Table II. Per Cent Mesquite Seedling Stem Kill Using 50% Acetone Carrier in Dip Applications[a]

Compound	Mg./Liter 50	12.5	25	50	50	100
	30 Days after Treatment				17 Days after Treatment	
2,4,5-Trichlorophenoxyacetic acid ester of						
DL-2-(2,4,5-Trichlorophenoxy)-1-propanol	71	15	29	51		
2-(2,4-Dichlorophenoxy)ethanol	70	16	30	56	46	61
2-(2,4,5-Trichlorophenoxy)ethanol	58	8	31	57	47	41
DL-1-(2,4,5-Trichlorophenoxy)-2-propanol	55			53		
DL-2-(2,4,5-Trichlorophenoxy)propionic acid ester of						
2-(2,4,5-Trichlorophenoxy)ethanol	42	14	34	43		
DL-2-(2,4,5-Trichlorophenoxy)-1-propanol	37	10	25	47		
DL-1-(2,4,5-Trichlorophenoxy)-2-propanol	10			9		
2,4-Dichlorophenoxyacetic acid ester of						
2-(2,4,5-Trichlorophenoxy)ethanol	4					
DL-1-(2,4,5-Trichlorophenoxy)-2-propanol	3					
2-(2,4-Dichlorophenoxy)ethanol	3					
DL-2-(2,4,5-Trichlorophenoxy)-1-propanol	2					
2-(2,4-Dichlorophenoxy)ethanol ester of 2-methyl-4-chlorophenoxyacetic acid	4					
2-(2,4-Dichlorophenoxy)ethanol ester of DL-2-(2,4-dichlorophenoxy)propionic	2					
2-(2,4-Dichlorophenoxy)ethanol ester of 4-chlorophenoxyacetic acid	1					
DL-2-(2,4,5-Trichlorophenoxy)propionic acid		4	22	38		
2,4,5-Trichlorophenoxyacetic acid		6	8	29	49	53
2,4-Dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid (1 to 1 mixture)					43	48

[a] All concentrations on a phenoxyalkyl parent acid equivalent basis.

initial response to the application. Final evaluations of the field plots will not be possible until the fall of 1959.

The considerable herbicidal activity of this group of compounds on mesquite suggests that further evaluations should be made on a variety of weed and crop plants. They may have possible uses in foliage or soil applications for the control of unwanted vegetation; to some extent this has already been indicated by preliminary evaluations of other investigators (*1*, *4*).

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PESTICIDE RESIDUES

Quantitative Determination of Arsenic Residues in Plant Materials

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A total analytical method is presented for the microdetermination of arsenic in plant material as finally measured by means of the molybdenum blue reaction. The procedure is based on a wet-ashing process of the material to be analyzed, followed by distillation in special apparatus. From 1 to 60 γ of arsenic per sample can be determined by direct reading from a single standard curve. This method is especially valid for determination of arsenic residues in any crop after preharvest treatment with arsenical compounds. Some typical data are given from field experiments using the organic arsenic-containing Tuzet as a fungicide on apple trees.

MICROAMOUNTS OF ARSENIC such as are found in plant material after fungicidal or insecticidal treatment require especially sensitive analytical methods, as, for example, in investigations of mechanisms of action or of the ultimate disposition of the arsenic in plant tissues and systems. It is accordingly important to be able to demonstrate magnitudes of arsenic-containing residues in harvested commodities. The analytical method, of course, should respond to arsenic in any compound or oxidation state.

Extensive literature shows that the molybdenum blue reaction is generally used for the quantitative microdetermination of arsenic as well as phosphorus. However, the molybdate method cannot be used directly to determine trace amounts of arsenic in biological materials because of the influence of phosphorus upon arsenic determinations. All published procedures for the determination of arsenic therefore include an ashing process, with subsequent differential separation of the arsenic for quantitative measurement.

