Laboratory Method for Determining the Rate of Volatilization of Insecticides from Plants

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A direct measurement method for evaluating the rate of volatilization of insecticides was developed. The all-glass collection system consisted of a glass chamber which contained the treated surface. Glass tubing was used to connect the chamber to two gas washing-absorption bottles and ethylene glycol was utilized as the scrubbing solvent. A series of experiments were performed to test the suitability of benzene as a solvent for extracting lindane, dieldrin, and tetradifon from ethylene glycol; recoveries ranging from 95 to 99% were obtained for lindane and 97 and 98% for dieldrin and tetradifon, respectively. The ability of ethylene glycol to retain 50 and 500 μg. of lindane at different temperatures and flow rates was evaluated; the recoveries were between 92 and 96%. Preliminary data are given, using the collection system, for the determination of the rate of loss of lindane from both leaf and glass surfaces as influenced by temperature and humidity.

Several factors which influence the persistence of an insecticide on a plant include volatilization, penetration, hydrolysis, oxidation and reduction, type of plant, and rate of plant growth (Coffin, 1964). Although these factors exert their influence simultaneously, it is often desirable to evaluate each of them separately to describe the mechanism of insecticide dissipation adequately.

Numerous studies have been reported regarding the dissipation of chlorinated insecticides as influenced by climatic conditions. Much of this work, however, has dealt with the toxicity of residues (Burgess and Sweetman, 1949; Chisholm *et al.*, 1949; Gaines and Dean, 1949; Gaines and Mistric, 1951; Kalkat *et al.*, 1961; Mistric and Martin, 1956; Teotia and Dahm, 1950); thus, the nature of these investigations has necessitated the use of bioassay techniques, often in conjunction with indirect chemical determinations (Decker *et al.*, 1950; Gannon and Decker, 1958; Lichtenstein and Medler, 1958), involving the analysis of treated surfaces at different time intervals. Other indirect methods, designed to measure the rate of loss of insecticides from such substrates as glass, paper, wood, etc., have been reported (Chisholm and Koblitsky, 1947; Fleck, 1944; Kalkat *et al.*, 1961; Lyon and Davidson, 1965). These methods, however, have generally employed gravimetric procedures using milligram quantities of the insecticides.

To evaluate effectively the rate of volatilization of insecticides from plants, it was considered necessary to develop a direct measurement method in which all of the insecticide present, including the parent compound and any metabolites which vaporized from the treated surface, could be quantitatively recovered and analyzed.

This paper describes the collection apparatus, together with the procedures developed for the extraction of three insecticides from the scrubbing solvent, and includes preliminary data obtained using this system for the evaluation of the rate of loss of lindane from leaf and glass surfaces as influenced by temperature and humidity.

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MATERIALS AND METHODS

Insecticides. The insecticides used were analytical grade standards and included lindane (gamma isomer of 1,2,3,4,5,6-hexachlorocyclohexane), dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4*a*,5,6,7,8,8*a*-octahydro-1, 4-*endo*-*exo*-5,8-dimethanonaphthalene), and tetradifon (*p*-chlorophenyl 2,4,5-trichlorophenyl sulfone).

Reagents. Reagent grade acetone, benzene, petroleum ether, diethyl ether, and practical grade acetonitrile were redistilled prior to use.

Ethylene glycol, reagent grade. Several peaks resulting from solvent contamination were detected by GLC (EC detector) under conditions where benzene extracts of the ethylene glycol were highly concentrated. Additional purification of the ethylene glycol, by extracting the solvent with benzene prior to use, has proved satisfactory under such conditions.

Collection Apparatus. The all-glass collection system (original chamber furnished by F. A. Gunther) is shown in Figure 1. A 5-gallon borosilicate glass jug, severed approximately 3 inches from the bottom, was used as the chamber to contain the treated plant or glass plate. The edges of the chamber were ground to provide a good mesh between the two sections. The neck of the chamber was modified to accept a dome-shaped top, which contained two standard-taper holes to accommodate air inlet and outlet tubing. The scrubbing system consisted of two gas washing-absorption bottles (Kontes Glass Co., Vineland, N. J.), each containing a fritted glass disk; these bottles were connected in tandem. This system was similar to that reported by Abbott *et al.* (1966), but ethylene glycol was used as the scrubbing solvent rather than *N,N*-dimethylformamide.

