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.

Acknowledgment

The authors gratefully acknowledge ths assistance of Doreen Lockwood in conducting the dietary feeding studies; K. J. Olson for the intubation feeding; S. E. Sadek for micropathology consultation; T. A. Hymas and Mark Norris for portions of the data on large animals, fowl, and dogs; and C. E. Wade for the blood and brain cholinesterase activity determinations. The skin irritation and sensitization tests on human subjects were conducted for The Dow Chemical Co. by Industrial Bio-Test Laboratories, Inc., Chicago, Ill., under the direction of J. C. Calandra.

Literature Cited

(1) Barker, S. B., *J. Biol. Chem.* **152,** 453 (1944).
(2) Bruce, R. B., Howard, J. W., Elsea, J. R., J. AGR. FOOD CHEM. **3,** 1017 (1955).
(3) Dow Chemical Co., Midland, Mich., Agr. Chem. Development Bull. No. **114,** "Trolene for Cattle Grub Control," May 1958.
(4) Draize, J. H., *Food, Drug, Cosmetic Law J.* **10,** 722 (1955).
(5) Dubois, K. P., *A.M.A. Arch. Ind. Health* **18,** 488 (1958).
(6) *Federal Register* **21,** 8104 (Oct. 23, 1956).
(7) Finney, D. J., "Probit Analysis," 2nd ed., p. 131, Cambridge Univ. Press, Cambridge, Eng., 1952.
(8) Frawley, J. P., Fuyat, H. N., Hagan, E. C., Blake, J. R., Fitzhugh, O. G., *J. Pharmacol. Exptl. Therap.* **121,** 96 (1957).
(9) Frawley, J. P., Hagan, E. C., Fitzhugh, O. G., *Ibid.*, **105,** 156 (1952).
(10) Hamblin, D. O., Marchand, J. F., "Cholinesterase Tests and Their Application in the Field," Bull., American Cyanamid Co., March 1951.
(11) Hazleton, L. W., Holland, E. G., *Arch. Ind. Hyg. Occupational Med* **8,** 399 (1953).
(12) Hornstein, I., Sullivan, W. N., Murphy, R., *J. Econ. Entomol.* **51,** 408 (1958).
(13) Knapp, F. W., Terharr, C. J., Roan, C. C., *Ibid.*, **51,** 361 (1958).
(14) Leng, M. L., The Dow Chemical Co., Midland, Mich., private communication, July 1958.
(15) McCollister, D. D., Hollingsworth, R. L., Oyen, F., Rowe, V. K., *A.M.A. Arch. Ind. Health* **13,** 1 (1956).
(16) McGregor, W. S., Bushland, R. C., *J. Econ. Entomol.* **49,** 86 (1956).
(17) Martin, Herbert, "Guide to Chemicals Used in Crop Protection," p. 136, Can. Dept. Agr. Bull., 3rd ed., October 1957.
(18) Michel, H. O., *J. Lab. Clin. Med.* **34,** 1564 (1949).
(19) Moyle, C. L. (to The Dow Chemical Co.), U. S. Patent **2,599,516** (June 3, 1952).
(20) Plapp, F. W., Casida, J. E., J. AGR. FOOD CHEM. **6,** 622 (1958).
(21) Radeleff, R. D., Woodward, G. T., *J. Econ. Entomol.* **50,** 249 (1957).
(22) Roth, A. R., Eddy, G. W., *Ibid.*, **50,** 244 (1957).
(23) Weil, C. S., *Biometrics* **8,** 343 (1952).
(24) Williams, M. W., Fuyat, H. N., Frawley, J. P., Fitzhugh, O. G., J. AGR. FOOD CHEM. **6,** 514 (1958).

Received for review March 26, 1959. Accepted July 9, 1959. Division of Agricultural and Food Chemistry, 135th Meeting, ACS, Boston, Mass., April 1959.

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.

Information has been lacking on the magnitude and fate of insecticide residues on tobacco. TDE and endrin residues on green tobacco during priming time are above 50 and 10 p.p.m., respectively. These residues are dissipated about 45% during processing. Auction market tobacco contains approximately 37 p.p.m. of TDE and 1.8 p.p.m. of endrin. An average of 13 γ of TDE and 0.2 γ of endrin are found per commercial cigarette. TDE and dehydrochlorinated TDE have been measured in the mainstream smoke of commercial cigarettes at 1.6 and 1.4 γ per "smoked cigarette." This information suggests that further education is needed on the application of these insecticides to minimize the residues. Studies on newer insecticides should be made with a view toward producing flue-cured tobacco which will yield insecticide-free cigarette smoke.

radation of the parent insecticides during tobacco processing and smoking.

PERSISTENCE OF TDE AND ENDRIN RESIDUES ON GREEN, FLUE-CURED, AND AGED TOBACCOS

Materials and Methods

Reagents. The reagents used in the modified Schechter-Haller method (*9*, *26*) for DDT, the dechlorination–sulfanilic acid–phenyl azide method (*5*) for endrin, and the horizontal quartz tube combustion–potentiometric titration method for total organic chlorine (*1*, *18*) were used. The following additional reagents were needed:

Purified *n*-pentane. Purify by distilling commercial grade *n*-pentane over sodium metal in an all-glass apparatus, discarding a 5% forecut and 15% bottoms.

Acetone, reagent grade, ACS

Dry ice.

Hyflo Super Cel, Johns-Manville Co.

Magnesium oxide, Fisher Sea-sorb 43, Fisher Scientific Co.

Attaclay, Attapulgus Minerals and Chemical Corp.

Aluminum oxide, Baker & Adamson No. 1236. Allied Chemicals & Dye Corp.

Silicic acid, Baker & Adamson No. 1169. Allied Chemicals & Dye Corp.

Apparatus. The apparatus described in the modified Schechter-Haller method for DDT (*9*, *26*), the dechlorination–sulfanilic acid–phenyl azide method for endrin (*5*), and the horizontal quartz tube combustion–potentiometric titration method for total organic chlorine (*1*, *18*) were used. The following additional apparatus was needed:

Dry ice bath.

Büchner funnels, glass, medium porosity, ST 19/38 bottom male joint, vacuum take-off, 30-ml. capacity.

Test tubes, 25 × 200 mm., ST 19/38 top female joint.

Chromatographic columns, 18 mm. inside diameter × 350 mm., ST 19/38 bottom male joint, vacuum take-off.

Field Sampling and Subsampling. Fifty or more leaves of green and flue-cured tobacco, 50-leaf samples of flue-cured tobacco from commercial auction markets, and 5-pack samples of commercial cigarettes were taken for analysis. The leaf samples were reduced in particle size using a Hobart cutter, and laboratory subsamples of 300 grams for green tobacco and 200 grams for cured tobacco were taken. The analytical samples from cigarettes consisted of the tobacco removed from 100 cigarettes, and it weighed from 73 to 128 grams.

Extraction. The laboratory subsamples were tumble-extracted end-over-end at 50 r.p.m. for 60 minutes with *n*-hexane at 3 to 1 (ml. per gram) ratio for green tobacco and at a 10 to 1 ratio for the cured and cigarette tobacco. The extracts were filtered and concentrated (in Danish-Kuderna concentrators fitted with water-cooled Friedrichs condenser heads) on an 85° C. water bath, to approximately 10 ml. The concentrated extracts were transferred to screw-capped vials and stored, at −20° C., until analyzed.

