Semiautomated Determination of Di-Syston in Granular Formulations

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The semiautomated determination of Di-Syston, *O,O*-diethyl *S*-[2-(ethylthio)ethyl] phosphorodithioate, in granular clay formulations has been made more specific. Interference from naturally occurring phosphorus in the clay carrier, a source of difficulty in the previously reported method, is eliminated, and the direct determination of Di-Syston in the presence of Dasanit, *O,O*-diethyl *O*-[4-(methylsulfinyl)phenyl] phosphorothioate, is now possible. The new method is essentially equivalent to the previous one with respect to precision and manpower requirement. The procedure involves prewetting the granular samples with water containing a small amount of surfactant, extracting the Di-Syston with 2-methoxyethanol–water (10:1), hydrolyzing the Di-Syston to ethylthioethylthiol, and measuring the hydrolysis product colorimetrically after allowing it to react with a sulfhydryl reagent, 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB).

Di-Syston (thiodemeton or disulfoton), *O,O*-diethyl *S*-[2-(ethylthio)ethyl] phosphorodithioate, is a product of Bayer AG of Leverkusen, West Germany, and is licensed to Chemagro Agricultural Division of Mobay Chemical Corporation in the U.S. and Canada. It is a systemic insecticide, and its formulations are used for control of sucking insects and mites on plants. The most commonly used formulations are a spray concentrate and impregnated granules. A new granular formulation, first registered in 1973, contains both Di-Syston and Dasanit, *O,O*-diethyl *O*-[4-(methylsulfinyl)phenyl] phosphorothioate.

Granular formulations are produced by a number of manufacturers under contract to Chemagro. This practice effects savings in freight costs but produces severe demands on analytical control because of the thousands of individual batches involved.

Prior to the 1968–1969 season, the active ingredient (AI) content of Di-Syston granular formulations was monitored by a manual hydrolysis method of analysis. The thousands of samples from 1 month's production imposed a severe burden on the analytical laboratory, since the working time required for each Di-Syston analysis was 40 min. Therefore, a method more suitable for mass production of data was required.

During the 1968–1969 season, a semiautomated procedure based on total phosphorus was developed and put into use at Chemagro (Talbott et al., 1972). This utilized a granular formulation of known Di-Syston content as a reference standard. In order to cancel interference from naturally occurring phosphorus in the clay carrier, it was necessary that the standard be prepared from the same type of clay as contained in the sample. Several such standards were required, corresponding to the various types of clays used in commercial production.

In late 1972, a search was begun for a new, more specific semiautomated procedure for Di-Syston. Some previously used clays which caused little interference with the phosphorus method were not available in sufficient amounts for the 1973 production season, and other clays were substituted. Some of these caused interferences that were much too high to risk using a granular reference standard, as a slight mis-match was magnified into significant error. Specificity for Di-Syston was also necessitated by the development of the new formulation which contains both Di-Syston and another organophosphorus pesticide, Dasanit, as the total phosphorus method obviously could not distinguish between the two active ingredients. The new method for Di-Syston is based on hydrolysis to ethylthioethylthiol, followed by reaction of the latter compound with Ellman's sulfhydryl reagent, 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) (1959). The use of this reagent in an automated procedure was patterned after the method of Levine et al. (1965).

The average working time for the new Di-Syston method is 17 min per sample. The increase over the 13-min average for the phosphorus method is considered well justified by the increase in specificity and the elimination of dependence on laboratory-formulated granular control standards.

EXPERIMENTAL SECTION

Apparatus. The AutoAnalyzer I system (Technicon Instruments, Tarrytown, N.Y.) consisted of two proportioning pumps, a sampler II, an oil bath containing a 2.0 mm i.d. × 20 ft glass coil, equipped with a proportional temperature controller, a 2.0 mm i.d. × 10 ft glass coil, a colorimeter equipped with selenium detectors and 420-nm filters, and a recorder equipped with absorbance scale chart paper. Disposable plastic sample trays and 250-ml disposable plastic flasks (urine bottles) from Scientific Products Division of American Hospital Supply Corp. were used.

