Analysis of Guthion Insecticide

C. J. COHEN, W. R. BETKER, D. M. WASLESKI, and J. C. CAVAGNOL
Research Department, Chemagro Corp., Kansas City, Mo.

Guthion has been analyzed by three methods: caustic hydrolysis to form anthranilic acid which is diazotized, coupled with *N*-(1-naphthyl)-ethylenediamine, and the resultant purple color measured at 555 mμ; caustic hydrolysis to form the sodio O,O-dimethyl phosphorodithioate which is complexed with cupric ion in acid and the absorbance measured at 420 mμ; and infrared measurement of P=S stretching vibration at 654 cm.$^{-1}$ (15.25 microns). These methods of analysis are discussed and compared.

GUTHION, [*O,O* - dimethyl *S* - 4-oxo - 1,2,3 - benzotriazin - 3-(4*H*)-ylmethyl phosphorodithioate] is a broad spectrum insecticide which is effective against Coleoptera, Diptera, Homoptera, Hemiptera, Lepidoptera, and various mite species. It is in the same oral toxicity range as parathion, but has a safety factor of lower dermal and inhalation toxicity. Guthion, also known as BAY 17147 and Gusathion, has been marketed as spray concentrates, a wettable powder, and a dust.

The technical material is a brown waxy solid with a melting point of approximately 65° to 68° C., but in the pure state is white and melts at 73° to 74° C. It is soluble in most organic solvents except aliphatic hydrocarbons.

In choosing a suitable method for production control, several routes appeared promising. Wollenberg and Schrader (*9*) described a colorimetric method using phenyl-1-naphthylamine in an acid medium. Giang has hydrolyzed Guthion in base to give sodio *O,O*-dimethyl phosphorodithioate (*3*) from which a yellow copper complex has been formed (*6*). Giang and Schechter (*1*) reported a method in which Guthion was treated with acid to form formaldehyde which was determined colorimetrically with chromotropic acid. However, this method was not intended for production control. Bratton and Marshall (*2*) reported that sulfanilamide was diazotized and coupled to form a purple complex. Similarly, anthranilic acid also forms a purple complex. In addition to these methods, an infrared procedure has been investigated in the authors' laboratory.

Experimental

Apparatus. Buret, automatic filling, Teflon stopcock, 50 ml.

Cells, round or rectangular, borosilicate glass, 2 cm.

Colorimeter, capable of measuring at 420 mμ, 2-cm. path.

Paper, filter, Whatman No. 541, 11 cm.

Shaker, mechanical.

Spectrophotometer, infrared, capable of scanning 14.3 to 16.0 microns.

Timer, with minute and second hands.

Reagents. Buffer Solution No. 1. Add reagents in order described: In 4 liters of water dissolve 815 grams of ammonium acetate. Add 400 ml. of concentrated hydrochloric acid and mix thoroughly. Dilute to 8 liters and mix.

Buffer Solution No. 2. Proceed as for Buffer No. 1 except add 0.80 gram of potassium bromate before diluting to 8 liters and mixing.

Copper Sulfate Pentahydrate, 23% solution, aqueous.

1,2-Dimethoxyethane, b.p. 83° to 85° C.

Ethanol, 2B anhydrous.

Guthion, m.p. 73° to 74° C. (Chemagro Corp., Kansas City, Mo.). Crude technical material was crystallized from benzene, then from methanol after charcoal treatment, and finally from methanol.

N-(1-naphthyl)-ethylenediamine Dihydrochloride. Dissolve 1 gram in 100 ml. of distilled water, add 2 grams of charcoal, shake, and filter. Prepare fresh daily.

Sodium hydroxide, 3.0*N* in methanol (120 grams NaOH/liter), carbonate-free.

Procedure

Anthranilic Acid Method. Dissolve a 0.4-gram sample of Guthion in 25 ml. of dioxane in a 250-ml. volumetric flask and dilute to volume with 2-propanol. Pipet a 5-ml. aliquot into a 100-ml. volumetric flask and dilute to volume with 2-propanol. Pipet a 5-ml. aliquot of this last dilution into a 100-ml. volumetric flask, add 5 ml. of 1*N* sodium hydroxide, and let the solution stand 20 minutes after mixing. Acidify with 6 ml. of 3*N* hydrochloric acid, add 1 ml. of 0.25% sodium nitrite solution and let the solution stand 10 minutes. Destroy the excess sodium nitrite with 1 ml. of 5% ammonium sulfamate. After 10 minutes, add 4 ml. of *N*-(1-naphthyl)-ethylenediamine dihydrochloride solution, swirl, and let the solution stand 25 minutes to permit color development. Dilute to volume and read the absorbance at 555 mμ after 5 minutes against a reagent blank. Prepare a calibration curve using recrystallized Guthion.

