Table I. Recovery Data

	Added, P.P.M.	Recovered, P.P.M.[a]	Recovery, %
Broccoli	0.52	0.45	87
	0.50	0.41	82
	0.50	0.49	98
	0.50	0.47	94
	0.50	0.37	74
	0.50	0.40	85
	0.10	0.11	110
Cauliflower	0.50	0.37	74
	0.50	0.36	72
	0.55	0.41	74
	0.51	0.44	86
	0.55	0.41	75
Lettuce	0.10	0.09	90
	0.96	1.10	115
Av.			86

[a] All values given are corrected for background interferences due to untreated samples.

Crop Interference. Interference coloration produced by untreated samples of lettuce range from 0 to 0.1 p.p.m., broccoli from 0.05 to 0.25 p.p.m., and cauliflower 0.2 to 0.4 p.p.m. The higher interference values obtained from broccoli and cauliflower were from samples received overripe, in flower, and in some cases somewhat dehydrated. Samples in marketable condition gave the lower interference values.

Bruce (*2*) has shown that interfering materials in the benzene strip solutions of broccoli, cabbage, and cauliflower can be removed by adding 10 grams of a mixture of anhydrous sodium sulfate, Attapulgas clay, and Filter-Cel (2 to 2 to 1 by weight) per 100 ml. of strip solution. The purifying agents are removed by filtering the mixture through a rapid paper. Consistently good recoveries of Spergon were obtained when amounts as low as 0.06 p.p.m. were added to untreated samples.

Acknowledgment

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Literature Cited

(1) Agricultural Chemicals Research Laboratory, Naugatuck Chemical, Division United States Rubber Co., Bethany, Conn., Bethany Information Sheet No. **42,** pp. 2–4, December 1952.
(2) Bruce, R. B., "Determination of Spergon Residues on Cauliflower, Cabbage, and Broccoli," Western Division, Hazelton Laboratories, Palo Alto, Calif., March 1958, unpublished report.
(3) Burchfield, H. P., McNew, G. L., *Phytopathology* **38,** 4, 299–306 (1948).
(4) Cunningham, H. S., Sharvelle, E. G., *Ibid.*, **30,** 4 (1940).
(5) Eddins, A. H., Florida, Univ. Agr. Expt. Sta. (Gainesville) Bull. **492,** March 1952.
(6) Schoene, D. L., Tate, H. D., Brasfield, T. W., *Agricultural Chemicals*, **IV,** No. 11, 24 (1949).
(7) Sharvelle, E. G., Young, H. C., Jr., Sheme, B. F., *Phytopathology* **32,** 944–52 (1942).
(8) Ter Horst, W. P. (to United States Rubber Co.), U. S. Patent **2,349,771** (May 23, 1944).
(9) Waters, W. A., "Chemistry of Free Radicals," p. 75, Clarendon Press, Oxford, Eng., 1946.

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ALGICIDE MEASUREMENT

The Microdetermination of 2,3-Dichloro-1,4-naphthoquinone (Phygon) in Water

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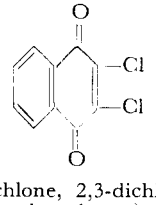
A spectrophotometric method for the determination of micro amounts of Phygon in water is described. The insoluble matter in the water is removed by filtration, the filtrate is adjusted to an acid pH by the addition of phosphoric acid, and the Phygon is distilled from the filtrate. Chloroform extraction of the distillate concentrates the Phygon, and the evaporation of the chloroform to a small volume results in a further concentration. The Phygon in the chloroform is detected by its ultraviolet absorption spectrum. Correction for background of the absorption data reduces the error introduced by other ultraviolet absorbing materials. This method is applicable in the range of 8 to 250 parts per billion, with an average recovery of 86%.

PHYGON (*5*) has been used widely as a foliage spray to control diseases caused by fungi on a variety of food crops.



Phygon (Dichlone, 2,3-dichloro-1,4-naphthoquinone)

The quinone structure of Phygon and its successful use as a fungicide led Fitzgerald, Gerloff, and Skoog (*1*) and Fitzgerald and Skoog (*2*) at the University of Wisconsin to include it in their screening tests for toxicants of bloom producing blue-green algae.

Since this work of Fitzgerald and Skoog, the application of Phygon as an algicide and submersed aquatic weed regulant has received wide and successful use. Some of its uses have been in recreational lakes, farm ponds, irrigation reservoirs, swimming pools, industrial water, and recirculating systems.