The usual molybdate reagent imposes certain definite restrictions—i.e., choice of reducing agent, the amount of acid used, and the concentrations of other reagents and foreign ions present. The influence of foreign ions is essentially eliminated in the present procedure, and the acid effect is discussed in detail

below. An excess of the molybdate reagent must be avoided, as this reagent itself can be reduced to molybdenum blue. The literature indicates that most analysts have used hydrazine sulfate as the reducing agent of choice. Use of this compound results in a completely clear and colorless blank solution; also, the intensity of the final molybdenum blue from arsenic-containing samples is independent of the time of heating when this reagent is present and when maximum color intensity is achieved (*14*). Older procedures for arsenic are often characterized by poor reproducibility and recoveries because the above variables were neither recognized nor understood. An interesting study recently published by Hoffman (*7*) on organic arsenicals in feeds shows the "spectrum" of results obtained from standard samples by 18 collaborators with a method based upon arsine evolution.

Among the many possible micromethods for arsenic, the one based upon the work of Magnuson and Watson (*12*) seemed most adaptable to routine residue determinations. However, their method contained some uncertainties (cf. *1*, *13*), which have now been evaluated and compensated.

In the present procedure, arsenic in the plant tissues is converted to the quinquevalent form by the usual wet ashing with a nitric-sulfuric acid mixture, then separated practically quantitatively from interfering substances by distillation as a hydrobromic acid reaction product in special apparatus; the intense molybdenum blue reaction for arsenic is then applied to the distillate. A standard curve for the range 1 to 60 γ of arsenic need be prepared only once, for the recoveries are constant and the interferences nil. This method is simple, fast, and applicable to both organic and inorganic arsenic-containing compounds, and thus is adapted to the routine analyses of large numbers of dissimilar substrates.

Reagents

Potassium bromide, 30% aqueous solution of the C.P. salt, filtered before dilution to volume.

Molybdate reagent, 1% solution. Mix 20 ml. of reagent grade concentrated sulfuric acid with 80 ml. of water; dissolve 1 gram of C.P. ammonium molybdate tetrahydrate in 50.0 ml. of this dilute sulfuric acid solution, then dilute to 100.0 ml. with water. Prepare fresh every third day.

Hydrazine sulfate, 0.05% aqueous solution. Recrystallize this C.P. salt from water before use. Prepare fresh every third day.

Special Apparatus

A special distillation head is required to trap the arsenic(V) bromide (*1*, *12*) distilled from the wet-ashed analytical sample. This head is essentially that of Chaney (*3*) and Chaney and Magnuson (*4*), whose original diagram and text explanation were not sufficiently clear as to either construction or operation. A detailed diagram of the slightly modified complete distillation apparatus is therefore shown in Figure 1.

The lip, *L*, is constructed to return all the condensate to the boiler through the capillary, *K*, which is 1 mm. in inside diameter and 10 cm. long. Baffle plate *S* prevents the scrubbing solution contained in *F* from spattering into the capillary condensate-return. The tube ascending from the boiler is widened as shown into a chamber, *R*, which in similar apparatus (*12*) contained a second baffle plate; this second baffle plate is unnecessary in the present distillation head. In operation sufficient distilled water is placed in the U-shaped trap, *F*, to close the bend; the short section with indentations (Hempel column) increases the efficiency of scrubbing of the vapor stream containing the volatile arsenic compound. Vapors passing through the trap are condensed and returned to the boiler by *L* and *K*. Capillary *K* must not be wider than 1 mm., or the resistance of the water in *F* will cause the ascending vapors to pass through this capillary rather than through the scrubbing solution.

To distill the arsenic compound the external temperature applied to the boiler flask must be between 450° and 480° C., as measured with a thermoelement just touching the bottom apex of the flask; lower temperatures result in incomplete recoveries of arsenic. Equipment successfully and routinely used for this purpose includes a 220-volt Simon-Müller vertical muffle oven regulated by a variable transformer to achieve the constant desired temperature. The boiler flask is placed directly upon an asbestos ring on the already hot oven.

All accessory glassware must be boiled with concentrated nitric acid before use to extract interfering substances from the glass; the distillation head is cleaned by several blank runs.

Procedure

Preparation of Sample. The analytical sample must represent the average of the larger sample of plant material submitted. The amount of analytical sample selected for analysis depends upon the nature of the crop but should contain not more than 100 γ of arsenic; thus, 10 to 20 grams of green leaves or 20 to 100 grams of fresh, aqueous fruits or other plant material with a high water content are optimum for the present procedure. At least one arsenic-free control sample is essential.