Infrared Method. Weigh accurately the equivalent of 0.4 gram of Guthion into a 100-ml. volumetric flask, dissolve, and dilute to volume with dimethoxyethane. Mechanically shake 25% wettable powder for 5 minutes with 50 ml. of dimethoxyethane. Fill a 0.5-mm. KBr infrared absorption cell and scan the spectrum from 700 to 625 cm.$^{-1}$ (14.3 to 16.0 microns). Measure the peak height at 654 cm.$^{-1}$ (15.25 microns) using a base line drawn between the minima near 14.3 and 16.0 microns (700 and 625 cm.$^{-1}$). Prepare a calibration curve from the absorbances of a series of standard solutions. For spray concentrates, the standard solutions must contain the proper amount of spray concentrate ingredients.

Copper Complex Method. Weigh accurately the equivalent of 0.12 gram of Guthion into a 100-ml. volumetric flask, add about 70 ml. of ethanol, and swirl until dissolved. Mechanically shake 25% wettable powders for one hour. Dilute to volume with ethanol and mix thoroughly. Pipet 5 ml. into a 100-ml. volumetric flask and dilute to volume with methanol. Mix thoroughly. Pipet 10 ml. into a 250-ml. separatory funnel to which 10 ml. of methanol has been added.

From this point, start samples individually at five-minute intervals. Pipet 3.0 ml. of 3.0*N* methanolic NaOH into the separatory funnel. Stopper and swirl to assure mixing but do not allow stopper to be wetted. Let stand 10 minutes. Add 100 ml. of buffer solution No. 2 with a graduate, shake vigorously to assure mixing, and allow to stand for 20 minutes (decomposed samples require

further handling as shown later). From an automatic filling buret, add exactly 50 ml. of chloroform. (The total time allowed after addition of copper sulfate until the reading of the solution must not exceed 2.0 minutes.) Add 10 ml. of copper sulfate solution and immediately shake vigorously for 30 seconds. Allow layers to separate and filter the chloroform layer through a Whatman 541 filter into a 2-cm. cell and read immediately at 420 mμ against a blank of chloroform.

With decomposed samples or samples of very low per cent Guthion, additional oxidizing agent may be required. Proceed as follows:

Carry out the copper complex procedure but use an appropriate amount of buffer solution containing no potassium bromate (buffer solution No. 1). Prepare separately a 1% solution of potassium bromate. Add 100 ml. of buffer solution No. 1 and 1 ml. of bromate solution. To another aliquot of the sample repeat the procedure, but add 3 ml. of bromate solution. If the absorbance of this aliquot is the same or lower than the first disregard it and use the original value. If the second value is higher, repeat the procedure using 5 ml. of bromate solution. This procedure is repeated until a maximum absorbance value is obtained.

Standard for Copper Complex Method. Weigh accurately between 0.11 and 0.13 gram of purified Guthion into a 1-liter volumetric flask, add 100 ml. of ethanol, and swirl to dissolve. Dilute to volume at 25.0° C. with methanol and mix thoroughly. Pipet 4-, 5-, and 6-ml. aliquots into 250-ml. separatory funnels containing 16, 15, and 14 ml. of methanol, respectively. Prepare two sets of these aliquots, and run one set at the beginning and the second at the end of the run. The corresponding absorbances are averaged and plotted against milliliters aliquoted, in such a manner that three significant figures can be read without interpolation of graph divisions.

Calculations

$$K = \frac{\text{wt. of std.} \times 200 \times 100}{1000 \text{ ml.}} = \frac{\text{wt. of std.} \times 20}{}$$

$$\text{Guthion, \%} = \frac{\text{ml. from graph}}{\text{wt. of sample}} \times K$$

Discussion

The first method used for the quality control of Guthion was alkaline hydrolysis to form anthranilic acid followed by diazotization and coupling (*1*) with *N*-(1-naphthyl)-ethylenediamine dihydrochloride as shown in Figure 1. The purple complex has a maximum absorbance at 555 mμ.

