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-benzo-triazin-3(4H)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(4H)-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 con-

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%

taining 10% oxygen analog would contribute only 0.2% positive error.

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.

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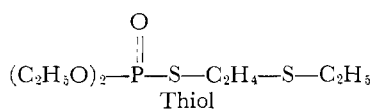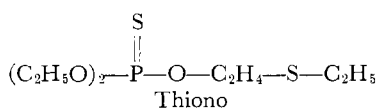(9) Wollenberg, O., Schrader, G., Angew. Chem. **68,** 41 (1956).

ANALYTICAL METHOD

Determination of the Isomer Ratio of Systox

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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).

$$(C_2H_5O)_2{-}\overset{\overset{S}{\|}}{P}{-}O{-}C_2H_4{-}S{-}C_2H_5$$
Thiono

$$(C_2H_5O)_2{-}\overset{\overset{O}{\|}}{P}{-}S{-}C_2H_4{-}S{-}C_2H_5$$
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 (7) 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

zero-order in the 0 to 50% isomerization range and first-order in the 50 to 100% isomerization range. Fukuto and Metcalf (3) in 1954 using P[32]-labeled Systox and paper chromatography to separate the isomers, reported a first-order reaction over the entire range. Muller and Goldenson (10) in 1956 applied NMR to study the isomerization and reported the reaction to be intermediate between first- and second-order.

Figure 1 shows the isomer ratio change of three samples of technical Systox of different initial isomer ratios kept at room temperature for one year. Infrared was used to determine the thiol isomer and the total Systox was determined by caustic hydrolysis. The thiono isomer was calculated as the difference between the thiol and total Systox and plotted logarithmically on the ordinate. Time in months is plotted linearly along the abscissa. The straight-line relationship indicates that the isomerization is first-order—i.e., a function of thiono isomer concentration.

In the manufacturing process the thiono isomer is synthesized and then isomerized by heating to an isomer ratio of 2 parts of thiono to 1 part of thiol. Heat greatly increases the rate of isomerization and at 120° C. the thiono isomer is completely converted to the thiol isomer in about 4 hours (3). Therefore, a rapid, simple method was sought for process control.

Henglein, Schrader, and Mühlmann (5, 6) and Hensel and Kessler (7) have described infrared measurements of the P=S and P=O absorption bands to calculate the isomer ratio. For technical Systox, a similar method has been used, which consists of determining the thiol isomer concentration by measuring the absorption of the P=O stretching band at 8.0 microns and determining the total Systox by hydrolysis. The difference between the thiol value and the total Systox value gives the thiono isomer concentration. When this method was applied to spray concentrates of Systox, interference from the emulsifiers was encountered.
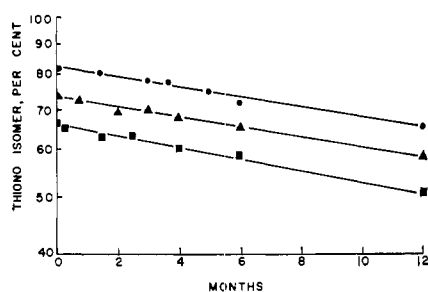
Hensel and Kessler (8) observed that the thiol isomer hydrolyzed much more rapidly at room temperature than the thiono isomer and described a method based on the different rates of cold alkaline hydrolysis. The thiol isomer calculated by the authors by this method was consistently higher than by infrared. Interferences from hydrolyzable impurities such as Sulfotepp account for this difference. The 5 hours needed for hydrolysis makes this method too long to be usable as a process control method.

The thiol Systox (9) has been determined by hydrolysis in an alkaline lead acetate solution, extracting the lead salt of the liberated mercaptan $Pb(SC_2H_4-SC_2H_5)_2$ with chloroform, acidifying, and determining the lead with EDTA or the mercaptan by iodine titration. The latter variation has been recommended by the Collaborative Pesticides Analytical Committee (2). The purpose of forming the lead salt is to prevent the oxidation of mercaptan to disulfide. However, in the iodometric method, if the liberated mercaptan is titrated within 5 minutes, the loss is negligible. In the present procedure the thiol isomer is determined by an iodometric titration of the mercaptan liberated immediately following a 5-minute hydrolysis in $2N$ methanolic sodium hydroxide.

Groves (4) described a method based on a bromide-bromate oxidation before and after alkaline hydrolysis. The thiono isomer required ten bromine equivalents before and after alkaline hydrolysis, while the thiol isomer required two bromine equivalents before and eight equivalents after alkaline hydrolysis. This method works well and may be used in place of the method described in this paper if a correction is made for Sulfotepp.