Reagents. All chemicals were ACS grade unless otherwise noted and all aqueous dilutions were made in distilled water. Buffer was prepared by dissolving 60 g of sodium borate decahydrate, 30 ml of concentrated hydrochloric acid, and 4 ml of 45% Dowfax 2A-1 in water and diluting to 1 l. Dowfax 2A-1 was obtained from Dow Chemical Co., Midland Division, as a 45% solution. A 0.1% solution of the Dowfax 2A-1 was prepared in water. DTNB was obtained from Aldrich, No. D21,820-0, and prepared fresh daily as a 0.11% solution in 1:1 methanol–water. Sodium hydroxide was 4.5 *N* in water and contained 1 ml of 45% Dowfax 2A-1 per liter. The extraction solvent was a mixture of 890 ml of water and 8 l. of practical or industrial grade 2-methoxyethanol (ethylene glycol monomethyl ether, EGME). The dilution solvent was a solution containing 300 ml of water, 150 ml of 0.1% Dowfax, and 2.7 l. of EGME.

Standard. A quantity of reference standard sufficient to last the season was prepared by compositing samples of a number of different batches of technical Di-Syston, all meeting manufacturing specifications. The purity of the mixture was established by quantitative infrared

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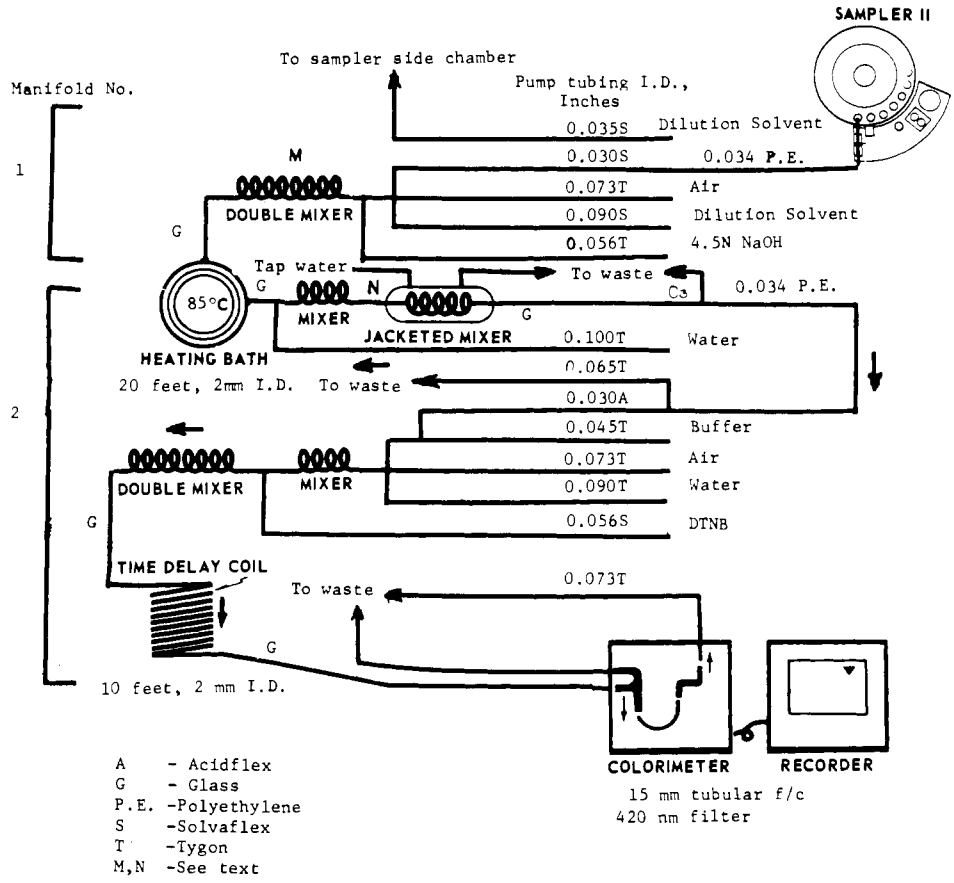

Figure 1. Flow diagram for Di-Syston determination.

spectrophotometric comparison with a known Di-Syston standard at 657 cm^{-1}, similar to the procedure in Zweig (1964).