The time of hydrolysis was investigated by hydrolyzing a sample of Guthion for 5, 10, 15, 30, and 60 minutes. The hydrolysis was essentially complete after 10 minutes. Maximum color development was obtained using 5 ml. of 1*N* sodium hydroxide and 6 ml. of 3*N* hydrochloric acid. The time necessary for the diazotization of the anthranilic acid formed was studied and although diazotization was complete in 2 minutes, the use of 10 minutes gave the best reproducibility in subsequent studies. Use of 2.5 and 5% solutions of ammonium sulfamate which destroys the excess nitrous acid was investigated. The 5% solution gave better reproducibility.

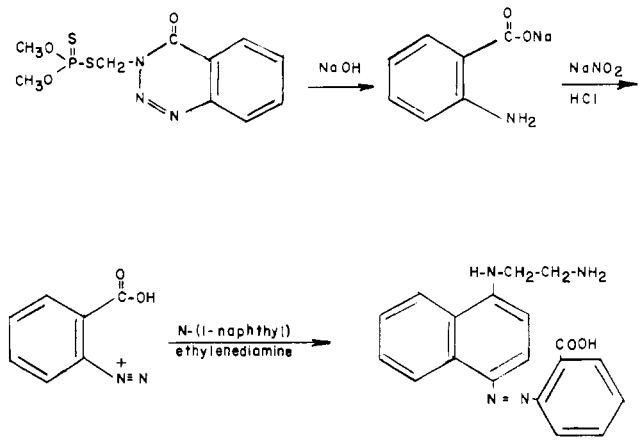


Figure 1. Anthranilic acid method

A 1% solution of *N*-(1-naphthyl)-ethylenediamine was chosen because lower concentrations gave inconsistent color development. Better reproducibility was obtained when the *N*-(1-naphthyl)-ethylenediamine solution was cleaned up with charcoal and prepared fresh daily. Also, a deeper color was developed when the solutions were undiluted during development time. Maximum color was developed by permitting the solution to stand for 25 minutes, then diluting to volume and reading the solutions after 5 minutes.

Benzene, ethanol, ether, and dioxane were evaluated with respect to the speed of dissolution of technical Guthion. Dioxane was the best of these solvents and was used in the final method.

Several of the known impurities previously isolated and identified in technical Guthion (*8*) were analyzed by the anthranilic acid method. These compounds and the extent of their interference on a molar basis are shown in Table I. The numbers represent the contribution as apparent per cent Guthion when an equivalent molar quantity of the compound is present as an impurity. A reproducibility study showed the 95% confidence interval to be ±2.5% relative (*5*).

The method described above is subject to interferences as shown in Table I, and infrared analysis was investigated as a more specific technique. Three peaks looked promising: 9.8 (P—O—C), 12.2 (unassigned), and 15.25 (P=S) microns as shown in Figure 2. The infrared scan of a sample that had been heat decomposed showed that the 15.25 (654 cm.$^{-1}$) peak had completely disappeared. This peak also had the flattest base line and, therefore, was chosen as the best peak for analytical purposes. The compounds in Table I were investigated for possible interference at 15.25 microns along with sodio *O,O*-dimethyl phosphorodithioate and its free acid. Of these compounds, only anthranilic acid,

Table I. Interference of Impurities in Anthranilic Acid Method

Compound	Interference Per Mole, %
Anthranilic acid	100 (standard)
Benzazimide	0.0
3-(Chloromethyl)benzazimide	100.0
3-(Hydroxymethyl)benzazimide	0.0
3-(Mercaptomethyl)-benzazimide	30.6
3-Methyl benzazimide disulfide	23.0
3-Methyl benzazimide sulfide	39.1
3-Methyl benzazimide	90.0
Oxygen analog (*O,O*-dimethyl *S*-4-oxo-1,2,3-benzotriazin-3(4*H*)-ylmethyl phosphorothioate)	100.0
O,O,S-Trimethyl phosphorodithioate	0.0

