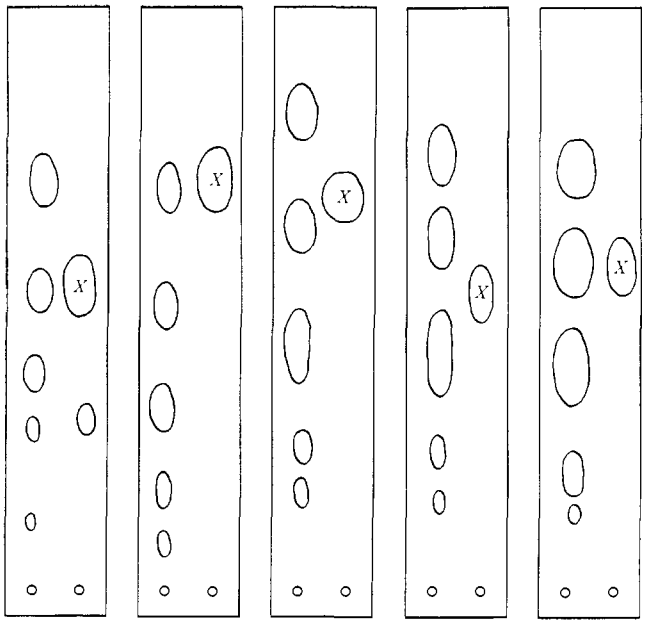

Figure 1. Mobilities of carbamate insecticides as observed in the chromatographic system

Approximately 0.5 hour is required for the solvent front to reach 15 cm. from the origin. The solvent is allowed to evaporate from the chromatogram and the dry chromatogram is sprayed with a 15% (w./v.) aqueous potassium hydroxide solution. The sprayed strip is then held over steam for 1 minute, followed by spraying with 1*N* acetic acid in methanol. After drying under an infrared lamp, a 0.1% (w./v.) methanolic *p*-nitrobenzenediazonium fluoborate solution is sprayed on the strip. The orange to red spot is intensified further by spraying with 15% (w./v.) aqueous sodium hydroxide solution. Gordon (*1*) has shown that a system of marker dyes gives convenient and reproducible reference positions. Thus a mixture of dyes, as described by Gordon (*1*), is also pipetted on the chromatogram prior to development. Spot movements are defined in relation to the standard dyes run simultaneously on the same strip. The dyes used in this chromatographic system are obtained from the coupling of diazotized 4-amino-2,5-diethoxybenzanilide plus 2-naphthol; diazotized 4-amino-2,5- diethoxybenzanilide plus 2-anilinoethanol; diazotized 4-benzoylamino - 2,5 - dimethoxyaniline, (Fast Blue RR), plus 2-anilinoethanol; and tetrazotized *o*-dianesidine (Naphthanil Diazo Blue B), plus 2-anilinoethanol. These dyes were used without further purification.

Figure 1 indicates the mobilities of the carbamate insecticides observed in the chromatographic system. Ten micrograms of each of the carbamates are easily detected.

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INSECTICIDE DETERMINATION

Diazotized Sulfanilic Acid Reagent for Endrin Analysis

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The colorimetrically undesirable side reaction between excess sulfanilic acid and diazotized sulfanilic acid can be prevented by addition of an excess of sodium nitrite, which is then destroyed by addition of ammonium sulfamate.

IN THEIR method for "Determination of Endrin in Agricultural Products and Animal Tissues," Bann and coworkers (*1*) employed a coupling reagent, prepared by mixing equal volumes of 0.5% sulfanilic acid in 50% acetic acid and 0.05% sodium nitrite solution. The reagent prepared in this manner contains a quantity of sulfanilic acid equal to 3.6 times the chemical equivalent of the sodium nitrite used. In our laboratory, diazotized sulfanilic acid was found to react with excess sulfanilic acid present, resulting in a strong background color. The intensity of the background color varied with the age of reagents and temperature.

Laboratory studies showed that an excess of sodium nitrite would diazotize sulfanilic acid completely, thus preventing the color-forming reaction. Further tests showed that if a slight excess of sodium nitrite was used in the diazotization of sulfanilic acid and the excess destroyed with ammonium sulfamate, there was little or no background color from the color-forming reagents.

Diazotized sulfanilic acid reagent is prepared as follows:

0.25% sulfanilic acid. Dissolve 0.25 gram of sulfanilic acid in 100 ml. of 60% glacial acetic acid in distilled water. Warm to 50° C. to aid solution of the acid.

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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