0.1% sodium nitrite. Dissolve 0.1 gram in 100 ml. of distilled water.

0.5% ammonium sulfamate. Dissolve 0.5 gram of ammonium sulfamate in 100 ml. of distilled water.

Add four volumes of the sulfanilic acid solution to five volumes of the sodium nitrite solution and mix thoroughly. Let stand for 5 minutes, add one volume of the ammonium sulfamate solution, and mix thoroughly. This reagent is stable for at least 4 hours. In practice fresh solutions have been prepared daily and the mixed reagent at least every 4 hours. These precautions may not be necessary.

Use of this reagent in the colorimetric determination of endrin practically eliminates the reagent background color.

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INSECTICIDE RESIDUES

Determination of Insecticide Residues on Green and Flue-Cured Tobacco and in Main-Stream Cigarette Smoke

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Trace amounts of insecticides presently recommended for control of tobacco insects, TDE and endrin, have been detected in commercial cigarettes and cigarette smoke. Newer insecticides are being investigated in a search for effective materials which do not leave a residue on cured tobacco and which yield a residue-free smoke. Procedures for determination of Guthion and Sevin residues in solvent extracts of tobacco and in main-stream cigarette smoke are described. Residue studies with Guthion and Sevin indicate that of the total residue on green tobacco at priming time only 27 and 12%, respectively, were detectable after flue-curing. Guthion and Sevin residues were detected in main-stream cigarette smoke at levels approximating 0.3% and 1% of that added to cigarettes prior to smoking. Tobacco treated at a recommended level of 0.5 pound of Guthion or 1.0 pound of Sevin per acre showed no measurable contamination of main-stream cigarette smoke.

FOUR INSECTICIDES, Guthion [(*0,0*-dimethyl *S*-[4-oxo-1,2,3-benzotriazin-3(4*H*)-ylmethyl] phosphorodithioate], SD 4402 (1,3,4,5,6,7,8,8-octachloro-3*a*,4,7,7*a*-tetrahydro-4,7-methanophthalan), Sevin (1-naphthyl *N*-methylcarbamate), and Thiodan (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin 3-oxide) have shown considerable promise in the control of insects attacking tobacco (*3*). Since insecticides presently recommended for the control of tobacco insects, TDE and endrin, have been detected in commercial cigarettes and cigarette smoke (*2*), these newer compounds are being investigated in search for effective insecticides which do not leave a residue on flue-cured tobacco and which will yield a residue-free smoke. Part I of this paper deals with determination of Guthion and its suspected biological degradation product, the oxygen analog of Guthion (Oxyguthion), and Part II deals with determination of Sevin on green and flue-cured tobacco and in main-stream cigarette smoke.

Experimental

Materials and Methods. The basic analytical method used in these studies for Guthion residues was evolved by Meagher *et al.* (*4*) for use in determining Guthion residues in crops. In this method, the intact Guthion or Oxyguthion molecule is hydrolyzed in an alkaline solution. Anthranilic acid formed in the process is diazotized and coupled with *N*-(1-naphthyl)-ethylenediamine dihydrochloride yielding a strong magenta color.

The basic method used for Sevin residues was that of Miskus, Gordon, and George (*5*) as modified by Whitehurst (*6*) and is based on the production of a blue color when the alkaline hydrolysis product of Sevin, 1-naphthol, couples with *p*-nitrobenzene diazonium fluoborate.

Reagents and Apparatus. The reagents and apparatus used for Guthion were previously described by Meagher *et al.* (*4*) and by Bowery *et al.* (*2*).

The reagents and apparatus used for Sevin were previously described by Miskus, Gordon, and George (*5*), Whitehurst (*6*), and Bowery *et al.* (*2*). The following additional reagents were needed:

Aluminum Oxide, Woelm (Alupharm Chemical Co., New Orleans, La.) acid (anionotropic) of activity grade 1.

Florisil (Floridin Co., Tallahassee, Fla.), 60-/100-mesh, dried overnight at 180° C. with 11% water added subsequently.

Field Sampling and Subsampling. Seventy-five fully grown green leaves, 600 to 1000 cured leaves, and 5-pack samples of experimental cigarettes were taken for analysis. The leaf samples were chopped in a Hobart cutter. The smoke samples were collected in acetone by the method set forth by Bowery *et al.* (*2*) and the smoke collected from 90 cigarettes constituted an analytical sample.

