Analytical Methods for Pesticides, Plant Growth Regulators, and Food Additives

Volume IV

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PESTICIDES OF INTERNATIONAL IMPORTANCE

Analytical Methods for PESTICIDES, PLANT GROWTH REGULATORS, AND FOOD ADDITIVES

Edited by
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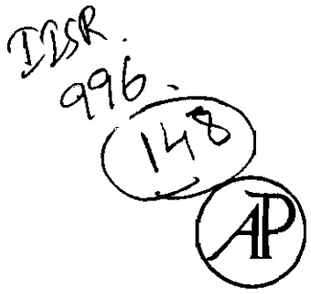
Volume IV HERBICIDES



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PREFACE

Ever since 1959, when herbicides and plant growth regulators were considered "pesticides" for the enforcement of United States food and drug laws (Colley Amendment), the problem of residues from such compounds in raw agricultural crops has become of paramount importance. Since many tolerances for herbicides are zero or "no residue" strictly owing to accepted agricultural practices—for example, for preemergent herbicides—the residue methods have to be of a greater sensitivity than those for compounds with a finite tolerance.

In Volume IV of this treatise thirty different herbicides and plant growth regulators which at present are widely used are covered in detail. Experimental methods for formulation and residue analyses have been described by experts or originators of the methods. Where alternate procedures have been developed by different laboratories, the combined effort by several authors does not necessarily imply seniority of authorship (Chapters 11 and 23). Where one analytical method is recommended for several herbicides, the chapters have been combined, for example, Chapter 16 covering monuron, diuron, and neburon.

Again, as in Volumes II and III of this treatise, other valuable data for each compound are included, such as chemical and biological properties, history of development, methods of synthesis, and modified residue methods for different crops.

The Editor wishes to thank all of the contributors to this volume for meeting their deadlines promptly and having adhered to the rigid format which such a multi-authored treatise demands.

GUNTER ZWEIG

Weizmann Institute of Science
Rehovoth, Israel
June, 1964

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Analytical Methods for Pesticides, Plant Growth Regulators, and Food Additives

Volume IV

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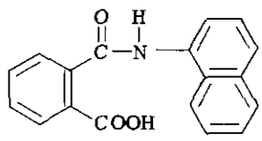
Alanap

J. R. LANE



N-1-Naphthylphthalamic acid

I. GENERAL

A. EMPIRICAL FORMULA

$C_{18}H_{13}O_3N$ (Mol. wt. 291.29).

B. ALTERNATE NAMES

N-1-Naphthylphthalamic acid, NPA, Alanap. Alanap is the registered trademark of the U. S. Rubber Co.

C. SOURCE OF ANALYTICAL STANDARD

Naugatuck Chemical Division, United States Rubber Company, Naugatuck, Connecticut.

D. BIOLOGICAL PROPERTIES

The oral LD_{50} for rats is in excess of 8.2 gm/kg of body weight. When administered as the water-soluble sodium salt of *N*-1-naphthylphthalamic acid, the oral LD_{50} for rats is indicated to be ca. 1.9 gm/kg of body weight. No adverse effects have been observed in the handling of large quantities of Alanap in the laboratory and in the field.

