Toxicological Studies of *O,O*-Dimethyl-*O*-(2,4,5-trichlorophenyl) Phosphorothioate (Ronnel) in Laboratory Animals

DONALD D. McCOLLISTER, FRITZ OYEN, and VERALD K. ROWE

Biochemical Research Laboratory, The Dow Chemical Co., Midland, Mich.

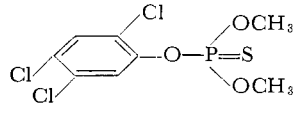
Ronnel is an organic phosphorus compound offered as a systemic for control of the cattle grub. It is also available as a spray for use against flies, roaches, screw worms, and other pests. Studies with laboratory animals were undertaken to ascertain handling precautions and for the evaluation of safety for proposed uses. Ronnel is low in acute toxicity by oral administration or skin absorption, it exerts but slight irritating effects on the skin or eyes, and is not a skin sensitizer. No morphological changes resulted from long term feeding studies on rats at 15 mg. per kg. per day, nor in dogs at 10 or 25 mg. per kg. per day. Plasma cholinesterase levels were depressed to a greater extent than those of RBC or brain, although substantial amounts of enzyme remained even after the feeding of large dosages of ronnel. No unusual handling precautions appear necessary. Potentiation is not of significance. Ronnel is shown to be safe for use as recommended.

RONNEL IS THE COMMON name which has been assigned to *O,O*-dimethyl-*O*-(2,4,5-trichlorophenyl) phosphorothioate by the American Standards Association. Former code names were Dow ET-57 and ET-14. This substance is highly effective as a systemic insecticide for cattle grub control (*3,16, 22*) and is available commercially for this use under the name of Trolene. As Korlan, ronnel is available as a residual spray for the control of flies in barns and in other premises (*3, 12, 13*). Korlan formulations have shown promise, when applied to livestock for ectoparasite control (*12, 13*). A number of veterinarians have found these preparations to be effective against Demodectic mange in dogs (*3*).

The toxicological information already reported included observations on cattle during extensive evaluation for grub control (*3, 21*). The metabolism of the compound has been studied by Plapp and Casida (*20*). The following studies were undertaken to obtain additional toxicological data, to recommend safe handling practices in manufacturing, formulating, and field use of the material, and to assist in the evaluation of safety for proposed uses from the standpoint of public health.

Material Description

O,O-Dimethyl-*O*-(2,4,5-trichlorophenyl) phosphorothioate has the following structural formula:



The synthesis of ronnel has been patented (*19*) and the description of a manufacturing process has been given by Martin (*17*).

Ronnel of 99.4% purity has been prepared. This material is a white powder with a melting point of 40.97° C. and a vapor pressure of 0.0008 mm. of mercury at 25° C. It is quite soluble in most good organic solvents, such as acetone, carbon tetrachloride, diethyl ether, methylene chloride, and toluene. However, it is practically insoluble in water (0.004 gram per 100 grams at 25° C.).

The samples of ronnel used in the first toxicological studies on laboratory animals were dependent on the purity of the 2,4,5-trichlorophenol used in the process. The purity ranged from 89 to 95% for this raw material. Later, the final products were submitted for analyses by infrared techniques. The test material used in some of the early acute toxicity experiments was 94 ± 3% pure. However, all other studies, including the dietary feeding and potentiation, employed a white powder material with a melting point of 40.41° C. and designated purity of 98 ± 2%.

In vitro, ronnel has little, if any, inherent effect on cholinesterase activity, whereas the oxygen analog is a potent cholinesterase inhibitor having about 1000 times the activity of the sulfur compound. The oxygen analog has been used as the basis for an assay method for residues of ronnel employing bromine oxidation followed by a manometric procedure with flyhead cholinesterase (*14*).