Green leafy material is coarsely chopped and thoroughly mixed, and weighed subsamples are placed directly in 500-ml. Kjeldahl flasks for subsequent wet ashing. Fruit samples must be large enough so that the analytical sample can be obtained by using a quarter of each fruit submitted; these quarters of fruits are pulped in a Waring Blendor, and subsamples are weighed and placed in Kjeldahl flasks as above.

Wet Ashing. Two small glass beads are placed in each Kjeldahl flask containing the analytical subsample, then 5.0 ml. of concentrated sulfuric acid (reagent grade) followed by 10 to 15 ml. of concentrated nitric acid (reagent grade) are added with digestion in the usual manner. Additional amounts of nitric acid may be necessary to achieve complete oxidation of the plant material, with 5- to 10-ml. increments at first and then 1- to 2-ml. increments as complete oxidation is approached; there must be excess nitric acid in the final digestion mixture. As soon as this mixture becomes clear and colorless, it is heated strongly until copious fumes of sulfur trioxide appear, so that no traces of nitric acid will be retained in the solution. Nitric acid interferes strongly with the formation of the molybdenum blue complex and results in lower extinction values. Addition of perchloric acid is not necessary; if it is used to accelerate the ashing process, it must be volatilized from the digestion mixture to the extent that perchlorates do not precipitate upon cooling (*1*). The loss of sulfuric acid during the fuming operation must not exceed 40% of the initial amount added or arsenic will also be lost (*12*).

Distillation. After being cooled to room temperature, the strong acid solution in the Kjeldahl flask is transferred with a measuring pipet to the two-necked distillation flask (Figure 1), and additional concentrated sulfuric acid is added to make exactly 5 ml. of acid in the distillation flask; this precaution is essential to permit distillation at constant acid concentration. Kjeldahl flask and pipet are then rinsed four times with 5-ml. portions of distilled water, these rinsings are added to the distillation flask, two glass beads are introduced, and the flask is carefully heated over a gas flame to dense fumes of sulfur trioxide, again to concentrate the acid. After the solution cools to room temperature, 7.0 ml. of distilled water is added, and the apparatus as shown in Figure 1 is assembled with 2.0 ml. of the potassium bromide solution in the dropping funnel. Upon heating as explained under Special Apparatus, clearly visible condensed water quickly appears in trap *F*, whereupon 6.0 ml. of distilled water is added to this trap through the top of the distillation head, which is then immediately fitted with the reflux condenser. The potassium bromide solution is next blown into the distillation flask by means of a quick

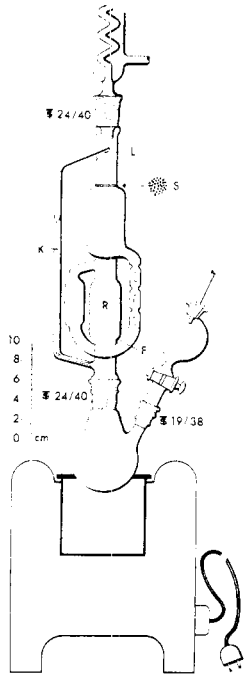


Figure 1. Diagram of distillation apparatus

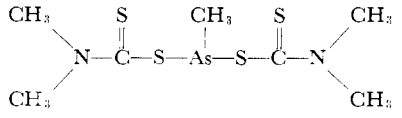
puff of air so as to overcome the overpressure within the distillation system, and the dropping funnel stopcock is immediately closed.

Ordinarily, after 4 to 6 minutes of vigorous distillation, faint but definitely visible vapors appear in the lower chamber, *R;* at this point the distillation is quickly stopped by lifting the distillation head with condenser from the flask so that the contents of trap *F* cannot be drawn back into the distillation flask. After removal of the condenser, the contents of the trap are carefully poured through the upper opening of the head into a 50-ml. volumetric flask, and the head is rinsed three times with 5-ml. portions of distilled water; these rinsings are also added to the volumetric flask.

Development of Color. To the trap solution in the 50-ml. volumetric flask are added 8.0 ml. of 1*N* sulfuric acid solution, 4.0 ml. of ammonium molybdate solution, and 4.0 ml. of hydrazine sulfate solution; after careful but thorough mixing and dilution to volume, the flask is immersed to the mark in a boiling water bath for exactly 10 minutes, then cooled to room temperature. The resultant intense blue solution of the molybdenum blue complex is stable for at least 24 hours, as reported by previous workers (*8, 15*), and is measured preferably at $\lambda_{max.}$ (840 mμ) in a 5-cm. cell.