Alanap is a selective herbicide and growth regulant applied as a spray to the soil rather than as a contact herbicide to the weeds. (Exceptions: careless weed and cocklebur, which are very sensitive to post-emergence application.) Both mono- and dicotyledonous weed growth are retarded for a considerable time, or are killed. For maximum weed control, Alanap must be applied to the prepared soil surface im-
~~~

mediately or within 48 hours after the crop is planted. Since it has little effect on most emerged weed seedlings, application must be made before the weed seed germinates.

Alanap controls various annual broadleaf weeds and grasses such as pigweed, carpet weed, purslane, lambsquarter quickweed, ragweed, chickweed, cocklebur, velvetleaf foxtail, crabgrass, barnyard grass, goosegrass, jungle-rice, sprangletop, sandbur, windmill grass, stinkgrass, and cup grass; also ground cherry in asparagus, cotton (irrigated), soybeans, and nursery stock (Feldman and Reynolds, 1957).

E. History

The preparation of the *N*-aryl phthalamic acids is described in Beilstein (1929). Use of these compounds and their derivatives as plant growth regulants and phytocides is described by Hoffmann and Smith (1949) and in the inventions of Smith and Hoffmann (1951a,b); Feldman and Smith (1955); Smith and Feldman (1955, 1956, 1959); and Smith and his co-workers (1956a,b). The materials described give typical growth regulatory responses including prevention of fruit drop and formation of seedless fruit.

F. Physical Properties

Alanap, a nonvolatile white crystalline solid (m.p. 185°C, $d^{20/4}$ 1.40) is almost insoluble in water but slightly soluble in acetone, benzene, and alcohol. The alkali salts are very soluble in water (ca. 30 gm/100 ml solution). Decomposition occurs in solutions above a pH of 9.5.

G. Chemical Properties

1. method of synthesis

N-1-Naphthylphthalamic acid is prepared by reacting equimolecular amounts of phthalic anhydride and 1-naphthylamine in benzene, kerosene, toluene, or xylene at room temperature. The commercial method of preparation is described in the invention of Smith and Hoffmann (1951b).

2. *chemical reactions*

N-1-Naphthylphthalamic acid forms aqueous soluble salts with a variety of basic materials, is decomposed by strong acid or alkali, and by temperatures above 180°C.

H. Formulations

Alanap-3, a liquid concentrate, contains 23.7% (by weight) of the

sodium salt of *N*-1-naphthylphthalamic acid (equivalent to 22% of the active *N*-1-naphthylphthalamic acid).

Alanap-10G, a dry granular material, contains 10% (by weight) of the active *N*-1-naphthylphthalamic acid on an Attaclay base.

II. ANALYSIS

A. Formulations—Alanap 3

1. Review of methods

Potentiometric titration did not distinguish between the sodium salt of *N*-1-naphthylphthalamic acid and small amounts of sodium phthalate. Caustic hydrolysis of the sodium salt of *N*-1-naphthylphthalamic acid and distillation of 1-naphthylamine followed by colorimetric determination of the amine did not distinguish between any free 1-naphthylamine present and amine from the *N*-1-naphthylphthalamic acid itself. Incomplete distillation of the amine leads to poor sensitivity and reproducibility.

2. Recommended method

a. *Principle*

Nitrogen in *N*-1-naphthylphthalamic acid and free 1-naphthylamine is converted to ammonium sulfate by digestion with concentrated sulfuric acid in the presence of a catalyst and a salt to elevate the boiling temperature. Ammonia is liberated by the addition of strong alkali and quantitatively absorbed in boric acid solution. The ammonia is then titrated with standard acid (Furman, 1939). The total nitrogen found from Alanap plus any free 1-naphthylamine is calculated as Alanap (I).

A second portion of the sample is extracted with ether to dissolve free 1-naphthylamine. The absorbance of the extract, measured at 325 mμ, is used to calculate the percent of free 1-naphthylamine as Alanap (II). The % Alanap (I) — (II) gives actual % Alanap.

b. *Reagents*

Boric acid solution, 4% aqueous.

Kel-Pac Powder No. 2 (Kansas City Lab Supply Company, Westport Road, Kansas City, Mo.).

Methyl purple indicator (Fleisher Chemical Co., Washington 4, D.C.).

Sodium hydroxide, 40% solution (to afford high concentration with a minimum heat of solution), 0.1 *N*.

Sulfuric acid, concentrated and 0.10 *N*.

Zinc metal, mossy.
Ether, diethyl, A.R.

c. *Apparatus*

Bulbs, connecting and scrubber, modified Davisson (Will Scientific Inc., Item 20576).

Burettes, 50-ml, in 0.1-ml graduations.

Digestion and distillation apparatus, Kjeldahl.

Flasks, Kjeldahl, 800-ml.

Flasks, receiving, 500-ml, Erlenmeyer, wide-mouth.

Stoppers, rubber, Kjeldahl, 800-ml size (Fisher Scientific Co. Item 14-149-A).

Tubes, delivery, glass, 18 mm I.D., with exit orifice constricted to 3 mm.

Spectrophotometer, Beckman Model DU.

d. *Experimental Procedure—N-1-Naphthylphthalamic Acid*

Weigh ca. 0.9 gm of Alanap-3 (or 1.5 gm of Alanap 10-G) on an analytical balance. Transfer the sample to an 800-ml Kjeldahl flask. Add 1 Kel-Pac No. 2 (open the end of the Kel-Pac and empty the contents into the digestion flask). Add 30 ml of concentrated sulfuric acid. Swirl to mix the contents of the flask thoroughly. Place the flask on the digestion unit and apply heat. Digest the contents of the flask until disintegration of the sample is complete, as indicated by a clear green solution and continue digestion for about 2 hours. (Using the approximation of 1-hour digestion for every 5 minutes of heating required to turn the contents a clear green color.)

Cool the flask and add 250 ml of distilled water. Mix well and cool the solution in an ice-water bath. To a 500-ml wide-mouth Erlenmeyer flask add 75 ml of boric acid solution, 75 ml of distilled water and 8 drops of the indicator.

Attach a delivery tube to the distillation nnit and elevate the Erlenmeyer receiving flask so the tip of the tube is immersed to a maximum depth in the receiving solution. Turn on the condenser water and heaters. Without shaking the cooled Kjeldahl flask, add five pieces of mossy zinc metal, then 100 ml of 40% NaOH, allowing it to run slowly down the inside neck of the flask so two layers form. Quickly attach the flask to the Davisson trap on the distillation unit.

Thoroughly mix the contents by swirling the Kjeldahl flask while holding the stopper connection in place. Distill 200 ml for a total volume of 350 ml in the receiving flask.

Lower the receiving flask so the tip of the delivery tube is no

longer immersed, then discontinue heating the Kjeldahl flask. Allow the flasks to remain in this position until the water in the trap has been sucked back into the Kjeldahl flask.

Remove the receiving flask and delivery tube. Rinse the tube down, inside and out, with distilled water collecting the washings in the receiving flask.

Titrate the distillate with 0.10 *N* H_2SO_4. (The color change is green to purple with an intermediate gray tinge. True purple is considered the end point, while the gray color serves as a warning.) Carry out a reagent blank analysis daily exactly as described above.

e. *Determination of Free 1-Naphthylamine*

Weigh ca. 0.5 gm of Alanap-3 (ca. 1.0 gm of Alanap 10-G) on an analytical balance. Transfer the sample to a beaker and add 30 ml of 0.1 *N* NaOH to dissolve Alanap-3 (or wet Alanap 10-G). Transfer the sample (filter Alanap 10-G through a Whatman No. 4 filter paper) to a separatory funnel. Rinse the beaker with 20 ml of 0.1 *N* NaOH and add the washings to the separatory funnel. Extract the aqueous solution twice with 45 ml of ether. Combine the ether extracts and adjust the volume to 100 ml. Mix well and measure the absorbance at 325 mμ in 1-cm cells. Carry out a reagent blank analysis and use it as the reference solution

f. *Calculation*

$$\% \text{ Total (uncorrected) Alanap (I)} = \frac{29.1 \times (A - B) \times N}{S.W. \text{ in Grams}}$$

$$\% \text{ 1-Naphthylamine calculated as \% Alanap (II)} = \frac{0.733 \times \text{Absorbance}_{325\ m\mu}}{S.W. \text{ in Grams}}$$

$$\% \text{ Alanap (I)} - \% \text{ Alanap (II)} = \text{Actual \% Alanap}$$

where A = titer of sample, milliliters
B = titer of reagent blank analysis, milliliters
N = normality of sulfuric acid (ca. 0.10 N)
S.W. = sample weight

g. *Sensitivity*

Results are accurate to within 1% of the Alanap content. The precision is generally within ±0.05%. Duplicate analyses are made on all samples.

3. DISCUSSION OF METHOD

The Kjeldahl method for the determination of organic nitrogen is satisfactory for the assay of *N*-1-naphthylphthalamic acid in Alanap-3 or

10-G. Free 1-naphthylamine, the only possible source of additional nitrogen in the sample, is also determined and subtracted.

B. Residue Analysis

1. Review of Methods

Smith and Stone (1953) developed a sensitive and specific colorimetric test to determine Alanap residues in food crops. Using a special compact distillation apparatus, the crop sample (1 to 5 gm) is boiled in 30% NaOH. Under these conditions, Alanap hydrolyzes readily, the 1-naphthylamine liberated distills out and is trapped in acetic acid. Addition of diazotized sulfanilic acid produces an intense red coloration at a pH of 3 (specific for naphthylamine). All naturally occurring amines produce a yellow color with this reagent. Recoveries of 1 p.p.m. are obtained from a variety of fleshy crops.

When field samples treated at the recommended dosages indicated no residue at the 1-p.p.m. sensitivity, it was desirable to increase the sensitivity of the method to 0.10 p.p.m. to see if a residue might be detected at this level. Increasing the sample size alone did not provide increased sensitivity with crops such as peanuts, soybeans, cottonseed, and dried plants. These crops gave heavy foaming and intense interference.

In order to obtain 0.1-p.p.m. sensitivity with these crops, Lane and his associates (1958) made several modifications of the method of Smith and Stone (1953), including a much larger sample size, solvent extraction of the 1-naphthylamine from interference in the distillate and use of a base-line technique calculation.

2. Recommended Method

a. *Principle*

In this modification of the method by Lane *et al.* (1958) Alanap hydrolyzes in boiling 40% NaOH to liberate 1-naphthylamine which distils. Boiling the distillate drives off potential interferences. The 1-naphthylamine partitions from the distillate into hexane and subsequently into dilute mineral acid. Adjustment of the pH to 3 (for maximum color intensity) and addition of diazotized sulfanilic acid produces a red color with 1-naphthylamine and a weak yellow color with small amounts of natural interfering materials. Absorbance measurement at three wavelengths and calculation by a base-line technique calculation (as discussed by Newell *et al.*, 1958) minimizes the effects of interference and variation of the background between samples.

b. *Reagents*

Sulfanilic acid; saturated 30% acetic acid solution. (<1%)

c. *Apparatus*

Adapter, reducing, with top 24/40 outer and bottom 45/50 inner ℱ joints.

Bulb, cylindrical connecting, Kjeldahl, with 24/40 inner ℱ joints on both ends.

Condenser, West-type, 300-ml jacket length, with top 20/40 outer and bottom 24/40 inner ℱ joints.

Flask, round-bottomed, 5-liter, with 45/50 ℱ joint.

Tube, connecting, 105°, with a top outer 24/40 ℱ joint.

d. *Experimental Procedure*

i. *Analysis.*

Place a 200-gm sample of the ground material, 1000 ml of 40% NaOH, 5 gm of paraffin wax, 2 ml of Antifoam A, and 1 gm of zinc granules in a 5-liter round-bottomed flask.

Swirl the flask to wet the sample thoroughly and place in a heating mantle. Clean and lubricate each joint with glycerol (except the water-condenser connections). Join securely and position a 400-ml beaker or flask as the receiver. While cooling the condenser with rapid water flow, heat the flask with a Variac setting of about 120. When an inch of foam has formed on the surface of the flask's contents, turn down the Variac setting to 70 until distillation begins. If the foam climbs to the neck of the flask, place a wet cloth on the exposed surface of the flask. The foam will subside in a few seconds. In a short time the foam will remain at a low level. Increase the heating slowly until a Variac setting of 110 is reached and distillation will proceed rapidly.

Collect 200 ml of distillate. Rinse the condenser with 50 ml of hexane and combine with the distillate. (Here samples may be stored overnight.) Add 50 ml of distilled water to the distillate plus hexane and boil until the volume is reduced to 200 ml. Cool the boiled distillate to room temperature and transfer to a 500-ml separatory funnel. Rinse the emptied receiver with 100 ml of hexane and add to the 500-ml separatory funnel. Stopper and shake the separatory funnel briskly for 1 minute. Allow the layers to settle for 2 minutes, and draw off and discard the aqueous layer. Rinse the retained hexane layer once with 100 ml of distilled water and draw off and discard all traces of the water layer.

To the washed hexane layer in the separatory funnel, add 4.5 ml of

distilled water and one drop of concentrated hydrochloric acid. Shake the funnel thoroughly for 1 minute. Test the wet, ground-glass stopper for the pH of aqueous phase with 0.0 to 1.5 pH range Hydrion paper to assure a pH of 1.0 or below. If the pH is higher, add a second drop of concentrated hydrochloric acid and shake thoroughly. Let the solution stand for 2 minutes before drawing the aqueous layer into a 10-ml mixing cylinder. At this point, samples may be stored for as long as 24 hours before developing the color.

To the acid solution in the mixing cylinder add dropwise, with mixing, 10% NaOH (containing 0.01 gm phenolphthalein/100 ml) until basic. Add 3 ml of glacial acetic acid followed by ten drops of coupling reagent (prepared by mixing equal volumes of sulfanilic acid solution and 0.12% sodium nitrite solution 5 to 30 minutes prior to use). Dilute the solution in the mixing cylinder to 10 ml with distilled water, mix well, and measure the absorbance at 480, 534, and 600 mμ in a Beckman DU spectrophotometer in 1-cm cells 30 minutes after the addition of coupling reagent.

ii. *Discussion of Interference.*

Interference should be nondetectible in all crops (Table I). Where Alanap is present the characteristic red color appears. Where Alanap is not present the color solution has a yellowish, almost colorless tinge (Fig. 1). NaOH in excess of that needed to neutralize the HCl, just prior to the addition of the color-forming reagent, causes a strengthening of a yellow background color after the dye bas formed. If natural interference (yellow) appears in an analysis, calculations similar to those used by Lane *et al.* (1958) may be substituted. The appearance of interference color, however, is the exception and is usually caused by deviation in analytical technique. Newell *et al.* (1958) discuss the base line technique for minimizing the effect of interference on data.

iii. *Sensitivity of the Method.*

Table I shows actual recovery data from a series of individual untreated samples fortified with 0.10 p.p.m. of Alanap before distillation. Interference from the various crops is less than 0.02 ppm; 0.03 ppm of Alanap is detectible.

iv. *Recoveries.*

Recoveries of Alanap from the eight crops listed in Table I show a low of 0.06 p.p.m. and a high of 0.13 p.p.m. with an average recovery of 88% from 28 determinations.

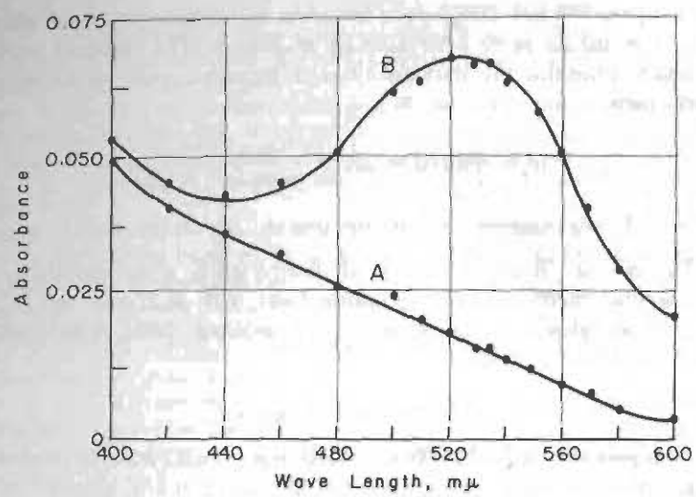


FIG. 1. Typical absorption spectra used for deriving equations. A, untreated soybeans; B, untreated soybeans +15 μg of Alanap.

TABLE I
RECOVERIES OF ALANAP ADDED TO UNTREATED FOOD CROPS

Crop	Added, p.p.m.	Determinations, No.	Recovered p.p.m.[a]	Recovery, %	Recovery, Ave.
Beans	0.08	3	0.06 to 0.08	78 to 98	91
Melons	0.10	1	0.073	73	73
Peaches	0.10	2	0.08 to 0.10	80 to 100	90
Sweet Potatoes	0.10	5	0.07 to 0.13	70 to 130	88
White Potatoes	0.10	3	0.09 to 0.114	90 to 114	95
	0.20	1	0.18	90	90
Cottonseed	0.10	4	0.06 to 0.12	60 to 120	83
(+25 gm zinc)	0.10	4	0.07 to 0.11	70 to 110	90
Peanut vines	0.10	1	0.08	80	80
Soybeans	0.10	4	0.089 to 0.11	89 to 110	100

[a] Corrected for interference from untreated samples.

v. *Calculations.*

Prepare a standard curve by processing 0, 10, 20, and 30 μg of Alanap (0.05, 0.10, and 0.15 p.p.m.) through the given analytical procedure and plot a curve of net absorbance vs micrograms of Alanap.

$$\text{Net absorbance} = \text{Abs.}_{534\ m\mu} - \left(\frac{\text{Abs.}_{480\ m\mu} + \text{Abs.}_{600\ m\mu}}{2}\right)$$

Compare the net absorbance found in an unknown sample analysis to the standard curve to determine μg of Alanap. The standard curve is sufficiently reproducible that an average equation may be derived for general use.

$$\text{P.p.m. Alanap} = 323 \times \frac{\text{Net absorbance}}{\text{Sample wt. (gm)}}$$

3. APPLICATION OF THE METHOD TO DIFFERENT CROPS

The method is applicable to all fleshy fruits and vegetables; also the following field dried crops: cotton bolls, ginned cotton and cotton-seed; whole plants; soybeans with and without pods, whole plants; peanuts and whole plants.

4. DISCUSSION OF METHOD

Zinc granules are added to the basic sample digestion to protect the 1-naphthylamine from being oxidized by components present in the sample. Without zinc, recoveries of 0.1 p.p.m. Alanap are low. Paraffin wax (household) and Antifoam A in combination are the most successful antifoam agents for use in the basic digestion and distillation of all dried plants, seeds, and nuts. No antifoam agents are used in the analysis of peaches, sweet and white potatoes.

For watery crops the following reduced size of distillation system is generally adequate. A 200-gm crop sample, 600 ml of 40% NaOH, and 1 gm of zinc granules are distilled in a 2-liter round-bottomed flask attached to the described distillation apparatus. The 200 ml of distillate are diluted with water, boiled, extracted, and the analysis completed as given in the procedure.

Recently, it was found that Soxhlet extraction of oily nut or seed meal removes all oil, but does not remove *N*-1-naphthylphthalamic acid. The residual oil-free meal foams less, and the fortified samples give better recovery in analysis than do the whole meal samples. As an example, 200 grams of oily nut or seed meal (in a 60×180 mm thimble) are Soxhlet-extracted with 500 ml of hexane or petroleum ether for 2 hours at a rate of one siphoning per 4 minute interval. After drying, the oil-less meal powder is transferred to a 2- or 3-liter flask and 1000 ml of 40% NaOH and antifoam agents are added. The analysis is completed as indicated in the method given previously.

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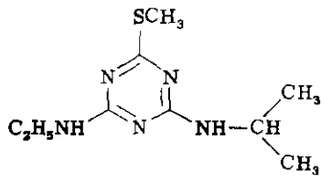
E. KNÜSLI



2-Methylthio-4-ethylamino-6-isopropylamino-*s*-triazine

I. GENERAL

A. EMPIRICAL FORMULA

$C_9H_{17}N_5S$ (Mol. wt. 227.33).

B. ALTERNATE NAMES

Trial number G 34 162.

C. SOURCE OF ANALYTICAL STANDARD

J. R. Geigy S.A., Basel, Switzerland; Geigy Agricultural Chemicals, Ardsley, New York.

D. BIOLOGICAL PROPERTIES

1. HERBICIDAL ACTIVITY

Ametryne is a pre- and post-emergence herbicide for general and selective use. The potential fields for its selective application include sugar cane, small grains, peanuts, and soybeans.

2. TOXICITY

Acute oral toxicity: LD_{50} for mouse 965 mg/kg; rat 1110 mg/kg.

Subchronic toxicity: A series of rats fed for 90 days with a daily oral administration of 20 and 200 mg Ametryne 50 W/kg was comparable in all behaviors with the controls.

Cutaneous toxicity: A 5% suspension of Ametryne or Ametryne 50 W in gum arabic applied for 5 consecutive days to rats caused no signs of adsorptive toxicity.

E. History

H. Gysin and E. Knüsli are co-inventors of Ametryne on Swiss Patent 337,019 (to J. R. Geigy S.A., Basel, Switzerland). See also Gysin and Knüsli (1960).

F. Physical Properties

Melting point: 84–86°C.

Solubility in water at 20°C: 0.0185% (185 p.p.m.); in organic solvents, high.

Colorless, not combustible, not explosive, not corrosive.

UV-spectra: See Fig. 1.

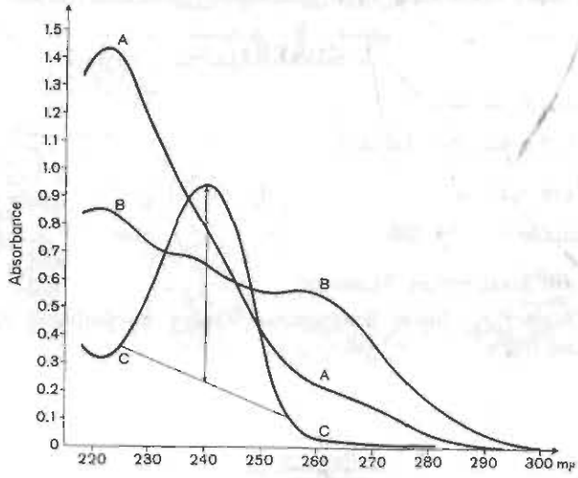


Fig. 1. UV-Spectra of: A, Ametryne in a 0.5% aqueous borax solution; B, Ametryne in 1 *N* sulfuric acid (cation); C, product of hydrolysis (2-hydroxy-4-ethylamino-6-isopropylamino-*s*-triazine) in 1 *N* sulfuric acid (cation). (Concentration of Ametryne = 8 μg/ml in each solution.)

G. Chemical Properties

1. Method of Synthesis

Ametryne is synthesized by reacting 2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine (Atrazine) with methyl mercaptan in the presence of an equivalent of sodium hydroxide; or by reacting 2-mercapto-4-ethyl-

amino-6-isopropylamino-*s*-triazine with a methylating agent in the presence of sodium hydroxide.

2. PROPERTIES

It is stable in neutral, slightly acidic or basic media; it hydrolyzes to the herbicidally inactive 2-hydroxy-4-ethylamino-6-isopropylamino-*s*-triazine in acid or basic media, especially at higher temperatures.

H. FORMULATIONS

Ametryne is available as a 50% wettable powder and a 25% emulsifiable solution.

II. ANALYSIS

A. FORMULATION ANALYSIS

The methods (Suter, 1959) and their accuracy are identical with those described for Prometryne, as are the respective interferences. (See Chapter 18 on "Prometryne.") However, the following different equations have to be used:

$$\% \text{ of Ametryne} = \frac{a \times 227.34 \times 100}{w_1 \times 10{,}000} = \frac{a \times 2{,}273}{w_1} \qquad (1)$$

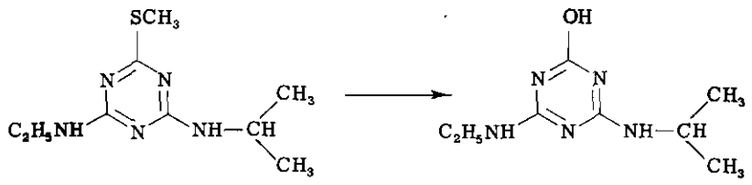
$$\% \text{ of Ametryne} = \frac{(50.0 - b) \times 227.34 \times 100 \times 1.015}{w_2 \times 10{,}000} = \frac{(50.0 - b) \times 2.308}{w_2} \qquad (2)$$

B. RESIDUE ANALYSIS

The method (Delley, 1961) and its peculiarities are analogous to those described for Prometryne (see Chapter 18 on "Prometryne") with the following differences:

1. PRINCIPLE

The respective scheme is as follows:



2. EXTRACTION

Quantitative extraction is achieved by extracting three times with 25 ml of 0.1 *N* HCl.

3. DISTRIBUTION COEFFICIENTS IN DIFFERENT SOLVENT SYSTEMS

In the course of the development of the present procedure, some approximate distribution coefficients at analysis conditions (concentration in organic phase: concentration in aqueous phase) were found and are shown in Table I.

TABLE I
DISTRIBUTION COEFFICIENTS OF AMETRYNE IN SEVERAL SOLVENT SYSTEMS[a]

Organic phase	0.1 *N* NaOH	Water	0.001 *N* HCl	0.01 *N* HCl	0.1 *N* HCl	1 *N* HCl
n-Pentane	24	19	2.3	0.24	0.02	—
Ether, diethyl	>80	>80	—	6.6	0.5	0.09
CH_2Cl_2	>80	>80	—	35	5.4	13
$CHCl_3$	>80	>80	>80	58	11.4	58

[a] Concentration in organic phase: concentration in aqueous phase.

It seems that by means of methylene chloride and chloroform the active ingredient is partly extracted from 1 *N* HCl in the form of Ametryne hydrochloride.

REFERENCES

Delley, R. (1961). Anal. Lab., J. R. Geigy S.A., Basel, Switzerland, unpublished work.

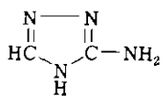
Gysin, H., and Knüsli, E. (1960). *Advan. Pest Control Res.* 3, 289–358.

Suter, R. (1959). Anal. Lab., J. R. Geigy S.A., Basel, Switzerland, unpublished work.

3

3-Amino-*s*-Triazole

G. L. Sutherland



I. GENERAL

A. Empirical Formula

$C_2H_4N_4$ (Mol. wt. 84.1).

B. Alternative Names

Cytrol, Amino Triazole Weedkiller, Amitrole, Weedazol.

C. Source of Analytical Standard

Agricultural Division, American Cyanamid Company, Princeton, New Jersey.

D. Biological Properties

Aminotriazole shows herbicidal activity against a wide variety of plants. The acute oral LD_{50} (male albino rats) is approximately 25 gm/kg.

E. History

The synthesis of aminotriazole was described by Curtius and Lang in 1888. Its use as a herbicide was developed by Amchem Products and is covered in U. S. Patent 2,670,282.

F. Physical Properties

Melting point: 150–153°C (technical).

Solubility: About 28 gm in 100 gm of water at 23°C; 53 gm in 100 gm of water at 53°C; 26 gm in 100 gm of ethanol at 75°C; insoluble in nonpolar solvents.

G. Chemical Properties

1. Method of Synthesis

The compound is formed by the interaction of formic acid and aminoguanidine.

2. CHEMICAL REACTIONS

Aminotriazole reacts with acids to form salts; with aldehydes and ketones to form aldimines and ketimines; and with di- and trivalent metal salts to form complexes.

H. FORMULATIONS

Aminotriazole is sold as a 50% soluble powder and liquid formulation consisting of an equimolar solution with ammonium thiocyanate.

II. ANALYSIS

A. FORMULATION ANALYSIS

1. RECOMMENDED METHOD

a. *Principle*

Solid formulations of 3-amino-*s*-triazole are extracted with dimethyl formamide and an aliquot of the extract is diluted with water. Excess standard acid is added and the solution is titrated with standard alkali. The volume of the titrant consumed between the first and second inflections is a measure of the aminotriazole content.

b. *Reagents*

N,N-Dimethylformamide (DMF), Reagent Grade.
Hydrochloric acid, 0.5 *N*.
Sodium hydroxide, 0.5 N, standardized.

c. *Special Apparatus*

Powder funnel.
Filter funnel, fritted-glass, medium porosity.
pH Meter, Beckman Model G or equivalent, equipped with glass-calomel electrode.

d. *Experimental Procedure*

i. *Sample Preparation.*

Accurately weigh 10.000 ± 0.001 gm of sample and quantitatively transfer to a 100-ml glass-stoppered Erlenmeyer flask. Add 60 ml of DMF and shake for 2 to 3 minutes. Allow the inert material to settle and decant the supernatant liquid into a 100-ml volumetric flask.

The 3-amino-*s*-triazole will completely dissolve and the inert material will remain in suspension.

Repeat the extraction of the inert material with three 10-ml portions of DMF. Shake well after each addition and transfer the supernatant to a 100-ml volumetric flask. Dilute to volume with DMF and mix well.

Vacuum filter 40–50 ml of the solution through a glass filter funnel of medium porosity.

Pipette a 25-ml aliquot of the filtrate into a 400-ml beaker containing 50 ml of distilled water and 40 ml (graduated cylinder) of a 0.5 *N* HCl.

Using a Beckman Model G pH Meter (see Note below) equipped with a glass-calomel electrode system, and while stirring mechanically, titrate the solution with 0.5 *N* NaOH rapidly from a burette until a pH of 1.9 is reached, and then in 0.5-ml increments (recording the pH after each increment) until a pH of 3.5 is obtained. Again add NaOH rapidly until a pH of 6.5 is reached, and then dropwise to a pH of 7.5 (second equivalence point).

ii. *Interferences.*

There should be no interference in the soluble powder formulation for which the method is intended.

iii. *Calculations.*

Determine the first inflection point (pH approximately 2.50–2.90) using the following technique:

Plot pH vs. milliliters of 0.5 *N* NaOH. Connect all the points and draw a straight line through the most number of points comprising the inflection of the curve (Fig. 1).

Determine the equivalence point (E_1) of the inflection as follows:

Find the pH corresponding to points *A* and *B*, (see Fig. 1) where the curve departs from the straight line. The value

$$E_1 = \frac{\text{pH for } A + \text{pH for } B}{2}$$

From the plot, read the milliliters of 0.5 *N* NaOH corresponding to E_1.

Let C = milliliters of 0.5 *N* NaOH required to titrate to the first equivalence point.

Let D = total milliliters of 0.5 *N* NaOH required to titrate to a pH of 7.5 (second equivalence point).

Then % 3-AT = $(D\text{-}C) \times N$ of NaOH $\times$ 3.36.

Note. Check the pH meter used in the analysis at pH 4.0 and pH 7.0, using standard buffers.

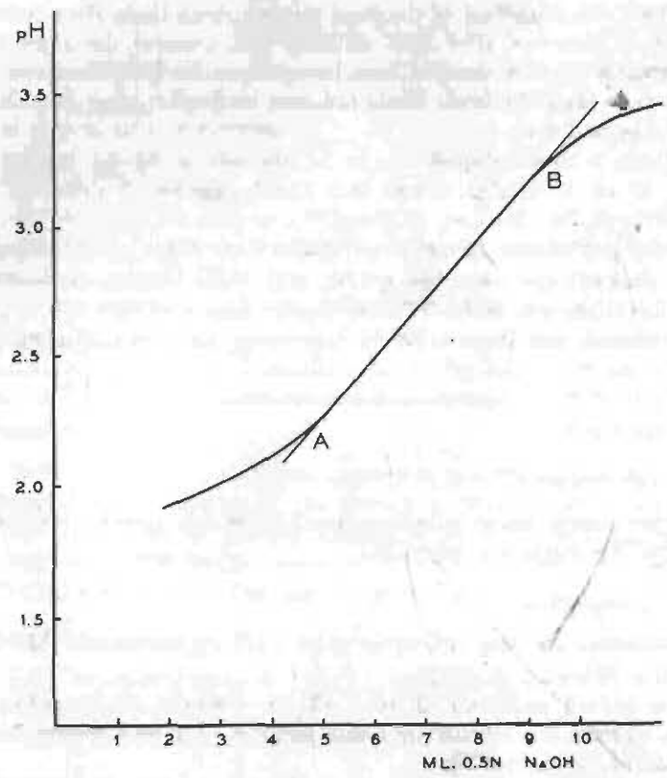


FIG. 1. Titration curve for formulation analysis of 3-amino-*s*-triazole.

2. DISCUSSION OF METHOD

Aminotriazole, having an amino group, forms salts with acids and can be readily titrated. Since it is possible for the diluents to have an appreciable titre, the extraction with DMF is employed.

3. MODIFICATION OF RECOMMENDED METHOD

The liquid formulation with ammonium thiocyanate do not require extraction; begin with the addition of the hydrochloric acid.

B. RESIDUE ANALYSIS

1. REVIEW OF METHODS

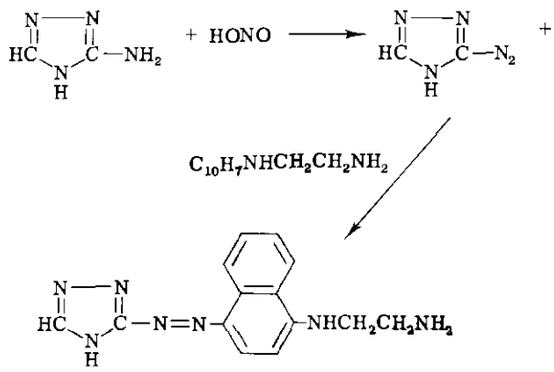
Aminotriazole residues in soil and in some crops have been determined by treatment with nitroprusside reagent to give a color measured

at 636 mμ. A second method utilized the color (485–500 mμ) of the product from chromotropic acid and aminotriazole treated with nitrous acid. More recently, nitrous acid-treated aminotriazole has been coupled with *N*-(1-naphthyl)ethylenediamine and the color determined at 455 mμ (Burke and Storherr, 1961).

2. RECOMMENDED METHOD

a. *Principle*

The chromophoric moiety in the naphthylethylenediamine procedure has not been clearly established. However, it can be speculated that the reaction proceeds as follows:



b. *Reagents*

Acetonitrile, Industrial Grade. Redistill, collecting the 81–82°C fraction.

Amberlite 120 (H^+) resin, 16–50 mesh. Treat with excess 5% NaOH solution and wash with H_2O until the washings are neutral to universal pH test paper. Treat with excess 5*N* HCl and wash with H_2O until the washings are neutral. Drain and store in a closed container. Save the used resin for reactivation and reactivate as for new resin.

3-Amino-s-triazole (3-AT) standard solution. Accurately weigh 100.0 mg of the pure material (m.p. 157–158°C) and dilute to 500 ml with water. Dilute a 5-ml aliquot to 100 ml with water. 1 ml = 10 μg.

Sulfuric acid solution. Concentrated H_2SO_4, Reagent Grade, + H_2O (3 + 1).

Sodium nitrate, reagent grade; 0.5% aqueous solution. Prepare fresh daily.

Sulfamic acid, reagent grade; 5% aqueous solution.

N-(1-Naphthyl)ethylenediamine dihydrochloride. Reagent grade, 1% aqueous solution. Prepare fresh daily.

Nuchar-C 190N decolorizing charcoal. Activate as follows: Heat, with stirring, 400 ml of charcoal with 100 ml of H_2O and 300 ml of conc. H_2SO_4 for 1–2 hours on a steam bath. Filter through a large fritted glass Büchner funnel, and wash with hot water until the filtration is neutral to methyl orange. Dry in a 130°C forced-draft oven for 48 hours, cool, and shake to powder.

c. *Apparatus*

Blender, one-quart capacity.
Chromatographic columns.
Spectrophotometer, Beckman DU or equivalent, with 1-cm cells.
Shaker.
Erlenmeyer flasks, 1000-ml, with ground-glass joint.

d. *Experimental Procedure*

i. *Sample Preparation.*

Blend 200 gm of chopped vegetable crop, or 100 gm of ground grain or meal, for 4 minutes with 20 gm of Celite 545 diatomaceous silica and 300 ml of 95% ethanol USP (use 80% ethanol for ground grain or meal). Filter the mixture on a large Büchner funnel, using two "Sharkskin" papers. Wash the blender cup and filter with 100–200 ml of 70% ethanol. Quantitatively transfer the crop extract to a 1-liter Erlenmeyer flask, add 10 ml of 30% hydrogen peroxide, heat gently for 30 minutes on a steam bath, and cool.

ii. *Resin Adsorption and Desorption of 3-AT.*

(Procedure may be interrupted at any stage prior to color development.)

Add 40 gm of resin to the cooled extract, stopper, and shake for 30 minutes. Decant the liquid through a short chromatographic column containing a glass-wool plug. Wash the resin in a flask with three 30-ml acetone washes, decanting each wash through the column; then wash the flask and column with five 50-ml portions of water, discarding the washings. Combine all the resin particles by extruding the glass-wool plug from the column into the original resin flask. Add 50 ml of H_2O and 20 ml of concentrated ammonium hydroxide, heat for 30 minutes on a steam bath, decant the hot solution through a clean 200-mm chromatographic column containing a glass-wool plug, and collect the filtrate and subsequent washes in a 400-ml beaker. Wash the flask and column by

decantation, using four 25-ml portions of 2 *N* ammonium hydroxide. Wash the column, which now contains most of the resin, with 30 ml of 2 *N* ammonium hydroxide. Concentrate the filtrate and washings to 10–12 ml.

iii. *Acetonitrile Clean-up.*

To the cooled ammoniacal concentrate, add 50 ml of acetonitrile and stir; add 4–5 gm of Celite 545 diatomaceous silica and stir; and finally filter mixture through an 18.5-cm S & S No. 588 folded filter paper. Collect the filtrate and subsequent washings in a 400-ml beaker. Wash the reaction beaker and filter with three 50-ml portions of 90% acetonitrile-water (9 + 1). Evaporate the combined filtrate and washings to 20 ml using moderate heat and moderate air jet. Add 10 to 20 ml of water and concentrate to about 20 ml. Repeat two to three times, until no odor of acetonitrile is detectable. Cool the solution and dilute to 20 ml.

iv. *Acid Digestion and Clean-up.*

Add 3 ml of sulfuric acid solution to the cooled concentration extract, add two glass beads, cover with a watch glass, and boil gently on a hot plate for 10 minutes. (At the end of the 10-minute acid-digestion period, the volume should be about 10 ml. The volume can be brought to 10 ml in 10 minutes on a hot plate set at medium heat.) Cool the acid solution, add 50 ml of water and 1.0 gm of charcoal, cover with the same watch glass, and boil vigorously for 10 minutes on the hot plate. Filter the hot mixture through an 18.5-cm S & S No. 588 folded filter paper and collect the filtrate in a 400-ml beaker. Wash the reaction beaker and filter with 200 ml of hot 0.75% sulfuric acid (2 ml of H_2SO_4 solution dilution to 200 ml), followed by 50 ml of the hot water wash.

Concentrate the combined filtrate and washings to 10–15 ml (if a yellowish color reappears in the 10–15 ml concentrate, add about 50 mg of charcoal and heat to boiling), cool, and dilute up to 25 ml with water. Shake the mixture thoroughly and filter through an 11-cm S & S No. 589 Blue Ribbon paper. Take 5-ml aliquots for color development.

v. *Color Development*

To two separate 25-ml Erlenmeyers, add 5-ml sample aliquots, 1 ml of water, 3 ml of sulfuric acid (3 + 1) and swirl; then add 0.5 ml of 0.5% $NaNO_2$, swirl, and set aside for 10 minutes. Add 0.5 ml of 5% sulfamic acid to each flask and blow across the mouth of flask to eliminate the oxides of nitrogen (or shake in the 25-ml flask for 2 minutes, if preferred). To one sample flask, add 0.5 ml of 1% *N*-(1-naphthyl)ethylenediamine dihydrochloride solution and swirl; to the other flask add

0.5 ml of water and swirl; and set both flasks aside for 5 minutes. Read the sample solution and blank solution against water at 455 mμ. Subtract the blank from the sample reading. Using a standard curve for 3-AT, determine the amount of 3-amino-*s*-triazole in the 5-ml aliquot.

vi. *Interferences*

Interference of ethanol and ethyl acetate, if present as glassware contaminants in the color reactions, is noted.

vii. *Sensitivity.*

Control values on a variety of crops were no higher than 0.02 p.p.m. In no instance did the crop controls exhibit absorption curves characteristic of those from aminotriazole.

viii. *Recovery.*

Recoveries with a variety of crops were generally in the range of 75–90%.

ix. *Preparation of Standard Curve.*

In a series of 25-ml Erlenmeyer flasks prepare 5-ml standards containing 0, 5, 10, 15, and 20 μg of 3-AT respectively. To each flask, add 4 ml of H_2SO_4 solution (3 + 1) and swirl; then add 0.5 ml of 0.5% $NaNO_2$, swirl, set aside 10 minutes, and then proceed exactly as for "sample determination." Draw the standard curve through points obtained by plotting absorbances versus micrograms of 3-AT.

3. APPLICABILITY OF RECOMMENDED METHOD TO DIFFERENT CROP OR FOOD MATERIALS

Crop controls vary from crop to crop, but very little within a crop. The absorptive capacity for the ion exchange resin varies with the crop, but the amount of resin has been set high enough to accommodate the crops evaluated.

4. DISCUSSION OF METHOD

a. *Preliminary Clean-up with Acetonitrile*

Celite has been found particularly successful in reducing control values, as has the sulfuric acid digestion with charcoal clarification.

b. *Adsorption of 3-AT on Resin*

Twelve grams of resin originally used were inadequate for the adsorption of 3-AT from fresh or frozen vegetable extracts and because

of this, recoveries were low. When the resin weight was increased to 18 gm, recoveries from fresh or frozen vegetable extracts to which 3-AT had been added at a level of 0.187 p.p.m. ranged from 80 to 90%. However, 18 gm were insufficient for extracts of canned vegetables; the yields were only 50%. Again, when the amount of resin was increased to 36 gm for canned vegetable extracts, recoveries were similar to those obtained for fresh or frozen produce with 18 gm of resin. To insure complete adsorption, therefore, the final method was revised to specify 40 gm of resin for use with extracts of all products, regardless of type or processing.

c. *Clean-up*

The ideal clean-up procedure should leave only the compound being investigated; this compound should be recovered unchanged and in 100% yields. There are few pesticide methods that approach this ideal, mainly because of the lack of specificity of solvents used in the clean-up process.

While it is not proposed as a universal pesticide solvent, the writers wish to emphasize the excellent ability of acetonitrile to partition pesticides. Investigation showed 3-AT to be slowly soluble in absolute acetonitrile and readily soluble in acetonitrile containing small quantities of water. Further experiments with 90% acetonitrile (9 parts of CH_3CN and 1 part of water) demonstrated conclusively that all 3-AT remained in solution while most of the proteins, amino acids, and carbohydrates retained by the resin were precipitated. Celite 545 diatomaceous silica was used merely to bind the precipitate and withhold it from solution. Consequently, 90% acetonitrile in conjunction with Celite 545 diatomaceous silica was employed for a preliminary cleanup.

d. *Digestion and Clarification*

The final step (the sulfuric acid digestion with charcoal clarification) is also a prerequisite. Impurities that react with sulfuric acid must be decomposed and removed from the solution prior to the acid color development reaction. Since 3-AT is stable in sulfuric acid solution, this digestion type of clean-up works very well.

It was found experimentally that the charcoal must be activated before use. Recoveries of pure 3-AT, beginning at the charcoal clarification of an acid solution, have been 95% or higher.

Recoveries of 3-AT from pure solution (no crop) carried through the entire procedure have been 90% or better.

Recoveries range from 72 to 92% for vegetables, regardless of the type of processing (fresh, frozen, or canned). Blanks for the control samples of the same vegetables are reasonably low. However, although the

absorbances of the control sample are consistent within any one crop, there is a marked difference from crop to crop. The apparent 3-AT found in control crops ranged from 0.003 to 0.02 p.p.m. The method does not obviate the need for control crop blanks.

The chromogenic reaction of 3-AT with *N*-(1-naphthyl)ethylenediamine dihydrochloride is extremely sensitive. When the procedural modifications are used with the color development reaction, as little as 1 μg of 3-AT in 40 gm of crop (0.025 p.p.m.) will show the characteristic absorption curve for 3-amino-*s*-triazole.

Both ethyl alcohol and ethyl acetate will react with the color development reagents. Neither solvent will come through the clean-up procedures, but improper cleaning of glassware, color development flasks, or pipettes could conceivably cause high absorbance readings.

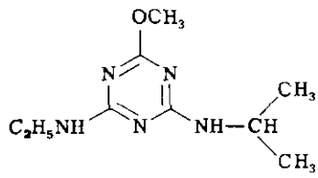
Reference

Burke, J., and Storherr, R. W. (1961). *J. Assoc. Offic. Agr. Chemists.* 44, 196.

4

Atratone

E. KNÜSLI



2-Methoxy-4-ethylamino-6-isopropylamino-s-triazine

I. GENERAL

A. EMPIRICAL FORMULA

$C_9H_{17}N_5O$ (Mol. wt. 211.27).

B. ALTERNATE NAMES

Trial number G 32 293, Primatol B (trade name for general use).

C. SOURCE OF ANALYTICAL STANDARD

J. R. Geigy S.A., Basel, Switzerland; Geigy Agricultural Chemicals, Ardsley, New York.

D. BIOLOGICAL PROPERTIES

1. HERBICIDAL ACTIVITY

Atratone is a pre- and post-emergence herbicide for general and selective use. A potential field for selective application is sugar cane.

2. TOXICITY

Acute oral toxicity: LD_{50} for mouse 905 mg/kg; rat 2400 mg/kg.

Subchronic toxicity: A series of rats fed for 90 days with a daily oral administration of 10 and 200 mg Atratone 50 W/kg or 20 µl Atratone 25 E/kg was comparable in all behaviors with the controls.

E. HISTORY

H. Gysin and E. Knüsli are co-inventors on Swiss Patent 337,019 (to J. R. Geigy S.A., Basel, Switzerland); see also Knüsli (1958).

F. Physical Properties

Melting point: 94–96°C.

Solubility in water at 20°C: 0.18% (1800 p.p.m.); in organic solvents. high.

Colorless, not combustible, not explosive, not corrosive.

UV-spectra: analogous to Prometone (see this volume, Chapter 17).

G. Chemical Properties

1. method of synthesis

Atratone is synthesized by reacting 2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine (Atrazine) with methanol in the presence of an equivalent of sodium hydroxide.

2. properties

It is distillable; stable in neutral, slightly acidic or basic media; it hydrolyzes to the herbicidally inactive 2-hydroxy-4-ethylamino-6-isopropylamino-*s*-triazine in acid or basic mediums, especially at higher temperatures.

H. Formulations

Atrazine is available as a 50% wettable powder and a 25% emulsifiable solution.

II. ANALYSIS

A. Formulation Analysis

The methods and their accuracy (Suter, 1959) are identical with those described for Prometone, as are the respective interferences (see this volume, Chapter 17 on "Prometone"). The percentage of Atratone is obtained by the following equation:

$$\% \text{ Atratone} = \frac{a \times 211.3 \times 100}{w \times 10{,}000} = \frac{a \times 2.113}{w}$$

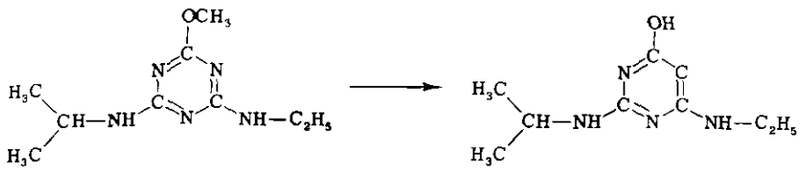
B. Residue Analysis

1. recommended method—spectrophotometric method

a. *Principle*

In this method (Delley, 1960) Atratone is extracted with methylene chloride. As the compound is relatively soluble in water, the residue of the methylene chloride extract can be taken up in water. Re-extract with methylene chloride. Due to the basic properties of Atratone, the compound can be extracted with 0.1 *N* sulfuric acid. Re-extract once more with methylene chloride, and finally hydrolyze the

residue with 0.1 *N* sulfuric acid, warming the solution on the water bath.



The cation of the hydrolysis product shows a maximum absorption at 240 mμ, which is used for the spectrophotometric determination.

b. *Reagents*

Methylene chloride, distilled.
Sodium hydroxide, 2%.
Sulfuric acid, 1 *N*.
Sulfuric acid, 0.1 *N*.
Sodium acetate, 1 *N*.
Acetic acid, 0.001 *N*.

All the above mentioned aqueous reagents are extracted with methylene chloride.

c. *Experimental Procedure*

i. *Extraction and Pretreatment.*

Extract the material to be tested with methylene chloride. Transfer the methylene chloride extract into a 400-ml beaker and evaporate the solvent. Dissolve the residue in a few milliliters of methylene chloride and add 200 ml of water. Place on a water bath and evaporate the methylene chloride. The Atratone moves into the aqueous solution and separates from the fats and waxes. After cooling, decant the aqueous fraction through a blue-band filter into a separatory funnel. Wash the beaker and the filter with water. Add 10 ml of 2% NaOH and extract three times with 30 ml of methylene chloride. Each of these three methylene-chloride extracts has to be washed with 200 ml of water in a second separatory funnel; all three extracts contain water-soluble and methylene chloride soluble compounds extracted from the investigated material, including Atratone.

ii. *Isolation.*

Combine the three methylene chloride extracts (90 ml) and extract three times with 100-ml portions of 0.1 *N* H_2SO_4, as shown in the following scheme. During this operation the basic Atratone moves into the acid layer.

Process

Second extraction with 40 ml of methylene chloride

↓

First extraction with 40 ml of methylene chloride

↓

90 ml of the re-extract

↓

Separatory funnel 1 (500 ml) — contains at the beginning of the process 100 ml of 0.1 N H_2SO_4

↓ CH_2Cl_2

Separatory funnel 2 (250 ml) — contains at the beginning of the process 100 ml of 0.1 N H_2SO_4

↓ CH_2Cl_2

Separatory funnel 3 (250 ml) — contains at the beginning of the process 100 ml of 0.1 N H_2SO_4

↓ CH_2Cl_2 to be discarded

As can be seen from the scheme, after the extraction in the first separatory funnel, the methylene chloride layer is drained into the second separatory funnel where it is again shaken, separated, and drained into the third separatory funnel, shaken and separated. The methylene chloride layer is discarded. Wash the three separatory funnels successively with two 40-ml portions of methylene chloride.

Care has to be taken not to keep the Atratone dissolved for an extended period in the 0.1 N H_2SO_4, because losses might occur due to hydrolysis. Consequently, collect as soon as possible all the sulfuric acid layers in the separatory funnel 1 and buffer the solution by adding 50 ml of 1 N sodium acetate. Rinse the separatory funnels 2 and 3 thoroughly with distilled water so as to eliminate the last traces of sulfuric acid. Then add to each of the two separatory funnels 100 ml of 0.001 N acetic acid and proceed according to the following scheme:

20 ml of methylene chloride

↓

20 ml of methylene chloride

↓

30 ml of methylene chloride

↓

Separatory funnel 1 — contains 300 ml of 0.1 N H_2SO_4 + 50 ml of 1 N sodium acetate

↓ CH_2Cl_2

Separatory funnel 2 — contains 100 ml of 0.001 N acetic acid

↓ CH_2Cl_2

Separatory funnel 3 — contains 100 ml of 0.001 N acetic acid

CH_2Cl_2 containing Atratone

The above scheme indicates how the Atratone is extracted from the buffered aqueous solution by one 30-ml portion and two 20-ml portions of methylene chloride. Wash each methylene chloride extract separately and successively with the diluted acetic acid in the two successive separatory funnels, draining the extract always into the same clean 100-ml ground-glass stoppered Erlenmeyer flask. Evaporate the solvent completely on the water bath at about 50°C, applying vacuum. To insure continuous boiling during evaporation, a capillary tube of the type used for melting point determinations can be inserted with the opening at the bottom of the Erlenmeyer flask. The residue is a thin film which contains the Atratone. Care has to be taken to avoid any unnecessary heating because of the volatility of Atratone in this form.

iii. *Hydrolysis and Determination.*

As described for Prometone (this volume, Chapter 17).

iv. *Discussion of Interferences.*

Other 2-alkoxy- and also 2-alkylthio-4,6-diamino-*s*-triazines may interfere.

v. *Sensitivity.*

The limit of determination is about 5 μg.

vi. *Calculation.*

As described for Prometone with an instrument factor of 124.

2. DISCUSSION OF METHOD

a. *Examples of Values Found*

TABLE I
EXTINCTION VALUES FOR ATRATONE[a]

	E^{225}	E^{240}	E^{255}	Δ	$\Delta_{corr.}$
Blank value of the reagents	0.066	0.029	0.032	−0.020	—
8 μg of Atratone	0.111	0.105	0.039	+0.030	0.050
40 μg of Atratone	0.221	0.451	0.076	0.302	0.322
80 μg of Atratone	0.376	0.888	0.131	0.635	0.655

[a] E^x = Extinction at x mμ using a Beckman DU spectrophotometer.

Following the procedure, the extinction values, shown in Table I, and distribution coefficients in different solvent systems (see Table II) were obtained.

TABLE II
DISTRIBUTION COEFFICIENTS FOR ATRATONE IN SEVERAL SOLVENT SYSTEMS

Organic phase	Aqueous phase	Concentration in org. phase / Concentration in aq. phase
$CHCl_3$	0.1 *N* H_2SO_4	0.74
$CHCl_3$	0.01 *N* H_2SO_4	5.1
$CHCl_3$	Water	~400
CH_2Cl_2	0.1 *N* H_2SO_4	0.3
n-pentane	0.01 *N* HCl	0.007
n-pentane	Water	1.1

Because the characteristics of Atratone differ essentially from those of Prometone, a similar but still different extraction and isolation is required. If one tries for instance to determine Atratone following the method prescribed for Prometone, only about 60% of the theoretical amounts results.

b. *Rate of Hydrolysis for Atratone*

Atratone, when hydrolyzed with 1 *N* sulfuric acid on the boiling water bath, has a half-life of about 10 to 15 minutes.

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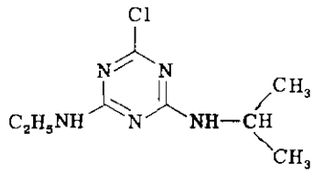
5

Atrazine

E. KNÜSLI



2-Chloro-4-ethylamino-6-isopropylamino-*s*-triazine

I. GENERAL

A. EMPIRICAL FORMULA

$C_8H_{14}ClN_5$ (Mol. wt. 215.69).

B. ALTERNATE NAMES

Trial number G 30 027. Atrazine-Geigy; Primatol A (trade names for general use). Atrazine-Geigy; Gesaprim (trade names for selective use).

C. SOURCE OF ANALYTICAL STANDARD

J. R. Geigy S.A., Basel, Switzerland; Geigy Agricultural Chemicals, Ardsley, New York.

D. BIOLOGICAL PROPERTIES

1. HERBICIDAL ACTIVITY

Atrazine is a pre- and post-emergence herbicide suitable for general and selective use. Its actual and potential fields of selective application are: corn, sorghum, millet, vine, asparagus, sugar cane, pineapple, fruit trees, and summer fallow.

2. TOXICITY

Acute oral toxicity: LD_{50} for mouse 1750 mg/kg; for rat 3080 mg/kg; for rabbit 750 mg/kg.

Chronic toxicity: A series of rats fed for 2 years with daily oral amounts of 2, 20, 200 p.p.m. (related to food intake) of Atrazine 50 W was comparable in all respects to the controls.

Cutaneous toxicity: A 5% suspension of Atrazine 50 W in gum arabic applied for 5 consecutive days to rats caused no signs of adsorptive toxicity.

E. History

H. Gysin and E. Knüsli are co-inventors of Swiss Patents 329,277 and 342,784 (to J. R. Geigy S.A., Basel, Switzerland). See also "Triazine als Herbizide," 4th Int. Congress of Crop Protection, Hamburg, 1957.

F. Physical Properties

Melting point: 173–175°C.
Solubility in water at 0°C: 0.0022% (22 p.p.m.);
27°C: 0.007% (70 p.p.m.);
85°C: 0.032% (320 p.p.m.);
diethyl ether at 27°C: 1.2% (12000 p.p.m.);
methanol at 27°C: 1.8% (18000 p.p.m.);
n-pentane at 27°C: 0.036% (360 p.p.m.);
chloroform at 27°C: 5.2% (52000 p.p.m.).
pK-value in water at 22°C: 1.68.
Colorless, not combustible, not explosive, not corrosive.
UV spectra: practically identical with those given for "Simazine" (see Chapter 23 of this volume).

G. Chemical Properties

1. Method of Synthesis

Atrazine is synthesized by reacting cyanuric chloride with one equivalent of ethylamine and isopropylamine in the presence of an acid acceptor.

2. Properties

It sublimates at higher temperatues; is stable in neutral, slightly acidic or basic media; it is hydrolyzed to the herbicidally inactive 2-hydroxy-4-ethylamino-6-isopropylamino-*s*-triazine in acidic or basic media, especially at higher temperatures.

H. Formulations

Atrazine is available as a 80% wettable powder; a 50% wettable powder; and in 4, 8, and 20% granules.

II. ANALYSIS

A. Formulation Analysis

1. POTENTIOMETRIC METHOD FOR WETTABLE POWDERS

The method (Suter, 1958–1959) is analogous to that given for simaziue. However, the appropriate equation for atrazine is:

$$\% \text{ Atrazine} = \frac{(a - b) \times 215.7 \times 100}{w \times 10{,}000} \quad (1)$$

A possible contaminant of 2,4-dichloro-6-ethylamino-*s*-triazine simulates a higher content of atrazine than is effectively present. The method does not differentiate between other 2-chloro-4,6-diamino-*s*-triazines like 2-chloro-4-amino-6-ethylamino-*s*-triazine, 2-chloro-4-amino-6-isopropylamino-*s*-triazine, 2-chloro-4,6-bis-ethylamino-*s*-triazine and 2-chloro-4,6-bis-isopropylamino-*s*-triazine.

2. SUPPLEMENTARY METHOD FOR WETTABLE POWDERS

The method (Suter, 1958–1959) is the same as that described for simazine with the appropriate equation:

$$\% \text{ Atrazine} = \frac{c \times 215.7 \times 100}{w \times 10{,}000} \quad (2)$$

2-Ethylamino-4,6-bis-isopropylamino-*s*-triazine or 2-hydroxy-4-ethylamino-6-isopropylamino-*s*-triazine are inclnded in the value obtained. 2,4-Dichloro-6-ethylamino-*s*-triazine does not respond to the titration with perchloric acid.

If the values following Eq. (1) and Eq. (2) are different, the approximate contents of 2,4-dichloro-6-ethylamino-*s*-triazine or 2-ethylamino-4,6-bis-isopropylamino-*s*-triazine are calculated as follows:

Value Eq. (1) higher than Eq. (2):
Eq. (2) = content of atrazine

$$\frac{[\text{Eq. (1)} - \text{Eq. (2)}] \times 193.04}{215.7}$$

= content of 2,4-dichloro-6-ethylamino-*s*-triazine (Mol. wt. 193.04) (3)

Value Eq. (2) higher than Eq. (1):
Eq. (1) = content of atrazine

$$\frac{[\text{Eq. (2)} - \text{Eq. (1)}] \times 238.36}{215.7}$$

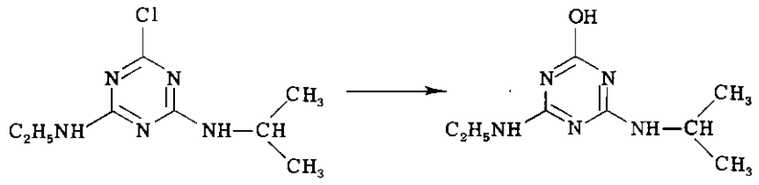
= content of 2-ethylamino-4,6-bis-isopropylamino-*s*-triazine (Mol. wt. 238.36) (4)

2,4-Dichloro-6-ammo-*s*-triazines or 2,4,6-tris-amino-*s*-triazines are not differentiated.

B. Residue Analysis

The methods follow, in general, those given for "Simazine" (see this volume, Chapter 23).

1. SPECTROPHOTOMETRIC METHOD



In the column clean-up atrazine and hydroxyatrazine behave like simazine and hydroxysimazine (Gigger *et al.*, 1961) (see Chapter 23 on "Simazine," Section II,C,2).

