Table I. Recovery of PCNB from Alfalfa–Clover–Bird's-foot Trefoil Forage

Added, P.P.M.	Recovery, %
0.2	130
0.2	120
0.2	110
0.5	98
1.0	83
2.0	100
2.0	76
2.0	78.5

Check (average of 4 analyses), 0.34 p.p.m.

Table II. Determination of PCNB on Forage[a]

Days after Application	Residue, P.P.M. Spectrophotometric	Polarographic
7	32.0	30.0
	2290.0	1950.0
	725.0	744.0
	416.7	522.0
21	15.6	12.8, 13.2
	62.0	69.6
	3.6	7.7
	31.3	70.0, 73.5
	2.4	4.6, 3.8
	48.8	79.8, 78.0
35	1.8	3.3, 3.3
	35.6	35.2, 38.0
	18.8	17.4, 17.4
	14.3	11.9
	28.8	18.0, 18.6
	49.0	43.5

[a] Dry subsamples of same field sample.

that the outside diameter of the sample portion of the cell was reduced to 18 mm. to accommodate a smaller sample. A stopcock was attached to the base of the sample holder to facilitate cleaning. The entire sample was added to the H-cell and deoxygenated for 10 minutes with nitrogen. The polarogram was determined over the range of 0.00 to −1.15 volts against a saturated calomel electrode. The diffusion current was measured manually with a Fisher Elecdropode, which has a sensitivity range of 0.01 to 0.00001 equivalent per liter. The half-wave potential is −0.47 volt.

Standard Curve. Prepare a standard curve by adding 2-, 3-, 10-, 20-, and 30-ml. portions of a hexane solution of PCNB (5 μg. per ml.) to a series of 125-ml. acetylization flasks. Distill off the hexane through a Snyder column and remove the last traces with a gentle stream of air. Add 2.5 ml. each of the sodium acetate and acetic acid solutions and 5 ml. of isopropyl alcohol. Reflux for 5 minutes, cool, filter, and record the polarogram as in the analysis of forage.

Results and Discussion

The method was used to recover PCNB added to forage before extraction. Table I shows the recoveries obtained.

On May 6, 1957, PCNB was applied to clover-timothy forage plots for control of Sclerotinea crown rot. The rate of application was 75 pounds per acre, which was about 10 times the recommended rate. The PCNB was applied as a 75% wettable powder in 400 gallons of water per acre from a hand sprayer. The object was to study the disappearance of the fungicide with time after a very high rate of application. Samples were taken for residue analysis 7, 21, and 35 days after application. Table II shows PCNB found by the method of Ackermann *et al.* (*1*) and by the polarographic procedure.

Although very good agreement was obtained between the two methods, large differences can be observed among samples from the same field treatment. This variability might be due to errors in calibration of the hand sprayer, lack of agitation of the spray formulation during application, and variations in plant composition (differences in the percentage of weeds present) at harvest. Unfortunately, a satisfactory sample of check material from this experiment could not be obtained.

The half-wave potential shifted to a limit of about −0.53 volt during analysis of forage material. The magnitude of this shift was roughly proportional to the size of the sample. This did not affect interpretation of the polarogram, as no other waves occur in this region.

Acknowledgment

This work was done in cooperation with D. A. Roberts, who conducted the field experiment.

Literature Cited

(1) Ackermann, H. J., Baltrush, H. A., Berges, H. H., Brookover, D. O., Brown, B. B., J. AGR. FOOD CHEM. **6,** 747–50 (1958).
(2) Lingane, J. J., Laitinen, H. A., *Ind. Eng. Chem., Anal. Ed.* **11,** 504 (1939).
(3) Ordas, E. P., Smith, V. C., Meyer, C. F., J. AGR. FOOD CHEM. **4,** 444 (1956).
(4) Webster, J. G., Dawson, J. A., *Analyst* **77,** 203–5 (1952).

Received for review January 28, 1960. Accepted August 1, 1960.