Depending upon the species of algae and type of aquatic weeds that are to be controlled, doses from 0.03 to 0.75 p.p.m. have been effective (*4*). Excellent control of algae growth in the recirculating cooling towers at the Naugatuck Chemical plants at Naugatuck, Conn., and Baton Rouge, La., has been obtained over a 2-year period with a concentration of 0.25 p.p.m. This level is maintained by daily additions of Phygon and routine analysis of the cooling tower water by the method described herein.

Phygon has a strong ultraviolet absorption spectrum in chloroform (Figure 1). Micro quantities [parts per billion

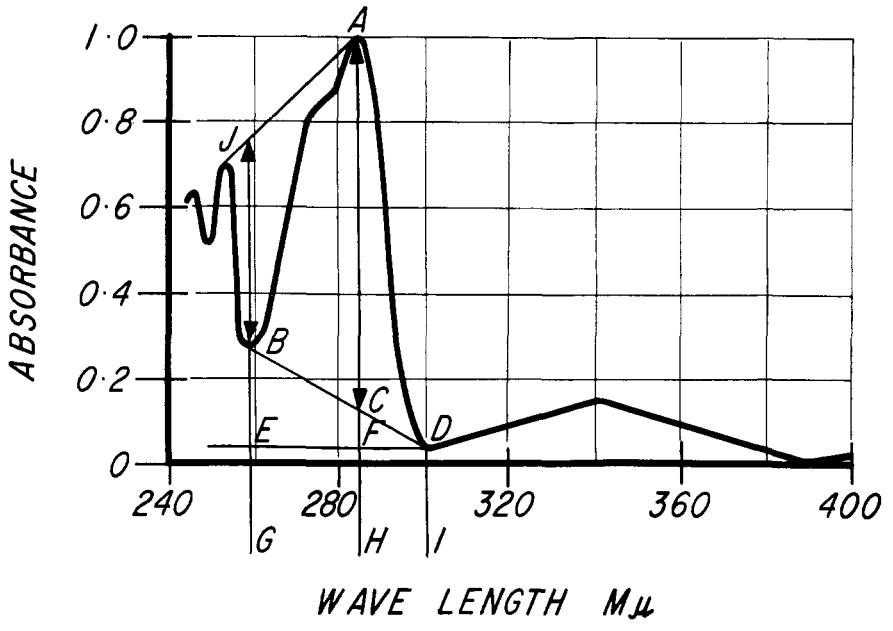


Figure 1. Ultraviolet absorption spectrum of Phygon in chloroform (0.0119 gram per liter) showing geometric relationships used in derivation of Equations 1 and 2

(p.p.b.)] of Phygon could be detected in water samples by steam distillation of the sample, chloroform extraction of the distillate, concentration of the chloroform extract, and measurement of the absorbance at selected wave lengths.

Analytical Method

Equipment and Reagents. Beckman DU spectrophotometer equipped with 1-cm. matched silica cells.

Büchner fritted-glass disk fine porosity funnel (Macalaster Bicknell, New Haven, Conn.).

5-liter distilling flask, single-necked 45/50 ⅋ joint (Macalaster Bicknell, Catalog No. 14181).

45/50 to 24/40 male to female ⅋ reducing adapter tube (Macalaster Bicknell, Catalog No. 40954).

24/40 ⅋ double male connecting bulb Kjeldahl (Macalaster Bicknell, Catalog No. 31096).

24/40 ⅋ West-type condenser, 300 mm. (Macalaster Bicknell, Catalog No. 9446).

All reagents used were of analytical reagent grade.

Clean all the glassware that will come in contact with the sample by rinsing thoroughly with ample portions of acetone, chloroform, 3A alcohol, and distilled water, in the order listed.

Analytical Procedure

Filter about 4500 ml. of the sample through a fine porosity, sintered-glass filter with vacuum. Measure out 4000 ± 25 ml. of the filtered sample into a 5-liter, round-bottomed distilling flask. Add 600 grams of sodium chloride and 5 ml. of phosphoric acid, assemble steam distillation apparatus, and distill 1500 ± 25 ml. of the sample into a 2-liter separatory funnel. Add 50 ml. of chloroform and shake vigorously for at least 1 minute. Allow the chloroform layer to separate, and drain it off into a 150-ml. beaker containing about 15 grams of anhydrous sodium sulfate. Dry the chloroform and decant into a second beaker. Extract the sample with a second 50-ml. portion of chloroform. Dry the chloroform in the same beaker as before and combine with the first extraction. Evaporate the mixture to at least 25 ml. Cool to room temperature and measure its volume in a 25-ml. graduate. Transfer to a 1-cm. silica cell and measure the absorbance at 253.8 mμ (slit width 1.0 mm.), 259.0 mμ (slit width 0.8 mm.), 283.5 mμ (slit width 0.56 mm.), and 299.0 mμ (slit width 0.50 mm.).