Procedure. Samples were prepared by mixing the bulk sample with a wide spatula in a beaker of appropriate size, to ensure homogeneity, and weighing 3.0 ± 0.01 g of Di-Syston 15% granules or 6.0 ± 0.01 g of Dasanit + Di-Syston 7.5–7.5% granules into a 250-ml disposable flask. Five milliliters of 0.1% Dowfax was pipetted into each flask, and the capped flasks were shaken on a mechanical shaker for 15 min. Standards and conditioning solution were prepared by weighing 0.45 ± 0.0001, 0.52 ± 0.0001, and 2 ± 0.05 g of Di-Syston reference standard of known purity into each of three 250-ml disposable flasks labeled X_1, X_2, and X_3, respectively. Each flask contained 5 ml of 0.1% Dowfax, previously added by pipet. Extraction solvent (100 ml) was pipetted into all flasks, which were then capped and shaken mechanically for 1 hr.

With the AutoAnalyzer apparatus set up according to the flow diagram in Figure 1, portions of conditioning solution, standards, and sample extracts were placed sequentially on the sampler tray. The conditioning solution, X_3, was placed in the first two cups, and standard X_2 in the next four and last two cups of the run. Standard X_1 was placed in the first cup following the first four X_2 cups and before the last two X_2 cups. Also, one cup each of X_1 and X_2 standards was placed at intervals of 5–10 samples. The sampler was operated with a 30 sample/hr cam having a 2:1 sample to wash ratio. The probe was a 1.4 mm o.d. × 75 mm glass capillary attached to a length of 0.034 in. i.d. polyethylene tubing. Except where noted in Figure 1, the pump tubing and transfer lines were of the same type of tubing.

The sample extracts from the cups and the wash solution (dilution solvent) in the side chamber were sampled alternately with the sample probe, mixed with diluting solvent and 4.5 *N* sodium hydroxide, and passed through the heating bath held at 85 ± 0.02°C. The resulting hydrolysate was further diluted with water and cooled in a water-jacketed mixing coil. A portion of each diluted hydrolysate was diluted further with water and buffer solution to about pH 8. The DTNB reagent was mixed into the solution and the color developed in a 10 ft glass coil at ambient temperature. The absorbance of the 2-nitro-5-thiobenzoate anion was measured in a 15-mm flow cell at 420 nm in the colorimeter. When interpreting the chart, the X_3 conditioning peaks, the first three X_2 standard peaks, and the last X_2 peak were rejected. Net absorbances were obtained by subtracting the base line from the standard and sample peak maxima. If the absorbances of either the two X_2 or the X_1 standards bracketing a set of samples differed by more than 3.0% of their average, the bracketed sample absorbances were discarded and that portion of the run was repeated, using fresh portions of standards and sample extracts. The Di-Syston content of the sample was calculated by comparing the sample absorbance with the average standard absorbances in a two-point equation where *A* represents

$$K_1 = [(W_{S_2} - W_{S_1}) \times P]/(S_2 - S_1)$$

$$K_2 = S_1K_1 - W_{S_1}P$$

$$\text{Di-Syston, \%} = (AK_1 - K_2)/W$$

the sample absorbance, *W* the sample weight, S_1 and S_2 the absorbances of the two standards, W_{S_1} and W_{S_2} the corresponding weights of standards, and *P* the percentage purity of the reference standard.

RESULTS AND DISCUSSION

After initially optimizing the extraction, hydrolysis, and color development portions of the new sulfhydryl method,

Table I. Comparison of Precision for Phosphorus and Sulfhydryl AutoAnalyzer Determinations of Di-Syston on Oil-Dri Clay (before Optimizing Sulfhydryl Conditions)

	AI, %							
	Phosphorus				Sulfhydryl			
Sample	Observation		Mean	σ^2	Observation		Mean	σ^2
1	15.1	15.3	15.2	0.02	15.1	15.3	15.2	0.02
2	15.0	15.2	15.1	0.02	14.4	14.7	14.6	0.05
3	15.3	15.2	15.2	0.01	14.7	14.8	14.8	0.01
4	15.0	15.3	15.2	0.05	14.4	14.8	14.6	0.08
5	15.2	15.5	15.4	0.05	14.9	15.1	15.0	0.02
7	15.5	16.1	15.8	0.18	15.5	15.9	15.7	0.08
7	16.0	16.0	16.0	0.00	15.4	15.9	15.6	0.13
			Av 15.4	0.055			Av 15.1	0.065
			$\sigma = \pm 0.2$				$\sigma = \pm 0.3$	
			95% C.L. = ±0.4%				95% C.L. = ±0.6%	

a reproducibility problem was encountered. This was traced to inadequate temperature control related to the mercury thermoregulator in the heating bath. Replacement of the thermoregulator with a Fisher Proportional controller eliminated the temperature cycling and improved the reproducibility.

