H. E. Smalley,* H. R. Crookshank,¹ and R. D. Radeleff

Activated charcoal was evaluated as an agent for preventing residues of the organophosphorus insecticide, ronnel, in sheep. Ronnel was mixed into the feed at a level of approximately 1000 ppm and fed for 84 days. One group received only this ration; for a second group, 5% of activated charcoal was mixed into the ronnel-contaminated feed; the third group was given oral drenches of a charcoal slurry twice weekly. Three control groups were under the same regimen as above, except for deletion of

R onnel [0,0-dimethyl-0-(2,4,5-trichlorophenyl)phosphorothioate] is one of the organophosphorus pesticides and is used for the systemic control of the larvae of the cattle grubs, *Hypoderma bovis* and *H. lineatum*. Its uses are not restricted to systemic application; it is effective as a contact insecticide as well. Ronnel is unusual in that it is one of the few organophosphorus insecticides which leave appreciable residues in meat and milk of treated animals, precluding its use in lactating animals and requiring a delay of 63 days for beef cattle from time of treatment to slaughter. Crookshank and Smalley (1970) showed that a single oral dose (100 mg/kg) of ronnel in sheep excreted completely only after about 21 days, as determined by omental fat residue analyses.

Activated charcoal has been used in medicine for a number of years, primarily as an antidote in acute poisonings. Decker *et al.* (1968) studied the relative adsorbency by activated charcoal of a wide variety of drugs and other chemicals often found in the home. Wilson *et al.* (1968) reported that activated plant charcoal could increase the elimination of residues of the chlorinated hydrocarbon pesticide, dieldrin, to more than twice the natural rate in the dairy cow.

The revival of interest in therapeutic usage of activated charcoal and its use to reduce residues of dieldrin in food animals prompted us to explore its efficacy in reducing the residues of other pesticides.

This study reports the effect of orally-administered, activated charcoal of plant origin on the residue levels of ronnel in the omental fat of sheep.

MATERIALS AND METHODS

Thirty-six commercial medium wool lambs were used. The sheep were examined, identified, and treated for internal parasites. Three ewes and three wethers were randomly allotted by weight to each of six treatment groups.

The protocol called for continuously feeding ronnel to sheep, treating some with activated charcoal, and measuring fat residue levels during and after the administration until the excretion of ronnel was completed. ronnel. Omentectomies were performed at intervals and fat samples were analyzed by gas-liquid chromatography. Activated charcoal with the ronnel-contaminated feed reduced residue levels to 10% of those in sheep not receiving charcoal. Oral drenches of activated charcoal did not significantly reduce the residue levels, but did retard elimination slightly. The rate of excretion of residues at the termination of the test was not affected by continued administration of charcoal.

The six groups were established as follows: Group I— Control—Normal diet. Group II—5% charcoal in the diet. Group III—Normal diet and drenched twice weekly with an equivalent amount of charcoal in 1 l. water slurry. Group IV—Ronnel mixed in the diet. Group V—Ronnel mixed in the diet with the addition of 5% activated charcoal. Group VI—Ronnel mixed in the diet and drenched twice weekly with charcoal slurry.

The period of continuous administration of ronnel (12 weeks) was chosen to approximate the average length of time lambs would remain in the feedlot, plus a 3-week period expected for excretion of residues.

The basal diet for the first 9 weeks consisted of 30% ground sorghum grain, 55% cottonseed hulls, 10% cottonseed meal, and 5% molasses. The sheep were slowly brought up to full ration well before the start of the experiment. During the last 22 days of the experiment the grain content was increased to 37.5% and the cottonseed meal to 12.5%, with a compensating decrease in cottonseed hulls. Salt and water were available at all times.

Crystalline ronnel of 92.5% purity (maximum purity available from the manufacturer, Dow Chemical Company, Midland, Mich.) was used. It was added to the total diet at a level of approximately 1000 ppm by dissolving 50 g of ronnel in 200 ml of chromatographic grade acetone and spraying the solution over 45.4 kg of feed spread on a plastic sheet. The acetone was allowed to evaporate at room temperature, then the feed was thoroughly mixed. Activated charcoal (Darco S-51, Atlas Chemical Industries, Inc., Wilmington, Del.) was added at the level of 5% of the total diet by mechanical mixing.