PART I. GUTHION RESIDUES

Analytical Procedure

Extraction. Laboratory subsamples of 100 grams of chopped green or flue-cured tobacco were blended for 3 minutes with acetone at a 1:1 and 6:1 (milliliters per gram) ratio, respectively. The acetone filtrates were reduced in volume in Danish-Kuderna concentrators. The cured tobacco concentrates were dewaxed (*2*). Both green and dewaxed cured acetone concentrates were diluted to 100 ml. with acetone and then with

400 ml. of 1.25% HCl. The acidified aqueous mixtures were allowed to stand for 1 hour. The aqueous mixtures were first extracted six times with 50-ml. portions of hexane to remove the Guthion fraction and then six times with 50-ml. portions of chloroform to remove the Oxyguthion fraction.

The acetone-smoke samples were concentrated to approximately 10 ml. by using Danish-Kuderna concentrators. The acetone-smoke concentrates were dewaxed (*2*) and excess acetone was removed by placing the samples in a 65° C. water bath under a filtered air manifold. The air-dried smoke concentrates were taken up with 25 ml. of acetonitrile (saturated with hexane) and diluted with 75 ml. of water. The aqueous smoke mixtures were extracted with hexane to remove the Guthion fraction and with chloroform to remove the Oxyguthion fraction as previously described.

Cleanup. The hexane fractions of both leaf and smoke samples containing Guthion were chromatographed on glass columns, Shell design (*2*), using 150-mm. adsorbent beds of Attaclay–Hyflo Super Cel, 2:1 w./w. Guthion was eluted from the columns with 350 ml. of 25% chloroform in hexane.

Chloroform fractions of leaf samples containing Oxyguthion were chromatographed on Shell columns using 35-mm. adsorbent beds of adsorption alumina (Fisher Scientific Co.). Oxyguthion was eluted with 125 ml. of chloroform.

Chloroform fractions of smoke samples containing Oxyguthion were evaporated to dryness under a filtered air manifold in a 65° C. water bath and taken up with acetone sufficient to make a slurry with 12 grams of Hyflo Super Cel. The excess acetone was removed by air drying, and the Hyflo Super Cel–smoke mixtures were placed under vacuum over calcium chloride for 16 hours to remove any residual acetone. The Hyflo Super Cel–smoke mixtures were packed into Shell columns and the Oxyguthion was eluted with 250 ml. of warm distilled water (55° C.). The aqueous eluates were extracted six times with 50-ml. portions of chloroform to remove Oxyguthion. Chloroform extracts were rechromatographed on Shell columns using 35-mm. adsorbent beds of adsorption alumina. Oxyguthion was eluted with 125 ml. of chloroform.

In order to remove any interfering amino compounds that still remained after chromatography, all of the Guthion and Oxyguthion fractions were transferred to separatory funnels with 25 ml. of benzene and shaken for 5 minutes with 10 ml. of 3*N* HCl. The acid layer was discarded and benzene and any emulsion were passed through 2-inch beds of anhydrous sodium sulfate into 25 × 250 mm. collection tubes.

Hydrolysis. The benzene phases were then concentrated to 10 ml., mixed with 10 ml. of 0.5*N* KOH in 2-propanol, and were allowed to stand at room temperature for 20 minutes. This treatment converts Guthion and Oxyguthion to anthranilic acid. Hydrolysis was stopped at the end of 20 minutes by addition of 8 ml. of 3*N* HCl. The hydrolysis mixtures were extracted twice with 25-ml. portions of distilled water. The aqueous phases containing anthranilic acid were transferred to 100-ml. Erlenmeyer flasks. Colored interferences persisting at this point were eliminated by adding 0.5 gram of zinc dust and refluxing for 2 hours.