Acute Oral Toxicity

Small Animals. The test substance was given by intubation in single oral doses as a solution or suspension in corn oil to eight animal species. All of the animals that survived were observed until it was certain they had fully recovered from any toxic effects (usually about 2 weeks). Data for these acute oral tests are outlined in Table I along with the LD_{50} values calculated by the Weil (*23*) modification of the method of Thompson. Excessive dosages of ronnel result in symptoms characterized by various degrees of salivation, tremors, diarrhea, pinpoint pupils, and respiratory distress. These symptoms are those of a cholinergic nature commonly associated with organic phosphate compounds.

Large Animals. No LD_{50} has been determined for cattle or other large domestic animals. Radeleff and Woodward (*21*) found that dosages of 400 mg. per kg. of body weight, in cattle, produced adverse reactions, but were not fatal. Ronnel has been administered to thousands of cattle in doses of 110 to 150 mg. per kg. of body weight without producing harmful effects, except in some isolated cases where other factors have been involved. Calves and pregnant cows tolerated ronnel as well as the other cattle. Oral doses of 400 mg. per kg. have not caused adverse effects in sheep and goats under the conditions observed to date. Horses tolerated 110 mg. of ronnel per kg. of body weight.

Cholinesterase Activity in Animals Following Single Oral Doses

Rats and Dogs. Predosing cholinesterase values were obtained on samples of blood obtained from the tail tip of rats and an ear vein of dogs. Then, the animals were given single oral doses of ronnel by intubation. The dosages employed, number of animals per dose, and cholinesterase activities measured in

Table I. Acute Oral Toxicity of Ronnel

Species	Sex	No. of Animals	No. of Dosage Levels	LD_{50} (G./Kg.) 19/20 Confidence Limits
Rats	M[a]	80	7	1.74 (1.40 to 2.17)
Guinea pigs	M	24	6	3.14 (2.41 to 4.18)
Rabbits	Both	32	8	0.64 (0.40 to 0.99)
Mouse	F	15	3	2.14 (1.54 to 2.99)
Dog[b]	Both	4	1	>0.5
Duck	Both	32	8	>4.0
Chickens	M	21	5	>5.0
Turkeys	Both	28	4	0.50 (0.34 to 0.75)

[a] Range-finding LD_{50} for female rats >2000 mg./kg.
[b] Higher doses invariably cause emesis in the dog.

Table II. Cholinesterase Activity Following Single Doses of Ronnel

Species and Dose, G./Kg.	Animals, No.	Time, Days	Cholihesterase Activity, % "Control" Plasma	Red Blood Cells
Dogs, 0.5	2	0	100	100
		1	47	110
		7	68	110
		14	80	102
Rats, 1.0	10	0	100	100
		1	23	74
		7	68	93
Rats, 0.5	2	0	100	100
		1	46	110
Rats, 0.25	2	0	100	100
		1	57	99

plasma and in red blood cells following the administration of ronnel are given in Table II. At no time during the 2-week observation period did either the rats or the dogs appear to have been affected adversely as judged by gross appearance and behavior. However, both dogs were noted to have passed soft stools about 3 to 4 hours after being fed. The plasma cholinesterase values were decreased, but the red blood cell cholinesterase, on the other hand, was not affected significantly except in rats at the high dosage level.

Cholinesterase Method

The method employed in all the cholinesterase measurements, reported in this paper, for plasma, red blood cell, and brain tissues was the American Cyanamid Co. modification (*10*) of the electrometric procedure of Michel (*18*). An incubation temperature of 30° C. was used. The substrate buffers for rat and dog red cells were those described by Frawley *et al.* (*9*). Using this prodedure, normal values for male rat plasma, red blood cell, and brain cholinesterase activity averaged about 0.19, 0.49, and 0.79 Δ pH unit per hour, respectively. Corresponding values for the female rats were 0.54, 0.44, and 0.81 Δ pH unit per hour.

Skin and Eye Contact

Rabbits. When a small amount of the powdered material was placed directly on the eyeball of a rabbit, there was evidence of slight pain and transient irritation of the conjunctival membranes. There was no corneal injury and the rabbit eye appeared entirely normal within 48 hours.