Cleanup. "DEWAXING." At the time of analysis, the extracts were removed from cold storage and allowed to warm up to 25° C., transferred to 100-ml. volumetric flasks, and made up to volume with *n*-hexane. Analytical aliquots, equivalent to 100 grams of green tobacco and to 25 grams of cured and cigarette tobacco, were pipetted into test tubes. The tubes were placed in a 60° C. water bath under a filtered air manifold and evaporated to near dryness. The residues were taken up in a minimum of hot acetone. The tubes and a supply of wash acetone were then chilled in a dry ice–acetone bath, at −75° C., for 30 minutes. The cold acetone-insolubles precipitated out while the TDE and endrin residue components remained in solution, and the insolubles were filtered off through 1 gram of Hyflo Super Cel using medium porosity sintered glass Büchner funnels. The acetone filtrates were placed in a 45° C. water bath under a filtered air manifold and evaporated to near dryness. The concentrates were taken up in 10 ml. of *n*-hexane, and the evaporation was repeated twice to remove the last traces of acetone. For endrin analyses of cured and cigarette tobacco, an additional dewaxing utilizing cold *n*-pentane was necessary. The "wax-free" samples were then ready for subsequent adsorption column chromatography.

CHROMATOGRAPHY. Tobacco samples that were to be analyzed for TDE and endrin by means of their total organic chlorine content were chromatographed on Shell (*22*) columns using adsorbent beds of 200 mm. of Attaclay mixture (Attaclay and Hyflo Super Cel, 2 to 1, w./w.) and magnesia mixture (Fisher Sea-sorb 43 and Hyflo Super Cel, 2 to 1, w./w.) respectively. TDE was eluted from the Attaclay column with 150 ml. of *n*-hexane, and the endrin was eluted from the magnesia column with 500 ml. of *n*-hexane.

Tobacco samples that were to be analyzed for their TDE content by the colorimetric method of Schechter-Haller were chromatographed on glass columns 350 mm. long and with 18 mm. in inside diameter, packed with three zones of adsorbent material. The lower zone was 150 mm. of silicic acid mixture (silicic acid and Hyflo Super Cel, 4 to 1, w./w.). The middle zone was 75 mm. of Attaclay mixture. The upper zone consisted of 75 mm. of aluminum oxide. The columns were packed dry under 5 pounds of vacuum with gentle tamping and prewashed with 100 ml. of *n*-hexane. The samples were added, and the TDE was eluted from the column with 150 ml. of *n*-hexane at a flow rate of approximately 2 ml. per minute.

The tobacco samples that were to be analyzed for their endrin content by colorimetric dechlorination–sulfanilic acid–phenyl azide method were chromatographed on Shell columns packed with two zones of adsorbent material. The lower zone was 250 mm. of Florisil (200-mesh, or up, activation temperature 1200° F.). The upper zone was 125 mm. of magnesia mixture. The columns were packed dry under 5 pounds of vacuum with gentle tamping and prewashed with 100 ml. of *n*-hexane. The samples were added and eluted from the column with *n*-hexane using the procedure set forth by Bann *et al.* (*5*). The final analytical sample for cigarette

tobacco consisted of the pooled eluates from four 25-gram chromatographed samples.

Eluates from all columns were collected and concentrated to approximately 10 ml. in Danish-Kuderna concentrators. The "clean" extracts were then ready for analysis.

Analysis. Samples were analyzed for total organic chlorine utilizing a Shell-Braun horizontal quartz tube combustion furnace in conjunction with a Beckman Model K automatic titrator. The combustion procedure followed the method described by Agazzi *et al.* (*1*, *3*), and the titration procedure used was that of Ewart and coworkers (*10*).

Samples were analyzed for TDE and endrin residues utilizing the modified Schechter-Haller method (*9*, *26*) and the procedure of Bann, Lau, and Potter (*5*).

Persistence of TDE and Endrin Residues on Green Tobacco. The 1954 tests were made to study the persistence of TDE and endrin sprays and dusts applied for hornworm control, and to study sampling procedures for TDE and endrin residues. TDE and endrin sprays and dusts were applied on 0.20-acre plots which were replicated three times. Six untreated guard rows separated each plot. The sprays were applied with a Hahn high-clearance sprayer equipped with Tee Jet, hollow cone nozzles adjusted to deliver 25 to 40 gallons of solution per acre, depending upon the size of the plants. The dusts were applied with a Hudson rotary hand duster delivering 20 to 40 pounds of dust per acre. Total organic chlorine analyses were made from samples taken from the second, fourth, and sixth priming (harvest). One leaf from each of 10 plants per plot was sampled 1 to 2 and 5 to 7 days after treatment for all three primings. In the second priming, analysis was also made of the leaves sampled 15 days after treatment. Only the laminae of the leaves were analyzed, and the results are reported on a dry basis.

The 1956 tests were made to study the effect of plant growth on the persistence of TDE and endrin residues. TDE and endrin were applied to green tobacco just prior to buttoning (early stages of inflorescence) with a high clearance sprayer at the rate of 1.0 and 0.4 pound of active ingredient per acre, respectively. Seven hollow cone nozzles per row, delivering 40 gallons per acre directed the spray on the tobacco in such a way that the entire plant was thoroughly covered. Leaves that were full-grown, but not necessarily ripe, half-grown or quarter-grown at the time of application were marked. Each leaf category was primed at intervals ranging from 0 to 21 days after treatment. Fifty-leaf samples were taken from the full-grown leaves, 70 from the half-grown leaves, and 100 from the quarter-grown leaves. All analyses were made on the uncured leaves, using colorimetric methods. In addition to the chemical analyses, the leaves were also subjected to bioassay tests using tobacco hornworms as the indicator. Five, one-quarter leaf sections from the desired treatment were cut out and placed in pint ice cream cartons. Five third- or fourth-instar hornworms collected from untreated tobacco were placed in each carton, and five cartons were used for each test. Thus 25 larvae were used per treatment, except with the tests made 17 days after application. In this case the field populations of larvae were low, and only 15 larvae were used in the quarter-grown leaf treatments and 12 larvae in the half-grown treatments. Mortality was recorded after 72 hours.

Persistence of TDE and Endrin Residues on Processed Tobacco. These tests were made in 1955 to determine the persistence of TDE and endrin residues during the various grower and manufacturing operations. Seven applications of TDE and endrin at 1.0 and 0.4 pound of active ingredient per acre were applied over a 3-week period to 0.5-acre unreplicated plots with a high clearance sprayer delivering 40 gallons of solution per acre. The number of applications intentionally exceeded normal recommendations for several reasons. It seemed desirable to ensure a high residue so that dissipation might be followed easily; and as this tobacco was to be used in the initial tests to trace TDE and endrin degradations, during the cigarette smoking process it was felt that high residues would facilitate such studies.

At the time of priming, which was immediately after the seventh application, 200 green leaves were randomly sampled, extracted, and analyzed by colorimetric methods. The remainder of the harvest was barn flue-cured (during which the temperature was slowly elevated to 160 to 170° F. over a period of several days). At the end of the curing, 200-leaf samples were again withdrawn from each treatment and prepared for analysis. In addition, 20-pound samples of the remaining tobacco in each treatment were delivered to each of two tobacco companies for commercial processing. After the leaf samples had been redried, and stripped or shredded, 5-pound subsamples were reclaimed for analysis. The remaining 15 pounds of each treatment were subjected to commercial aging in hogsheads. The treatments were separated from each other and from the other tobacco in the hogsheads by means of polyethylene sheets. At the end of the first year and again after 2 years of aging, 5-pound samples of each treatment were analyzed. After 2 years of aging the final 5 pounds of aged tobacco were made into cigarettes.