Diazotization and Coupling. Zinc was removed from the reaction mixtures by filtration. Two 20-ml. aliquots, *A* and *B*, were taken from each of the Guthion and Oxyguthion filtrates. Twenty milliliters of a blank solution (mixture containing 20 ml. of 2-propanol, 0.6 gram of KOH, and 1.2 ml. of HCl per 100 ml. of water) were added to each aliquot to establish the proper media for optimum color production. Using a split-blank procedure, similar to that used by Averell and Norris (*1*) for parathion residues, the aliquots were diazotized with 1 ml. of 0.25% sodium nitrite. The excess nitrite was destroyed at the end of a 10-minute reaction period by the addition of 1 ml. of 2.5% ammonium sulfamate. The diazotized anthranilic acid in aliquots *A* were coupled with 2 ml. of 1% *N*-(1-naphthyl)-ethylenediamine dihydrochloride yielding a strong magenta-colored complex. Aliquots *B*, from which the coupling reagent was omitted, allowed correction for residual color in the extracts. This correction was found to be small and could be neglected in most cases.

The absorption spectra of the color complex produced from Guthion and Oxyguthion were essentially identical. Guthion had a maximum at 547 mμ, and Oxyguthion had a maximum at 550 mμ. This color reaction was found to be linear within the range of 2 to 1000 γ of added Guthion or Oxyguthion.

Results and Discussion

In order to facilitate detection of the main Guthion-residue components that might be present in green and flue-cured tobacco and main-stream cigarette smoke, the foregoing procedure was applied to a series of 100-gram insecticide-free green and flue-cured leaf tobacco samples and to acetone extracts of smoke from 90 experimental insecticide-free cigarettes.

Based on the addition of 0, 100, 200, 400, and 600 γ each of authentic Guthion and Oxyguthion to 100-gram samples of check green and flue-cured tobacco, Guthion recovery averaged 55% with no interferences from Oxyguthion present. Oxyguthion recovery averaged 74% with interference from Guthion equivalent to approximately 16% of any Guthion present. In order to increase present reliability in interpretation of Guthion and Oxyguthion recoveries as well as Guthion interference in the Oxyguthion fraction, standard curves were developed for 0 to 600 γ of Guthion and Oxyguthion in the presence of each other as well as in the presence of 100 grams of green and flue-cured tobacco. This permitted accurate determination of Guthion and Oxyguthion content of field samples of green and flue-cured tobacco and a readily available method for making a correction for "false" Oxyguthion derived from the Guthion source. This inability to extract all of the Guthion from the acidified aqueous mixture with hexane occurred only in the presence of tobacco extractives. This difficulty occurred with non-dewaxed green tobacco samples as well as with dewaxed flue-cured tobacco samples. Repeated studies without these extractives indicated that complete recovery of Guthion was possible with hexane.

Using 5-cm. cells and absorbance values of not less than 0.10, the "apparent" Guthion and Oxyguthion background or check values for green and flue-cured tobacco amounted to 15 γ or 0.15 p.p.m. based on the 100-gram samples.

Based on the addition of a mixture of 0, 30, 300, and 3000 γ each of authentic Guthion and Oxyguthion to a series of acetone smoke concentrates, Guthion recovery averaged 88% with approximately 4% interferences from Oxyguthion present. Oxyguthion recovery averaged 95% with approximately 2% interferences from the Guthion present. The apparent Guthion and Oxyguthion background or check values amounted to 0.42 γ of Guthion and 0.58 γ of Oxyguthion per smoked cigarette.

Leaf Studies. The tobacco plots were unreplicated, two rows wide, and 400 plants long. Three to five border rows separated treatments. Sprays (0.5 pound per acre) were applied with a Hahn Hi-Boy at 21 gallons per acre using seven hollow cone nozzles per row. Dusts (0.75 pound per acre) were applied with a Hudson, rotary hand duster in which each side of the plant was dusted with one outlet held at the uppermost leaves and one outlet at the center leaves. The plots were primed (harvested) at 0, 1, 3, 7, and 14 days after treatment.

Results of the analysis of the green and flue-cured samples are shown in Table I. These data show the field dissipation of residue due to weathering. In addition, they indicate that there is an average of 8% of Guthion residue remaining after flue-curing.

