[CONTRIBUTION FROM THE BUREAU OF ENTOMOLOGY AND PLANT QUARANTINE, U. S. DEPARTMENT OF AGRICULTURE]

The Vapor Pressure of Phenothiazine[1]

BY O. A. NELSON AND L. E. SMITH

During recent years considerable experimental work has been done with phenothiazine for the purpose of establishing the value of this compound as an insecticide. It has been tested as a stomach poison against a great variety of insects,[2] but thus far the most extensive field tests have been aimed at the substitution of this compound for arsenicals for the control of the codling moth larva.

When freshly applied, phenothiazine is more toxic to this insect than lead arsenate at the same dosage. After a few days, however, the deposit loses much of its toxicity. This loss may be due to loss of deposit due to lack of adhesive properties or to decomposition in sunlight. Although the rate of vaporization of phenothiazine is very low, nevertheless it has been suggested that the loss in toxicity might be due to the evaporation of the smaller particles from the surfaces of the sprayed fruits or leaves. For this reason the vapor pressure and the rate of evaporation of this compound were determined.

Method.—It was evident that the vapor pressure of phenothiazine was much too low to be determined by the static method. An apparatus was designed, therefore, whereby the vapor pressure could be determined by the air-saturation method. The apparatus, a modification of the one used by Vanstone,[3] is shown in Fig. 1.

The saturator, about 14 inches long, was loosely packed with glass wool and powdered phenothiazine and suspended in a constant-temperature bath. Packing it in this manner reduced to a minimum the possibility of channeling and also provided an enormous surface, thus facilitating saturation of the air with the compound. The exit tube of the saturator was electrically heated to prevent condensation of the vapors before they reached the thin-walled condenser, cooled in an ice-salt mixture. The weight of the phenothiazine condensed was determined by a colorimetric method developed in the Division of Insecticide Investigations, which is based on the transformation of the compound into a red product by treatment with bromine. The limit of accuracy of this method was 1.0 microgram of phenothiazine, representing an error of less than 5% in most of the determinations. This method is essentially the same as the one described by Eddy and DeEds.[4] For the lower temperatures 11.8 liters of air was drawn through the aspirator, while for the higher temperatures only 2 liters was required. The rate of flow of the air through the apparatus was controlled by means of the capillary tip at the outlet tube from the aspirator.

In determinations of vapor pressure by the air-saturation method it is imperative that the air drawn through or over the sample be completely saturated with the compound under investigation. That the air was saturated with respect to the phenothiazine was evidenced by the observation that air could be drawn through the saturator at a rate twice that used in the vapor-pressure determination without lowering the results.

In order to verify the results obtained in the 14-inch saturator and only one condenser, a second series of vapor pressure determinations was made in which the saturator consisted of three, and later five, 10-inch glass tubes, filled as described above, and heated in an electrically controlled air furnace, and two condensers instead of one. Not a trace of phenothiazine was obtained in the second condenser, thus proving complete absorption in the former experiments. The results of this series are given in Table I, series 2.

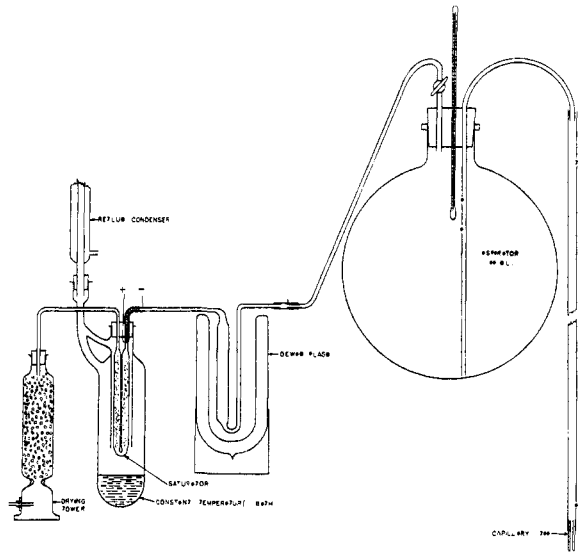

Fig. 1.

Experimental Results

After the weight of the compound vaporized in a known volume of air had been determined, the vapor pressure was readily calculated from Dalton's law of partial pressures. The expression used by Vanstone, correcting the volume of air for temperature and pressure, is

$$\text{V. P.} = \frac{wV_cTP\ 760}{273pV + 760wV_cT}$$

(1) Original manuscript received November 29, 1940. Not copyrighted.

(2) L. E. Smith, *U. S. Dept. Agr., Bur. Entomol. Plant Quarantine, Entomol. Tech. Cir.*, E-399 (1937) (mimeo.).