FUNGICIDE RESIDUES

Colorimetric Estimation of Dodecylguanidine Acetate Residues

W. A. STELLER, K. KLOTSAS, E. J. KUCHAR,[1] and M. V. NORRIS

Central Research Division, American Cyanamid Co., Stamford, Conn.

THE FUNGICIDE *n*-dodecylguanidine acetate, Cyprex 65W [American Cyanamid's 65% dodine (coined name) formulated as a wettable powder], has been proved useful for control of specific plant diseases. It is especially effective against apple scab [*Venturia inaequalis* (Cke.) Wint.], pear scab (*Venturia pirina* Aderh.), and cherry leaf spot (*Coccomyces hiemalis* Hig.) Additional uses are being developed.

[1] Present address, Olin Mathieson Chemical Corp., New Haven, Conn.

In conjunction with the field research which established its utility, a satisfactory analytical method was required to obtain data on the rate of disappearance of *n*-dodecylguanidine acetate (dodine) residues and to establish that its use would not result in hazardous residues at harvest.

A method was developed for determining dodine residues on apples using a surface extraction procedure to remove the chemical from the fruit. Subsequently, Hamilton and Szkolnik (*7*) reported local penetrant properties for the fungicide into apple leaves. When this property of dodine was first recognized, a macerate extraction procedure for total residues was developed to evaluate the efficacy of the surface extraction technique for determining total dodine residues.

Many high molecular weight nitrogenous bases have been shown to form salt-like addition products with acidic dyes in buffered aqueous solution (*1, 4, 12*). These addition products partition favorably into water-immiscible organic solvents, such as chloroform and benzene.

A colorimetric method has been developed for the determination of *n*-dodecylguanidine acetate (dodine) residues in plant materials. A long-chain base cation, dodecylguanidine, is made to complex with an anionic dye, bromocresol purple, in a buffered aqueous alcohol solution. This complex is then extracted into chloroform. The bromocresol purple, proportional in amount to the DDG, is next extracted from the chloroform into aqueous alkali. The absorbance of this alkaline phase is measured spectrophotometrically at 590 mμ and is used to calculate the quantity of dodine present. The method is applicable to determining residues of dodine varying from 0.2 to 2.6 p.p.m. in a 50-gram sample. Residues from apples, apple leaves, pears, and sour cherries have been measured.

The dye equivalent of the nitrogenous base can then be extracted into aqueous alkali and its absorbance read at the proper wave length. Dodecylguanidine (DDG) was found to form with bromocresol purple a complex which could be extracted satisfactorily into chloroform. The analytical procedure which has been developed for determining residues of dodecylguanidine utilizes this principle.

Surface residues are extracted by tumbling a representative fruit sample with methanol; total residues, by macerating the fruit sample with a 2 to 1 (v./v.) mixture of methanol and chloroform. After a preliminary cleanup of the extract, the DDG is complexed with bromocresol purple in a buffered water-alcohol solution and the complex is then extracted into chloroform. The chloroform solution is shaken with aqueous alkali which allows the bromocresol purple equivalent to the DDG to be extracted completely into the aqueous alkaline phase. The absorbance of this phase at 590 mμ is a measure of the dodine present. Calibration curves prepared by the procedure obey Beer's law between 10 and 130 γ of dodine. The method (except for the leaf modification) is applicable to determining concentrations of dodine from 0.2 to 2.6 p.p.m. in a 50-gram sample. In some instances, the range has been extended by varying the sample size or by diluting the final alkaline indicator phase with 0.05*N* sodium hydroxide before measuring the color intensity.

The applicability of the method was tested by analyzing samples fortified with known amounts of the fungicide. These results are shown in Tables I through III. To create surface residue, a methanolic solution of dodine was dripped on the sample and the solvent was allowed to evaporate overnight before analysis. For total residue, a methanolic solution of dodine was added during maceration and the macerate was allowed to stand at least 30 minutes before analysis.