Volatilization Studies. Garden beans, *Phaseolus vulgaris* (Top Crop variety), were germinated and grown for approximately 2 weeks in a sterilized sandy soil in the green-

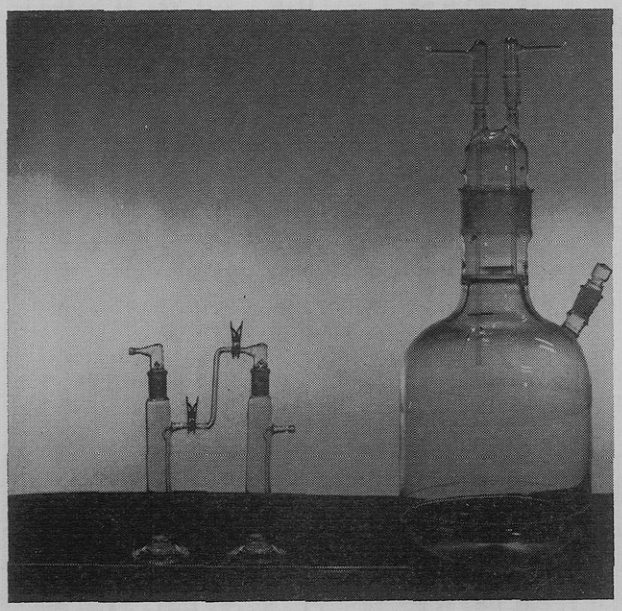

Figure 1. Glass collection apparatus used in volatilization studies

house. The plants were then transferred to aluminum foil–covered Erlenmeyer flasks containing a modified Hoagland's nutrient solution. This solution was changed daily for 4 days preceding the experiments. At this stage of growth, the primary leaves were approximately 85 mm. in length. The plant selected for treatment (uniformity in size being a critical factor) was removed from the nutrient culture and the roots were thoroughly rinsed with distilled water. A Teflon collar, slightly larger than the flask opening, was fitted to the plant. This collar served a dual purpose as a support for the plant and as a barrier to minimize vapor exchange to the root water. The plant was then placed in a 250-ml. Erlenmeyer flask containing distilled water.

Lindane was selected primarily because of its stability and high vapor pressure. It was dissolved in acetone and 50 μl. (250 μg.) were applied in small drops to each primary leaf with a 50-μl. Hamilton syringe A precision of ±0.9% was obtained in applying 100-μl. volumes of lindane using this syringe. At the same time, a standard was prepared containing an equal quantity of the insecticide.

The treated plant was placed in the base of the chamber and the upper section placed on the lower one and secured by means of nylon filament tape. The glass chamber was placed in a growth chamber (Model CEL 37-14, Sherer-Gillett Co., Marshall, Mich.) and connected to the scrubbers by means of glass tubing with ball and socket joints. The use of metal clamps provided an airtight fit between the various sections. Each scrubber contained 150 ml. of ethylene glycol. The experiments were continued for 72 or 96 hours under dark conditions, with sampling of the first scrubber being performed every 24 hours. A hygrothermograph was used to monitor the temperature and relative humidity within the growth chamber. The volume of air flowing through the system was maintained at about 650 ml. per minute by use of a flowmeter connected to a small vacuum pump.

A borosilicate glass watch glass (75-mm. diameter) was used in those studies involving the volatilization of lindane from a glass surface. As with the plant experiments, 100 μl. (500 μg.) of the insecticide were applied in small drops to the surface.

At each sampling period, the lindane present in the scrubber solvent was extracted with benzene as follows: The ethylene glycol was transferred to a 500-ml. separatory funnel, and the scrubber was rinsed with 50 ml. of the solvent followed by 75 ml. of benzene. The mixture was extracted and after the two phases had separated, the lower phase was drained into a second separatory funnel containing 50 ml. of benzene. The extraction procedure was repeated three more times; the lower layer was discarded, and the benzene phase was washed twice with distilled water and then dried through a column containing anhydrous sodium sulfate (reagent grade, granular). At the termination of the experiment (after 96 hours), a similar procedure was followed for the second scrubber. The above procedure was also used for extracting dieldrin and tetradifon from ethylene glycol (Table I).

The glass chamber and associated tubing were rinsed with acetone, and each sample was diluted to its respective volume. The acetone was removed by evaporating an aliquot of the sample to near dryness under vacuum. Benzene was added to the flask and the solution was then transferred to a separatory funnel and washed with distilled water. Moisture was removed by passing the solution through anhydrous sodium sulfate.

The insecticide residue remaining on the plant was extracted by blending the finely sliced tissue in a mixture of acetonitrile-benzene (2 to 1) which was contained in a small Waring Blendor. This mixed-solvent method was a modified version of the 2-propanol–benzene method of Thornburg (1963). The plant pigments were removed from the tissue extract by passing the mixture through a Florisil (60- to 80-mesh activated at about 650° C. and held in foil-covered bottles at 110° C.) column.