Sulfotepp (tetraethyl dithionopyrophosphate), a by-product of the synthesis, was found to be present in Systox, usually in the 1 to 3% concentration range. The presence of Sulfotepp was confirmed by gas chromatography. Sulfotepp is easily hydrolyzed, consuming two equivalents of base. It will also be oxidized by bromide-bromate consuming 16 equivalents. Therefore, all of the methods described need a correction for Sulfotepp. A GLC method is described for determining Sulfotepp in Systox.

Experimental

Apparatus. Gas chromatograph, F & M Model 810 equipped with a thermal conductivity detector and two 6 foot $\times \frac{1}{4}$ inch stainless steel columns packed with Carbowax 20M, 10% on Chromsorb W, 60/80-mesh.

Reagents. Hydrochloric acid, $0.1N$ aqueous, standardized.

Iodine solution, $0.1N$. Dissolve 26 grams of iodine and 50 grams of potassium iodide in 2 liters of water. Standardize against arsenic trioxide.

Sodium hydroxide, $0.1N$ aqueous

Sodium hydroxide, $2N$ in methanol

Sulfotepp

Systox, thiol isomer, 98%

Systox, thiono isomer, 94%

Procedure. TOTAL SYSTOX BY HYDROLYSIS. Weigh to the nearest 0.1 mg. a sample containing 0.5 gram of Systox into a 250-ml. Erlenmeyer flask having a ground-glass joint. Add 20 ml. of isopropyl alcohol and pipet in 50 ml. of $0.1N$ sodium hydroxide. Attach the flask to a reflux condenser and reflux for 1 hour for technical Systox or 4 hours for Systox spray concentrates. Cool, then quantitatively transfer the contents of the flask to a 400-ml. beaker. insert a glass and a calomel electrode system connected to a pH meter, and titrate with standardized $0.1N$ hydrochloric acid until a pH of 6.0 is reached. Titrate until this pH remains constant for at least 3 minutes. Carry a blank through the procedure, omitting the sample. For spray concentrates make a blank consisting of the proper proportions of all spray concentrate ingredients, omitting only the Systox.

FREE ACID. Weigh to the nearest 1 mg. a sample containing 2 grams of Systox into a 250-ml. beaker. Add 25 ml. of isopropyl alcohol and 100 ml. of carbonate-free water and titrate as above.

THIOL ISOMER BY IODINE TITRATION. Weigh to the nearest 1 mg. a sample containing 1 gram of Systox into a 125-ml. iodine flask. Add 25 ml. of $2N$ methanolic sodium hydroxide, stopper, swirl, and allow the flask to stand for 5 minutes. Add 25 ml. of glacial acetic acid and immediately titrate with $0.1N$ iodine solution to the first faint permanent yellow color.

THIOL ISOMER BY INFRARED. Weigh to the nearest 0.1 mg. a 0.3-gram sample of technical Systox into a tared 25-ml. volumetric flask. Dilute with carbon disulfide and reweigh. Calculate the weight per cent, W. Similarly weigh and dilute a sample of pure thiol isomer Systox, W_2. Run the spectrum of each from 7.3 to 8.5 microns in a 0.2-mm. NaCl cell with a cell of equal thickness filled with carbon disulfide in the reference beam. Measure the absorbance of the 7.95-micron peak above a base line drawn between the minima near 7.5 and 8.4 microns. Let the sample absorbance be A and the standard absorbance be B. It has been established that the absorptivity of the thiono isomer at 7.95 microns is 11.2% that of thiol. Therefore, a correction factor is necessary.

SULFOTEPP BY GLC. Make the following settings on the chromatograph:

Column temperature, ° C.	200
Detector temperature, ° C.	200
Injection port temperature, ° C.	200
Bridge current, ma.	200
Recorder, mv.	1
Helium flow, ml./min.	60

Inject 2.00 μl., Y, of pure Sulfotepp and set attenuator to $X32$. Sulfotepp has a retention time of about 6.5 minutes. After the Sulfotepp has cleared the column, inject 2.00 μl., V, of technical Systox or 5.00 μl. of Systox spray concentrates at an attenuation of $X2$. (Systox follows Sulfotepp by about 1 minute.) Measure the areas of the sample, A, and standard, B.