Results and Discussions

Sampling. At the outset of the persistence studies a sampling study was conducted to determine the TDE and endrin residue load on green tobacco as related to insecticides, times and rates of application, leaf position (hence, exposure), and over-all sampling error. Leaf samples consisting of 10 upper and 10 lower leaves were taken at random from each of the three replications, 1 day and 7 days after each of three applications. Duplicate field samples were taken for several of the treatments. Each 10-leaf sample was extracted separately. Extracts from samples taken 7 days after treatment were combined, and the combined 30-leaf sample extracts were analyzed. The entire sampling study consisted of 182 separate analyses. Statistical analysis of the data showed that: separate analysis of each application plus a combined analysis indicated that significant differences between materials, between leaf position, and between applications occurred throughout. The effects of the materials and the positions were in turn affected by the application date—the effects were not the same from date to date. The statistical analysis was computed on the logarithms of the concentration, in parts per million, assuming that the errors would be proportional to the response rather than constant regardless of the amount being measured. The sampling errors, in parts per million, were computed as percentage of the response rather than as constants. The basic sampling error per 10-leaf sample measured from true duplicates averaged over several materials, positions, and replications was 46%. Based on the 10-leaf sample the sampling error to be expected for the composite 30-leaf sample would be 24%. The observed sampling error was 22%. This agreement was good enough to permit the prediction of the sample sizes which would be required for any desired degree of precision. The sampling error appeared to increase with the increase in time from application to sampling. This effect was related to the added effect of climate and other factors on the distribution of the residue and indicated that the problem of obtaining an accurate estimate increases if a period of time had elapsed from the application date. This time effect was, however, in part compensated by the decrease in residue over time; hence, the actual sampling error in parts per million remained appreciably small when only small amounts of residue persisted.

This sampling study shows that in order to work at an acceptable error range of 15 to 20%, composite samples consisting of approximately 50 individual tobacco leaves would be required. Thus, in subsequent tests, samples consisted

Table I. Recoveries[a,b,c] of TDE and Endrin from Untreated Green and Cured Tobaccos and Cigarettes

	TDE						Endrin					
	Total Chloride Method			Colorimetric Method			Total Chloride Method			Colorimetric Method		
Sample Type	Added, p.p.m.	Found, p.p.m.	Recovery, %	Added, p.p.m.	Found, p.p.m.	Recovery, %	Added, p.p.m.	Found, p.p.m.	Recovery, %	Added, p.p.m.	Found, p.p.m.	Recovery, %
Green tobacco	3.0	2.7	92	0.1	0.09	91	2.0	1.8	91	0.1	0.08	89
	30.0	27.0	90	1.0	0.94	94	20.0	19.0	95	1.0	0.92	92
	300.0	282.0	94	10.0	9.00	90	200.0	186.0	93	10.0	9.40	94
Cured tobacco	3.0	2.5	85	0.2	0.17	87	2.0	1.7	86	0.3	0.25	85
	30.0	26.4	88	2.0	1.70	85	20.0	17.0	85	3.0	2.58	86
	300.0	258.0	86	20.0	17.60	88	200.0	176.0	88	30.0	25.50	85
Cigarette tobacco	3.0	2.4	82	0.2	0.16	84	2.0	1.6	83	0.3	0.24	81
	30.0	24.9	83	2.0	1.60	80	20.0	16.2	81	3.0	2.40	82
	300.0	240.0	80	20.0	16.60	83	200.0	168.0	84	30.0	25.20	84

[a] TDE and endrin added prior to extraction.
[b] Values shown are means of three determinations.
[c] Values corrected for apparent toxicant value in untreated check.

of at least 50 leaves taken at random from experimental plots, curing barns, and auction markets. Commercial cigarette tobacco, being a highly randomized entity itself, imposed no limitations on sampling procedures, therefore, 100 cigarettes were arbitrarily taken as an adequate sample.

Extraction. The operational efficiency in extracting TDE and endrin from different tobacco types was established by fortification of untreated samples prior to solvent equilibration and the recoveries are indicated with other procedural efficiencies in Tables I and II. The dangers of using such a localized addition procedure for studying extraction efficiency have been pointed out by Gunther (*13*), and are emphasized in the authors' studies of the TDE content of cured tobacco *vs.* cigarette tobacco. Early workers (*17, 28*) with tobacco were unable to get any significant correlation between the TDE residue load found on treated tobacco after curing and on similar tobacco after it had been processed into cigarettes. They invariably found a much higher residue load on the cigarette tobacco. Data on the TDE residue load on cured tobacco taken from experimental plots intentionally treated with a high dosage level and the TDE level found on cigarette tobacco processed from this same cured tobacco revealed an apparent rise of 109% in TDE level from the cured tobacco to the cigarette tobacco. The only difference between the cured tobacco (whole or partially broken leaves) and the cigarette tobacco (shredded) was one of physical consistency. Therefore, this 109% increase suggested a TDE-tobacco "wax" absorption picture similar to that occurring with other organic insecticides and waxy or oil-containing plant surfaces. Carman (*7*) brought this out in his treatment of subsurface residues on apples and citrus.

A much more complete extraction of the TDE residue occurred when the leaf tissue was cut up (as in the case of the cigarette tobacco), thus exposing the subsurface tissue to the solvent action of the *n*-hexane, as compared to the slight breaking of the leaf tissue when the cured tobacco was tumble-extracted for regular surface extraction. To explore this point further, additional replicates of the cured whole leaf tobacco were secured from the experimental material. From this, a first subsample was subjected to gentle laving with a stream of *n*-hexane. This wash was collected and designated as the surface extract. The same tobacco sample was then cut up in a Hobart cutter to a consistency similar to that of cigarette tobacco, and then tumble-extracted with *n*-hexane for 60 minutes and designated as the subsurface extract. A second subsample was subjected to the usual 60-minute tumble-extraction with no more than the usual amount of leaf breakage, and the extract from this sample was designated as the normal extract. A third subsample was also cut up in the Hobart cutter without any previous surface laving, and then tumble-extracted for 60 minutes and designated the total extract.

Table II. Procedural Efficiences[a,b] for the Determination of TDE and Endrin on Green and Cured Tobaccos and in Cigarettes

		TDE, Standard Deviation, P.P.M.		Endrin, Standard Deviation, P.P.M	
Sample Type	Sample Size, G.	Total chloride method	Colorimetric method	Total chloride method	Colorimetric method
Green tobacco	100	0.21	0.01	0.17	0.02
Cured tobacco	25	0.85	0.04	0.68	0.08
Cigarettes	25	0.85	0.04	0.68	0.08

[a] Toxicant added after extraction.
[b] All dewaxing and chromatographic procedures were found to be 100% efficient within the standard deviation of the method for the sample type.

The results of the analysis of these subsample extracts indicated that the residue level in the total extract of the cured tobacco was in close agreement with the value previously found for the cigarette tobacco. Also, the normal extract value was in excellent agreement with the value previously obtained for cured tobacco. Finally, the surface extract value and the subsurface extract value added up to the same amount as that found in the total extract. This indicated that TDE and endrin residues (confirmed by similar tests) on cured tobacco are both surface and subsurface in nature, being absorbed by the waxy materials on the surface of the leaf, and that maceration of the leaf tissue is necessary to approach satisfactory solvent equilibration.

Cleanup. *n*-Hexane extracts of cured and cigarette tobacco carry a high level of wax which was not apparent in the green leaf samples. This wax seriously interfered in total organic chlorine and colorimetric analyses causing creeping of the sample in the quartz tube combustion procedure, high blanks in silver nitrate titration, as well as high blanks and off-colors in colorimetric determinations. This waxy residue resisted efforts to isolate it from TDE and endrin by means of oxidation, saponification, steam distillation, and solvent partition. The procedure finally adopted was based on the work of Fairing *et al.* with methoxychlor residues (*11*) and consisted of the precipitation of the waxes from cold acetone and cold pentane (−75° C.) with separation from the TDE and endrin by filtration, as previously described.