A blank comparison solution is prepared from a mixture of the above quantities of reagents diluted to 50 ml. and subsequently heated in the manner described above. It is advisable occasionally to prepare blank distillation samples, which should show no arsenic content when evaluated against the blank comparison solution; if arsenic is present, either the glassware or the reagents are contaminated.

Standard curves are prepared in the above manner from solutions of pure sodium arsenate as well as from samples of Urbazid carried through the entire procedure. Urbazid comprises 20% of the formulation Tuzet and confers an arsenic content of 4.5% to it (Farbenfabriken Bayer, A.G., Germany):



Urbazid

Methylarsine-bis(dimethyldithiocarbamate)

(22.67% arsenic)

Results and Discussion

Distillation. The form in which the arsenic is distilled in the present general procedure is not known. Magnuson and Watson (*12*) assume that it is probably distilled as "an unstable arsenic pentabromide which decomposes on passing through the water in the trap."

Heating by a coil- or furnace-type electric heater is best in this temperature range. Liquid metal baths contain lead, tin, and other metals which could contaminate the analysis; a fumeless high-boiling silicone bath might be substituted satisfactorily, however. Heating mantles are not recommended for the present purpose because they overheat the upper walls of the boiler flask and thus violently evaporate the potassium bromide solution, subsequently added through the side neck of the flask, to contaminate the trap contents. The heater must be at temperature when a distillation is begun.

Microdistillation. After the fuming operation 7 ml. of water is added to the cooled sulfuric acid reaction mixture. Magnuson and Watson (*12*) used only 5 ml. of water for the present purpose, but they used an additional 2 ml. later to rinse the potassium bromide solution from the dropping funnel. As this solution contains excess potassium bromide in both procedures, it is actually not necessary to rinse the dropping funnel; a total of 7 ml. of water must be added to the distillation flask as described because the proportion of water at this stage is critical (cf. Influence of Acid Concentration).

When very small amounts of arsenic are present, the above volumes of liquids added to the distillation head as described under Distillation may be halved with only 3 ml. of trap liquid and two or three 2-ml. washes of water; the volumetric flask should be of 25-ml. capacity, according to Magnuson and Watson (*12*) and Bartlet, Wood, and Chapman (*1*). In the present work, however, such small volumes of water were found to be critical for micro quantities of arsenic.

Reduction. In other laboratories stannic chloride and reduced molybdic acid have been used for this purpose. Some objections against the use of these two reducing agents have been raised, however. For example, stannic chloride was reported as not contributing to the stability of the molybdenum blue (*14, 18*) and may also tend to produce a cloudy final solution (*19*); disadvantages of the reduced molybdic acid ("molybdenum blue reagent") are the necessity for a longer heating period to develop color (*5, 19, 21*) and an undesirable complexity in the preparation of the reducing solution (*19, 21*).

Other reducing agents that have been used for the microdetermination of phosphorus through the molybdenum blue reaction include 1-amino-2-naphthol-4-sulfonic acid, hydroquinone, and *p*-methylaminophenol sulfate, as reviewed in detail by Klement (*10*) and Lange (*11*). These reagents are not applicable for the determination of arsenic as they are generally used with sulfite or bisulfite salts, whereby arsenic ion can be reduced to arsenious ion which, of course, can no longer yield the molybdenum blue complex (cf. *19, 20*). Eastoe and Eastoe (*6*) have reported the successful use of a mixture of sodium metabisulfite, sodium sulfite, and aminonaphtholsulfonic acid as reducing agent in the determination of arsenic, however.

Hydrazine sulfate is the reducing agent of choice for maximum sensitivity and stability of the final colored complex (*14*); the present procedure will detect about 1 γ of arsenic per sample.

Color Evaluation. The molybdenum blue absorption curve between 600 and 900 mμ shows maximum absorption at 840 mμ, but lack of suitable equipment has occasioned doubtful use of wave length regions as low as 620 mμ for assay purposes with, however, decreased sensitivity. In the present work a Zeiss photocolorimeter Elko II with filter S 75 (maximum transmittance begins at 750 mμ) and a 5-cm. cell proved satisfactory.

