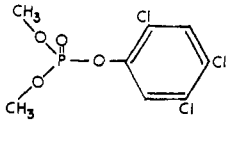
A gas chromatograph equipped with a flame photometric detector provided a highly sensitive method of determining residues of ronnel (*O,O*-dimethyl *O*-2,4,5-trichlorophenyl phosphorothioate) and the oxygen analog of ronnel (dimethyl 2,4,5-trichlorophenyl phosphate) in animal tissues. With extraction and cleanup, 0.002 ppm of ronnel and 0.005 ppm of the oxygen analog could be detected, and the recoveries were 75–95% for ronnel and 80–100% for the oxygen analog.

Webley (1961), Smith and Thiegs (1962), and Claborn and Ivey (1965, 1964) described methods of detecting ronnel (*O,O*-dimethyl *O*-2,4,5-trichlorophenyl phosphorothioate), an insecticide that is promising for control of external parasites on livestock. However, a method of determining the residues of the oxygen analog is needed for a thiophosphate such as ronnel. Consequently, a method of the required sensitivity was developed for determining both compounds from the same sample which can also be used to analyze for residues in all body tissues. This method makes use of a gas chromatograph equipped with a Melpar flame photometric detector. It will accurately detect residues of ronnel and its oxygen analog (dimethyl 2,4,5-trichlorophenyl phosphate) in the body tissues as low as 0.002 ppm and 0.005 ppm, respectively. The structural formulas for ronnel (I) and its oxygen analog (II) are as follows.



(I)



(II)

MATERIALS AND METHODS

Reagents and Equipment. All solvents redistilled in glass. Silicic acid, Mallinckrodt's 100-mesh powder, analytical reagent grade, heated 16 hr at 225° C, cooled, 20% water added, and allowed to equilibrate. Glass wool, dichloromethane–hexane extracted and dried. Chromatographic column, Kontes technical glassware drawing no. 11416-B with 24/40 joints. Gas chromatograph, Micro-Tek Model 160 equipped with a Melpar flame photometric detector.

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. Carrier gas was prepurified nitrogen adjusted to a flow rate of 75 ml per min (exhaust). The column was heated isothermally at 200° C, the injector at 240° C, and the detector at 170° C. Hydrogen and oxygen flowing to the detector were adjusted to 200 and 30 ml per min, respectively. At these conditions, the retention time for ronnel was about 1.35 min and for its oxygen analog was about 1.3 min. A series of standard solutions of ronnel in hexane ranging with concentrations of ronnel from 0.01 to 0.08 μg per ml and of the oxygen analog ranging from 0.02 to 0.08 μg per ml was prepared. Ten microliters of each concentration were 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. Six-tenths nanogram of ronnel in 10 μl of hexane gave a response of 25–28% Full Scale Deflection and 0.6 ng of the oxygen analog in 10 μl of hexane gave a response of 15–18% FSD.

Procedures for extraction of tissues and partitioning with acetonitrile have been described by Claborn and Ivey (1965).

Cleanup of Extracts. For the cleanup of ronnel and the oxygen analog, a chromatographic column was prepared by adding, in order, a plug of glass wool, 2.5 cm of sodium sulfate, 12 g of silicic acid, 2.5 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 hexane. Three 10-ml portions of hexane were used to transfer the sample extract to the column and the column was then washed with 50 ml more solvent. The receiver was changed and the ronnel was eluted with 260 ml more of hexane at a flow rate of about 200 ml per hr. The receiver was changed again and the column was washed with 35 ml of 3:1 dichloromethane–hexane. The oxygen analog was then eluted with 90 ml of the same solvent into a fresh receiver (NOTE: Each lot of silicic acid must be calibrated to determine correct volume of eluting solvent), a glass bead was added, the solvent was evaporated, and the last 2–3 ml was removed with the aid of an aspirator at room temperature. The residue was dissolved in 5 ml of hexane, stoppered tightly, and held for gas chromatography. The eluate containing the ronnel was condensed to 5–10 ml, quantitatively transferred to a 100-ml Erlenmeyer flask with hexane, and evaporated to dryness as described for the oxygen analog. Then this residue was dissolved in 5 ml of hexane and stoppered tightly. The residues of ronnel and its oxygen analog were determined by injecting a 10-μl aliquot of each into the gas chromatograph and comparing peak heights with standards of about the same concentration.

