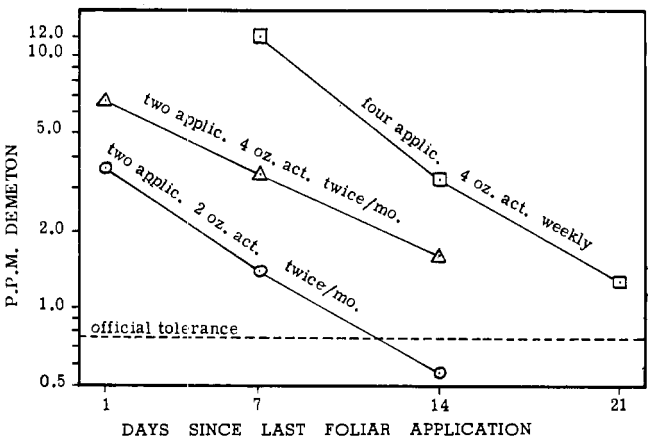

Figure 2. Demeton residue persistency on lettuce

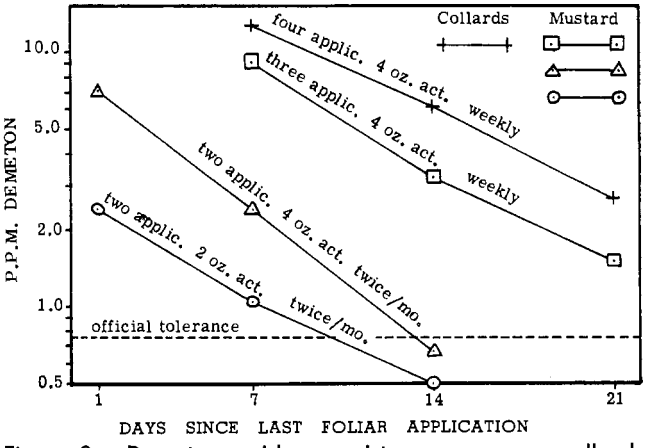

Figure 3. Demeton residue persistency curves on collards and mustard greens

when applied to leafy vegetables, deteriorates or detoxifies with time by a number of processes. Some of these processes such as evaporation, adsorption, and breakdown are probably taking place simultaneously (*1*).

As the translocation of demeton moves more preferentially through the xylem (*1*, *8*), it will move quite rapidly up into young, actively growing plants following absorption through the roots. According to Tietz (*6*), the active ingredient of demeton is translocated in the plants tested through the xylem following an application of the region of the roots, whereas following a leaf treatment it is transported chiefly in the phloem. He also states that the demeton is translocated through the medium of the transpiration stream to shoot organs growing above the ground. As a result of foliar applications, therefore, limited translocation and diffusion could occur in the leaves, primarily in the peripheral regions. There is also the possibility that some of the foliar applied systemic fell on the soil and was eventually absorbed through the roots and carried into the plant through the transpiration stream.

There are of course many intangibles and uncontrollable variables involved in any pesticide residue experiment in the field. A given crop, maturing rapidly under a comparatively high mean temperature, might be expected to absorb and translocate demeton rather readily in comparison with the same crop growing more slowly under cooler temperatures. There are also, however, the variables of sunlight, rainfall, and wind and their interactions which would tend to break down a foliar-applied systemic phosphate—as a conventional phosphate insecticide—until actual penetration of the plant itself was achieved.

In dealing with systemic insecticides applied to edible portions of leafy vegetables, the following factors should be kept in mind as far as possible excess residues remaining on the marketable crop: heterogeneity of leaf surface, very high surface to fresh weight ratio, dosage level in active ingredient, number of and interval between applications, interval or waiting period following the last application and harvest, possibility of systemic entering plant through roots as well as limited access provided by leaf contact, slower rate of detoxification and breakdown after penetration of the plant, stage and rate of plant growth at application time and the highly important, but largely uncontrollable, climatic factors.