Other wave lengths that have been used include 740 (*1*), 725 (*4*), 700 (*16*), 690 (*12*), 660 (*4*), 625 (*17*), and 620 (*8*) mμ. With the technique presented here, use of a suitable spectrophotometer allowing molybdenum blue evaluation at 840 mμ would expedite detection of very small amounts of arsenic. A detailed absorption curve from 600 to 900 mμ affords the extinction values of interest shown in Table I.

Kingsley and Schaffert (*9*) report 860 mμ as the absorption maximum for the molybdenum blue complex. The difference between this and the above value can be ascribed to the proportional compositions of the reagents in general use, established by Boltz and Mellon (*2*).

Table I. Molecular Extinction Values for Molybdenum Blue Complex at Several Wave Lengths

λ, Mμ	ε
620	7,572
625	7,705
660	8,507
690	8,962
700	9,230
725	10,140
740	11,610
750	12,660
840	25,415

Influence of Acid Concentration. The intensity of color of the molybdenum blue complex is dependent upon acid concentration. When the amount of acid per final unit solution is too small, the blue color is too dark because of color formation from the molybdate itself, and with excess acid the color fades. Between these extremes lies a region where small fluctuations in acid concentration are without effect.

To illustrate, in Figure 2 are extinction values for 26.6- and 39.9-γ quantities of arsenic plotted against increasing amounts of acid per usual sample; on the abscissa are recorded milliliters of increment 1*N* sulfuric acid solution (or 1*N* sodium hydroxide solution required to neutralize these 50-ml. preparations to phenolphthalein). With this method of arsenic determination, reproducible values are therefore attainable only in the region of the plateau, shown in Figure 2, occurring between 20 and 30 meq. of acid per 50-ml. sample.

Careful adherence to the procedural details presented above affords the correct acid concentration in the final 50-ml. volumetric flask, and this amount of acid is derived from:

1. The hydrobromic acid distilled from the distillation flask into the trap; this amounts to between 2 and 3 meq. Magnuson and Watson (*12*) report 2.5 to 4.0 meq. and Maren (*13*) reports 1.3 to 3.6 meq. Bartlet, Wood, and Chapman (*1*) have stated that the above ranges of values could result from the amount of water added before the distillation as related to the temperature of distillation.
2. The acid content of the hydrazine sulfate reagent.
3. The sulfuric acid content of the molybdate reagent.
4. The addition of 1*N* sulfuric acid solution before the development of the molybdenum blue color.

Point 1 is therefore not involved in the preparation of the arsenate-containing standard solution. As determined by titration with 1*N* alkali, the molybdate reagent mixture (points 2 and 3) contains 14.6 meq. of acid, as shown in Figure 2. These combined acid concentrations are too small for stability of the final molybdenum blue dye, however, and 8.0 ml. of 1*N* sulfuric acid solution must be added (point 4) to achieve the region of minimum influence of acid concentration upon reproducibility and stability of the final dye.

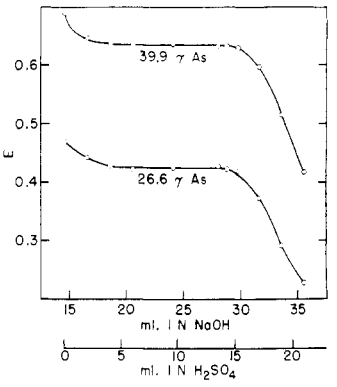


Figure 2. Effect of acid concentration on color intensity

The distillation must therefore be conducted in the manner specified and within the time specified. If it is conducted at a higher temperature or is prolonged, so much acid may be distilled into the trap that the other reagents and the supplemental acid addition before color development may exceed the critical region around 30 meq. of acid. In case of doubt, it is possible to determine by the back-titration of an aliquot of a questioned final solution whether the correct acid concentration had been achieved, with correction to 50 ml. of sample and reference to the data graphed in Figure 2.

Influence of Foreign Ions. The influence of foreign ions upon the formation of the molybdenum blue complex has been studied by several investigators; tin from canning containers adversely affects the reaction (*1*) also. With the present method the influence of foreign ions is essentially eliminated. It has been shown in these laboratories that interferences are not caused by mixtures of:

Arsenate–copper sulfate–acetic acid
Arsenate–lead acetate
Arsenate–calcium oxide
Arsenate–zinc sulfate

The heavy metals in these mixtures were in the same proportions as the corresponding components of Schweinfurter Green, lead arsenate (dilead orthoarsenate), simple calcium arsenate, and Tuzet, respectively.