Ronnel, in the dry powdered form, was applied repeatedly—10 continuous applications in 14 days—under a gauze bandage to the intact skin of the rabbit. Upon 24-hour contact, only a very slight hyperemia of the skin was produced. Repeated, prolonged contact caused a moderate degree of reddening and scaliness, but no edema.

Skin absorption studies were conducted using a modified Draize technique (*4*) in which ronnel as a solution in corn oil was applied under an impervious cuff for a period of 24 hours to the shaven trunks of rabbits. One of 12 rabbits died at a dosage of 1.0 gram per kg., six of 12 died at 2.0 grams per kg., and eight of eight rabbits succumbed at a dosage of 4.0 grams per kg. of the test material.

Skin Irritation and Sensitization

Human Subjects. A total of 50 human subjects—30 men and 20 women—were employed to evaluate the primary skin-irritating and skin sensitizing properties of ronnel. Each subject received three patch applications per week, with at least 24 hours intervening, for a total of nine applications in a 3-week period. Two weeks after the ninth application, a single challenge application was made.

The gauze of each patch was saturated with ronnel in the form of a 10% suspension in sesame oil applied to the flexor surface of the upper arm of the subject. Between applications, the site was examined and scored for the degree of reaction present according to a severity system of 0, 1, 2, 3, 4. Of a total of 450 scorings, there were 444 zeros, 4 fours, and 2 threes.

Of the grade 3 or 4 reactions observed, one occurred after seven applications, three after eight applications, and two after the ninth application. This may have occurred as the result of a fatiguing action. From the challenge patch readings, there was no evidence that the test material possessed skin sensitizing properties.

Table III. Acute Oral Toxicity of Ronnel to Male Rats in Paired Combination with Other Organic Phosphate Compounds

Mixture of Ronnel with:	LD_{50} G./Kg. Found	LD_{50} G./Kg. Expected	Ratio Expected: Found
Malathion	0.89	1.90	2.1
Diazinon	1.83	1.07	0.6
Systox	0.020	0.026	1.3
Methyl parathion	0.071	0.067	0.9
EPN	0.28	0.90	3.2
Parathion	0.054	0.099	1.8
Phosdrin	0.010	0.014	1.4
Guthion	0.145	0.250	1.7
Trithion	0.165	0.129	0.8
Shradan	0.012	0.012	1.0

Joint Toxicity Studies by Acute Oral Feeding

In 1956, the U. S. Food and Drug Administration announced that it would require additional scientific testing to obtain official tolerances for sprays and dusts containing organic phosphate insecticide used on food crops (*6*). This was deemed necessary for the purpose of determining potentiation which was defined as an increase in toxicity which occurs when some of the compounds are used together. Frawley *et al.* (*8*) report a tenfold increase above the predicted toxicity for EPN (*O*-ethyl-*O*-*p*-nitrophenyl phenylphosphonothioate) - malathion mixtures given in single oral doses to rats. Williams *et al.* (*24*) report further work by the Division of Pharmacology, Food and Drug Administration in which paired combinations of five organic phosphate insecticides were fed to dogs for six weeks. As judged by plasma and red cell cholinesterase levels, potentiation was not observed for any of these pairs at permitted residue tolerance levels in the diet. Thus, it was concluded that no public health problem would be presented by this phenomenon, even with EPN-malathion mixtures, at such levels in the human diet.

In the present study, the LD_{50} values for male rats were determined for each organic phosphate material individually using a minimum of five dosage levels and five rats per level. Additional animals were given single doses by intubation of mixtures containing 50% by weight of

ronnel and 50% of each of the other phosphate materials as a solution or suspension in corn oil.

The LD_{50} values for each mixture were compared with the expected LD_{50} values, calculated from the LD_{50} obtained on the individual components and the proportion of each in the mixture. These calculations have been described by McCollister (*15*) and Dubois (*5*), and are based upon suggestions by Finney (*7*) for materials having similar joint toxic action. These data and the ratios of expected to found LD_{50} are presented in Table III.