The cations of the hydrolysis products of atrazine and simazine both show a maximum absorption of 240 mμ.

The method (Delley, 1958) has been successfully applied for the determination of residues in corn, grapes, and soil.

2. COLORIMETRIC METHOD

(See also Chapter 23 on "Simazine," Section II,C,4, "Colorimetric Method.")

3. RESIDUE ANALYSIS BY BIOASSAY

For general remarks see Chapter 23 on "Simazine," "Residue Analysis by Bioassay." In a Mexican silt loam, Clark soybeans were found to be suitable for the bioassay of atrazine. The useful range lies in the range from 0.5 p.p.m. upward (Talbert, 1960–1961).

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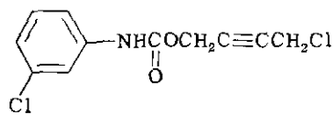
Talbert, R. E. (1960–1961). Univ. of Missouri, Columbia, unpublished work.

6

Carbyne

T. R. Hopkins



4-Chloro-2-butynyl *N*-3-chlorophenylcarbamate

I. GENERAL

A. Empirical Formula

$C_{11}H_9O_2NCl_2$ (Mol. wt. 258.19).

B. Alternative Names

Barban, S-847. The name Carbyne is the registered trademark of the Spencer Chemical Company.

C. Source of Analytical Standard

Spencer Chemical Company, Kansas City, Missouri.

D. Biological Properties

Carbyne is a highly selective herbicide recommended for the post-emergence control of wild oats (*Avena fatua* and *Avena ludoviciana*) in wheat, barley, flax, peas, sugar beets, rapeseed, mustard, and safflower. Sugar beets, sunflower, and safflower are extremely resistant to Carbyne; and rates as high as 8 lb/acre do not affect their growth. Wild rye (*Secale cereale*), ryegrass (*Lolium multiflorum*), blackgrass (*Alopecurus myosuroides*), and certain members of the *Polygonum* species (such as smartweed, lady's thumb, and wild buckwheat) are also controlled by Carbyne.

Carbyne is classified as a relatively safe material. The LD_{50} of the active ingredient in albino rats is 1.35 gm/kg. With albino rabbits, the acute percutaneous LD_{50} is 23 gm/kg. Guinea pigs displayed no skin sensitization to a challenge after repeated exposure.

E. History

Carbyne was first discovered in 1955 by T. R. Hopkins and J. W. Pullen of the Spencer Chemical Company. The material was field tested by several investigators in the United States and Canada in 1958. The synthesis and utility of the active ingredient was described in a patent (Hopkins and Pullen, 1959), and more specific details of the synthesis of the active component and several of its closely related analogues have been published (Hopkins *et al.*, 1959). A discussion of the variables affecting the usage of this herbicide has appeared recently (Hoffmann *et al.*, 1960).

F. Physical Properties

1. The active ingredient is a white crystalline solid melting at 75–76°C with solubility characteristics as given in Table I.

TABLE I
Solubility of Barban at 25°C in 100 gm of Solvent

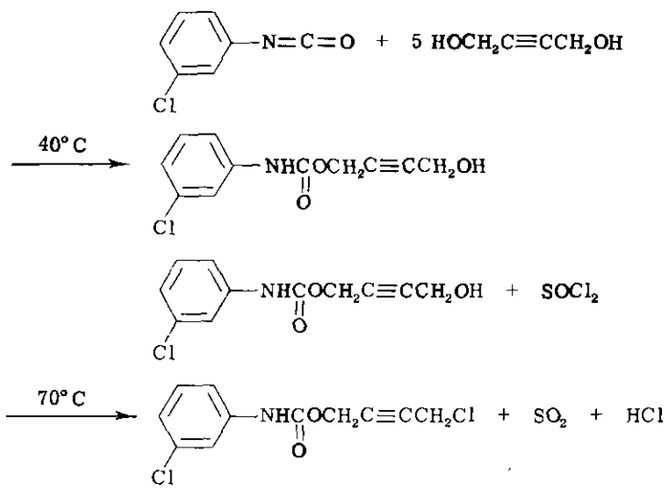
Solvent	Solubility
n-Hexane	0.2 gm
2,2,4-Trimethylpentane	0.3 gm
n-Dodecane	0.1 gm
Kerosene	0.5 gm
Benzene	37.0 gm
Toluene	29.5 gm
Xylene	20.6 gm
Isopropylbenzene	7.3 gm
n-Butylbenzene	13.1 gm
Ethylene dichloride	54.6 gm
Isophorone	47.0 gm
Water	1.1 mg
Glycerol formal	45.0 gm
Propylene glycol	15.0 gm

G. Chemical Properties

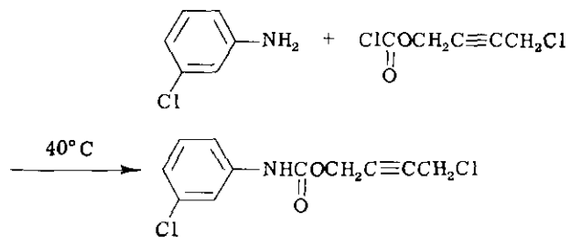
1. synthesis

The carbamate may be prepared in high yields by the following methods:

a. Reaction of an isocyanate and a diol followed by a nucleophilic chlorination.



b. Reaction of a chloroformate with an amine.



2. CHEMICAL PROPERTIES

The carbamate is decomposed at temperatures above its melting point (75°C), and HCl is evolved. Hydrolysis at the carbamate linkage occurs with acid or base. The half-life in 1 *N* caustic soda is 0.97 minutes at 25°C, and under mildly basic conditions the terminal chlorine is liberated. Under acid conditions, the terminal chlorine is stable and hydrolysis results in the formation of 3-chloroaniline salts.

H. Formulations

Carbyne is an emulsifiable concentrate containing 1 lb/gal of barban in an organic solvent with an emulsifier and a corrosion inhibitor. It is a dark amber solution with a specific gravity of 1.01 and a viscosity of 7.27 cp at 25°C. The formulation turns to a viscous gum at −45°C

without prior crystallization. Upon warming, the gum returns to its original form. It is stable in the presence of mild steel, galvanized iron and tin plate; although corrosion at the liquid-air interface may occur upon prolonged storage. Phenolic-lined cans, glass containers, or aluminum tanks are recommended for continuous service.

II. ANALYSIS

A. Formulation Analysis—Recommended Method

The principle of this method (Bombaugh and Bull, 1961) is to separate the active ingredient from the materials of formulation by adsorption on silica gel, followed by elution with appropriate mixtures of ethylene dichloride and *n*-hexane. The concentration of the active ingredient in the eluent is measured quantitatively at 277.5 mμ.

1. Reagents

n-Hexane, Phillips spectrograde (or equivalent).

Ethylene dichloride. Distilled until spectrally clear in the region 260 mμ through 320 mμ when compared with water.

Eluting Solution 1. Prepare a mixture of 1 volume of ethylene dichloride to 2 volumes of *n*-hexane. Pass the mixture through a 2.5 cm × 60-cm column of fully activated alumina.

Eluting Solution 2. Prepare a mixture of 2 volumes of ethylene dichloride to 1 volume of *n*-hexane. Pass through a 2.5 cm × 60-cm column of fully activated alumina. Wöelm neutral alumina—Brockman Grade I—obtained from Alupharm Chemical Co., New Orleans, La., is preferred.

Silica gel, chromatographic grade, 30 to 80 mesh—activated by heating for 24 hr at 200°C.

Ottawa sand.

2. Apparatus

Ultraviolet spectrophotometer. Cells of 2-cm path length.

Chromatographic column—1-cm diameter × 20 cm long (see Fig. 1).

3. Experimental Procedure

a. Calibration of a Carbyne Sample Solution is carried out in the following manner: A solution containing 0.0100 gm of Carbyne in 100 ml of eluting Solution 2 is prepared. The ultraviolet absorbance of this solution at 277.5 mμ, using a 2-cm cell and the eluting Solution 2 as a reference, is determined. The absorptivity (a) is then calculated as

$$a = \frac{A}{cl}$$

where A = absorbance
c = concentration, grams per liter
l = cell path length, centimeters

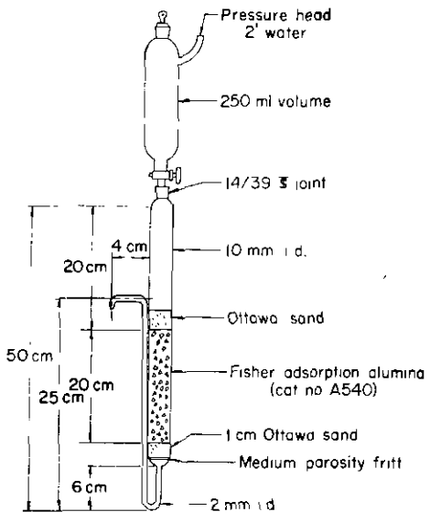


FIG. 1. Chromatographic column for formulation analysis of Carbyne (Bombaugh and Bull, 1961). Reprinted with permission of the American Chemical Society.

b. A 2-gm ± 0.1 gm sample of Carbyne is transferred to a previously weighed 100-ml volumetric flask and the exact amount of Carbyne added is determined as follows: About 50 ml of ethylene dichloride is added, the flask stoppered and shaken vigorously to effect a homogeneous solution. The solution is diluted to volume with more ethylene dichloride, stoppered and thoroughly mixed. A 10-ml portion of the solution is transferred onto a previously prepared chromatographic column and eluted with 175 ml of eluting solution 1. A cell full of the final portion of the eluent is collected and examined by its UV spectrum to insure that no active ingredient has been eluted. If an absorbance in excess of 0.01 is observed, elute with 25 ml of additional eluting solution 1 and recheck this eluent. The column is then eluted with 225 ml of eluting Solution 2, and the entire eluent is collected in a 250-ml volumetric flask. The solution is diluted to volume with additional eluting Solution 2 and mixed thoroughly. The UV absorbance is determined at 277.5 mμ in a

2-cm cell using eluting Solution 2 as a reference. The sample is scanned from 400 mμ to 200 mμ. On the same chart, a strip is run (recording for at least three divisions) with the instrument set at exactly 300 mμ. The chart is backed-up and a strip is run (recording at least three divisions) at exactly 277.5 mμ. The operating wavelength is always approached from the long wavelength side to avoid any "back lash" error. The absorbance value at 300 mμ is subtracted from the absorbance value at 277.5 mμ. The difference in absorbance is then used as a measure of the active ingredient in solution. The absorbance of the solution and the previously determined value for the absorptivity of the active ingredient is used to calculate the per cent of the active ingredient present.

c. The calculation is made as follows:

$$\% \text{ Active ingredient} = \frac{25A}{a \times l \times g}$$

where 25 = dilution factor

A = absorbance of the eluent solution

a = absorptivity of pure Carbyne in $\frac{(\text{Absorbance} \times \text{liters})}{(\text{gm} \times \text{cm})}$

l = cell length, centimeters

g = formulation loaded on the column, grams

d. The silica gel is evaluated as follows: A chromatographic column, as described above, is loaded with the silica gel. A 10-ml quantity of Carbyne sample solution is pipetted onto the column. This sample is eluted with eluting Solution 1 and collected as seven separate 25-ml portions. The column is then eluted with eluting Solution 2 and collected as nine separate 25-ml portions. The absorbance maximum at 277.5 mμ is read and recorded. The absorbance vs. the milliliters eluted is plotted and a differential chromatograph of the sample is obtained. A silica gel of proper activity should pass all of the formulation ingredients and other impurities without passing the active ingredient when eluted with eluting Solution 1. In turn, all of the active ingredient must be eluted with eluting Solution 2.

e. The method affords a precision of 0.45%. An analysis of variance shows that the photometry introdnces a standard deviation of $\pm$0.14% while the chromatography introduces a standard deviation of $\pm$0.43%. Replicate analyses of several known samples show a recovery of 98.9%. Provision is made to correct partially the interfering absorbance of the constituents of formulation by subtracting the absorbance at 300 mμ from the absorbance at 277.5 mμ.

B. Residue Analysis

1. REVIEW OF METHODS

A colorimetric procedure for the determination of 3-chloroaniline from the herbicide isopropyl *N*-(3-chlorophenyl)carbamate (CIPC) has been published (Gard and Rudd, 1953) and is based on the formation of a complex with hypochlorite and phenol. Since this procedure led to variable results, another method was developed.

2. RECOMMENDED METHOD

a. The analytical method for determining Carbyne residues is based on a colorimetric determination of the 3-chloroaniline moiety (Riden and Hopkins, 1961). In this procedure, the active ingredient is isolated from plant tissues, hydrolyzed, and the 3-chloroaniline is collected by steam distillation, diazotized, and coupled with *N*-(α-naphthyl)ethylenediamine. The resulting dye is then determined colorimetrically. This is a modification of previously published methods (Bratton *et al.*, 1939; Werner, 1939) and is similar to those methods used for the determination of monuron (q.v.) (Bleidner *et al.*, 1954), acetanilide (Brodie and Alexrod, 1948), parathion (Averell and Norris, 1948), and certain sulfa drugs (Ekert, 1943).

b. *Reagents*

Ethylene dichloride. Analytical grade; distilled and the portion boiling at 83–85°C retained.

Potassium nitrite, analytical grade.

Ammonium sulfamate, Matheson Coleman & Bell; m.p. 180–182°C.

N-(α-Naphthyl)ethylenediamine dihydrochloride. Eastman, recrystallized twice from 6 *N* HCl.

Cellulose, Whatman standard cellulose powder.

c. *Apparatus*

Waring Blendor.

Spectrophotometer.

Cells, 1-cm path length.

d. *Experimental Procedure*

i. *Sample Preparation.*

Crops are harvested by the agronomic procedure normally employed for each individual crop. Green tissue is kept frozen until

analyzed. Mature food portions are utilized as such. Each sample is stored in an individual polyethylene bag until it is analyzed.

ii. *Method.*

Samples of the green tissue are chopped into small pieces, weighed and ground in a Waring Blendor for 3 to 5 minutes with sufficient ethylene dichloride to form a thin paste. The homogenate is filtered through a glass wool plug in a funnel; the tissue is reprocessed in the blender with fresh ethylene dichloride, filtered and washed twice with fresh ethylene dichloride, with the extracts and washes kept separate. When this procedure is used with mature sugar beets or green peas, an emulsion is formed necessitating a change in the solvent system to isopropyl alcohol or acetonitrile. The slurry is then filtered through cheesecloth and processed as described above.

Samples of grain are homogenized with ethylene dichloride in the Waring Blendor, the solvent is removed in a basket-head centrifuge at 2000 to 2500 r.p.m. and the solids are washed twice with fresh ethylene dichloride. All plant tissues are recovered and saved for an assay of "water soluble 3-chloroaniline-contaiuing substance."

The extracts are freed from interfering substances by treatment on a column consisting of a mixture of 1 part of Celite, 2 parts of anhydrous sodium sulfate and 2 parts of Attaclay, by weight (Lane, 1958).

The extracts, followed by the first wash, second wash, and fresh ethylene dichloride, are passed through the columns coutaining 20 gm of the mixture per 100 ml of extract. A 30 × 500-mm column is used for large samples (20 to 200 gm) and a 20 × 300-mm column for smaller samples. The mixture is packed in the column with firm tamping and is supported by a 1-inch layer of sea sand on glass wool. From 3 to 5 psi of air prcssure may bc used, but care must be taken to prevent the column from runniug dry, as shrinkage will occur, ruining subsequent column washing.

The column effluents are combined and evaporated in a 300-ml flask at 30° to 50°C with the aid of a gentle stream of filtered air. To all flasks, except those containing flax residues, are added 100 ml of 10% aqueous NaOH; and the flask's contents are refluxed for 1 hour under a condenser. Flax residues, being oily, require 20% NaOH and 2 hours of reflux time. The condenser is then rinsed thoroughly into the flask and a one-piece distillation head-coudenser is placed on the flask. The 3-chloroaniline resulting from the hydrolysis of barban is then steam-distilled into a 50-ml volumetric flask, containing 5 ml of 1 *N* HCl, until a total volume of about 40 ml is obtained.

One milliliter of a 1% potassium nitrite solution is added to the

volumetric flask, mixed well, and allowed to stand for 10 minutes at ambient temperature. One milliliter of 2% ammonium sulfamate is then added to destroy the excess nitrous acid. After 3 minutes, 1 ml of a 0.1% solution of *N*-(α-naphthyl)ethylenediamine dihydrochloride is added, the solutions are brought to volume, and the absorbance at 550 mμ is measured. When extracts of older plants are analyzed, interfering dyes may result from *o*-aminoacetophenone formed by the alkaline hydrolysis of tryptophan (Bleidner, 1954). These dyes may be removed by chromatography on a cellulose column using a 1:15 (v/v) mixture of glacial acetic acid and 1 *N* HCl as the developer. This solvent mixture was more suitable than 1 *N* HCl alone.

A water slurry of Whatman standard cellulose powder is used to prepare a 10 × 360-mm chromatographic column. The dyes to be separated are placed on this column in 1 *N* HCl. The addition of 100 to 125 ml of a 1:15 (v/v) mixture of glacial acetic and 1 *N* HCl elutes the dye formed by the *o*-aminoacetophenone, while the dye from 3-chloroaniline moves only about one-third the length of the column. A 1:1 (v/v) mixture of glacial acetic acid and 1 *N* HCl quantitatively removes the 3-chloroaniline dye. The dye is collected in a 50-ml volumetric flask, diluted to volume with glacial acetic acid and the absorbance is measured at 550 mμ. The acetic acid lends stability to the system, the colors remaining unchanged for a least 3 days.

After the plant tissues have been extracted with etbylene dichloride, they are retained and air-dried to remove the solvent. A sample of material is weighed (about 50 gm) and placed in a 1-liter flask. Six hundred milliliters of 10% aqueous NaOH is added and the mixture is refluxed for about 2 hours. If foaming occurs, Dow-Corning Antifoam A is added. The condenser is turned downward and about 75 ml of the distillate is collected in a volumetric flask containing about 5 ml of 1 *N* HCl. If the distillate is colored, it is made basic and redistilled.

The final distillate is diazotized, coupled, brought to volume (100 ml), chromatographed and the absorbance at 550 mμ is measured; all the steps being carried out as described above.

The total residue is to be considered as a sum of the organic and "water-soluble" components.

iii. *Interferences.*

Tryptophan in plant tissues is hydrolyzed to *o*-aminoacetophenone (Bleidner, 1954). When this material is diazotized and coupled with the *N*-(α-naphthyl)ethylenediamine, a dye is formed which interferes with the 3-chloroaniline determination. This dye is removed by chromatography on a cellulose column as a routine part of the analytical method.

iv. *Sensitivity.*

The sensitivity of the method is dependent on the background encountered in the procedure. The absorbance readings of the color-developing reagents are identical to those obtained when distilled water is used. Untreated green plants provide absorbances of 0.004 to 0.010 when analyzed.

Harvested grain from untreated plants gives readings of 0.000 to 0.008. The quantities of tissue used, from 10 to 200 gm, have no effect on the background. These readings represent an apparent background of 0 to 2 μg of active ingredient. A sensitivity of twice the background is considered valid and is, thus, 0.01 to 0.02 p.p.m. of the active ingredient for a 100 to 200-gm sample of plaut material.

v. *Recovery.*

Extraction studies of the active ingredient from green tissues and final harvested food samples show recoveries of 68 to 102%. These studies have been run on wheat, barley, peas, flax, sugar beets, safflower, rapeseed, sunflower, and several forage crops.

vi. *Standard Curve.*

A standard curve plotting absorbance versus micrograms/50 ml for both 3-chloroaniline and the active ingredient is presented in Fig. 2.

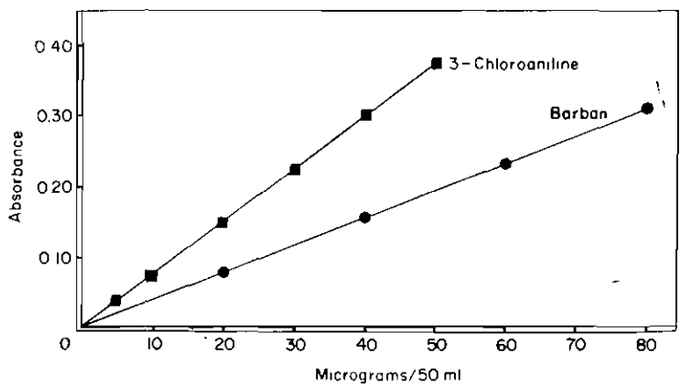


FIG. 2. *Standard curves for 3-chloroaniline and barban* (Riden and Hopkins, 1961). Reprinted with permission of the American Chemical Society.

vii. *Sample Calculations.*

$$c = \frac{a - b}{w}$$

where c = amount of barban, parts per million
a = barban in sample, micrograms
b = barban in check (background), micrograms
w = sample weight, grams

a and b are derived from Fig. 2 whereby absorbancies of the sample and check are converted to micrograms.

3. DISCUSSION

Barban is converted to ethylene dichloride-insoluble components in all plants studied. These 3-chloroaniline-containing materials are water-soluble. The discovery of these new substances allows a means of studying the metabolism of the herbicide. Separate methods of analysis for the organic soluble and insoluble fractions have thus been developed.

The decline studies with Carbyne on all crops examined to date show a rapid disappearance of the active ingredient even under the adverse conditions of overdosage and late application. Crops sprayed at the recommended time lose over 99% of the applied material within 2 weeks. Four weeks after application the residues are 0.01 p.p.m. or less. In all cases, the residues found in food portions of the crops are below 0.01 p.p.m., which is the sensitivity of the analytical method. This is true even when rates fourfold greater than the recommended ones are used.

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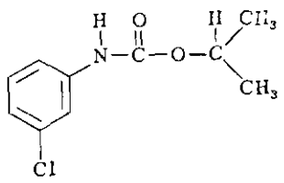
CIPC

L. N. GARD AND C. E. FERGUSON, JR.



Isopropyl *N*-(3-chlorophenyl) carbamate

I. GENERAL

A. EMPIRICAL FORMULA

$C_{10}H_{12}O_2NCl$ (Mol. wt. 213.67).

B. ALTERNATIVE NAMES

Chloro-IPC, 3Cl-IPC, and isopropyl ester of chlorocarbanilic acid.

C. SOURCE OF ANALYTICAL STANDARD

Pittsburgh Plate Glass Co., Chemical Division, Agricultural Chemical and Ammonia Sales, One Gateway Center, Pittsburgh 22, Pennsylvania.

D. BIOLOGICAL PROPERTIES

1. HERBICIDAL APPLICATIONS

CIPC, in its use as a herbicide, has come to be recognized as an extremely valuable agricultural chemical. It possesses a distinct biological activity, particularly toward narrow-leaved annual plants when applied to the soil during pre-emergence or post-emergence of the plant. It controls the growth of many narrow-leaved species, including annual bluegrass (*Poa annua*), barnyard grass (*Echinochloa crusgalli*), purslane (*Portulaca oleracea*), chickweed (*Stellaria media*), smartweed (*Polygonum pennsylvanicum*) and crabgrass (*Digitaria* Spp.). A number of other narrow-leaved species show intermediate susceptibility to the action
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of this chemical. CIPC possesses a greater biological activity than the related unchlorinated compound isopropyl *N*-phenylcarbamate (IPC) (q.v.).

2. SPROUT INHIBITOR

CIPC is an effective sprout inhibitor for white potatoes during the storage period, under properly controlled conditions. Rhodes *et al.* (1950) reported that isopropyl *N*-phenyl carbamate (IPC) markedly reduced sprouting of stored potato tubers and that it was superior to the methyl ester of naphthaleneacetic acid (MENA) for this purpose.

Marth and Schultz (1952) and Heinze *et al.* (1955) found that under certain conditions CIPC was more effective than IPC, MENA, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and maleic hydrazide as a sprout inhibitor.

3. FRUIT THINNING OF PEACHES AND PRUNES

Chemical thinning of peaches and prunes at early stages of growth can be achieved by treating the fruit-bearing tree with spray containing CIPC in concentrations ranging from 125 to 400 p.p.m. Aqueous sprays for this purpose are prepared from emulsifiable concentrate formulations and are applied by pressurized equipment to entire trees where thinning is desired. The time of application found most effective is after full bloom and when the fruit is developed to a size of 1–2 cm in diameter (Marth and Prince, 1953; Mitchell *et al.*, 1958).

4. TOXICITY

The acute oral toxicity studies conducted in 1953 by the Industrial Hygiene Foundation of America, Inc., Pittsburgh, Pa. for Pittsburgh Plate Glass Company indicated that the LD_{50} for rats was between 5 and 7.5 gm per kilogram of body weight. Similar tests with rabbits showed that the LD_{50} for rabbits is approximately 5 gm per kilogram of body weight. A chronic toxicity study on CIPC was conducted (Larson *et al.*, 1960) and showed that CIPC is of a low order of toxicity. Recently (1960) the U. S. Food and Drug Admiuistration, and tbe U. S. Department of Agriculture have permitted a tolcrance of 50 p.p.m. of CIPC on potatoes involved in interstate commerce. This very high tolerance applied to potatoes only, and cannot be interpreted as being applicable to other food crops at the present time.

E. History

Biological effects of the esters of carbanilic acid (carbamates) were reported by Friesen in 1929. In 1945 Templeman and Sexton published

the results of plant growth studies with carbanilic esters, and reported that the isopropyl ester (IPC) effectively retarded the growth of oats.

Experimental field work eventually led to commercial application of IPC for weed control in legumes, strawberries, spinach, and lettuce. Commercial experience soon showed IPC to have two limitations: under field conditions its performance was variable and inconsistent, and its residual life in the soil was too short for most crop applications.

Much of the early work in weed control with carbamates emphasized IPC. In 1951, Witman and Newton reported the more toxic effect of CIPC as compared with IPC. Since then, extensive work has been done with CIPC as a herbicide applied in various forms both at pre-emergence and post-emergence for the control of narrow-leaved species.

Some of the crops grown in soil receiving CIPC treatment for weed control and analyzed for residues of the herbicide are given in Table I.

TABLE I
TREATED CROPS ANALYZED FOR CIPC RESIDUES

Crop	Herbicidal treatment (CIPC)	References
Lettuce	Pre-emergence	Gard *et al.*, 1954
Sugar beets	Pre-emergence	Gard *et al.*, 1954
Onions	Pre-emergence	Gard *et al.*, 1954
Peanuts	Pre-emergence	Gard *et al.*, 1954
Cottonseed	Pre-emergence	Gard *et al.*, 1954
Spinach	Pre-emergence	Gard *et al.*, 1954
Strawberries	At onset of dormancy	Gard and Reynolds, 1957
Sweet potatoes	Layby	Gard and Reynolds, 1957
Grapes	Annually—spring	Gard and Reynolds, 1957
Tomatoes	Layby	Gard and Reynolds, 1957
Carrots	Post-emergence	Gard and Reynolds, 1957
Celery	Pre-transplant and Post-transplant	Gard *et al.*, 1959
Rice	Pre-emergence	Gard *et al.*, 1959
Peas	Pre-emergence	Gard *et al.*, 1959
Lima beans	Pre-emergence	Gard *et al.*, 1959
Snap beans	Pre-emergence	Gard *et al.*, 1959
Soybeans	Pre-emergence	Gard *et al.*, 1959
Alfalfa	Post-emergence	Gard, unpublished
Broccoli	Post-emergence	Gard, unpublished

F. PHYSICAL PROPERTIES

Melting point: 38 to 40°C.
Boiling point: 247°C (decomp.).
Specific gravity at 30°C: 1.180.
Refractive index $n^{20}D$ (supercooled): 1.5395.

Specific heat: 0.45 cal/gm/°C.
Flash point: 175°C.
Fire point: 175°C.
Vapor pressure, 25°C: 10^{-5} to 10^{-6} mm Hg (extrapolated).
Solubility in water at 25°C: 89 p.p.m.
Solubility in organic liquids: Soluble in alcohols, hydrocarbons, chlorinated hydrocarbons, ketones, esters, and anhydrous NH_3.

G. Chemical Properties

1. Methods of Synthesis

Two important methods for synthesizing CIPC are presented. The first method involves the reaction of equimolar quantities of isopropyl alcohol with *meta*-chlorophenyl isocyanate [Eq. (1)].

$$ClC_6H_4NCO + C_3H_7OH \rightarrow ClC_6H_4NHOCOC_3H_7 \quad (1)$$

The second method of synthesis involves the reaction of equimolar quantities of isopropyl chloroformate with *meta*-chloroaniline in a sodium hydroxide medium [Eq. (2)].

$$ClC_6H_4NH_2 + C_3H_7OCOCl \xrightarrow{NaOH} ClC_6H_4NHOCOC_3H_7 + NaCl + H_2O \quad (2)$$

2. Chemical Reactions

Being an ester, CIPC is slowly hydrolyzed under acidic and basic conditions to yield *meta*-chloroaniline, CO_2, and isopropyl alcohol. This reaction forms the basis for analytical procedures applicable to the technical product, its formulations, and crop residue tests. CIPC is subject to degradation in the presence of strong oxidizing agents, but is not readily acted upon by mild organic intermediates and solvents. On heating to temperatures of approximately 250°C, decomposition occurs to form chlorophenyl isocyanate and isopropyl alcohol.

H. Formulations

Granular applications of the herbicide are made by a broadcast type of operation from formulations containing 5, 10, and 20% of active ingredient using either hand-operated or mechanically operated equipment. Spraying applications are conducted with emulsifiable concentrates of CIPC.

The liquid formulations containing CIPC used expressly for the inhibition of sprouting of white potatoes during storage are formulated under the trade name of "Sprout-Nip." One type of application involves the use of an aqueous emulsion wherein the potatoes are treated by a

dipping process. Another type of application involves the spraying of a nonaqueous formulation on the potatoes in storage bins with equipment capable of atomizing or vaporizing the formulation directly into very minute particles resembling fog.

Various aspects of the different formulations of CIPC are covered under U. S. Patent 2,695,225.

II. ANALYSIS

A. Formulation Analysis

1. Review of Methods

a. *Total Chlorine Analysis*

In the case of technical CIPC and formulations, both liquid and solid, containing no chloro compounds as vehicles or diluents, appraisal of the active ingredient may be achieved by determining the total chlorine content by Parr bomb fusion with sodium peroxide, followed by chlorine analysis by Volhard titration. Other modes of sample decomposition suitable for determining total chlorine in organo-compounds may also be applicable to CIPC and its formulations (see Volume I, Chapter 11).

b. *Nitrogen Analysis*

An approximation of the purity of the technical product and the active ingredient concentration of formulations can be obtained by a total nitrogen analysis, provided the associated components of the mixture contain no nitrogen compounds. This method of assay analysis is not recommended, however, in view of the comparatively low concentration level of nitrogen in the compound.

c. *Hydrolysis Procedures*

CIPC is susceptible to hydrolysis by both acids and alkalies to yield carbon dioxide, isopropyl alcohol, and 3-chloroaniline. Tbis hydrolysis reaction provides the basis for the analytical methods most commonly used for assaying the technical product and formulations.

2. Recommended Procedure

a. *Principle*

Hydrolysis methods are recommended for the analysis of the technical product and its formulations. Acidic hydrolysis is the preferred technique since the reaction goes to completion within one hour.

It is based on the measurement of the carbon dioxide resulting from acidic hydrolysis of the sample with an excess of a sulfuric-phosphoric acid mixture. The carbon dioxide evolved during hydrolysis is absorbed in a measured excess of standardized sodium hydroxide solution and determined titrimetrically. Granular formulations are extracted with methylene dichloride in a Soxhlet apparatus prior to hydrolysis in order to separate the CIPC from the granular base which, if present, would introduce serious bumping problems during the hydrolysis reaction. Emulsifiable concentrates are hydrolyzed directly without prior separation. In some cases, the hydrolysis is carried out in an alkaline environment under reflux conditions during an overnight reaction period. In this case also, the measurement of the CO_2 formed during hydrolysis is the basis of the method. The CO_2 is collected chemically as the carbonate in the alkaline mixture during the reaction. At the termination of the hydrolysis, the CO_2 is liberated from the mixture by dilute acid treatment and measured titrimetrically by the same procednre and apparatus used for the acidic hydrolysis.

The results obtained by both acidic and alkaline hydrolysis procedures indicate that the precision of the method is $\pm 0.05\%$ carbon dioxide, or $\pm 0.25\%$ expressed as CIPC. The calculated accuracy of the method is 99% or better.

b. *Reagents*

The acid hydrolysis mixture is prepared by mixing 9 ml of conc. H_2SO_4 with 91 ml of phosphoric acid. Other reagents are: standardized 1 *N* HCl and 1 *N* NaOH solutions, 10% $BaCl_2$ solution, and 0.5% phenolphthalein indicator solution.

c. *Apparatus*

The apparatus employed for the acid hydrolysis of the sample is shown in Fig. 1.

All the air drawn through the system by an aspirator pump (H) is scrnbbed thoroughly with a dilute solution of potassium hydroxide (A) to remove residual CO_2, and is then used to sweep the reaction flask (C) during and after hydrolysis of the sample. The gas is passed through a water-cooled condenser (F) where condensable materials are returned to the reaction flask. As tbe gases leave the condenser, they are drawn through the NaOH absorber solution (E), and inert gases are vented through the aspirator system (H). The phosphoric-sulfuric acid hydrolysis mixture is introduced to the sample through the dropping funnel (B). Heat is applied to the reaction flask by the Bunsen burner. The vacuum on the system is regulated by the stopcock (G). After the

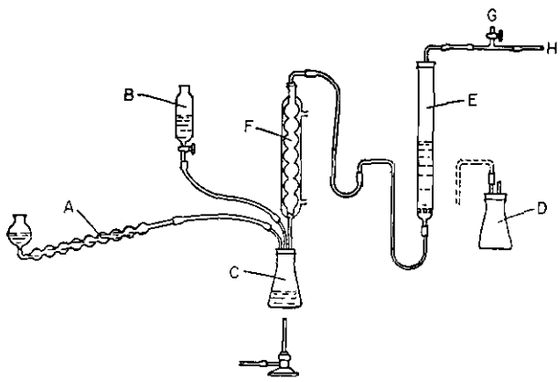

FIG. 1. Hydrolysis and carbon dioxide evolution assembly. A, Meyer sulfur bulb; B, acid reservoir; C, reaction flask; D, receiver flask; E, absorption tower; F, condenser; G, stopcock; and H, aspirator connection.

reaction is complete, the absorber solution is transferred to the receiver flask (D).

d. *Experimental Procedure*

The experimental procedure provides for the analysis of both the technical product and its formulations.

i. *Sample Preparation—Granular Formulations.*

Assemble a Soxhlet apparatus in the customary manner. Weigh 25 gm of granular sample containing 5% CIPC into the paper extraction thimble and place in the Soxhlet. Use correspondingly smaller samples accurately weighed for formulations containing larger amounts of CIPC. Extract the sample in a cellulose thimble with 125 ml of methylene dichloride for 2 hours to separate the CIPC from the carrier.

At the end of the extraction period, remove the flask containing the extract and cool to room temperature. Transfer the extract to a 250-ml wide-mouth Erlenmeyer reaction flask and evaporate the solvent by boiling gently on a steam bath. When a few milliliters of solvent remain in the flask, remove from the steam bath and pass a slow stream of filtered air over the surface of the liquid in the flask and complete the removal of solvent at room temperature. After the solvent has been expelled, proceed with the hydrolysis and carbon dioxide measurement in the manner described for the technical product.

ii. *Technical Product.*

Weigh 2.5 to 3.0 gm of the technical or purified CIPC sample to

0.1 mg, or 5 gm of the 47% emulsifiable concentrate, and transfer to a 250-ml wide-mouth Erlenmeyer reaction flask. Add 5 ml of distilled water, and four or five glass beads to reduce bumping during hydrolysis, and connect the flask to the apparatus as shown in Fig. 1.

Charge the CO_2 absorption tower (E) with 40.0 ml of standardized 1 *N* NaOH and add four drops of *n*-butanol. Apply suction to the system by adjusting the water aspirator so that air is drawn slowly and continuously through the air scrubber (A). Add 30 ml of the phosphoric-sulfuric acid mixture to the reaction flask containing the sample. Heat this flask gently, intermittently at first, until the sample has melted and the initial evolution of gas has ceased. Adjust the Bunsen burner to permit slow, even boiling of the sample mixture, while maintaining a continuous flow of air through the air scrubber. Continue this process for one hour to insure complete reaction.

Disconnect the absorption tower from the aspirator connection and reflux condenser, and transfer the absorber solution to a 500-ml receiver flask (D), by the proper manipulation of suction applied to the receiver flask. Rinse the tower free of the NaOH solution with distilled water. Dilute the absorber solution in the flask to approximately 300 ml with distilled water, add 50 ml of 10% barium chloride, and mix vigorously for approximately 1 minute. Without filtering off the precipitated $BaCO_3$, add 2 ml of phenolphthalein indicator solution and titrate the excess NaOH with standardized 1 *N* HCl.

Process a blank determination in the manner described above, employing water and the acid hydrolysis mixture. Compute the net volume (milliliters) of the standardized NaOH solution consnmed and correct for the blank.

iii. *Interfering Substances.*

Materials other than CIPC that liberate CO_2 under the conditions of the test cause interference in the method and appropriate correction must be applied.

iv. *Calculation.*

The formula for calculating the analytical results is given in the following equation:

$$\% \text{ Isopropyl } N\text{-(3-chlorophenyl) carbamate (CIPC)} = \frac{\text{ml of NaOH (net)} \times N \times 0.1068 \times 100}{\text{Sample wt.}}$$

B. Residue Analysis

Analytical methods for determining residue concentrations of CIPC in treated crop samples fall into two general categories: (1) those in-

volving hydrolysis techniques, either in cases where the CIPC is extracted with an appropriate solvent such as methylene dichloride or petroleum ether (Gard and Rudd, 1953), or by the direct hydrolysis treatment of the bulk of the sample without extractive treatment; (2) those involving instrumental measurement of the CIPC directly on the sample extract without hydrolysis.

The basis of the methods falling into the first of these categories is the measurement of the 3-chloroaniline hydrolysis product by sensitive spectrophotometric methods. The choice of the appropriate extraction technique and hydrolysis conditions for a particular product application is difficult to predict in advance and will vary from product to product. The method to be used is dependent on the inherent interferences which exist with the product and which respond to the complete analytical method.

The direct hydrolysis of the bulk of the sample without extractive separation of the CIPC is confined exclusively to an alkaline hydrolysis technique. Acidic hydrolysis results in serious charring and decomposition problems. In cases where the direct alkaline hydrolysis of the sample is applicable, the procedure to be followed would be the same as that applied to sample extracts as described in the "General Hydrolysis Procedure."

An extractive and direct measurement method for determining the CIPC content by an infrared instrumental technique was developed by the authors in conjunction with K. S. Dress and R. H. Shupe of the Infrared Lahoratory, Pittsburgh Plate Glass Company, Chemical Division (Ferguson *et al.*, 1963). This infrared method is less sensitive than spectrophotometric methods involving 3-chloroaniline measurements and is applied exclusively to the analysis of samples which contain CIPC in amounts exceeding 0.1 mg in a 200-gm sample. An example of this type of application is the analysis of potatoes treated for sprout inhibition for which a 50-p.p.m. tolerance of CIPC was recently set.

1. RECOMMENDED METHOD—ALKALINE HYDROLYSIS METHOD

a. *Principle*

The method involves extractive separation of CIPC from the macerated or ground sample with a nonaqueous solvent. After separation and evaporation of the solvent to concentrate the CIPC, the residue which remains is hydrolyzed in a comparatively strong alkaline medium to yield 3-chloroaniline. The resulting 3-chloroaniline is steam-distilled from the alkaline solution and is determined in the distillate by a suitable spectrophotometric procedure capable of measuring micro amounts of 3-chloroaniline.

Two analytical methods are generally used for measurement of the 3-chloroaniline. One method involves the formation of a blue-colored complex of the 3-chloroaniline with hypochlorite and a phenol-ammonium hydroxide reagent (Gard and Rudd, 1953; Jacobs, 1949). The second method involves the spectrophotometric measurement of the rose-red colored complex formed after diazotization of 3-chloroaniline and coupling with *N*-1-naphthylethylenediamine dihydrochloride (Montgomery and Freed, 1959).

b. *Reagents*

Strong alkali solution for the hydrolysis operation is prepared in 50% (w/v) strength from Reagent Grade sodium hydroxide. Other required reagents for the hydrolysis and distillation steps include: petroleum ether, Dow-Corning Antifoam-Type A, 95% ethanol, and Celite filter aid (Johns-Manville).

Reagents required for the phenol-ammonia-hypochlorite spectrophotometric measurement method include 0.36 *M* HCl, and calcium hypochlorite solution, prepared by treating 5 gm of calcium hypochlorite (70% available chloriue) with 95 ml of distilled water and heating to 60°C while stirring. The resulting mixture is filtered through a Whatman No. 40 filter paper and the clear filtrate stored in a glass-stoppered bottle for not more than 10 days.

The phenol-ammonia reagent is prepared fresh at the time of use by diluting 5 ml of NH_4OH (sp. gr. 0.90) with 95 ml of distilled water and dissolving 5 gm of pure phenol in the solution. The phenol found especially applicable for this use may be obtained as a U.S.P. grade from Oronite Chemical Company, San Francisco, California.

Reagents required for the diazotization-dye coupling spectrophotometric method include 2% sodium nitrite solution, prepared fresh daily; 10% sulfamic acid solution, prepared fresh every 3 days; 2% *N*-1-naphthylethylenediamine dihydrochloride solution, prepared fresh daily; approximately 6 *M* HCl; and 1 *M* HCl.

c. *Apparatus*

The hydrolysis and distillation apparatus used is shown in Fig. 2. Cooling water is supplied and regulated to the condensers in such a fashion that during hydrolysis the assembly functions in a total reflux manner. After the hydrolysis period is complete, the flow of cooling water in the reflux condenser is discontinued and is directed through the downward condenser, thus providing for the collection of distillate. By this arrangement it is unnecessary to rearrange or disconnect any of the items of equipment after the testing procedure has commenced.

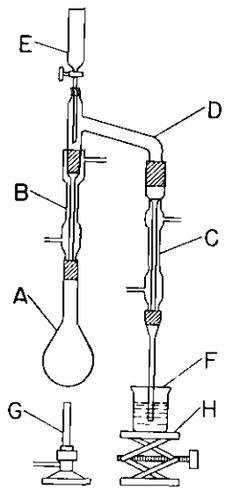

FIG. 2. Hydrolysis and distillation assembly. A, 500-ml Kjeldahl Flask, with 24/40 ℻ joint; B, reflux condenser, West-type, with 24/40 ℻ joint; C, distilling condenser, West-type, with 24/40 ℻ joint; D, side-arm distilling head, with 24/40 ℻ joints and thermometer joint with 10/30 ℻ joint; E, 125-ml dropping funnel, with 10/30 ℻ joint; F, receiver beaker; G, Bunsen burner; and H, Lab Jack.

Other items of apparatus include: a spectrophotometer equipped with 1-cm and 5-cm cells, a centrifuge which accommodates 250-ml sedimentation bottles, and a blending or grinding device capable of macerating the sample to fine pulp.

d. *Experimental Procedure*

i. *Sample Preparation.*

A sufficient amount of sample is macerated to provide 200 gm for each test. Fresh foods such as tomatoes and peas containing substantial amounts of inherent water are macerated and blended in their own juices. Dried foods and dry products, such as alfalfa hay and rough rice, are ground and macerated in a dry state.

Sample processing in either case is conducted by placing somewhat more than the 200-gm sample required for the test into the blender and macerating or grinding to a fine, uniform pulp.

ii. *Extraction Procedure—Aqueous Macerate.*

After maceration, a 200-gm portion of aqueous macerate is weighed into a 500-ml separatory funnel.

One hundred and fifty milliliters of petroleum ether is added to the separatory funnel, and the funnel is shaken thoroughly for one minute to extract any herbicide into the ether layer. The slurry is then transferred to 250-ml sedimentation bottles and centrifuged at about 1800 r.p.m. for 10 minutes to separate the layers. The top ether layer is withdrawn with the aid of a suction pipette and transferred to another 500-ml separatory funnel. The water-pulp layer is returned to the first separatory funnel and re-extracted a second and then a third time, using 100 ml of petroleum ether each time. The ether layers are combined in the separatory funnel and the water-pulp fraction is discarded.

About 75 ml of a 50% v/v ethanol-water wash solution is added to the separatory funnel containing the combined ether extracts, and the mixture is shaken thoroughly to wash the ether solution. After separation of the layers (a little crystalline NaCl may be added to aid in breaking any emulsions which may form), the alcohol-water layer is transferred to a second separatory funnel containing 50 ml of petroleum ether and is extracted a second time. The alcohol-water layer is again extracted in a third separatory funnel containing 50 ml of petroleum ether. After this third extraction, the alcohol-water layer is discarded. The successive washing of the ether layers is repeated three additional times, after which the petroleum ether layers are combined for further treatment.

iii. *Extraction Procedure—Nonaqueous Macerates.*

A 200-gm portion of macerate from dried samples containing a comparatively low level of moisture is weighed into an 800-ml beaker, and sufficient petroleum ether is added to thoroughly wet the sample and leave a layer of solvent on the surface. The slurry is allowed to leach overnight, after which it is filtered by decantation through a Whatman No. 41 filter paper utilizing suction and a Büchner funnel. An additional portion of petroleum ether is added to the beaker containing the pulp, and is allowed to leach for one additional hour, stirring the slurry occasionally. After leaching, the slurry is decanted through the original filter, finally transferring all the powder to the funnel and rinsing the solids retained on the filter with two 50-ml portions of petroleum ether. The filtrates and washings are combined and reserved for further treatment, and the pulp is discarded.

iv. *General Hydrolysis Procedure.*

The petroleum ether extracts are combined in a 500-ml hydrolysis flask (Kjeldahl type) fitted with a 24/40 T glass joint and evaporated to dryness under reduced pressure by connecting the flask to a water-

aspirator pump and immersing the flask in a bath of warm water (ca. 40°C).

When the evaporation of the ether is complete, the flask is disconnected from the suction line, 100 ml of distilled water and 2 to 3 drops of anti-foam (Dow Corning Type A) are added, and the flask is connected to the distillation apparatus. The distillation receiver (a 125-ml beaker) is charged with 5 ml of 0.36 *M* HCl if the phenol-ammonia-hypochlorite procedure is followed, or 5 ml of 6 *M* HCl if the diazotization dye-coupling method is followed. The outlet tube of the distillation condenser must dip below the liquid level of the acid in the receiver beaker.

Fifty milliliters of the 50% NaOH solution are added through the dropping funnel into the distillation flask followed by 25 ml of distilled water. The solution is warmed gently with a Bunsen burner, and allowed to reflux for 1.5 hours. At the end of this hydrolysis period, the cooling water to the reflux condenser is shut off, and the sample is allowed to distill from the distillation flask, the vapors condensing in the downward condenser and being collected in the distillation receiver beaker. Thirty milliliters of distillate are collected in the receiver beaker.

After collection of the distillate, 0.2 gm of the Celite filter-aid is added and mixed thoroughly to clear the distillate and remove any turbidity which may be present as a result of volatile diverse materials which may have steam-distilled. Treatment of cloudy distillates with Celite has been found to be quite effective for removal of the substances causing this turbidity (Gard *et al.*, 1959). After this Celite treatment, the solution is filtered through a 7-cm Whatman No. 42 filter paper. The filtrate is collected in a clean, dry 150-ml beaker if the phenol-ammonia-hypochlorite method is to be followed. The filtrate is collected directly in a 50-ml volumetric flask if the diazotization dye-coupling method is to be followed. In either case, the washing of the filter is accomplished with a minimum of water so that the total volume of distillate and washings does not exceed 40 ml.

v. *Phenol-Ammonia-Hypochlorite Spectrophotometric Method.*

To develop the color with the 3-chloroaniline collected in the filtered distillate, two drops of 5% calcium hypochlorite solution are added and the solution is allowed to digest at room temperature for 5 minutes with intermittent mixing. After this reaction, the solution is heated to boiling and allowed to boil vigorously for 30 seconds on an electric hot plate. The solution is removed from the hot plate and 5 ml of the phenol-ammonia reagent is added immediately to the hot solution. This reagent is added beneath the surface of the hot solution with a pipette,

with the tip portion of the pipette functioning as a stirring rod for mixing during the addition of the phenol-ammonia reagent. The mixture is allowed to digest without accelerated cooling for 15 minutes for full color development.

Near the end of this digestion period the solution is diluted to exactly 45 ml with distilled water. A suitable portion of the diluted solution is transferred to the 5-cm cell of the spectrophotometer and the transmittance (650 mμ) is measured using distilled water in the reference cell.

vi. *Diazotization Dye-Coupling Spectrophotometric Method.*

To the filtrate from the Celite treatment in the 50-ml volumetric flask add 1 ml of 2% sodium nitrite solution, mix thoroughly, and allow to stand 20 minutes for complete diazotization. Add 1 ml of 10% sulfamic acid solution, mix thoroughly, and allow to stand for 15 minutes for complete destruction of the excess nitrite. After decomposition of the nitrite is complete, add 5 ml of 2% *N*-1-naphthylethylenediamine dihydrochloride solution, dilute to volume (50 ml) with 1 *M* HCl and mix thoroughly. After 90 minutes, measure the transmittance of the solution at 540 mμ using distilled water in the reference cell of the spectrophotometer.

vii. *Interfering Substances.*

In all food crops tested by the analytical methods a small but finite interference expressed as CIPC has been found. No attempt has been made to identify the particular components in each sample which contribute to this interference. Presumably, these interfering substances possess some chemical structure resembling aniline and respond to the analytical method. Each crop analyzed has been evaluated for its own peculiar interference and corrections made during the analysis of "recovery" and treated crop samples for this interference. Establishment of valid and justified interference correction values necessitates testing a minimum of five replicates to permit statistical analysis and to verify the consistency of this correction.

viii. *Sensitivity.*

The practical lower limit of detection by both analytical methods described, as applied to crop analysis, is approximately 0.01 mg of CIPC, or 0.05 p.p.m. based on a 200-gm crop sample. It must be emphasized that this practical lower limit of sensitivity is greatly dependent upon the magnitude of the naturally occurring interfering substances present in the crop samples which respond to the analytical method.

ix. *Recovery.*

In general, recovery analyses are conducted for each crop by adding CIPC to samples of the untreated crop in amounts approximating the concentration expected in the treated crop samples. The CIPC is added in a solvent identical to that used in the extraction step of the analysis and is added to the weighed portion of the sample before extraction. Recovery analyses must be performed for each crop studied in order to prove the validity of the analytical method for that particular crop. In the authors' laboratory the calculated average recovery has ranged between 80% and 100% for a substantial number of crop analyses (Gard *et al.*, 1959; Gard and Reynolds, 1957; Gard and Rudd, 1953).

x. *Calibration of Spectrophotometer and Preparation of Standard Curve, Phenol-Ammonia-Hypochlorite Method.*

Calibration of the spectrophotometer is conducted by measuring aliquots representing 0.00 to 0.05 mg of 3-chloroaniline dissolved in dilute hydrochloric acid, according to the color development steps of the analytical procedure. Absorbancy values for the standards are obtained with a spectrophotometer at 650 mμ, with 5-cm cells and distilled water in the reference cell. Typical data are given in Table II.

TABLE II
CALIBRATION OF SPECTROPHOTOMETER FOR 3-CHLOROANILINE[a]
PHENOL-AMMONIA-HYPOCHLORITE METHOD

3-Chloroaniline (mg)	Transmittance at 650 mμ (%)
0.00	98
0.01	82
0.02	68
0.03	57
0.04	47
0.05	39

[a] NOTE: 3-Chloroaniline is the hydrolysis product of CIPC.

xi. *Calibration of Spectrophotometer and Preparation of Standard Curve, Diazotization Dye-Coupling Method.*

Calibration of the spectrophotometer is conducted by measuring aliquots representing 0.00 to 0.100 mg of 3-chloroaniline dissolved in 1 *M* HCl according to the color development steps of the analytical procedure. Optical transmittance values for the standards are obtained

with a spectrophotometer at a wavelength of 540 mμ utilizing both 1-cm and 5-cm cells and distilled water as reference. Typical transmittance data are given in Table III.

TABLE III

CALIBRATION OF SPECTROPHOTOMETER FOR 3-CHLOROANILINE[a] DIAZOTIZATION DYE-COUPLING METHOD

3-Chloroaniline (mg)	Transmittance at 540 mμ, %	
	1-cm cell	5-cm cell
0.000	97.5	95.0
0.002	94.0	80.0
0.004	90.0	68.5
0.006	86.0	58.0
0.008	84.5	49.0
0.010	82.5	40.0
0.012	79.0	36.0
0.015	76.0	28.5
0.018	72.0	23.0
0.020	71.0	—
0.030	60.5	—
0.040	52.5	—
0.050	44.0	—
0.060	38.0	—
0.070	30.0	—
0.080	27.0	—
0.100	19.5	—

[a] NOTE: *3-Chloroaniline is the hydrolysis product from* CIPC.

xii. *Sample Calculation.*

The CIPC content of the sample under examination is calculated from the following equation:

$$\text{p.p.m. CIPC} = \frac{\text{mg of 3-chloroaniline (net)}^{a,b} \times 1.67 \times 1000}{\text{Sample wt.}}$$

where a = value obtained from calibration curve

b = correction for interference in the method due to unknown compounds in the sample which respond to the test

2. DISCUSSION OF THE METHOD AND ITS APPLICABILITY TO DIFFERENT CROPS

In general terms, the analytical methods described can be considered as being applicable to a wide variety of different crops (Table I). However, in specific applications technical modifications are nearly always required to resolve individual interference problems and handling

techniques which characterize each crop. These problems must be dealt with on an individual basis.