In the event trace amounts of interfering substances codistill into the trap solution, a second distillation in exactly the same manner has proved useful.

Range of Method. Under the specified conditions and with the recommended volumes and cell, between 1 and 60 γ of arsenic in the 50 ml. of final solution (0.02 to 1.2 p.p.m.) are directly determinable from a standard curve. The detection of amounts of arsenic in the 1-γ region, however, has a comparatively high degree of uncertainty, and repeated analyses with greater amounts of plant sample can be accommodated by shorter cells or by dilution, if necessary, as described below.

The Lambert-Beer law holds strictly only for the range 1 to 10 γ of arsenic; beyond 10 γ the relationship between extinction and the amount of arsenic exhibits a slight deviation from linearity (see Figure 3). If the amount of arsenic in a sample is more than 60 γ—i.e., the extinction value is not directly determinable—the final solution should be read in a cell of shorter light path or a suitable aliquot should be diluted with a measured volume of the blank solution. With either alternative the amount of arsenic actually present in the measured sample can be determined only by referring the extinction value so obtained to the standard curve; this arsenic value is then related to the parent sample in terms of absolute thickness of cell or degree of dilution. Corresponding calculations from the extinction value itself afford incorrect arsenic values, as illustrated with two examples

1. Exactly 60 γ of arsenic (as sodium arsenate) was treated to develop the molybdenum blue complex as described. Aliquots of this deep blue solution were added to increasing amounts of the blank solution, then measured in a 5-cm. cell.
2. Similarly, the blue solution from 61.2 γ of arsenic in 50 ml. of water was measured in cells of decreasing thickness.

Table II shows some of the theoretical amounts of arsenic established in the dilution series and some of the different light paths used. The third column shows the micrograms of arsenic existing in each solution as established by use of the standard curve as recommended. The last two columns illustrate improper use of calculations from the standard curve. Column 5 contains extinction values calculated by multiplying the measured extinction value by the dilution and the light path factor, respectively. Column 6 contains the resultant arsenic content of each solution as read from an extrapolated standard curve. Values in column 6 increase with decreasing arsenic content of the sample.

When the present method is used within the range 1 to 60 γ of arsenic, it is possible to detect 0.1 p.p.m. in a sample of 10 grams of plant material, or 0.01 p.p.m. in 100 grams of sample; the upper limits lie between 0.6 and 6.0 p.p.m., respectively.

Standard Curves. As photometric evaluations afford only relative values by direct measurement, the arsenic content of a given sample is derived from a series of standard reference curves, prepared from definite amounts of arsenic in both

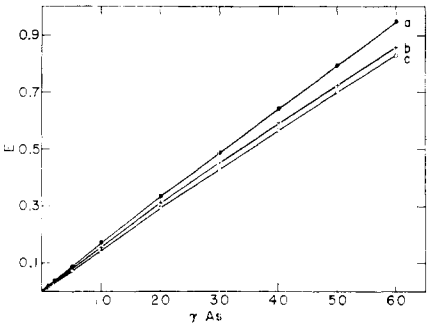


Figure 3. Standard curves

a. Sodium arsenate, primary standard
b. Sodium arsenate, distilled
c. Urbazid, ashed and distilled

the presence and absence of plant material. In the present investigation, these curves also included arsenic derived from purified sodium arsenate (24.01% arsenic) and from the typical arsenic-containing pesticide Urbazid (22.67% arsenic).

With Sodium Arsenate. Graduated amounts of purest Merck sodium arsenate ($Na_2HAsO_4.7H_2O$) were treated with the color-developing reagents as described, but without ashing and distillation, to give solutions containing 8.0 ml. of 1.N sulfuric acid, 4.0 ml. of molybdate reagent, and 4.0 ml. of hydrazine sulfate solution, and then were diluted with distilled water to 50.0 ml. each. The use of 8.0 ml. of the 1.N sulfuric acid solution here makes it unnecessary to add the other 3 meq. of acid that would be present in a distillation sample or over-all blank sample because the region of the color stability permits a leeway of 10 meq.; this 8.0 ml. of acid places the sample on the plateau (see Figure 2). After the required heating for 10 minutes, the intensities of the resulting colors are read and plotted as extinction values to yield curve *a*, Figure 3.