Table I. Recovery of Dodine from Apples

Sample Wt., Grams	Dodine, P.P.M. Added	Dodine, P.P.M. Found	Recovery, %
	Surface Residues		
33	0	0.14	...
67	0	0.07	...
100	0	0.09	...
33	0.67	0.64	96
	0.67	0.64	96
	1.0	0.80	80
	1.0	0.80	80
	1.0	0.80	80
	1.0	0.81	81
	6.55	5.80	89
67	0.39	0.34	87
100	0.13	0.11	85
	Total Residues		
50	0	0.1[a]	...
100	0	0.06[b]	...
50	0.24	0.21	87
	0.32	0.23	72
	0.54	0.38	70
	0.59	0.62	105
	0.76	0.55	72
	1.18	1.13	96
	2.50	2.10	84
100	0.12	0.14	117
	0.10	0.07	70
	0.20	0.18	90
	0.30	0.23	77

[a] Average of 5 analyses with standard deviation of 0.023.
[b] Average of 5 analyses with standard deviation of 0.013.

Table II. Recovery of Dodine from Apple Leaves and Cherries

Sample Wt., Grams	Dodine, P.P.M. Added	Dodine, P.P.M. Found	Recovery, %
	Apple Leaves		
10	0	0.66	...
	0	0.47	
20	0	0.37	...
30	0	0.32	...
20	0.5	0.60	120
30	0.5	0.43	86
10	1.0	1.8	180
20	1.3	1.2	92
10	2.6	2.6	100
20	2.6	1.9	73
	3.0	2.7	90
10	3.9	3.4	87
	3.9	3.4	87
	5.2	4.4	85
	5.2	4.9	94
	6.5	5.2	80
	Cherries		
50	0	0.07[a]	...
	0.13	0.16	123
	0.13	0.16	123
	0.26	0.21	81
	0.26	0.21	81
	0.26	0.18	69
	0.52	0.37	71
	0.52	0.39	75
	0.52	0.32	62
	0.52	0.32	62
	0.78	0.48	62
	0.78	0.48	62
	0.78	0.55	71
	0.78	0.58	74
	1.04	0.66	63
	1.04	0.80	77
	1.04	0.76	73
	1.04	0.75	72
	1.04	0.70	67
	1.04	0.65	63
	1.04	0.71	68
	1.30	0.86	66
	1.30	0.90	69
	1.30	0.85	65

[a] Average of 11 analyses with standard deviation of 0.025.

Procedure

Reagents. Dodecylguanidine Acetate (Dodine), purified sample, obtainable from Products Laboratory, Agricultural Division, American Cyanamid Co., Box 672, Princeton, N. J.

Bromocresol Purple Reagent (high purity material is essential). Three lots purchased thus far from Hartman and Leddon Co., Philadelphia, Pa., have been satisfactory without purification. Different lots from other suppliers often produced calibration curves with varying slopes. Related impurities, such as cresol red and bromocresol green, are believed responsible for these effects. Reproducible calibration curves could not be prepared from samples in which cresol red was detected. A new calibration curve should be prepared when-

Table III. Recovery of Dodecylguanidine Acid Phthalate from Apples

Sample Wt., Grams	Dodecylguanidine Acid Phthalate, P.P.M. Added	Dodecylguanidine Acid Phthalate, P.P.M. Found	Recovery, %
50	0	0.23	...
	0	0.31	...
	0	0.27	...
	0	0.27	...
	0.26	0.19	73
	0.26	0.24	92
	0.52	0.44	85
	0.52	0.50	96
	0.78	0.79	101
	0.78	0.59	76
	1.04	1.00	96
	1.30	1.29	99
	1.30	1.27	98
	1.30	1.11	85

ever a different lot of bromocresol purple is put into use.

A completely reliable purification technique has not been developed but the following recrystallization procedure (*8*) has proved suitable in most cases: Add 15 grams of bromocresol purple to 500 ml. of toluene and heat to 85° C. while stirring constantly; filter off the insoluble material and allow the toluene solution to cool slowly to room temperature while stirring constantly; filter off the bromocresol purple crystals and allow them to air-dry.