Calculate the parts per billion of Phygon in the sample from the equations:

$$(3.41\ A_{283.5} - 2.09\ A_{299.0} - 1.32\ A_{259.0}) \times \text{ml.} \quad (1)$$

and

$$(5.19\ A_{253.8} - 6.31\ A_{259.0} + 1.11\ A_{283.5}) \times \text{ml.} \quad (2)$$

where ml. is the volume of chloroform concentrate and A is the absorbance. Good agreement between Equations 1 and 2 assures that any interferences have been minimized, and Phygon is the actual constituent being measured.

Discussion of Analytical Method

In view of the great sensitivity of this method and the small concentrations being determined, errors can be caused by small amounts of other aromatic compounds. All equipment must be scrupulously clean. Insoluble foreign materials are removed by filtration and no Phygon is lost because, at the concentrations usually encountered, it is completely soluble.

The addition of sodium chloride increases the efficiency of the Phygon distillation by increasing the pot temperature during reflux.

A small amount of phosphoric acid is added to keep the Phygon from decomposing during the distillation. Phosphoric acid is nonvolatile under experimental conditions. In alkaline and neutral solutions Phygon hydrolyzes to form 2-hydroxy-3-chloro-1,4-naphthoquinone (*3*). The intensity of the orange-red color produced by this hydrolysis was too weak for spectrophotometric measurements.

Derivation of Equations 1 and 2

From Figure 1:

$$AC = AH - CH = AH - CF - FH$$

as triangles BED and CFD are similar

$$\frac{CF}{BE} = \frac{FD}{ED}$$

Then

$$AC = AH - \frac{FD \cdot BE}{ED} - FH$$

and

$$AC = AH - \frac{FD}{ED}(BG - EG) - FH$$

where AH = absorbance at 283.5 mμ
FD = 299.0 mμ − 283.5 mμ = 15.5 mμ
ED = 299.0 mμ − 259.0 mμ = 40.0 mμ
BG = absorbance at 259.0 mμ
$EG = FH = DI$ = absorbance at 299.0 mμ

Substituting:

$$AC = A_{283.5} - \frac{15.5}{40.0}(A_{259.0} - A_{299.0}) - A_{299.0}$$

Solving:

$$AC = A_{283.5} - 0.3875\ A_{259.0} - 0.6125\ A_{299.0}$$

As the concentration of Phygon is proportional to the height AC:

$$\text{Parts per billion} = \frac{250\ k\ AC \times \text{ml.}}{1000}$$

Substituting:

$$\text{Parts per billion} = 0.25\ k\ A_{283.5} - 0.9688\ k\ A_{259.0} - 0.1531\ k\ A_{299.0} \times \text{ml.}$$

Using a purified sample of Phygon and solving for k

$$\text{Parts per billion} = 3.41\ A_{283.5} - 2.09\ A_{299.0} - 1.32\ A_{259.0} \times \text{ml.} \quad (1)$$

where ml. = ml. of chloroform concentrate.

Equation 2 is developed similarly using height BJ.

Table I. Comparison of Data Calculated by Base-Line Technique vs. Direct Absorption Calculation

Curve	$253^3/_4$ Mμ, P.P.B./Ml.	$283^1/_2$ Mμ, P.P.B./Ml.	Equation 1	Equation 2	Average
A	+2.53	+0.47	+0.33	−0.29	+0.02
B	+2.17	+0.79	+0.11	−0.10	+0.01
C	+2.14	+1.17	−0.02	+0.06	+0.02