Another curve is required to validate the efficiency of the ashing and distillation operations. A graduated series of amounts of sodium arsenate is carried through the entire procedure, and the resulting extinction values are plotted as in curve *b*, Figure 3; comparison of curves *a* and *b* indicates an average loss of arsenic during the combined ashing and distillation steps of about 8%.

With Urbazid. A purified sample of Urbazid was dissolved in concentrated sulfuric acid at several concentrations, and aliquots were ashed and distilled as described; the molybdenum blue colors were measured and plotted to give curve *c*, Figure 3.

Comparison of curve *c* with curve *b* indicates an arsenic loss of about 5% when this compound is substituted for sodium arsenate in the over-all procedure; this apparent loss of arsenic may be ascribable to volatility of the sample of Urbazid. Curve *c*, however, demonstrates proportionality between amount used and amount found, so that quantitative accuracy is assured if sample data are referred to curve *c*. A comparison of curve *c* with the data in Table III shows further that in the presence of plant material the recovery of arsenic is practically complete. Table III includes both sets of values up to 60 γ of arsenic per sample for purposes of comparison.

Table II. Apparent Arsenic Recovery after Serial Dilution with Increasing Amounts of Blank Solution and Different Light Paths

	Arsenic Content				
			Calculated		
	Measured		From γ	From Extinction Value	
γ in Sample	E × 10³	γ	value, γ	E × 10³	γ
60.0	967.8	60.0	...	...	...
40.0	646.1	39.6	59.4	969.2	60.7
20.0	330.7	19.8	59.4	992.1	62.2
10.0	168.6	10.0	60.0	1011.6	63.5
Light Path, Cm.					
5.002	977.6	61.2	...	...	...
1.997	401.6	24.3	60.9	1006	63.1
0.996	204.0	12.1	60.8	1024	64.3
0.479	100.8	6.1	61.4	1035	65.0
0.101	19.3	1.25	61.9	1099	69.2

Table III. Comparison of Arsenic Recovery from Urbazid with and without Addition of 10 Grams of Chopped Beet Leaves per Sample

		Urbazid Found[a]		
Arsenic Equiv. Added, γ	Leaf Background[a] Arsenic, γ[c]	Arsenic, alone, γ[d]	Arsenic plus leaves γ[d]	Net[b] Arsenic
1.0	1.7	1.35	2.8	1.1
1.0	1.5	...	2.75	1.25
3.0	...	3.0	...	...
5.0	1.3	5.0	6.0	4.7
5.0	1.5	...	7.3	5.8
10.0	1.5	10.05	12.2	10.7
20.0	1.5	20.0	22.5	21.5
30.0	3.7	29.8	33.9	30.2
40.0	4.35	40.0	45.15	40.8
50.0	...	51.0	...	...
60.0	4.35	60.6	65.2	60.85

[a] Average of duplicate determinations.
[b] Arsenic found corrected for appropriate background value.
[c] Read from curve *b*, Figure 3.
[d] Read from curve *c*, Figure 3.

Table IV. Arsenic Residues in Apples after Treating with Tuzet (0.1%)

(Date of last spray July 19, 1957)

	Date		Arsenic, P.P.M.	
Variety	Picked	Analyzed	4 applications	10 applications
James Grieve	Aug. 20	Sept. 20	0.11	0.19
Cox Orange Ren.	Sept. 30	Oct. 15	0.10	0.21
Jonathan	Oct. 18	Nov. 15	0.20	0.25
Champ. Ren.	Oct. 25	Dec. 30	0.05	0.1

Conversion Factors. The information presented is based upon pure arsenic as the product of calculation. To convert these values to arsenic trioxide, to Urbazid, or to Tuzet equivalents the following factors are used:

As_2O_3 = As × 1.32
Urbazid = As × 4.41
Tuzet = As × 22.1

Field Data

In Table IV are presented some typical values for arsenic residues in four varieties of apples to illustrate magnitudes encountered in current practice.

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A bibliography of applications of the molybdenum blue reaction to the microdetermination of arsenic is available from the authors upon written request.