The following screening test was found useful for detecting the presence of cresol red in bromocresol purple: Dissolve 50 mg. of sample in toluene and pass the solution through a 1 × 15 cm. column containing activated Florisil (magnesium silicate gel from Floridin Co., Tallahassee, Fla., 60- to 100-mesh; activate by slurrying with 3*N* hydrochloric acid, wash free of chloride with water, and heat at 790° C. for 4 hours). Elute the column with 5% butanol in toluene and extract the eluate with aqueous alkali (10 ml.). A red-colored aqueous solution having an absorption peak at 572 mμ indicates the presence of cresol red (*6*). If the absorbance exceeds 0.100 at 572 mμ, the material should be recrystallized.

Bromocresol Purple Solution. Dissolve 0.4 gram of bromocresol purple in 75 ml. of 0.01*N* sodium hydroxide, adjust to pH 6.0 to 6.1 with sodium hydroxide or hydrochloric acid, and dilute to 500 ml. with distilled carbon dioxide–free water. Store in a stoppered brown bottle and allow to age at least 24 hours before use. This solution is generally stable for at least 1 month.

Buffer Solution, pH 5.5. Dissolve 15.2 grams of disodium hydrogen phosphate ($Na_2HPO_4 . 7H_2O$) and 74.0 grams of sodium dihydrogen phosphate ($NaH_2PO_4 . H_2O$) in distilled carbon dioxide–free water and dilute to 1 liter with water.

Special Apparatus. Beckman Model DU Spectrophotometer (or equivalent) with 1-cm. cells.

Waring Blendor (or equivalent), 1-quart size.

Tumbling Machine, motor-driven rollers geared to produce agitation at about 40 r.p.m.

Surface Residues on Apples

Calibration Curve. Dissolve approximately 32.5 mg. of purified dodine in 250 ml. of synthetic grade methanol (99.85% minimum) in a volumetric flask. Transfer a 25-ml. aliquot to another 250-ml. volumetric flask and dilute to volume with methanol (1 ml. is equivalent to about 13 γ of dodine). Using this standard solution, take aliquots of 0, 1, 2, 3, 4, and 5 ml. through the following procedure.

Place each aliquot in a 400-ml. beaker, dilute to 100 ml. with methanol, and add 50 ml. of water, 30 ml. of 30% sodium chloride solution, and 1 ml. of concentrated hydrochloric acid. Adjust the pH to about 5.5 with 4*N* sodium hydroxide. Add 20 ml. of buffer and 20 ml. of bromocresol purple solution and readjust the pH to 5.5 by means of a pH meter (the pH rises to 6.0 to 6.5 probably because of the change in the ionization of the phosphate salts by the alcohol present).

Transfer the solution to a 500-ml. separatory funnel and extract the DDG–bromocresol purple complex into chloroform (U.S.P. grade) by shaking for 2 minutes with each of two 50-ml. portions of this solvent. Shake the combined chloroform layers with 20 ml. of 0.05*N* sodium hydroxide for 1 minute to remove all free and combined bromocresol purple. Recomplex the DDG which remains in the chloroform as the free base by shaking this phase for 3 minutes with 5 ml. of the bromocresol purple solution and 20 ml. of the buffer solution. Extract any excess free bromocresol purple from the chloroform by shaking with 15 ml. of buffer for 1 minute. Transfer the chloroform layer to a clean, dry 250-ml. separatory funnel and shake for 2 minutes with 20 ml. of 0.05*N* sodium hydroxide (measured by pipet).

Read the absorbance of the aqueous layer at 590 mμ in a 1-cm. cell using 0.05*N* sodium hydroxide as a reference. Plot micrograms of dodine *vs.* corresponding absorbance (corrected for a reagent blank). Twenty micrograms of dodine produces a color having an absorbance of about 0.170. The calibration curve between 10 and 65 γ is essentially a straight line with a slope of 10.2 (absorbance *vs.* milligrams of dodine).