Acknowledgment

The authors are grateful to the Chemagro Corp. of New York and Pittsburgh, Pa., for furnishing outdated, human blood plasma, as the source of cholinesterase used in this study, and 21.2% demeton standard reference.

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Received for review May 22, 1957. Accepted April 18, 1958. Divisions of Agricultural and Food Chemistry and Analytical Chemistry, 131st Meeting, ACS, Miami, Fla., April 1957. Florida Agricultural Experiment Station Journal Series No. 621.

PESTICIDE RESIDUES

Determination of Diphenylamine Residues on Apples

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DIPHENYLAMINE has shown promise as an effective agent for the prevention of scald on apples. This report presents two procedures that are useful for the determination of this compound as a residue on fruit.

Methods similar to those presented here have been described. Ponomarenko (*3*) described a colorimetric procedure for the determination of diphenylamine in air by coupling with diazotized sulfanilic acid in acid solution. Clements and Harrow (*1*) presented a method for the determination of diphenylamine in dyes based on ultraviolet absorption.

Analytical Procedure

Special Reagents. Petroleum ether. Reagent grade, purified by allowing each gallon to percolate through a column 2.6 × 76 cm. of silica gel (Davison Chemical Co. 20–200 mesh). The first 100 ml. is discarded.

n-Heptane. Purify 99% *n*-heptane (Phillips Petroleum Co.) in the same manner as the petroleum ether.

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Residues in apples are determined by blending apple samples with 90% methanol in a Waring Blendor. The slurry is extracted with petroleum ether. Diphenylamine is then extracted from the petroleum ether into concentrated hydrochloric acid as the hydrochloride. Dilution of the hydrochloric acid solution with water yields the free amine, which is re-extracted into petroleum ether. The petroleum ether solution is analyzed by the colorimetric procedure presented here. Mean recovery of 94% of diphenylamine was obtained when the amine was added to apples in concentrations of 0.1 p.p.m. or greater.

Diazotized 2,4-dinitroaniline. Described by Titus, Ulick, and Richardson (*4*). Diazotize 1.0 gram of 2,4-dinitroaniline with 0.5 gram of sodium nitrite each in 5.0 ml. of concentrated sulfuric acid. While stirring continuously, add dropwise 20 ml. of 85% phosphoric acid over a period of 1 hour. Decompose the excess nitrite with urea and dilute to 100 ml. with 85% phosphoric acid. Dilute 8.0 ml. of this stock to 100 ml. for the reagent. Store under refrigeration.

Preparation of Sample for Analysis. Determine total diphenylamine content as follows: Grind samples of control apples—approximately 200 grams—in a Waring Blendor with 90% aqueous methanol, and dilute the slurry formed to 500 to 1000 ml. with 90% methanol. Transfer aliquots of this slurry, representing 30 to 40 grams of apple, to 250-ml. centrifuge bottles, and add 50 ml. of petroleum ether. Shake the bottle for 1 minute and then centrifuge. Transfer the upper petroleum ether layer to an Erlenmeyer flask, and extract the lower layer twice more with 25-ml. portions of petroleum ether. Combine these petroleum ether extracts and evaporate on the steam bath to approximately 10 ml. Transfer this solution quantitatively to a 50-ml. glass-stoppered, graduated centrifuge tube with a total of 10 ml. of petroleum ether. Add exactly 4.0 ml. of concentrated hydrochloric acid, and stopper the tube and shake it for 1 minute. This procedure converts the diphenylamine to the hydrochloride, which is completely extracted into the concentrated acid.

Draw off the petroleum ether layer by means of an aspirator, taking care not to disturb the lower layer. Add petroleum ether again to the 25-ml. mark, shake the tube for 30 seconds, and remove the upper layer again. Immerse the tube in boiling water for 10 minutes, then cool, and add petroleum ether to the 25-ml. mark and shake the tube again in the manner described above. Remove the upper layer again with the aspirator and dilute the hydrochloric acid solution to 50 ml. with distilled water. Blow off the petroleum ether remaining with a gentle stream of air. Add exactly 10.0 ml. of petroleum ether and extract the diphenylamine from the aqueous layer by shaking the tube vigorously for 1 minute. Take aliquots of the upper layer for analysis.