Two outstanding examples of technical modifications often required in the analytical procedure because of the particular nature of the sample involved are as follows: (1) The use of a different solvent other than petroleum ether for the extraction of the sample, and (2) the use of dilute sulfuric acid for the hydrolysis step in the method. Other modifications of the method to improve recoveries and reduce interferences may be required and can only be determined by experimentation (see also Volume I, Chapter 5).

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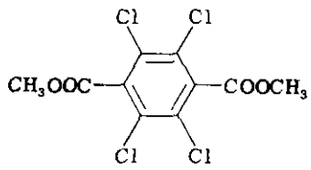
8

Dacthal

H. P. Burchfield and Eleanor E. Storrs



Dimethyl 2,3,5,6-tetrachloroterephthalate

I. GENERAL

A. Empirical Formula

$C_{10}H_6O_4Cl_4$ (Mol. wt. 332.15).

B. Alternative Names

Tetrachloroterephthalic acid, dimethyl ester, DAC-893. The name Dacthal is the registered trademark of the Diamond Alkali Company.

C. Source of Analytical Standard

Diamond Alkali Co., Agricultural Chemicals, Research Center, P. O. Box 348, Painesville, Ohio.

D. Biological Properties

Dacthal is a pre-emergence herbicide which has little post-emergence effect on most crops or weeds. It is particularly promising for the control of crabgrass in turf and certain weeds in crops. Lambsquarters, crabgrass, witch grass, purslane, chickweed, and foxtail are controlled at application rates of 4 to 8 pounds per acre; while pigweed, annual blue grass and Johnson grass may require higher rates. Common ragweed, mustards, Jimson weed, Galinsoga, and smartweed are resistant at the rates commonly used. Dacthal has given good control of annual grassy weeds in turf, ornamentals, field and forage crops, vegetables, and fruit plantings.

The acute oral toxicity (LD_{50}) of Dacthal to male albino rats is reported to be greater than 3.0 gm per kilogram of body weight. The

acute dermal toxicity (LD_{50}) to albino rabbits is reported to be greater than 10.0 gm per kilogram of body weight. A single application of 3 mg to the eyes of albino rats produced a mild degree of irritation which completely subsided in 3 hours. Information on chronic toxicity was not available at the time of writing.

E. History

Dacthal is the trademark of Diamond Alkali Co. for dimethyl 2,3,5,6-tetrachloroterephthalate. Its use as a herbicide was disclosed in U. S. Patent 2,923,634 and its selective biological activity described by Limpel *et al.* (1962), and Harris *et al.* (1960). It was first tested as a pre-emergence herbicide by various agricultural experiment station workers in 1959.

F. Physical Properties

Pure Dacthal is a white crystalline solid, of very low vapor pressure, which melts at 156°C. Its solubility in water is less than 0.5 p.p.m. and it is sparingly soluble in acetone, benzene, cyclohexanone, dioxane, tetrahydrofuran, xylene, and petroleum solvents. It is essentially odorless.

G. Chemical Properties

Dacthal can be prepared by the chlorination of *p*-xylene, and conversion of the reaction products to 2,3,5,6-tetrachloroterephthaloyl chloride. The latter compound is then caused to react with methanol to yield the dimethyl ester.

Dacthal is thermally stable and is not hydrolyzed easily by aqueous acid. However, it will dissolve readily in conc. H_2SO_4 and on warming it liberates tetrachloroterephthalic acid. Saponification of the methyl groups by alcoholic KOH is slow, and, on prolonged refluxing, some of the ring halogen is eliminated. 2,3,5,6-Tetrachloroterephthalic acid is a strong organic acid because of the electronegativity of the four halogen atoms and two carboxyl groups substituted in the ring. Consequently, the dimethyl ester is an alkylating agent. This is the basis for the colorimetric method used for residue analysis.

H. Formulations

Dacthal is marketed as wettable powders containing 50% of the active ingredient (W-50) for the control of crabgrass in turf, and 75% of the active ingredient (W-75) for the control of crabgrass and annual weeds in vegetables and strawberries.

Formulations for experimental work only include 20/40 mesh gran-

ules containing 1.5% of the active ingredient (G-1.5) for tests on turf and ornamentals; and 30/60 mesh granules containing 5% of the active ingredient (G-5) for limited applications on established fruit crops.

Dacthal has been marketed for use on lawns under the name RID.

II. ANALYSIS

A. Formulation Analysis

Methods for formulation analysis have not yet been released by the manufacturer. Determination of total chlorine will not give highly accurate results, since compounds may be present as impurities which contain variable amounts of halogen.

For approximate results, the colorimetric method described in Section II,B can be used. However, methods of this type are usually not considered accurate enough for the determination of major components.

B. Residue Analysis

1. REVIEW OF METHODS

Micro amounts of Dacthal can be determined by gas chromatography (Storrs and Burchfield, 1962), or colorimetrically by reaction with γ-(4-nitrobenzyl)-pyridine (Schuldt *et al.*, 1961). Gas chromatographic methods can be applied to the analysis of Dacthal residues in crops. (See Volume I, Chapter 9 for details.)

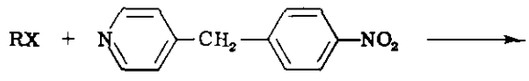
2. RECOMMENDED METHOD–COLORIMETRIC METHOD FOR THE DETERMINATION OF DIMETHYL TETRACHLOROTEREPHTHALATE

a. *Principle*

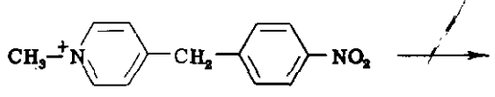
Pesticides such as Dyrene, and simazine, which contain strongly electronegative groups can be determined colorimetrically by causing them to react with pyridine to form quaternary bases. In alkaline solution, the pyridine ring opens to form a highly colored anil of glutaconic dialdehyde. The ease with which the pyridine ring is cleaved is dependent upon the electronegativity of the entering group. Thus, cyanogen chloride gives a color with pyridine and organic base alone, while the *s*-triazines and similar materials require the presence of an alkali metal hydroxide or a quaternary base. On the other hand, the heterocyclic rings of compounds such as methyl pyridinium chloride are not cleaved at all under the normal conditions of temperature and pressure.

The ring halogens of Dacthal are unreactive, so colorimetric analy-

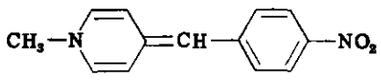
sis by the usual modifications of the pyridine-alkali reaction is not possible. However, Dacthal, since it is the ester of a strong acid, is an active methylating agent and will react with pyridine derivatives to form quaternary salts such as methyl pyridinium iodide. As noted above, the rings of these compounds are not cleaved readily, but colors can be developed by using a pyridine derivative which can be dehydrated to yield a chromophore. Thus, a number of alkylating agents (I) have been shown to form colored products (IV) when caused to react with γ-(4-nitrobenzyl)-pyridine (II) as described by Epstein *et al.* (1955) and reviewed by Burchfield and Schuldt (1958). The overall reaction is as follows:



I II



III



IV

The color is developed by dehydrating the quaternary base (III) in the presence of triethylamine. Methylation of the reagent (II) is carried out in an autoclave at a temperature of 120°C. Pyridine and triethylamine are added subsequently to develop the color. The absorption maximum is at 565 mμ (Fig. 1). Some color is developed when the reaction product is dissolved in pyridine alone, but absorbances are not reproducible. Color intensity and reproducibility are enhanced greatly on addition of triethylamine. Readings should be made shortly after adding this reagent, since the color fades on standing.

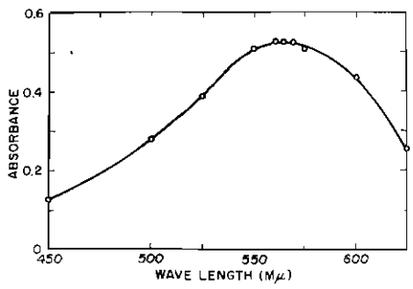


FIG. 1. Absorption spectrum of reaction product of dimethyl tetrachloroterephthalate with γ-(4-nitrobenzyl)-pyridine and triethylamine in pyridine solution.

b. *Reagents*

The following solvents or reagents are required: Reagent Grade pyridine (Mallinckrodt Chemical Works), Reagent Grade triethylamine (Eastman), Clark and Lubs pH 4 phthalate buffer (Beckman Instrument Co.), analytical Reagent Grade acetone (Mallinckrodt Chemical Works), Reagent Grade methylene chloride, neutral Norit A decolorizing carbon (Fisher Scientific Co.), commercial grade acetonitrile, Reagent Grade *n*-heptane, anhydrous sodium sulfate, and grade I Woelm acid aluminum oxide (Aluphram Chemicals, New Orleans, La.).

The γ-(4-nitrobenzyl)-pyridine used in preparing the reagent can be obtained from Mathieson, Coleman, and Bell. The reagents used in the analytical determinations are a 5% solution of γ-(4-nitrobenzyl)-pyridine in analytical Reagent Grade acetone (w/v) and a 50% solution of triethylamine in pyridine (v/v).

c. *Apparatus*

An autoclave operated at 120°C and 18 psig pressure is useful for carrying out the reaction. However, a home pressure cooker will also serve satisfactorily. Special laboratory glassware which is needed includes 1,000-ml and 250-ml separatory funnels, 4-inch conical funnels, fritted funnels, medium-porosity Büchner-type fritted funnels, filter flasks with side tubes, quart jars with aluminum-lined lids, and 22-mm O.D. × 28-cm glass columns with stopcocks. Since the alkylation reaction is carried out in 125-ml Erlenmeyer flasks, a large supply of these is desirable. Other apparatus, in addition to common laboratory glassware, includes an interval timer, a source of clean dry air, aluminum foil, Whatman No. 802 filter paper, and a 1-gallon Waring Blendor, model CB-4.

Any suitable colorimeter or spectrophotometer employing 1-cm cells can be used for absorbance measurements.

d. *Experimental Procedure*

i. *Extraction from Plant Material or Soil.*

For soft succulent products such as vegetables and berries, the sample (200 gm) and 500 ml of methylene chloride are added to a Waring Blendor and macerated for 2 minutes. Without stopping the blendor, an additional quantity of methylene chloride (500 ml) is added, and the blending is continued for an additional 3 minutes. The homogenate is then transferred to a 1,000-ml separatory funnel. After the phases have separated, the lower layer is drawn off and saved for analysis.

Hard fibrous tissues are prepared for extraction by running them through a food chopper. For the extraction of such materials as well as for soils, the sample (200 gm) is placed in a 500-ml glass-stoppered Erlenmeyer flask, and 250 ml of methylene chloride is added. The flask is then shaken for 2 hours on a mechanical shaker. The contents are filtered through four thicknesses of cheesecloth. The filtrate is reserved for analysis.

ii. *Clean-up Method.*

Aliquots of the methylene chloride extract (250 ml) are transferred to a 400-ml beaker and evaporated to dryness under a current of clean dry air. Acetonitrile (50 ml) is then added and the contents of the beaker mixed well with a stirring rod. The solution is transferred to a 250-ml separatory funnel, and the residue remaining in the beaker is washed with *n*-hexane (25 ml) which is then added to the acetonitrile in the separatory funnel. The funnel is stoppered and shaken for a period of 1 minute. When the phases separate, the lower phase (acetonitrile) is transferred to a second separatory funnel and extracted with 25 ml of *n*-heptane for 1 minute. This process is repeated until a total of three extractions have been made. The acetonitrile layer is then placed in a 250-ml beaker and evaporated to dryness under a stream of clean, dry air. A hot-water bath (50°C) is used to facilitate the evaporation of the solvent. The residue is dissolved in methylene chloride (50 ml) and 2 tablespoons of decolorizing charcoal (Norit-A) are added. The mixture is stirred thoroughly, and the solution is permitted to remain in contact with the absorbant for 15 minutes with occasional stirring. It is then vacuum-filtered through fritted-disk funnels (medium porosity) into a 250-ml suction flask. The beaker and funnel are washed with two 15-ml portions of methylene chloride. The filtrate is transferred to a 250-ml beaker, and the suction flask is rinsed with methylene chloride (20 ml). The combined filtrate and washing is reserved for further clean-up by chromatography on alumina.

(a) *Preparation of chromatographic column.* A plug of glass wool is placed in the bottom of a 22-mm O.D. × 28-cm column and forced into place with a solid glass rod. The column is packed with activated alumina to a height of 4 inches. During packing, each inch of material is compacted as it is added to the column. When all of the alumina has been added, 1 inch of anhydrous sodium sulfate is placed on top of it. The column is preconditioned by passing methylene chloride (150 ml) through it. The level of the methylene chloride should not be allowed to drop below the top of the sodium sulfate layer. Columns can be conditioned in advance but should be used the same day.

(b) *Chromatography.* The methylene chloride solution containing Dacthal is percolated through the column, and the Dacthal is thus adsorbed on the activated alumina. After all of the solution has been transferred to the column, the beaker which contained the Dacthal solution is washed with 25 ml of a 2% solution of acetone in methylene chloride (v/v). The washings are also percolated through the column. As soon as the meniscus of the methylene chloride reaches the top of the sodium sulfate layer, the sides of the column are washed with a 2% solution of acetone in methylene chloride (2 to 3 ml). This procedure is repeated three times. Each time, the liquid level is allowed to reach the sodium sulfate layer before adding the next portion of solvent. A fourth portion (25 ml) is added to the column directly. The solvent which passes through the column up to this point is discarded. When the 25-ml rinse reaches the sodium sulfate layer, an additional 100 ml of a 2% acetone solution in methylene chloride is added to the column. The Dacthal is eluted from the column with this portion of solvent. When the last of this solvent has passed into the sodium sulfate layer, the eluate is collected for analysis.

iii. *Measurement of Dacthal.*

γ-(4-Nitrobenzyl)-pyridine reagent (2 ml) is added to the eluate from the column, and the solution is evaporated to dryness at 50°C in a hot-water bath under a stream of clean, dry air. Beckman pH 4 phthalate buffer diluted to half-strength (2 ml) is added, and the mouth of the flask is covered with aluminum foil. The flasks are placed in an autoclave at 120°C (18 psig) for 1 hour and are then removed and cooled. The aluminum covers are removed and the contents evaporated to dryness under a stream of clean, dry air. Additional buffer (1 ml) and pyridine (8 ml) are added to each flask, and they are swirled until all the solid dissolves. Triethylamine reagent (1 ml) is added. The contents of the flasks are mixed thoroughly, and the absorbance is measured at 565 mμ against a reagent blank which is processed in the same manner

as the sample. The readings should be made as soon as possible after adding triethylamine.

iv. *Interferences.*

Any compound which can function as an alkylating agent will give a positive test in this reaction. This includes alkyl halides, alkyl sulfates, and ethylenimines. Other esters of terephthalic acid give colored products under these conditions, but, in general, the response is not as intense as with dimethyl tetrachloroterephthalate. The color decreases when fewer chlorine atoms are substituted in the aromatic ring, or when the methyl groups of the ester are replaced by higher alkyl groups. In general, modifications which would be expected to reduce the alkylating power of the terephthalate ester result in lower intensities. Compounds which respond sufficiently to the color test to be classed as potential interferences include dimethyl trichloroterephthalate, methyl butyl tetrachloroterephthalate, monomethyl tetrachloroterephthalate, and dimethyl dichloroterephthalate. The color intensity decreases in the order given.

Thorough clean-up of the sample is required to obtain reliable results. Extracts of soils and crops which have not been subjected to chromatography often yield purple colors corresponding to a few micrograms of Dacthal when it is known that this compound has not been applied. Therefore, chromatography on alumina is required to eliminate this interference. The nature of the materials in the extracts giving rise to this effect have not been established.

v. *Recovery.*

Dacthal added to various crops at rates ranging between 0.25 and 3.75 p.p.m. has been analyzed with acceptable results (Table I). Recoveries varied from a low of 74% to a high of 104%.

Recovery of dimethyl tetrachloroterephthalate from dry soil was found to be in the rauge of 80 to 90% of the amount added.

In a study in which the chemical was applied to the soil at rates of 10 and 20 lb of active ingredient per acre, periodic samples were taken for one year to determine the residual amount of chemical present. After one year, 1.8 and 10.3% of the initial dose remained in plots treated at the 10-lb rate, and 4.2 and 13.1% remained at the 20-lb rate.

vi. *Sensitivity.*

The sensitivity depends upon a number of factors, including sample size, dilution factor, final volume of the solution on which absorbance measurements are made and the ratio of background to color intensity. These will vary with the individual crop and modification of the method

TABLE I
RECOVERY OF DIMETHYL TETRACHLOROTEREPHTHALATE ADDED TO VARIOUS PLANT HOMOGENATES AND SOIL[a]

Sample material	Added, p.p.m.	Found, p.p.m.	Recovery, %
Cottonseed (nondelinted)	0.25	0.215	86.0
	0.50	0.43	86.0
Cottonseed (delinted)	0.25	0.232	92.8
	0.50	0.518	103.6
Cottonseed oil	1.25	1.10	88.0
	2.50	2.12	84.8
	3.75	3.18	84.8
Onions	0.25	0.235	94.0
	0.50	0.405	81.0
	1.00	0.975	97.5
Sweet potatoes	0.25	0.18	74.0
	0.50	0.41	82.0
	1.00	0.74	74.0
Soil	0.35	0.203	81.2
	0.50	0.414	82.8
	1.00	0.913	91.3
	1.25	1.005	80.4

[a] All of the above recovery values are means of a minimum of triplicate determinations.

used for analysis. A solution containing the reaction product obtained from 1 μg of Dacthal per milliliter has an absorbance of 0.18. Residues as low as 0.01 p.p.m. have been detected in crop samples.

vii. *Standard Curve.*

A solution containing 1000 p.p.m. of pure Dacthal in acetone is prepared. From this stock, solutions containing 10, 20, 30, and 40 μg per milliliter are prepared by diluting with acetone. An aliquot of each solution (1 ml) is added to duplicate 125-ml Erlenmeyer flasks, and the solvent is evaporated under a current of clean, dry air. Color is developed as described in Section II,B,2,d,iii; and micrograms of compound per milliliter of solution is plotted against absorbance (Fig. 2).

3. APPLICABILITY OF RECOMMENDED METHOD TO DIFFERENT CROP OR FOOD MATERIALS

Cottonseed (nondelinted), cottonseed (delinted), cottonseed oil, onions, sweet potatoes, strawberries, and a number of other crops have

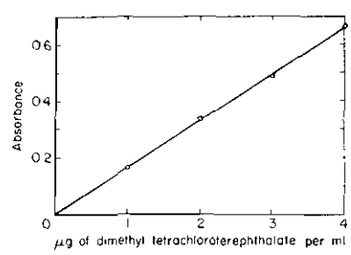


FIG. 2. Standard curve for the estimation of dimethyl tetrachloroterephthalate.

been treated with Dacthal and analyzed for residue. Satisfactory results were obtained in all cases. The method is equally applicable to the determination of Dacthal in soil.

4. DISCUSSION OF METHOD

This method provides a general technique for the analysis of compounds having alkylating power and undoubtedly should be useful for the determination of other pesticides having this function. It is particularly useful when the alkylating group is a hydrocarbon radical such as the methyl group, since other pyridine-alkali procedures (Burchfield and Schuldt, 1958) are not applicable. Although this general method has been used previously for the analysis of alkyl halides and ethylenimines (Epstein *et al.*, 1955), the reaction conditions described here are unique.

In early experiments, extremely variable results were obtained when dimethyl tetrachloroterephthalate was treated with a 5% solution of γ-(4-nitrobenzyl)-pyridine in acetone in a boiling water bath for periods up to 2 hours. It appeared that the color was more reproducible when the residue was dissolved in pyridine rather than the acetone used by previous workers. However, some batches of reagent did not yield colored products. In cases where color was obtained, increasing the time of heating enhanced the color intensity, but the sensitivity was not satisfactory. The addition of the Beckman phthalate buffer (pH 4) to the reactants before heating in the water bath resulted in reproducible results, but the sensitivity was still poor. When buffers with higher or lower pH values were tried, the color intensity decreased. Evidently, variable amounts of impurities in the reagent caused the erratic results obtained in earlier experiments, since highly purified reagent did not yield strong colors in the absence of buffer. Phthalate buffers were superior to phosphate buffers for this purpose. Beckman pH 4 buffer gave the highest color intensity. When this buffer was used together with a 5% solution of reagent in pyridine, the color intensity was found to

increase with time of heating. This suggested the use of higher temperatures. Therefore, the flasks were placed in an autoclave for 30 minutes at a temperature of 120°C. Under these conditions, the color intensity increased fivefold. Time studies showed that 60 minutes in the autoclave was optimum. Subsequent work showed that the color intensity at 565 mμ was virtually independent of pH between values of 3 and 5. Similarly, dilution of the buffer to ⅕ strength did not reduce the absorbance. Therefore, the Beckman pH 4 phthalate buffer at half strength was used in all subsequent work. Varying the concentration of the reagent between 3 and 10% also failed to produce significant changes in intensity. Consequently, a 5% solution of the reagent in acetone was selected arbitrarily.

The reaction product formed on autoclaving was found to be stable. Pyridine and triethylamine were added to samples immediately after heating and to samples held at room temperature 5 hours after heating. No significant difference in color intensity was observed. Therefore, it is permissible to allow samples to stand in the dry state at least 5 hours before developing the color. The reaction product was also found to be stable in the pyridine solution. However, once the triethylamine is added, the absorbance should be read as soon as possible, since the color fades rapidly.

5. MODIFICATION OF METHODS OR ALTERNATE METHODS

No modifications of this method have been published. Dacthal can be measured by gas chromatography, but results on application to residue analysis have not been published.

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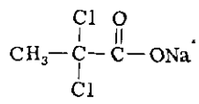
Dalapon (Sodium)

GRANT N. SMITH AND E. H. YONKERS



Sodium 2,2-dichloropropionate

I. GENERAL

A. EMPIRICAL FORMULA

$C_3H_3O_2Cl_2Na$ (Mol. wt. 165).

B. ALTERNATE NAME

Sodium α,α-dichloropropionate. The name dalapon is the foreign trademark of the Dow Chemical Company.

C. SOURCE OF ANALYTICAL STANDARD

Agricultural Chemical Research Center, The Dow Chemical Company, Midland, Michigan.

D. BIOLOGICAL PROPERTIES

Dalapon is a growth regulator which is used to control undesirable grasses. It is actively absorbed and translocated by both foliage and roots.

Dalapon (sodium salt) is quite low in acute oral toxicity for all the species studied. The results of feeding single doses of dalapon to various species of animals are given in Table I.

E. HISTORY

Dalapon was first introduced by The Dow Chemical Company (U. S. Patent 2,642,354) in the summer of 1953 to state and federal experiment stations for limited field testing. The next year, a commercial product was made available for railroads and certain other nonagricultural applications. Since 1955, dalapon has been shown to be useful in
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TABLE I
DALAPON FEEDING STUDY RESULTS

Species	Sex	gm/kg of acid equivalent LD_{50}	19/20 Confidence limits
Rat	Male	8.12	7.36 to 8.95
Rat	Female	6.59	5.99 to 7.26
Mouse	Female	>4.0	—
Guinea pig	Female	3.36	2.40 to 4.72
Rabbit	Female	3.36	2.00 to 5.65
Chick	Mixed	5.66	—
Cattle	Steers	>4.0	—

agricultural applications for the control of noxious grasses in a wide variety of commercial crops. The tolerance of many crops to dalapon has now been established.

F. PHYSICAL PROPERTIES

Melting point; 193–197°C.

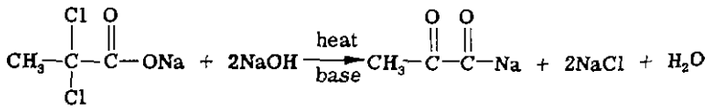
Solubility; Sodium α,α-dichloropropionate is soluble in water. The sodium salt is not readily soluble in most common solvents except alcohols, e.g.; ethanol, 18.5 gm/100 gm; methanol, 82.7/100 gm.

Specific gravity (25°/25°) and pH of water solutions:

	Sp. Gr.	pH
1%	1.0049	5.7
10%	1.0547	5.5
20%	1.1145	5.6
50%	1.2978	6.0

G. CHEMICAL PROPERTIES

In alkali solutions and temperatures higher than 120°C, α-dehalogenation occurs according to the following equation:



This reaction will also occur at lower temperatures. The rate of reaction is markedly affected by temperature and alkalinity.

II. RESIDUE ANALYSIS

A. Review of Methods

Various methods have been developed by The Dow Chemical Company for the analysis of dalapon in a wide variety of crops. Most of these procedures are modifications of the original method developed for the determination of dalapon in sugar cane (Smith *et al.*, 1957). The method consisted of three main steps. Dalapon was extracted from the plant with water; or, in the case of sugar cane, the juice was expelled by a commercial operation. Dalapon was then separated from interfering substances by solvent extraction, reverse-phase column chromatography, and heavy metal precipitation of interfering materials. It was then converted to pyruvic acid which was in turn determined by the dinitrophenylhydrazine method (Friedemann and Haugen, 1943).

The original procedure (Smith *et al.*, 1957) has been modified for use with various crops by using alcohol or water solutions containing protein-precipitating agents for the initial extraction of the Dalapon.

The major modification which has been introduced has been the use of a Dowex-1 ion exchange column to further separate pyruvic acid, formed by the hydrolysis of dalapon, from other interfering substances.

Most biological materials contain keto acids or related compounds which will interfere with the colorimetric procedure used for the determination of pyruvic acid. Dalapon is isolated from some of these interfering substances by the extraction procedure, reverse-phase chromatography and the copper-lime precipitation technique. Some of the interfering substances are however carried through the procedure. These must be removed by ion exchange chromatography and solvent distribution of the dinitrophenylhydrazones.

B. Recommended Method

1. PRINCIPLE

This method is applicable to the determinatiou of 0.1 p.p.m. or more of dalapon in raw sugar cane juice using 250 to 500-gm samples.

Dalapon is separated from naturally occurring interfering substances by means of solvent extraction, chromatography, and heavy metal precipitation. It is then hydrolyzed to pyruvic acid and the pyruvic acid detected as the 2,4-dinitrophenylhydrazone (Friedemann and Haugen, 1943).

2. REAGENTS

Ethyl ether, C.P. The ether should be tested with the dinitrophenylhydrazine reagent to be sure that it is free of interfering substances.

Hydrochloric acid, C.P.

Sodium hydroxide, 5 *N*.

2,4-Dinitrophenylhydrazine reagent. Dissolve 200 mg of 2,4-dinitrophenylhydrazine (Eastman white label) in 100 ml of 2 *N* HCl. Shake until completely dissolved; filter and store in a refrigerator when not in use.

Benzyl alcohol, C. P.

Hy-Flo Super-Cel, siliconized. Heat 5 kg of Johns-Manville Hy-Flo Super-Cel overnight in an oven at 100°C. Cool and add to 15 liters of 60–70°C Skelly Solve, containing 100 ml of dimethyldichlorosilane Lot. No. T-352 (Dow-Corning Corporation). Shake the slurry for several minutes, then partially evacuate the container to remove air bubbles from the slurry. Repeat this operation several times. Remove the solvent by filtration and wash the filter cake several times with fresh solvent. Dry the filter cake on a steam bath until fairly free of solvent. Heat overnight in an oven at 100°C. Store in a closed bottle when not in use.

Hydrochloric acid, 0.1 *N* saturated with benzyl alcohol.

Distilled water, saturated with benzyl alcohol.

Copper sulfate, 20%.

Calcium hydroxide, C.P.

Toluene, C.P.

Sodium carbonate, 10% solution filtered to remove insoluble materials.

Sodium bicarbonate, C.P.

3. APPARATUS

Continuous ether liquid-liquid extractor (Fig. 1).

Chromatographic columns 24 × 350-mm.

Volumetric flasks, 10, 25, and 50-ml.

Centrifuge.

Centrifuge tubes, 15 and 50-ml.

Erlenmeyer flasks, 125-ml.

Transfer pipettes, 1, 2, 3, 5, and 10-ml.

Autoclave.

Separatory funnels, 60-ml.

Beckman Spectrophotometer.

Ion exchange tube (Fig. 2).

Gradient elution equipment (Fig. 3).

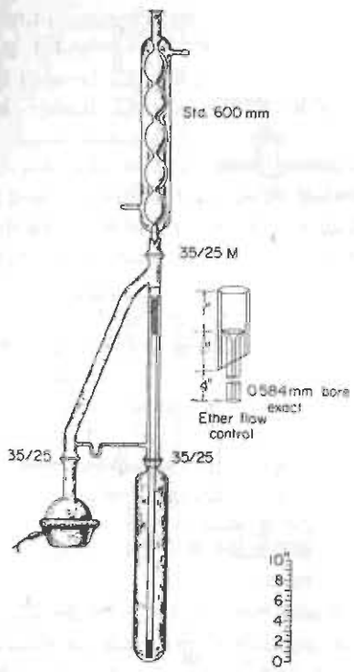


FIG. 1. Continuous ether extractor (Smith *et al.*, 1957).

4. EXPERIMENTAL PROCEDURE

a. *Sample Preparation*

Prepare duplicate samples for analysis according to the following procedure:

To a 250 to 500-gm sample of raw sugar cane juice expressed from cane in a standard sugar mill, add 15 ml of conc. HCl and sufficient distilled water to dilute the sample to approximately 1500 ml. The solution should be acid to Congo Red paper.

Transfer the solution to the continuous extraction apparatus. Assemble the apparatus using a 250-ml distillation flask.

Add sufficient ethyl ether to the system to permit a continuous operation and so that approximately 150 ml of ether will be in the distillation flask. Boiling chips should be added to the distillation flask to prevent bumping.

Extract the solution for 8 hours at a constant rate. Transfer the ether from the distillation flask to a 500-ml separatory funnel. Extract

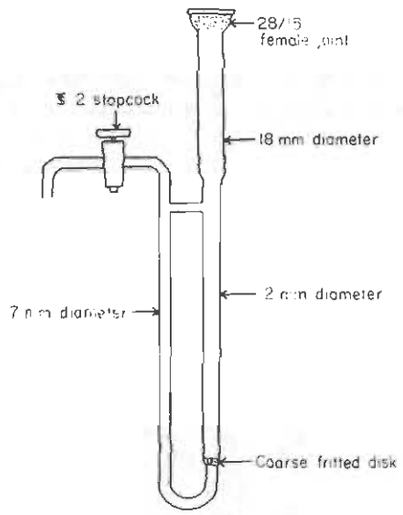


FIG. 2. Ion exchange column.

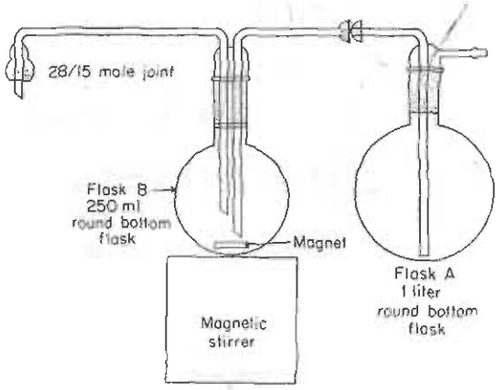


FIG. 3. Gradient elution equipment.

the ether solution once with 10 ml of 5 *N* NaOH and once with 5 ml of distilled water (see Note 1).

Combine the water phases and make the solution acid to Congo Red paper by the addition of conc. HCl. Add 15 ml of 2,4-dinitrophenylhydrazine reagent, shake, and allow to stand at room temperature for 1 hour.

Prepare a chromatographic column as described below. Transfer

the solution to the chromatographic column and allow it to pass slowly into the column, using a slightly positive air pressure for this operation. After the solution has entered the column, introduce 75 ml of 0.1 *N* HCl saturated with benzyl alcohol. This should be added in small aliquots of 10 to 20 ml.

Follow the acid solution with approximately 125 ml of distilled water saturated with benzyl alcohol. As soon as the water is added to the column, the effluent from the column is collected in 10 ml fractions. Ten fractions are collected (see Note 2). Each fraction is analyzed as follows:

To each fraction, add 0.5 ml of 20% $CuSO_4$ solution and 0.5 gm of $Ca(OH)_2$. Shake vigorously for 1 minute and allow to stand for 15 minutes with occasional shaking.

The precipitate is removed by centrifuging; and is washed twice with 10 ml of distilled water.

The original solution and the two wash portions are combined and placed in a 125-ml Erlenmeyer flask. Add conc. HCl nntil the solution is slightly acidic. Solid sodium bicarbonate is added until a small quantity of solid bicarbonate remains. Cover each flask with aluminum foil.

At this point, the fractions from each duplicate set of samples are divided into two sets. The fractions from one sample are nsed to estimate tissue blank and are not hydrolyzed. The other fractions are used to estimate combined tissue blank and 2,2-dichloropropionic acid.

The fractions that are to be hydrolyzed are placed in an autoclave and heated for 50 minutes at 120–125°C (15 psi), then cooled. All fractions are then analyzed according to the following procedure:

Concentrated hydrochloric acid is added to each solution until it is acidic to Congo Red paper. Transfer the solutions to 60-ml separatory funnels, using a small amount of distilled water to insure complete transfer. Two separatory fnnnels must be provided for each solution.

To each solution, add 2 ml of 2,4-dinitrophenylhydrazine reagent, mix thoroughly, and allow to stand for 5 minutes at room temperature. Add 10 ml of toluene to each solution and shake vigorously for 1 minute. Allow the layers to separate. Transfer the water phase to the second separatory funnel provided for each solntion. Save the toluene layer by allowing it to remain in the first separatory funnel.

Extract the water phase again with 10 ml of toluene by shaking vigorously for 1 minute. Allow the layers to separate, discard the water phase, and transfer the tolnene layer to the first separatory funnel.

Extract the combined toluene layers with 5 ml of 10% sodium carbouate solution and then with 3 ml of the same solution. Combine the sodium carbonate solutions and place in a 10-ml volumetric flask. Dilute

to volume with 5 *N* NaOH. Mix thoroughly and allow to stand for 10 minutes. Filter the solutions through Reeve Angel No. 804 12.5-cm fluted filters. Read the solutions in the Beckman Spectrophotometer at 440 mμ against distilled water.

The difference between the absorbance readings of the hydrolyzed and unhydrolyzed samples is proportional to the amount of 2,2-dichloropropionic acid present in the samples. This is estimated by referring to a standard curve prepared by using dilute solutions of 2,2-dichloropropionic acid.

b. *Preparation of Chromatographic Column*

Mix 20 gm of siliconized Super-Cel with 16 ml of benzyl alcohol (mix thoroughly). Add distilled water saturated with benzyl alcohol with mixing until a thin slurry is obtained. Transfer the slurry to a 500-ml filtering flask and evacuate until the slurry is largely free of air bubbles.

Introduce the slurry into a 24 × 350-mm chromatographic column and pack uniformly by use of air pressure and a packing rod. This operation is best accomplished by repeatedly allowing a portion of the liquid phase to pass from the column, then packing with the rod. The column should be rotated as it is packed so that the material in the column will have a uniform consistence.

c. *Standard Curve*

Weigh out several samples of 2,2-dichloropropionic acid and dilute with distilled water to give solutions ranging in conceutratious from 10 to 100 μg per milliliter of solution. Pipette 10-ml aliquots of each solution into 125-ml Erlenmeyer flasks and add 25 ml of distilled water. Add solid sodium bicarbonate until a small amount of solid bicarbonate remains.

Cover the flasks with aluminum foil and autoclave for 50 miuutes at 120–125°C (15 psi). After cooling, acidify the solutions with concentrated hydrochloric acid until acidic to Congo Red paper.

Add 2 ml of dinitrophenylhydrazine reagent. Allow the resulting solution to react for 5 minutes at room temperature. Extract this solution with two successive 10-ml portions of toluene. Combine the toluene layers and extract with one 5-ml portion and one 3-ml portion of 10% Na_2CO_3. Combine the sodium carbonate solutions and transfer them to a 10-ml volumetric flask. Dilute to volume with 5 *N* NaOH solution, mix, and filter if necessary. Fifteen minutes after addition of the sodium hydroxide, measure the absorption at 440 mμ against distilled water and correct for the reagent blank.

The data obtained from these analyses can be used to construct a standard curve which follows Beer's law.

NOTE 1: Sufficient sodium hydroxide should be used to insure that the extracting solution is basic after the acids in the ether solution have been neutralized.

NOTE 2: After becoming familiar with this procedure, it will be possible to discard all but the two or three samples containing the dichloropropionic acid.

C. MODIFICATION OF METHOD

In those methods in which an ion exchange column is employed, the following procedure is employed starting with the effluent from the reverse phase chromatographic column.

Transfer the effluent to a 250-ml Erlenmeyer flask, cover with aluminum foil, and hydrolyze in an autoclave for 50 minutes at 120°–125°C (15 psi). Cool and add enough distilled water to dissolve any excess benzyl alcohol.

Prepare an ion exchange column as follows: Add ion exchange resin to the ion exchange tube (Fig. 2) to make a packed column 130-mm high. To pack the column, nse sufficient air pressure to create a flow rate of 2.5 ml per minute. Wash the column with distilled water until the effluent is neutral to Hydrion wide-range paper (see Note 3).

Force the hydrolyzed effluent through the ion exchange resin with the same amount of pressure used to pack the column. Wash the column with 25 ml of distilled water. Discard the effluent and wash the water from this column. Add approximately 2 ml of distilled water to the top of the ion exchange column and carry out a gradient elution with hydrochloric acid, increasing the concentratiou from 0 to approaching 1 *N*. Flask A of the gradient elution equipment (Fig. 3) contains 1 *N* hydrochloric acid. Flask B contains 200 ml of distilled water and a magnetic stirring bar encased in polyethylene. Start the magnetic stirrer and apply pressure to flask A until the eluting liquid is about to enter the ion exchange tube, then attach the ion exchange tube to the elution equipment. Increase the pressure to obtain a flow rate of 2.5 ml per minute and collect 60 ml of effluent.

Transfer the effluent from the ion exchange column to a 125-ml separatory funnel and couple with 2 ml of 2,4-dinitrophenylhydrazine reagent for exactly 5 minutes at room temperature. Extract with two 10-ml portions of tolueue. Combine the tolueue extracts and extract with one 5-ml portion and one 2-ml portion of 10% sodium carbonate. Combine the sodium carbonate extracts in a 10-ml volumetric flask and dilute

to volume with 1.5 *N* NaOH. Mix thoroughly and allow to stand for 15 minutes. Filter the solution through a Reeve Angel No. 804 12.5 cm fluted filter paper. Measure the optical density against water at 440 mμ using a Beckman Model *DU* spectrophotometer and cuvettes with a 10-mm light path. Obtain the micrograms of 2,2-dichloropropionic acid corresponding to the experimental optical density from a standard curve.

NOTE 3: To prepare the ion exchange resin wash 200 gm of Dowex 1-X8, 200–400 mesh, with 1 liter of 1.5 *N* NaOH. Decant the NaOH solution and wash with 6 1-liter portions of distilled water. Soak in 1 liter of 3 *N* HCl overnight. Decant the HCl and wash with 3 1-liter portions of distilled water. Slurry with 750-ml distilled water and store in a stoppered bottle.

In most of the modifications of the original procedure (Smith *et al.*, 1957), either the copper-lime precipitation procedure or the ion-exchange column is used to decrease the tissue blank. The modifications used with various crops are shown in Table II.

TABLE II

SUMMARY OF BLANKS AND RECOVERIES OF DALAPON ADDED TO VARIOUS AGRICULTURAL COMMODITIES

Crop	Method Cu-Lime	Used ion exchange	Results blank (range)	Average recovery p.p.m.	Range recovery determined p.p.m.
Alfalfa hay	Yes	No	1–4	83%	0.4–120
Animal fat	No	Yes	0.3–1.3	92%	0.5–5
Animal muscle	No	Yes	0.7–1.5	80%	1–10
Apples	No	No	<1	>80%	0.5–5
Cottonseed	No	No	1–4	83%	5–20
Milk	No	No	0.04–0.05	99%	0.1–1.5
Oranges	Yes	No	0.1–0.3	88%	0.2–5.0
Pineapple	No	No	0.5	91%	0.5–5
Peas	Yes	No	1–3	90%	0.1–12
Potatoes	No	No	0.5–1.0	93%	0.5–10
Sugar cane (juice)	Yes	No	0.03–0.04	77%	0.1–1.0
Sugar beets	No	Yes	0.02–0.1	90%	0.1–1.5
Trefoil hay	Yes	No	2.0–4.5	88%	20–280

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Smith, G. N., Getzendaner, M. E., and Kutschinski, A. H. (1957). *J. Agr. Food Chem.* **5**, 675.

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DEF

D. MacDougall

$(CH_3CH_2CH_2CH_2S)_3P{=}O$

S,S,S-Tributyl phosphorotrithioate

I. GENERAL

A. Empirical Formula

$C_{12}H_{27}OS_3P$ (Mol. wt. 314.5).

B. Alternative Names

B-1776. The name DEF is the trademark of the Chemagro Corporation.

C. Source of Analytical Standard

Chemagro Corporation, Box 4913, Hawthorn Road, Kansas City 20, Missouri.

D. Biological Properties

DEF is a very effective defoliant for cotton. After an application of DEF at the rate of 1 to 2 lb of the active ingredient per acre, 90 to 95% defoliation is usually obtained in 5 to 7 days. The conditions which appear to favor defoliation are: (a) nearly depleted nutrient and moisture supply, (b) warm temperatures ranging between 60° and 90°F, (c) adequate moisture in the air, (d) application of the chemical when the plants are physiologically mature and the leaves are ready to abscisss, and (e) complete spray coverage. DEF has also been found to be effective for the defoliation of some ornamentals such as roses.

The acute oral LD_{50} of DEF active ingredient is 325 mg/kg to female rats. The acute dermal LD_{50} of DEF to rats is in the vicinity of 1,000 mg/kg. Rats exposed to air concentrations of DEF of up to 3,804 µg/liter did not die after an exposure period of 60 minutes.

E. History

DEF is a defoliant developed by the Ethyl Corporation and the Pittsburgh Coke and Chemical Company. It is manufactured by Chem-
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agro Corporation and sold under licenses from the Pittsburgh Coke and Chemical Company and the Phillips Petroleum Company. The method for defoliation, employing DEF as the principle active ingredient, is described and protected by U. S. Patents 2,841,486 and 2,965,467. A process for preparing DEF active ingredient is protected by U. S. Patent 2,943,107.

F. Physical Properties

Colorless to pale yellow liquid.
Melting point; less than −25°C.
Boiling point; 150°C at 0.3 mm pressure.
Specific gravity; 1.057 at 20°C.
Solubility; DEF is soluble in aliphatic, aromatic, and chlorinated hydrocarbon solvents as well as in alcohols.

G. Chemical Properties

1. method of synthesis

DEF is prepared by reacting phosphorus trichloride with butyl mercaptan to form *S,S,S*-tributyl phosphorotrithioite. This product is then oxidized to phosphorotrithioate. The chemical reactions involved are shown below.

$$PCl_3 + 3C_4H_9SH \rightarrow (C_4H_9S)_3P + 3HCl$$

$$(C_4H_9S)_3P + \text{oxygen (air)} \rightarrow \underset{\text{DEF}}{(C_4H_9)_3PO}$$

2. chemical reactions

DEF is relatively stable to both acids and heat. It hydrolyzes slowly under alkaline conditions.

H. Formulations

DEF is marketed as a spray concentrate formulation containing 6 lb of active ingredient per gallon, or as a dust containing 7.5% of the active ingredient.

II. ANALYSIS

A. Formulation Analysis

1. review of methods

The most satisfactory method for the analysis of DEF formulations is by means of infrared absorption. The DEF determination is based

on the absorption due to the P=O group at 8.33 μ. For the analysis of spray concentrate formulations, the emulsifying agents must be removed since they interfere with the infrared absorption pattern. This is accomplished by passing the formulation through a column of acid-washed alumina. The DEF is eluted from the column with carbon tetrachloride. For the analysis of 7.5% dusts, an extraction is carried out in the Bailey-Walker extractor with carbon tetrachloride for 16 hours. After the extraction or clean-up, the final infrared spectrophotometric determination is the same for both formulations.

2. RECOMMENDED METHOD

a. *Principle*

The method is based on the absorbance of the P=O group at 8.33 μ.

b. *Reagents*

Alumina. Acid-washed, standardized as described below.

Carbon tetrachloride, Reagent Grade.

DEF. Analytical standard, obtained from Chemagro Corporation.

DEF. Spray concentrate standard, obtained from Chemagro Corporation.

c. *Apparatus*

Bailey-Walker extractors.

Chromatography tube. 20-mm diameter × 200-mm length, fitted with a coarse sintered-glass disk.

Infrared spectrophotometer.

Water bath, thermostated to 25°C.

d. *Experimental Procedure*

i. *Sample Preparation.*

For the analysis of DEF spray concentrate, it is necessary to prepare a column of acid-wasbed alumina. For this purpose weigh out 10 gm of acid-washed alumina. Pour this into a chromatography column which has been previously filled one-quarter to one-half full with carbon tetrachloride. Allow the alumina to settle and the excess carbon tetrachloride to drain tbrough the column. When the level of the carbon tetrachloride drops to within a few millimeters of the surface of the alumina, discard the carbon tetrachloride and place a 100-ml volumetric flask under the column. Pipette 5 ml of the spray concentrate onto the top of the alumina, being careful not to disturb the surface. Record the

temperature of the sample in degrees centigrade. After the level of the liquid has dropped to the surface of the alumina, elute three times with successive 30-ml portions of carbon tetrachloride. Place the volumetric flask, containing the eluent, iu a water bath at 25°C for 15 minutes. Dilute to volume with carbon tetrachloride at 25°C. Mix thoroughly.

For the analysis of 7.5% DEF dusts, extract 10 gm of the dust in the Bailey-Walker extractor with 40 to 45 ml of carbon tetrachloride for 16 hours. Transfer the extract to a 50-ml glass-stoppered volumetric flask. Rinse with 5 ml of carbon tetrachloride. Place the flask in a water bath at 25°C for 15 minutes and dilute to volume with carbon tetrachloride at 25°C. Mix thoroughly.

The directions given for the infrared absorbance measurement of DEF solutions apply to the Perkin-Elmer Model 21 infrared spectrophotometer. Fill a 0.15-mm sodium chloride infrared absorption cell with the carbon tetrachloride solution containing DEF. Place this in the sample beam. Fill a variable-path length infrared absorption cell with carbon tetrachloride. Set this cell to the same thickness as the cell in the sample beam and place it in the reference beam. Set the slit width to 175 μ, the response to 1, auto suppression to 0. Adjust the gain to its optimum setting at 8.33 μ. Close the shutter slowly and accurately set the pen to the 0 transmission line. At 8.0 μ, adjust the 100% adjustment to give between 95% and 100% transmission. Run the spectrum from 7.9 to 8.9 μ in about 1.5 minutes. Draw a base line between the minima at 8.0 and 8.8 μ. Measure the height of the 8.33 μ peak above this base line in absorbance units. Let this value = A_{sample}.

In analyzing spray concentrate formulations, re-run the determination using the standard DEF spray concentrate formulation obtained from the Chemagro Corporation. The concentration of DEF in the unknown is calculated from comparison with a standard spray concentrate.

In analyzing the DEF dust formulations, weigh out two 0.35-gm samples of the analytical standard DEF. Place these in 10-ml volumetric flasks. Dilute to volume with carbon tetrachloride at 25°C. Mix thoroughly. Determine the infrared absorbance on these solutions as instructed above. Determine the concentration of DEF in the unknown by comparison with the absorbance of the standard.

ii. *Calculations.*

Let A_{std} = absorbance of the standard spray concentrate or the standard DEF depending upon whether spray concentrate or dust is being analyzed.

Calculate K value as shown below:

$$K = \left(\frac{A_{\text{std. No. 1}}}{\text{Wt. Std. No. 1}} + \frac{A_{\text{std. No. 2}}}{\text{Wt. Std. No. 2}} \right) \Big/ 2$$

$$\% \text{ DEF in sample} = \frac{A_{\text{sample}} \times 100}{K \times \text{Wt. of sample}}$$

$$\text{Pounds of DEF/gallon at } 20^\circ\text{C} = \frac{(A_{\text{sample}} \times 1669)[1.000 + D(T - 20)]}{K}$$

where D = coefficient of cubical expansion = 0.0013
T = temperature at which original samples were measured, degrees C

3. DISCUSSION OF METHOD

The infrared spectrophotometric procedure will be interfered with by any other compound which contains a P=O group or other groups which absorb at 8.33 μ. However, practical experience in analyzing DEF over a period of several years has shown that problems of this sort are minimal. The standard deviation of the method is slightly more than 1% of the measured value.

A method of residue analysis for DEF is now under study.

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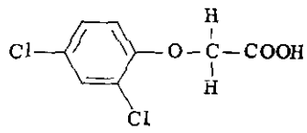
11

2,4-Dichlorophenoxyacetic Acid

R. P. Marquardt, H. P. Burchfield, E. E. Storrs, and Arthur Bevenue[1]



I. GENERAL

A. Empirical Formula

$C_8H_6O_3Cl_2$ (Mol. wt. 221.04).

B. Alternate Name

2,4-D acid.

C. Source of Analytical Standard

Bioproducts Center, The Dow Chemical Company, Midland, Michigan.

D. Biological Properties

2,4-Dichlorophenoxyacetic acid and related compounds have such outstanding and unusual herbicidal properties that they have become some of the leading synthetic plant hormones. By selective action they are highly toxic to most broad-leaved plants and relatively nontoxic to monocotyledonous plants; thus they are frequently used as weed killers. These phenoxy acids are also important for such selective uses as the prevention of preharvest fruit drop, the production of seedless fruit, and the regulation of the growth of plants.

E. History

Shortly after Pokorny (1941) reported the preparation of 2,4-dichlorophenoxyacetic acid, investigators in England and at the

[1] Sections I and I,A are by R. P. Marquardt; II,B by H. P. Burchfield and E. E. Storrs; and II,C by Arthur Bevenue.

Boyce Thompson Institute for Plant Research in the United States proved the growth-regulating properties of the aryloxyalkylcarboxylate series of componnds. In 1942, Zimmerman and Hitchcock first described the use of 2,4-dichlorophenoxyacetic acid as a plant growth regnlator. Many of the discoveries which resnlted from the research on these compounds during the war years were not published until after the war because of their military value.

Since the war, the manufacture of 2,4-dichlorophenoxyacetic acid and related compounds and their use as weed killers and plant growth regulators have grown tremendously. Other widely nsed members of this family of herbicides are 2,4,5-trichlorophenoxyacetic acid (2,4,5-T acid), 2-(2,4,5-trichlorophenoxy)propionic acid (Silvex), 2-methyl-4-chlorophenoxyacetic acid (MCP acid), 4-chlorophenoxyacetic acid, and 2,4-dichlorophenoxyethylsulfuric acid (Sesone). 2,4-Dichlorophenoxyacetic acid especially is a multimillion pound per year chemical in manufacture and use. The amine salts and esters are generally used to obtain desired solubilities and volatilities.

F. Physical Properties

Melting point: 138°C (Pokorny, 1941); 139–140°C (uncorr.) (Synerholm and Zimmerman, 1945); 140–141°C (Zimmerman, 1943).

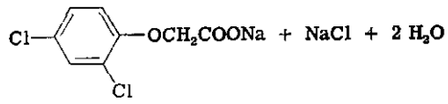
Solubilities (grams of 2,4-D acid/100 g solvent at 25°C). Data by Kaufman (1954):

Water	0.089
Ethanol	129.9
Ethyl ether	24.34
n-Heptane	0.111
Toluene	0.67
Xylene	0.58

White, crystalline material, stable and nonhygroscopic.

G. Preparation

2,4-Dichlorophenoxyacetic acid is nsually prepared by reacting 2,4-dichlorophenol and monochloroacetie acid in aqueous sodium hydroxide:



The related compounds are prepared in a similar manner.

II. RESIDUE ANALYSIS

A. Colorimetric Analysis of 2,4-D Acid and Related Compounds

1. REVIEW OF METHODS

A bioassay method involving the use of plants has been reported (Swanson, 1946). Two methods measuring the ultraviolet absorbancy of 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid have been used by Warshowsky and Schantz (1950) and by Gordon and Beroza (1952); however, these methods were not capable of detecting microgram quantities of the two acids. A sensitive colorimetric test for phenoxyacetic acids reported by Freed (1948) involves heating the phenoxyacetic acids with chromotropic acid (4,5-dihydroxy-2,7-naphthalenedisulfonic acid) in concentrated sulfuric acid. A quantitative adaptation of this test was used by Marquardt and Luce for determining 2,4-dichlorophenoxyacetic acid in milk (1951) and in grain and seed (1955); however, these two methods were limited to the determination of phenoxyacetic acids (phenoxypropionic acids do not give a color test with chromotropic acid) and occasionally gave high blanks when interfering materials were difficult to remove. Finally, a basic analytical scheme involving cleavage with pyridine hydrochloride of the ether linkage common to the phenoxy acids was developed by Marquardt and Luce (1961).

Pyridine hydrochloride is an acidic onium salt, especially in the fused state. Prey (1941) showed that the molten compound cleaves the phenyl ethers. It was found that with the same conditions pyridine hydrochloride cleaves the phenoxy acids. The phenol derivatives liberated by the cleavage are determined colorimetrically as measures of the original phenoxy acids.

2. DETERMINATION OF 2,4-DICHLOROPHENOXYACETIC ACID IN SUGAR CANE JUICE

a. *Principle*

The 2,4-D acid is extracted from a sample of sugar cane juice with chloroform. After suitable clean-up, cleavage of the ether linkage in 2,4-

dichlorophenoxyacetic acid with pyridine hydrochloride produces 2,4-dichlorophenol, which is determined colorimetrically as a measure of the original compound.