Determination. Extract a representative sample of apples with a suitable measured volume of methanol by rolling in a sealed bottle for 1 hour. Filter the extract through filter paper and wash the paper with 10 ml. of methanol. For analysis, take a measured portion of the methanol extract which will contain 20 to 60 γ of dodine. Dilute to 100 ml. with methanol and add 1 ml. of concentrated hydrochloric acid. (If an aliquot larger than 100 ml. is taken, add 1 ml. of concentrated hydrochloric acid and concentrate to 100 ml. on a steam bath.) Add 80 ml. of 10% sodium chloride and extract the aqueous-alcohol phase twice with 50-ml. portions of carbon tetrachloride, shaking each time for 1 minute. Discard the carbon tetrachloride layers.

These extractions remove plant material which would cause considerable emulsion difficulty later in the procedure. If the second portion of carbon tetrachloride is not clear or if an interface persists between the two phases, extract the aqueous-alcohol phase a third time with carbon tetrachloride. Continue from that point in the calibration curve procedure beginning, "Adjust the pH of the aqueous-alcohol solution to about 5.5 with 4*N* sodium hydroxide," etc. Measure the absorbance of the alkaline indicator phase, and obtain the dodine equivalent from the calibration curve prepared in a similar manner.

Total Residues in Apples and Cherries

Calibration Curve. Follow the procedure described for the preparation of the calibration curve for surface residues on apples through the point where the DDG–bromocresol purple complex is extracted into chloroform from 220 ml. of the methanol-water phase. Then shake the combined chloroform layers twice with 25-ml. portions of the 5.5 buffer solution, the first for 30 seconds and the second for 1 minute. The buffer washes at this point are necessary to lower the control value (apparent micrograms of dodine in untreated samples) to an acceptable level.

Transfer the chloroform layer to another separatory funnel and shake for 2 minutes with 20 ml. of 0.05*N* sodium hydroxide. Recomplex the DDG in the chlorofrom layer by shaking for 3 minutes with 5 ml. of the bromocresol purple solution, 20 ml. of the buffer solution, 25 ml. of methanol, and 10 ml. of 30% sodium chloride. Transfer the chloroform to another separatory funnel, and shake for 1 minute each with 3 succesive 15-ml. portions of the buffer solution. Transfer the chloroform layer to a clean, dry separatory funnel, and shake for 2 minutes with 20 ml. of 0.05*N* sodium hydroxide (measured by pipet).

Read the absorbance of the aqueous alkali at 590 mμ in a 1-cm. cell using 0.05*N* sodium hydroxide as a reference. Prepare a calibration curve by plotting the absorbance *vs.* the micrograms of dodine. The reagent blank is negligible in this procedure. Twenty micrograms of dodine produces a color having an absorbance of about 0.150. The calibration curve between 10 and 65 γ is essentially a straight line with a slope of 9.4 (absorbance *vs.* milligrams of dodine).

Determination. Macerate a representative sample of apples or pitted cherries in a Waring Blendor with 2-to-1(v./v.) methanol-chloroform using the ratio of 400 ml. of solvent per 100-gram sample. Filter the pulp from the extract by suction using two pieces of No. 1 Whatman filter paper. Wash the pulp by slurrying it with 2-to-1 methanol chloroform using the ratio of 100 ml. of solvent per 100-gram sample. (Wash cherry pulp a second time with an additional 100 ml. of methanol per 100-gram sample.)

Measure the volume of the extract and transfer a portion equivalent to a 50-gram sample to a 400-ml. beaker. Add 1 ml. of concentrated hydrochloric acid and several small glass beads, and evaporate to 50 ml. on a steam bath. Stir occasionally until boiling begins to avoid loss due to superheating. Add 30 ml. of aqueous 30% sodium chloride and 100 ml. of methanol. Allow the solu-

tion to cool and transfer it to a 500-ml. separatory funnel.

Extract gently with 50 ml. of carbon tetrachloride by inverting the separatory funnel and returning it to the upright position six to eight times. Discard the carbon tetrachloride layer and repeat the extraction with another 50 ml. of carbon tetrachloride. Allow the phases to separate and discard the carbon tetrachloride. Gentle agitation with carbon tetrachloride is essential at this point to prevent the formation of a stable emulsion. Two extractions usually remove sufficient emulsifying materials so that vigorous shaking is possible for further extractions. Continue to extract with 50-ml. portions of carbon tetrachloride until the carbon tetrachloride extract is clear and colorless. Do not draw off the interphase until after the final extraction.