Dietary Feeding Studies

Large Animals. Feeding studies on cattle (*3*) indicate that ronnel may be fed continuously, at 5 mg. per kg. per day, for at least 240 days with no evidence of ill effect. However, higher levels fed continuously to calves may produce mild, transitory respiratory symptoms. In older animals, a daily dose of 20 mg. per kg. was well tolerated for eight months and produced only a slight depression in weight gain. Levels of 0.05% (approximately 28.8 mg. per kg. daily) in the diet of swine retarded growth slightly over a 16-day period. A level of 0.025% (approximately 14.4 mg. per kg. daily) was well tolerated by the swine when fed for 18 days.

Dogs. A female mongrel dog, about 2 years of age, was given ronnel mixed in one can of dog food, at one feeding, daily. Dosage was calculated to administer 25 mg. per kg. per day of the test material. The experiment was terminated after approximately 11 months during which the animal appeared to be in excellent health and general condition.

Microscopic examination of tissue sections for 12 different organs revealed no evidence of any adverse effect resulting from the 25 mg. per kg. per day doses of ronnel for an 11-month period. Plasma, red cell, and brain cholinesterase activity were found to be 29, 58, and 25% of normal, respectively.

Female beagle hounds approximately 6 months old were purchased from a commercial kennel in November 1956, and kept for a 2-month conditioning period in the laboratory before starting an experiment. Then, one dog per dosage level was placed on diets containing 0, 300, 100, 30, or 10 p.p.m. of ronnel. Ground Purina Laboratory Chow served as a control and basic ration for this experiment.

Throughout the entire experimental period all of the dogs appeared to be in excellent condition and exhibited normal behavior and appetites. Food consumption and body weight records indicated that the dogs, on the average, consumed the test compound in amounts of 10, 3, 1, or 0.3 mg. per kg. per day from the diets containing 300, 100, 30, or 10 p.p.m., respectively. All the dogs maintained a normal pattern of growth. Hematological findings, final body and organ weights, and terminal blood urea nitrogen (*7*) values showed no significant deviations from the control. Microscopic examination of the stained sections of the heart, lungs, liver, kidneys, spleen, pancreas, adrenals, thyroid, sciatic nerve, lymph node, urinary bladder, gall bladder, and skeletal muscle revealed no evidence of effects detrimental to the animals which could be attributed to the test compound.

Plasma and red cell cholinesterase measurements showed a considerable fluctuation when compared on a periodic basis. However, the general pattern (Table IV) indicated no significance to changes in plasma or red blood cell cholinesterase activity in the dogs that received the equivalent of the 3, 1, or 0.3 mg. per kg. per day of ronnel.

Rat Diet—Procedure. In August 1956, groups of male and female rats were started on diets containing ronnel in amounts calculated to administer 50, 15, 5, 1.5, or 0.5 mg. per kg. per rat per day. This was accomplished by adjusting the percentage of the test material in the diets, weekly, for the first 13 weeks on the basis of changing body weight and food consumption records. The *A* series of rats—10 of each sex per group—were started on an experiment designed to terminate after approximately 3 months. A second, or *B* series—five of each sex per group—were included for special studies of blood and brain cholinesterase activity and the microscopic examination of tissues during a recovery period following the 3-month test. At the same time, a *C* series of rats—20 males and 20 females per group—were started on the same dosage levels for a lifetime—2 year—dietary feeding study, and a *D* series—10 males and 10 females per group—of rats were begun so that animals would be available for sacrifice and examination after 1 year and 18 month intervals of the 2-year experiment.

The experimental diets were prepared by mixing thoroughly finely ground ronnel with ground Purina Laboratory Chow. Four groups of male and female rats—a total of 45 rats of each sex—were maintained on the basic ration only and served as control groups for these experiments.