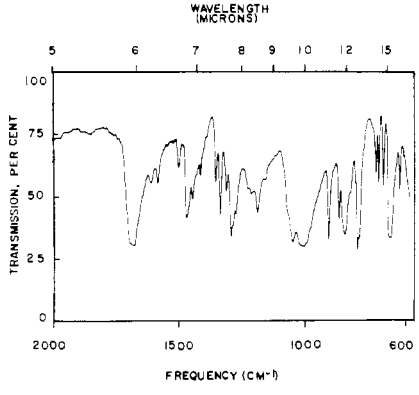


Figure 2. IR spectrum of Guthion

O,O,S-trimethyl phosphorodithioate, and sodio *O,O*-dimethyl phosphorodithioate interfered. However, these compounds are present only in trace amounts in technical Guthion.

Several solvents were investigated. Acetone, dioxane, and acetonitrile did not give linear plots when absorbance was plotted against concentration, but carbon disulfide, ether, and dimethoxyethane were satisfactory in this respect. The 95% confidence limit of the method is ±2.0% relative.

Another method, a variation of the malathion procedure (*3*), adapted for the control analysis of Guthion, was based on the caustic hydrolysis of Guthion to sodio *O,O*-dimethyl phosphorodithioate, the acidification to the acid, and the formation of a copper complex which was extracted into an organic solvent, as shown in Figure 3. The complex had an intense yellow color with a maximum absorbance at 420 mμ in chloroform. This solvent was used because it readily extracted the complex from water, and its density afforded ready separation from the aqueous phase.

The effect of pH on the formation and extraction of the complex was investigated by adding measured amounts of acetic acid (hydrochloric acid for lower pH's) and aqueous ammonia to the hydrolyzate. The resultant pH was measured on a standard pH meter after adding copper sulfate and extracting with chloroform (Figure 4). The upper portion of this curve may be considered as two plateaus—from pH 1.75 to 2.5 and pH 4.5 to 5.25. The procedure was modified to include a buffer so that the pH of the hydrolyzate had a pH 2.1 at the extraction step. The use of this buffer gave reproducible results. However, when copper sulfate was omitted from the procedure (essentially a sample blank), absorbances equivalent to 1 to 3% of the apparent Guthion in the sample were found. These blank values were too high and variable to permit use of this procedure as a method.

The blank value could be reduced to 1% consistently if the hydrolysis solution was treated with 4.0 ml. of ammonium hydroxide and was permitted to stand for 20 minutes before acidification. However, the addition of concentrated aqueous ammonia necessitated a lengthy titration with standardized hydrochloric acid to attain the desired pH, which was considered impractical for a routine method.

The 4.5 to 5.25 pH plateau of the pH-absorbance curve was also investigated. After hydrolysis, 5 ml. of concentrated hydrochloric acid and 15 ml. of 68% ammonium acetate were added to attain a pH of 4.65. Absorbance values at this pH were reproducible. Sample blank values amounted to 0.5%.

A sample of Guthion, formulated as a wettable powder (27.1% by infrared analysis), gave 24.3% by this revised procedure. As reducing materials present in the hydrolyzate can convert cupric ion to the cuprous form and thereby reduce complexation, the use of oxidizing agents was investigated. Doubling and halving the amount of ferric chloride recommended by Ware for the determination of malathion (*7*) had no effect on the value obtained; omission of ferric chloride had no effect. Such reagents as sodium hypochlorite and iodine immediately consumed the dithio acid formed (no color formation). Even less than equivalent amounts of these reagents reacted immediately to oxidize proportional amounts of acid present.

In the analysis of Guthion, 25% wettable powder formulations, 1 ml. of a 1.0% aqueous solution of potassium bromate added to the acidified hydrolyzate increased the absorbance by 8%. Figures 5 and 6 show the effect of various amounts of the 1.0% potassium bromate solution on formulations. Because concentrations of Guthion are not the same, absorbances between samples cannot be compared directly. Since the addition of potassium bromate solution in the analysis of purified Guthion gives an increase in absorbance, at least a portion of the reducing interfering compounds are formed by the hydrolysis of Guthion. The addition of 1 ml. of 1% potassium bromate solution was incorporated into the procedure. A test procedure using various amounts of potassium bromate solution to determine maximum absorbance is suggested for badly decomposed samples.