Transfer the aqueous methanolic extract to a 400-ml. beaker, and adjust the pH to 5.5 with 4*N* sodium hydroxide. Add 20 ml. of buffer and 20 ml. of bromocresol purple solution and readjust the pH to 5.5 by means of a pH meter. Transfer the solution to a 500-ml. separatory funnel. Extract with an additional 50-ml. portion of carbon tetrachloride to remove completely the last trace of that solvent which separates as an oily phase when the water concentration in the aqueous phase is increased. Discard the carbon tetrachloride. Extract the DDG–bromocresol purple complex into chloroform by shaking with each of two 50-ml. portions of this solvent for 2 minutes. Continue from that point in the calibration curve procedure which reads, "Then shake the combined chloroform layers with 25 ml. of the 5.5 buffer solution," etc. Measure the absorbance of the alkaline indicator phase and obtain the dodine equivalent from the calibration curve prepared by a similar procedure.

Total Residues in Apple Leaves

Calibration Curve. Carry aliquots of 0, 1, 2, 3, 4, and 5 ml. of the diluted standard solution (13 γ per ml.) through the following procedure: Dilute the aliquot in a 500-ml. separatory funnel with 100 ml. of methanol, 100 ml. of sodium chloride solution, and 5 ml. of 10*N* sodium hydroxide solution. Extract the DDG from this solution by shaking for 1 minute with each of six 50-ml. portions of technical grade carbon tetrachloride (because of an unfavorable distribution, approximately 10 to 15% of the DDG is lost at this point as it still remains in the aqueous phase after six extractions).

To the combined carbon tetrachloride layers, add 50 ml. of methanol and 1 ml. of concentrated hydrochloric acid, and mix well. Then add 50 ml. of aqueous 20% sodium chloride solution and shake for 2 minutes. Transfer the carbon tetrachloride layer to another separatory funnel, add methanol, hydrochloric acid, and aqueous sodium chloride, and extract as before. Combine the two aqueous-alcohol phases containing the DDG; shake for 30 seconds with each of two 50-ml. portions of carbon tetrachloride and discard the carbon tetrachloride. Add 5 ml. of 10*N* sodium hydroxide and 50 ml. of chloroform to the aqueous-alcohol phase. Shake for 1 minute and transfer the chloroform to a clean 250-ml. separatory funnel. Repeat the extraction of the aqueous-alcohol phase with another 50-ml. portion of chloroform. Combine the two chloroform extracts; add 20 ml. of 0.05*N* sodium hydroxide and shake for 1 minute.

Transfer the chloroform layer to another 250-ml. separatory funnel. Add 20 ml. of the buffer and 5 ml. of the bromocresol purple solution, and shake for 3 minutes. Transfer the chloroform layer to another 250-ml. separatory funnel, and wash it with two 15-ml. portions of the buffer solution by shaking with each for 1 minute. Then transfer the chloroform phase to a clean, dry 250-ml. separatory funnel. Add 20 ml. of 0.05*N* sodium hydroxide (measured by pipet) and shake for 2 minutes. Read the absorbance of the aqueous phase at 590 mμ in a 1-cm. cell using 0.05*N* sodium hydroxide as a reference. Prepare a calibration curve by plotting the absorbance *vs.* micrograms of dodine.

A working calibration curve can also be prepared by the following condensed procedure: Dilute the standard solution aliquot with methanol, aqueous sodium chloride solution, and alkali, as described in the above procedure, and then shake with each of two 50-ml. portions of chloroform for 1 minute. Continue as described above beginning with, "Combine the two chloroform extracts; add 20 ml. of 0.05*N* sodium hydroxide and shake for 1 minute."

The longer procedure is more representative of the one actually used for analysis of plant extracts. Results based upon a calibration curve prepared by the shorter procedure will be approximately 20% lower, because the curve does not compensate for successive small losses of DDG which occur when the samples are carried through the longer multiextraction procedure.