Early samples in the 1973–1974 season were analyzed by the phosphorus method as well as the sulfhydryl method. Since the samples were for manufacturing control purposes, these analyses were generally run only once unless the result failed to meet product specifications. Data on one early series having replicates by both methods are shown in Table I. The ±0.2% σ value and the 95% confidence level of ±0.4% found for the phosphorus method matched the precision previously reported (Talbott et al., 1972). The 95% confidence level of ±0.6% for the new sulfhydryl method, however, appeared broad enough to warrant further investigation of the method for improvement of the reproducibility.

One problem which contributed to the initially high 95% confidence limits for the sulfhydryl method was found to be a nonlinear absorbance curve with a negative absorbance intercept. The use of two standards which bracketed the sample concentration range was sufficient to correct this.

There was a tendency for the standard peaks to start low and then build to a maximum after the fourth or fifth standard. This was alleviated by inserting, ahead of the regular standards in the run, a "conditioning solution" consisting of a relatively high concentration of standard Di-Syston in the usual solvent.

It was found that several hours' usage caused the Solvaflex and Tygon tubing to harden, with deleterious effects on reproducibility. The peaks shown in Figure 2 represent the two standards and the conditioning solutions, run with aged tubing. The carryover from the conditioning solution to the first four cups of the high standard becomes evident on comparing the first two well-defined peaks with the next set of four for this standard. The following low standards were also affected by the preceding high standard.

Both high sampling rates and large differences in concentration between adjacent cups also resulted in carryover and error. If the sample concentration fell more than 12% outside the range used for the two standards, 0.44–0.53 g/100 ml, the effect on adjacent peaks was seen to be quite significant at sampling rates greater than 10 samples per hour. A comparison of 10, 20, and 30 samples per hour rates is shown in Figure 3. The approximate concentrations were 2.0, 0.52, 0.45, and 0.26 g/100 ml for samples 1, 2, 3, and 4, respectively. Peaks 5 and 6 are replicates of 2. At the 10 samples per hour rate, peaks 5 and 6 are identical and peak 2 is very slightly higher, amounting to about 0.7% relative on an AI basis. At 20 samples per hour, peak 2 is elevated significantly by carryover from peak 1, giving a 2.4% relative error. At 30 samples per hour, not only is peak 2 elevated but also peak 5 is depressed by dilution carried over from peak 4. Maintenance of proper sample sizes minimized this problem and permitted operation at the high rate. Obviously the precision and accuracy can be expected to be best with the base-line separation obtained at the 10

Table II. Comparison of Precision, Di-Syston Reference Standards Absorbances

Observations, χ_1 standard	Observations, χ_2 standard
0.560	0.660
0.560	0.650
0.565	0.652
0.570	0.655
0.565	0.649
0.567	0.660
0.565	0.660
Av 0.564	0.655
	0.655
$\sigma = \pm 0.004$	0.652
95% C.L. = ±0.008	0.662
	0.662
	Av 0.656
	$\sigma = \pm 0.005$
	95% C.L. = ±0.010

Table III. Precision of Optimized Sulfhydryl Method for Di-Syston 15% Granular

	AI, %			
Sample	Day 1	Day 2	Day 3	Mean
1	15.7	15.4	15.7	15.6
2	16.1	15.9	15.8	15.9
3	16.1	16.1	15.8	16.0
4	16.0	15.7	15.7	15.8
5	15.8	15.6	15.5	15.6
6	16.3	16.6	16.3	16.4
7	14.3	14.2	14.6	14.4
8	12.0	11.9	11.4	11.8
9	15.7	15.8	15.7	15.7
10	15.4	15.4	15.2	15.3
11	12.4	12.4	11.8	12.2
12	15.4	15.1	14.9	15.1
13	15.2	15.1	14.7	15.0
14	15.5	15.3	15.1	15.3
15	15.4	15.6	15.2	15.4
	Av 15.1	15.1	14.9	15.0
		$\sigma = \pm 0.2$		
		95% C.L. = ±0.4		