Gas Chromatography. Analyses were made with an Aerograph Hy-Fi Model 600-B equipped with an electron-capture detector containing a 250-mc. tritium source. A Model 328 isothermal controller was used to maintain a constant oven temperature. The recorder employed was a 1-mv. Leeds and Northrup Model H, equipped with a Disc Integrator. A 3-foot × $^1/_{16}$-inch (I.D.) borosilicate glass column containing 5% Dow 11 silicone grease on Chromosorb W (60- to 80-mesh) was utilized. The operating parameters for the analysis of lindane were: column

Table I. Suitability of Benzene as a Solvent for Extracting Three Insecticides from Ethylene Glycol

Insecticide	Quantity Added, μg.	Extractions Required	Per Cent Recovery and Deviation from Mean
Lindane	1	4	99.0[a] ± 2.0
Lindane	10	4	98.7[a] ± 0.8
Lindane	100	4	96.7[a] ± 3.1
Lindane	1000	4	95.3[a] ± 5.7
Dieldrin	40	3	97.0[b]
Tetradifon	500	3	98.3[b]

[a] Average of duplicate samples.
[b] Single sample.

temperature, 182° C.; detector temperature, ca. 190° C.; injector temperature, ca. 195° C.; carrier gas flow rate, 20 ml. per minute of prepurified nitrogen.

For the studies involving lindane, the peak heights of the samples (analyzed in triplicate) were compared with those of the standards. Peak areas were calculated by use of the recorder integrator for the dieldrin and tetradifon analyses.

RESULTS AND DISCUSSION

A series of experiments were performed to test the suitability of benzene as a solvent for extracting lindane, dieldrin, and tetradifon from ethylene glycol. As shown in Table I, satisfactory recoveries were obtained for all insecticides. Under these experimental conditions, the highest recoveries for lindane, together with the lowest deviations, were obtained at the 1- and 10-μg. levels.

The ability of ethylene glycol to retain 50 and 500 μg. of lindane at different temperatures and flow rates was evaluated (Table II). The retention of lindane apparently was not affected to any degree as the concentration and environmental conditions were altered.

The rate of volatilization of lindane from leaf and glass surfaces was evaluated at temperatures of 16° and 27° C.

Table II. Retention of Lindane in Ethylene Glycol at Different Concentrations, Temperatures, and Flow Rates over 24 Hours

Lindane Added, μg.	Temperature, ° C.	Flow Rate, Ml./Minute	Per Cent Recovery and Deviation from Mean
50	25	650	92.8[a]
50	25	1350	95.8[a]
500	16	650	95.3[b] ± 2.2
500	27	650	91.7[b] ± 1.1

[a] Single sample.
[b] Average of duplicate samples.

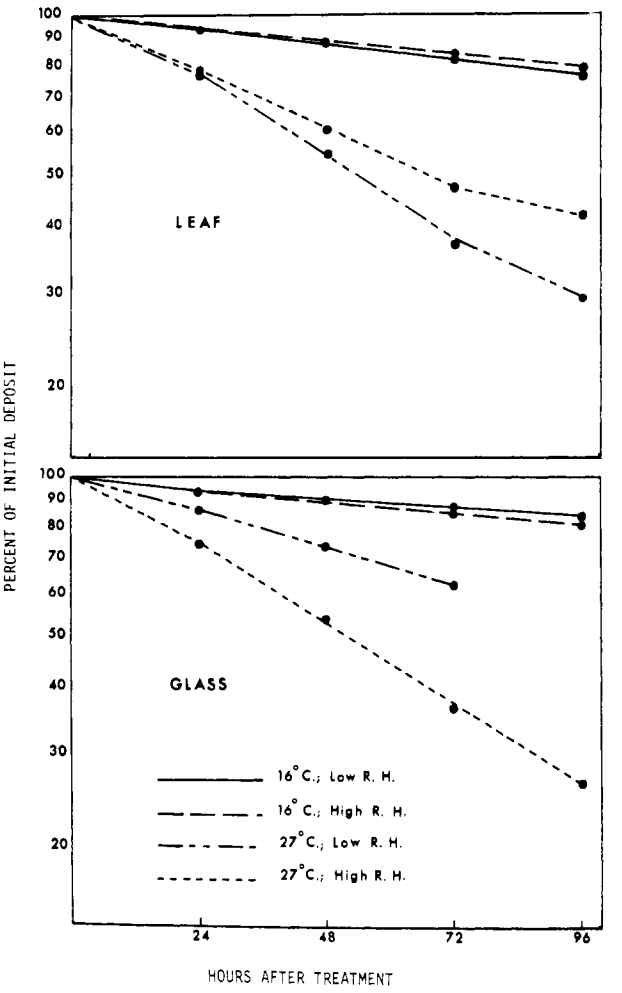

Figure 2. Rate of loss of lindane from leaf and glass surfaces as influenced by temperature and humidity

Table III. Effect of Temperature and Humidity upon Volatilization of Lindane from Leaf and Glass Surfaces with an Air Flow of 650 Ml. per Minute