All available known impurities and hydrolysis products of Guthion (*8*) were evaluated for interference in the method as shown in Table II. A 1-ml. aliquot of a 0.1% ethanolic solution (about twice the Guthion concentration) of the compounds was added to an aliquot of pure Guthion calculated to give an absorbance of 0.350. Potassium bromate solution was omitted from the procedure.

A negative interference was found when the thiol isomer [*O,S*-dimethyl *S*-4-oxo-1,2,3-benzotriazin-3(4*H*)-ylmethyl phosphorodithioate], sulfur, sodium sulfide, *O,O,S*-trimethyl phosphorodithioate, and 3-(mercaptomethyl)benzazimide were added. The addition of 5 ml. of 1.0% potassium bromate solution in the procedure eliminated the negative interferences.

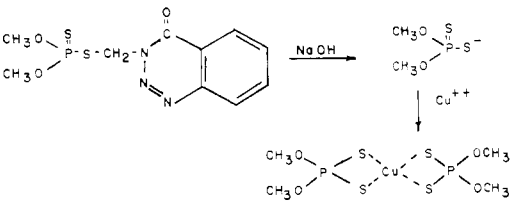


Figure 3. Copper complex method

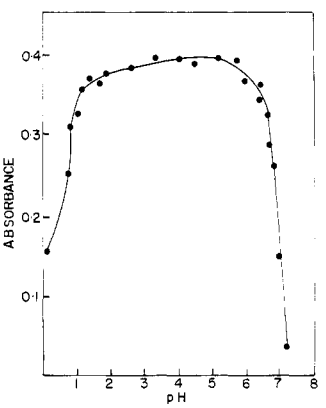


Figure 4. Variation of absorbance with pH

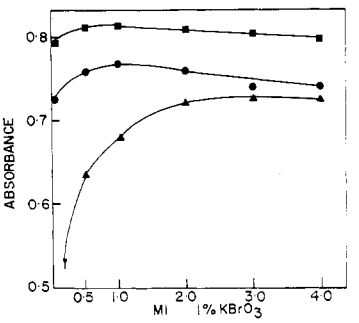


Figure 5. Effect of bromate oxidation on chelate formation, standard and technical

■ Fresh technical
● Standard
▲ Old technical (ca. 30%)

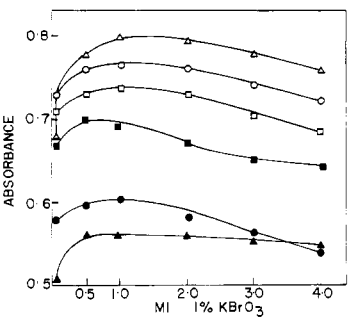


Figure 6. Effect of bromate oxidation on chelate formation, standard and various formulations

△ Fresh spray concentrate
○ Standard
□ Fresh 40% Premix
■ Fresh liquid concentrate
● Fresh 25% wettable powder
▲ Old 25% wettable powder

Table II. Effect of Impurities, Decomposition and Hydrolysis Products on Copper Complex Method

	Abs.
Guthion	0.350
Compound added (2 × Guthion concn.)	
Anthranilic acid	0.342
Benzazimide	0.347
Benzimide	0.349
Benzoic acid	0.350
3-(Chloromethyl)benzazimide	0.348
Desmethyl Guthion, sodio *O,O*-methyl *S*-4-oxo-1,2,3-benzotriazin-3(4*H*)ylmethyl phosphorodithioate	0.343
Dimethyl disulfide	0.352
Formaldehyde	0.350
3-(Mercaptomethyl)benzazimide	0.295
Methyl anthranilate	0.349
Methyl benzazimide disulfide	0.347
Methyl benzazimide sulfide	0.350
N-Methyl anthranilic acid	0.345
O,O,S-Trimethyl phosphorodithioate	0.331
Oxygen analog of Guthion	0.362
Phosphoric acid	0.351
Salicylic acid	0.351
Sodium sulfide	0.320
Sulfur	0.222
Thiol isomer of Guthion	0.230

The oxygen analog [*O,O*-dimethyl *S*-4-oxo-1,2,3-benzotriazin-3(4*H*)-ylmethyl phosphorothioate] gives a slight positive interference. However, since the concentration used was twice that of the Guthion the interference would be negligible—i.e., a sample containing 10% oxygen analog would contribute only 0.2% positive error.