Table I. Guthion (G) and Oxyguthion (OG) Residues on Green and Flue-Cured Tobacco

Guthion Treatment, Actual/Acre	Sampling Interval, Days	Green Tobacco, P.P.M.[a]		Cured Tobacco, P.P.M.[a]		Recovery, after Flue-Curing, %		OG/G Ratio	
		G	OG	G	OG	G	G + OG	Green	Cured
Dust 0.75#/A.	0	76.0	...	5.5	20.7	7.3	27.3	...	3.8
	1	50.9	ND[b]	...	...	...	...	0.00	...
	3	9.2	1.9	0.7	1.6	7.7	20.8	0.02	2.3
	7	3.7	1.4	0.5	1.4	13.5	37.3	0.38	2.8
	14	ND	ND	0.3	1.3	...	...	0.00	4.3
					Mean	9.5	28.5	0.10	3.3
Spray 0.50#/A.	0	75.0	15.3	10.3	27.9	13.8	42.4	0.20	2.7
	1	44.5	7.0	...	...	...	...	0.16	...
	3	31.5	2.8	1.3	3.5	4.2	14.0	0.16	2.7
	7	18.5	2.8	1.1	3.5	6.0	21.6	0.15	3.2
	14	9.2	4.2	0.6	2.7	6.6	24.7	0.45	4.5
					Mean	7.6	25.7	0.21	3.2

[a] P.p.m. reported on dry-stemless basis.
[b] None detected at sensitivity level of 0.15 p.p.m.

Table II. Guthion and Oxyguthion in Main-Stream Smoke from Treated Cigarettes

Cigarettes Content, γ/Cigarette	Smoke Content, γ/Smoked Cigarette[a,b]	Recovery, %
Guthion		
300	0.63	0.21
150	0.41	0.27
3	0.00	...
Oxyguthion		
3000	0.57	0.019
300	0.04	0.013
30	0.00	...

[a] Values shown are means of three determinations.
[b] Corrected for: Av. background of apparent Guthion and Oxyguthion for check smoke of 0.42 and 0.58 γ/smoked cigarette.

The relationship between the Oxyguthion content of green tobacco and its counterpart after curing is difficult to assess. The ratio of Oxyguthion (OG) to Guthion (G) in the flue-cured tobacco is twenty-five times greater than this ratio in the green tobacco. This indicates that there may be some conversion of Guthion to Oxyguthion during the flue-curing process. Further studies on this point are now under way. Due to this apparent conversion, the total Guthion complex remaining after flue-curing averages 27%.

Smoke Studies. Experimental cigarettes manufactured from a blend of insecticide-free flue-cured burley and aromatic tobaccos to approximate commercial cigarettes were infiltrated with acetone solutions of Guthion and Oxyguthion. The acetone was evaporated from the cigarettes by air-drying for 48 hours. Analysis of cigarette tobacco indicated that Guthion and Oxyguthion were not dissipated during this pre-smoking period.

Guthion was added to a series of cigarettes to produce levels of approximately 3, 150, and 300 γ of Guthion per cigarette. Oxyguthion was added to another series of cigarettes to produce levels of approximately 30, 300, and 3000 γ of Oxyguthion per cigarette. These sets of treated cigarettes were smoked as previously described and the acetone-trapped smoke analyzed for Guthion and Oxyguthion content.

Table II shows that recovery of Guthion in the acetone-trapped main-stream smoke of Guthion-treated cigarettes was 0.21% from the 300-γ level cigarettes, 0.27% from the 150-γ level cigarettes, and was at background level for the 3-γ level cigarettes. Recovery of Oxyguthion in the acetone-trapped main-stream smoke of the Oxyguthion-treated cigarettes was 0.019% from the 3000-γ level cigarettes, 0.013% from the 300-γ level cigarettes, and was at background for the 30-γ level cigarettes.

Since the chloroform fraction of the smoke from Guthion-treated cigarettes and the hexane fraction of the smoke from Oxyguthion-treated cigarettes should yield a color complex value of the same magnitude as the background, the appearance of appreciable color in these fractions led to the following observations:

Analysis of the chloroform or so-called Oxyguthion fraction of smoke produced from Guthion-treated cigarettes indicated levels of the color complex equivalent to 0.08% of the added Guthion. Analysis of the hexane or so-called Guthion fraction of smoke produced from Oxyguthion-treated cigarettes indicated levels of the color complex equivalent to 0.03% of the added Oxyguthion. It is felt that this color production may be due to formation of benzazimide derivatives other than benzazimide itself or hydroxymethyl benzazimide. Meagher *et al.* (*4*) discussed possible degradation products of Guthion that can cause interference with the basic method.