Determine surface residues by placing samples of apples (2 to 4 kg.) in a large wide-mouthed, screw-cap bottle, add 200 ml. of petroleum ether, and mechanically rotate the bottle for 5 minutes. Pour off the solvent, add an additional 200 ml. of petroleum ether, and rotate the bottle again for 5 minutes. Combine the two washes, dilute to a suitable volume, and take an aliquot—usually 25 ml.—for analysis. Wash this aliquot once with an equal volume of 50% aqueous methanol and then carry through the procedure described above, beginning with the addition of 4.0 ml. of concentrated hydrochloric acid.

Colorimetric Procedure. To an aliquot of 5.0 ml., or less, of a petroleum ether solution of diphenylamine in a test tube, add 0.5 ml. of diazotized 2,4-dinitroaniline reagent and 0.4 ml. of acetonitrile (reagent grade). Extract the diphenylamine into the reagent by shaking vigorously for 30 seconds. Immerse the tube in a boiling water bath for 10 minutes, then add 4.5 ml. of 85% phosphoric acid and mix thoroughly. After cooling, read the absorbance in a spectrophotometer at 530 mμ against a blank treated in the same way.

Standard curves prepared by this procedure indicate that the absorbance is proportional to concentrations over the range of 1.0 to 20.0 γ of diphenylamine and follows Beer's law.

Ultraviolet Procedure. Prepare the sample as described above, except that the final extraction is made into 10.0 ml. of *n*-heptane instead of petroleum ether. Read the absorbance of the heptane solution at 265, 282, and 300 mμ. Calculate the concentration of diphenylamine by means of the equation:

$$C = 10.04\,(2.235\,A_{282} - 1.150\,A_{265} - 1.083\,A_{300})$$

where C is the concentration of diphenylamine in micrograms per milliliter, A_{282}, A_{265}, and A_{300} are the absorbances at 282, 265, and 300 mμ, respectively. This equation is valid only for the spectrophotometer used and, therefore, the correction factors must be recalculated for other instruments.

Discussion

An absorbance curve of the color formed, presented in Figure 1, shows that maximum absorbance is found at 530 mμ.

Table I. Variation of Absorbance with Volume of Acetonitrile Added

(Color developed with 12.2 γ of diphenylamine in 5.0 ml. of petroleum ether plus 0.5 ml. of reagent)

Acetonitrile, Ml.	A_{530} mμ
0.05	0.452
0.10	0.515
0.20	0.571
0.40	0.580
0.60	0.551
1.00	0.540
1.50	0.510

Table II. Recovery of Diphenylamine Added to Control Apples

Variety	Diphenylamine Added, P.P.M.	Diphenylamine Found, P.P.M.	Recovery, %
TOTAL RESIDUES			
Rome Beauty	0.143	0.139	97.2
	0.286	0.276	96.5
	0.572	0.500	87.5
	1.43	1.32	92.4
Jonathan	0.156	0.184	118
	0.625	0.522	83.5
	1.56	1.39	89.0
	3.12	2.98	95.5
SURFACE RESIDUES			
Rome Beauty	0.125	0.116	93.0
	1.25	1.16	93.0
	2.50	2.21	88.4

Table III. Comparison of Results Obtained by Colorimetric and Ultraviolet Methods

Sample No.	Colorimetric, P.P.M.	Ultraviolet, P.P.M.
3A	12.7	12.8
3B	12.0	12.0
12	0	0
13A	24.2	23.2
14	0	0
15A	18.5	16.7
15B	21.2	21.7

In preliminary work, diphenylamine is not completely extracted into the phosphoric acid solution of the reagent from petroleum ether. The use of acetonitrile in combination with the reagent, however, gives complete extraction. The effects of adding varying quantities of acetonitrile are presented in Table I. These results indicate that 0.4 ml. is the optimal amount for extraction and color development.