Determination. Disintegrate 50 grams of apple leaves (without solvent) in about 10-gram portions in a Waring Blendor. Extract the leaves by tumbling mechanically for 1 hour in a 1-quart jar with 500 ml. of methanol. Separate the extract from the pulp by filtering through paper. Transfer an aliquot of the extract which will contain 10 to 60 γ of dodine to a 500-ml. separatory funnel, dilute to 100 ml. with methanol (if an aliquot of more than 100 ml. is taken for analysis, evaporate to 100 ml.), and add 100 ml. of 20% aqueous sodium chloride solution and 5 ml. of 10*N* sodium hydroxide solution. Continue as described for preparation of the calibration curve beginning, "Extract the DDG from this solution by shaking," etc. Measure the absorbance of the alkaline indicator phase at 590 mμ and obtain the dodine equivalent from the calibration curve prepared by a similar procedure.

Results and Discussion

All recovery and field sample data reported in this paper have been corrected for control values—i.e., the amount of "apparent" dodine found when untreated samples are carried through the analytical procedure. Appropriate controls should be run for each type of material being analyzed.

The data reported in Table III were obtained using the acid phthalate rather than the acetate salt of dodecylguanidine. As expected, the procedure proved equally applicable for both salts, and the calibration curves were identical when equimolar concentrations were used as a basis for comparison.

The recovery data shown in Tables I (total residues) and III were obtained by a slight modification of the procedure recommended. Methanol and sodium chloride were not added at the point in the procedure which reads, "Recomplex the DDG in the chloroform layer by shaking for 3 minutes with 5 ml. of the bromocresol purple solution, 20 ml. of the buffer solution, 25 ml. of methanol, and 10 ml. of 30% sodium chloride." During subsequent work addition of methanol and sodium chloride helped to eliminate an occasional erratic value obtained during preparation of the calibration curve. These erratic values were a result of the dodecylguanidine–bromocresol purple complex forming an insoluble film between the aqueous and chloroform layers which could not be extracted quantitatively into the chloroform layer. This difficulty was not encountered during analysis of apples, and either procedure was found applicable.

When residues are quite low it may occasionally be desirable to analyze extracts representing 100 grams of sample. To do this, the aliquot is acidified with 2 ml. of concentrated hydrochloric and evaporated to 100 ml. on a steam bath. Nine grams of sodium chloride and 100 ml. of methanol are added, and the solution is extracted with carbon tetrachloride and analyzed as described for total residues. Control values for samples of 100 grams were generally of the same magnitude (parts per million) as those for 50 grams.

A comparison of surface *vs.* total residues found on field sprayed apples (Table IV) indicates that the more simplified surface residue method may be adequate for measuring total residue levels at harvest.

Typical rate of disappearance data is shown in Table V. Surface and total residues were determined on McIntosh apples sampled from trees receiving no dodine after second, third, fourth, and fifth cover sprays, respectively. In general, the data indicate a fair degree of uniformity in the rate of disappearance of residues. The value of 0.9 p.p.m. of total residue found on the one harvest sample (Tables IV and V) taken 56 days after the last spray is believed due to inadequate sampling, and this value therefore is not considered typical. Rate of disappearance studies made on McIntosh, Cortland, and Red Delicious apples have indicated no significant differences among varieties.

The recoveries obtained from cherry samples fortified with dodine are lower than those from apples. Some material appears to be present in cherries which has a slightly adverse effect on the partitioning of the DDG–bromocresol purple complex between the solvents involved. The recommended calibration curve procedure for total residues in both apples and cherries does not include the series of lengthy preliminary cleanup extractions required when sample extracts are analyzed. Incorporation of these preliminary extractions in the calibration curve procedure would automatically produce recoveries 15 to 25% higher than those reported. A similar situation has been mentioned in connection with residues on apple leaves. The residue data shown in Table II for apple leaves, however, were obtained by use of a calibration curve which included these cleanup extractions.