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FUNGICIDE RESIDUES

Colorimetric Method for the Determination of Dyrene Residues in Plant Material

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An analytical method for the microdetermination of the fungicide, 2,4-dichloro 6-(o-chloroanilino)-s-triazine (Dyrene) as a spray residue involves acid hydrolysis of the compound to form o-chloroaniline, which is diazotized and coupled with N-1-naphthylethylenediamine to produce a magenta color which is measured spectrophotometrically at 540 mμ. The method has been used for the determination of Dyrene residues in celery, onions, potatoes, and tomatoes.

THE IMPORTANCE OF DYRENE as a fungicide required the development of an accurate method for the determination of its residues on crops.

A colorimetric method developed by Burchfield and Storrs (*2*) at the Boyce Thompson Institute has been used to determine trace amounts of Dyrene and related compounds. The method depends upon the reaction of Dyrene with pyridine and measurement of the color developed when the solutions are made alkaline with aqueous sodium hydroxide. Quaternary pyridinium salts are first formed, which apparently undergo ring opening to form intensely colored Schiff bases. The color fades rapidly, but reproducible results can be obtained if the absorbance is read 2 minutes after addition of the alkali and compared to standards run under the same conditions.

An alternative method, reported in this paper, has been developed at the Pittsburgh Coke and Chemical, and Chemagro laboratories. It involves the acid hydrolysis of the Dyrene to form *o*-chloroaniline, which is diazotized and coupled with *N*-1-naphthylethylenediamine to yield an intensely colored azo dye. The color formed is essentially the same as in the Averell-Norris method for parathion (*1*).

In the development of a satisfactory method for the determination of Dyrene residues, a study was made to determine the optimum conditions for hydrolysis and color development. With pure solutions of hydrolyzed Dyrene, the color reaches a maximum in 10 minutes and is stable for at least another hour. However, the color develops more slowly in the presence of some plant extracts, taking up to 30 minutes to reach a maximum.

In view of this, all solutions should be measured for absorption one hour after addition of the coupling reagent. The color produced has an absorption maximum at 540 mμ (Figure 1). The absorption spectrum is identical with that obtained with *o*-chloroaniline, and Beer's law is obeyed over the range of 0 to 3.5 γ per ml. of final solution (Figure 2).

To determine the optimum conditions for the hydrolysis of Dyrene, hydrolyses were carried out in both acid and alkaline solutions and the efficiency of the hydrolysis reaction was measured by the colorimetric procedure. In these studies 215 γ of Dyrene in 10 ml. of isopropyl alcohol were refluxed on a steam bath for periods ranging from 0.5 to 5 hours with either 5 ml. of 5*N* hydrochloric acid or 7 ml. of 10*N* hydrochloric acid. An identical experiment was carried out using either 5 ml. of 5*N* potassium hydroxide or 10 ml. of 5*N* potassium hydroxide. In each case, an appropriate amount of acid or base was added to give an excess of 25 meq. of hydrochloric acid when the color was developed.

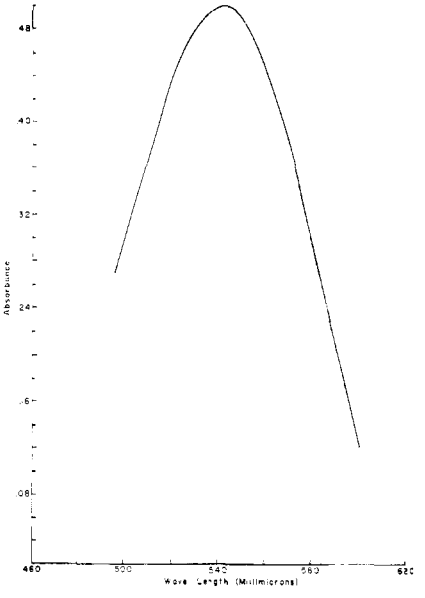

Figure 1. Absorption spectrum developed in determination of Dyrene

Complete hydrolysis of 215 γ of Dyrene should produce 100 γ of *o*-chloroaniline. Table I indicates that very little color develops when alkaline hydrolysis is used. The theoretical amount of color (based on *o*-chloroaniline) is obtained after 4 or 5 hours of refluxing with 5 ml. of 5*N* hydrochloric acid. At the higher