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Application of Oscillographic Polarography to Determination of Organic Phosphorus Pesticide Residues*

By RAYMOND J. GAJAN (Division of Food, Food and Drug Administration, Washington 25, D.C.)

Some organic phosphorus pesticides have been determined successfully by polarography (1-7). However, in most cases the methods were used to determine the pesticide in commercial formulations. Little has been reported on the polarographic determination of microgram amounts of these pesticides such as those encountered in pesticide residue analysis.

Many of the methods used for the determination of these pesticides are nonspecific. They are based on cholinesterase assay (8-12), radioactive tracer analysis (13, 14), total phosphorus (15-17), and paper chromatography (18-24).

In this paper we will discuss the polarography of Systox, Di-Syston, and Thimet and some of their oxidation products, and will show that polarography combined with

paper chromatography can be used to increase the specificity of analysis of these compounds.

Experimental

Apparatus

(a) *Cathode ray polarograph*.—Model K1000/Polarotrace, manufactured by Southern Analytical Ltd., Surrey, England, was used. A polarogram is traced every 7 seconds on a cathode ray-oscilloscope.

(b) *Silver wire electrode*.—A No. 22 silver wire. Deposit electrically a thin coating of AgCl on the wire before use (25).

Reagents

(a) *Tetraethyl ammonium hydroxide*.—10% aqueous solution (Eastman No. 2078).

(b) *Petroleum ether*.—Redistilled, 30-60°C.

(c) *Chloroform*.—Redistilled, $61.2 \pm 0.1^\circ\text{C}$.

(d) *Ethyl acetate*.—Reagent grade, redistilled, $77.1 \pm 0.1^\circ\text{C}$.

(e) *Electrolyte solution*.—2% aqueous solution of tetraethyl ammonium hydroxide; prepare fresh daily.

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(f) *Standard pesticide solutions*.—Prepare standard solutions containing 50 mmg of pesticide chemicals per ml in ethyl acetate or petroleum ether.

Preparation of Standard Curves

Transfer aliquots of standard solutions containing from about 10 to 50 mmg (depending upon polarographic properties of compound) to 50 ml Erlenmeyer flasks, and evaporate carefully to dryness by means of an air jet. For all chemicals discussed in this paper, except Systox-thiol-sulfone, add 4 ml of electro-

lyte solution and let stand 5 minutes. For Systox-thiol-sulfone add 4 ml of electrolyte and polarograph immediately. Transfer the electrolytic solution to a polarographic cell, bubble prepurified N₂ through the solution for 5 minutes, and polarograph between $-0.4V$ and $-1.0V$ versus either a silver wire or mercury pool reference electrode.

Discussion of Polarography

Table 1 describes the peak potentials expressed in volts and wave heights expressed