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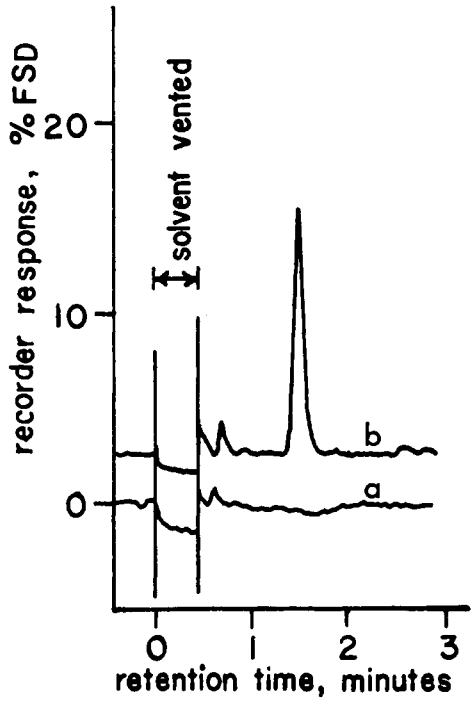

Figure 1. Chromatogram of extracts from omental fat: a, untreated tissue; b, tissue + 0.01 ppm ronnel

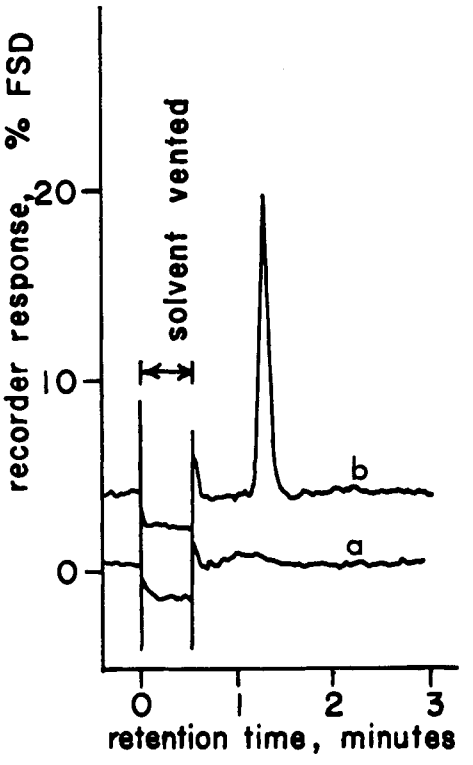

Figure 3. Chromatogram of extracts from muscle: a, untreated tissue; b, tissue + 0.01 ppm ronnel

DISCUSSION

Recovery Experiments. The efficiency of the overall procedure was tested by adding known amounts of ronnel and its oxygen analog to control samples of various tissues before blending. The recovery of ronnel and ronnel oxygen analog from fortified control tissues is shown in Table I.

The recoveries of the oxygen analog from fat were consistently high, ranging from 107–115%, so tests were made to find the explanation. The residue from fat samples proved to increase the sensitivity of the determination. For example, no peaks were visible in the control samples at the retention time of the oxygen analog, but when the residues from control samples were spiked with the oxygen analog, an equivalent