		Per Cent Recovery[a]							
		Glass				Leaf			
	Time Interval, Hours	16° ± 1° C.		27° ± 2° C.		16° ± 1° C.		27° ± 2° C.	
		60 ± 5% R.H.	70 to 100% R.H.	40 ± 5%, R.H.	60 to 80% R.H.	60 ± 5% R.H.	70 to 100% R.H.	40 ± 5% R.H.	60 to 80% R.H.
1st scrubber	24	6.3	6.3	13.3	25.1	6.3	6.4	23.7	20.8
	48	10.0	10.8	25.9	45.7	12.1	11.5	45.1	39.1
	72	12.7	15.2	37.4	63.0	18.0	16.3	63.0	52.5
	96	16.1	19.2	...	73.4	23.9	21.0	70.6	58.1
2nd scrubber	96	None detected	None detected	None detected	None detected	None detected	Trace	None detected	Trace
Entire plant	96	...	...	...	...	63.0	63.2	21.2	35.7
Root water	96	...	...	...	...	0.1	0.1	0.3	0.4
Glass plate	96	75.0	73.2	47.5	18.4	...	...	...	...
Chamber	96	1.1	0.6	2.7	0.8	1.3	0.7	0.7	0.4
Tubing	96	0.4	Trace	0.1	0.3	0.1	0.4	0.1	0.1
Total		92.6	93.0	87.7	92.9	88.4	85.4	92.9	94.7

[a] Based on 500 μg. applied.

in combination with relative humidity ranges of 60, 70 to 100, and 40, 60 to 80%, respectively. With the exception of one 72-hour experiment, all others were continued for 96 hours. One plant was used for each experiment. The total quantity of insecticide recovered (Table III) was satisfactory; however, more variation was evident when lindane was applied to leaves than when applied to glass. This increased variation for the plant samples may be due to incomplete extraction of that portion of the insecticide bound by cellular components. Only small amounts of the insecticide were found on the walls of the chamber or tubing. In six of the eight experiments conducted, lindane was not detected in the second scrubber, which indicates that ethylene glycol is very efficient in retaining a highly volatile insecticide such as lindane.

As was anticipated, the rate of loss of lindane (Table III) from both glass and leaf surfaces increased as the temperature was raised. As the humidity increased, the loss from a glass surface increased; however, the reverse situation occurred with the plants at both temperatures. The nature of the substrate affected the rate of volatilization (Figure 2). The disappearance rate from a glass surface was essentially linear throughout the 96-hour period, analogous to a first-order decay curve. At 16° C., the rate of loss from bean leaves was also linear; however, at 27° C., the rate was approximately linear up to 72 hours, after which the loss occurred at a diminishing rate. These curves (Figure 2) are similar to the degradation and persistence curves described by Gunther and Blinn (1955).

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LITERATURE CITED

Abbott, D. C., Harrison, R. B., Tatton, J. O'G., Thomson, J., *Nature* **211,** 259 (1966).
Burgess, A. F., Sweetman, H. L., *J. Econ. Entomol.* **42,** 420 (1949).
Chisholm, R. D., Koblitsky, L., *Agr. Chem.* **2** (9), 35 (1947).
Chisholm, R. D., Nelson, R. H., Fleck, E. E., *J. Econ. Entomol.* **42,** 154 (1949).
Coffin, D. E., *Residue Rev.* **7,** 61 (1964).
Decker, G. C., Weinman, C. J., Bann, J. M., *J. Econ. Entomol.* **43,** 919 (1950).
Fleck, E. E., *J. Econ. Entomol.* **37,** 853 (1944).
Gaines, J. C., Dean, H. A., *J. Econ. Entomol.* **42,** 429 (1949).
Gaines, J. C., Mistric W. J., Jr., *J. Econ. Entomol.* **44,** 580 (1951).
Gannon, N., Decker, G. C., *J. Econ. Entomol.* **51,** 8 (1958).
Gunther, F. A., Blinn, R. C., "Analysis of Insecticides and Acaricides," Interscience, New York, 1955.
Kalkat, G. S., Davidson, R. H., Brass, C. L., *J. Econ. Entomol.* **54,** 1186 (1961).
Lichtenstein, E. P., Medler, J. T., *J. Econ. Entomol.* **51,** 222 (1958).
Lyon, W. F., Davidson, R. H., *J. Econ. Entomol.* **58,** 1037 (1965).
Mistric, W. J., Jr., Martin, D. F., *J. Econ. Entomol.* **49,** 757 (1956).
Teotia, T. P. S., Dahm, P. A., *J. Econ. Entomol.* **43,** 864 (1950).
Thornburg, W. W., "Analytical Methods for Pesticides, Plant Growth Regulators, and Food Additives." Vol. **1,** pp. 87–108, Academic Press, New York, 1963.

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