b. *Reagents*

Filter-aid. Hyflo Super-Cel, a Celite product, diatomaceous silica (Johns-Manville Co.).

Phosphotungstic acid solution. Dissolve 200 gm of phosphotungstic acid (approximately $P_2O_5 \cdot 24WO_3 \cdot xH_2O$) in water and dilute the solution to 500 ml. Disregard any insoluble material in the solution.

Hydrochloric acid, concentrated.

Hydrochloric acid, dilute. Dilute 60 ml of concentrated hydrochloric acid to 1 liter with water.

Chloroform, redistilled. Center 90% cut of technical grade chloroform.

Pyridine hydrochloride, practical grade.

Ammonium hydroxide, approximately 1 *N*. Dilute 70 ml of concentrated ammonium hydroxide (28%) to 1 liter with water.

Skellysolve F. A 35° to 60°C petroleum fraction (Skelly Oil Co.).

Ammonium hydroxide, approximately 0.05 *N*. Dilute 50 ml of 1 *N* ammonium hydroxide to 1 liter with water.

Buffer solution. Dissolve 300 gm of dibasic potassium hydroxide in water and dilute the solution to 1 liter. This solution should have a pH of 9.1 ± 0.1 (check with a pH meter). If necessary, adjust the pH by adding a small amount of monobasic potassium phosphate or tribasic potassium phosphate.

4-Aminoantipyrine, 1.0% solution. Dissolve 1.00 gm of 4-aminoantipyrine in water and dilute the solution to 100 ml. Store the reagent in an amber bottle in a dark place. Make a fresh solution weekly.

Potassium ferricyanide, 2.0% solution. Dissolve 2.00 gm of potassium ferricyanide in water and dilute the solution to 100 ml. Store the reagent in an amber bottle in a dark place. Make a fresh solution weekly.

2,4-Dichlorophenoxyacetic acid (2,4-D acid), commercial product assaying 99+%.

Methanol, A.C.S. grade.

c. *Apparatus*

Evaporative concentrator, Kuderna-Danish, with 500-ml upper flask and 25-ml lower flask (Fig. 1).

Peanut oil bath. Regulated to a temperature range of 207° to 210°C.

Wire holder. For suspending the 25-ml flask of the Kuderna-Danish evaporative concentrator in the peanut oil bath.

Steam distillation apparatus (Fig. 2).

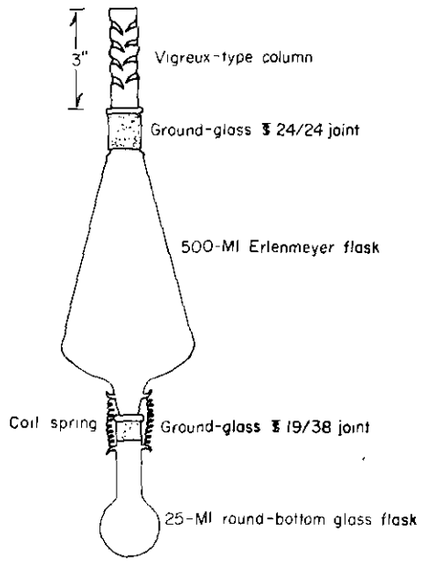


FIG. 1. Kuderna-Danish evaporative concentrator.

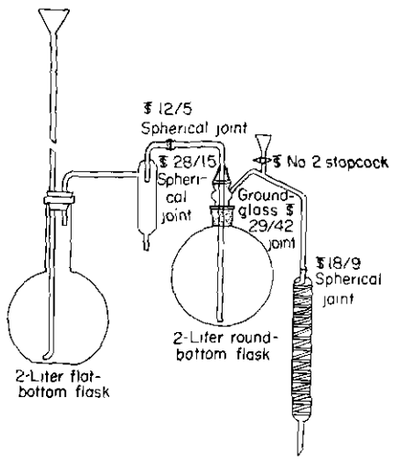


FIG. 2. Steam distillation apparatus.

Coleman spectrophotometer. Model 14, equipped with 4-cm absorption cells. Any photometer measuring light transmittance at 515 mμ should be suitable.

d. *Experimental Procedure*

i. *Sample Preparation and Procedure.*

Weigh 150 gm of sugar cane juice in a 250-ml beaker. Add 5.0 gm of filter-aid, 10 ml of phosphotungstic acid, and 10 ml of conc. HCl. Stir the mixture in the beaker occasionally for 15 minutes.

Filter the solids on a 7-cm Büchner funnel and wash with three 25-ml portions of dilute HCl. Discard the solids. Pour the combined solution of filtrate and washings into a 500-ml separatory funnel.

Place a few boiling chips in the Kuderna-Danish evaporative concentrator. Extract the 2,4-D acid from the solution with three 50-ml portions of chloroform. Combine the extract solutions in the concentrator and attach the Vigreux column.

Using a steam bath, evaporate the chloroform. When the liquid level is in the 25-ml flask of the concentrator, remove the Vigreux column and continue the evaporation. After all of the chloroform has evaporated, blow a gentle stream of air in the concentrator for 1 minute.

Disconnect the 25-ml flask from the concentrator and add 10 gm of pyridine hydrochloride to the residue. Using the wire holder, suspend the flask with contents in a peanut oil bath kept at a temperature of 207–210°C. After 10 minutes, swirl the contents of the flask to make a homogeneous solution and then keep the flask suspended in the oil bath for an additional 50 minutes. Cool the flask and contents to room temperature.

Dissolve the pyridine hydrochloride by filling the flask with water from a 150-ml portion of water. Add the aqueous solution to 150 ml of water and 10 ml of conc. HCl in the 2-liter round-bottomed flask of the steam distillation apparatus. Wash the 25-ml flask with the remainder of the 150-ml portion of water, adding the washings to the solution in the 2-liter flask. Steam-distill 500 ml of distillate into a 1-liter Erlenmeyer flask containing 25 ml of 1 *N* NH_4OH.

Pour the distillate into a 1-liter separatory funnel and wash it three times with 50-ml portions of Skellysolve. Discard the washings.

Acidify the washed distillate with 5 ml of conc. HCl. Extract the 2,4-dichlorophenol with three 50-ml portions of Skellysolve and combine the extract solutions in a 250-ml separatory funnel. Wash the solution with three 25-ml portions of water and discard the washings.

Extract the 2,4-dichlorophenol from the washed Skellysolve solution with one 10.0-ml and two 5.0-ml portions of 0.05 *N* NH_4OH and combine the extract solutions in a 50-ml volumetric flask. Add 20.0 ml of buffer solution (pH 9.1) and 1.0 ml of 1.0% 4-aminoantipyrine solution and mix well. Add 1.0 ml of 2.0% potassium ferricyanide solution and

again mix well. After 1 minute, dilute to volume with water and mix well. Fill a 4-cm absorption cell with the solution. Three minutes after the addition of the potassium ferricyanide, determine the absorbance with a spectrophotometer at 515 mμ, using water as a reference liquid.

Determine the micrograms of 2,4-D acid represented by the absorbance by referring to the standard calibration curve. Subtract any apparent 2,4-D acid found in the control sugar cane juice and correct for the per cent recovery of 2,4-D acid obtained from the juice.

Calculate parts per million of 2,4-D acid, as based on the weight of the juice sample:

$$\text{p.p.m. of 2,4-D acid} = \frac{\text{micrograms in sample}}{150}$$

ii. *Preparation of Standard Calibration Curve.*

Prepare standard solution I as follows: Dissolve 0.100 gm of 2,4-D acid in about 50 ml of methanol and dilute the solution with methanol to 1 liter. Concentration: 100 μg of 2,4-D acid per milliliter.

Prepare standard solution II by diluting 15.0 ml of standard solution I to 200 ml with methanol. Concentration: 7.5 μg of 2,4-D acid per milliliter.

Prepare standard solution III by diluting 30 ml of standard solution I to 200 ml with methanol. Concentration: 15 μg of 2,4-D acid per milliliter.

Pipette 0, 1.0, 2.0, 5.0, 10.0, 15.0, and *20.0* ml of standard solution *II* and 15.0 and 20.0 ml of standard solution III into respective 25-ml flasks of the Kuderna-Danish evaporative concentrator. Proceed with the known amount of 2,4-D acid in each flask as follows:

Add a few boiling chips and assemble the evaporative concentrator. Using a steam bath, evaporate the methanol. After all of the methanol has evaporated, blow a gentle stream of air in the concentrator for 1 minute.

Disconnect the 25-ml flask from the concentrator and add 10 gm of pyridine hydrochloride. Continue the determination as described in the procedure.

Prepare a standard calibration curve by plotting the data on graph paper. Beer's law is followed over the range from 0 to 300 μg of 2,4-D acid.

iii. *Recovery.*

Prepare standard solution A as follows: Dissolve 0.100 gm of 2,4-D acid in 25 ml of I *N* NH_4OH and dilute the solution with water to 1 liter. Concentration: 100 μg of 2,4-D acid per milliliter.

Prepare standard solution B by diluting 15.0 ml of standard solution A with water to 200 ml. Concentration: 7.5 μg of 2,4-D acid per milliliter.

Prepare standard solution C by diluting 30.0 ml of standard solution A with water to 200 ml. Concentration: 15.0 μg of 2,4-D acid per milliliter.

Weigh 150 gm of nontreated sugar cane juice in a 250-ml beaker. Using standard solution B or C, add a known amount of 2,4-D acid in the same range as for the standard calibration curve.

Continue the determination as directed in the procedure.

Determine the micrograms of 2,4-D acid represented by the absorbance reading by referring to the standard calibration curve and subtract any apparent 2,4-D acid content found in the control juice. Calculate the per cent recovery obtained.

Recovery data obtained from known amounts of 2,4-D acid added to nontreated sugar cane juice are shown in Table I. Analysis of the nontreated juice itself showed no apparent 2,4-D acid content.

TABLE I
RECOVERY OF 2,4-D ACID FROM SUGAR CANE JUICE

Added, p.p.m.	Found, p.p.m.	Recovery, %
0.050	0.0450	90
0.050	0.0425	85
0.10	0.100	100
0.10	0.100	100
0.25	0.220	88
0.25	0.222	89
0.50	0.460	92
0.50	0.460	92
0.75	0.692	92
0.75	0.702	94
1.00	0.919	92
1.00	0.914	91
1.00	0.943	94
1.50	1.485	99
1.50	1.354	90
2.00	1.984	99
2.00	1.907	95

3. DETERMINATION OF PROPYLENE GLYCOL BUTYL ETHER ESTER OF 2-(2,4,5-TRICHLOROPHENOXY)PROPIONIC ACID IN SUGAR CANE JUICE

a. *Principle*

The propylene glycol butyl ether ester of 2-(2,4,5-trichlorophenoxy)-propionic acid[2] is extracted from a sample of sugar cane juice with carbon

[2] The commercial product is the active ingredient in Kuron (registered trademark of the Dow Chemical Co.) herbicide formulations.

tetrachloride. After suitable clean-up, cleavage of the ether linkage in the ester with pyridine hydrochloride produces 2,4,5-trichlorophenol, which is determined colorimetrically as a measure of the original compound.

b. *Reagents*

The following reagents are in addition to, or supplant reagents already listed:

Methanol, dilute. Mix equal volumes of methanol and water.

Carbon tetrachloride, redistilled. Center 90% cut of technical grade carbon tetrachloride.

Buffer solution. Dissolve 200.0 gm of dibasic potassium phosphate and 20.0 gm of monobasic potassium phosphate in water and dilute the solution to 1 liter. This solution should have a pH of 7.8 ± 0.1 (check with a pH meter). If necessary, adjust the pH by adding a small amount of dibasic potassium phosphate or monobasic potassium phosphate.

4-Aminoantipyrine, 0.3% solution. Dissolve 0.30 gm of 4-aminoantipyrine in water and dilute the solution to 100 ml. Store the reagent in an amber bottle in a dark place. Make a fresh solution weekly.

Potassium ferricyanide, 1.0% solution. Dissolve 1.0 gm of potassium ferricyanide in water and dilute the solution to 100 ml. Store the reagent in an amber bottle in a dark place. Make a fresh solution weekly.

Propylene glycol butyl ether ester of 2-(2,4,5-trichlorophenoxy)propionic acid. May be obtained from Bioproducts Center, The Dow Chemical Company, Midland, Michigan.

c. *Apparatus*

Same as already listed.

d. *Experimental Procedure*

i. *Sample Preparation and Procedure.*

Weigh 150 gm of sugar cane juice in a 400-ml beaker. Add 150 ml of methanol, 10 gm of filter-aid, and 10 ml of phosphotungstic acid solution. Stir the mixture in the beaker occasionally for 30 minutes.

Filter the solids on a 7-cm Büchner funnel and wash with three 25-ml portions of dilute methanol. Discard the solids. Pour the combined solution of filtrate and washings into a 1-liter separatory funnel. Add 150 ml of water and 10 ml of conc. HCl hydrochloric acid to the solution.

Place a few boiling chips in the Kuderna-Danish evaporative concentrator. Extract the propylene glycol butyl ether ester of 2-(2,4,5-trichlorophenoxy)propionic acid from the solution in the separatory funnel with three 50-ml portions of carbon tetrachloride. Combine the extract solutions in the concentrator and attach the Vigreux column.

Continue the determination as in the procedure for 2,4-D acid until ready to add the buffer solution.

Pipette 20.0 ml of buffer solution (pH 7.8) and 1.0 ml of 0.3% 4-aminoantipyrine solution into the 150-ml volumetric flask and mix the solution well. Add 1.0 ml of 1% potassium ferricyanide solution and again mix well. After 1 minute, dilute to volume with water and mix well. Fill a 4-cm absorption cell with the solution. Three minutes after the addition of the potassium ferricyanide, determine the absorbance with a spectrophotometer at 505 mμ, using water as a reference liquid.

Determine the micrograms of propylene glycol butyl ether ester of 2-(2,4,5-trichlorophenoxy)propionic acid represented by the absorbance reading by referring to a standard calibration curve. Subtract any apparent ester found in the sugar cane juice and correct for the percent recovery of the ester obtained from the juice. Calculate parts per million, as based on the weight of the juice sample.

ii. *Preparation of Standard Calibration Curve.*

Prepare standard solutions of propylene glycol butyl ether ester of 2-(2,4,5-trichlorophenoxy)propionic acid with methanol in the same way as described for 2,4-D acid.

Obtain the absorption data on known amounts of the ester in the same manner as described for 2,4-D acid but using the procedure for the ester.

Prepare a standard calibration curve by plotting the data on graph paper. Beer's law is followed in the range from 0 to 300 μg of the propylene glycol butyl ether ester of 2-(2,4,5-trichlorophenoxy)propionic acid.

iii. *Recovery of Propylene Glycol Butyl Ether Ester of 2-(2,4,5-Trichlorophenoxy)propionic Acid Added to Sugar Cane Juice.*

Weigh 150 gm of sugar cane juice in a 400-ml beaker. Using the standard methanol solutions already prepared, add a known amount of propylene glycol butyl ether ester of 2-(2,4,5-trichlorophenoxy)propionic acid in the same range as for the staudard calibration curve.

Continue with the determination as described in the procedure for the ester.

Calculate the percent recovery in the same manner as with 2,4-D acid in sugar cane juice.

Recovery data obtained by the authors are shown in Table II. Analysis of uontreated juice showed an apparent ester content of 0.028 p.p.m.

TABLE II

RECOVERY OF PROPYLENE GLYCOL BUTYL ETHER ESTER OF 2-(2,4,5-TRICHLOROPHENOXY)PROPIONIC ACID FROM SUGAR CANE JUICE

Added, p.p.m.	*Found, p.p.m.*	*Recovery, %*
0.050	0.033	66
0.050	0.047	94
0.10	0.087	87
0.10	0.082	82
0.25	0.224	90
0.25	0.247	99
0.50	0.461	92
0.50	0.492	98
0.75	0.651	87
0.75	0.618	82
1.00	0.839	84
1.00	0.879	88
1.50	1.320	88
1.50	1.356	90
2.00	1.834	92
2.00	1.834	92

4. APPLICABILITY OF THE BASIC ANALYTICAL PROCEDURE TO DIFFERENT AGRICULTURAL PRODUCTS

The basic analytical scheme, in which the respective phenols produced by cleavage of the phenoxy acids with pyridine hydrochloride are determined colorimetrically as measures of the original herbicides, is generally applicable to all types of agricultural materials. Extraction and clean-up procedures will have to be modified, of course, and colorimetric or other methods adapted for the phenols involved.

5. DISCUSSION

Chloroform is used in the procedure for 2,4-dichlorophenoxyacetic acid because carbon tetrachloride, employed in the same manner, does not extract this acid quantitatively.

Carbon tetrachloride is used in the procedure for propylene glycol butyl ether ester of 2-(2,4,5-trichlorophenoxy)propionic acid because it quantitatively extracts 2-(2,4,5-trichlorophenoxy)propionic acid yet extracts less of other materials along with it. However, chloroform can be used if on occasion it should be the preferred solvent.

Since pyridine hydrochloride is very hygroscopic, do not expose it to the air more than necessary. However, avoid desiccating the com-

pound, for the small amount of water already present appears to promote proper cleavage of the phenoxy acids.

B. Gas Chromatographic Analysis of 2,4-D in Milk Using Internal Standards

1. RECOMMENDED METHOD

a. *Principle*

In this method (Storrs and Burchfield, 1961) milk is acidified and hydrolyzed enzymatically with pepsin to degrade the proteins. The 2,4-D and lipoidal materials are then removed by liquid-liquid extraction with diethyl ether. The 2,4-D is separated from the lipids by partition between hexane and acetonitrile; the 2,4-D appears in the acetonitrile phase. The solvent is evaporated, and the residue is methylated with BF_3-methanol (Metcalfe and Schmitz, 1961) or diazomethane.

The residue obtained after methylation is then injected into a gas chromatograph equipped with a 6-foot silicone column operated at 201°C. The methyl ester is eluted in about 6 minutes. Known amounts of 4-chlorophenoxyacetic acid and 2-chloro-4-bromophenoxyacetic acid are added to the milk immediately before extraction to serve as internal standards (Storrs and Burchfield, 1962). This permits calculation of the amount of 2,4-D in the milk from peak-area ratios, even though all of the steps in preparing the sample for chromatography are not quantitative. The background from milk constituents, which are not completely removed during the clean-up step, is substantially eliminated by using a microcoulometric detector which is selectively sensitive to halogen (Coulson *et al.*, 1960) (see also Volume I, Chapter 9).

b. *Reagents*

4-Chlorophenoxyacetic acid used as an internal standard can be obtained from Eastman. 2-Cbloro-4-bromophenoxyacetic acid is synthesized from 4-bromophenoxyacetic acid by chlorination with sodium hypochlorite using the general method of Hopkins and Chisholm (1946). BF_3-methanol reagent (Applied Science Laboratories, Inc.) is used to esterify the acids. Pepsin N.F. Powder (Merck and Co.) is used to hydrolyze the milk prior to extraction. The magnesium sulfate used to dry the ether extracts is washed with an ether solution of *p*-toluenesulfonic acid and then with dry ether until neutral. It is dried and stored in a tightly capped bottle until used.

c. *Apparatus*

Milk is hydrolyzed in 1-liter Erlenmeyer flasks on a Gyrotary shaker (New Brunswick Scientific Co.). All-glass liquid-liquid extractors equipped with individual Glas-Col heaters and powerstats are used for the extraction of milk. Five hundred-milliliter separatory funnels are used for partition of extracts between hexane and acetonitrile, and 200-ml Erlenmeyer flasks for evaporation of the solvents and for methylation of acids. Extractions of the esterified residues are carried out in 300-ml separatory funnels, and 200-ml Erlenmeyer flasks are then used for drying the ether extracts and evaporation of the solvent. A small tube drawn to a closed capillary tip is used for the final evaporation of the solvent and concentration of the residne.

A gas chromatograph equipped with a microcoulometric titration cell for the detection of halides (Dohrmann Instrument Co., Model C-100) is used for the separation and quantitative analysis of the esters of 2,4-D and the internal standards.

d. *Experimental Procedure*

i. *Sample Preparation.*

A sample of milk (350 ml) which has been frozen and then warmed to room temperature is placed in a 1-liter Erlenmeyer flask. 2-Chloro-4-bromophenoxyacetic acid (70 μg) and 4-chlorophenoxyacetic acid (35 μg) are added as internal standards. Concentrated HCl (4.5 ml) is added while swirling the flask, followed by 2 gm of pepsin. The flask is swirled until all of the pepsin dissolves. The mouth is then covered with a film of plastic such as Saran wrap, and the flask is placed on a rotary shaker adjusted to a slow speed for 15 hours at 40°C. After digestion is complete anhydrous sodium sulfate (50 gm) is added, and the sample is boiled for 2 minutes and cooled to room temperature. It is then extracted with diethyl ether in a liquid-liquid extractor for 20 hours. At the end of the extraction period, the ether layer is dried over magnesium sulfate (10–15 gm). The ether extract is then filtered through Whatman No. 2 filter paper and the solvent evaporated under a stream of clean, dry nitrogen with the flask warmed over a water bath at 65°C. The residue is taken up in four 25-ml portions of hexane and added to a 500-ml separatory funnel. The flask is then rinsed with four 25-ml portions of acetonitrile which is also added to the funnel. The milk extract is partitioned between the two layers by shaking vigorously. When the phases separate, the hexane is drawn off with an aspirator. The acetonitrile

solution is extracted with three 50-ml portions of hexane, and the upper layers are drawn off and discarded. The acetonitrile layer is shaken vigorously with a fourth portion of hexane (50 ml), and the lower layer transferred quantitatively to a 200-ml Erlenmeyer flask. The acetonitrile is evaporated slowly to complete dryness under a gentle stream of clean, dry nitrogen while warming the flask in a water bath at 85 to 90°C.

The flask is cooled to room temperature and a small amount of acetonitrile (1–2 ml) is added. The flask is swirled to dissolve the residue, diethyl ether (10 ml) is added and the solution filtered through Whatman No. 2 filter paper into a 125-ml Erlenmeyer flask. The flask is rinsed with three 25-ml portions of ether, and the combined filtrate and washings are evaporated in a water bath at 85 to 90°C.

The residue is methylated by adding the BF_3-methanol reagent (5 ml) and boiling for 4 miuutes on a steam bath. The reaction mixture is quenched with distilled water (100 ml) and extracted with two successive portions of diethyl ether (100 ml, then 40 ml) in a separatory funnel. The ether extract is neutralized with $NaHCO_3$ and dried over magnesium sulfate. The dried extract is decanted quantitatively into another flask and evaporated slowly at room temperature to a small volume, then transferred qnantitatively to a small tube with a capillary tip. The remaining ether is evaporated, and the residue (20–50 μl) contained in the capillary tip is ready for injection into the chromatograph.

ii. *Chromatography.*

A Dohrmanu Instruments Co. chromatograph with the injection block at a temperature of 230°C and the column at a temperature of 201°C is used. The carrier gas is nitrogen with an inlet pressure of 20 psig and the outlet at atmospheric pressure. A 6-ft $\times$ ¼-in. O.D. aluminum column packed with 20% Dow-Corning High Vacuum silicone grease on acid-washed Chromosorb is used.

Oxygen is introduced into the combustion tube to burn organic compounds to CO_2 and H_2O, while halogen-containing compounds are burned to CO_2, H_2O, and HX. Some elemental halogen may be produced if insufficient hydrogen is present. Only one-half the detector response is obtained from elemental halogen as from HX, since hypohalide as well as halide are produced when the halogen reacts with water. To prevent this as much as possible, the oxygen is bubbled through water or 0.5 *M* ammonium hydroxide prior to entering the combustion tube to add a source of hydrogen to the gas stream.

A portion of the milk extract (5 to 20 μl) is injected into the chromatograph, and the effluent from the column is allowed to vent into the

atmosphere for 1 minute to elute volatile components. The vent valve is then closed and the sample passed through the combustion tube. Methyl 4-chlorophenoxyacetate is eluted first, followed by the methyl esters of 2,4-D and 2-chloro-4-bromophenoxyacetate. If 2,4,5-trichlorophenoxyacetic acid is present, the methyl ester is eluted after methyl 2-chloro-4-bromophenoxyacetate.

iii. *Interferences*

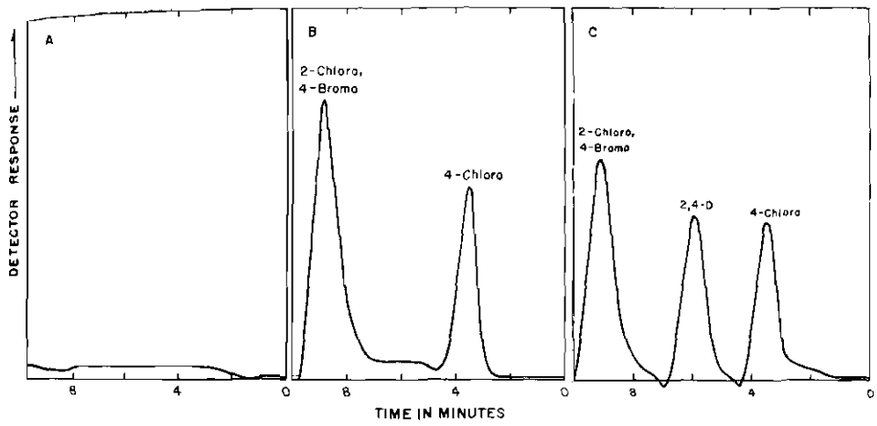


FIG. 3. Recovery of 2,4-D from milk using 4-chlorophenoxyacetic acid and 2-chloro-4-bromophenoxyacetic acid as internal standards. A, milk background without 2,4-D or standards. B, standards without 2,4-D added to milk. C, standards with 2,4-D added to milk.

The only interferences other than normal milk background (Fig. 3A) would be other halogen-containing compounds with the same retention volume as 2,4-D methyl ester.

iv. *Sensitivity.*

Using the procedure described above, as little as 0.01 p.p.m. of 2,4-D can be determined on chromatographing ¼ to ⅓ of the residue remaining on extraction of 350 ml of milk.

v. *Recovery and Calculations.*

Recovery need not be quantitative when an internal standard is added to the sample before extraction (Burchfield and Storrs, 1962). 2-Chloro-4-bromophenoxyacetic acid and 4-chlorophenoxyacetic acid are suitable standards for the analysis of 2,4-D, since the former compound emerges from the gas chromatograph just before 2,4-D and the latter

just after it. Moreover, these two compounds have chemical properties similar to those of 2,4-D and behave in a parallel manner during extraction, clean-up, and esterification. Thus, when the internal standards are added to milk free of 2,4-D, and the sample is processed and chromatographed, two peaks are obtained, one for each standard (Fig. 3B). However, when 2,4-D is present, a third peak appears (Fig. 3C). The amount of 2,4-D present in the milk can be calculated from the ratio of the area of this peak to that of either standard by the relation

$$p = (A/A_o)p_oR$$

Where p is parts per million of 2,4-D, p_o the parts per million of standard added to the milk, A the area of the 2,4-D peak, A_o the peak area of the standard, and R an empirical correction factor determined by adding a fixed amount of standard and various amounts of 2,4-D to uncontaminated milk. Either standard can be used, but a separate value for R must be determined for each.

The amount of 2,4-D in the milk can also be interpolated from a curve in which peak area ratios are plotted against micrograms of 2,4-D. The relationship obtained in the absence of milk is shown in Fig. 4.

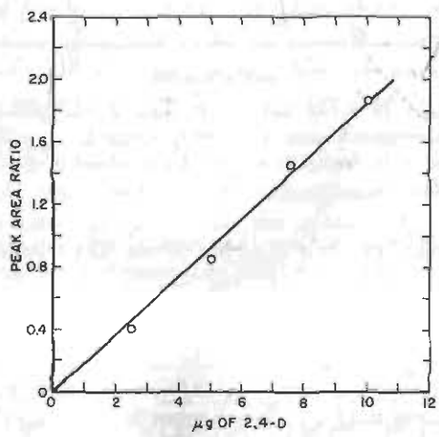


FIG. 4. Relation between micrograms of 2,4-D and peak area ratio using 2-chloro-4-bromophenoxyacetic acid as an internal standard in the absence of milk.

A linear relation is also obtained when 2,4-D and standard are extracted from milk in the range of 0 to 140 μg of 2,4-D in 350 ml of milk (Fig. 5).

The internal standard method is convenient, since it eliminates the need for quantitative recovery at each step and injection of the entire sample into the gas chromatograph. It also provides a safeguard against artifacts occurring during analysis, which might otherwise go undetected.

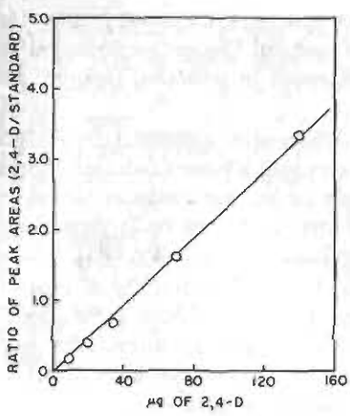


FIG. 5. Relation between micrograms of 2,4-D added to milk and peak area ratio using 70 µg of 2-chloro-4-bromophenoxyacetic acid per 350 ml of milk (0.2 p.p.m.) as an internal standard.

For example, analyses carried out on a series of putrified milk samples indicated that no 2,4-D was present. However, the standards could not be detected either, indicating that substances were present in the putrified milk which bound compounds of the 2,4-D type and prevented their extraction. Without the standards, it would have been concluded, perhaps erroneously, that no 2,4-D was present. The use of internal standards also provides a convenient check on mechanical errors during routine analyses. If the peaks representing them are much smaller than usual, or are missing from the chromatogram entirely, it may be possible that manipulative errors were made.

2. APPLICABILITY TO DIFFERENT FOOD CROPS

This particular method was devised for the analysis of milk. However, there is no reason why it could not be applied to other dairy products and crops, with appropriate changes in methods used for extraction. However, a separate calibration curve should be run for each food product in which a constant amount of standard and variable amounts of 2,4-D are added and recovered. This involves no more work than demonstrating recovery by conventional analytical techniques, since in this case also, the validity of the method must be established for each food product.

3. DISCUSSION OF METHOD

It seems probable that many of the difficulties encountered in pesticide residue analysis could be reduced by use of the internal stand-

ard method coupled with gas chromatography. Ideally, the standard should have physicochemical properties ideutical to those of the pesticide except for a difference in retention volume great enough to permit good peak resolution.

The following criteria are suggested in selecting a standard: (1) The *molecular structure and physicochemical properties* of the standard should approach those of the pesticide as closely as possible. (2) The retention volumes of the two compounds should be as close as possible while still permitting baseline resolution. (3) The experimentally determined value R should be as close to unity as possible. (4) The standard should not be a compound that is likely to be found as a major impurity in the pesticide. (5) The standard should not be another pesticide or have the same retention volume as known pesticides that might be carried through the analysis with it.

4. MODIFICATION OF METHODS

Where laboratory safety is not a problem, the methyl esters can be obtained in higher yield and in a shorter time by substitution of diazomethane for BF_3-methanol. Tbe residue after the final evaporation is taken up in ether containing 10% methanol (3 ml) and methylated by passing diazomethane through the solution for 20 minutes, as described in Burchfield and Storrs (1962). The solvent is evaporated and the residue is ready for injection into tbe gas chromatograph.

The amount of milk residue injected is quite large, so that the injection block becomes contaminated and the column overloaded with continued use. When the peaks become asymmetric, cleaning of the block and changing of the column is indicated. However, quantitative results, under otherwise unsatisfactory conditions, can be obtained when internal standards are used, since the ratios of peak areas remain constant even though their absolute magnitudes may vary from injection to injection. It is probable that column life could be prolonged by further clean-up of the milk extract by liquid-solid chromatography, but this has not been necessary in any of the work carried out to date.

C. GAS CHROMATOGRAPHIC ANALYSIS OF 2,4-D IN DRY CROPS

1. RECOMMENDED METHOD

a. *Principle*

In this method (Bevenue *et al.*, 1962) crops of low moisture content are partially rehydrated with water followed by extraction with an acidified organic solvent mixture. After several base-acid extractions the

2,4-D is treated with diazomethane to convert it to its corresponding methyl ester. The methyl ester is determined with a microcoulometric gas chromatograph by measuring the areas of the peaks, recorded on a strip chart recorder calculating by means of an equation derived from Coulomb's law. Because of the specificity of the microcoulometric detector, only halide components will be recorded on the chromatogram. The principle of gas chromatography and microcoulometric detection is described in Volume I, Chapter 9.

b. *Reagents*

Sulfuric acid solution, 10% (v/v).

Ethyl alcohol, 95%.

Ethyl ether.

Petroleum ether, 30–60°C, redistilled.

Benzene, redistilled.

Sodium bicarbonate, 3% aqueous solution (w/v).

Solvent mixture A: 10 ml 10% sulfuric acid + 15 ml 95% ethyl alcohol + 25 ml petroleum ether + 75 ml ethyl ether.

Solvent mixture B: Etbyl ether-petroleum ether (30–60°C) v/v.

Diazomethane. A 100-ml distilling flask is fitted with a dropping funnel and an efficient condenser set downward for distillation. The condenser is connected to two receiving flasks in series, the second of which contains 20–30 ml of ether. The inlet tube of the second receiver is dipped below the surface of the ether and both receivers are kept cooled in an ice bath. Add 5 gm of KOH to the distillation flask and dissolve in 8 ml of water. Add 25 ml of 95% ethyl alcohol to the KOH solution. Heat the flask containing the alkali solution to 65°C. Add through the dropping funnel over a period of about 25 minutes a solution of 7.0 gm of "Diazald" (*N*-methyl-*N*-nitroso-*p*-toluenesulfonamide, Aldrich Chemical Co., Milwaukee 10, Wis.) in about 130 ml of ethyl ether. The distillation rate should equal the rate of addition. When the dropping funnel is empty, slowly add 20 ml of ethyl ether and continue distillation until the condensate becomes colorless.

c. *Apparatus*

Dohrmann microcoulometer gas chromatograph, Model 100. Six-foot, ¼ inch O.D. aluminum or stainless steel spiral column containing 20% Dow-11 silicone grease on acid-washed Chromosorb P.

Wiley mill, equipped with a 2-mm screen.

Shaker, gyrotory (New Brunswick Scientific Co., New Brunswick, N. J.).

Buchler, batch model, evaporator.

d. *Experimental Procedure*

i. *Sample Preparation.*

Crop samples of low moisture content are ground in a Wiley mill to pass a 2-mm screen. Leafy or grasslike materials of higher moisture content are macerated by hand or in a food chopper. The meal or shredded material (25–50 gm) is transferred to a 500-ml Erlenmeyer flask, 25 ml of water are added and the contents mixed until the water is dispersed throughout the sample material. Solvent mixture A (150 ml) is added and the flask is agitated on a mechanical shaker for 1 hour. Grass-like materials will require 5 to 6-fold amounts of the solvent mixture A. Materials with high fat content may be preferentially mixed with the water and solvent mixture A in a Waring Blendor prior to the 1-hour shaking period.

The slurry is filtered through Whatman No. 1 filter paper on a Büchner funnel and the residue is washed twice with 20 to 25 ml aliquots of solvent mixture B. The filtrate and washings are combined. The filtrate is transferred to a separatory funnel, 75 ml of bicarbonate solution are added and the contents are carefully mixed. Two additional 50-ml amounts of bicarbonate solution are added, the flask is shaken vigorously, and the two phases are allowed to separate completely. The aqueous lower layer is retained. This solution is washed twice with 50-ml aliquots of petroleum ether and the ether phase discarded. Any flocculent precipitate present in the water phase may be removed by filtration through a plug of glass wool. The bicarbonate solution is acidified with 10% H_2SO_4 to about pH 3 and extracted with three 50-ml aliquots of ethyl ether. The ether layer is concentrated in a flash evaporator to about 10 ml and transferred to a 25-ml conical type centrifuge tube with benzene. One to two milliliters of diazomethane solution are added and the esterification is completed, usually within 10 minutes, as judged by the persistence of the yellow color due to excess diazomethane. The contents are transferred with benzene to a 6.5-ml calibrated hematocrit tube and, using a warm-air stream, the volume of the concentrate is reduced to a known amount (100–250 μl). Suitable aliquots of the sample are injected into the gas chromatograph apparatus.

ii. *Chromatography.*

Optimum chromatographic conditions are an injection block temperature of 250°C, column temperature 210°C, combustion zone temperature 825°C, and nitrogen and oxygen flow rates of 100 ml/minute.

The Dohrmann instrument is designed so that the chromatographic eluents are combusted to CO_2, H_2O, and halogen. A microcoulometer cell

connected to the combustion zone will determine the amount of halogen present by continuous automatic titration with internally generated silver ions. A permanent record of the results are produced on a strip chart recorder.

Sample sizes for injection into the gas chromatograph will vary from 10 μl to 50 μl (1–20 gm of original sample material) depending upon the amount of 2,4-D present in the sample. The amount of 2,4-D found in the sample is calculated by means of the following equation:

$$\text{Micrograms 2,4-D} = \frac{\text{(Peak area)(Recorder sensitivity)(35.5 gm/eq.)(60 sec/min)} \times (10^6)(10^{-3}V/\text{mv})(10^2)}{\text{(Sensitivity range, ohms)(\% Cl in 2,4-D)(96,500 coulombs/eq.)}}$$

Where the peak area is in square inches.

If the recorder sensitivity and chart speed each are unity, the above equation can be reduced to

$$\text{micrograms 2,4-D} = \frac{\text{(Area)(2210)}}{\text{(Ohms)(32.1\%)}}$$

For 1 to 5 μg of the 2,4-D methyl ester, optimum sensitivity is 64 ohms.

iii. *Interferences.*

Interferences will be limited to the possible presence of naturally occurring halides or halogen-containing pesticide contaminants having the same retention times as 2,4-D methyl ester.

iv. *Sensitivity.*

The lower limit of detection is 0.1 p.p.m. and may be as low as 0.05 p.p.m. if the background response is practically nil.

v. *Recovery.*

The average recovery from crop materials, such as lima beans, corn grain, corn and sorghum stovers, and walnuts will be about 80–85%. This can be determined by adding known amounts of 2,4-D to a plant material which is known to have no history of 2,4-D contamination. Gas chromatograph column efficiency for the methyl ester of 2,4-D was 95–105%.

2. DISCUSSION

With crops of low moisture content, it is important to rehydrate the sample material prior to extraction with the acidic organic solvent mixture; otherwise, 2,4-D recoveries will be very poor.

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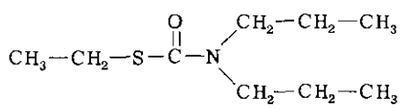
12

Eptam

G. G. Patchett, G. H. Batchelder, and J. J. Menn



Ethyl di-*n*-propylthiolcarbamate

I. GENERAL

A. Empirical Formula

$C_9H_{19}ONS$ (Mol. wt. 189).

B. Alternative Names

The Weed Society name is EPTC. Its former designation was Stauffer experimental compound R-1608. The name Eptam is the registered trademark of the Stauffer Chemical Company.

C. Source of Analytical Standard

Stauffer Chemical Company, Richmond Research Center, 1200 South 47th Street, Richmond, California.

D. Biological Properties

Eptam is a selective herbicide highly effective against a number of annual grasses and broadleaf weed species. A literature abstract of crop tolerance and weed susceptibility was compiled by Antognini (1959). Most susceptible weed species are controlled at a dosage of 3 pounds active per acre; a dosage of 6 pounds per acre may be used where more resistant weeds are present with a tolerant crop such as control of nutgrass (*Cyperus* spp.) in potatoes. Eptam is also effective in controlling certain perennial grasses such as Johnson grass (*Sorghum halepense*), Quack grass (*Agropyron repens*), and Bermuda grass (*Cynodon dactylon*), when the stems and rhizomes are thoroughly cut up and mixed in the soil with a disk or rotary tiller prior to treatment.

Eptam must be incorporated into the soil immediately after appli-

cation for optimum results. The acute oral LD_{50} for male albino rats is 1,630 mg/kg and the acute dermal LD_{50} for albino rabbits is about 10,000 mg/kg.

E. History

Eptam was originally synthesized and developed at the research laboratories of Stauffer Chemical Company (Tilles and J. Antognini, 1959).

F. Physical Properties

Boiling points:

232°C (760 mm)	167°C (100 mm)
127°C (20 mm)	112°C (10 mm)

Solubility: Eptam is miscible with most organic solvents including hexane, benzene, xylene, and methyl alcohol. Its solubility in water is 375 p.p.m. at 20°C.

Vapor pressure: 0.035 mm at 25°C (by extrapolation of the vapor pressure curve from 100 to 1 mm).

Refractive index: N_D^{30} 1.4750.

Density: d_4^{30} 0.9546.

G. Chemical Properties

1. Method of synthesis

React 1 mole of ethyl chlorothiolformate with 1.05 moles of di-*n*-propylamine and 1.25 moles of aqueous sodium hydroxide. Two other methods of synthesis of Eptam and 264 other thiolcarbamates have been described (Tilles, 1959).

2. Chemical stability

Eptam is very stable to hydrolysis in dilute acidic and basic solutions. Less than 5% hydrolysis was observed when 100 p.p.m. Eptam was stored for 300 days at 20°C in 0.1 *N* HCl, H_2SO_4, NaOH, or distilled water. Hydrolysis is complete in 10 minutes in 96% H_2SO_4 at 85°C, but the rate falls off abruptly at lower acid strengths.

H. Formulations

Eptam is formulated as an emulsive liquid containing 6 pounds of the active ingredient per gallon and designated Eptam 6-E; and as a granular product containing 5% of the active iugredient aud designated Eptam 5-G. More concentrated granular products may also be used iu some areas.

II. ANALYSIS

A. Formulation Analysis

1. REVIEW OF METHODS

Two methods are available for technical and formulated Eptam. A specific method based upon determination by gas chromatography is preferred, but a general method based upon Kjeldahl nitrogen is useful in control analyses where other nitrogen-containing materials are absent. Classical Kjeldahl methods are suitable provided techniques are used which minimize Eptam loss during the acid digestion.

2. RECOMMENDED METHOD

a. *Principle*

Eptam is determined by gas chromatography with reference to a sample of known composition. The liquid emulsive formulation is injected directly into the gas chromatograph, whereas the granular products are first extracted with a benzene-methanol-water system (see also Volume I, Chapter 9).

b. *Apparatus*

Gas chromatograph, Beckman GC-2 or equivalent.

Column, 6-ft, ¼-inch aluminum packed with Dow Corning silicone high vacuum grease (20%) on acid washed Chromosorb.

Beckman Liquid Sampler syringe or equivalent.

c. *Procedure*

i. *Sample Extraction.*

Weigh 10 gm of 5% Eptam granular into a 125-ml glass-stoppered Erlenmeyer flask; add 15.0 ml of anhydrous methanol, 1.0 ml of water, and 25.0 ml of benzene. Stopper the flask and shake for 1 minute; allow to stand for 5 minutes; add 50 ml of water and shake for 1 minute. An emulsion usually forms, but it can be broken with the addition of 1 or 2 ml of methanol. After the emulsion is broken, swirl to extract the excess methanol from the benzene phase. Dry a portion of the benzene phase with anhydrous sodium sulfate and stopper tightly for subsequent analysis.

Chloroform saturated with water has recently been found to effect complete extraction of Eptam from the granular product, and is more rapid.

ii. *Gas Chromatography.*

With a precision syringe, inject 10 or 20-μl aliquots of Eptam liquid emulsive or the extracted granular Eptam into the column after equilibration at 160°C and 30 psi helium (use lower gauge pressure in instruments not containing capillary restrictors). The typical retention time is 8 minutes for Eptam under these conditions. Measure the area of the recorded peak and refer it to the area of a reference standard, chromatographed under identical conditions. The reference standard should be similar to the sample in concentration of Eptam and solvent used. Results from eighteen injections showed the relative standard deviation to be $\pm 1.2\%$.

B. Residue Analysis

1. Review of Methods

Eptam residues have been determined by radiotracer techniques (Fang and Theisen, 1959), by gas chromatography (Hughes and Freed, 1961), and by colorimetry (Batchelder and Patchett, 1960). The gas chromatographic method has been mainly applied to the analyses of soil samples, but the method may also be applied to the analyses of some crop samples. For routine crop sample analyses, the colorimetric method is preferred due to its proven reliability and low background values for a wide range of sample types. Where equipment is available, a gas chromatographic method employing the microcoulometric detector for sulfur may be employed.

Under normal usage, Eptam leaves no detectable residue in crops dne to its rapid metabolism as determined by the radiotracer studies of Fang and Theisen (1959).

2. Recommended Method—Colorimetric Method

a. *Principle*

Eptam is hydrolyzed in sulfuric acid to di-*n*-propylamine which is converted to the yellow complex of cupric dithiocarbamate having an absorption peak at 440 mμ (Batchelder and Patchett, 1960). Eptam is separated from the sample extractives by steam distillation, the colored materials formed on reaction with sulfuric acid are removed by benzene extraction of the aqueous amine hydrochloride solution, and any remaining color is compensated for by measurement before and after color formation.

b. *Special Reagents*

Copper-ammonium reagent. Dissolve 1.0 gm of cupric sulfate pentahydrate in 5.0 ml of water and dilute to 250 ml with concentrated ammonium hydroxide.

Eptam standard solution. 100 μg per milliliter of isoöctane.

Hodag FD-62 silicone antifoam solution, Hodag Chemical Co., Chicago, Illinois.

c. *Experimental Procedure*

i. *Sample Preparation.*

Macerate 400 gm of the crop sample with 1000 ml of water and transfer to a 4-liter wide-monthed Erleumeyer flask. Add 25 ml of glacial acetic acid and steam distill the Eptam into a 500-ml separatory funnel with about 400 ml of distillate. Where foaming is a problem, substitute 10 ml of Hodag FD-62 antifoam for the acetic acid. Add 5 drops of conc. HCl to the distillate and extract the Eptam from the aqueous phase with two 30-ml portions of isoöctane. Combine the isoöctane extracts, which represent 400 gm of sample, and store them for subsequent hydrolysis and colorimetry.

ii. *Colorimetry.*

Add a 30-ml aliquot of isoöctane extract, representing 200 gm of the sample, into a 40-ml centrifuge tube. Add 1 ml of 96% H_2SO_4, shake vigorously and allow to settle. Discard the isoöctane phase by aspiration with a vacuum suction tube. The Eptam partitions into the sulfuric acid and is then hydrolyzed by heating for 20 minutes in a glycerol bath at $85 \pm 1°C$. Roll the tube twice dnring this period to rinse the sides with the hot acid. Cool the tube in an ice bath and dilute the cold acid cautiously to 20 ml with water.

Add a drop of 1% phenolphthalein and neutralize cautiously with 50% NaOH while swirling in the ice bath. Add three extra drops of NaOH and 10 ml of benzene, shake to extract the di-*n*-propylamiue and discard the aqueous phase. Add 15 ml of water and one drop of phenolphthalein, acidify with conc. HCl and add three extra drops. Shake to extract the amine from the benzene and discard the benzene phase whicb contains any interfering color. Add an additional 10 ml of benzene, shake and discard the benzene phase. Add one drop of phenolphthalein and make the mixture basic with 50% NaOH and add two extra drops. Add 10.0 ml of benzene, shake and transfer about 4 ml of the benzene layer to a test

tube containing a gram of anhydrous sodium sulfate. Shake until the benzene extract is clear and pour into a 1-cm cell and determine the background absorbance at 440 mμ against a benzene blank with a Beckman DU spectrophotometer (or equivalent). Return the benzene to the centrifuge tube containing the major portion of benzene and the dilute sodium hydroxide. Add 0.5 ml each of cupric ammonium reagent and carbon disulfide. Shake immediately and continuously for 4 minutes. Dry an aliquot of the benzene phase as before and read the absorbance at 440 mμ within 10 minutes. Use the same cell for determining absorbance values before and after color formation to cancel the effect of cell corrections.

iii. *Interferences.*

Nonbasic organic compounds which steam distill and yield a secondary amine when heated with sulfuric acid could interfere with the Eptam colorimetry. Naturally occurring interferences have only been encountered in turnips and were removed by column chromatography. Tillam can interfere, but could be separated from Eptam by gas chromatography.

iv. *Sensitivity.*

Four micrograms of Eptam can be detected by the method. This is equivalent to 0.02 p.p.m. for most crops.

v. *Recovery.*

An average of 85% recovery has been effected when Eptam was added in the range of 0.05 to 0.5 p.p.m. to a wide variety of crops including alfalfa, apples, asparagus, dried beans, beets, clover, corn, onions, peppers, potatoes, prunes, spinach, strawberries, sugar beets, tobacco, and tomatoes. Recoveries are frequently 10 to 20% lower for very oily crops such as safflower seeds.

vi. *Standard Curve.*

A linear response is effected for Eptam in the range of 4 to 400 μg with typical absorbance values of 0.012 and 0.947 respectively. The reagent background has a typical absorbance value of 0.003.

vii. *Sample Calculations.*

Determine the amount of Eptam in the sample by referring the net absorbance (the difference between absorbance before and after the color formation) to a standard curve prepared by processing aliquots of the Eptam standard solution in a similar manner.

3. APPLICABILITY OF RECOMMENDED METHOD TO DIFFERENT CROP OR FOOD MATERIALS

Dried or starchy crops which present mechanical problems in the steam distillation extraction method can be extracted with hexane (Batchelder and Patchett, 1960), or with hexane-isopropanol (Gutenmann and Lisk, 1960). Foaming is reduced with crops such as snap-beans, safflower seeds, cabbage, tomatoes, sugar beet tops, apples, and tobacco by the addition of Hodag FD-62 prior to steam distillation. Crops with a very high oil content may have to be limited to 100 gm or less to effect good steam distillation recovery. Turnips are the only crop tested that interfere with the colorimetry. Turnip background values of 3 to 24 p.p.m. were reduced to less than 0.02 p.p.m. by chromatography on Florisil, eluting the interferences with 1.5% ether and eluting the Eptam with 3% ether in isoöctane.

4. DISCUSSION OF METHOD

The relative standard deviation of the colorimetric method is ±3.4%. During color formation, the solution temperature should be 20 to 25°C for optimum results. The reaction may not be completed at lower temperatures and side reactions can occur at higher temperatures. After addition of reagents, a delay in starting the 4-minute shaking period can also result in side reactions. The color formed in the absence of sample extractives is normally stable after drying, but slow fading has been observed with some samples.

5. MODIFICATION OF METHOD

The colorimetry can be significantly simplified when applied to soil samples which normally do not form any significant discoloration after digestion with sulfuric acid. The colorimetry can be carried out directly after neutralizing the sulfuric acid where a slight background color is not objectionable.

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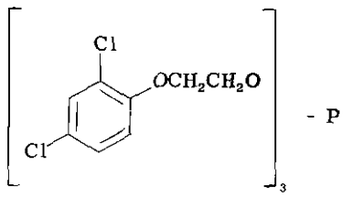
13

Falone

J. R. Lane



Tris-(2,4-dichlorophenoxyethyl) phosphite

I. GENERAL

A. Empirical Formula

$C_{24}H_{21}O_6PCl_6$ (Mol. wt. 649.39).

B. Alternate Names

3Y9, 2,4-DEP. Falone is the registered trademark of the U. S. Rubber Company.

C. Source of Analytical Standard

Naugatuck Chemical Division, U. S. Rubber Company, Naugatuck, Connecticut.

D. Biological Properties

Falone is an effective pre-emergence herbicide for corn, peanuts, strawberries, and white potatoes for which it has been registered by the U. S. Department of Agriculture. It has also shown promise on asparagus, nursery turf (specifically crabgrass control) and for maintaining weed control in "summer fallow" in western areas, as described by Feldman (1958).

Weeds controlled by Falone include pigweed, lambsquarter, ragweed, purslane, cocklebur, chickweed, foxtail, barnyard grass, crabgrass, goosegrass, and Florida pusley. Crops reported sensitive to either pre- or post-emergence application are beets, broccoli, cabbage, canta-

loupe, carrot, lettuce, mangel, mustard, onion, pea, pepper, pumpkin, radish, rutabaga, snapbean, soybean, squash, Swiss chard, tobacco, tomato, turnip, and watermelon.

Soil moisture, temperature, and orgauic content influence the biological action of Falone. Like most pre-emergence herbicides, it requires moisture for activation. However, muck soils reduce the weed control.

The acute oral toxicity (LD_{50}) of Falone is estimated to be 0.85 $\pm$ 0.14 gm/kg of body weight for the albino rat. In subacute (90 day) toxicological feeding studies carried out by the Food and Drug Research Laboratories, Inc., on 80 weanling albino rats, no abnormalities resulted from the ingestion of 0.0085 or 0.0425% Falone in the diet for 90 days, and the highest dose level (0.2125%) elicited mild adverse effects on growth, and on kidney and adrenal weights without morphological changes in these organs. No adverse effects have been noted in the handling of Falone in formulation or field operations.

E. History

Falone is one of a group of new chemicals, the poly (chlorophenoxyethyl) phosphites, which were initially prepared and found to be biologically active by Harris and Feldman (1958) as described in their patent. It is particularly useful as a pre-emergence herbicide.

F. Physical Properties

Pure Falone is a white waxy solid. The technical grade is a nonvolatile viscous brown oil which is soluble in all proportions with aromatic hydrocarbons but only to the extent of 1 part in 100 parts of Stoddard solvent. It is insoluble in water but hydrolyzes in the presence of water. Under normal anhydrous storage conditions, it is stable indefinitely.

G. Chemical Properties

1. method of synthesis

Phenol is reacted with chlorine gas to produce 2,4-dichlorophenol which is subsequently reacted with ethylene oxide to produce 2,4-dichlorophenoxyethanol. Reaction of the 2,4-dichlorophenoxyethanol with phosphorus trichloride in a hydrocarbon solvent with an amine as a sequestering agent produces Falone.

2. chemical reaction;

When exposed to aqueous acids or bases, Falone hydrolyzes slowly to 2,4-dichlorophenoxyethanol and phosphorous acid.

H. Formulation

Falone 44-E is an emulsifiable concentrate containing 44% by weight of active ingredient.

II. ANALYSIS

A. Formulation Analysis

1. REVIEW OF METHODS

Hooker Chemical Corp. (1959) and the Virginia-Carolina Chemical Corp. (1959) describe analytical methods of assay for trialkyl and dialkyl hydrogen phosphites. The methods utilize the property of trialkyl phosphites to react rapidly with halogen and of dialkyl phosphites to react slowly under more drastic conditions to produce an addition product.

Stone (1960) adapted the above tests for tri- and dialkyl hydrogen phosphites to the assay of Falone which may also contain small amounts of bis-2,4-dichlorophenoxyethyl phosphite.