Because the chromatographic elution patterns were seriously affected by varying amounts of wax in extracts, the use of analytical samples of a definitive size with their wax content eliminated or reduced to a somewhat standard level was imperative. Attaclay columns were found adequate for pigment removal from *n*-hexane extracts of green tobacco when their TDE content was determined by the combustion chlorine

Table III. Background Levels of Apparent TDE and Endrin in Purified Extracts of Untreated Tobaccos

Sample Type	Sample Size, G.	Average Background, P.P.M. Apparent TDE: Total chloride method	Apparent TDE: Colorimetric method	Apparent Endrin: Total chloride method	Apparent Endrin: Colorimetric method
Green tobacco	100	2.2	0.05	1.7	0.05
Cured tobacco	25	7.0	0.20	5.4	0.28
Cigarettes	25	7.0	0.20	5.4	0.28
Pooled "clean" eluates					
Cured	100	...	...	...	0.07
Cigarettes	100	...	...	...	0.07

Table IV. Residues[a,b] of TDE and Endrin on Green Tobacco

Treatment	Lb./Acre	Days after Treatment: 2nd Priming, 1	2nd Priming, 5	2nd Priming, 15	4th Priming, 1	4th Priming, 7	6th Priming, 2	6th Priming, 7
TDE, spray	1.0	460	69	25	196	89	68	52
Dust	3.0	2667	277	86	167	82	582	149
Endrin, spray	0.2	58	11	4	42	7	27	5
	0.4	91	18	6	27	9	29	13
Dust	0.4	316	33	..	31	10	114	6

[a] P.p.m. reported on a dry-stemless basis.
[b] Total organic chlorine method.

procedure. However, changes which occur in the constituents of leaves as they are cured and subsequently processed into cigarettes necessitated the use of combinations of adsorbents to give color-free eluates without loss of insecticide.

After experimentation with numerous materials and variations in column lengths, a column satisfactory for use in the colorimetric determination of TDE residues on green, cured, and cigarette tobacco was developed. This was a three-zone column of alumina, Attaclay mixture, and silicic acid mixture. Such a column proved to retain the pigments from *n*-hexane extracts equivalent to 100 grams of green tobacco, and to 25 grams of cured and cigarette tobacco, while permitting the elution of up to at least 20,000 γ of TDE from the column with *n*-hexane with an efficiency of 100%.

During early studies of endrin residues on green tobacco, prior to the introduction of the specific photometric method for the endrin molecule by the Shell Development Co. (*2*), many adsorbents were utilized to obtain color-free eluates. The majority of the adsorbents which were found successful for TDE cleanup were found either to adsorb both the tobacco interferences and the endrin or neither. Magnesia mixture was found to be useful with green tobacco samples up to 100 grams, retaining the green tobacco pigments, and permitting endrin elution with 500 ml. of *n*-hexane. However, magnesia mixture alone was not satisfactory for use with extracts of cured and cigarette tobacco, and following the lead of the endrin photometric method (*2*), a two-zone column of magnesia mixture and Florisil was adopted. This magnesia-Florisil column would retain tobacco interferences from *n*-hexane extracts equivalent to 25 grams of cured and cigarette tobacco. However, this sample size was not large enough to give sufficient sensitivity to the detection of endrin in cigarettes. In studies on the relative contribution to the endrin color reaction from nonendrin sources, combining pooled "clean" check eluates did not significantly increase the nonendrin color production. This indicated that the tobacco interferences had been reduced to a minimum using acetone and pentane dewaxing, followed by magnesia-Florisil chromatography. Pooling eluates, at least by a factor of 4, did not exert an additive effect from nonendrin color production sources. Thus, using a pooled sample consisting of four 25-gram clean eluates, the sensitivity for detecting endrin in cured and cigarette tobacco was increased fourfold. During the evaluation of this type of column, the activation temperature and the shelf age of the Florisil had a pronounced effect on the elution point of endrin. Bann *et al.* (*5*) pointed out the necessity of calibrating this type of column as to magnesia and Florisil batch, as well as the total amount of extractives placed on the column.

Analysis. The procedural efficiencies and the analytical manipulations involved in the determination of chlorinated hydrocarbon insecticides by means of the horizontal quartz tube combustion chlorine method, as well as by the determination of DDT, TDE, and related compounds by the Schechter-Haller colorimetric method, and of endrin by the dechlorination–sulfanilic acid–phenyl azide colorimetric method on other crop substrates have been adequately treated by various workers (*4, 13, 22, 26*).

By using the extraction and isolation techniques previously described, these methods have been adapted to the analyses of tobacco samples. The procedural efficiencies for these techniques are shown in Tables I and II. The background levels of apparent TDE and endrin in untreated green, cured, and cigarette tobacco are shown in Table III. These results show that the methods can be extended to a different material, tobacco, and that recovery, sensitivity, and the successful transition from total chlorine methods to more specific colorimetric methods permit the investigation of the magnitude and fate of TDE and endrin residues on tobacco as it is processed by the growers and manufacturers and smoked by the consumer.

Persistence of TDE and Endrin Residues on Green Tobacco. Table IV gives the residues of TDE and endrin remaining on green tobacco 1 to 2, 5 to 7, and 15 days after treatment. These residues are high in comparison to those found on other crops. Although considerably reduced by rainfall, growth, and weathering, the residues were still at a high level after 5 to 7 days. The magnitude of these residues on tobacco which was harvested immediately following treatment was very high. Thus, it is recommended that growers treat their tobacco immediately after priming rather than just before. Table V shows the dissipation of TDE and endrin following applications to green leaves at different stages of growth. The quarter-grown leaves had the highest residue initially because these leaves, being near the top of the plant, received spray from three nozzles. Five to 10 days after treatment the leaves that had been full grown when sprayed retained the highest residue. Because these leaves were already full grown, any reduction in the residue level was due solely to weather factors. In the case of the smaller leaves, growth subsequent to treatment diluted the residues. Worms feeding on the tobacco were controlled fairly well through the 11th day with TDE and through the 17th day with endrin. The biological data and chemical data were in fairly good agreement except for conflicting data obtained with 6 p.p.m. of endrin at 17 and 21 days after treatment.

Persistence of TDE and Endrin Residues on Processed Tobaccos. Table VI shows the comparative stability of TDE and endrin residues at various stages in the commercial processing of tobacco. The samples from the two companies were in very close agreement, and the figures shown are averages of the two tests. About 40% of both TDE and endrin disappear during

Table V. Residues[a,b] of TDE[c] and Endrin[d] Persisting on Green Tobacco and Their Toxicity to Hornworms

Days after Application	½ Grown Leaves				½ Grown Leaves				Full Grown Leaves	
	Hornworm				Hornworm					
	TDE, p.p.m.	Mortality, %	Endrin, p.p.m.	Mortality, %	TDE, p.p.m.	Mortality, %	Endrin, p.p.m.	Mortality, %	TDE, p.p.m.	Endrin, p.p.m.
Hours, 4	518		317		465		193		361	125
Days										
1	286		86		215		62		175	54
3	713	88	70	100	156	85	47	96	120	20
5	78		25		117		38		105	20
11	73	76	14	92	64	68	8	100	97	17
17	56	0	7	75	60	9	6	78	92	14
21	25	3	4	13	48	5	6	38	61	11

[a] P.p.m. reported on a dry-stemless basis.
[b] Colorimetric methods.
[c] Applied at 1 lb./acre.
[d] Applied at 0.4 lb./acre.

curing. Subsequent to curing, however, the insecticides remain at approximately the same level through redrying and aging. These figures suggest that the residue level on tobacco purchased from the grower, at the commercial auction markets, will not be altered except as it may be diluted in the blending with other tobaccos and additives in cigarette manufacture.