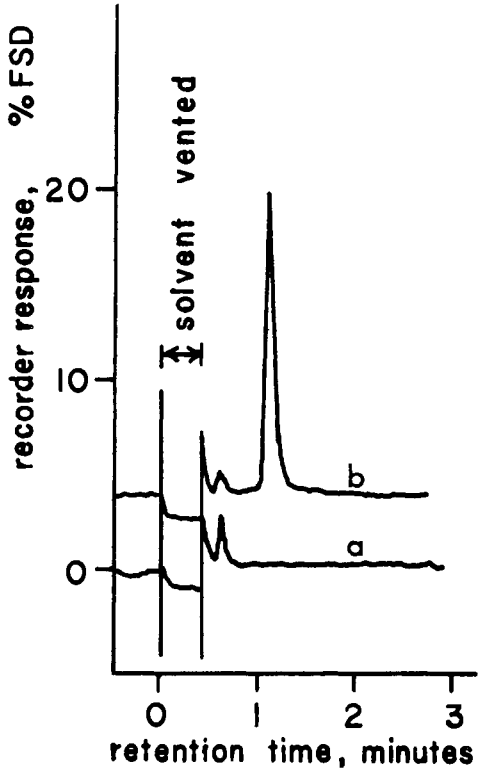

Figure 2. Chromatogram of extracts from omental fat: a, untreated tissue; b, tissue + 0.015 ppm ronnel oxygen analog

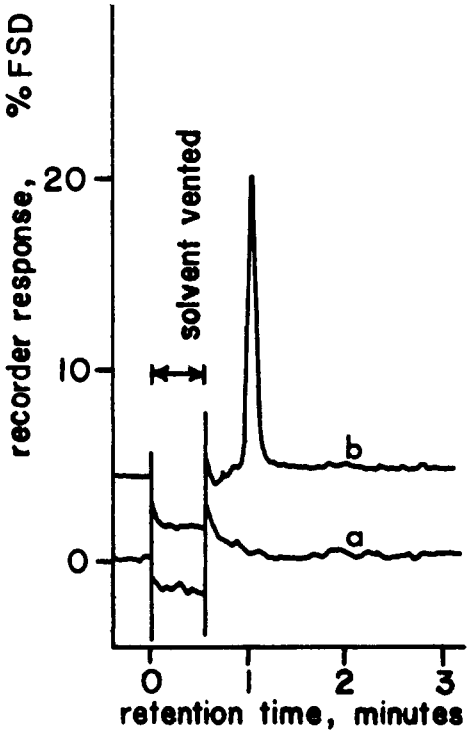

Figure 4. Chromatogram of extracts from muscle: a, untreated tissue; b, tissue + 0.015 ppm ronnel oxygen analog

Table I. Recovery of Ronnel (0.01 ppm) and Ronnel Oxygen Analog (0.015 ppm) from Various Body Tissues[a]

Tissue	Ronnel added (ng)	Ronnel found (ng)	% Recovery	O_2 analog added (ng)	O_2 analog found (ng)	% Recovery
Omental fat	200	154	77	300	285–300	95–100
Muscle	200	162	81	300	288	96
Kidney	200	166	83	300	282	94
Liver	200	190	95	300	282	94
Heart	200	164	82	300	279	93
Spleen	200	162	81	300	288	96
Brain	200	150	75	300	240	80

[a] Control values were < 0.002 ppm and 0.005 ppm, respectively, for ronnel and ronnel oxygen analog.

amount had greater sensitivity than the standard. This error was eliminated by using control samples spiked with the standard solution after processing for the standards. The difficulty did not arise with other tissues. Figures 1–4 are chromatograms showing recoveries of ronnel and its oxygen analog from tissue.

Sensitivity. With the input attenuator at 10^3, the output attenuator at 16, and the bucking range at 10^{-8}, 0.1 ng of ronnel in 10 μl of hexane gave a response of 4–5% FSD, and 0.2 ng of the oxygen analog gave a response of 5–6% FSD. The control samples showed no peaks at the retention time for ronnel or the oxygen analog; however, a 5–6-min wait between injections of the oxygen analog was necessary because two peaks (5–10% FSD) eluted at this point. At the conditions described, 0.1 ng of ronnel and 0.2 ng of the oxygen analog were readily detected and 0.002 ppm of ronnel and 0.005 ppm of the analog could be detected in the body tissues.

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