During the course of the experiment, the animals were observed frequently for changes in general appearance. Records were kept of body weights, mortality, and average daily food consumption. Whenever possible, failing animals were sacrificed when moribund in an effort to ascertain the cause of the impending death.

Plasma and red cell cholinesterase activities were determined for the special groups of five male and female rats after 25, 39, 54, 67, and 97 days of experiment. After 105 days on experimental diets, the *A* series of rats were fasted overnight, weighed, killed by decapitation, and examined at autopsy. The lungs, heart, liver, kidneys, spleen, testes, and brain were removed and weighed. Hematoxylin and eosin stained sections of these organs, as well as the adrenals and pancreas, were prepared for microscopic examination. At this time, all the rats in the *B* series were placed on the basic ration only. Blood and brain cholinesterase determinations were made at intervals in order to follow the recovery of any decreased activity of the enzyme. Tissues for microscopic examination were obtained at intervals of 30 and 45 days on this recovery regimen.

From the *D* series of animals, tissues for microscopic examinations were obtained by sacrifice at the end of 12- and 18-month periods. Organ weights and brain cholinesterase values were determined. Additional plasma and red cell cholinesterase activity measurements were made in the long term tests after intervals of 243, 355, 416, 514, 562, 605, 647, and 695 days.

At the end of the 2-year experimental period, all of the surviving rats from the *C* series were fasted overnight, weighed, killed by decapitation, and examined at autopsy as previously described for the 105-day experiment.

Results of 105-Day and Recovery Rat Studies. Throughout the experimental period of 105 days, all of the rats appeared normal and in good general condition. Mortality records and measurement of average food consumption indicated no evidence of ill effect. Growth of the experimental groups was closely comparable to that of the control groups. Final average body and organ weights showed no significant changes from the control animals. Microscopic examination of the tissues from the experimental and the control rats revealed no evidence of adverse effects attributable to ronnel in any of the groups of rats that received the 15, 5, 1.5, or 0.5 mg. per kg. per day levels of the test material. However, in the male and female rats at the 50 mg. per kg. per day level, there was some evidence in the liver of a slight granular degeneration or cloudy swelling

Table IV. Over-all Blood Cholinesterase Activity—One-Year Dietary Feeding to Dogs

Ronnel, Mg./Kg./Day	Cholinesterase, % of Control	
	Plasma	Red cell
25	29	58
10	45	64
3	82	120
1	100	86
0.3	100	100

Table V. Over-all Cholinesterase Activity

(Two-year dietary feeding to rats, 5 of each sex per group)

Ronnel, Mg./Kg./Day	Sex	Cholinesterase, % "Control" Plasma	RBC	Brain
50	M	68	53	39
	F	32	50	42
15	M	73	76	66
	F	28	71	68
5	M	87	92	91
	F	39	95	92
1.5	M	105	98	96
	F	58	91	98
0.5	M	116	104	101
	F	85	107	100

of the parenchymal cells of the entire lobule. In the kidneys, there appeared to be some cloudy swelling and vacuolation of the renal tubular epithelium with very slight interstitial nephritis. Similar changes to those described were observed in the control animals but, in the opinion of the pathologist, not quite to the same degree.

The results of the blood and brain cholinesterase determinations are presented below in combination with those measurements made over the entire two-year study.

In the *B* series of rats placed on the control ration following the 105-day administration of ronnel in the diet, all of the rats, including those that had received the 50 mg. per kg. per day level, showed essentially complete recovery of any depressed cholinesterase activity within 6 to 8 weeks. Microscopic examination of the tissues from the 50 mg. per kg. per day animals sacrificed after 30 and 45 days on the recovery ration showed no evidence of any residual pathological effects.

Results of 2-Year Rat Studies. The groups of male and female rats maintained for two years on the diets administering 50, 15, 5, 1.5, or 0.5 mg. of ronnel per kg. of body weight per day appeared normal in general appearance and behavior. Growth curves, mortality records, hematological data, and food consumption records showed no significant differences from the control groups. Organ weight averages obtained from rats after the 12-, 18-, or 24-month periods showed some variations, but nothing consistent with dosage or of significant degree.