MAGNITUDE OF TDE AND ENDRIN RESIDUES ON AUCTION MARKET TOBACCO AND IN CIGARETTES

Materials and Methods

The TDE and endrin residue analyses were conducted in accordance with the colorimetric methods previously described. The method used for endrin analysis is specific. The TDE method, however, is sensitive to TDE and other insecticides, such as DDT, methoxychlor, and perthane. Of these others only DDT is used in tobacco culture. DDT is used primarily for budworm control; the applications are generally made only to the upper bud region of the plant 15 or more days before priming. The method of Rosenthal *et al.* (*25*), specific for TDE, shows that over 90% of the TDE-DDT complex found on United States tobacco is TDE. Accordingly, the results reported will be expressed as TDE.

Commercial Auction Market Samples. These samples were taken in a similar fashion each year—namely, two leaves were removed from each of 25 or more randomly selected baskets of flue-cured tobacco irrespective of grade or priming, and the 50-leaf sample was combined for each market. Samples from each warehouse were analyzed separately for their TDE and endrin residue.

Commercial Cigarettes. In 1955, four popular brands of cigarettes were purchased from retail outlets in seven widely separated locations in the United States. In 1956 and 1957, domestic cigarettes (including the brands sampled in 1955) were purchased from a commercial wholesaler in Raleigh, N. C., and, in addition, some foreign cigarettes were obtained. The tobacco removed from five packs of each brand type constituted the analytical sample.

Table VI. Effect of Processing on TDE and Endrin Residues[a] on Tobacco

Spray Treatment	Lb. per Acre	Times Treated	Residue, P.P.M. 1 Day after Treatment	Cumulative % Loss[b] after Indicated Process				
				Flue-cured	Stemmed or shredded and redried	Aged, Years 1	Aged, Years 2	Cigarette manufacture
TDE	1.0	7	784	41	43	44	44	44
Endrin	0.4	7	100	42	45	47	47	47

[a] P.p.m. reported on dry-stemless basis.
[b] % loss from 1-day residue.

Table VII. TDE and Endrin Residues[a] Found on Samples of Tobacco from Commercial Auction Markets

Belt	TDE, P.P.M.			Endrin, P.P.M.	
	1956	1957	1958	1957	1958
Border	68.0	41.3	61.2	1.5	2.9
Eastern	40.8	31.8	44.1	2.0	2.3
Middle	15.0	21.4	34.9	1.2	0.9
Old	10.0	15.5	32.9	0.7	0.6
All location mean	39.0	28.6	43.7	1.5	2.2
Range	1.0–140.2	7.8–58.4	14.0–114.0	0.0–3.4	0.0–7.4
Number of markets	30	26	56	26	46

[a] P.p.m. reported on dry-stemless basis.

Results and Discussion

Table VII shows the magnitude of TDE and endrin residues on tobacco from commercial auction markets. This tobacco is representative of that which is now in commercial storage, undergoing aging prior to cigarette manufacture. The values are grouped according to the Tobacco Belt and indicate that the residues are highest on tobaccos from the Border and Eastern Belts and lowest from the Middle and Old Belts. The residue level seems to parallel the relative importance of hornworms and budworms in the respective belts. The results in 1956 and 1958 were quite similar; residues of TDE were slightly lower in 1957. The results in 1958 indicated a slightly greater use of insecticide. In order for the results to be truly comparable, it would have been necessary to have the samples balanced for primings. The random sampling scheme did not, of course, ensure balanced representation. The comparison of 1956 and 1958 is felt to be more valid because the surveys were made when approximately similar primings were marketed. The higher residue levels found in 1956 and 1958 as compared with those of 1957 are explained as the results of heavier infestations of hornworms during these years with consequent increased use of TDE and endrin.

The TDE level on the four popular brands of cigarettes sampled in 1955 was about 12.5 p.p.m. There was no difference among brands, but there were slight differences between locations. The location means are not felt to be significantly different as it is known that cigarettes manufactured in a given time period are distributed throughout the United States in a random fashion.

Table VIII shows TDE residue levels in 1956 and 1957. There was considerable range among the brand types tested (4.7 to 17.9 p.p.m. TDE), although the four brands that were analyzed in 1955 once again were similar (about 12 p.p.m. TDE). Foreign brands of cigarettes also contained detectable quantities of the TDE-DDT complex, although one brand of 100% Turkish tobacco did not contain TDE or DDT. Table VIII also indicates the endrin residue level of five brands of regular cigarettes.

Table VIII. TDE Residues[a] on Commercial Cigarettes

Sample	No. of Brand-Types Sampled	TDE, P.P.M. Mean	TDE, P.P.M. Range
1956			
Regular-USA	7	11.2	8.9–13.8
1957			
Regular-USA[b]	7	13.4	12.2–15.7
King-USA	10	13.0	8.0–17.2
Filter-King-USA	13	11.7	8.6–15.7
Mixed-Canadian[c]	12	8.1	4.2–13.5
Austrian[c]	3	8.6	5.3–12.4
Turkish	1	0.0	

[a] P.p.m. reported on a dry-stemless basis.
[b] Five brand types sampled for endrin residues had a range of 0.08 to 0.29 p.p.m and a mean of 0.16 p.p.m.
[c] TDE-DDT complex, mainly DDT.

THE MAGNITUDE AND FATE OF TDE RESIDUES IN CIGARETTE SMOKE

As studies indicated that TDE and endrin residues could be detected on commercial tobacco and in commercial cigarettes, the next logical step was to determine the fate of these insecticides subjected to the high temperatures (in excess of 800° C.) of a cigarette during smoking. This was done by the isolation and identification of the insecticide residue components from the main-stream smoke of experimental and commercial cigarettes.

While it had been previously shown by Haag (*16*) that cigarettes processed from TDE-treated tobacco yielded main-stream smoke with an organic chlorine content higher than that from untreated cigarettes, the nature of the organic chlorine component(s) was not determined. The modified colorimetric procedure of Schechter-Haller was used in the determination of the nature of the TDE residue components in cigarette smoke. By this method, TDE yields an intense blue color and its known or theoretically possible degradation products produce red colors. Compounds that react in this method are designated as either blue SH (Schechter-Haller) positive or red SH positive.

Materials and Methods

In order to facilitate the detection of any TDE residue components that might be present in the main-stream smoke, experimental cigarettes carrying a high level (440 p.p.m. TDE) were used in the initial studies. These cigarettes were manufactured from the TDE-treated tobacco which had undergone commercial flue-curing, redrying, and aging (Table VI). Commercial cigarettes were sampled after the proper analytical scheme for the detection of TDE residue components in the main-stream smoke of experimental cigarettes had been established.

Smoke Collection. Smoke samples were collected from experimental and commercial cigarettes by means of an L&M Model 3 cigarette smoker (*20*). The apparatus is designed to smoke 15 cigarettes in rotation, taking from each a 35-ml. puff of 2-second duration, once every minute. A cigarette is considered smoked after eight puffs.