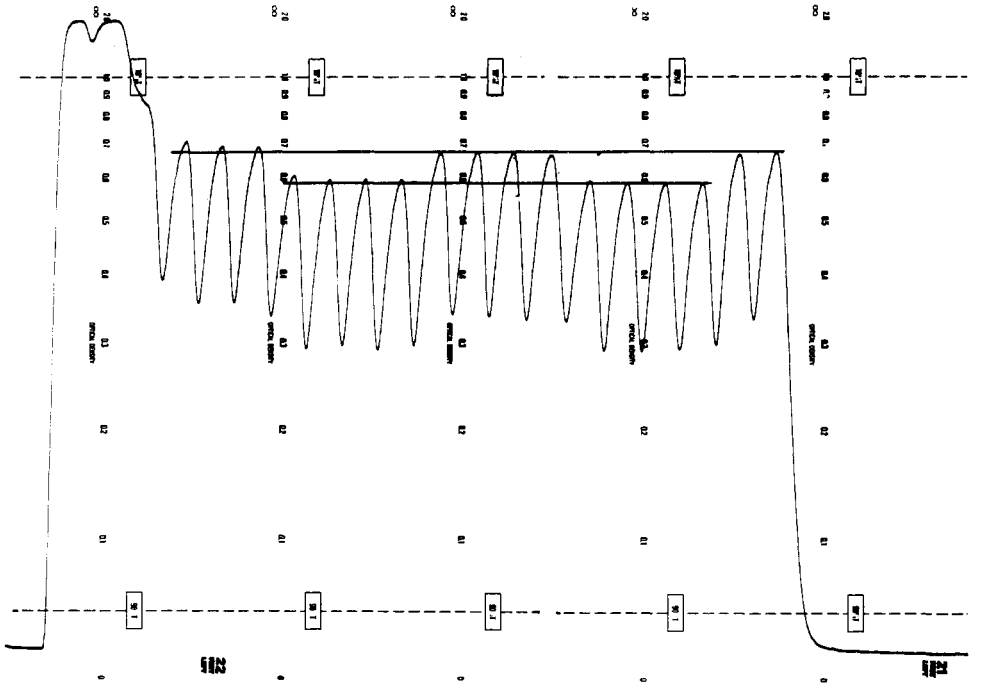

Figure 2. Effect of stretched and/or hardened tubing in manifolds.