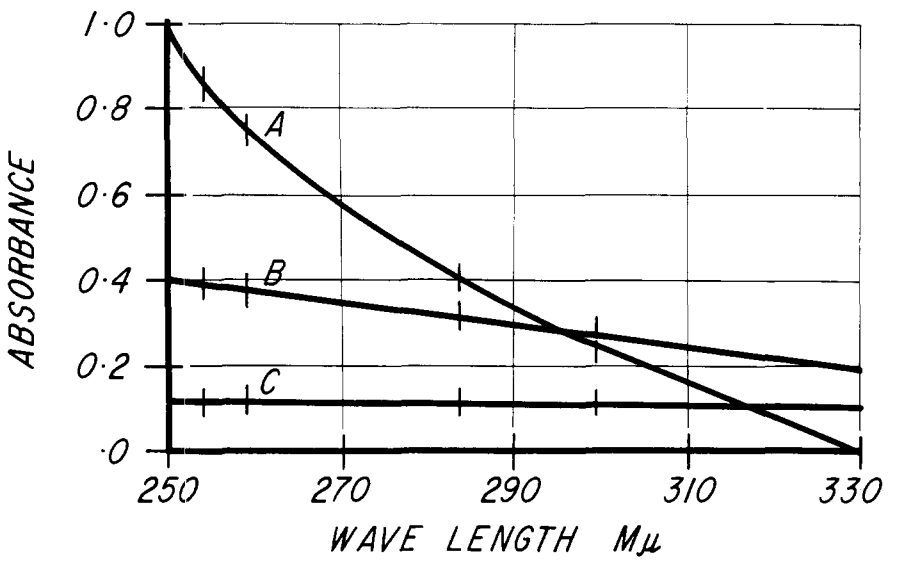


Figure 2. Theoretical spectra of possible interferences caused by the presence of foreign materials in water samples

As the foreign materials that could interfere with the accuracy of this method are unknown, it was necessary to reduce the error due to unknown backgrounds to an absolute minimum.

With the hypothetical spectra of backgrounds as shown in Figure 2, the base-line technique reduced background error more than the absorbance ratio method usually employed in spectrophotometric analyses. The data in Table I show that the base-line method is much less sensitive to foreign materials which may accompany the Phygon into the chloroform extract.

Discussion of Results

As shown in Table II, the Phygon recovery from solutions of known concentrations between 8 to 250 parts per billion is 80% or better. The Phygon is not completely recovered because of two factors: It hydrolyzes slightly even in acid solution, and the steam distillation is not continued long enough to approach 100% recovery.

Solutions of 500 and 750 parts per billion were run, but the recoveries dropped to 70%. The recovery of such high concentration solutions can be improved by collecting a larger distillate and continuing with the normal procedure.

Table II. Recoveries of Known Percentages of Phygon in Water between 0 to 250 Parts per Billion

Added, P.P.B.	Recovered, P.P.B.	Recovery, %
0 (Blank)	0.7	...
8.6	7.6	88
10.4	11	106
25	20	80
50	40	80
100	85	85
250	201	80

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Literature Cited

(1) Fitzgerald, G. P., Gerloff, G. C., Skoog, Folke, *Sewage and Ind. Wastes* **24,** No. 7, 888 (1953).
(2) Fitzgerald, G. P., Skoog, Folke, *Ibid.*, **26,** No. 9, 1136 (1954).
(3) Gullstrom, D. K., Burchfield, H. P., *Anal. Chem.* **20,** 11174 (1948).
(4) Naugatuck Chemical, Division of United States Rubber Co., Naugatuck, Conn., Booklet No. **46.**
(5) Ter Horst, W. P. (to United States Rubber Co.), U. S. Patent **2,349,772** (May 23, 1944).

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GROWTH REGULATOR AND HERBICIDE RESIDUES

Extension of the Residue Methods for 1,2-Dihydro-3,6-pyridazinedione (Maleic Hydrazide) and *N*-1-Naphthylphthalamic Acid (Alanap)

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REPORTED PROCEDURES for the determination of 1,2-dihydro-3,6-pyridazinedione [maleic hydrazide (*1, 2*)] (*7*) and *N*-1-naphthylphthalamic acid [Alanap (*4*)] (*5*) are satisfactory when applied to certain food crops at the residue concentrations described. However, interfering components in tobacco preclude the determination of maleic hydrazide residues in this crop by the literature method. Modification of the method is necessary.

The original method has been used successfully in the Naugatuck Chemical laboratories for several years on a wide variety of plant material. It consists of an alkaline digestion of the sample, rapid steam distillation of the sample with zinc as a reductant, and the determination of the hydrazine in the distillate by means of the yellow color formed with *p*-dimethylaminobenzaldehyde.

When maleic hydrazide in amounts up to 200 p.p.m. is added to untreated tobacco, and the tobacco is analyzed by the original procedure given above, a strong red interference coloration is developed, but very little *p*-dimethylaminobenzalazine—the yellow coloration produced by maleic hydrazide.