Table IX. Chromatographic Elution Patterns of SH Positive Material of Extracts from Experimental Cigarette Smoke

Consecutive 10-Ml. Fractions	Column 1 Alumina-Attaclay-Silicic Crude Extract, SH Color	Column 2 Alumina-Attaclay-Florisil Eluate from Column 1, SH Color	Column 3 F-20–Alumina Eluate from Column 2, SH Color
1	Orange-red	Colorless	Orange[a]
2	Orange-red	Light orange	Orange
3	Orange-red	Pink	Orange
4	Red	Red	Light orange
5	Red	Orange-red	Light orange
6	Red	Orange-red	Light orange
7	Brown	Colorless	Pink[b]
8	Brown	Colorless	Pink
9	Brown	Colorless	Pink
10	Off-purple	Colorless	Colorless
11	Off-purple	Light blue[c]	Colorless
12	Off-purple	Blue	Colorless
13	Blue	Blue	Colorless
14	Blue	Blue	Colorless
15	Blue	Blue	Colorless
16	Blue	Blue	Colorless
17	Blue	Light blue	Colorless
18	Blue	Colorless	Light pink[d]
19	Blue	Colorless	Pink
20	Off-pale blue	Colorless	Pink
21	Off-pale blue		Red
22	Off-pale blue		Red
23	Colorless		Red
24	Colorless		Red
25	Colorless		Pink
26			Pink
27			Light pink
28			Colorless
29			Colorless
30			Colorless

[a] Fractions 1–6 maximum 323 mμ, found in check cigarette smoke.
[b] Fractions 7–9 maximum 510 mμ, found on F-20 alumina, removed by pentane prewashing.
[c] Fractions 11–17 maximum 593 mμ, same as TDE.
[d] Fractions 18–27 maximum 541 mμ, same as deHCl-TDE.

Tobacco smoke is an aerosol consisting of millions of liquid-solid particles per cubic centimeter of gaseous phase. In preliminary studies, the aerosol phase of the main-stream smoke was collected by the use of an alpha-cellulose filter trap consisting of a 25 × 150 mm. glass tube packed with 2.5 grams of SW-40-A Solka-Floc α-cellulose to a depth of 80 mm. Previous work indicated that when the filter is connected to the mouth piece of the smoker it removed over 99% of the liquid-solid aerosol phase of the main-stream smoke under the smoking conditions previously indicated. In later studies the main-stream smoke was collected by means of a series of three gas washing bottles each containing 350 ml. of acetone.

Isolation of the TDE Residue Components. Analytical samples consisted of the cellulose—or acetone—trapped aerosol phase of the main-stream smoke from 90 cigarettes. The cellulose was removed from the filter tube and extracted with two successive 250-ml. portions of acetone. The acetone-cellulose extracts and the acetone trapping solutions were concentrated to approximately 10 ml. using Danish-Kuderna concentrators. The acetone smoke con-

centrates were transferred to 150-ml. beakers, mixed with 12 grams of aluminum oxide, and the excess acetone was removed by placing the beakers in a 65° C. water bath under a filtered air manifold. The alumina slurries were placed under vacuo over calcium chloride for 16 hours to remove the residual acetone and water. The alumina-smoke complex was thoroughly mixed and quantitatively transferred to glass chromatographic columns as the upper zone of the three-zone (alumina–Attaclay–silicic acid) column previously discussed. The columns were eluted with 250 ml. of *n*-pentane with the results shown in Table IX, column 1.

Preliminary studies on eluates from these columns involving the Schechter-Haller reaction indicated the possibility of both blue SH and red SH positive material being present. In order to separate the blue and red SH positive materials additional cleanup was required. A second set of these eluates was concentrated to 10 ml. using Danish-Kuderna concentrators. The pentane concentrates were then subjected to cold (−75° C.) acetone and pentane dewaxing as previously described. The dewaxed pentane concentrates were placed on chromatographic columns similar to the three-zone column, but in which Florisil was substituted for the silicic acid mixture. The columns were packed dry under gentle vacuum and prewashed with 150-ml. of *n*-pentane. The samples were added and the columns were eluted with 200 ml. of *n*-pentane. The blue and red SH positive materials were eluted from this type of column with the results shown in Table IX, column 2. Thus the use of the alumina-Attaclay-Florisil column in conjunction with the alumina–Attaclay–silicic acid column gave enough resolution of the blue and red SH positive materials that it was possible to collect all the red SH positive material in the first 80 ml. of pentane from the second column, while all the blue SH positive material was collected in the next 120 ml. This material was tentatively identified as TDE.

Resolution of the Red SH Positive Material. Various analogs of TDE, either known or theoretically possible as degradation products, yield red SH positive colors (*12*, *23*, *26*, *27*). In order to elucidate the nature of the heat-induced red SH positive degradation products of TDE in cigarette smoke, further fractionation was accomplished by means of the procedure of Sternburg and Kearns (*27*). A set of pentane fractions from the alumina-Attaclay-Florisil column containing the red SH positive material was subjected to this method. This demonstrated that of the six TDE derived compounds—1-chloro-2,2-bis(*p*-chlorophenyl)-ethylene, bis(*p*-chlorophenyl)methane, 4,4′-dichlorobenzophenone, 4,4′-dichlorobenzhydrol, *p*-chlorophenylacetic acid, and *p*-chlorobenzoic acid—that could be contributors to the red SH positive material in cigarette smoke, only the 1-chloro-2,2-bis(*p*-chlorophenyl)ethylene, the dehydrochlorinated ethylene derivative of TDE (dehydrochlorinated TDE) was present to any measureable extent.

Table X. TDE Residue Components in Experimental Cigarettes and Cigarette Smoke

Sample[a]	TDE,[b] P.P.M.	deHCl-TDE,[b] P.P.M.
Cigarettes	440	0
Main-stream smoke	82	90
Butts	460	0
Ash	0	0

[a] Original green tobacco treated with an exaggerated level of TDE.
[b] Based on weight of tobacco consumed during smoking.

Resolution of Dehydrochlorinated TDE and Check Smoke Interferences. In conducting these procedures, samples of smoke from cigarettes containing TDE-treated tobacco and smoke from untreated (check) cigarettes were analyzed concurrently. Once it had been established that dehydrochlorinated TDE was the only detectable contributor to the red SH positive material from the TDE source, it was no longer necessary to apply the Sternburg procedure to the smoke samples. The red SH positive fraction from the alumina-Attaclay-Florisil column could be interpreted as dehydrochlorinated TDE. There was, however, an unknown component in check smoke which gave an orange color after being subjected to the Schechter-Haller reaction and it resisted all earlier efforts of clean-up and separation. This material would appear concurrently with dehydrochlorinated TDE in the separation scheme and seriously interfered with the sensitivity range of any proposed dehydrochlorinated-TDE detection for commercial cigarettes. Therefore, the entire red SH positive fraction was subjected to an additional purification step to free it of the smoke interference. A set of pentane fractions from the alumina-Attaclay-Florisil column containing the red SH positive material was concentrated and placed on a 10-cm. column of *n*-pentane prewashed activated alumina (Alcoa F-20, 80 to 200 mesh) and eluted with 300 ml. of *n*-pentane. The elution pattern is shown in Table IX, column 3. These data show that all the non-TDE–derived red SH positive material can be discarded with the first 150 ml. from the column, while the next 150 ml. contained the red SH positive dehydrochlorinated TDE.

Results and Discussion

Experimental Cigarettes. Main-stream smoke was collected from experimental cigarettes carrying a high level of TDE (440 p.p.m.). In addition to the smoke collections from these experimental cigarettes, the ash and butts were also collected. The smoke, ash, and butts were analyzed colorimetrically for their TDE content. The results of these analyses are shown in Table X. They show that, of the TDE in the cigarette, approximately 40% was moved into the main-stream smoke by the high temperature of the burning tip of the cigarette. Approximately half of the 40% was tentatively identified as unchanged parent TDE and the other half as the dehydrochlorinated ethylene derivative of TDE (dehydrochlorinated TDE). There was little or no indication that the volatilized TDE or dehydrochlorinated TDE was condensed in the butt as the residue of this tobacco remained relatively constant.