(3) Ernest Vanstone, *J. Chem. Soc.*, **97**, 429 (1910).

(4) C. W. Eddy and Floyd DeEds, *J. Food Research*, **2**, 2059 (1937).

wherein

V. P. = vapor pressure of phenothiazine, in mm.
w = weight of phenothiazine condensed, in grams
V_c = specific volume of phenothiazine = 22.41 liters/gram-mole = 0.1126 liter per gram
P = pressure of atmosphere, in mm.
p = pressure of air in aspirator, in mm.
V = volume of air aspirated, in liters
T = absolute temperature of air in aspirator

Table I gives the experimental data obtained in this investigation, together with the vapor pressures as calculated by means of the above equation.

TABLE I

EXPERIMENTAL DATA FOR VAPOR PRESSURE OF PHENOTHIAZINE

Temp., °C.	Replications, number	Vapor pressure, mm.	Standard error, mm.
	Series 1		
63	7	0.00008	0.00002
66	3	.00014	.00003
78	6	.00026	.00003
100.5	3	.0016	.0001
111	10	.0038	.0002
121.5	4	.0089	.0004
	Series 2		
78	4	0.0002	
90	1	.00064	
96.5	1	.0014	
99	3	.0018	
100	3	.0019	
110	2	.0037	
112	1	.0042	
121	2	.0085	

Over limited ranges of temperature and pressure the relation between these factors can be expressed by the equation $\log p = 9.265 - 4490.0/T(\text{abs.})$. By means of this equation the vapor pressures of phenothiazine were calculated at 10-degree intervals from 40 to 140°. These data are recorded in Table II.

TABLE II

CALCULATED VAPOR PRESSURES OF PHENOTHIAZINE

Temp., °C.	Vapor pressure, mm.	Temp., °C.	Vapor pressure, mm.
40	0.000008	100	0.0016
50	.000023	110	.0035
60	.000058	120	.0070
70	.00015	130	.013
80	.00032	140	.025
90	.00081		

Rate of Vaporization of Phenothiazine

The rate at which phenothiazine vaporized was determined in two series of experiments, in which glass plates were dusted with finely divided phenothiazine. In the first series two glass plates with areas totaling 384 sq. cm. were placed inside a 4-inch glass tube, which in turn was kept at 45° in a constant-temperature air thermostat. A gentle current of air was drawn over the dusted surfaces to carry off the phenothiazine vapors. The runs were continued for two to four days. An absorption bulb filled with ethyl alcohol and kept at ice–salt temperature was inserted in the air line for three to six hours, and the weight of phenothiazine was determined by the colorimetric method. To determine whether all the phenothiazine was absorbed, a second absorption bulb was inserted in the line, and air was drawn through for four hours. No phenothiazine was found in the second bulb.

In the second series of experiments, the finely divided phenothiazine was dusted on 45 by 50 mm. cover glasses, which were then placed at an angle of about 45° in echelon arrangement on a wire rack in the air thermostat at 45°. A moderate current of air was drawn over the dusted surfaces for ninety-six hours. The loss in weight of phenothiazine was determined by direct weighing of the cover glasses, before and after evaporation, on a micro balance sensitive to 0.002 mg.

Table III gives a summary of the results obtained in these experiments.

TABLE III

RATE OF VAPORIZATION OF PHENOTHIAZINE

Series	Replications	Rate of evaporation μg./sq. cm./hr.	Probable error of the mean μg./sq. cm./hr.
1	11	0.019	0.002
2	8	.027	.0027
Average		.023	.0013

The average particle size of the sample used in these experiments, as determined by the air-permeation method,[5] was 3 microns diameter.

The method used in determining the rate of vaporization was designed to yield results comparable to those obtained under field conditions, where the insecticide is sprayed or dusted on the surfaces of fruits and leaves. The weight of phenothiazine per unit area of glass surface in these experiments was about five times the amount applied to fruits or foliage, and because of this difference in density of phenothiazine particles there might possibly be a slight difference in rate of evaporation. The difference is believed to be small, however, and the rates recorded here are considered good estimates of the rate of loss of this compound due to vaporization in actual use.

The original weight of phenothiazine on the glass plates was approximately 200 micrograms per square centimeter. As the average rate of loss amounted to only 0.023 microgram per square centimeter per hour, the loss over a period

(5) E. L. Gooden and C. M. Smith, *Ind. Eng. Chem., Anal. Ed.*, **12**, 479 (1940).

of one hundred hours would be slightly more than 1%.

Summary

The vapor pressure of phenothiazine within the temperature range of 63–121° and the rate of evaporation of the finely powdered compound at 45° have been determined.