Conclusions

Residue studies with Guthion on tobacco indicate that of the total residue on green tobacco at priming time only 27% was detectable after flue-curing despite apparent conversion of some Guthion to Oxyguthion. Residue recovered from main-stream cigarette smoke was less than 0.3%.

To place these findings on practical terms in relation to Guthion usage as an insecticide for control of tobacco insects and the amount of Guthion residue complex reaching the smoker, the following conclusions have been made:

The residue load on tobacco sprayed at the recommended levels (0.5 pound per acre) of Guthion, primed the same day it was treated and flue-cured, approximated 10 p.p.m. of Guthion and 28 p.p.m. of Oxyguthion. Residual Guthion levels twenty times greater and Oxyguthion levels two hundred times greater than these would be required before measurable contamination of main-stream cigarette smoke could be detected by the analytical method used in these studies. Priming tobacco 1 week after treatment would increase this margin by a factor of 8.

Thus, it is felt that Guthion can be used for insect control on flue-cured tobacco and cigarettes could be produced with essentially no detectable Guthion or Oxyguthion in main-stream smoke.

PART II. SEVIN RESIDUES

Analytical Procedure

Green Tobacco. Extraction. One hundred-gram subsamples of chopped green tobacco were blended with 400 ml. of methylene chloride. The methylene chloride filtrates were reduced in volume under vacuum (*6*) in a Danish-Kuderna concentrator to approximately 50 ml. The concentrates were transferred to 250-ml. Erlenmeyer flasks and dried with anhydrous sodium sulfate for 30 minutes with intermittent shaking. The dried concentrates were transferred to 100-ml. volumetric flasks and diluted to volume with methylene chloride.

Cleanup. Twenty-five milliliter aliquots were transferred to fresh 250-ml. Erlenmeyer flasks, placed in a 40° C. water bath, and evaporated just to dryness under a gentle stream of nitrogen. The residues were taken up in 10 ml. of acetone and diluted with 100 ml. of a mix-

Table III. Sevin Standard Curves for Green and Flue-Cured Tobacco and Main-Stream Cigarette Smoke

Sevin Added, γ	Absorbance of Standard[a] Alone	And 25 grams green tobacco	And 25 grams flue-cured tobacco	And 90 cigarette smoke concentrate
0	0.00	0.05	0.09	0.21[b]
10	0.18	0.23	0.19	...
20	0.37	0.42	0.38	...
25	0.46	...	...	0.51[c]
30	0.55	0.60	0.57	...
40	0.75	0.80	0.76	...
50	0.93	0.98	0.95	0.61[c]
75	...	...	...	0.83[c]

[a] Values shown are means of three determinations.
[b] No Sevin peak at 575 mμ.
[c] Positive Sevin peak at 575 mμ.

Table IV. Sevin Residues on Green and Flue-Cured Tobacco[a,b]

Location	Dosage and Formulation/Acre	Interval from Last Application to Sampling, Days	Sevin on Tobacco, P.P.M. Green	Cured	Recovery after Flue-Curing, %
Greenville	1-85%WP	1	132.4	13.7	11.0
		4	29.4	...	...
		8	12.8	...	...
		11	10.1	...	...
		15	10.1	...	...
Rocky Mt.	1-36M	4 hr.	154.4	...	...
Rocky Mt.	1-85%WP	4 hr.	158.2	...	...
Greenville	1-36M	4 hr.	132.4	15.8	12.0
Greenville	1-85%WP	4 hr.	158.2	15.3	9.7
Clayton	0.8-85%WP	4 hr.	114.3	14.5	12.7

[a] Corrected for: Av. background of apparent Sevin for check green and flue-cured tobacco of 0.1 and 0.37 p.p.m.
[b] P.p.m. reported on dry-stemless basis.