Mortality records showed that most of the deaths were due to respiratory diseases, ear infection, and other spontaneous occurrences. A total of 22 rats from the group of 240 rats started on the 2-year study developed tumors. All tumors occurred after 18 months on the experiment and involved two males with subcutaneous growths and 16 females with tumors of the mammary glands. In the other instances, there were one parotid, one uterine, and two mesentery tumors seen. None of these by type, location, or dosages were in any way related to the repeated dietary ingestion of ronnel.

Microscopic examination of the tissues from the small *D* groups of rats sacrificed at intervals of 12 and 18 months, and the larger *C* groups sacrificed after 2 years, revealed no effects detrimental to the rats, attributable to the long-term feeding of ronnel, except for both sexes which had received the 50 mg. per kg. per day dosage. In the livers of these animals, there was evidence of central lobular, granular degeneration, and some necrosis of the parenchymal cells. In the kidneys, there was evidence of cloudy swelling of the tubular epithelium together with interstitial nephritis. None of these reported observations were considered irreversible, particularly in view of the chronicity of exposure to ronnel, by dietary administration.

No evidence of an adverse effect occurred in the groups of male and female rats maintained for 2 years on diets administering 15, 5, 1.5, or 0.5 mg. per kg. per day of ronnel as judged by general appearance and behavior, growth, mortality, food consumption, periodic hematological examination, average body and organ weights, and gross and microscopic examination of the tissues.

Plasma and red blood cell samples were examined for cholinesterase activity a total of 12 to 14 times during the 2-year dietary feeding period. Brain cholinesterase activity was determined in tissue samples obtained at autopsy after the 105-day, 12-month, 18-month, and 2-year periods. The over-all pattern of results is presented in Table V with the data for each group of rats expressed as the per cent of activity shown by the control groups. Plasma cholinesterase, particularly in the female rats, was affected to the greatest extent. No significant inhibition is indicated for either male or female rats that received 5, 1.5, or 0.5 mg. per kg. per day of ronnel in so far as red blood cell or brain cholinesterase activity were concerned.

Significance of Results

Handling Hazards. The results of the studies reported here indicate that ronnel is low in acute and subacute oral toxicity. The probability that human subjects or livestock would ingest sufficient amounts of the material to cause serious toxic effects is slight. However, it is recommended that ronnel or its formulations be kept in closed containers where it is unavailable to livestock and out of reach for children.

If eyes are contaminated, they should be washed for several minutes with flowing water. Because of the action of other ingredients, formulations of ronnel may present a lesser or greater hazard to the eyes depending upon specific compositions.

This material does not present a problem from skin absorption under ordinary handling conditions. However, prolonged exposure to relatively large areas of the skin may result in the absorption of toxic amounts. Precautions should be taken to avoid prolonged or repeated skin contact with ronnel. Protective clothing may be advisable in specific circumstances. Good care and cleanliness should be sufficient to avoid difficulties.

Potentiation. Precise statistical methods for judging the significance of these data are not available. Whether any of the pairs exhibit significant potentiation is rather a matter of toxicological opinion. Based upon past experience and present judgment of the importance of this phenomenon to the evaluation of safety, it is concluded that potentiation is of no practical significance under ordinary conditions of field application and use of ronnel as recommended.

Dietary Feeding. In the repeated dietary feeding studies, rats tolerated rather well the dietary administration of ronnel in amounts up to 50 mg. per kg. per day (equivalent to 1000 p.p.m.). No gross symptoms of toxicity caused by the inhibition of cholinesterase activity were seen, at any time, during the study. The same conclusions apply to the dogs studied at the 10 and 25 mg. per kg. per day levels—300 and 1000 p.p.m. Changes observed by microscopic examination in livers and kidneys of rats were slight in degree as concluded from critical pathological examinations.