The results obtained show that the loss in toxicity of phenothiazine when used for the control of insect pests is not due to evaporation.

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Tracer Studies with Radioactive Hydrogen. Some Experiments on Photosynthesis and Chlorophyll

BY T. H. NORRIS, S. RUBEN AND M. B. ALLEN

Since nothing is known regarding the role of chlorophyll in green plant photosynthesis, there has been, and still is, considerable speculation on the subject. The many theories that have been proposed may be divided into two classifications: (1) chlorophyll participates as a reducing agent (hydrogen donor), (2) chlorophyll merely acts as a sensitizer (as certain dyes function on photographic plates, for example). The first category is by far the larger and we may cite a few of the more interesting proposals found in this group.

Dixon and Ball[1] have suggested that chlorophylls *a* and *b* are involved in a reversible oxidation–reduction cycle in which chlorophyll *a* (GH_2) is oxidized to *b* (GO).

$$GH_2 + CO_2 + \text{light} \longrightarrow GO + 1/n(H_2CO)n \quad (1)$$

and *b* in turn is reduced to *a* as follows[2]:

$$GO + H_2O + \text{light} \longrightarrow GH_2 + O_2 \quad (2)$$

The discovery that the chlorophylls contain a readily oxidizable group led Conant[3] and coworkers to propose that the pigment might act as a two electron reducing agent.

$$12GH_2 + 6CO_2 \xrightarrow{\text{enzyme}} 12G + C_6H_{12}O_6 + 6H_2O \quad (3)$$

in a dark reaction,[3] chlorophyll being regenerated by a photochemical process.[4]

$$12G + 12H_2O + \text{light} \longrightarrow 12GH_2 + 6O_2 \quad (4)$$

Stoll[4] and Willstätter[5] independently consider chlorophyll to function as a hydrogen donor in a photochemical reaction producing monodehydrochlorophyll (GH). This free radical is supposed to revert to the original dye via another photochemical process.

$$GH + H_2O + h\nu \longrightarrow GH_2 + OH \quad (5)$$

Franck[6,7] has a somewhat similar point of view, but has formulated the chlorophyll regeneration as

$$GH + R'OH + h\nu \longrightarrow GH_2 + R'O \quad (6)$$

where R'OH is different from water.

It occurred to us that using radioactive hydrogen[8] H^3, as a tracer it might be possible to learn whether or not chlorophyll is participating in photosynthesis as a donor of hydrogen. If photosynthesis is allowed to proceed for a sufficiently long time in water containing HTO[9] chlorophyll containing T should be formed[10] if the idea underlying equations 2, 4, 5, 6 is correct.

Experimental

Eleven cc. of the unicellular green alga *Chlorella pyrenoidosa* was suspended in 220 cc. of 0.05 *M* potassium bicarbonate solution containing HTO and strongly illuminated for three hours. During this period 2.5×10^{-3} mole of oxygen was evolved and a simple calculation shows that if the donor scheme is correct each chlorophyll should have been oxidized and reduced at least 100 times.[10a] The algae were centrifuged and the chlorophyll removed by exhaustive extraction with 95% acetone. All operations from the end of the illumination to the final burning of the chlorophyll were performed in strict darkness. In order to facilitate operations in the dark, the volumes of all solutions and vessels were carefully predetermined. To remove a lower layer from a separatory funnel, an evacuated

(1) Dixon and Ball, *Sci. Proc. Roy. Dublin Soc.*, **16**, 435 (1922).

(2) For an attempt at an experimental check on this proposal *cf.* Ruben, Frenkel and Kamen, *J. Phys. Chem.*, **46**, 710 (1942).

(3) Conant, Dietz and Kamerling, *Science*, **73**, 268 (1931).

(4) Stoll, *Naturw.*, **20**, 955 (1932); **24**, 53 (1936).

(5) Willstätter *ibid.* **21** 252 (1933)

(6) Franck and Herzfeld, *J. Phys. Chem.*, **45**, 978 (1941).

(7) Franck and Gaffron, "Advances in Enzymology," I, Inter-Science Publishers, Inc., New York, N. Y., 1941, p. 215.

(8) Alvarez and Cornog, *Phys. Rev.*, **56**, 613 (1939).

(9) We use the symbol T for H^3 (*cf.* Libby and Barter, *J. Chem. Phys.*, **10**, 184 (1942)).

(10) The possibility of an isotope separation will be discussed below.

(10a) Since the quantum yield is 0.1–0.08 (*cf.* Manning, Stauffer, Duggar and Daniels, THIS JOURNAL, **60**, 266 (1938), and Emerson and Lewis, *Am. J. Bot.*, **28**, 789 (1941)).