ture of 0.1% of ammonium chloride and 0.2% of phosphoric acid which coagulated much of the tobacco constituents, but left the Sevin residue in solution (*6*). Two milliliters of 12*N* HCl were added, the mixtures shaken, and allowed to stand for 1 hour. The coagulant mixtures were filtered into separatory funnels through 30-ml. capacity glass Büchner funnels packed with Hyflo Super Cel previously wet with fresh coagulating mixture. The flasks and funnels were rinsed with 50 ml. of 10% methanol. The filtered aqueous mixtures were extracted three times with 50-ml. portions of methylene chloride. The methylene chloride extracts were combined and concentrated, as previously described, to approximately 5 ml. The concentrates were chromatographed on 18-mm. (inside diameter) columns using 21-cm. beds of Florisil. The columns were eluted with 5% acetone in methylene chloride. The first 20 ml. from the columns were discarded and the next 50 ml. were collected. The eluates were concentrated to 5 ml. under vacuum and rechromatographed on 10-cm. beds of acid alumina (Woelm). The alumina columns were eluted with 25% acetone in methylene chloride, discarding the first 50 ml. and collecting the next 150 ml.

Color Development. Eluates from the alumina columns were concentrated under vacuum to approximately 2 ml., placed in a 40° C. water bath, and taken to dryness under a gentle stream of nitrogen. After the addition of 2 ml. of 1*N* KOH in methanol, the reaction mixtures were allowed to stand for 2 minutes. One-milliliter portions of fluoborate indicator (10 mg. per 25 ml. of CH_3OH) were added and the developing blue dye transferred to 10-ml. volumetric flasks, diluted to volume with methanol, and read as soon as possible at 575 mμ on a Spectronic 20 Bausch and Lomb.

Flue-Cured Tobacco. Extraction. Twenty-five-gram subsamples of chopped flue-cured tobacco were blended with 250 ml. of hexane (redistilled over metallic sodium). The hexane filtrates were reduced in volume to approximately 5 ml. under vacuum and were taken to dryness under nitrogen as previously described. Residues were taken up with 10 ml. of acetone and dewaxed (*2*). The dewaxed, flue-cured tobacco samples were then subjected to the cleanup and color development procedures previously described for green tobacco samples.

Main-Stream Cigarette Smoke. Cleanup. The acetone-smoke samples were concentrated and dewaxed using the procedures previously described under green and flue-cured tobacco. The dewaxed concentrates were taken to dryness under nitrogen and transferred to separatory funnels with 50 ml. of acetonitrile and diluted to 350 ml. with water. The aqueous mixtures were extracted three times with 50-ml. portions of petroleum ether (saturated with acetonitrile). The petroleum ether extracts were combined, passed through anhydrous sodium sulfate, and concentrated under vacuum to approximately 5 ml. The concentrates were coagulated and chromatographed as previously described for green tobacco samples. The samples were then freed from interfering naturally occurring phenolic compounds. This was accomplished by transferring the nitrogen-dried eluates from the acid alumina columns into 50 ml. of methylene chloride and washing three times with 50-ml. portions of 10% aqueous methylamine. Traces of methylamine were removed from the methylene chloride by washing twice more with 25 ml. of water. The methylene chloride solutions were dried with anhydrous sodium sulfate. Color was developed as previously described.

Results and Discussion

In order to facilitate detection of the Sevin residue that might be present on green and flue-cured tobacco and mainstream cigarette smoke, standard curves were prepared by applying the foregoing procedures to a series of insecticide-free green and flue-cured leaf tobacco samples and to acetone extracts of smoke from insecticide-free cigarettes to which were added 0, 10, 20, 30, 40, and 50 γ of authentic Sevin.

Table III shows results of these analyses. Recoveries of known amounts of Sevin added prior to extraction averaged 92% for green tobacco, 92% for flue-cured tobacco, and 55% for cigarette smoke. The apparent Sevin background or check value for green and flue-cured tobacco amounted to 0.1 and 0.37 p.p.m. based on 25-gram samples. The apparent Sevin background or check value amounted to 0.49 γ per "smoked" cigarette for smoke samples.

Leaf Studies. Leaf samples were taken from tobacco plots utilized for insect control and flavor evaluation studies (*3*). One series of plots was primed 1, 4, 8, 11, and 15 days after treatment. The others were sampled only at the 1-day interval. Results of the analysis of green samples have been previously published (*3*) and the flue-cured results are shown in Table IV. These data show the field dissipation of residue due to weathering and that 9.7 to 12.7% of the Sevin residue remains after flue-curing.