2. RECOMMENDED METHOD

a. *Principle*

In this method (Stone, 1960) the per cent of tris-(2,4-dichlorophenoxyethyl) phosphite is determined by titration of the sample dissolved in alcoholic benzene-pyridine solution with alcoholic iodine solution to a yellow endpoint. The per cent bis-2,4-dichlorophenoxyethyl phosphite is then determined by reacting the same sample with excess alcoholic iodine solution for 15 minutes followed by titration of the unreacted iodine with alcoholic sodium thiosulfate solution. The milliliters of titrating solutions used are substituted in equations to calculate each component.

b. *Reagents*

Benzene, A.R.

Iodine in methanol (no potassium iodide), 0.10 *N*.

Methanol, absolute A.R.

Potassium acetate, potassium iodide solution (120 gm of potassium acetate (anhydrous) and 32 gm of potassium iodide dissolved in 640 ml of methanol).

Pyridine, A.R.

Sodium thiosulfate in aqueous methanol, 0.07 *N* (150 ml of H_2O/1000 ml of methanol).

c. *Apparatus*

Microburette, 10-ml capacity.

d. *Experimental Procedure*

Per cent Tris-(2,4-dichlorophenoxyethyl) phosphite. Weigh on an analytical balance 2.0 gm of Falone 44-E and transfer into a 125-ml iodine flask. Add 5 ml of benzene, 2 ml of pyridine and 5 ml of methanol. Swirl to mix and titrate rapidly with 0.10 *N* alcoholic iodine from a 50-ml burette to the first fleeting yellow endpoint.

$$(RO)_3P + I_2 \rightarrow (RO)_3PI_2$$

Calculation:

$$\% \text{ Tris-(2,4-dichlorophenoxyethyl) phosphite} = \frac{\text{meq. } I_2 \times 32.45}{\text{Sample weight (g)}}$$

Per cent Bis-(2,4-dichlorophenoxyethyl) phosphite. To the same sample add 20 ml of potassium acetate-potassium iodide solution and ca. 10.00 ml of 0.10 *N* iodine solution to provide an excess of iodine. Stopper and swirl the flask. Allow a 15-minute reaction period, then titrate the unreacted iodine with 0.10 *N* sodium thiosulfate solution from a 10-ml microburette. The endpoint is a change of the iodine-brown or yellow to a cloudy colorless solution.

$$(RO)_2POH + I_2 \rightarrow (RO)_2POI + HI \cdot \text{pyridine}$$

Calculation:

$$\% \text{ Bis-(2,4-dichlorophenoxyethyl) phosphite} = \frac{\text{meq. } I_2 - \text{meq. } Na_2S_2O_3 \times 23.0}{\text{Sample weight (g)}}$$

3. DISCUSSION OF METHOD

The alcoholic 0.10 *N* sodium thiosulfate solution is standardized daily against known 0.10 *N* iodine. The alcoholic 0.10 *N* iodine is then standardized against the sodium thiosulfate solution. The method is suitable for the analysis of Falone technical and 44-E.

B. Residue Analysis

1. REVIEW OF METHODS

Pre-emergence application of Falone suggested that a residue, if any, would be extremely small in treated crops. Prior art offered no residue test specific for such chemicals with a sensitivity of 0.1 p.p.m. Lane

(1961) developed a new color test specific for compounds containing halogen-substituted phenoxy or naphthoxy groups; and, under the prescribed clean-up, specific for Falone and its distillable hydrolysis product 2,4-dichlorophenoxyethanol (DCPE). The test is based on the following color reactions.

Dimethylnaphthidine (I) dissolved in concentrated sulfuric acid containing a small amount of potassium nitrate reacts immediately to form an intense stable purple coloration (II). If 1 to 5 μg of DCPE (III) are also present, they are nitrated to IV, thereby increasing the reactivity of the chlorine atoms. IV then reacts with II, causing the intensity of the purple color to decrease in proportion to the concentration of DCPE, in the ratio of ca 0.3 absorbance unit per microgram of DCPE (Fig. 1).

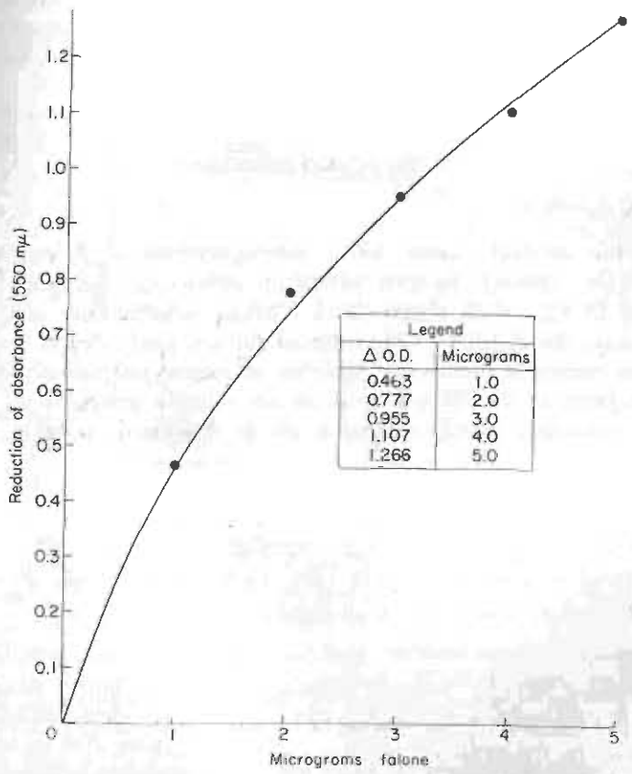
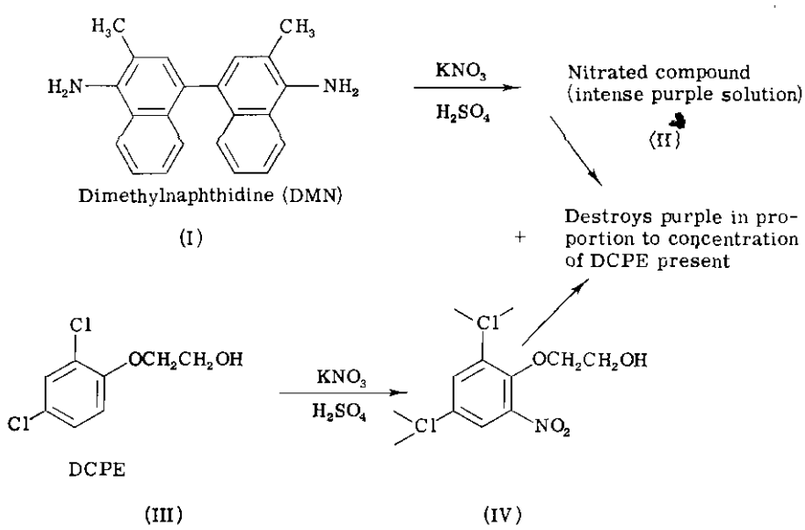


FIG. 1. Standard curve for Falone. Color reaction conditions: boiled H_2SO_4 (conc.); 8 drops nitrate reagent; DMN reagent to produce initial purple of 1.7; 30-minute reaction period.



2. RECOMMENDED METHOD

a. *Principle*

In this method (Lane, 1961) microquantities of Falone are hydrolyzed in refluxing aqueous potassium carbonate, liberating 3 molecules of DCPE which steam distill. Carbon tetrachloride extracts the DCPE from the distillate. Concentrated sulfuric acid extracts the DCPE from the carbon tetrachloride. Addition of nitrate and dimethylnaphthidine reagents to the sulfuric acid extract initially produces an intense purple coloration which, during a 20 to 30-minute reaction period, lessens in intensity proportionally to the microquantity of DCPE present (1 to 5 μg).

The net absorbance (the difference in absorbance at 550 mμ between an untreated sample analysis and a fortified or treated sample analysis) is compared to a standard curve (Fig. 1) to determine the micrograms of residue.

b. *Reagents*

Standard solution. Prepare an acetone or ether solution of 50 μg of Falone per milliliter. (The content of technical material is assumed to be equivalent to the chlorine content as determined by a total chlorine analysis.)

Dimethylnaphthidine (DMN) reagent. Place 0.010 gm of 3,3-dimethylnaphthidine and 30 ml of conc. H_2SO_4 in a clean, dry, 30-ml

dropping bottle. Seal with the ground-glass pipette and swirl occasionally for an hour to dissolve the dimethylnaphthidine completely. Prepare fresh daily 1 hour before initial use.

Nitrate reagent. Add 1.0 gm of potassium nitrate and 30 ml of conc. H_2SO_4 to a clean, dry, 30-ml dropping bottle. Swirl occasionally to dissolve completely and seal with the ground-glass pipette. This reagent is stable for a week.

Nitromethane, practical grade (colorless).

Sulfuric acid, A.R. boiled. Boil 300-ml portions plus two carborundum chips in clean, dry Kjeldahl flasks on a digestion rack for 0.5 hour. Store the cooled acid in a clean, dry acid bottle.

c. *Apparatus*

Use no lubricant on any ground-glass stopper, joint, or stopcock of any equipment used.

Distillation apparatus (Fig. 2).

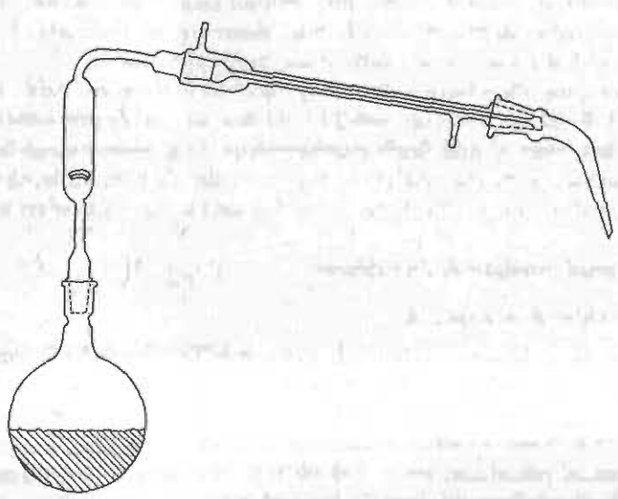


FIG. 2. Distillation apparatus for Falone residue analysis.

Heating mantle, hemispherical type, 1000-ml capacity.

Flask, round-bottomed, short-necked, 24/40 ℉ joint, 1000-ml.

Kjeldahl connecting bulb, Iowa State-type, fitted with 24/40 inner ℉ joints on both ends.

West-type condenser, drop-tip 24/40 ℉ joints, 300-ml jacket length.

Bent adapter, 24/40 ℉ joint.

Soxhlet extraction apparatus, (500-ml capacity boiling flask).

Bottles, dropping with ground-in pipette, 30-ml capacity.

Hypodermic syringe, with needle, 0.25-ml capacity, 0.01-ml graduations.

Centrifuge tubes, conical shape, 50 and 100-ml graduated, with glass stoppers.

Lapping and grinding compound, grease-mixed, Grit No. 600 (extremely fine), Clover Grade 4-A.

d. *General Experimental Procedure*

Because of procedural differences in the analysis of the various crops, a complete analytical procedure for all crops is described, which is divided into sections (Section e below). Those sections necessary for the analysis of a specific crop are given with the crop in the following list.

Corn ears and whole peanuts. Use Soxhlet extraction A, nitromethane partition, distillation, clean-up of distillate B, extraction of distillate, color formation and calculation.

Corn stalks, peanut plants, and peanut hay. Use Soxhlet extraction B, nitromethane partition, distillation, clean up of distillate A and B, extraction of distillate, color formation, and calculation.

Asparagus, cranberries, potatoes, and strawberries. Add a 50-gm sample, 175 ml of 30% (by weight) potassium carbonate solution, one drop of Antifoam A, and Carborundum chips to a 1-liter round-bottomed flask. Proceed with the analytical sections: distillation, clean-up of distillate B, extraction of distillate, color formation, and calculation.

e. *Individual Analytical Procedures*

i. *Soxhlet Extraction A.*

To a 43 × 123-mm Whatman extraction thimble add 5 gm of anhydrous sodium sulfate followed by 50 gm of sample, and cover with another 5 gm of anhydrous sodium sulfate. Soxhlet-extract the thimble in a medium-sized apparatus attached to a 500-ml boiling flask containing 350 ml of petroleum ether (30–60°C). The sample is extracted for 2 hours at a rate of one siphoning every 3 minutes.

ii. *Soxhlet Extraction B.*

To a 60 × 180-mm Whatman extraction thimble add 10 gm of anhydrous sodium sulfate, followed by 50 gm of sample, and cover with another 10 gm of anhydrous sodium sulfate. Soxhlet-extract the thimble in a large-sized apparatus attached to a 1-liter boiling flask containing 500 ml of petroleum ether. The sample is extracted for 3 hours at a rate of one siphoning every 4 minutes.

iii. *Nitromethane Partition.*

Add the petroleum ether extract to a dry, 500-ml separatory funnel containing 40 ml of nitromethane (practical grade). Stopper, shake vigorously for 30 seconds, and allow 5 minutes for separation of layers.

Draw off the lower (nitromethane) layer into a 1-liter round-bottomed flask. If the nitromethane layer contains flocculent matter, filter it through a Whatman No. 4 filter paper before transfering to the flask. Add 200 ml of distilled water, one drop of Antifoam A, and Carborundum chips.

Place the flask in a 1-liter heating mantle and attach to the distillation apparatus (Fig. 2). With the Powerstat set at 90 units, distill, collecting nitromethane and water (immiscible layers) in a 100-ml graduated centrifuge tube until 40 ml of water (top layer) have been collected. Discard the distillate. Remove and cool the distilling flask to room temperature, add 65 gm of granular potassium carbonate, and swirl to dissolve.

iv. *Distillation.*

Place the flask in a 1-liter heating mantle and attach to the distillation apparatus (Fig. 2) which is steamed to clean prior to each use. Set the Powerstat at 85 units and with a rapid condenser water flow, collect 100 ml of distillate.

v. *Clean-up of Distillate A.*

Collect the distillate in a marked 250-ml Erlenmeyer flask, and boil away ca. 20 ml to remove volatile interferences. Cool to 25°C.

vi. *Clean-up of Distillate B.*

Omit clean-up for potatoes.

Acidify the distillate with 20 drops of conc. H_2SO_4, extract with 30 ml of nitromethane in a 250-ml separatory funnel, and draw off the lower nitromethane layer into a 1-liter round-bottomed flask containing 130 ml of distilled water and Carborundum chips. Attach the flask to the distillation apparatus (Fig. 2) and distill and discard the nitromethane plus 30 ml of water (top layer). Cool the residue in the distilling flask to 30°C for extraction.

vii. *Extraction of Distillate.*

After clean-up, acidify the distillate with 20 drops of conc. H_2SO_4 and extract with 40 ml of carbon tetrachloride in a 250-ml separatory funnel. Filter the lower carbon tetrachloride layer through a

Whatman No. 4 filter paper into a clean, dry 50-ml conical centrifuge tube fitted with a ground-glass stopper. Add 2 gm of anhydrous sodium sulfate, stopper the centrifuge tube, shake for 30 seconds, and allow to stand for 10 minutes. Decant the dried carbon tetrachloride and filter through a Whatman No. 4 filter paper into a dry 60-ml separatory funnel. Add 3.0 ml of conc. H_2SO_4 from a 25-ml burette, stopper and shake vigorously for 30 seconds. Swirl gently and let stand for 20 minutes for layer separation. Draw off the lower, concentrated sulfuric acid layer into a dry 5-ml volumetric flask.

viii. *Color Formation.*

First, prepare a reagent blank to determine the intensity of purple available from a given amount of freshly prepared DMN reagent. Add 3.0 ml of conc. H_2SO_4 to a dry 5-ml volumetric flask followed by 8 drops of nitrate and 10 drops of DMN reagents. Horizontally agitate the volumetric flask to mix the contents thoroughly and allow to stand for 30 minutes to simulate an actual analysis. Then, measure the reagent blank at 550 mμ in a 1-cm cell, using water as the reference in a Beckman Model DU spectrophotometer. Use the absorbance value thus obtained to estimate the number of drops of DMN reagent needed to produce an initial optimum purple intensity of 1.6 to 1.7 absorbance units in ensuing sample analyses.

Add the color reagents to a set of analyses to ensure similar reagent activity. Carry an untreated sample, an untreated sample fortified with 0.1 p.p.m. of Falone and duplicate analyses of one or two accompanying treated samples together with a reagent blank analysis through the prescribed analytical procedure to obtain the concentrated sulfuric extracts in the 5-ml volumetric flasks. Carefully add eight drops of the nitrate reagent to each volumetric flask, followed by the number of drops of DMN reagent necessary (as estimated above) to provide a purple intensity of 1.6 to 1.7 absorbance units. (The drops of the reagents added should fall directly to the bottom of the 5-ml flasks without touching the neck.) Agitate the volumetric flasks to mix the contents and let stand for 30 minutes. Transfer the color solutions to an equal number of clean, dry 1-cm cells and measure together at 550 mμ, using water as the reference.

ix. *Preparation of Sample.*

Dry crops—i.e., corn ears and stalks; whole peanuts, plants, and hay—are ground to a meal in a Wiley Mill No. 2. Watery crops—i.e., asparagus, cranberries, potatoes, and strawberries—are ground to a soup in a blender, and analyzed immediately, or frozen in sealed glass jars

and stored. Asparagus and potatoes exhibit less interference if they are analyzed immediately after processing.

f. *Discussion of Interference*

After the 20-minute period for the phase separation of sulfuric acid from carbon tetrachloride in the final extraction, the sulfuric acid extracts of a set of crop analyses should be light yellow, of equal intensity and possibly have a very slight turbidity, which will clear during the color reaction. If one analysis appears to be noticeably more intensely yellow or to have a greater turbidity than the others, satisfactory interference removal was not obtained, and it is discarded. The sulfuric acid extract is drawn off very slowly into the 5-ml volumetric flask to insure that none of the carbon tetrachloride layer comes with it. A drop of carbon tetrachloride inadvertently passed on with the sulfuric acid produces a turbidity which will persist and make the absorbance measurement unreliable. All colored solutions must be crystal clear for reliable absorbance measurements. With the prescribed clean-up, practically no naturally occurring substances from the crops analyzed remain at the color formation step to interfere.

g. *Sensitivity*

The method is sufficiently sensitive to detect 0.03 p.p.m. of Falone in the eight crop products (Table I). Hydrolysis of Falone in aqueous

TABLE I
RECOVERIES OF FALONE ADDED TO UNTREATED CROPS

Crop	Added p.p.m.	No. of detns.	Recovered p.p.m.[a]	Recovery %	Ave. recovery %
Asparagus	0.10	2	0.07,0.08	71, 78	75
Corn ears	0.10	22	0.06–0.10	63–100	74
Corn stalks	0.10	9	0.05–0.10	50–100	68
Cranberries	0.10	2	0.07,0.07	66, 72	69
Peanuts, whole	0.10	23	0.07–0.09	51–92	71
Peanut plants	0.10	14	0.05–0.10	50–100	76
Potatoes	0.10	7	0.07–0.10	68–97	87
Strawberries	0.10	13	0.06–0.10	60–100	75

[a] Corrected for interference from untreated samples.

potassium carbonate, distillation of the DCPE and clean-up as described isolates DCPE from similar compounds such as 2,4-D, 2,4-dichlorophenol, or the sodium salt of 2,4-dichlorophenoxyethyl sulfate, which would produce a similar color reaction with the reagents as DCPE.

h. *Recoveries*

Recoveries of 0.10 p.p.m. in 92 determinations from the various crop products average 74% with a high of 100% and a low of 50% (Table I).

i. *Standard Curve*

Prepare a standard curve (Fig. 1) by processing aliquots of Falone standard solution as described below.

To a 1-liter round-bottomed flasks containing 175 ml of 30% potassium carbonate solution, add appropriately graduated microquantities of Falone (i.e., 1, 2, 3, 4, and 5 μg) as an acetone solution of the technical grade. (The content of technical material is assumed to be equivalent to the chlorine content as determined by a total chlorine analysis.)

The 1-liter flask is attached to the distillation apparatus (Fig. 2) and 100 ml of the distillate is taken. The analyses are then completed as described in the procedure beginning with the section entitled "Extraction of Distillate" (Section vii above).

j. *Calculation*

Determine the micrograms of Falone in the sample by referring the net absorbance (the difference between the absorbance of an untreated sample and a treated or fortified sample) to a standard curve (Fig. 1).

3. APPLICATION OF RECOMMENDED METHOD TO DIFFERENT CROPS

Since the crops analyzed by this method include berries, fodder crops, grain, green vegetables, nuts, and tubers, it is expected that a large number of related crops can be analyzed equally successfully if the clean-np technique given for a similar described crop is followed.

4. DISCUSSION OF METHOD

Traces of water in the concentrated sulfuric acid extracts or in the color reagents will inhibit the color reaction. Extreme caution must be observed to provide dry 60-ml separatory funnels, 30-ml dropping bottles for reagents, 50-ml centrifuge tubes, 5-ml volumetric flasks, 1-cm cells for absorbance measurements, and a 25-ml burette for dispensing the 3-ml aliquots of sulfuric acid for the extraction of the carbon tetrachloride. The above glassware is cleaned with warm water, followed by an acetone rinse and dried in an oven at 100°C. When beginning the analysis, a bottle of boiled sulfuric acid is fitted with a screw-on type plastic adapter to hold an automatic 100-ml graduated dispensing burette. All sulfuric acid needed for reagents and analyses are drawn, as needed,

from this system to minimize contamination of the acid by air moisture.

No lubricant is used on any stopcocks or other ground glass joints due to the possible contamination of the organic solvents and sulfuric acid which contact the joints. To insure that the unlubricated stopcocks of the separatory funnels are leakproof and turn easily, the stopcocks are ground into their joints with very fine grade, grease-mixed, valve grinding compound. The compound is removed with successive rinses of hexane, acetone, and water.

a. *Color Formation*

An initial purple intensity of ca. 1.7 absorbance units is necessary to be able to accurately detect 5 μg (0.10 p.p.m.) of DCPE. Five micrograms of DCPE cause a lessening in intensity of ca. 1.2 absorbance units in an analysis. Natural interference from the various crop products analyzed cause a reduction of 0.0 to 0.2 units.

When the purple color of nitrated DMN in an analysis is weakened by reaction to 0.5 absorbance units, and the total original concentration of about 200 μg of DMN is 80% consumed, the rate of reaction slows considerably, as is noted in the standard curve with 5 μg of DCPE.

A reaction period of 20 to 30 minutes for complete color change is most suitable to obtain high recoveries from Falone-fortified samples and a near straight line for the major portion of the standard curve.

Increasing or decreasing the number of drops of nitrate reagent will likewise increase or decrease the speed with which completion of the desired color change is obtained. If the color reaction is completed in 15 minutes or less, the nitrate content is usually so strong that it will cause untreated sample analyses to show increased interference levels and analyses of fortified samples to show lower recoveries. Although eight drops of nitrate reagent was found satisfactory for many sets of analyses, more or fewer drops of nitrate reagent may be needed to obtain a maximum color change in a 20 to 30-minute period. The color reaction (as indicated by fortified samples) appears to be completed 5 minutes faster in asparagus and strawberry analyses than in standard analysis and 5 minutes slower in peanuts and corn analyses. Plant constituents apparently aid or reduce the ability of the nitrate reagent to produce the desired reaction. Reagents, sulfuric acid, the room temperature and humidity are sometimes responsible for small variations in the rate of reaction.

All sulfuric acid and reagents should be at a room temperature of 70 to 85°F for standard and crop analyses. Low temperatures of reagents reduce the rate of reaction, and conversely higher temperatures increase it.

b. *Nitromethane as a Partitioning Solvent*

Nitromethane appears to be extremely selective in partitioning Falone from petroleum ether extracts of corn meal. A single extraction with nitromethane removes essentially all Falone from the petroleum ether, yet extracts no fats or waxes.

c. *Preparation of Fortified Samples*

One-tenth ml (5 μg) of Falone standard solution dispensed from a 0.25-ml hypodermic syringe is added to a 50-gm sample. For dry crops, Falone is added to the sample in the Soxhlet thimble prior to extraction. To watery crops, the material is added to the sample in the 1-liter flask prior to distillation.

Sulfuric acid, A.R. in the 10-pound bottle is usable in this test. However, the relatively larger concentration of nitrate in this quality of sulfuric acid will produce the following noticeable changes in the general analysis. The DMN reagent will quickly become intensely purple with the formation of nitrated DMN due to the nitrate present. Use of this sulfuric acid for extracting and preparing both DMN and nitrate reagent requires about one-quarter the number of drops of nitrate reagent for the color reaction as is needed with the boiled acid. Interference from crop analyses are slightly higher and more variable. The standard curve is similar in shape but reduced in size by about 20%.

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IPC

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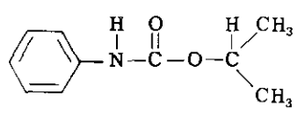

Isopropyl *N*-phenylcarbamate

I. GENERAL

A. Empirical Formula

$C_{10}H_{13}O_2N$ (Mol. wt. 179.22).

B. Alternative Names

IPC, isopropyl *N*-phenyl urethane, and isopropyl ester of carbanilic acid.

C. Source of Analytical Standard

Pittsburgh Plate Glass Company, Chemical Division, Agricultural Chemical and Ammonia Sales, One Gateway Center, Pittsburgh 22, Pa.; and Food Machinery and Chemical Corporation, Fairfield Chemicals, 411 Lexington Ave., New York 17, N. Y.

D. Biological Properties

1. herbicidal activity

IPC has economic value as a herbicide or plant growth regulator. It possesses biological activity, particularly toward narrow-leaved annual plants when applied to the soil during pre-emergence periods. The list of particular plants which can be controlled by this chemical includes annual bluegrass (*Poa annua*), wild oats (*Avena fatua*), rattail fescue (*Festuca myuros*), and ryegrass (*Lolium multiflorum*). There are a number of other narrow-leaved species which are susceptible to IPC, some of which may be classified as showing intermediate susceptibility (Freed, 1951).

The biological mechanism by which the phenyl carbamates function can be described briefly as being due to a disturbance in the normal division of the plant cells. Ennis (1948), Doxey (1949), and Ivens and Blackman (1950) agreed that the phenyl carbamates as a class cause a decrease in the rate of cell division. The selectivity of certain species which are resistant to IPC may be due to an antidote substance, or substances, produced by these species which in some way depress the activity of the herbicide (Ries, 1953).

A more detailed discussion of the character and performance of IPC may be obtained by referring to the chapter on CIPC (in this volume) since the chemical and biological activity of both componnds are essentially the same.

Food crops on which various formulations of IPC have shown promising weed control include: sugar beets, peas, mint, and salad greens such as spinach, kale, turnips, and cabbage. As a post-emergence treatment, it has been particularly effective in controlling annual grasses normally associated with clover, alfalfa, and perennial grasses being grown for seed. In addition to its uses on harvested crops, IPC is effective for weed control in ornamental plantings such as flower and shrubbery beds.

2. ACUTE TOXICITY

a. *Oral Administration*

Acute toxicity tests conducted for IPC in 1949 by the Industrial Hygiene Foundation of America, Inc., Pittsburgh, Pa., for Pittsburgh Plate Glass Company suggest that the acute oral toxic level of IPC is very low—the single oral LD_{50} for rats is approximately 5 gm/kg. The chief toxic manifestation was anesthesia which increased in depth with increasing dosage.

b. *Inhalation Exposure*

The Industrial Hygiene Foundation found that the inhalation of IPC dust by rats produced anesthesia at much lower dosage levels than occurred as a result of acute oral administration. A dust concentration of 25 mg/cu meter produced light anesthesia in four hours, which represented a calculated intake of 0.6 mg or a dosage of 5 mg/kg assuming 100% retention of the dust. Varying degrees of anesthesia are produced by the inhalation of IPC, the depth depending on the dosage, and the earliest signs of lightheadedness or sluggishness should provide an adequate warning of excessive dosage.

E. HISTORY

Biological effects of esters of carbanilic acid (carbamates) were

reported by Freisen in 1929. Templeman and Sexton (1945) published results of plant growth studies with carbauilic acid esters, and reported that the isopropyl ester (IPC) effectively retarded the growth of oats.

Experimental field work eventually led to commercial application of IPC for weed control in legumes, strawberries, spinach, and lettuce. Commercial experience soon showed IPC to have two limitations: (1) under field conditions its performance was variable and inconsistent, and (2) its residual life in the soil was too short for most crop applications.

Much of the early work in weed control with carbamates emphasized IPC. In 1951, Witman and Newton reported the more toxic effect of CIPC as compared with IPC.

Some of the crops grown in soil receiving post- or pre-emergence herbicidal treatment with IPC which have been analyzed for residues of the herbicide at the time of harvest are listed in Table I.

TABLE I
TREATED CROPS ANALYZED FOR IPC RESIDUE

Crop	Treatment	Reference
Peas	Pre-emergence	Gard and Reynolds, 1957
Strawberries	Dormant	Gard *et al.*, 1959; Montgomery and Freed, 1959
Sugar beets	Pre-emergence	Gard *et al.*, 1959
Spinach	Pre-emergence	Gard *et al.*, 1959
Flax	Post-emergence	Gard and Ferguson, unpublished results
Lettuce	Post-emergence	Bissinger and Fredenburg, 1951

F. PHYSICAL PROPERTIES

The following data are taken from unpublished information obtained in the authors' laboratory, Chemical Division of Pittsburgh Plate Glass Co., Barberton, Ohio.

Melting point, 87–88°C (sublimes).

Specific gravity at 20°C, 1.09.

Solubility in water at 20°C, 250 p.p.m.

Solubility in organic solvents: Readily soluble in esters, alcohols, ketones, and chlorinated solvents. Limited solubility in aliphatic and aromatic hydrocarbons.

G. CHEMICAL PROPERTIES

1. METHODS OF SYNTHESIS

Two important methods for synthesizing IPC are shown. The first method involves the reaction of equimolar quantities of isopropyl alcohol and phenyl isocyanate, as shown in Eq. (1):

$$C_6H_5NCO + C_3H_7OH \rightarrow C_6H_5NHOCOC_3H_7 \quad (1)$$

The second method of synthesis involves the reaction of equimolar quantities of isopropyl chloroformate and aniline in a sodium hydroxide medium as shown in Eq. (2):

$$C_6H_5NH_2 + C_3H_7OCOCl \xrightarrow{NaOH} C_6H_5NHOCOC_3H_7 + NaCl + H_2O \qquad (2)$$

2. CHEMICAL REACTIONS

Since IPC is a carbamic acid ester, it is essentially neutral in action and does not corrode common metals to any appreciable extent. It is slowly hydrolyzed under basic or acidic conditions to yield aniline, carbon dioxide, and isopropyl alcohol. IPC is slightly volatile due to its measurable vapor pressure at room temperature.

H. Formulations

During exploratory and development work with IPC, several methods for applying the herbicide to crops were evolved. Among the various types of formulations are wettable powders, dusts, granular formulations, and sprays prepared from emulsifiable concentrates. These formulations are applied for weed control during either the pre-emergence or post-emergence period.

Technical IPC having a minimum assay of 98% is available from various sources. Emulsifiable concentrates are customarily available as 0.4 lbs of IPC per imperial gallon, and 2 lbs and 3 lbs of IPC per U.S. gallon. Wettable powders are available as 75% and 50% IPC. In addition, a 95% dust-base product, a 10% pelletized IPC, a 10% granular IPC, and a 3% IPC-gypsum base mix are commercially available.

Emulsifiable concentrates and wettable powders are applied to the crop or soil with appropriate spray type equipment. Granular and other solid formulations are customarily applied by a broadcast type of operation, using either hand-operated or mechanically operated equipment. The granular or dry formulations are often quite applicable to crops grown in hot, dry climates where volatility of the IPC is a problem, or where liquid emulsions have proven too sensitive or toxic.

II. ANALYSIS

A. Formulation Analysis

1. REVIEW OF METHODS

a. *Hydrolysis Procedures*

IPC is susceptible to hydrolysis by both acids and alkalies to yield

carbon dioxide, isopropyl alcohol, and aniline. This hydrolysis reaction provides the basis for the analytical method generally used for determining the purity of the technical product and the IPC content of formulations. In 1951, Gard described an analytical procedure based on the measurement of the carbon dioxide evolved during the hydrolysis.

b. *Total Nitrogen Analysis*

An approximation of the purity of the technical product, and to a lesser extent, the active ingredient concentration of formulations may be achieved by a total nitrogen analysis providing the associated components of the mixture contain no nitrogen-bearing compounds. A total nitrogen determination, however, is not a recommended method for determining the purity of the technical product or the active ingredient of formulated mixtures, although this approach may be used as an alternative.

2. RECOMMENDED METHOD

Hydrolysis methods are recommended for the analysis of the technical product and its formulations. Acidic hydrolysis and simultaneous collection of the liberated carbon dioxide during the reaction period is preferred (Gard, 1951), since the reaction is completed within 1 hour. In some cases, the hydrolysis is carried out in an alkaline environment under reflux conditions during an overnight reaction period. In this case, the carbon dioxide is collected chemically in the alkaline mixture as the carbonate during the hydrolysis step. At the termination of the hydrolysis period, the carbon dioxide is liberated from the mixture by dilute acid treatment and measured titrimetrically by the same procedure and in the same apparatus as used for the acidic reaction.

The results obtained by both acidic and alkaline hydrolysis procedures indicate that the precision of the method is $\pm$ 0.05% carbon dioxide, or $\pm$ 0.20% expressed as IPC. The calculated accuracy of the method is 99% or better.

The complete analytical method as applied to the analysis of IPC is identical in all respects to that which is used for the analysis of CIPC formulations with the exception of the calculation. All of the principles, reagents, apparatus, and analytical steps as described in the chapter on CIPC (specifically the section on formulation analysis) may be applied without modification (see this volume). The formula for calculating the analytical results in terms of IPC is:

$$\% \text{ Isopropyl } N\text{-phenylcarbamate (IPC)} = \frac{\text{ml of NaOH (net)} \times N \times 0.08961 \times 100}{\text{Sample wt.}}$$

B. Residue Analysis

Analytical methods for determining residue concentrations of IPC in treated crop samples fall into two general categories: (1) those involving hydrolysis techniques, either in cases where the IPC is extracted with an appropriate solvent, snch as methylene dichloride or petroleum ether (Bissinger and Fredenburg, 1951; Gard and Rudd, 1953), or by direct hydrolysis of the sample without extractive treatment (Montgomery and Freed, 1959; Gard and Ferguson, unpublished work); and (2) those involving instrumental measurement of the IPC directly on the sample extract without hydrolysis (Gard and Ferguson, unpublished work).

The principle upon which the hydrolysis technique depends is that of making a colorimetric measurement of the aniline distilled from the sample or sample extract after alkaline or acid hydrolysis. The two methods for the color development are the (1) phenol-ammonia-hypochlorite method described by Gard and Rudd (1953) and (2) the diazotization-dye coupling method proposed by Montgomery and Freed (1959).

The choice of an appropriate extraction technique and hydrolysis conditions for a particular product application is indeed difficult to predict and will vary from product to product, depending on the inherent interferences which exist with the product and which respond to the complete analytical method. Some control of the interference levels can be achieved through the use of one or the other of the spectrophotometric methods for the measurement of the aniline, or by making modifications in the hydrolysis or extraction steps.

The direct hydrolysis of the bulk of the sample without extractive separation of the IPC is confined exclusively to an alkaline hydrolysis treatment. Acidic hydrolysis results in serious charring and decomposition problems. In cases where the direct hydrolysis of the sample is applicable, the procedure to be followed would be the same as that applied to sample extracts.

In view of the foregoing discussion, it is apparent that each crop or sample undertaken for IPC residue tests involves a considerable amount of exploratory and investigative work to ascertain the optimum analytical conditions which yield nominal interference, or blank values, for the untreated sample; and, at the same time, provide reasonable and satisfactory recovery values for IPC when added in known amounts to portions of the untreated control samples.

The analytical methods for determining microamounts of IPC as residue in crop samples are identical in all respects to those for deter-

mining CIPC in similar crop samples excepting the wavelength at which transmittance measurements are made for the diazotization dye-coupling method (555 mμ) and the calculation. Refer to Volume II, for a discussion of the principles of the method, reagents, apparatus, and experimental procedure. Aniline is the hydrolysis product of IPC and must be substituted in the discussion for 3-chloroaniline referred to in the chapter on CIPC. The formula for calculating the IPC residue in crops is:

$$\text{p.p.m. IPC} = \frac{\text{mg of aniline} \times 1.94 \times 1000}{\text{Sample wt.}}$$

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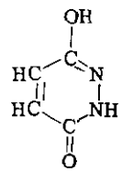
15

Maleic Hydrazide

J. R. Lane



I. GENERAL

A. Empirical Formula

$C_4H_4O_2N_2$ (Mol. wt. 112).

B. Alternative Names

MH, 6-hydroxy-3-(2H)-pyridazinone. MH is the registered trademark of the U. S. Rubber Company.

C. Source of Analytical Standard

Naugatuck Chemical Division, U. S. Rubber Company, Elm Street, Naugatuck, Connecticut.

D. Biological Properties

The following data are taken from Zukel (1957):

Prevents growth of suckers on tobacco plants.

Retards grass growth along highways, airfields, in unused areas.

Prevents sprouting of potatoes and onions in storage.

Stops runner growth of strawberries to increase yield.

Extends life of cnt roses.

Slows down tree growth under utility lines.

Reduces frost damage to citrus trees.

Plant processes affected by maleic hydrazide include photosynthetic activity, osmotic pressure, a resistance to wilting, and reduced water loss by transpiration.

Maleic hydrazide ion is absorbed by an aqueous route through the

leaf cuticle from both the upper and lower surfaces of the leaf and moves slowly through the cuticle and mesophyll but freely through the phloem. Translocation appears in both an upward and downward direction from the point of application. Maleic hydrazide inhibits cell division without affecting cell expansion.

E. History

Maleic hydrazide was first synthesized by Curtius and Foesterling (1895). Its use as a plant growth regulant and herbicide was discovered by Schoene and Hoffmann (1949) in the laboratories of the Naugatuck Chemical Division of the U. S. Rubber Company. The commercial process for manufacturing this chemical is described by Harris and Schoene (1951). The uses and applications of maleic hydrazide and its derivatives are described by Hoffmann and Schoene (1952), Zukel and Harris (1952), and Schoene and Zukel (1957) in patents assigned to the U. S. Rubber Company.

F. Physical Properties

Maleic hydrazide is a white, nonvolatile crystalline solid (M.P., 298°C). The solubility of maleic hydrazide at 25°C is 2.4 gm/100 gm of dimethylformamide and <1 gm/100 gm of solvent in water, ethanol, acetone, and xylene.

The compound has an acidity comparable to acetic acid and readily forms salts with basic materials.

Maleic hydrazide salt of:	Solubility in Water at 25°C (gm/100 gm solution)
Diethanolamine	70
Potassium	40
Sodium	20

G. Chemical Properties

In the method of synthesis developed by Harris and Schoene (1951) maleic anhydride and a mineral acid salt of hydrazine are reacted in a slightly acidic, aqueous solution. Maleic hydrazide precipitates.

Maleic hydrazide is comparable in acidity to acetic acid and forms neutral water soluble salts with a variety of basic substances. It is very stable as a dry solid or in acidic, neutral, or basic aqueous solution. Boiled in 50% aqueous sodium hydroxide solution, it is unchanged. However, the addition of zinc granules reduces maleic hydrazide to succinic hydrazide which is easily hydrolyzed in aqueous caustic liberating hydrazine. Heating small quantities of maleic hydrazide several hours in concentrated sulfuric acid produces hydrazine sulfate.

H. Formulation

MH-30 is a water-soluble liquid containing the diethanolamine salt of maleic hydrazide equivalent to 30% maleic hydrazide. One gallon contains 3 pounds as maleic hydrazide.

II. ANALYSIS

A. Formulation Analysis (MH-30)

1. Recommended method for the analysis of MH-30

a. *Principle of the Method*

MH-30 is a solution of an amine (diethanolamine) salt of maleic hydrazide in water. The maleic hydrazide content is determined by titration with alcoholic sodium hydroxide using an alcoholic medium to repress the ionization of the amine solvent during the titration.

b. *Reagents*

Ethanol, anhydrous 2-B or 3-A.
Sodium hydroxide, standard 0.1 *N* alcoholic solution.

c. *Apparatus*

Erlenmeyer flasks, 250-ml.
Burettes, 50-ml.

d. *Procedure*

i. *Sample Preparation.*

Weigh 1 to 2 gm of the sample on an analytical balance into a 250-ml Erlenmeyer flask and add 150 ml of anhydrous ethanol. Titrate with *N*/10 NaOH to the thymophthalein end point (colorless to blue).

ii. *Calculation.*

$$\% \text{ Maleic hydrazide} = \frac{\text{Ml of NaOH} \times \text{normality} \times 11.2}{\text{Sample weight in grams}}$$

B. Residue Analysis

1. Review of methods

The original test for the determination of maleic hydrazide residues was developed by Wood (1953). The test consisted of boiling micro-quantities of maleic hydrazide in strong potassium hydroxide solution,

containing granular zinc as a reductant. Under these conditions, hydrazine is quantitatively liberated, steam-distilled with nitrogen gas and trapped in an acidic solution of *p*-dimethylaminobenzaldehyde. A special compact distillation apparatus was designed to insure maximum recovery of hydrazine.

To overcome problems of excessive foaming and high interference encountered in some crops with Wood's (1953) original procedure, Lane and his co-workers (1958) modified the apparatus and employed a base-line technique type of calculation. Expansion of the revised method produced Method G-114-A (Lane, 1960) sufficiently detailed to provide a working laboratory procedure for most crops. An AOAC collaborative study of this method by five laboratories (Lane, 1963) showed fine reproducibility of analysis.

2. RECOMMENDED METHOD—METHOD G-114-A

a. *Principle*

In this method (Lane, 1960) the sample is submitted to a caustic digestion to drive off volatile interferences. Addition of granular zinc followed by distillation expels the hydrazine liberated from the maleic hydrazide by the combined action of the zinc and caustic. The hydrazine is swept out in a stream of nitrogen gas and is reacted in an aqueous acid solution of *p*-dimethylaminobenzaldehyde forming a yellow color which is measured spectrophotometrically.

b. *Reagents*

DAB reagent. Dissolve 2 gm of *p*-dimethylaminobenzaldehyde in 100 ml of 1 *N* H_2SO_4. The reagent is stable.

Ferrous chloride, A.R., $FeCl_2 \cdot 4H_2O$ (greenstone chips—do not use rust-brown or yellow chips).

Grease, high-vacuum (silicone), Dow-Corning.

Hydrochloric acid, ca. 1 *N* solution.

Sodium hydroxide pellets, A.R.

Paraffin wax, household.

Zinc, granules, 10-mesh.

c. *Apparatus*

Maleic hydrazide distillation apparatus (Wood, 1953), equipped with 300-ml distillation flasks, double-thickness with thermometer well. (Obtainable from Macalaster Bicknell Company, 181 Henry Street, New Haven, Connecticut.)

Tank of dry nitrogen, with a Hoke pressure valve.

Spectrophotometer, Beckman Model DU, equipped with 1-cm cells.

Centrifuge tubes, conical, beaded rim, 50-ml, graduated in 1-ml divisions.

Wire gauze squares, 5-inch with 4-inch diameter asbestos center.

Thermometers, 90–220°C (Tinius Olsen No. 718636, yellow bak (or equivalent).

Hot plate, electric, Chromalox, Hevi Duty or equivalent.

Ring, extension, cast iron with 4.5 inch I.D.

d. *Experimental Procedure*

i. *Processing of Samples.*

For watery crops homogenize the whole sample to a soup in a blender. To store for a period of time retain ca. 200 gm of the homogenate in a sealed jar below 0°C. For root crops and others which grind with difficulty, chop into small pieces with a knife, and add a measured 10–25% H_2O as required to facilitate homogenation. To process a sample containing a number of large specimens, take two diagonally opposite eighth sections from each specimen for homogenization to reduce volume requirements. For a large sample of small specimens, a randomly selected subsample is adequate. To analyze a frozen homogenate, thaw completely and mix well before sampling.

ii. *Procedure.*

Transfer a sample of the ground crop (see Table I) to a 300 ml

TABLE I

ANALYTICAL SPECIFICATIONS FOR INDIVIDUAL CROPS

Crop	Sample weight gm	Precook temperature	Antifoam agents
Apples	5	160°C	0.5 gm wax after precook
Cranberries	8	180°C	0.5 gm wax + 10 drops Antifoam A after precook
Grass root dust	1	160°C	0.5 gm wax + 2 drops Antifoam A before precook
Lemon juice	50	160°C	0.5 gm wax after precook
Lemon skins	8	160°C	0.5 gm wax after precook
Onions	3	160°C	10 drops Antifoam A
Peaches	5	160°C	1 gm wax + 10 drops Antifoam A after precook
Potatoes	2	160°C	1 ml Wesson oil before precook
Potato chips	1	160°C	None
Tobacco dust	1	160°C	3 gm wax + 10 drops Antifoam A before precook
Twig dust	3	160°C	0.5 gm wax after precook

distilling flask. Dry the socket neck joint and add 50 gm of NaOH *pellets, 40 ml of distilled water, and antifoam agents as indicated* in Table I. Add 1 ml of a nonvolatile mineral or silicone oil to the thermometer well and insert the thermometer. Heat the flask on a high temperature hot plate and swirl every 30 sec until the pellets dissolve and steady boiling begins. When the temperature reads 160°C (cranberries, 180°C; *see* Table I), *remove the flask and let cool 5 min.* Wipe the socket joint clean, dry, and add 0.5 gm of ferrous chloride and 4 ml (5 gm) of zinc granules.

Quickly grease the socket joint with a light film of high-vac silicone grease and attach the flask to the apparatus (Fig. 1). Center the flask *firmly on an asbestos impregnated 5-inch iron gauze square secured* by a support ring. Place 4 ml of DAB reagent in a 50-ml centrifuge tube (ice-cooled) and immerse the condenser tip. Adjust the nitrogen flow to produce 3 bubbles/sec in the receiver. With a rapid flow of condenser water, heat the flask with a Bunsen burner, centering the tip *of the outer cone of the flame on the iron gauze. When boiling begins,* adjust the distance of the burner, so the foaming contents fill ca. 75% of the flask. Distill until the thermometer reads 173°C; then (while distillation continues) slowly add water from the reservoir until the

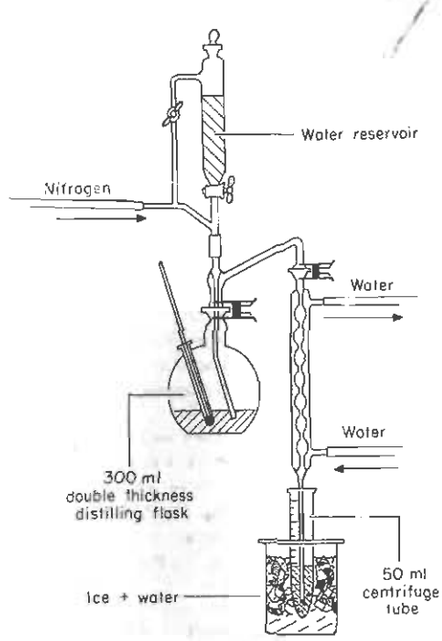


FIG. 1. Maleic hydrazide distillation apparatus.

temperature drops to 168°C. Discontinue the water addition and allow distillation to proceed until the temperature again reaches 173°C. Continue the water addition and distillation in this manner until the receiver contains 40 ml. Remove the receiver tube.

Note: If, during the distillation, the aqueous contents of the receiver become turbid or a slight precipitate appears, the addition of one to two drops of conc. H_2SO_4 will make the solution crystal clear again. Where wax is found in the distillate, it is removed by filtering the distillate through a coarse-porosity sintered-glass funnel before making absorbance measurements.

Record the volume of distillate obtained and measure the absorbance in 1-cm cells on a Beckman Model DU Spectrophotometer at 430, 460, and 490 mμ, using as reference 4 ml of DAB reagent diluted to 40 ml with distilled water. Determine the net absorbance (see Section II,B,2,d,v).

When the distillation has been completed, remove the hot distilling flask from the apparatus with heat-resistant gloves, place on a bench top, remove thermometer, and seal the well with a small cork. Rinse the nitrogen-water inlet tube free of caustic with dilute hydrochloric acid, followed by water. Then (wearing heat-resistant gloves and safety glasses) pour out the molten contents of the distilling flask into an iron can in the sink to trap the zinc granules. Rinse the emptied flask three times with water and pour the water rinses into the zinc trapping can. Then rinse the flask two times with dilute hydrochloric acid to loosen any remaining encrusted caustic and zinc granules, fill with dilute hydrochloric acid and let stand until the next use. Then rinse with water several times before use.

Note: Careful removal of all zinc with HCl from every flask after each distillation is absolutely necessary because any residual zinc will cause undesired destruction of maleic hydrazide in the caustic precook of the next analysis. Due to the etching effect of the boiling sodium hydroxide solution on the inner walls of the flask, a flask may be expected to last approximately 30 distillations before a leak develops in the wall of the flask or of the thermometer well. In either case, the flask must be thrown away.

iii. *Standard Solution.*

Dissolve 0.010 gm of purified maleic hydrazide in 1000 ml of 0.01 *N* NaOH to obtain 10 μg of maleic hydrazide per ml of solution.

iv. *Standard Curve.*

To clean 300 ml distilling flasks add 50 gm of NaOH pellets, 40 ml of clear water, and standard solution (iii) equivalent to 0, 5, 10, 20, 30,

50, 100, 150, and 200 μg of maleic hydrazide. Precook, distill, and measure the absorbance as in Section B,2,d,ii.

Determine the net absorbance for each standard analysis (Section B,2,d,v), and plot the net absorbance against μg of maleic hydrazide to obtain the standard curve (Fig. 2).

v. *Calculation.*

The net absorbance of each analysis is determined by:

$$\text{Net absorbance} = \text{Abs.}_{480\ m\mu} - \left(\frac{\text{Abs.}_{430\ m\mu} + \text{Abs.}_{490\ m\mu}}{2}\right) \times \frac{\text{Total ml. of color solution}}{40}$$

To determine the μg of maleic hydrazide, compare the net absorbance to the standard curve.

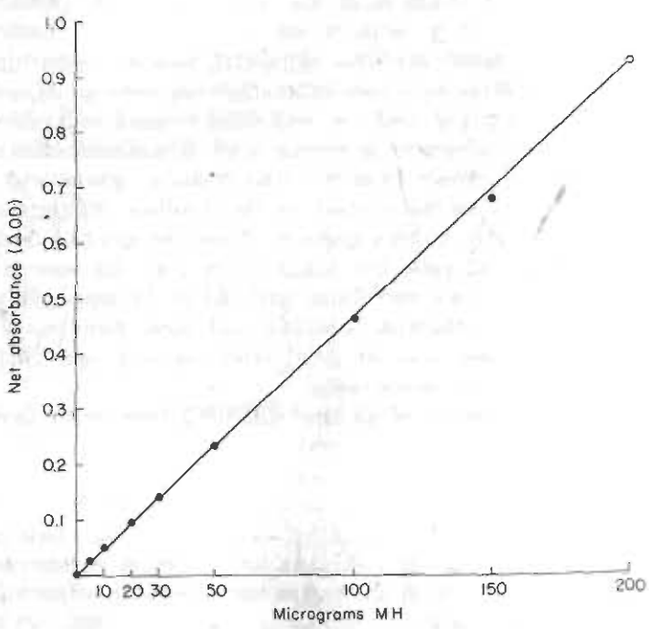


FIG. 2. Recovery curve of distilled maleic hydrazide standards.

From the slope of the standard curve (i.e., net absorbance vs. microquantity of maleic hydrazide) a factor is derived for converting the information from the standard curve into a simple general equation. From the standard curve:

$$\text{Micrograms} = \text{Net absorbance} \times K_{(\text{a constant})} \tag{2}$$

for any point along the straight line curve and transposing;

$$K = \frac{\text{Micrograms}}{\text{Net absorbance}} \tag{2a}$$

The K thus obtained may be multiplied by the net absorbance [Eq. (1)] found in any analysis to give micrograms of maleic hydrazide [Eq. (2)] and the micrograms found divided by the sample weight in grams equal parts per million.

$$\text{p.p.m. of Maleic hydrazide} = \frac{\text{Micrograms}}{\text{Sample wt. in grams}} \tag{3}$$

vi. *The Corrected Standard Curve and Factor for Crops.*

For rapidity of analysis, some laboratories prepare a so-called corrected standard curve, i.e., a curve similar to the Standard Curve (iv) in which every standard analysis also contains the recommended weight of the crop and antifoam agents (Table I). The curve thus obtained incorporates correction for percent recovery in the slope, making supplementary recovery data usually unnecessary.

The factor K is obtained from the slope of the curve as under Standard Curve (*iv*). In a collaborative study for maleic hydrazide residues (Lane, 1963) five independent laboratories showed close agreement in the preparation of the standard curve as well as of a corrected curve for white potatoes. The latter averaged 85% recovery in the 5–150 μg range when compared to the standard curves.

3. APPLICABILITY OF RECOMMENDED METHOD TO DIFFERENT CROPS OR FOOD MATERIALS

Using one or more of the Analytical Specifications described in Table I, almost any crop or animal tissue is analyzable.

4. DISCUSSION OF METHOD

Under the analytical conditions described in Section II,B,d,ii, all crops produce a single weak pink or red interference coloration, whereas maleic hydrazide produces a yellow coloration. By the base line technique employed, the calculation automatically equates the interfering red coloration essentially to 0 p.p.m. as shown by Lane and co-workers (1958) while at the same time giving a true value of the maleic hydrazide residue present. Interference from untreated samples of most crops (calculated as parts per million of maleic hydrazide) fall within the range of -1 to $+1$ p.p.m. All net absorbance data need only be compared to the primary standards curve if treated samples are corrected for per cent recovery as determined by the analysis of fortified samples.

Five parts per million of maleic hydrazide is quantitatively recoverable from most crops (as determined by fortified samples) where a 1-gm

sample is used for analysis. Where sample size is 10 to 50 gm, 1 p.p.m. may be quantitatively determined. Recoveries of 80 to 100% are obtained consistently from all crops fortified with 5 to 200 p.p.m. An experienced analyst can complete ten analyses iu a day by weighing and precooking all samples, distilling one after another and finally making all absorbance measurements and calculations.

Wrapping the distilling arm of the apparatus tightly in asbestos gauze ribbon insulates the distilling hydrazine-steam from heat loss and condensation prior to reaching the cold condenser. More consistent and somewhat higher recoveries of maleic hydrazide are obtained.

The p-dimethylaminobenzaldehyde color reagent forms a yellow dye (similar to that formed with hydrazine) with certain caustic distillable basic materials such as aniline, the mononitroanilines and monosubstituted hydrazines. Unless removed hy caustic precook, these materials or their formation or liberation by the action of aqueous caustic on other pesticides containing such groups as a portion of the molecule will indicate a false high maleic hydrazide residue content.

5. MODIFICATION OF METHOD

Anglin aud Mahon (1958) also noted certain inadequacies in the original method of Wood (1953) and modified the method by discarding the cook, utilizing a completely different type of distillation apparatus and a technique of solvent extraction for the removal of certain interferences from the distillate.

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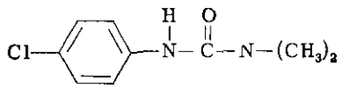
Monuron, Diuron, and Neburon

W. K. Lowen, W. E. Bleidner, J. J. Kirkland and H. L. Pease

I. GENERAL

A. Monuron—3-(*p*-Chlorophenyl)-1,1-dimethylurea



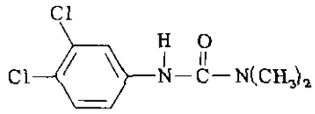
1. Empirical formula

$C_9H_{11}ON_2Cl$ (Mol. wt. 198.7).

2. Alternative names

CMU, N-*p*-chlorophenyl-*N'*-*N'*-dimethylurea.

B. Diuron—3-(3,4-Dichlorophenyl)-1,1-dimethylurea



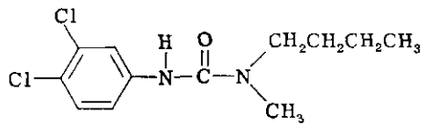
1. Empirical formula

$C_9H_{10}ON_2Cl_2$ (Mol. wt. 233.1).

2. Alternative name

DCMU, N-3,4-dichlorophenyl-N'-N'-dimethylurea.

C. Neburon—1-*n*-Butyl-3-(3,4-dichlorophenyl)-1-methylurea



1. Empirical formula

$C_{12}H_{16}ON_2Cl_2$ (Mol. wt. 275.2).
~~~