Table 1. Peak potentials and wave heights of pesticides

Pesticide	Formulas	Peak Potential (Ag:AgCl Silver Wire) at 25°C (Volts)	I_p/C
Systox-thiol	$\begin{array}{c} \text{C}_2\text{H}_5\text{O} \quad \text{O} \quad \text{H} \quad \text{H} \\ \diagdown \quad \parallel \quad \quad \\ \text{P} - \text{S} - \text{C} - \text{C} - \text{S} - \text{C}_2\text{H}_5 \\ \diagup \quad \quad \quad \\ \text{C}_2\text{H}_5\text{O} \quad \text{H} \quad \text{H} \end{array}$	-0.65	.766
Systox-thiol-sulfoxide	$\begin{array}{c} \text{C}_2\text{H}_5\text{O} \quad \text{O} \quad \text{H} \quad \text{H} \quad \text{O} \\ \diagdown \quad \parallel \quad \quad \quad \uparrow \\ \text{P} - \text{S} - \text{C} - \text{C} - \text{S} - \text{C}_2\text{H}_5 \\ \diagup \quad \quad \quad \\ \text{C}_2\text{H}_5\text{O} \quad \text{H} \quad \text{H} \end{array}$	-0.58	.488
Systox-thiol-sulfone	$\begin{array}{c} \text{C}_2\text{H}_5\text{O} \quad \text{O} \quad \text{H} \quad \text{H} \quad \text{O} \\ \diagdown \quad \parallel \quad \quad \quad \uparrow \\ \text{P} - \text{S} - \text{C} - \text{C} - \text{S} - \text{C}_2\text{H}_5 \\ \diagup \quad \quad \quad \quad \downarrow \\ \text{C}_2\text{H}_5\text{O} \quad \text{H} \quad \text{H} \quad \text{O} \end{array}$	-0.64	.048
Systox-thiono	$\begin{array}{c} \text{C}_2\text{H}_5\text{O} \quad \text{S} \quad \text{H} \quad \text{H} \\ \diagdown \quad \parallel \quad \quad \\ \text{P} - \text{O} - \text{C} - \text{C} - \text{S} - \text{C}_2\text{H}_5 \\ \diagup \quad \quad \quad \\ \text{C}_2\text{H}_5\text{O} \quad \text{H} \quad \text{H} \end{array}$	-0.55	.057
Systox-thiono-sulfoxide	$\begin{array}{c} \text{C}_2\text{H}_5\text{O} \quad \text{S} \quad \text{H} \quad \text{H} \quad \text{O} \\ \diagdown \quad \parallel \quad \quad \quad \uparrow \\ \text{P} - \text{O} - \text{C} - \text{C} - \text{S} - \text{C}_2\text{H}_5 \\ \diagup \quad \quad \quad \\ \text{C}_2\text{H}_5\text{O} \quad \text{H} \quad \text{H} \end{array}$	-0.70	.017
Systox-thiono-sulfone	$\begin{array}{c} \text{C}_2\text{H}_5\text{O} \quad \text{S} \quad \text{H} \quad \text{H} \quad \text{O} \\ \diagdown \quad \parallel \quad \quad \quad \uparrow \\ \text{P} - \text{O} - \text{C} - \text{C} - \text{S} - \text{C}_2\text{H}_5\text{O} \\ \diagup \quad \quad \quad \quad \downarrow \\ \text{C}_2\text{H}_5\text{O} \quad \text{H} \quad \text{H} \quad \text{O} \end{array}$	No wave	—
Thimet	$\begin{array}{c} \text{C}_2\text{H}_5\text{O} \quad \text{S} \quad \text{H} \\ \diagdown \quad \parallel \quad \\ \text{P} - \text{S} - \text{C} - \text{S} - \text{C}_2\text{H}_5 \\ \diagup \quad \quad \\ \text{C}_2\text{H}_5\text{O} \quad \text{H} \end{array}$	-0.80	.097
Di-Syston	$\begin{array}{c} \text{C}_2\text{H}_5\text{O} \quad \text{S} \quad \text{H} \quad \text{H} \\ \diagdown \quad \parallel \quad \quad \\ \text{P} - \text{S} - \text{C} - \text{C} - \text{S} - \text{C}_2\text{H}_5 \\ \diagup \quad \quad \quad \\ \text{C}_2\text{H}_5\text{O} \quad \text{H} \quad \text{H} \end{array}$	-0.62	.067

as microamperes of current measured (I_p) divided by the concentration in micrograms per ml (C), I_p/C , of the parent pesticide compounds and four degradation products of Systox which were polarographed under the conditions described above. The peak potentials obtained in cathode-ray polarograms are characteristic for any given ion or group, and are found to be about 0.05 volt more negative than the half-wave potential for the same reduction determined by conventional polarographs.

Straight line curves were obtained for all compounds polarographed and are shown in Fig. 1. We can see from Fig. 1 that the Systox-thiol and its sulfoxide gave larger waves, i.e., are more sensitive to polarography than are the thiono isomers. For both Systox-thiono and thiol isomers the parent isomer gives larger waves than its sulfoxide, which in turn gives larger waves than its sulfone.

Fig. 1—Standard curves for organic phosphorus pesticide residues. 1, Systox-thiol; 2, Systox-thiol-sulfoxide; 3, Systox-thiol-sulfone; 4, Systox-thiono; 5, Systox-thiono-sulfoxide; 6, Thimet; 7, Di-Syston.