Dilution of the reagent with phosphoric acid prior to heating, as was used by Titus, Ulick, and Richardson (*4*), gave a

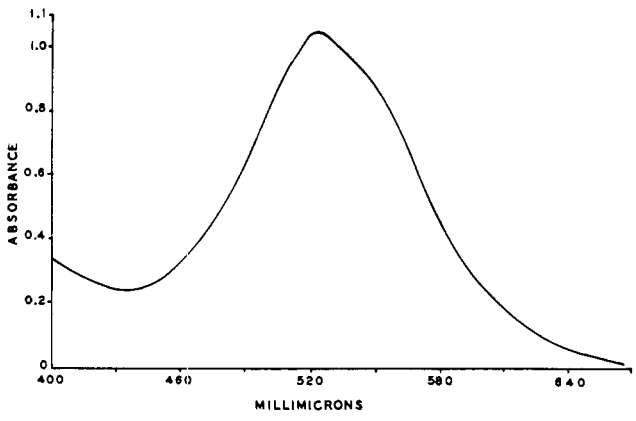

Figure 1. Spectral curve of color formed with 20.5 γ of diphenylamine

Volume, 5.0 ml. (Beckman DU spectrophotometer, 1.00-cm. cells)

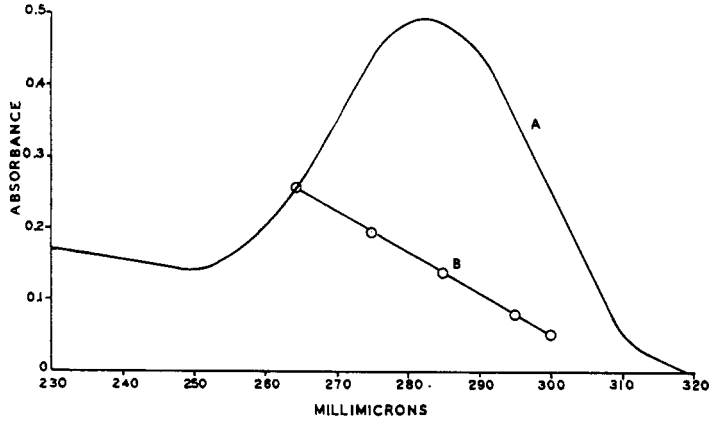

Figure 2. Spectral curves

A. Diphenylamine, 5 γ per ml.
B. Irrelevant materials from control apples in *n*-heptane

less intense color than the procedure described above. Increase in the time of heating did not increase the color intensity and the 10-minute period was arbitrarily chosen.

The use of volumes of petroleum ether solutions of diphenylamine larger than 5.0 ml. gave incomplete extraction into the reagent. Larger volumes of solution, of course, may be concentrated to 5.0 ml. or less and then extracted.

The ultraviolet absorption spectra of diphenylamine and of a solution obtained by carrying apples containing no diphenylamine (controls) through the ultraviolet procedure described are shown in Figure 2. The absorption spectra obtained from control apples show that the correction based on a straight line is valid. The equation used for the ultraviolet determination was calculated by the method described by Morton and Stubbs (*2*). A series of six solutions containing 0.80 to 8.00 γ of diphenylamine per milliliter of *n*-heptane as read at each of the three wave lengths, and the extinction coefficients for each were calculated. The average values were then used for calculation of the corrections in the above equations.

Various other procedures were attempted for the removal of interfering materials from apple extracts. Among these were distribution between petroleum ether and acetonitrile, saponification, steam distillation, and absorption on calcium hydroxide. The latter method appeared to offer some promise, but the process required more time than that described above.