Identification of TDE and Dehydrochlorinated TDE in Smoke from Commercial Cigarettes. In order to identify the blue and red SH positive material obtained in subjecting purified main-stream smoke from commercial cigarettes to the modified Schechter-Haller reaction as being specifically TDE and dehydrochlorinated TDE, several identification techniques were used. In addition to the tentative identification offered by their position in the isolation scheme, relative to that for authentic specimens of purified TDE and dehydrochlorinated TDE added to check smoke, spectral transmission curves of these isolated compounds were made using a Beckman DK-2 ratio recording spectrophotometer as shown in Figure 1. The absorption maxima for the blue SH positive material isolated from commercial cigarette smoke and the blue SH positive color obtained from authentic TDE were in close agreement at 593 mμ and 381 mμ. The absorption maxima for the red SH positive material isolated from commercial cigarette smoke and the red SH positive color from authentic dehydrochlorinated TDE were in close agreement at 541 mμ and 416 mμ. Main-stream smoke collected from cigarettes manufactured from a blend of insecticide-free flue-cured, burley and aromatic tobaccos processed through this same isolation and analytical scheme absorbed weakly at these wave lengths.

This close agreement of the absorption maxima between the blue SH and the red SH positive colors of cigarette smoke isolated compounds to those of authentic TDE and dehydrochlorinated TDE did not rule out, however, the possibility of similar colors being developed from other insecticide sources such as DDT, methoxychlor, and per-

thane. Of these compounds only DDT is used in tobacco culture. In order to rule out interferences from these other insecticides, the TDE and dehydrochlorinated TDE isolated from commercial cigarette smoke were subjected to a "confirming" reactions as described by Rosenthal (*25*). This reaction consisted essentially of treating the dehydrochlorinated derivative of TDE with 96% sulfuric acid with the subsequent formation of a "yellow color complex" measured on a spectrophotometer at 502.5 mμ. The use of the 96% sulfuric acid eliminated interference from perthane and methoxychlor, while absorbance of DDT under these conditions is very low. All of the TDE and dehydrochlorinated TDE isolated from commercial cigarette smoke reacted positively to the Rosenthal test, and quantitative estimation of the amounts present were of the same order of magnitude as those from the Schechter-Haller reaction.

At this stage of the investigation a preliminary report on smoke analysis was made by Mold and Walker (*21*) who obtained a clue as to the nature of one of the fractions they had obtained in the course of their study of the constituents of cigarette smoke. Mold and Walker (*21*) have since isolated TDE in the smoke produced from 34,000 cigarettes and offered infrared, ultraviolet, and melting point data to prove conclusively the presence of TDE in commercial cigarette smoke. They indicated that the level of TDE found approximated that reported by the authors (*6*). They published additional infrared data which likewise suggests the presence of dehydrochlorinated TDE in smoke.

These findings justified the application of the isolation and analytical scheme to the determination of TDE and dehydrochlorinated TDE in commercial cigarettes and in main-stream cigarette smoke. To establish the sensitivity level of this procedure main-stream smoke, collected from cigarettes manufactured from a blend of insecticide-free flue-cured, burley and aromatic tobaccos, was analyzed for its apparent TDE and dehydrochlorinated TDE content. Based on samples containing the smoke collected from 90 cigarettes, average background values of apparent TDE and dehydrochlorinated TDE were 0.1 γ of TDE and 0.05 γ of dehydrochlorinated TDE per smoked cigarette.

Commercial Cigarettes. One carton (200 cigarettes) each of 30 brand types of American cigarettes were purchased from a commercial wholesaler in Raleigh, N. C., in 1957. One hundred cigarettes from each carton were analyzed for their TDE content. An additional 90 cigarettes from each carton were subjected to the smoking process, and the collected smoke was analyzed for TDE and dehydrochlorinated TDE content. The results of these analyses are shown in Table XI, and indicate that, based on the amount of TDE in the cigarettes, approximately 12.9% is coming over in the main-stream smoke as parent TDE and 8.7% is dehydrochlorinated TDE.

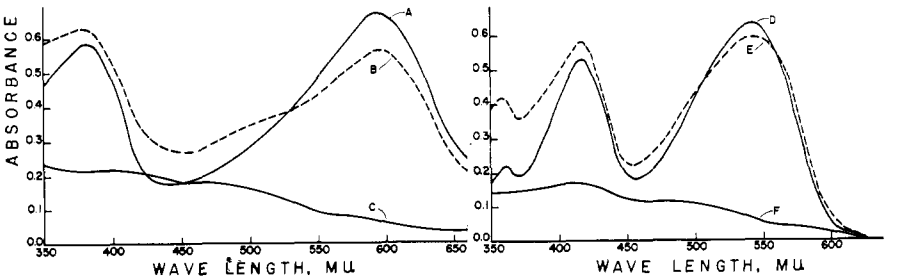

Figure 1. Absorption curves for TDE (left) and deHCl-TDE (right). Cigarette smoke isolates subjected to the Schechter-Haller reaction

A. Authentic TDE added to check cigarette smoke isolate
B. Commercial cigarette smoke isolate, blue SH positive fraction
C. Check cigarette smoke isolate, blue SH positive fraction
D. Authentic deHCl-TDE added to check cigarette smoke isolate
E. Commercial cigarette smoke isolate, red SH positive fraction
F. Check cigarette smoke isolate, red SH positive fraction

Table XI. TDE Residue Components in Commercial Cigarettes and Smoke

Sample	No. of Brand-types	γ/Cigarette and "Smoked Cigarette"[a]					
		Cigarette, TDE		Smoke			
				TDE		deHCl-TDE	
		Mean	Range	Mean	Range	Mean	Range
Regulars	7	12.7	11.2–15.2	1.6	1.5–1.7	1.4	0.9–2.6
Kings	10	14.7	9.7–20.6	1.7	1.5–1.8	1.3	0.7–1.5
Filter-Kings	13	10.3	7.8–13.4	1.6	0.7–1.9	0.6	0.3–0.8

[a] Corrected for: average background of apparent TDE for check cigarettes, 0.07 γ TDE/cigarette; average background of apparent TDE and deHCl-TDE for check smoke, 0.1 γ TDE and 0.05 γ deHCl-TDE/"smoked ciagarette."

Conclusions

The studies reported herein indicate that rather high levels of TDE and endrin residues may occur on green tobacco at priming. There is approximately a 40% loss of these residues during flue-curing, but the residues tend to go slightly subsurface and little or no additional loss occurs during commercial processing. There are indications that the TDE and endrin residue level is still high on flue-cured tobacco delivered to the commercial auction markets by growers and purchased by commercial companies for subsequent aging and processing into cigarettes. This suggests that growers have to be taught how to apply the insecticide to minimize the residues. Recent work at North Carolina State College (*15*, *19*) has shown that with reduced dosages and better placement and timing of applications, TDE and endrin residues can be reduced 50 to 60% with no appreciable increase in insect damage.

Dilution of the TDE and endrin residues found on commercial flue-cured tobacco by blending with other tobaccos and additives during commercial cigarette manufacture reduces the level of these insecticides in cigarettes. Appreciable dissipation and degradation of these residues occur during cigarette smoking. However, small but detectable amounts of TDE and dehydrochlorinated TDE are still found in the main-stream smoke of commercial cigarettes. These amounts, however, give no clue as to that retained by the smoker, because much or all of the TDE residue components may be exhaled. Studies on the magnitude and fate of the endrin residue components in cigarette smoke are under way in the authors' laboratory and will be reported at a later date.

Future work in the area of insecticide residues in cigarette smoke will consider the determination of the site, level, and fate of the insecticide-derived inhalation products in mammals.

As a long range plan we are investigating newer experimental insecticides (*14*) with a view toward producing flue-cured tobacco for cigarette manufacture with little or no insecticide residue present and which will yield an insecticide-free main-stream smoke.