At first it was thought that the sulfones were not readily polarographed under alkaline conditions—thus the reason for the smaller waves. However, it was later established that these compounds evidently reacted very rapidly with the electrolyte to form compounds that could not be polaro-

graphed under the conditions of the experiment. It was found that if the electrolyte solution was polarographed immediately after the Systox-thiol-sulfone was added, the polarographic wave attributed to this compound was obtained. On standing, this wave disappeared. A solution of thiono sulfone which did not give a polarographic wave was analyzed by paper chromatography and found to contain no organic phosphorus ester.

Other electrolytes, such as 0.01N NaOH, 0.01N KOH, and 1:1 mixture of 0.01N KOH:0.01N KCl, can also be used, but they are not as sensitive as the electrolyte of choice, 2% aqueous solution of tetraethyl ammonium hydroxide. No waves were obtained with 0.1N NaOH and 1N NaOH.

The general reaction for the alkaline hydrolysis of the thiol-Systox isomers may be written as shown in the equation (26):

The polarography of the sulfhydryl group ($-\text{SH}$), which is formed in this reaction, has been studied by several investigators (27-30). They found that the polarographic waves resulting from sulfhydryl-containing compounds are catalytic hydrogen waves. Thus, the large waves obtained from the Systox-thiol and Systox-thiol-sulfoxide may be due to these catalytic waves.

The waves obtained from the other compounds studied appear to be normal reduction waves. They are suitable for quantitative estimation, although the group involved in the reduction is not known.

While investigating the polarography of these pesticides, it was observed that six pesticide compounds tested were quite unstable in dilute solutions, e.g., a freshly prepared solution of Thimet (50 mmg/ml) in ethyl acetate gave one wave only, having a peak potential of -0.80 volts on being polarographed. A similar solution, after standing 3 weeks, gave two waves which had peak potentials of -0.80 volts and -0.65 volts. When the solution was polarographed after 9 weeks, two waves were obtained at -0.65 volts and -0.50 volts.

Table 2. Recovery of pesticides from paper chromatograms of known solutions

Pesticide	mmg Added	mmg Found	% Recovery
Systox-thiol	13.0	13.8	107.0
Systox-thiol-sulfoxide	20.0	19.4	97.0
Systox-thiol-sulfone	53.6	46.5	86.7
Thimet	20.0	16.7	83.5
Di-Syston	20.0	18.3	91.5

The above example suggests that when evaluating parent compounds it is advisable to use fresh reference solutions and check them frequently for decomposition.

Method of Analysis

Pesticide residues were extracted from the plant with acetonitrile and cleaned up with a special charcoal column, and the pesticide was subsequently identified by paper chromatography following the procedure of Getz (23). In this investigation, the pesticides were chromatographed with an aqueous system containing Epotuf, an epoxy resin, as the immobile phase. The mobile phase was a 40% aqueous solution of acetonitrile (24).

The paper chromatograms were prepared for polarographic analysis in the following manner: Divide (by marking) an 8 × 8" Whatman No. 3MM paper vertically into two parts. Transfer an aliquot of test solution to a spot on one half of the paper and a duplicate aliquot to a spot on the other. Place a known quantity of reference compounds on a spot on one half. After the chromatogram has been developed and dried, cut the paper at the dividing line. Treat the half bearing the reference compounds with the chromogenic spray (24). The spots obtained serve to identify the pesticides present and to indicate their location on the untreated half of the paper chromatogram. Place sections of the untreated paper chromatogram containing a pesticide in a 50 ml Erlenmeyer flask. Add 4 ml of electrolyte solution and let stand 5 minutes. Transfer the electrolyte solution to a polarographic cell, bubble prepurified nitrogen through the solution 5 minutes, and polarograph between -0.4 volts and -0.9 volts *versus* either a silver wire or mercury pool reference electrode. The microamperes of current thus obtained is compared to that obtained from a standard solution, which was prepared as follows: Transfer an aliquot of standard solution to a 50 ml Erlenmeyer flask and evaporate by means of

a soft air jet to dryness. Add a representative section of blank paper from the same chromatogram as the sample, add 4 ml of electrolyte solution, and proceed as described for the sample analysis.