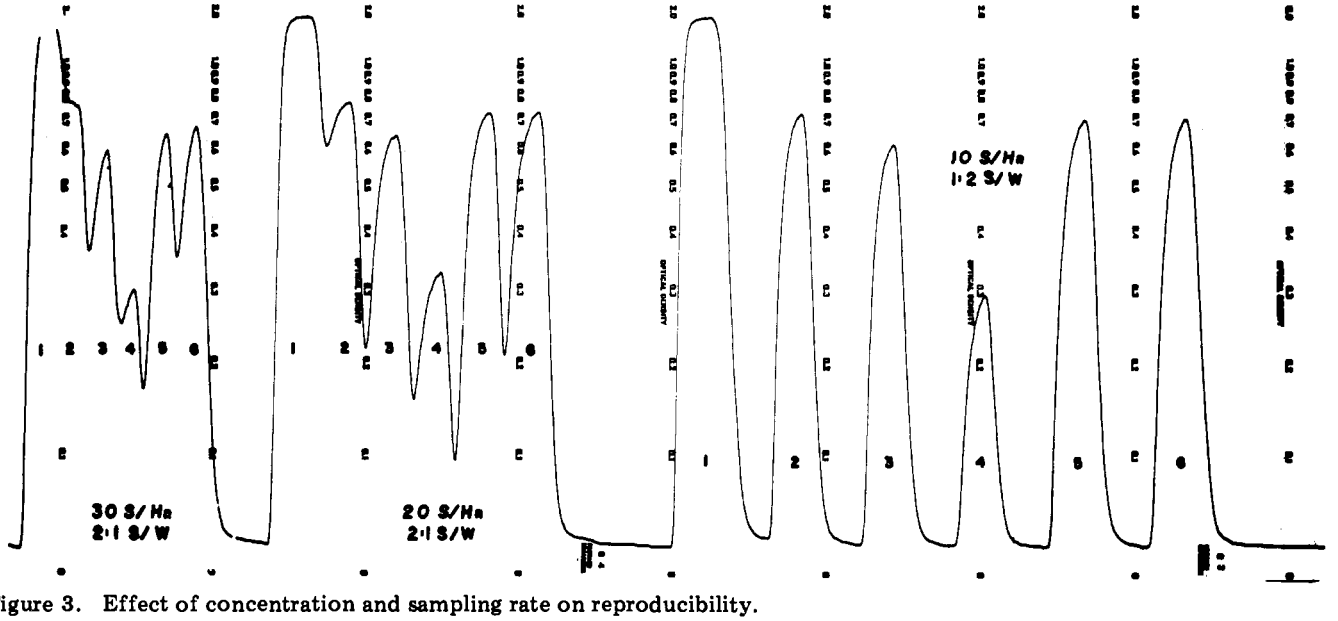

Figure 3. Effect of concentration and sampling rate on reproducibility.

Table IV. Accuracy and Precision of Analysis, Dasanit-Di-Syston 7.5-7.5 Granular

		Against Di-Syston standard				Against Di-Syston-Dasanit mixed standard			
				Std dev, % abs.				Std dev, % abs.	
Sample	Theory, %	AI found, %	Av	Precision	Accuracy	AI found, %	Av	Precision	Accuracy
1	7.04	6.95, 7.12, 7.19, 7.03	7.07	0.10	0.11	6.94, 7.09, 7.15, 7.03	7.05	0.09	0.09
2	8.04	8.18, 8.09, 8.02, 8.08	8.09	0.07	0.09	8.17, 8.06, 8.07, 8.02	8.08	0.06	0.08
3	9.04	9.06, 9.16, 8.93, 9.07	9.06	0.10	0.10	9.01, 9.08, 8.89, 9.09	9.02	0.09	0.10
		Overall	σ	±0.09	±0.10	Overall	σ	±0.08	±0.09
			95% C.L. =	±0.18	±0.20		95% C.L. =	±0.16	±0.18

samples per hour rate, but for routine control purposes this gain is usually not worth the loss in sample throughput.

Homogeneity of the hydrolysate going to the color development step was improved by inserting double mixing coils at points M and N in Figure 1 (before and after the heating bath, respectively). The peaks did not stabilize, however, until after adding a water-cooled mixing coil at point N. The effect is demonstrated in Figures 4a and 4b which show the performance before (a) and after (b) installing the water-cooled coil.

The benefits resulting from these modifications are demonstrated in Figure 5, which shows the peaks produced by the two standards and the conditioning solution in a typical run under optimum operating conditions. (The