Acknowledgment

The authors thank R. J. Monroe of the Department of Experimental Statistics for the statistical treatment of the sampling data, R. L. Baron and W. D. Brooks for the insecticidal applications, and Eileen Lecce and Irene Haithcock for their assistance in processing the many tobacco and cigarette smoke samples used in this study.

Literature Cited

(1) Agazzi, E. J., Peters, E. D., Brooks, F. R., *Anal. Chem.* **25,** 237 (1953).
(2) Agricultural Research Division, Shell Development Co., Analytical Method **ARMS-C-10/55** (1955).

(3) Analytical Department, Shell Development Co., Emeryville Method Ser. **EMS 1 × 10/53 aR** (1953).
(4) Assoc. Offic. Agr. Chemists, Washington, D. C., "Official Methods of Analysis," 7th ed., p. 382, 1950.
(5) Bann, J. M., Lau, S. C., Potter, J. C., J. AGR. FOOD CHEM. **6,** 196 (1958).
(6) Bowery, T. G., Guthrie, F. E., Division of Analytical Chemistry, Symposium on Methods for Analysis of Pesticide Residues, 131st Meeting, ACS, Miami, Fla., April 1957.
(7) Carman, G. E., Ewart, W. H., Barnes, M. M., Gunther, F. A., *Advances in Chem. Ser.* No. 1, 128 (1950).
(8) Chamberlin, F. S., Fahey, J. E., Special Rept. "Insecticide Residues on Shade-Grown Tobacco," U. S. Dept. Agr., Bur. Entomol. Plant Quarantine, 1951.
(9) Downing, G., Norton, L. B., *Anal. Chem.* **23,** 1870 (1951).
(10) Ewart, W. H., Gunther, F. A., Barkley, J. H., Elmer, H. S., *J. Econ. Entomol.* **45,** 578 (1952).
(11) Fairing, J. D., Warrington, H. P., *Advances in Chem.* Ser. No. **1,** 260 (1950).
(12) Ferguson, W. C., Kearns, C. W., *J. Econ. Entomol.* **42,** 810 (1949).
(13) Gunther, F. A., Blinn, R. C., "Analysis of Insecticides and Acaricides," Interscience, New York, 1955.
(14) Guthrie, F. E., Rabb, R. L., Bowery, T. G., *J. Econ. Entomol.* **52,** No. 5, in press.
(15) Guthrie, F. E., Rabb, R. L., Bowery, T. G., Lawson, F. R., Baron, R. L., *Tobacco Sci.* **3,** 65 (1959).
(16) Haag, H. B., unpublished rept. to Rohm and Haas Co., Medical College of Virginia, 1952.
(17) Hercules Powder Co., Wilmington, Del., Colorimetric Method for Toxaphene, I. M. No. 63, 1957.
(18) Hudy, J. A., Dunn, C. L., J. AGR. FOOD CHEM. **5,** 351 (1957).
(19) Lawson, F. R., Rabb, R. L., Guthrie, F. E., Bowery, T. G., "Studies of an Integrated Control System for the Hornworms that Attack Tobacco," unpublished manuscript.
(20) Liggett and Myers Tobacco Co., Durham, N. C., unpublished rept.
(21) Mold, J. D., Walker, T. B., *Tobacco Sci.* **1,** 161 (1957).
(22) O'Donnell, A. E., Neal, M. N., Weiss, F. T., Bann, J. M., DeCino, T. J., Lau, S. C., J. AGR. FOOD CHEM. **2,** 573 (1954).
(23) Ofner, R. R., Calvery, H. O., *J. Pharmacol. Exptl. Therap.* **85,** 363 (1945).
(24) Rauscher, W. H., *Ind. Eng. Chem., Anal. Ed.* **9,** 296 (1937).
(25) Rosenthal, I., Gordon, C. F., Stanley, E. L., J. AGR. FOOD CHEM. **7,** 486 (1959).
(26) Schechter, M. S., Soloway, S. B., Hayes, R. A., Haller, H. L., *Ind. Eng. Chem., Anal. Ed.* **17,** 704 (1945).
(27) Sternburg, J., Kearns, C. W., *J. Econ. Entomol.* **45,** 505 (1952).
(28) Townes, H. K., Entomology Department, North Carolina State College, unpublished data, 1952.

Received for review February 25, 1959. Accepted July 23, 1959. Division of Agricultural and Food Chemistry, 129th and 131st Meetings, ACS, Dallas, Tex., April 1956 and Miami, Fla., April 1957. Research supported partially by Southern Regional Research Project, S-22, and by the Shell Chemical Corp. and the Tobacco Industry Research Committee. Published with approval of the Director of Research as paper No. 1010 of the Journal Series.

PESTICIDE ACTIVITY AND STRUCTURE

Structure and Nematocidal Activity of Allylic and Acetylenic Halides

WILLIAM MOJE

Department of Soils and Plant Nutrition, University of California Citrus Experiment Station, Riverside, Calif.

Special techniques for estimating the dosage-response curves of some allylic and acetylenic halides using larvae of the citrus nematode, *Tylenchulus semipenetrans* Cobb, have been developed. The toxicities of these halides, as measured by the concentrations required to produce 50% inhibition of mobility, were found to be related to their reactivities in the S_N2 reaction with potassium iodide in acetone. A possible mode of action for these halides is suggested.

ORGANIC HALIDES are currently being used extensively as soil fumigants for the control of plant parasitic nematodes and other pathogenic soil organisms (*22*). The two most common types are the saturated aliphatic halides such as methyl bromide, ethylene dibromide, and 1,2-dibromo-3-chloropropane, and the 2,3-unsaturated alkyl halides such as 1,3-dichloropropene.

Although some of these have been utilized as nematocides for almost 15 years, very little is known concerning their mode of action. It has been suggested that these compounds act as narcotics and that physical properties such as vapor pressure, water solubility, and ability to dissolve wax are most important (*5, 21*). However, toxicological data were recently obtained which suggested that reactivity was involved (*29*). In a series of halides RX, where R was constant, the order of toxicity was RI>RBr>RCl. *cis*-1,3-Dichloropropene was more toxic than *trans*-1,3-dichloropropene and 2,3-unsaturated alkyl halides were more toxic than the corresponding saturated derivatives. In each case, nematocidal activity parallels the rate of reactivity of the halide in bimolecular nucleophilic displacement—S_N2—reactions. The present investigation was undertaken to study this relationship in more detail.

This report summarizes the techniques that were developed for the estimation of dosage-mortality curves using citrus nematode larvae and compounds which are toxicants at concentrations less than their solubilities in water. Results are also indicated for a number of allylic and acetylenic halides, and the relationship between the toxicities of these halides and their reactivities in the S_N2 reaction with potassium iodide in acetone.

Apparatus

VIALS. 25-ml. capacity with plastic snap-on caps (Merck, Sharpe and Dohme, Nos. 3352 and 6301). Drill a 27/32 inch diameter hole in half the caps.

FILTER CLOTHS. $1^1/_{32}$ inches in diameter. Drill from plastic cloth (Style PM-2711-C Polymax), 176 × 73 filaments per inch and a nominal diameter of 0.006 inch.

VIAL HOLDERS. Cut $^3/_4 \times 3 \times 31^1/_2$ inch hardwood lumber lengthwise, drill twelve $1^1/_4$-inch diameter holes on $2^1/_8$-inch centers $4^1/_{16}$ inches from each end, and line each hole with $^1/_{16}$-inch felt. Combine the two halves and through the $^3/_4$-inch side, drill three $^1/_4$-inch diameter holes, in the center and $1^1/_2$ inches from each end. Secure the two halves with $^3/_{16}$-inch stove bolts and wing nuts.

Petri dish holder and counting grid (Figure 1). Drill a $2^1/_8$-inch diameter hole in the center and four $^1/_8$-inch diam-