Figure 1. Isomerization of three samples of Systox at room temperature

Initial isomer ratio
● 5 to 1
▲ 3 to 1
■ 2 to 1

Calculations. Free acid, meq./gram =

$$\frac{\text{titer of sample, ml.} \times \text{normality}}{\text{sample weight, grams}}$$

GLC

$$\text{Sulfotepp, } \% = \frac{A \times \text{attenuation} \times Y \times 100 \times \text{specific gravity of Sulfotepp}}{B \times \text{attenuation} \times V \times \text{specific gravity of Systox}}$$

The specific gravities at 20° are: Sulfotepp 1.19; technical Systox 1.14; Systox spray concentrate 0.93.

HYDROLYSIS

$$\text{Hydrolyzables as Systox, } \% = 25.8 \frac{(\text{titer of sample, ml.} - \text{blank, ml.}) \times \text{normality}}{\text{sample weight, grams}}$$

where 25.8 is the millequivalent weight of Systox multiplied by 100 to convert to per cent.

Total Systox, % = hydrolyzables − (25.8 × free acid, meq./gram) − (1.60 × Sulfotepp, %)

where 1.60 is the ratio of the equivalent weights of Systox and Sulfotepp $\left(\frac{258}{161}\right)$.

IODOMETRIC

$$\text{Thiol isomer, } \% = \frac{25.8 \times \text{titer of sample, ml.} \times \text{normality of iodine}}{\text{sample weight, grams}}$$

INFRARED

$$\text{Thiol isomer, } \% = \frac{A \times W_2 \times 112.6}{B \times W_1} - $$

0.1257 × total Systox, %

$$\text{Isomer ratio} = \frac{\text{Systox, } \% - \text{thiol isomer, } \%}{\text{thiol isomer, } \%}$$

Results and Discussion

When known amounts of Sulfotepp were added to Sulfotepp-free Systox and injected into the gas chromatograph, recovery of Sulfotepp was quantitative.

The hydrolysis of the thiol isomer is believed to proceed as follows:

$$(C_2H_5O)_2—P(O)—S—C_2H_4SC_2H_5 + OH^- \rightarrow (C_2H_5O)_2—P(O)—O^- + HSC_2H_4SC_2H_5$$
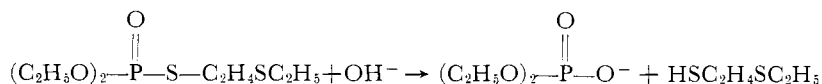
An extraction of the mercaptan as the lead salt is unnecessary, for the mercaptan can be titrated directly with standard iodine solution. The thiono isomer will not interfere, because it will not hydrolyze to give a mercaptan. A plot of per cent hydrolysis *vs.* time is shown in Figure 2.

The thiol isomer hydrolysis is completed in 5 minutes. The apparent interference from the thiono isomer is due to a small thiol contamination in the thiono isomer standard. The downward slope of the curve after 5 minutes is probably due to some air oxidation of the liberated mercaptan to disulfide. Since the sample

is hydrolyzed for only 5 minutes and then titrated immediately, this dropoff is negligible.

To determine isomer ratio by this method, the difference between the total Systox and thiol values is taken as the thiono isomer content. For process control of technical Systox, the total ester value was found to fall consistently between 95 and 100%. Using a total ester value of 97.5%, an isomer ratio determination can be run in 15 minutes.
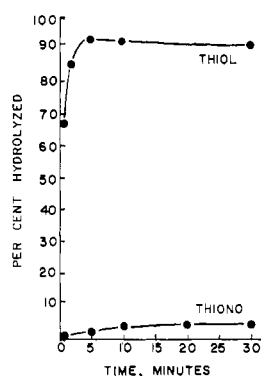

Figure 2. Hydrolysis of Systox with 2N NaOH in methanol

Mixtures of the two purified isomers were prepared and analyzed by this method (Table I). The values in the first two columns have been corrected for the purities of the samples used to make the mixtures as determined by hydrolysis, but the thiono isomer values have not been corrected for thiol content. It is nearly impossible to keep the thiono isomer free of thiol for any length of time and the small positive bias seen in Table I for the technical mixtures is due to a small thiol contamination in the thiono isomer.

Good agreement is shown between thiol added and thiol found. These

data also show that the method will work over an extended isomer ratio range. The iodometric method was reliable for both technical and spray concentrates, and did not require expensive infrared equipment, thus being suitable for use as a plant process control method for monitoring the isomer ratio.

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