The level of ronnel (1000 ppm) was greatly in excess of that which might ever be encountered in feed. The high level was selected to adequately challenge the efficacy of the charcoal as an adsorbent of ronnel. The 5% level for charcoal was selected to provide a maximum exposure in order to gain information on the effects of prolonged feeding on the biochemistry of the body. The latter observations will be reported separately.

Omental fat samples were taken from two animals in each of the six groups using the technique of Radeleff (1950) in accordance with the schedule in Table I. The ronnel levels in the omental fat were determined by gas-liquid chromatography essentially according to the procedure of Samuel (1966).

The use of "zero" or "0" in the text and in Table I represents levels below the sensitivity of our analytical procedure which was 0.05 ppm ronnel in the standard 2 g fat sample.

Veterinary Toxicology and Entomology Research Laboratory, U.S. Department of Agriculture, Agricultural Research Service, Veterinary Sciences Research Division, Post Office Drawer GE, College Station, Texas 77840

¹Animal Science Research Division, U.S. Dept. of Agriculture, and the Dept. of Animal Science and Biochemistry and Biophysics, Texas A&M University, College Station, Texas 77843

Table I.	Average Residue Levels (ppm) of Ronnel in Omental Fat of Sheep During 12 Weeks of Ronnel
	and/or Activated Charcoal Administration

Group	Ronnel in total diet, ppm	Charcoal in total diet	Pretreat- ment 2	weeks										
				Treatment						Posttreatment				
				3	4	5	8	9	10	3	4	5	6	7
Ι	0	0	0^a	0	0	0	0	1.4^{b}	0	^c	0			
II	0	5%	0	0	0	0	0	0	0		0			
III	0	Biweekly drench	0	0	0	0	0	0.2	0	0	•••	•••	• • •	
IV	1000	0	0	86	34	85	129	80	9 1		0.7	0.2	0	
v	1000	5%	0	3.5	5.0	6.0	9.5	10	14	1.3	0.7	0.3	0	
VI	1000	Biweekly drench	0	37	26	83	138	74	70	•••	2.1	0.7	0.4	0

^a The use of zero or 0 in the text and in Table I represents levels below the sensitivity of our analytical procedure which was 0.05 ppm ronnel in the andard 2 g fat sample. ^b Apparent contamination of sample. ^c ... indicates no samples taken. standard 2 g fat sample.

RESULTS AND DISCUSSION

The ronnel levels in the omental fat samples according to treatment groups are shown in Table I. While there were variations both between and among animals, it was obvious that the continuous feeding of the charcoal in the diet significantly reduced the level of ronnel in the omental fat, but that drenching twice weekly did not.

Crookshank and Smalley (1970) reported residues of 43.2 ppm 4 days after the oral administration of a single dose of 100 mg per kg of ronnel. These residues disappeared from the omental fat 3 weeks after administration. In the present experiment, the maximum level observed was 176 ppm after 8 weeks of feeding. The tissue levels of the ronnel-treated animals were relatively low 3 weeks after the addition of ronnel was stopped; however, one animal did not show a 0 level until after 6 weeks following removal from ronnel diet. Also, as noted in Table I, the lambs receiving the charcoal drench appeared to eliminate ronnel more slowly than the other treatment groups. No explanation is readily available for this finding.

As shown in Table I, two samples from control animals showed very minute quantities of ronnel in the omental fat. This residue was due to contamination of the samples during the surgical procedure.

LITERATURE CITED

Crookshank, H. R., Smalley, H. E., J. AGR. FOOD CHEM. 18(2), 326 (1970).

Decker, W. J., Combs, H. F., Corby, D. G., Toxicol. Appl. Pharma*col.* **13**, 454–460 (1968). Radeleff, R. D., *Veterin. Med.* **45**(3), 125 (1950). Samuel, B. L., *J.A.O.A.C.* **49**(2), 346–353 (1966).

Wilson, K. A., Cook, R. M., Emery, R. S., Fed. Proc. 27, 558 (1968).

Received for review August 7, 1970. Accepted November 17, 1970.