2. ALTERNATIVE NAME

N-3,4-dichlorophenyl-N′-*n*-butyl-N′-methylurea.

D. SOURCE OF ANALYTICAL STANDARD

The herbicidal compounds monuron, diuron, and neburon, are manufactured by E. I. DuPont de Nemours and Co., Inc., Industrial and Biochemicals Department, Wilmington, Delaware.

E. BIOLOGICAL PROPERTIES

The mammalian toxicity of these herbicides is low: The LD_{50}'s to rats are in the range of 3,000–4,000 mg/kg; they are not primary skin irritants, and they produce no allergic skin reactions.

F. PHYSICAL AND CHEMICAL PROPERTIES

These phenyl alkyl ureas are only sparingly soluble in hydrocarbons. They are stable toward oxidation and hydrolysis under normal conditions, but at elevated temperatures they can be hydrolyzed quantitatively. Much of their analytical chemistry is based on the latter reactions. Monuron can be prepared by the reaction of *p*-chlorophenyl isocyanate with dimethylamine (Bucha and Todd, 1951). 3,4-Dichlorophenyl isocyanate reacts with dimethylamine to give diuron. Neburon is synthesized by reacting 3,4-dichlorophenyl isocyanate with methylbutylamiue.

The following tabulation gives the melting points and water solubilities of the individual compounds:

	M.P.	Solubility, p.p.m.
Monuron	176–177°C	230
Diuron	158–159°C	42
Neburon	101.5–103°C	5

G. FORMULATIONS

These herbicidal compounds are available as wettable powder formulations (50–80%). In addition, diuron is marketed as a 28% aqueous suspension.

II. ANALYSIS

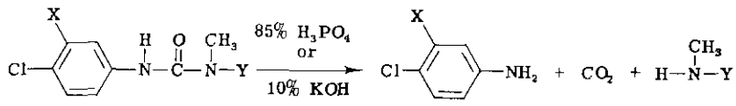
A. FORMULATION ANALYSIS

1. REVIEW OF METHODS

Formulations of monuron, diuron, or neburon can be assayed by a variety of methods. Because of the large number of chlorine-containing

pesticides on the market, methods based on total organic chlorine are not preferred. In well-defined systems, ultraviolet absorption of a methanol extract, or infrared absorption of an appropriate extract or of a potassium bromide disk (Kirkland, 1955) can be employed. However, the determination of fragments formed during quantitative hydrolysis (Lowen and Baker, 1952) is most generally applicable for assaying monuron, diuron, or neburon formulations.

Hydrolysis proceeds according to the following general reaction:



where X = H or Cl and Y = $-CH_3$ or $-C_4H_9$

The aliphatic amine thus formed can be distilled into a standard acid trap and the excess acid back-titrated. Similarly, the substituted aniline can be extracted into chloroform, and titrated directly with standard perchloric acid in glacial acetic acid. Other compounds which hydrolyze to give volatile and/or extractable bases interfere, but generally one of these approaches can be employed successfully. If both are equally applicable, hydrolysis in potassium hydroxide and determination of the aliphatic amine formed is preferred.

2. RECOMMENDED METHOD—ALIPHATIC AMINE PROCEDURE

a. *Reagents*

pH meter, Beckman Model H-2 or equivalent.

Sulfuric acid, standardized 0.1 *N*.

Sodium hydroxide, 0.1 *N* standardized.

Glycerol, USP or Reagent Grade.

Potassium hydroxide, 20% aqueous solution of Reagent Grade.

Silicone Defoamer, DC Antifoam A, Dow-Corning Corporation, Midland, Michigan.

b. *Apparatus*

Hydrolysis apparatus, Fig. 1.

c. *Procedure*

i. *Sample Preparation.*

Assemble the hydrolysis apparatus. Add 50 ml of 0.1 *N* H_2SO_4, accurately measnred from a burette, and 50 ml of methanol to the condensate beaker. Transfer an accurately weighed portion of the sample,

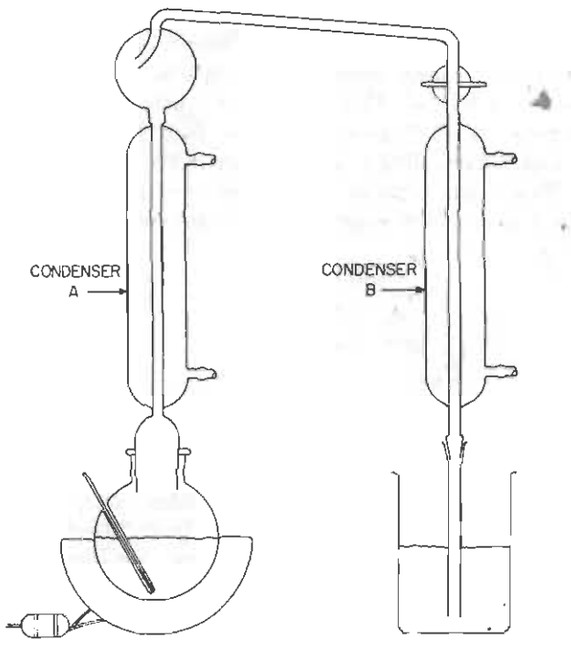


FIG. 1. Apparatus for hydrolyzing monuron, diuron, and neburon formulations.

containing 0.5 to 0.6 gm of the active ingredient, into the round-bottom flask. Add 15 ml of ethanol and swirl to disperse the sample. Add 100 ml of glycerol, several drops (6–8) of silicone defoamer, one or two boiling chips and 100 ml of 20% KOH solution, and immediately attach the reaction flask to condenser A. With cooling water flowing through each condenser independently, reflux the mixture for 3.5 hours.

Remove the cooling water from condenser A and distill the vapors into the standard acid trap until the pot temperature reaches 175°C. Adjust the heating rate so that this temperature is attained in 1.5 to 2.5 hours. Rinse condenser B and the connecting tube with methanol followed by distilled water, adding the rinsings to the beaker trap. Titrate the contents of the beaker with 0.1 *N* NaOH solution using a pH meter with glass-calomel electrodes, and determine the end point by a difference method rather than graphically.

ii. *Calculations.*

$$\% \text{ Monuron} = 19.87 \times \frac{(\text{ml HCl} \times N_{\text{HCl}}) - (\text{ml NaOH} \times N_{\text{NaOH}})}{\text{Sample wt. in gm}}$$

$$\% \text{ Diuron} = 23.31 \times \frac{(\text{ml HCl} \times N_{\text{HCl}}) - (\text{ml NaOH} \times N_{\text{NaOH}})}{\text{Sample wt. in gm}}$$

$$\% \text{ Neburon} = 27.52 \times \frac{(\text{ml HCl} \times N_{\text{HCl}}) - (\text{ml NaOH} \times N_{\text{NaOH}})}{\text{Sample wt. in gm}}$$

d. *Discussion*

The standard deviation of this procedure, based on the repeated analysis of a single sample on different days by different individuals, is about ± 1%, relative. Volatile, basic materials interfere with this method, as do compounds which yield volatile bases under these hydrolysis conditions.

3. RECOMMENDED METHOD—AROMATIC AMINE PROCEDURE

a. *Reagents*

Phosphoric acid, 85% Reagent Grade.
Chloroform, Reagent Grade.
Sodium hydroxide, 20%.
Hydrochloric acid, 3 *N*.
Phenolphthalein indicator solution.
Shaved ice.
Glacial acetic acid, Reagent Grade.
Perchloric acid in acetic acid, 0.1 *N*. Mix 8.5 ml of 72% perchloric acid (Eastman-White Label) with 300 ml of glacial acetic acid and add 20 ml of Reagent Grade acetic anhydride. Dilute to 1 liter with glacial acetic acid and allow to stand overnight to permit complete reaction of the acetic anhydride with the water present. Standardize by titrating, as described under "Procedure," against primary standard potassium acid phthalate (eq. wt. = 204.22), dissolved in 50 ml of glacial acetic acid and 25 ml of chloroform.

b. *Apparatus*

pH-millivolt meter. Leeds & Northrup line operated pH-indicator, or equivalent.
Electrodes, glass, methanol modified calomel, 2½″ sizes. The calomel electrode is a ground-glass sleeve type in which the aqueous potassium chloride is replaced with saturated methanolic potassium chloride.
Magnetic stirrer and stirring bar.

c. *Procedure*

i. *Sample Preparation.*

Weigh sufficient sample to contain 1.0–1.5 gm of the active in-

gredient into a 100-ml flask, add 10 ml of 85% H_3PO_4, connect a condenser to the flask and bring to a gentle boil. Continue to reflux for 3 hours. Cool and dilute to about 25 ml with distilled water.

Transfer the dilute hydrolyzate to a 500-ml separatory funnel containing 200 ml of shaved ice. Add about 25 ml of ice water, a drop of the phenolphthalein indicator, and neutralize by adding cold 20% NaOH in small increments. Add an additional 10 ml of 20% NaOH. Extract this cold, alkaline solution with one 25-ml portion of chloroform, followed by four 15-ml portions. Collect the chloroform phases in a 100-ml volumetric flask and dilute to volume. Pipette 25 ml of this solution into 50 ml glacial acetic acid contained in a 125-ml beaker. Add a magnetic stirring bar and place the beaker on stirrer platform. Immerse the electrodes, titrate with standard perchloric acid, and determine the end point graphically or by calculation.

ii. *Calculations.*

$$\% \text{ Monuron} = 19.87 \times \frac{\text{ml perchloric acid} \times N_{HClO_4} \times 4}{\text{Wt. sample in gm}}$$

$$\% \text{ Diuron} = 23.31 \times \frac{\text{ml perchloric acid} \times N_{HClO_4} \times 4}{\text{Wt. sample in gm}}$$

d. *Discussion*

The standard deviation of this method is approximately ±2% relative. Basic substances which can be extracted from aqueous medium by chloroform interfere with this technique, as do materials which yield such compounds when refluxed with phosphoric acid. In the event of such interferences, gas chromatographic resolution of the extract provides an unambiguous, but somewhat less precise method for analyzing the extract. The method is not suitable for precise assay of neburon, because small amounts of *n*-butyl methylamine are extractable with the aromatic amine.

B. Residue Analysis

1. Review of Methods

Residues of monuron, diuron, or neburon are readily determined by hydrolysis to the corresponding aromatic amines, which are isolated from the substrate by distillation and determined colorimetrically (Bleidner *et al.*, 1954), or by gas chromatography (Kirkland, 1962). Monuron yields *p*-chloroaniline (PCA), whereas 3,4-dichloroaniline (DCA) is obtained from diuron or neburon. These two anilines can be distinguished using liquid partition chromatography (Bleidner, 1954; Pease, 1962) or

by gas chromatography (Kirkland, 1962). Thus, monuron-diuron or monuron-neburon mixtures can be resolved and determined independently. However, if mixtures of diuron and neburon are encountered, only the total can be determined.

2. RECOMMENDED METHOD—COLORIMETRIC DETERMINATION

a. *Reagents*

Sodium hydroxide, 20%.

n-Hexane, acid-washed.

Dow-Corning Antifoam A.

p-Chloroaniline and 3,4-dichloroaniline, recrystallized from 70% ethanol.

Hydrochloric acid, 1 *N.*

Glacial acetic acid.

Sodium nitrite solution, 2%.

Sulfamic acid solution, 10%.

N-(1-Naphthyl)ethylenediamine dihydrochloride, 2% solution.

Whatman cellulose powder, 200 mesh, ashless.

b. *Special Apparatus*

Distillation-extraction apparatus, see Fig. 2.

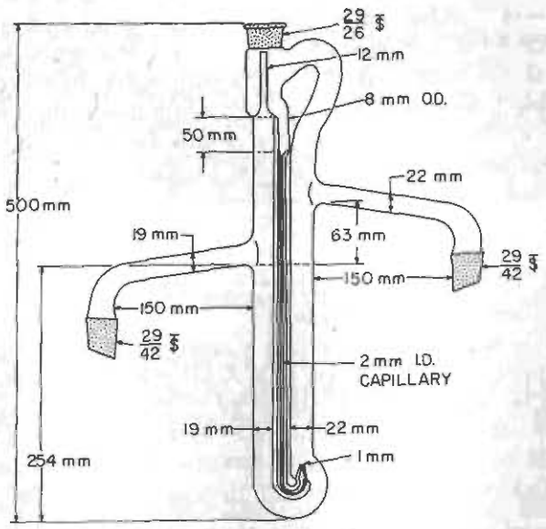


FIG. 2. Distillation-extraction apparatus for determining monuron, diuron, and neburon residues.

Monel metal flask, 2-liter round bottom.

Spectrophotometer.

Cellulose powder chromatographic column. Tightly pack dry Whatman cellulose powder by tamping into a 24 inch length of 15-mm O.D. glass tubing to a height of 20 inches. Use a penicillin assay disk as a packing support at each end of the column.

c. *Experimental Procedure*

i. *Sample Preparation.*

Extraction of monuron, diuron, or neburon residues is generally not applicable. The intact sample is introduced into the hydrolysis flask, where it is disintegrated. It is advisable to dice animal or plant tissue into ¼ to ½ inch cubes, but soil samples can be analyzed directly. While elaborate sample preparation is unnecessary, careful subsampling is required.

ii. *Colorimetric Determination.*

(a) *Hydrolysis and isolation of aniline.*[1] Place 100 to 500 gm of the sample in a 2-liter Monel metal flask; add several boiling chips, two teaspoons of Dow-Corning Antifoam A, and approximately 1000 ml of 20% NaOH. Fill the U-tube of the extraction apparatus to the level of the lower arm with distilled water, and attach the metal flask to the lower arm. Add 500 ml of acid-washed hexane to a 1-liter round-bottom flask, and attach to the upper arm. With a 24-inch water-cooled condenser in position, apply heat to both flasks at such a rate that approximately equal volumes of condensed hexane and water pass down the capillary. Allow the digestion-extraction to proceed for 12 hours.

At the completion of the digestion-extraction period, cool the hexane, and transfer it to a 1-liter separatory funnel, using several washings of acid-washed hexane. Extract the aromatic amines with six 15-ml portions of 1 *N* HCl. Collect the extract in a 100-ml volumetric flask, and dilute to volume with the 1 *N* acid.

(b) *Determination.* Transfer an aliquot of the aromatic amine extract, containing from 3 to 80 μg, to a 50-ml volumetric flask; and dilute to about 35 ml with 1 *N* HCl. Add 5 ml of glacial acetic acid, develop the color by diazotizing and coupling as described under *"Calibration,"* and dilute to volume with 1 *N* acid. Transfer 25 ml of the colored solution to a cellulose powder column and "develop" with five 250-ml portions

[1] Alternatively, the digestion-steam-distillation procedure suggested by Young and Gortner (1953) can be used. Their method requires less specialized equipment and elapsed time but more analyst's time.

of 1 *N* HCl, using suction to obtain a reasonable flow rate. Dyes obtained from naturally occurring interferences separate almost immediately, moving ahead of the *p*-chloroaniline and/or 3,4-dichloroaniline dyes, and washing through the column during development. The dye from monuron progresses to the lower half of the column, and the dye from diuron or neburon is retained in the upper portion of the column.

Drain the column, remove the suction, and cut the column to isolate the desired dyes. Insert new penicillin disks in the shortened sections, and elute the dye by passing 15 to 20 ml of a 1:1 (v/v) mixture of 1 *N* HCl and glacial acetic acid through the column. Collect the colored solution in a 25-ml volumetric flask and dilute to volume with the 1:1 mixture. Measure the absorbance at 560 mμ in 1-cm cells, using distilled water as the reference solution. Determine the number of micrograms of chlorinated aniline from the appropriate calibration curve, and calculate:

$$\text{p.p.m. Monuron} = \frac{(\mu\text{g of } p\text{-chloroaniline})(100)(1.56)}{(\text{wt. sample in gm})(\text{aliquot in ml})}$$

$$\text{p.p.m. Diuron} = \frac{(\mu\text{g of 3,4-dichloroaniline})(100)(1.44)}{(\text{wt. sample in gm})(\text{aliquot in ml})}$$

$$\text{p.p.m. Neburon} = \frac{(\mu\text{g of 3,4-dichloroaniline})(100)(1.70)}{(\text{wt. sample in gm})(\text{aliquot in ml})}$$

iii. *Calibration.*

Prepare standard solutions containing 5 μg of *p*-chloroaniline or 3,4-dichloroaniline per milliliter of 1 *N* HCl. Transfer aliquots containing from 5 to 80 μg of chlorinated aniline to separate 50-ml volumetric flasks, dilute each to approximately 35 ml with 1 *N* acid, and add 5 ml of glacial acetic acid. Pipet 1 ml of 2% sodium nitrite solution into each flask, mix, and allow to stand at room temperature for 15 minutes. Destroy any excess nitrite by adding 1 ml of 10% sulfamic acid solution, mixing, and allowing to stand for 10 minutes. Add 2 ml of 2% *N*-(1-naphthyl)-ethylenediamine dihydrochloride solution, measured by a pipette; dilute to volume with 1 *N* HCl; mix, and allow 15 minutes for full color development.

Determine the absorbance of each solution at 560 mμ using 1-cm cells and distilled water as the reference solution. Plot absorbance vs. chloroaniline concentration expressed as micrograms per 50 ml.

d. *Discussion*

This procedure has been applied successfully to soils, and to a wide variety of plant and animal tissues. Quantitative recoveries have been conclusively demonstrated at levels of 0.05 p.p.m. to at least 20 p.p.m.

in avocados, lemons, olives, grapefruit, apples, pears, oranges, pineapple, raspberries, gooseberries, blueberries, grapes, strawberries, wheat, grain, sorghum, corn, alfalfa, lotus hay, pasture grasses, tomatoes, asparagus, spinach, sweet corn, broccoli, radishes, peanuts, potatoes, celery, beans, peas, onions, beets, soybeans, sugar cane, refined sugar, molasses, cottonseed oil, milk, butterfat, chicken, beef, pork, dog, rat, and quail tissue. In certain substrates, such as cane sugar, the sensitivity has been extended to the parts-per-billion range.

The cellulose column partition step eliminates interferences obtained from naturally occurring substances, and the only contaminants which interfere with this procedure are those which give primary aromatic amines with R_f's closely approaching those of *p*-chloroaniline or 3,4-dichloroaniline on the cellulose column. Aniline, or compounds which hydrolyze to aniline, do not interfere. Unless contamination is suspected, it is unnecessary to run check determinations on untreated portions of the substrate being analyzed.

If sensitivities no better than 0.3 p.p.m. to 0.4 p.p.m. are required, the cellulose column treatment can generally be omitted. In this case, the absorbance of the diazonium dye solution is measured directly, the concentration of apparent chloroaniline is determined from the appropriate calibration curve, and the apparent residue in the sample is calculated. To estimate the actual monuron, diuron, or neburou level, it is necessary to determine the apparent residue in an untreated portion of the substrate, and deduct this "blank" value. Such blanks generally amount to a few tenths of a part per million.

3. RECOMMENDED METHOD—GAS CHROMATOGRAPHIC DETERMINATION

(See also Volume I, Chapter 9).

a. *Reagents*

Spectro-grade n-hexane, Phillips Petroleum Co., Bartlesville, Okla., or freshly distilled dichloromethane, Reagent Grade.

Sodium hydroxide, 50% (w/v), prepared from Reagent Grade material.

Ethanolic phenolphthalein solution, 0.1%.

b. *Special Apparatus*

Separatory funnels (250-ml) with Teflon stopcocks.

Gas chromatograph, model 300 or 500 programmed-temperature (F and M Scientific Corp., Starr Road and Rt. 1, P. O. Box 234, Avondale, Pa.) or equivalent, equipped with a 1-millivolt span, one second-response recorder.

Column—1 meter, ¼" O.D. stainless steel column of 20% (by weight) Apiezon "L" grease (James G. Biddle Co., Philadelphia, Pa.) on 60–80 mesh "Chromosorb" W, Johns Manville Corp., Manville, N. J., treated with 1% potassium hydroxide.

Agla micrometer syringe (Burroughs-Wellcome Co., Tuckahoe, N. Y.).

Flame ionization detector (optional). A suitable unit is available from F and M Scientific Corp., Avondale, Pa., and several other manufacturers. Operate as described in the instruction manual.

c. *Preparation of Column*

Place 10 ml of 1% methanolic KOH and 15 gm of 60–80 mesh "Chromosorb" W in a shallow evaporating dish. Add methanol and mix gently, to produce a "slush." Remove the solvent by heating on a steam or hot water bath, while gently and constantly stirring the mixture with a spatula. When elimination of the solvent appears complete, place the mixture in a vacuum oven at 100° for at least 2 hours.

Isolate the 60–80 mesh fraction by screening. Place 8.0 gm of this material in an evaporating dish and add 2.0 gm of Apiezon "L" grease which has previously been dissolved in 50–75 ml of boiling carbon tetrachloride. Remove the solvent as described above, and dry the mixture in a vacuum oven at 100°C for 2 hours. Pack the resulting dry mixture into the stainless steel tubing using vibration and tapping.

d. *Procedure*

i. *Hydrolysis-Distillation.*

Place up to 250 gm of the sample in a 1-liter round-bottom flask together with 10 ml of Antifoam A, 500 ml of 20% NaOH, and a few carborundum boiling chips. Attach a vertical condenser and heat at a gentle reflux for 2 hours. Arrange the condenser horizontally and distill slowly until 100 ml of distillate has been collected in a 250-ml separatory funnel containing 8 to 9 ml of Reagent Grade HCl.

ii. *Extraction.*

Wash the acidified distillate with two 50-ml portions of *n*-hexane or dichloromethane and discard the organic layers. Add two drops of phenolphthalein indicator solution and neutralize carefully with 50% NaOH. Add one drop of excess 50% NaOH, and cool the solution. Extract with four 20-ml portions of hexane or dichloromethane.

Using an instrument equipped with a thermal conductivity detector, the extract should be concentrated to slightly less than 5 ml by carefully

evaporating the solvent. Transfer the solution to a 5-ml volumetric flask, using a fine dropper to carry out hexane washes, dilute to volume, and chromatograph. (Should higher sensitivity be required, the sample may be similarly concentrated to 1 ml.)

When using a sensitive ionization detector, extract the basic distillate with five 19-ml portions of hexane, dilute to volume in a 100-ml volumetric flask, and chromatograph directly without concentration.

iii. *Chromatography.*

Condition the column by heating at 275°C for at least 24 hours. Then equilibrate, using the following conditions: vaporizer temperature —265°C; detector block—235°C; flow rate—50 cc helium/minute; initial column temperature—75°C; detector sensitivity—maximum. Using the micrometer syringe, inject a 0.50 ml aliquot of the hexane extract into the instrument evenly over a 2-minute period. Allow 2 minutes to elute the solvent, and then start the programmer, which is adjusted to increase the column temperature at the rate of 6.4°C/minute. (The solvent elution period and temperature programming rate can be varied to suit the solvent used and separation needed.) Under the specified conditions, the *p*-chloroaniline ("PCA") and 3,4-dichloroaniline ("DCA") peaks should emerge at approximately 180° and 214°C, respectively.

iv. *Calculations and Calibration.*

Determine the amount of PCA and/or DCA in the total extract from the following relationship:

μg Chlorinated aniline = peak ht. (mm)
$\times$ calibration factor (μg/mm/ml) $\times$ final extract volume (ml).

Calibration factors are determined daily by chromatographing a hexane standard. Using thermal conductivity, 0.50 ml of a solution containing about 40 μg per milliliter of PCA and DCA is employed. With flame ionization, a 0.8 μg per milliliter solution is used. Typical factors for PCA and DCA are 0.301 and 0.351 μg/mm/ml, respectively, for thermal conductivity detection. The concentrations of monuron and diuron in the original sample are calculated according to the equation:

$$\text{p.p.m. Monuron} = \frac{\mu\text{g of PCA in total extract} \times 1.56}{\text{Wt. of original sample in gm}}$$

$$\text{p.p.m. Diuron} = \frac{\mu\text{g of DCA in total extract} \times 1.44}{\text{Wt. of original sample in gm}}$$

$$\text{p.p.m. Neburon} = \frac{\mu\text{g of DCA in total extract} \times 1.70}{\text{Wt. of original sample in gm}}$$

e. Discussion

The sensitivity of the gas chromatographic method for determining monuron, diuron, or neburon residues is limited only by interferences from incompletely separated, unknown materials. This question of possible interferences must be answered for each sample matrix by analyzing untreated materials. Using the method herein described, interferences of less than 0.1 p.p.m. of apparent monuron, diuron, or neburon were found for okra, peanuts, pears, cucumbers, blueberries, corn fodder, carrots, and several types of soils.

Adequate sensitivity for most purposes can be obtained with conventional thermal conductivity detection. However, the use of a highly sensitive ionization detector is advantageous, because it generally eliminates the need for concentrating the extract before chromatographic analysis. Alternately, the more sensitive detector permits the analysis to be carried out on a smaller sample, or a smaller aliquot of extract concentrate. Figure 3 shows a chromatogram of the original hexane extract

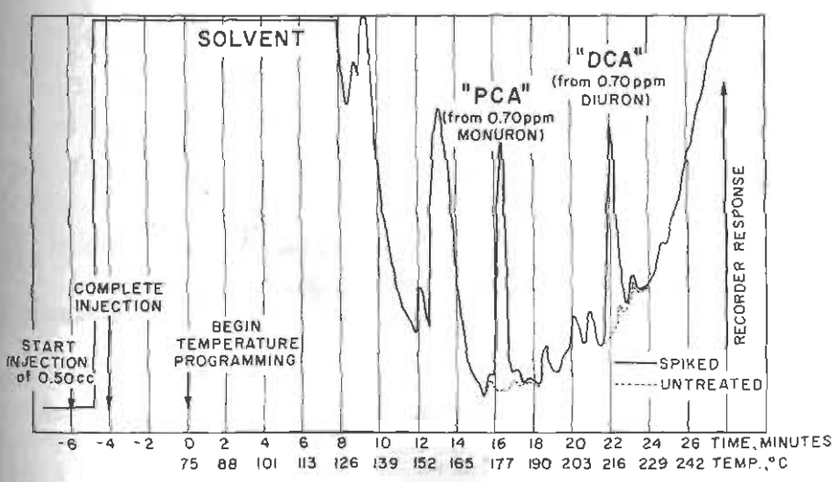


FIG. 3. Gas chromatography of extract from blueberries containing 0.7 p.p.m. monuron and 0.7 p.p.m. diuron.

from the hydrolysis-distillation of a 60-gm sample of blueberries fortified with 0.70 p.p.m. each of monuron and diuron. While a flame ionization detector was used to obtain this chromatogram, a similar scan could be obtained with a thermal conductivity detector by first concentrating the extract as described under "Procedure."

Using thermal conductivity detection, recovery of monuron, diuron, and neburon residues average about 85% of theory. The small losses occur during the final concentration step. Essentially quantitative recoveries are obtained when the original distillate extracts are analyzed directly, using the more sensitive ionization detector.

References

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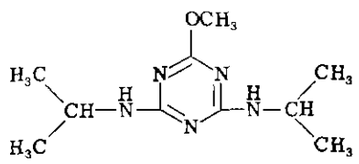
Prometone

E. KNÜSLI



2-Methoxy-4,6-bis-isopropylamino-*s*-triazine

I. GENERAL

A. EMPIRICAL FORMULA

$C_{10}H_{19}N_5O$ (Mol. wt. 225.92).

B. ALTERNATE NAMES

Trial number G 31 435; trade names for general use: Prometone-Geigy; Primatol O.

C. SOURCE OF ANALYTICAL STANDARD

J. R. Geigy S.A., Basel, Switzerland; Geigy Agricultural Chemicals, Ardsley, N. Y.

D. BIOLOGICAL PROPERTIES

1. HERBICIDAL ACTIVITY

Prometone is a pre- and post-emergence herbicide for general and selective use. The potential field for selective application is sugar cane.

2. TOXICITY

Acute oral toxicity: LD_{50} for mouse—2160 mg/kg; rat 2980 mg/kg.

Subchronic toxicity: A series of rats fed for 90 days with a daily oral administration of 20 and 200 μl Prometone 25E/kg was comparable in all behaviors with the controls.

Cutaneous toxicity: A 5% suspension of Prometone 25E in gum

arabic applied for 5 consecutive days to rats caused no signs of adsorptive toxicity.

E. History

H. Gysin and E. Knüsli are co-inventors of Prometone on Swiss Patent 337,019 (to J. R. Geigy S.A., Basel, Switzerland); see also Knüsli (1958).

F. Physical Properties

Melting point: 91–92°C.

Solubility in water at 20°C: 0.075% (750 p.p.m.). Solubility in benzene at 20°C: more than 25%. Solubility in chloroform, methanol, acetone: more than 50%.

Colorless, not combustible, not explosive, and not corrosive.

pK-value in water at 22°: 4.3.

UV spectra: see Fig. 1.

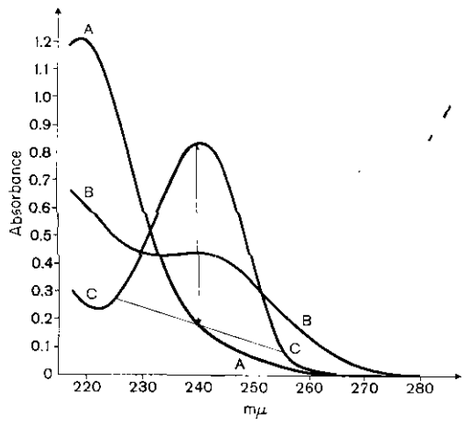


Fig. 1. UV Spectra of A, Prometone in water; B, Prometone in 1 *N* sulfuric acid (cation); and C, product of hydrolysis (= 2-hydroxy-4,6-bis-isopropylamino-*s*-triazine) in 1 *N* sulfuric acid (cation). (Concentration of Prometone = 7 μg/ml in each solution).

G. Chemical Properties

1. Method of Synthesis

Prometone is synthesized by reacting 2-chloro-4,6-bis-isopropylamino-*s*-triazine (see chapter on Propazine in this volume) with methanol in the presence of an equivalent of sodium hydroxide.

2. PROPERTIES

Prometone is distillable; stable in neutral, slightly acidic or basic media. It hydrolyzes to the herbicidally inactive 2-hydroxy-4,6-bis-isopropylamino-*s*-triazine iu an acidic or basic medium, especially at higher temperatures.

H. Formulations

Prometone is available as a 50% wettable powder and a 25% emulsifiable solution.

II. ANALYSIS

A. Formulation Analysis

1. RECOMMENDED METHOD FOR WETTABLE POWDERS

a. *Principle*

This method (Suter, 1959) is based on the titration of one isopropylamino group of Prometone with perchloric acid.

b. *Reagents*

Ether, Analytical Grade.
Glacial acetic acid, stabilized over CrO_3.
Perchloric acid, 0.1 *N* in glacial acetic acid.

c. *Experimental Procedure*

i. *Extraction.*

Weigh accurately 1,500 gm($=w$) of the sample to be tested into a 200-ml Erlenmeyer flask and add 100 ml of ether. Transfer to a water bath and keep gently boiling for about 2 minutes, then pass through a sintered-glass funnel (type 3G4) covered with a filter paper disk. Finally, wash the Erlenmeyer flask and the glass funnel with about 100 ml of ether.

ii. *Titration.*

Transfer to a 300-ml Erlenmeyer flask, evaporate the solvent, dilute the residue with glacial acetic acid and titrate with 0.1 *N* perchloric acid solution potentiometrically, or by using α-naphtholbenzein as indicator ($=a$ ml).

iii. *Discussion of Interferences.*

Other *s*-triazine-derivatives bearing at least two amino groups interfere.

iv. *Sensitivity.*

$\pm 0.5\%$.

v. *Calculation.*

$$\% \text{ Prometone} = \frac{a \times 225.29 \times 100}{w \times 10{,}000} = \frac{a \times 2.253}{w}$$

2. RECOMMENDED METHOD FOR EMULSIFIABLE SOLUTIONS

This method (Suter, 1959) is identical with the method described above for wettable powders. However, the sample (3 gm) is not extracted, but is directly dissolved in 100 ml of glacial acetic acid and titrated.

3. DISCUSSION

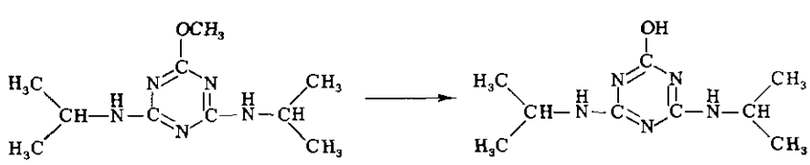
Optionally the methoxy group of Prometone can be determined by the classic method of Zeisel. A sample of wettable powder is extracted exhaustively with ether. The extracted material is dissolved in a mixture of phenol and propionic acid anhydride and demethylated with hydroiodic acid. The methyl iodide formed is absorbed in a measured excess of silver nitrate and the silver nitrate not consumed is back-titrated with 0.1 *N* potassium thiocyanate solution.