Diphenylamine is a weak base and forms the hydrochloride only in the presence of concentrated strong acids. Dilution of the acidic solution with 10 parts of water causes enough hydrolysis so that the free amine may be extracted into an organic solvent. That these extractions are quantitative is shown by the same standard curve being obtained by carrying diphenylamine solutions through these extractions and developing color, and by developing the color on the original petroleum ether solution of diphenylamine.

When the procedure as described was used without heating the concentrated hydrochloric acid solution in the water bath, high blank readings were obtained. Some interfering material was being carried through which produced a yellow color, and heating the concentrated hydrochloric acid solution at the point indicated eliminated this interference.

Recoveries of diphenylamine added to control apples (using the colorimetric procedure for determining total and surface residues) are shown in Table II. These recoveries varied from 82 to 118%, with a mean of 94.1%.

Table IV. Results from Determination of Diphenylamine Residues on Apples after Dipping in Solutions of Diphenylamine, Immediately after Dipping, and after Storage at 32° F.

Concentration, P.P.M.	Time in Storage, Days	Variety	Diphenylamine, P.P.M. Subsample A	B	C	Av.
Control		Arkansas	0	0	0	0
		Jonathan	0.04	0.02	0.02	0.03
		Grimes Golden	0.03	...	...	0.03
		R. I. Greening	0	0	0	0
		Cortland	0	0	0	0
		Delicious	0	0	0	0
1000	0	Rome Beauty	3.59	3.25	3.64	3.49
	0	Arkansas	3.44	3.22	3.04	3.23
	112	Arkansas	1.20	0.93	1.09	1.07
	142	Arkansas	1.27	1.63	1.70	1.53
	153	Rome Beauty	1.59	1.76	1.77	1.71
2000	0	Rome Beauty	9.74	8.33	7.84	8.64
	0	Jonathan	10.6	12.0	11.4	11.3
	112	Arkansas	3.88	4.15	3.48	3.83
	125	Rome Beauty	3.23	3.75	3.52	3.50
	142	Arkansas	2.43	3.15	3.32	2.97
	153	Rome Beauty	2.99	3.21	3.44	3.21
3000	0	Rome Beauty	12.7	11.95	12.20	12.3
	0	Grimes Golden	63.2	56.2	68.5	62.6
	125	Rome Beauty	4.43	5.16	5.12	4.90
	140	Delicious	4.02	4.21	4.80	4.34
	142	Arkansas	5.22	5.30	5.54	5.35
	142	Arkansas	6.54	4.44	6.35	5.78
	153	Rome Beauty	5.44	5.99	5.64	5.69

Table V. Results from Determination of Diphenylamine Residues on Apples Treated with Diphenylamine Wraps after Storage at 32° F.

Concentration, Mg. Wrap	Time in Storage, Days	Variety	Diphenylamine, P.P.M. Subsample A	B	C	Av.
0.75	108	Arkansas	0.55	0.52	0.47	0.51
	125	Rome Beauty	1.05	0.92	1.05	1.01
	142	Arkansas	0.03	0.03	0.07	0.04
	153	Rome Beauty	0.68	0.81	1.01	0.83
1.5	125	Rome Beauty	3.11	3.41	2.65	3.06
	142	Arkansas	2.11	1.88	2.16	2.05
	154	Rome Beauty	2.95	3.40	3.63	3.66
2.0	125	Rome Beauty	2.41	2.72	2.26	2.46
	154	Rome Beauty	3.55	3.28	3.85	3.56

Table VI. Results from Determination of Surface and Pulp Residues in Apples Treated by Dipping in Diphenylamine Solutions

Concentration, P.P.M.	Time in Storage, Days	Variety	Residue Found, P.P.M. Surface	Pulp	Surface + pulp	Residue Found, P.P.M., Whole Fruit Analysis
2000	112	Arkansas	1.81	1.90	3.71	3.83
	125	Rome Beauty	1.77	1.95	3.72	3.50
3000	125	Rome Beauty	2.19	1.93	4.12	4.90

A number of samples of apples treated by dipping in diphenylamine solution were analyzed by both methods. The results are presented in Table III, and indicate that both methods are in excellent agreement.