Also ronnel is a relatively weak inhibitor of cholinesterase, particularly in the sense that it affects predominantly the pseudo esterase of the plasma rather than the true acetylcholinesterase of the red blood cells upon both single and repeated oral feeding. By way of comparison, parathion (*9*) at 25 p.p.m. in the diet of rats for 2 weeks reduced both plasma and red cell cholinesterase activity to about 35%. Diazinon (*2*) in the rat diet for 4 weeks at 1000 p.p.m. was reported to have lowered red cell activity to zero, leaving plasma levels at 84% of normal. Malathion, on the other hand, at 1000 p.p.m. in a 2-year study (*11*) reduced rat plasma enzyme to zero, leaving red cell activity at 27%.

Technical grade malathion had a slight effect at 100 p.p.m. on both plasma and red cell cholinesterase, whereas, a purified sample was reported to have produced no significant inhibition when fed at the same dietary concentration.

Pertinent to these findings, Plapp and Casida (*20*) have postulated a metabolic pathway for ronnel in mammals. Two primary sites of hydrolytic attack were reported. Part of the molecule is believed to split with the formation of dimethylphosphorothioic acid, but to a greater extent the phosphorus-oxygen-methyl linkage is hydrolyzed, yielding stable *O*-methyl-*O*-hydrogen-*O*-(2,4,5-trichlorophenyl) phosphorothioate. These primary metabolites are excreted rapidly by the rat via urine. The metabolic pathway in a cow is believed similar to that postulated for rats, but with a slower rate of detoxication and excretion. This pattern of metabolism indicates no appreciable conversion of ronnel, in vivo, to the oxygen analog as it is commonly associated with certain other organic phosphate insecticides.

Experience in the therapeutic use of ronnel on cattle for grub control and the work on metabolism of Plapp and Casida (*20*) indicate that ronnel is absorbed rapidly into the blood stream upon oral administration and that its degradation products are readily excreted. When used as directed, no residue of ronnel remains in the tissues of cattle at the time of slaughter.

Ronnel does not present any unusual handling hazards in its manufacture and field use. Results of these studies indicate no public health hazard arising from ronnel as recommended for use in cattle grub control or as recommended for any other application.

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INSECTICIDE RESIDUES

Insecticide Residues on Tobacco

T. G. BOWERY, W. R. EVANS,[1] F. E. GUTHRIE, and R. L. RABB

Pesticide Residue Laboratory, Department of Chemistry and the Department of Entomology, North Carolina State College, Raleigh, N. C.

TDE [1,1-DICHLORO-2,2-BIS(*p*-CHLOROPHENYL)ETHANE] and endrin (1,2,3,4,10,10 - hexachloro-6,7 - epoxy - 1,4,4*a*,5,6,7,8,8*a* - octahydro - 1,4 - *endo* - *endo*-5,8-dimethanonaphthalene) are the major organic insecticides used on flue-cured tobacco for the control of the tobacco hornworm, *Protoparce sexta* (John). These two insecticides are usually applied more frequently and closer, in time, to the harvesting operation (priming) than other pesticides and, therefore constitute a greater potential hazard. In order to undertake a study of the residues of TDE and endrin, sampling procedures and analytical methods for the specific chemicals had to be evaluated, modified, and adapted to tobacco materials.

With the advent of organic insecticides into tobacco culture some residue studies (*8, 16, 28*) were made. The sodium reduction method (*4, 24*) for total organic chlorine was used. The values reported were inconsistent, the blanks were high and erratic, and the analytical sensitivity was poor. Total organic chlorine methods (*1, 18*) are now available for establishing the maximum amount of chlorinated insecticides that exist on tobacco. More specific colorimetric methods with greater sensitivity would be very valuable for providing information as to the possible extent of dissipation and deg-

[1] Present address, Department of Biochemistry, Purdue University, Lafayette, Ind.