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STUDIES IN PESTICIDE RESIDUES. 3. RONNEL RESIDUES IN THE PERIRENAL AND OMENTAL FAT OF CATTLE FOLLOWING DERMAL APPLICATION

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SUMMARY

Ronnel residues were determined in the perirenal and omental fat of cattle slaughtered at intervals of 1, 4, 7, 10, 13 or 16 days after one application of 2 gal of 0·1% insecticide. The maximum level (2·63 p.p.m.) was observed in the omental fat at 4 days and the minimum level (0·09 p.p.m.) in the perirenal fat at 16 days after treatment. After deposition was complete, residues were consistently higher in the omental than in the perirenal fat.

Each sample analysed by gas chromatography contained a compound which exhibited a retention time similar to that of a hydrolysis product of ronnel and was probably the known metabolite O-methyl-O-hydrogen-O-(2,4,5-trichlorophenyl) phosphorothioate.

I. INTRODUCTION

Ronnel (O,O-dimethyl-O-(2,4,5-trichlorophenyl) phosphorothioate), also known as Dow Et-57, Trolene, Korlan or Fenchlorphos, is a versatile and effective member of the organophosphorus group of insecticides. It has been used in the control of screwworms (Graham *et al.* 1959), flies (Roberts 1959; Eddy 1961; Johnson and Turner 1964), lice (Hoffman 1961) and mites (Foulk and Matthysse 1963) affecting poultry, and the larval stages of the cattle grub (McGregor and Bushland 1957). Trials by W. J. Roulston (unpublished data 1966) suggested that it might be of value in controlling a resistant strain of the cattle tick (*Boophilus microplus* Can.) recently discovered in the Brisbane Valley area (P. J. O'Sullivan, unpublished data 1966).

Ronnel has been shown to persist for more than 7 days in the subcutaneous fat of animals sprayed or orally dosed with the compound. Plapp and Casida (1958) dosed a cow with 100 mg ronnel per kg body-weight and found levels of ronnel plus metabolites to a total of 44 p.p.m. in subcutaneous fat 7 days after dosing. Millar (1965) found levels of this order in the subcutaneous fat of sheep 7 days after they were orally dosed with 100 mg ronnel per kg. He also found levels of 0·35 p.p.m. in the fat 7 days after sheep had been sprayed with 600 ml of 0·4% ronnel. No analyses were carried out at shorter intervals after treatments and no specific figures were given for the levels of unmetabolized ronnel in the tissues examined. However, he showed that the fat depots were the sites of highest concentration of unmetabolized ronnel and by a chromatographic technique confirmed that only small amounts of the metabolic products appeared in the fat.

In view of the possible use of ronnel on cattle in Queensland and the limited data on residues following its use, the persistence of this insecticide in the perirenal and omental fat of beef cattle was investigated. The results of the investigations are reported in this paper.

II. MATERIALS AND METHODS

Pesticide.—Spray fluid containing 0·1% ronnel was prepared from a 32% w/v emulsifiable concentrate supplied commercially under the trade name "Vallo Saef Jet."

Treatment and sampling.—Six Hereford steers with a weight range 788-924 lb were each treated with a single application of 2 gal of a 0·1% fluid applied as a spray from a low-pressure pumping system. Perirenal and omental fat samples were obtained from animals slaughtered at intervals of 1, 4, 7, 10, 13 or 16 days after treatment.

Sample preparation.—Samples of fatty tissue were comminuted, rendered at 55°C and allowed to filter through a glass-wool plug into a clean dry vessel.

Chemical analysis.—Ronnel was extracted from the tissue-free fat and quantitatively determined using modifications of the method of Claborn and Ivey (1965).

For extraction from fat, the fat (10 g) was dissolved in n-hexane (200 ml) and the ronnel partitioned into acetonitrile (4 x 50 ml). After back-washing with n-hexane (50 ml), the combined acetonitrile extracts were mixed with sodium sulphate solution (2% aqueous, 800 ml) and the mixture extracted with n-hexane (3 x 100 ml). The n-hexane extract was dried over anhydrous sodium sulphate, transferred with washings into a clean dry flask, and concentrated to 5 ml on a steam-bath under a steam of dry air.

For clean-up, a column (12 cm x 1·5 cm diam.) was prepared by pouring a slurry of florisil (15 g, 60–100 mesh deactivated with 9% water) in n-hexane onto a layer (2·5 cm) of anhydrous sodium sulphate, allowing this to settle and applying another layer (2·5 cm) of anhydrous sodium sulphate to the top of the column.