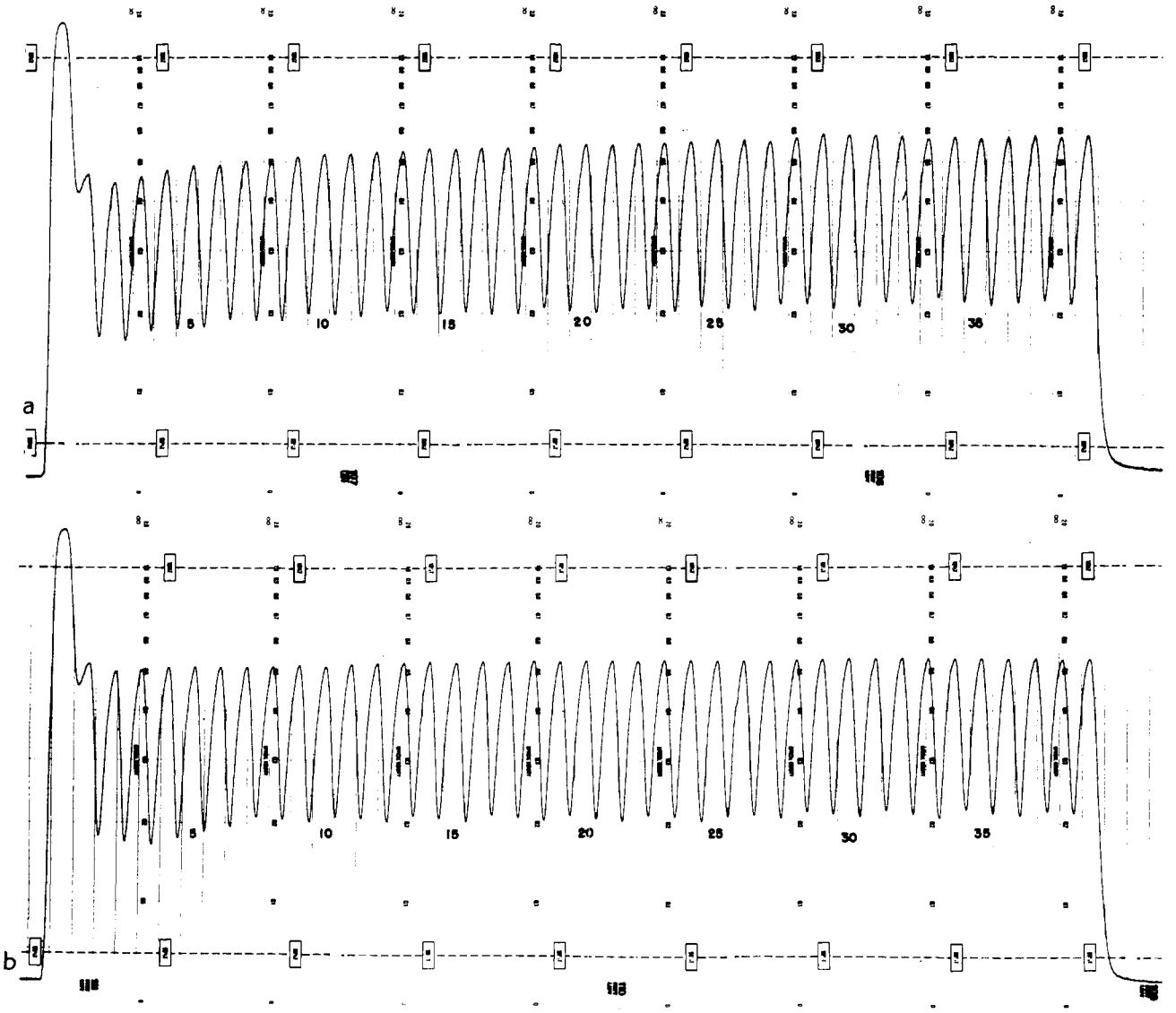

Figure 4. Effect of cooling the hydrolysate in Manifold 2: (a) no cooling; (b) with cooling.

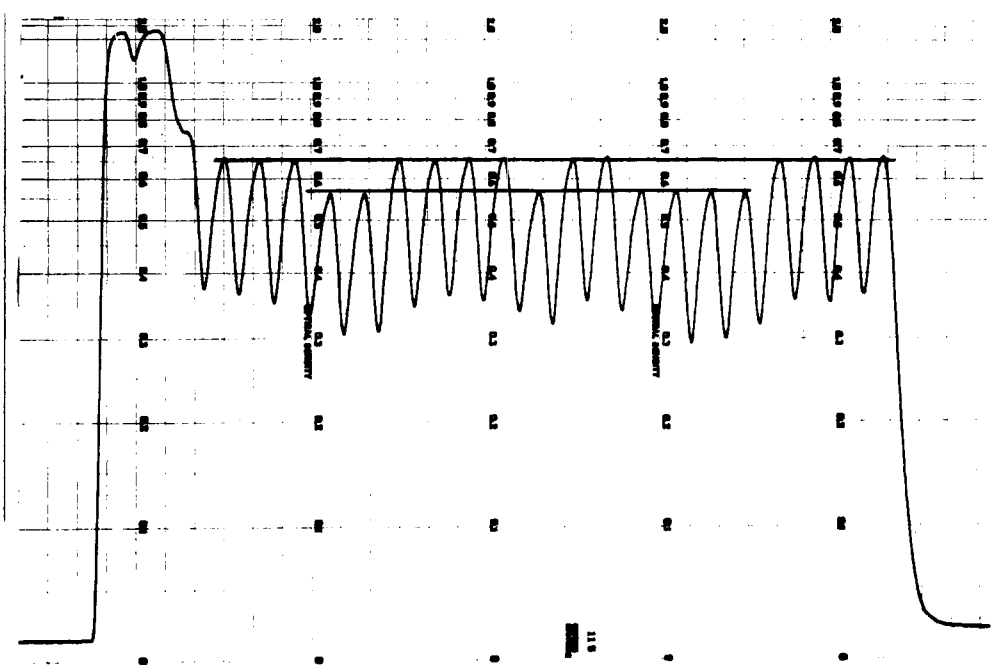

Figure 5. Typical reproducibility for standards at two different concentrations.