Table III. Reproducibility—25% Wettable Powder

	A_1	A_2	A_3
	26.0	26.9	(24.5)[a]
	26.8	26.6	26.9
	26.7	26.0	26.0
	27.0	26.1	26.2
	26.6	26.7	26.1
	26.3	26.8	26.2
	26.7	26.0	27.0
	26.4	26.7	26.7
Average	26.6	26.5	26.4

Over-all 2 σ actual = ±0.24
2 σ relative = ±0.96%

[a] Statistically eliminated.

Table IV. Comparison of Guthion Values by the Three Methods

Anthranilic Acid	Copper Complex	Infrared
86.0	80.0	81.5
89.6	81.0	79.9
91.7	81.7	79.6
90.2	82.1	84.1
88.6	81.4	83.3
89.6	79.7	78.9
95% confidence limits		
±2.1%	±1.0%	±2.0%

A sample of Guthion, 25% wettable powder, was submitted to the control laboratory for replicate analyses. The studies involved three analysts over a period of 30 days, and the results are shown in Table III. A comparison of results by the three methods on technical samples is shown in Table IV.

Literature Cited

(1) Averell, P. R., Norris, M. V., *Anal. Chem.* **20,** 753 (1948).
(2) Bratton, A. C., Marshall, E. K., *J. Biol. Chem.* **128,** 537 (1939).
(3) Giang, P. A., U. S. Dept. of Agr., Beltsville, Md., personal communication, August, 1956, Chemagro Rept. No. **1252.**
(4) Giang, P. A., Schechter, M. S., J. AGR. FOOD CHEM. **4,** 522 (1956).
(5) Kane, P. F., "Evaluation of Colorimetric Analysis," Chemagro Rept. No. **2194.**
(6) Norris, M. V., Vail, W. A., Averell, P. R., J. AGR. FOOD CHEM. **2,** 570 (1954).
(7) Ware, J. H., American Cyanamid Co., "Method for Determining Malathion."
(8) Wasleski, D. M., "Investigation of the Composition of Technical Guthion," Chemagro Rept. No. **9319.**
(9) Wollenberg, O., Schrader, G., *Angew. Chem.* **68,** 41 (1956).

Received for review September 20, 1965. Accepted March 3, 1966. Division of Agricultural and Food Chemistry, 149th Meeting, ACS, Detroit, Mich., April 1965.

ANALYTICAL METHOD

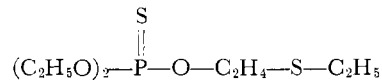
Determination of the Isomer Ratio of Systox

W. R. BETKER, C. J. COHEN, N. J. BEABER, and D. M. WASLESKI

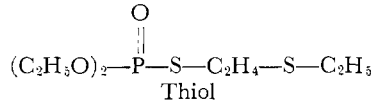
Research Department, Chemagro Corp., Kansas City, Mo.

The isomer ratio of Systox [demeton, O,O-diethyl O(and S)-2-(ethylthio)ethyl phosphorothioates] can be calculated by determining the total ester content by alkaline hydrolysis and the thiol isomer content by an iodometric titration of the mercaptan formed upon alkaline hydrolysis. The method is applicable to both technical and formulated materials. Sulfotepp (tetraethyl dithionopyrophosphate) will interfere if present and a method for determining Sulfotepp is described.

SYSTOX (demeton) is a systemic insecticide composed of two isomers, *O,O*-diethyl *O*-2-(ethylthio)ethyl phosphorothioate (thiono isomer), and *O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorothioate (thiol isomer).



Thiono



Thiol

The ratio of thiono to thiol isomer in technical Systox normally varies between 2 to 1 and 1 to 1. There is a difference in the chemical and biological properties of the two isomers, the thiol being three times more water-soluble (*1*) and more toxic than the thiono compound; the acute oral LD_{50} to male rats is 7.5 mg. per kg. for the thiono isomer and 1.5 mg. per kg. for the thiol isomer (*11*). It is, therefore, of importance to know the isomer ratio.

The isomerization of thiono to thiol isomer has been studied by several workers. Henglein and Schrader (*5*) in 1955 reported that the isomerization was