Calculate the results by the following equation:

$$\text{microamperes current from standard solution} \\ = \frac{\text{mmg pesticide in standard solution}}{\text{microamperes current from sample}} \\ \times \text{mmg pesticide in sample}$$

Results and Discussion

Experiments were conducted to check the recovery of the pesticides from paper chromatograms; results are shown in Table 2.

When the sample contains less than 10 mmg, as indicated by the paper chromatogram, the standard addition method described by Taylor (31) enables the analyst to have more certainty in identification and to determine small amounts of residue.

Plots of kale were sprayed with 1 lb/acre of either Systox, Di-Syston, or Thimet. The kale was harvested at various time intervals after spraying (designated as preharvest interval or PHI) and analyzed by the above method. The results were compared to those obtained by a paper chromatography method developed by Getz (24).

The results obtained from the field-sprayed kale are shown in Tables 3-5.

In interpreting the data presented in Tables 3-5, several concepts must be emphasized. The polarographic method of quantitative analysis, like many others, is at best comparative. The wave height obtained

Table 3. Comparison of residue values from eluted spots (Systox-thiol as standard) with values estimated from spots of a duplicate chromatogram

PHI* (day)	Systox-thiol	
	Polarographic (ppm)	Paper Chromatography (ppm)
0	1.33	1.0
2	0.60	0.8
4	—	0.6
15	—	0.0

* Preharvest interval.

Table 4. Comparison of values from eluted spots (Di-Syston as standard) with values estimated for spots of a duplicate chromatogram

PHI ^a (day)	Spots 1 and 2	
	Polarographic (ppm)	Paper Chromatography (ppm)
0	11.0	12
2	8.1	11
4	7.9	8
15	—	0.2

^a Preharvest interval.

from an unknown sample must be compared with the wave height obtained from a standard reference compound or a standard curve obtained from determining the standard under the same conditions. The only standard compounds we had were Thimet, Di-Syston, Systox (both thiono and thiol isomers), and the sulfoxide and sulfone derivatives of Systox. As we have previously indicated, we found that the various isomers and/or derivatives of the Systox compounds varied in sensitivity, that is, the wave heights obtained for the same amounts of the various isomers and derivatives varied as seen by the I_p/C values shown in Table 1. Where reference samples were available, as seen in Table 3, good agreement was obtained when comparing the results by paper chromatography and polarography.

In Table 4 and again in Table 5, none of the spots found on the paper compared with the parent Di-Syston and Thimet spots. The spots which were cut out and polarographed were, however, compared with the parent compounds because metabolites of these compounds were not available, and we therefore had no way of positively identifying the spot. The paper chromatograms of these spots were compared to the spots obtained by using the Systox derivatives as standards.

Summary

We have presented a new technique for the study and analysis of organic phosphorus pesticides and their residues. The combination of paper chromatography and polarog-

Table 5. Comparison of values from eluted spots (Thimet as standard) with values estimated for spots of a duplicate chromatogram

PHI ^a (day)	Total Polarographic for Spots 1 + 2 + 3 (ppm)	Total Paper Chromatography for Spots 1 + 2 + 3 (ppm)	Total Polarographic, All Other Spots (ppm)	Total Paper Chromatography, All Other Spots (ppm)
0	11.5	15.4	6.0	3.4
2	8.7	1.4	7.7	1.7
4	8.9	1.4	6.1	5.4
15	—	—	2.4	1.0

^a Preharvest interval.

raphy proves to be a useful tool in the study and analysis of these compounds. In limited trials, other pesticides not mentioned in this paper, such as parathion, EPN, Trithion, and Guthion, also have been analyzed by this technique with good results. The results obtained emphasized again the need for more basic studies in the isolation and identification of the various metabolites shown to be present by paper chromatography.

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