The concentrated n-hexane solution (5 ml) from the fat extraction process was quantitatively transferred to the column and ronnel was eluted with n-hexane at a flow rate of 1 ml/min. After discarding the initial fraction (75 ml) of eluent, the subsequent 200 ml was collected and concentrated to 10 ml on a steam-bath under a stream of air. The remaining solvent was removed with the air stream at room temperature.

Quantitative estimation.—Ronnel was quantitatively determined using a Hy-Fi Gas Chromatograph (Wilkens Instrument and Research Inc.) equipped with an electron capture detector and a pyrex column (10 ft x 1/16 in.) which was packed with 2% QF–1 on Chromosorb W 80–100 mesh (HMDS treated). The carrier gas was oxygen-free nitrogen at a flow rate of 50 ml/min and oven temperature was maintained at 176°C.

Under our conditions the retention time of ronnel, relative to gamma-BHC, was 1·66. Consequently, gamma-BHC was used as an internal standard for quantitative estimations. Standard solutions ranging from 0·2 to 1·0 p.p.m. ronnel were prepared, using as solvent n-hexane containing 0·05 p.p.m. of gamma-BHC, and were used to obtain a standard plot of the ratio (ronnel peak height/gamma-BHC peak height) against concentration of ronnel.

The solid residue from the column eluate was dissolved in a suitable volume (5–30 ml) of n-hexane containing 0·05 p.p.m. gamma-BHC. The volume was chosen to bring the concentration ratio of ronnel to BHC within the range of the standard graph. Aliquots of 2–3 microlitres were injected into the gas chromatograph and the concentration of ronnel in the original fat sample was determined using peak height ratios, the standard graph, and appropriate concentration factors.

Recoveries of added ronnel in the range of 0·1–3·0 p.p.m. were 95 to 80%. No blank reading was detectable.

III. RESULTS

Results of chemical analysis as p.p.m. ronnel in rendered fat, uncorrected for percentage recovery, are reported in Table 1. The figures represent individual animals. In the samples examined, the highest level of insecticide, 2·63 p.p.m., was found in the omental fat 4 days after spraying. After 16 days the level was less than 0·2 p.p.m.

TABLE 1

RONNEL RESIDUES IN PERIRENAL AND OMENTAL FAT FOLLOWING A SINGLE SPRAY TREATMENT OF CATTLE WITH 0·1% RONNEL

Interval between Treatment and Slaughter (days)	Ronnel (p.p.m.)	
	Perirenal Fat	Omental Fat
1	1·28	0·66
4	2·39	2·63
7	1·23	1·91
10	0·17	0·32
13	0·34	0·68
16	0·09	0·18

The gas chromatogram of each sample contained a second peak corresponding to a compound with retention time 2·18 relative to ronnel. The intensity of this peak increased proportionately as the intensity of the ronnel peak decreased. Hydrolysis of ronnel with ethanolic potassium hydroxide produced a mixture of products, one of which corresponded in retention time to the unknown compound.

IV. DISCUSSION

The data presented indicate that residues resulting from a single spray treatment with ronnel persist in the fat of cattle for at least 16 days. The analytical method used was specific for ronnel and the residue levels found do not include any metabolic products of the insecticide. Nevertheless, on gas-liquid chromatography an additional peak with retention time 2·18 relative to ronnel was observed in every sample. The concentration of this compound increased as the ronnel concentration in the fat sample decreased. A compound of identical retention time was prepared by hydrolysis of ronnel with ethanolic potassium hydroxide. It is considered that this compound could be O-methyl-O-hydrogen-O-(2,4,5-trichlorophenyl) phosphorothioate, which is known to be a product of the metabolism of ronnel (Plapp and Casida 1958; Millar 1965).

The fact that samples containing the highest residue levels were obtained on the fourth rather than on the first day after spraying indicates that percutaneous absorption may be delayed or that oral ingestion and storage of the pesticide resulted from licking during this period. In later samples, levels of ronnel were higher in the omental than in the perirenal fat.

The persistence of ronnel residues in bovine fat for at least 16 days after a single spray application indicates that a period between treatment and slaughter of more than 16 days is necessary before meat from treated cattle would be acceptable where a "zero tolerance" residue status is enforced.

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