The results obtained by the colorimetric method from the determinations of diphenylamine residues are shown in Tables IV and V. Table IV gives the results obtained from apples that were treated by dipping and Table V from wrapped apples. Two of the control samples contained negligible amounts, and the remainder contained no apparent diphenylamine. No corrections have been made for controls in calculating the results of treated apples.

Three samples of dipped apples were analyzed for surface and pulp residues, as well as total residue. The results, shown in Table VI, indicate that diphenylamine penetrates the skin, as approximately equal amounts were found on the surface and in the pulp.

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Received for review May 15, 1958. Accepted April 25, 1958. Work supported in part by the Biological Sciences Branch, Marketing Research Division, U. S. Department of Agriculture.

ANTIOXIDANT TOXICITY

Toxicological Studies on Sesamol

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Sesamol, a constituent of sesame oil, possesses antioxidant properties, and is at least partly responsible for the stability of this important oil. Sesamol can now be produced commercially and is a potentially useful antioxidant. Extensive toxicological investigations have shown that it is nonirritant to the skin and does not cause skin sensitization. Long-term feeding of twice recrystallized sesamol to rats had no effects on growth, mortality, or blood morphology. Deposits of pigment occurred in the kidneys of the female rats but were unrelated to the dietary levels of sesamol. A total of 20 proliferative lesions occurred in 134 rats fed sesamol. Sixteen of the lesions were benign, two were malignant, and two were questionable. No such lesions were found in the controls, nor in rats receiving the two lowest dosages of sesamol.

Chemical and physiological properties of sesame oil have been reviewed by Budowski and Markley (*4*). They stated that "sesame ranks ninth among the 13 vegetable oil crops which account for approximately 90% of the world's production of vegetable oils." They also pointed out that sesame oil "contains more unusual minor components and exhibits more unusual chemical and physiological properties than any other common edible oil. Some prominent characteristics of sesame oil are its stability on prolonged storage, its resistance to oxidative rancidity, and its ability to protect other fats against rancidity. These properties have been ascribed to the minor constituents—i.e., sesamin, sesamolin, and sesamol. In 1941, Olcott and Mattill (*13*) suggested that sesamol might be responsible for the stability of sesame oil and for its antioxidant properties.

Budowski, Menezes, and Dollear (*5*) demonstrated that the processing treatments of sesame oil released sesamol from its bound form (sesamolin) and resulted in increased stability of the oil. Because of its marked stability sesame oil is used in pharmaceuticals as a vehicle for fat-soluble substances, and finds extensive use in the food industry. Probably, the antioxidant properties of sesame oil are, in part at least, responsible for its value in certain pesticide formulations (*7, 8, 10, 11*) in which constituents such as rotenone and pyrethrin are subject to oxidative deterioration. The structures of sesamin and sesamolin have been proved recently (*2*). The relationship of sesamin, sesamolin, and sesamol, and the derivation of the latter by hydrolysis of sesamolin are shown in Figure 1. The isolation of the disaminyl ether from the hydrolyzate of sesamolin by Haslam and Haworth (*12*) suggests the formula shown for samin.

Sesamol is the methylene ether of oxyhydroquinone. From a structural point of view, sesamol probably would be a more effective antioxidant than either sesamolin or sesamin—both of which lack a free hydroxyl group. Budowski (*3*) found that sesamol possessed marked antioxidant activity in lard and vegetable oils, and that neither sesamolin nor sesamin had any appreciable antioxidant activity when tested by the active oxygen method. However, Gersdorff, Mitlin, and Beroza (*9*) found that sesamol is not active as a pyrethrin synergist, whereas sesamin and sesamolin are powerful synergists. These contradictory results suggest that a synergistic action toward pyrethrin in the presence of a biological factor may not be a simple antioxidant action. Observations on

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