conditioning solution produced the first two, very large peaks.) The reproducibilities of the absorbances of the two standards are shown in Table II. The 2σ values for the X_1 and X_2 standards were essentially the same, ±0.008 and ±0.010 absorbance unit, respectively. Table III lists the final results for a series of commercial, spray-coated samples, replicated on three separate days with fresh standards. The 95% confidence limits are ±0.4%, the same as yielded by the phosphorus method and significantly better than the earlier limits of ±0.6% shown in Table I.

To evaluate the applicability of the method to the new Dasanit + Di-Syston 7.5–7.5% granular formulation, three samples were prepared in the laboratory by the previously reported solution coating technique (Talbott et al., 1972). Di-Syston was applied at concentrations which were below, equal to, and above levels which might be expected from commercial production, and matching amounts of Dasanit were added. These samples were analyzed by four different analysts, using separate weighings of samples and standards.

The sample extracts were run against both Di-Syston and mixed Dasanit–Di-Syston reference standards. The data shown in Table IV indicate essentially no differences in accuracy and precision using either standard. Hence, for all practical purposes the Dasanit does not interfere with the Di-Syston. (The fact that the precision standard deviation is better than that shown in Table III is attributed to a higher degree of homogeneity in solution-coated material.)

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Overcrowding Factors of Mosquito Larvae. VII. Preparation and Biological Activity of Methyloctadecanes and Methylnonadecanes against Mosquito Larvae

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All structural isomers of 7-methyloctadecane and 8-methylnonadecane, two components of the overcrowding factors of mosquito larvae, were bioassayed for their biological activity against first- and fourth-instar larvae of *Culex pipiens quinquefasciatus* Say. Straight-chain alkanes from C_{11} to C_{20} were also evaluated for comparison. The branched-chain alkanes in general were more active than the straight-chain alkanes. Among the alkanes studied, 3-methyloctadecane and 3-, 4-, 7-, and 9-methylnonadecanes showed the greatest activity with LC_{50} 1–2 ppm and LC_{90} about or less than 10 ppm. These branched-chain alkanes showed greater activity than most petroleum hydrocarbon larvicides utilized in mosquito control today. At sublethal concentrations, the branched-chain alkanes showed good growth retarding activity against the immature stages of the mosquito.

Older larvae of the southern house mosquito, *Culex pipiens quinquefasciatus* Say, under overcrowded conditions elaborate a number of chemicals known as overcrowding factors which play a role in regulating mosquito larval populations. These factors manifest toxic and growth-retarding effects in younger larvae (Ikeshoji and Mulla, 1970a,b). In fractionating the overcrowded cultures of mosquito larvae, Ikeshoji and Mulla (1974) obtained a mixture of active components consisting of carboxylic acids and hydrocarbons which could be separated by gas chromatography. Mass spectrometric studies showed that the hydrocarbon fraction contained heptadecane, octadecane, 7-methyloctadecane, and 8-methylnonadecane.

Petroleum hydrocarbons have been known as mosquito-control agents for more than a half-century (Balfour, 1913; Takatsuki, 1917; Hagstrum and Mulla, 1968). These control agents, however, have been used as mixtures, and no efforts have been made to isolate and identify the active compounds. Moreover, the rates of application for satisfactory control of mosquitoes are quite high. Quraishi and Thorsteinson (1967) investigated toxicity of several pure alkanes and alkenes (both ranging from C_7 to C_{16}) against *Aedes aegypti* (L.) larvae and found that nonene was the only one which gave high mortality of larvae and pupae at the very high dosage of 500 ppm.

In studies on the isolation and identification of the overcrowding factors of mosquito larvae, Ikeshoji and Mulla (1974) reported the biological activity of pure hydrocarbons such as octadecane, 3-methyloctadecane, 3-methylnonadecane, and 9-methylnonadecane against young larvae of *C. p. quinquefasciatus*. These three branched-chain alkanes were the structural isomers of the naturally occurring 7-methyloctadecane and 8-methylnonadecane which were not available for bioassay at that time.

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