The methods described have been applied successfully by several independent investigators to the determination of residues of dodine in apples (*2, 3, 5, 9, 10*) and pears (*11, 13*).

Table IV. Comparison of Surface vs. Total Residues of Dodine on Field-Sprayed Apples

(Number of sprays, 11)

Days since Last Spray	Dodine Found, P.P.M.	
	Surface	Total
3	1.3	0.9
14	0.7, 0.7	0.5, 0.5
35	0.3, 0.3	0.3, 0.3
56	1.0, 1.0	0.9

Table V. Residues of Dodine on Apples from Trees in Field Spray Program

Rate of Application[a]	Last Spray Applied	No. of Sprays	Days since Last Spray	Dodine Found, P.P.M.
	SURFACE RESIDUES			
0.75	2nd cover 6/7/57	7	13	3.7
			22	0.3
			33	0.6
	Harvest		91	0
	3rd cover 6/18	8	12	1.2
			23	1.2
	Harvest		81	0
	4th cover 6/28	9	12	0.7
	Harvest		70	0.1
	5th cover 7/9	10	1	2.5
	Harvest		59	0.1
2	2nd cover 6/7/57	7	13	20.8
			22	2.6
			33	3.8
	Harvest		91	1.0
	3rd cover 6/18	8	12	5.0
			23	4.5
	Harvest		81	0.4
	4th cover 6/28	9	12	4.0
	Harvest		70	0.6
	5th cover	10	1	10.0
	Harvest		59	0.7
	TOTAL RESIDUES			
0.5	2nd cover 6/6/58	8	48	0.3
			69	0
			90	0
			111	0
	3rd cover 7/10	9	14	0.4
			35	0.4
			56	0.1
			77	0
	4th cover 7/21	10	66	0.2
	5th cover 7/31	11	3	0.4
			14	0.5
			35	0.2
			56	0.2
0.75	2nd cover 6/6/58	8	48	0.3
			69	0
			90	0
			111	0
	3rd cover 7/10	9	14	0.4
			35	0.4
			56	0.2
			77	0.1
	4th cover 7/21	10	66	0.2
	5th cover 7/31	11	3	0.9
			14	0.5
			35	0.3
			56	0.9

[a] Pounds of wettable powder (65% actual dodine) per 100 gallons.

Acknowledgment

The authors thank M. Paylor and S. Sandler for performing some of the analytical work and D. H. Palmiter, New York Hudson Valley Experiment Station, Poughkeepsie, for supplying samples in Table V.

Literature Cited

(1) Booth, R. E., *J. Am. Pharm. Assoc., Sci. Ed.* **44,** 568–70 (1955).
(2) Bowery, T. G., North Carolina State College, Department of Chemistry, Raleigh, N. C., private communication.
(3) Carroll, R. B., Greenwich, Conn., private communication.
(4) Durick, F., King, J. S., Ware, P. A., Cronheim, G., *J. Am. Pharm. Assoc., Sci. Ed.* **39,** 680–2 (1950).
(5) Frear, D. E. H., Pennsylvania State University, University Park, Pa., private communication.
(6) Furman, N. H., "Indicators," translation of 2nd German ed. by I. M. Kolthoff, pp. 126, 320, Wiley, New York, 1926.
(7) Hamilton, J. M., Szkolnik, M., *Phytopathology* **48,** 262 (1958).
(8) Lubs, H. A., Clark, W. M., *J. Wash. Acad. Sci.* **6,** 481–3 (1916).
(9) Mitchell, J. E., University of Wisconsin, Madison, Wis., private communication.
(10) Powell, D., University of Illinois, Urbana, Ill., private communication.
(11) Terriere, L., Oregon State College, Corvallis, Ore., private communication.
(12) Thomis, G. N., Kotionis, A. Z., *Anal. Chim. Acta* **14,** 457–65 (1956).
(13) Zweig, G., University of California, Davis, Calif., private communication.

Received for review December 9, 1959. Accepted July 28, 1960. Division of Agricultural and Food Chemistry, 136th Meeting, ACS, Atlantic City, N. J., September 1959.