Smoke Studies. Experimental cigarettes manufactured from a blend of insecticide-free, flue-cured burley and aromatic tobaccos were infiltrated with acetone solutions of Sevin. The acetone was evaporated from the cigarettes by air-

Table V. Recovery[a] of Sevin from Main-Stream Smoke of Treated Cigarettes

Cigarette, γ/Cigarette	Smoke, γ/Smoked Cigarette[b]	Recovery, %
Sevin		
50	0.50[c,d]	1.0
100	1.06[c,d]	1.0
150	1.81[c,d]	1.2

[a] Values shown are means of three determinations.
[b] Corrected for: Av. background of apparent Sevin for check smoke of 0.49 γ/smoked cigarette.
[c] Positive absorbance value, positive Sevin peak (blue) at 575 mμ.
[d] Based on smoke from 90 cigarettes.

drying for 48 hours. Analysis of the cigarette tobacco indicated that Sevin was not dissipated during this presmoking period.

Sevin was added to a series of cigarettes to produce levels of approximately 50, 100, and 150 γ of Sevin per cigarette. These sets of treated cigarettes were smoked as previously described (*2*) and the acetone-trapped smoke was analyzed for Sevin content. Table V shows approximately 1% recovery of Sevin from main-stream smoke of Sevin-treated cigarettes. It is highly probable that much of the Sevin is converted to a volatile material with loss of the detecting functional group. Future studies in this area are contemplated.

Conclusions

Residue studies with Sevin on tobacco indicate that of the total residue on green tobacco at priming time, only 12% was detectable after flue-curing. Residue recovered from main-stream cigarette smoke was approximately 1%.

To place these findings on practical terms in relation to Sevin usage in the field, as an insecticide, for the control of tobacco insects and the amount of Sevin residue reaching the smoker, the following conclusions have been made:

The residue load on tobacco sprayed at the recommended level (1 pound per acre) of Sevin, primed the same day as treated and flue-cured, approximated 15.2 p.p.m. of Sevin. Residual Sevin levels four times greater than these would be required to produce any measurable contamination of main-stream cigarette smoke. Priming tobacco 1 week after treatment would increase this margin by a factor of 10.

Thus, it is felt that Sevin can be used for insect control on flue-cured tobacco and produce cigarettes with essentially no detectable Sevin in main-stream smoke.

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FEED ADDITIVE RESIDUES

Determination of 3,5-Dinitro-*o*-toluamide (Zoalene) in Chicken Tissues

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An analytical procedure for the determination of zoalene in chicken tissues is described. Zoalene is extracted from the tissue with acetone and benzene, and chromatographed on an alumina column. The compound is determined colorimetrically by the addition of 1,3-diaminopropane in the presence of dimethylformamide. The average recovery from muscle tissue over the range of 0.1 to 4.0 p.p.m. was 77 ± 4%, and from liver tissue over the range of 0.2 to 4.0 p.p.m. it was 86 ± 5%.

ZOALENE (3,5-dinitro-*o*-toluamide) is a new drug which is effective in preventing caecal and intestinal coccidiosis of chickens. The metabolism of this compound in chickens was investigated in a feeding experiment (*3*) using C^{14}-labeled zoalene ($-C^*ONH_2-C^{14}$). When chickens were sacrificed while on feed containing the radioactive zoalene, two compounds were found in the tissues. The compounds were identified as 3,5-dinitro-*o*-toluamide and the metabolite, 3-amino-5-nitro-*o*-toluamide (ANOT).

Methods for the determination of zoalene and for the metabolite, ANOT (*4*), were developed to measure the residue of each compound in chicken tissues. This paper describes the method for the determination of zoalene in chicken tissue.

The determination of zoalene is based on the highly colored complex which the dinitro compound forms with 1,3-diaminopropane in the presence of dimethylformamide. The tissue is extracted with acetone and benzene and the extracts are combined in a separatory funnel and shaken. After the solution has separated into two phases, the aqueous phase is discarded. The acetone-benzene phase is concentrated and taken up in chloroform. The chloroform solution is passed through an alumina column which retains the zoalene but allows the fat and some other extraneous matter to pass through. The compound is eluted from the column with 80% ethyl alcohol which is subsequently evaporated to dryness. The residue is dissolved in a mixture of ethyl alcohol and dimethylformamide