B. Residue Analysis

1. RECOMMENDED METHOD—SPECTROPHOTOMETRIC METHOD

a. *Principle*

In this method (Delley, 1960) Prometone residue is extracted with *n*-pentane. Due to its basic properties, the compound may be easily extracted from *n*-pentane with dilute acid. From this aqueous acid solution the readily soluble compound can be re-extracted with chloroform. Because the hydrochloride is also soluble in chloroform, the chloroform extract can be washed with concentrated acid. Finally, the extracted residue is hydrolyzed with 1 *N* H_2SO_4 on a boiling water bath:



The cation of the hydrolysis product shows a maximum absorption at 240 mμ which is used for the spectrophotometric determination.

b. *Reagents*

n-Pentane, distilled.
Chloroform, distilled.
Hydrochloric acid, 18%.
Hydrochloric acid, 0.01 *N*.
Sulfuric acid, 1 *N*.

All the above mentioned aqueous reagents are extracted with some chloroform.

c. *Experimental Procedure*

i. *Extraction.*

Extract the material to be tested with *n*-pentane. Should Prometone be determined in an aqueous solution, make it basic with an excess of sodium hydroxide and extract 2 or 3 times with the same quantity of *n*-pentane. In case the extract should be concentrated, avoid complete evaporation because of the volatility of Prometone.

ii. *Isolation.*

Place 200 ml of the above *n*-pentane extract into a 500-ml separatory funnel and extract three times with 40 ml of 0.01 *N* HCl. Combine the hydrochloric acid extracts in a 200-ml separatory funnel and wash 3 times with 20 ml of chloroform. Wash the chloroform extracts separately and successively in a second 100-ml separatory funnel with 50 ml of 18% HCl. Finally, shake each chloroform extract in a third 100-ml separatory funnel with 50 ml of distilled water. After each portion of chloroform has been washed with 18% HCl and water, combine all of the extracts in a 100-ml Erlenmeyer flask with ground-glass stopper, and evaporate completely by means of a vacuum pump on a water bath kept at 50°C. To avoid any bumping, use a melting point capillary tube, with the opening dipping into the chloroform. Care has to be taken that the residue of Prometone is not exposed to high temperatures any longer than is absolutely necessary. (From a 50-ml beaker which contains a residue of 100 μg of Prometone, 80 μg will evaporate on the boiling water bath, within 30 minutes.)

iii. *Hydrolysis.*

To the dry residue in the 100-ml Erlenmeyer flask add exactly 10 ml of 1 *N* H_2SO_4, stopper the flask and place on a boiling water bath. While heating, release the stopper from time to time, then shake to

dissolve all sediments sticking to the glass, and continue heating for 3 hours. Cool and mix; then transfer the solution to a quartz cell for the determination.

iv. *Determination.*

Use a spectrophotometer Beckman DU, a hydrogen lamp, and quartz cells. Measure at wavelengths of 255, 240, and 225 mμ, and use a slit width of 0.7 mm. For reference, use a cell filled with distilled water.

v. *Discussion of Interferences.*

Other 2-alkoxy- and also 2-alkylthio-4,6-diamino-*s*-triazines may interfere.

vi. *Sensitivity.*

The limit of determination is about 2 μg. The accuracy depends on the different substrates analyzed and is in the range of 0.1 p.p.m.

vii. *Calculation.*

The extinction difference is calculated by using the following equation:

$$\Delta = E^{240} - \left(\frac{E^{255} + E^{225}}{2}\right)$$

From this extinction difference, Δ, subtract the extinction difference obtained in the *blank determination* of the same but untreated, Prometone-free material. Should no untreated material be available, the blank value of the reagents can at least be determined. The blank may be negative, in which case it is to be added instead of subtracted. With the corrected extinction difference, Δ, the quantity of the originally present Prometone can be calculated as follows:

$$\mu\text{g of Prometone} = 113 \times \Delta_{\text{corr.}}$$

The figure 113 is the instrument factor.

2. DISCUSSION OF METHOD

If the sample contains more than 150 μg of Prometone (E^{240} higher than 1.8), a larger amount of water is to be used for dilution. Such a dilution has to be considered in the final calculation.

The hydrolysis product remains perfectly stable in the dilute acid solution. Any other hydrolysis product formed before the extraction is not determined by the above described method.

Examples of extinction values are shown in Table I.

TABLE I
EXTINCTION VALUES FOR PROMETONE[a]

	E^{225}[b]	E^{240}	E^{235}	Δ
Blank value of the reagents	0.041	0.017	0.014	−0.011
50 μg of Prometone	0.231	0.592	0.092	+0.430
100 μg of Prometone	0.422	1.180	0.166	+0.886

[a] Using a Beckman DU Spectrophotometer. If another instrument is used, the figures have to be determined accordingly.
[b] E^x = Extinction at x mμ.

Distribution coefficients in different solvent systems: In the course of the development of the present procedure, some approximate distribution coefficients at analysis conditions (concentration in organic phase: concentration in aqueous phase) were found. These values are presented in Table II.

TABLE II
DISTRIBUTION COEFFICIENTS FOR PROMETONE IN SEVERAL SOLVENT SYSTEMS

		Distribution Coefficients
		Concentration in org. Phase / Concentration in aq. Phase
Organic phase	Aqueous phase	
$CHCl_3$	0.01 N H_2SO_4	20.00
$CHCl_3$	0.10 N H_2SO_4	3.00
$CHCl_3$	1.00 N H_2SO_4	0.30
$CHCl_3$	0.01 N HCl	18.00
$CHCl_3$	0.10 N HCl	5.50 (minimum)
$CHCl_3$	1.00 N HCl	20.00
$CHCl_3$	about 6.00 N HCl	35.00
n-pentane	water	9.00
n-pentane	1% CH_3COOH	0.40
n-pentane	0.001 N H_2SO_4	0.40
n-pentane	0.01 N H_2SO_4	0.05
n-pentane	0.01 N HCl	0.02
n-pentane	0.10 N H_2SO_4	0.03

Contrary to the chloroform/sulfuric acid distribution, there is a minimum in the case of chloroform/0.1 N hydrochloric acid. It seems that the hydrochloride is also soluble in chloroform. In fact, Prometone, after

extraction from hydrochloric acid (1:1), is present in a large amount in the form of the hydrochloride.

Hydrolysis rate of Prometone: Prometone when hydrolyzed with 1 *N* sulfuric acid on the boiling water bath has a half-life of 15 minutes.

References

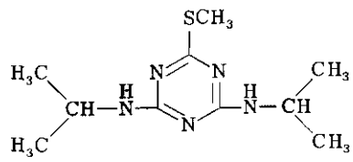
Delley, R. (1960). Anal. Lab., J. R. Geigy S.A., Basel, Switzerland, unpublished work.

Knüsli, E. (1958). *Phytiat.-Phytopharm.* 7, 85.

Suter, R. (1959). Anal. Lab., J. R. Geigy S.A., Basel, Switzerland, unpublished work.

Prometryne

E. KNÜSLI



2-Methylthio-4,6-bis-isopropylamino-*s*-triazine

I. GENERAL

A. EMPIRICAL FORMULA

$C_{10}H_{19}N_5S$ (Mol. wt. 241.35).

B. ALTERNATE NAME

Trial number G 34 161.

C. SOURCE OF ANALYTICAL STANDARD

J. R. Geigy S.A., Basel, Switzerland; Geigy Agricultural Chemicals, Ardsley, N. Y.

D. BIOLOGICAL PROPERTIES

1. HERBICIDAL ACTIVITY

Prometryne is a pre- and post-emergence herbicide for general and *selective use. The potential fields for selective application are: small* grains, potatoes, peanuts, soybeans, beans (*Phaseolus* spp.), peas, alfalfa, carrots and other Umbelliferae crops, and cotton.

2. TOXICITY

Acute oral toxicity: LD_{50} for mouse and rat 3,750 mg/kg.

Subchronic toxicity: A series of rats fed for 90 days with a daily oral administration of 100 mg Prometryne 50 W/kg was comparable in all respects with the controls.

Cutaneous toxicity: A 5% suspension of Prometryne in gum arabic applied for 5 consecutive days to rats showed no signs of adsorptive

toxicity. Prometryne 25E applied at the same rate caused very slight toxic symptoms.

E. History

H. Gysin and E. Knüsli are co-inventors of Prometryne on the Swiss Patent 337,019 (to J. R. Geigy S.A., Basel, Switzerland). See also the publication "Activity and Mode of Action of Triazine Herbicides," 4th Brit. Weed Control Conf., Brighton, 1958.

F. Physical Properties

Melting point: 118–120°C.

Solubility in water at 20°C: 0.0048% (48 ppm); in organic solvents, high.

Colorless, not combustible, not explosive, and not corrosive.

UV spectrum: same as for Ametryne (see this volume, Chapter 2).

G. Chemical Properties

1. Method of Synthesis

Prometryne is synthesized by reacting 2-chloro-4,6-bis-isopropylamino-*s*-triazine (Propazine; see this volume) with methylmercaptan in the presence of an equivalent of sodium hydroxide or by reacting 2-mercapto-4,6-bis-isopropylamino-*s*-triazine with a methylating agent in the presence of sodium hydroxide.

2. Properties

Prometryne is distillable; stable in neutral, slightly acidic or basic media; hydrolyzes to the herbicidally inactive 2-hydroxy-4,6-bis-isopropylamino-*s*-triazine in an acidic or basic medium, especially at higher temperatures.

H. Formulations

Prometryne is available as a 50% wettable powder and a 25% emulsifiable solution.

II. ANALYSIS

A. Formulation Analysis

1. Recommended Method for Wettable Powders

a. *Principle*

This method (Suter, 1960) is based on the titration of one alkylamino group of Prometryne with perchloric acid.

b. *Reagents*

Ether, Analytical grade.
Perchloric acid, 0.1 *N* in glacial acetic acid.

c. *Experimental Procedure*

i. *Sample Preparation.*

Weigh about 1 gm of the sample to be tested ($= w_1$) into a 200-ml Erlenmeyer flask and add about 70 ml of ether. Boil for a short while on a water bath and then filter the contents through a glass-sintered funnel of the 3 G 3 type covered with a round filter paper. Repeat this extraction procedure twice always using the remaining residue, and finally wash the Erlenmeyer flask and the glass funnel with ether.

Distill off the solvent. Dilute the solid residue in glacial acetic acid and titrate with 0.1 *N* perchloric acid potentiometrically ($= a$ ml), using 0.5-ml portions only near the end point.

ii. *Discussion of Interferences.*

2-Chloro-, 2-hydroxy-, 2-mercapto-4,6-bis-isopropylamino-*s*-triazine and any other triazine derivatives bearing at least two amino groups interfere. The major part of the three first-mentioned products will not be extracted however, due to their low solubility in ether.

iii. *Sensitivity.*

±0.5%.

iv. *Calculation.*

$$\% \text{ Prometryne} = \frac{a \times 241.37 \times 100}{w_1 \times 10{,}000} = \frac{a \times 2.414}{w_1} \qquad (1)$$

2. ALTERNATE RECOMMENDED METHOD FOR WETTABLE POWDERS

a. *Principle*

By boiling with diluted sulfuric acid, Prometryne is split into 2-hydroxy-4,6-bis-isopropylamino-*s*-triazine and methylmercaptan. The latter compound is distilled into an iodine solution and oxidized and the excess iodine is back-titrated. (Method of Suter, 1960.)

b. *Apparatus*

See Fig. 1.

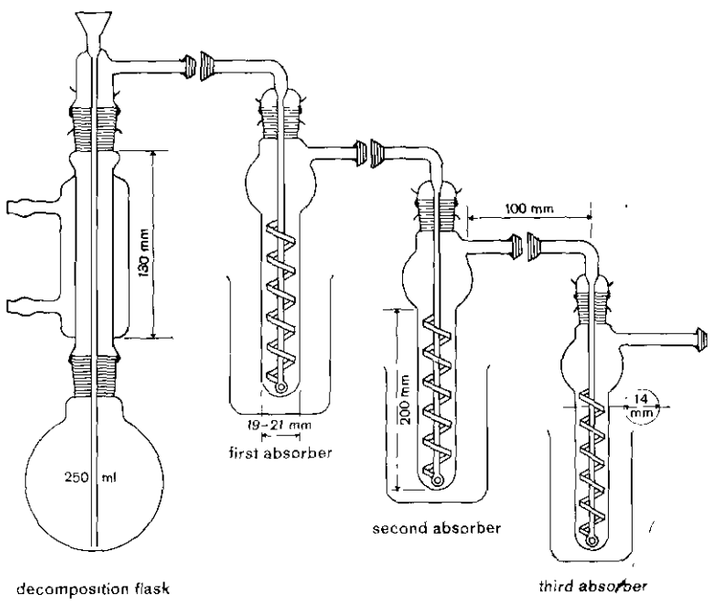


FIG. 1. Apparatus for the analysis of Prometryne formulations (all absorbers cooled by ice).

c. *Reagents*

First absorber to contain: 40 ml of 0.1 *N* iodine solution, 1 gm of solid potassium iodide, 3 drops of glacial acetic acid.

Second absorber to contain: 10 ml of 0.1 *N* iodine solution, 30 ml of distilled water, 1 gm of solid potassium iodide, 3 drops of glacial acetic acid.

Third absorber to contain: 25 ml of a 30% potassium iodide solution.

Ether, Analytical Grade.

Sodium thiosulfate solution, 0.1 *N*.

d. *Procedure*

i. *Sample Preparation.*

Extract 1 gm ($= w_2$) of the sample exhaustively with ether. Transfer the extract into the dry decomposition flask and evaporate cautiously to dryness. Assemble the apparatus. Connect the third absorber to a water pump, inserting ahead of it a screw compressor clamp and a

Woulf flask.[1] Regulate the air stream to about 5 bubbles per second. Now allow 50 ml of boiling 20% sulfuric acid to pass through the funnel tube into the decomposition flask and carefully heat to boiling over a gas flame. The solution should not rise into the funnel tube. Keep the contents of the flask boiling for 1 hour by constantly sucking air through the system.

Afterward, riuse the solutions of the three absorber flasks with distilled water quantitatively into a 600-ml beaker and titrate with 0.1 *N* sodium thiosulfate solution, using starch as indicator.

Amount of 0.1 *N* sodium thiosulfate solution used = *b* ml.

ii. Discussion of Interferences.

Other 2-methylthio-4,6-diamino-*s*-triazines interfere.

iii. Sensitivity.

±0.5%.

iv. Calculation.

$$\% \text{ Prometryne} = \frac{(50.0 - b) \times 241.37 \times 100 \times 1.015}{w_2 \times 10{,}000} = \frac{(50.0 - b) \times 2.450}{w_2} \quad (2)$$

1.015 = correction factor, since formation of methyl mercaptan is not quantitative.

B. Residue Analysis

1. Recommended Method—Spectrophotometric Method

a. *Principle*

In this method (Delley, 1961) the Prometryne residue is extracted with *n*-pentane. Due to the basic properties of Prometryne, the compound may be easily extracted with acid. From this aqueous acid solution the compound can be re-extracted with methylene chloride. The extract is then hydrolyzed with dilute acid on a boiling water bath:

[1] A Woulf flask is a type of trap, normally fitted with 2–3 necks and with a stop cock at the bottom, which protects the reaction vessel (in the given case, the absorber) from water which might be sucked back from the water pump.

The cation of the hydrolysis product shows a maximum absorption at 240 mμ, which is used for the spectrophotometric determination.

b. *Reagents*

n-Pentane, distilled.
Methylene chloride, distilled.
Hydrochloric acid, 0.1 *N*.
Sulfuric acid, 1 *N*.

c. *Experimental Procedure*

i. *Extraction and Isolation.*

Extract the material to be tested with 200 ml of *n*-pentane.

A system of three separatory funnels is used for the isolation of Prometryne. Pour the extract into the first separatory funnel and extract three times with 70-ml portions of 0.1 *N* hydrochloric acid. Wash the hydrochloric acid extracts, which now contain Prometryne, separately and successively in a second separatory funnel containing 100 ml of *n*-pentane. After separation, combine the three acid extracts in a third separatory funnel. From the combined hydrochloric acid extracts (about 210 ml), re-extract the Prometryne by shaking three times with 50-ml portions of methylene chloride. Evaporate carefully the three methylene chloride extracts successively using the same 100-ml ground-joint Erlenmeyer flask.

ii. *Hydrolysis.*

To the dry residue in the 100-ml Erlenmeyer flask add exactly 10 ml of 1 *N* sulfuric acid, stopper the flask and place it on a boiling water bath. While heating, release the stopper from time to time, and continue heating for 3 hours. Cool and mix. Then transfer the solution to a quartz cell for determination.

iii. *Determination.*

Use a spectrophotometer (Beckman DU), a hydrogen lamp, and qnartz cells. Measure at wavelengths of 255, 240, and 225 mμ, using a slit width of 0.7 mm. As reference use a cell filled with distilled water.

iv. *Discussion of Interferences.*

Other 2-alkylthio- and also 2-alkoxy-4,6-diamino-*s*-triazines interfere.

v. *Sensitivity.*

The limit of determination is about 2 μg. The recoveries in standard

determinations with addition of known amounts of Prometryne varied from 85–100%. The sensitivity depends on the different substrates and may range between 0.1 and 0.3 p.p.m.

vi. *Calculation.*

The extinctions measured are calculated by using the following equation:

$$\Delta = E_{240} - \left(\frac{E_{255} + E_{225}}{2}\right)$$

From this extinction difference, Δ, subtract the extinction difference obtained in the blank determination of the same but untreated, Prometryne-free, material. Should no untreated material be available, the blank valne of the reagents shonld at least be determined. The blank can be negative, in which case it is to be added instead of subtracted. From the corrected extinction difference, Δ_{corr}, the quantity of the originally present Prometryne can be calculated as follows:

$$\mu g \text{ of Prometryne} = 121\Delta_{corr.}$$

The figure 121 is the instrument factor of the Beckman DU used. Some

TABLE I
EXTINCTION VALUES FOR PROMETRYNE[a]

	E^{225}[b]	E^{240}	E^{255}	Δ
Blank value of the reagents	0.061	0.051	0.034	0.004
8.1 μg of Prometryne	0.082	0.127	0.036	0.068
40.5 μg of Prometryne	0.179	0.453	0.079	0.324
81.0 μg of Prometryne	0.322	0.890	0.130	0.664

[a] Beckman DU Spectrophotometer. If another type of instrument is used, the figures have to be determined accordingly.
[b] E^x = Extinction at x mμ.

extinction values obtained with this instrument are shown in Table I.

2. APPLICABILITY OF THE METHOD TO DIFFERENT CROPS

The method has been applied for the determination of residues in wheat, barley, peas, and beans.

3. DISCUSSION

a. *Distribution Coefficients in Different Solvent Systems*

In the course of the development of the present procedure, some approximate distribution coefficients at analysis conditions (concentra-

TABLE II
DISTRIBUTION COEFFICIENTS FOR PROMETRYNE IN DIFFERENT SOLVENT SYSTEMS[a]

Organic phase	0.1 *N* NaOH	Water	0.001 *N* HCl	0.01 *N* HCl	0.1 *N* HCl	1 *N* HCl
n-pentane	*>100*	*>100*	*8*	*0.9*	*0.09*	*0.008*
Ether	>100	>100	—	15	1.3	0.3
CH_2Cl_2	>100	>100	—	80	16	34
$CHCl_3$	>100	>100	—	>100	46	99

[a] *Concentration in organic phase:concentration in aqueous phase.*

tion in organic phase:concentration in aqueous phase) were determined. These are reported in Table II.

It seems that with methylene chloride and chloroform the active ingredient is partly extracted from 1 *N* hydrochloric acid in form of Prometryne hydrochloride.

b. *Comparative Behavior of Prometryne and Ametryne*

Compared with Ametryne, Prometryne's solubility in organic solvents is higher. Therefore, a larger volume of 0.1 *N* HCl in the otherwise similar analytical procedure has to be used.

The UV spectra of Prometryne are practically identical with those of Ametryne. Both substances hydrolyze at the same rate. When hydrolyzed with 1 *N* sulfuric acid on the boiling water bath, the half-life period is about 20 minutes; at room temperature it is about 1 month. During hydrolysis, a characteristic mercaptan odor is observed.

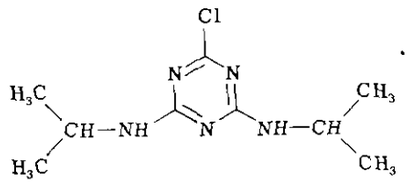
REFERENCES

Delley, R. (1961). Anal. Lab., J. R. Geigy S.A., Basel, Switzerland, unpublished work.

Suter, R. (1960). Anal. Lab., J. R. Geigy S.A., Basel, Switzerland, unpublished work.

Propazine

E. KNÜSLI



2-Chloro-4,6-bis-isopropylamino-*s*-triazine

I. GENERAL

A. EMPIRICAL FORMULA

$C_9H_{16}ClN_5$ (Mol. wt. 229.71).

B. ALTERNATE NAMES

Trial number G 30 028. Trade names for selective use: Propazine-Geigy; Gesamil.

C. SOURCE OF ANALYTICAL STANDARD

J. R. Geigy S.A., Basel, Switzerland; Geigy Agricultural Chemicals, Ardsley, N. Y.

D. BIOLOGICAL PROPERTIES

1. HERBICIDAL ACTIVITY

Propazine is a pre-emergence herbicide for general and selective use. The actual and poteutial fields of selective application are: sorghum, millet; carrots and other Umbelliferae crops; conifers.

2. TOXICITY

Acute oral toxicity: LD_{50} for mouse and rat more than 5,000 mg/kg.

Chronic toxicity: A series of rats fed for 180 days with a daily oral administration of 500 mg of Propazine 50 W/kg was comparable in all behaviors with the controls.

Cutaneous toxicity: A 5% suspension of Propazine or Propazine 50 W in gum arabic applied for 5 consecutive days to rats caused no signs of absorptive toxicity.

E. History

H. Gysin and E. Knüsli are co-inventors on Swiss Patents 329,277 and 342,784 (to J. R. Geigy S.A., Basel, Switzerland). See also "Introductory Remarks on the Chemistry and Herbicidal Properties of Triazine Derivatives," 3rd Brit. Weed Control Conf., Blackpool, 1956.

F. Physical Properties

Melting point: 212–214°C.

Solubility in water at 20°C: 0.00086% (8.6 p.p.m.); in organic solvents, low.

Colorless, not combustible, not explosive, and not corrosive.

UV spectra: practically identical with those given for "Simazine" (see Chapter 23 of this volume).

G. Chemical Properties

1. Method of Synthesis

Propazine is synthesized by reacting cyanuric chloride with two equivalents of isopropylamine in the presence of an acid acceptor.

2. Properties

Propazine sublimates at higher temperatures; stable in neutral, slightly acidic or basic media; hydrolyzes to the herbicidally inactive 2-hydroxy-4,6-bis-isopropylamino-*s*-triazine in acidic or basic media, especially at higher temperatures.

H. Formulations

Propazine is available as an 80% wettable powder; a 50% wettable powder; and as 0.5% granules.

II. ANALYSIS

A. Formulation Analysis

1. Potentiometric Method for Wettable Powders

a. *Principle*

This method (Suter, 1957–1959) is similar to that given for simazine (see this volume). However, the respective equation is:

$$\% \text{ Propazine} = \frac{(a - b) \times 229.72 \times 100}{w \times 10{,}000} \quad (1)$$

b. *Discussion of Interferences*

A contamination of 2,4-dichloro-6-isopropylamino-*s*-triazine simulates a higher content of Propazine than is effectively present. The method does not differentiate from the analogous 2-chloro-4,6-bis-amino-*s*-triazines, such as 2-chloro-4-amino-6-isopropylamino-*s*-triazine.

2. SUPPLEMENTARY METHOD FOR WETTABLE POWDERS

a. *Principle*

This method (Suter, 1957–1959) is similar to that described for simazine (see this volume). However, the respective equation is:

$$\% \text{ Propazine} = \frac{c \times 229.72 \times 100}{w \times 10{,}000} \quad (2)$$

b. *Discussion of Interferences*

The presence of similar 2-chloro-4,6-bis-amino-*s*-triazines, 2,4,6-tris-isopropylamino-*s*-triazine, or 2-hydroxy-4,6-bis-isopropylamino-*s*-triazine, is included in the value obtained. 2,4-Dichloro-6-isopropylamino-*s*-triazine does not respond to the titration with perchloric acid.

3. CONTENT OF 2,4-DICHLORO-6-ISOPROPYLAMINO-*s*-TRIAZINE OR 2,4,6-TRIS-ISOPROPYLAMINO-*s*-TRIAZINE

If the values obtained from Eqs. (1) and (2) are different, the approximate contents of 2,4-dichloro-6-isopropylamino-*s*-triazine or 2,4,6-tris-isopropylamino-*s*-triazine are calculated as follows:
If the value of Eq. (1) is higher than Eq. (2), then Eq. (2) = content of Propazine

$$\% \text{ 2,4-Dichloro-6-isopropylamino-}s\text{-triazine} = \frac{[\text{Eq. (1)} - \text{Eq. (2)}] \times 207.07}{229.72 \times 2} \quad (3)$$

If the value of Eq. (2) is higher than Eq. (1), then Eq. (1) = content of Propazine

$$\% \text{ 2,4,6-Tris-isopropylamino-}s\text{-triazine} = \frac{[\text{Eq. (2)} - \text{Eq. (1)}] \times 252.37}{229.72} \quad (4)$$

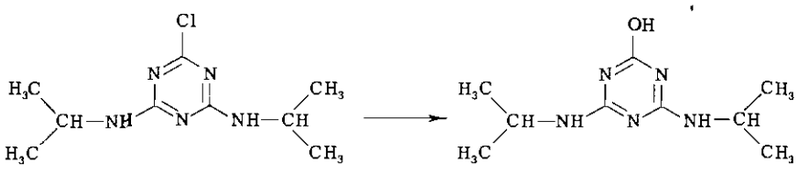
Discussion of interferences: The method does not distinguish between analogous 2,4-dichloro-6-amino-*s*-triazines or 2,4,6-tris-amino-*s*-triazines.

B. Residue Analysis

The methods follow, in general, those given for simazine (see this volume).

1. Spectrometric Method

See chapter on simazine, Section II,C,2, "Spectrometric method" (Delley, 1957).



The cation of the hydrolysis product shows a maximum absorption at 240 mμ, as does the cation of the hydrolyzation product of simazine.

a. *Column Clean-up*

In principle the column clean-up follows the one described for simazine (see this volume, Chapter 23 on simazine, Section II,C,2, c,ii). Instead of Woelm basic aluminium oxide, activity grade V, the more retentive grade IV has to be used (Gigger *et al.*, 1961).

b. *Sensitivity and Recovery*

The sensitivity of the method varies somewhat with different substrates but lies around 0.1 p.p.m. In absolute terms, quantities of 2 μg may be detected. The recoveries in standard determinations, with addition of known amounts of Propazine varied from 80–100%.

c. *Applicability of the Method to Different Crops*

The method has been successfully applied to the determination of residues in corn and millet.

2. Colorimetric Method

See Chapter 23 on simazine, Section II,C,4—"Colorimetric method" (Storrs and Burchfield, 1963).

References

Delley, R. (1957). Anal. Lab., J. R. Geigy S.A., Basel, Switzerland, unpublished work.

Gigger, R. P., Mattson, A., and Halama, P. (1961). Anal. Lab., Geigy Chem. Co., Ardsley, New York, unpublished work.

Storrs, E. E., and Burchfield, H. P. (1963). *Contribs. Boyce Thompson Inst.* In press.

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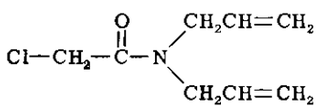
Randox

R. A. Conkin and L. S. Gleason



N-N-Diallyl-2-chloroacetamide (CDAA)

I. GENERAL

A. Empirical Formula

$C_8H_{12}ONCl$ (Mol. wt. 173.63).

B. Alternative Names

CP6343, CDAA.

C. Source of Analytical Standard

Monsanto Chemical Company, 800 North Lindbergh Blvd., St. Louis 66, Missouri. The name Randox is the registered trademark of the Monsanto Chemical Company.

D. Biological Properties

CDAA is a pre-emergence herbicide which controls the germinating seed of the following weeds: annual blue grass, carpet weed, crabgrass, green foxtail, purslane, yellow foxtail, barnyard grass, cheat, giant foxtail, pigweed, water grass, and stinkgrass (Hannah, 1954).

Randox has been granted label clearance for use in the following crops: cabbage (Wisc. & Fla.), canning peas, celery (Fla.), chrysanthemums (Calif.), dry beans (E. of Rocky Mountains), field or sweet corn, forage or grain sorghum, gladiolus (Calif.), lima beans (E. of Rocky Mountains), iris (Calif.), onions, popcorn, snap beans (E. of Rocky Mountains), soybeans (E. of Rocky Mountains), sugar cane (Fla.).

CDAA will not control established plants or plants originating from vegetative plant parts. Neither has this compound been known to control dormant seeds.
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The acute oral LD_{50} of CDAA for rats is 760 mg/kg of body weight. The MLD of Randox by skin absorption is 360 mg/kg for rats. On the basis of these tests, Randox is classed as moderately toxic to man. Randox is considered a severe skin irritant and a moderately severe eye irritant.

E. History

CDAA was discovered by Drs. John A. Speziale and Phillip C. Hamm while working for the Monsanto Chemical Company. The composition of matter and use properties of Randox are covered by U. S. Patent 2,864,683.

Randox was first granted label clearance in 1957 for use on nine crops. Since then, clearance has been granted for additional uses.

F. Physical Properties

Boiling point: 74°C at 0.3 mm Hg
Solubility:

Solvent	%	Solution point °C
Xylene	20	4
Kerosene	20	49
Chloroform	50	−50
Water	1.97	25
Ethyl alcohol	50	−18
Cyclohexanone	50	−50
Chlorobenzene	50	−50
Hexane	20	36

Refractive index (N_D^{25}): 1.4932
Appearance: Amber liquid at room temperature
Specific gravity: 1.088 at 25/15.6°C

G. Chemical Properties

1. Method of synthesis

The reaction of diallyl amine with chloroacetyl chloride in presence of dilute sodium hydroxide produces CDAA.

2. Chemical reactions

CDAA will decompose in the presence of mineral acids or bases.

H. Commercial Formulations

Randox is available in a 4 lbs/gallon emulsifiable concentrate and 20% concentration granules.

II. ANALYSIS

A. Formulation Analysis

1. REVIEW OF METHODS

Liquid or granular Randox formulations may be analyzed for their CDAA content by any standard method for the determination of total organic chlorine. Methods other than the one described below should be thoroughly evaluated with an analytical reference standard.

2. RECOMMENDED METHOD

a. *Principle*

The sodium-isobutyl alcohol reductiou of organic chlorine to inorganic chloride is the basis of this method. The organically bound chlorine is quantitatively liberated as chloride by refluxing with an alcoholic-sodium mixture; and is determined by titration with silver nitrate solution.

b. *Reagents*

Isobutyl alcohol, C.P., (low chlorides).
Metallic sodium, C.P.
Silver nitrate, standard solution, 0.1 *N.*
Methanol-water mixture, 50% v/v.
Nitric acid, 50% v/v.
Phenolphthalein indicator solution.

c. *Apparatus*

Condenser, Allihn-type.
Round-bottom flask; 100-ml.
pH indicator, Leeds & Northrop Model 7664 or equivalent.
Silver: potassium sulfate—mercurous sulfate electrode system or equivalent.

d. *Experimental Procedure*

Weigh accurately (±0.0001 gm) sufficient sample to provide 2.0-2.5 milliequivalents of chlorine, into a 100 ml round-bottomed flask. For

CDAA, 0.35–0.45 gm will suffice while Randox granules (20%) will require from 1.75–2.25 gm.

Add 20 ml of Reagent Grade isobutyl alcohol and 2 gm of metallic sodium cut into ¼-inch pieces. Connect the flask to a reflux condenser. Allow the sodium to react for 10 minutes without applying heat, then heat to reflux with an electric heating mantle. Shut off the heat 30 minutes after addition of the sodium. The mantle should be removed at this time.

Allow the reaction mixture to cool for 5 minutes. Add slowly, through the condenser, 20 ml of 1:1 methanol-distilled water mixture. Replace the heating mantle and heat to reflux for 5 minutes. Cool to room temperature and add 10 ml of acetone through the condenser. Remove the condenser, add three drops of 0.1% phenolphthalein indicator solution, and neutralize with a 1:1 nitric acid solution. The acid should be added in portions, with swirling after each addition. The final solution should be acid to Congo Red. Cool to room temperature.

Transfer the neutralized solution to a 250 ml beaker, rinsing the flask three times with distilled water (20 ml total) and three times with acetone (20 ml total). Place the beaker under the electrode system and titrate potentiometrically with 0.1 *N* silver nitrate.

Calculation:

$$\% \text{ Total chlorine in sample} = \frac{\text{ml } 0.1\ N\ AgNO_3 \times 0.35457}{\text{Wt. of sample}}$$

A correction for possible inorganic chlorides should be made when analyzing either the technical or formulated material. Weigh a 5.0-gm sample of technical or 20.0 gm of granules into a 250-ml beaker. Add 100 ml of acetone, (20 ml of distilled water plus 80 ml of acetone for granules) a drop of concentrated sulfuric acid and stir. Titrate under the same electrode system as above.

Calculation: See equation given above.

$$\% \text{ Total chlorine} - \% \text{ inorganic chlorine} = \% \text{ organic chlorine}$$
$$\% \text{ Organic chlorine} \times 4.8971 = \text{CDAA}$$

B. Residue Analysis

1. review of methods

Earlier residue studies were carried out by means of C^{14}-labeled material.

CDAA can be hydrolyzed by caustic to give the corresponding secondary amine. The amine may be recovered by distilling from an

alkaline solution. Reaction with cupric dithiocarbamate results in the formation of a highly colored complex.

2. RECOMMENDED METHOD

a. *Principle*

CDAA can be hydrolyzed by caustic to give the corresponding secondary amine. The amine may be recovered by distillation from an alkaline solution and trapping the free amine in dilute HCl (Dowden, 1938; Feigl, 1956; Hall *et al.*, 1951). Interferences extracted from crop samples are essentially eliminated by washing the solvent extract with dilute HCl. A further reduction of the background material is made by a solvent wash of the acidified distillate. This combination of washes effectively removes traces of free amines in the initial solvent extract and colored pigments that may steam distill with the free amine.

b. *Reagents*

Chloroform, USP.
Chloroform-carbon disulfide mixture, 1% CS_2 (v/v).
Sulfuric acid, 10% solution.
Carbon disulfide, A.R.
Ammonium hydroxide, A.R.
Copper sulfate pentahydrate, A.R.
Sodium hydroxide, 50% solution.
Sodium sulfate, anhydrous, granular.

Copper reagent. Dissolve 0.5 gm of copper sulfate in 10 ml of distilled water, and dilute to 500 ml with concentrated ammonium hydroxide.

Standard diallylamine Solution. Weigh 0.1000 gm of diallylamine into a 100-ml volumetric flask containing 5 ml of 0.1 *N* HCl. Dilute to volume with distilled water. Pipette a 1-ml aliquot (equivalent to 1000 μg) and transfer to a second 100-ml volumetric flask. Dilute to volume with distilled water. Each milliliter will contain 10 μg of diallylamine.

c. *Apparatus*

See Fig. 1.

d. *Experimental Procedure*

i. Sample Preparation.

Using a Waring Blendor or similar apparatus, grind a 300–500-gm sample of the crop. Vegetable crops are best handled by quick freezing the samples. This greatly aids grinding. Transfer the ground mass to a

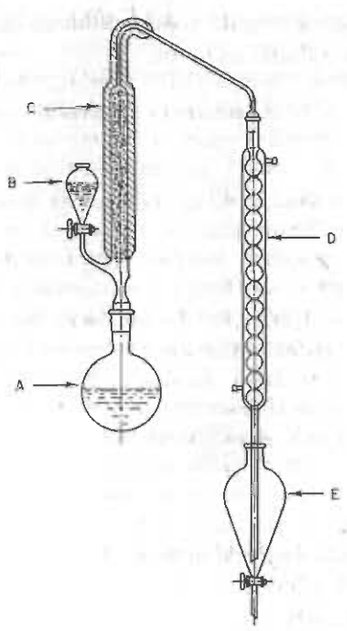


FIG. 1. Hydrolysis and distillation apparatus for Randox. (A) Round-bottom flask with glass joint; (B) sidearm funnel; (C) column packed with glass beads; (D) condenser; (E) collection funnel.

2000-ml wide-mouth jar, using a volume of chloroform equal to the sample weight (1 ml of the solvent to 1 gm of the sample). Do not macerate in the presence of chloroform, as a tight emulsion will form. Tumble the samples for 1 hour. Filter and discard the pulp. Transfer the filtrate to a separatory funnel and wash with three 100-ml portions of 10% (v/v) HCl. Draw off the chloroform (lower) layer into a suitably sized bottle for storage. Store in a refrigerator until ready for use. Discard the dilute HCl washes.

Measure an aliquot of the chloroform equivalent to 100 gm of the crop sample and transfer to a 250-ml round-bottom flask. Add 25 ml of 10% H_2SO_4 and place the flask on the steam bath. Add two or three carborundum boiling stones to prevent bumping. Evaporate the chloroform in the presence of the sulfuric acid.

Connect the flask to a take-over distillation unit equipped with a separatory funnel side-arm arrangement. Immerse the condenser tip in 5 ml of 0.1 *N* HCl contained in a 250-ml separatory funnel. (Keep the tip immersed at all times during and after the introduction of caustic.) (See Fig. 1.)

Introduce through the side-arm separatory fuunel, 30 ml of 50% NaOH. Swirl, or gently agitate the mixture as the caustic is slowly added, to prevent the sudden liberation of excess heat. Distill the liberated amine into the collection funnel, keeping the tip of the condenser beneath the surface of the dilute acid at all times. Collect 40 to 50 ml of the distillate, which is usually sufficient for quantitative recovery of amine.

Remove the collection funnel, cool to room temperature, and proceed with the color formation steps as outlined in the section under "Preparation of Standard Curve." Convert the absorbance at 435 mμ to micrograms of diallylamine by use of the standard curve.

ii. *Preparation of Standard Curve.*

Pipette 1, 4, 7, and 10-ml aliquots of the standard diallylamine solution and transfer to 125-ml separatory funnels. Dilute with distilled water to 50 ml. Add 10 ml of chloroform-carbon disulfide mixture, 5 ml of copper reagent, and 1 to 2 ml of 50% NaOH. Shake for 3 minutes, allow to separate. Draw off the chloroform (lower) layer into a 25-ml Erlenmeyer flask and dry over 1–2 gm of sodium sulfate until the colored solution is free of cloudiness. Occasional swirling may be necessary.

Decant a portion of the dry solution into a cuvette and measure the absorbance at 435 mμ using distilled water as a reference. Any standard spectrophotometer may be used for this purpose.

iii. *Calculation.*

$$\text{p.p.m. CDAA} = \frac{\text{Micrograms diallylamine}}{\text{Aliquot sample weight (grams)} \times 0.56}$$

Correct the parts per million of CDAA for per cent recovery as determined from a series of untreated crop samples to which known amounts of CDAA were added.

3. APPLICABILITY OF METHOD TO DIFFERENT CROP OR FOOD MATERIALS

The method is applicable to a variety of vegetable crops with no special difficulties.

4. DISCUSSION OF METHOD

The use of chloroform as the extraction solvent often leads to difficulties with the formation of tight emulsions. The use of anhydrous granular sodium sulfate in amounts up to that equal to the sample weight will greatly aid in breaking emulsions that may form during the tumbling or shaking step. It is also possible to place the sample in a deep freezer overnight. Filtration is accomplished as the sample warms to room temperature.

Reaction times for the formation of the colored complex may vary with the volume and temperature of the distillate. A maximum of 5 minutes is allowed for shaking, using a wrist-action mechanical shaker. The time and volume should be adjusted to give maximum consistent recoveries. Instrumental readings should be made without delay as the color is subject to fading in a few minutes. Excessive cloudiness in the chloroform layer is normally removed by drying over a gram or two of anhydrous sodium sulfate.

5. MODIFICATION OF METHOD

The validity of results following modification of the method should be verified by the use of an analytical reference sample.

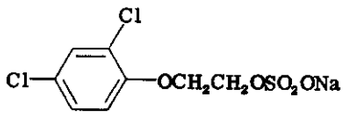
REFERENCES

Dowden, H. D. (1938). *Biochem. J.* **32**, 455–459.
Feigl, F. (1956). "Spot Tests in Organic Analysis." Elsevier, New York.
Hall, S. A., Stohlman, J. W., and Schechter, M. S. (1951). *Anal. Chem.* **23**, 1866.
Hannah, L. H. (1954). *Proc. N. Central Weed Control Conf.* pp. 73–75.

21

Sesone

H. A. STANSBURY, JR.



Sodium 2,4-dichlorophenoxyethyl sulfate

I. GENERAL

A. EMPIRICAL FORMULA

$C_8H_7O_5SCl_2Na$ (Mol. wt. 309.1).

B. ALTERNATIVE NAMES

Formerly "CRAG HERBICIDE I" (Union Carbide Chemical Company). The name Sesone is the registered trademark of the Union Carbides Chemical Company.

C. SOURCE OF ANALYTICAL STANDARD

Union Carbide Chemicals Company, 270 Park Avenne, New York 17, N. Y.

D. BIOLOGICAL PROPERTIES

Sesone has the unique property of being nonphytotoxic itself; yet, in contact with microorganisms in the soil, it is converted to the active seed toxicants, 2,4-dichlorophenoxyethanol and 2,4-dichlorophenoxyacetic acid (Carroll, 1952; Isleib and Aldrich, 1953). Sesone is used as a preemergence herbicide on strawberries (Denisen, 1953; Isleib and Aldrich, 1953), peanuts, and certain established woody ornamentals (Finn *et al.*, 1954; Kramer and King, 1954).

The LD_{50} of Sesone, determined by single oral doses to rats, is 1.4 gm/kg. In the 4-hour rabbit-belly-skin irritation test, a 10% aqueous solution gave a highly irritative action. A 1% aqueous solution on the rabbit eye was harmless, but an excess of 5% aqueous solution caused corneal necrosis.

E. History

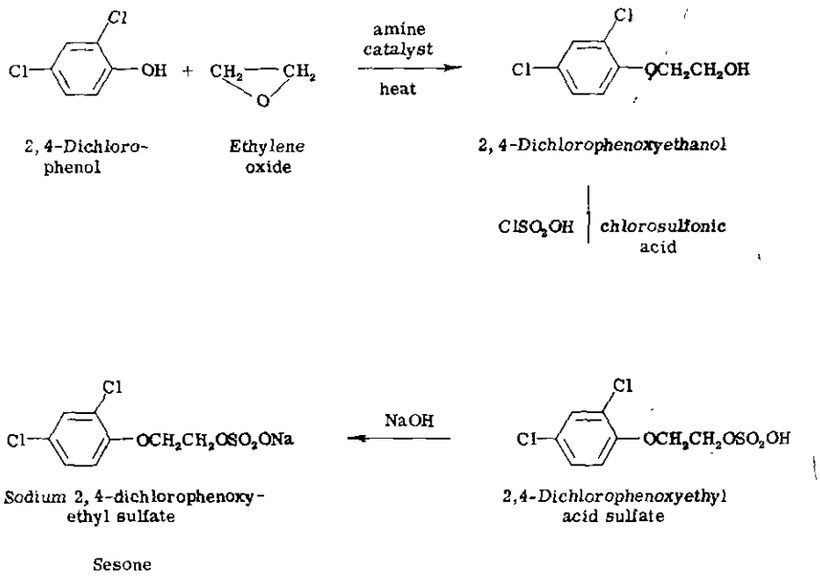
Sesone was first prepared by Lambrech in the research laboratories of the Union Carbide Chemicals Company (Lambrech, 1951). King *et al.* (1950) first reported on its herbicidal activity. Later work by Carroll (1952) and Vlitos (1952, 1953) showed that the herbicidal activity was dependent upon an acidic soil containing microorganisms.

F. Physical Properties

The pure salt is a colorless, crystalline, nonvolatile solid of melting point 170°C. It is soluble in distilled water up to 25% by weight, but is only 4% soluble in hard water containing 260 p.p.m. of calcium carbouate due to the formation of the slightly soluble calcium salt. Sesone is insoluble in common organic solvents except methanol.

G. Chemical Properties

1. Method of Synthesis



2. Chemical Reactions

Sesone is cleaved to 2,4-dichlorophenoxyethanol and sodium acid sulfate by aqueous acids. Some cations, such as calcium and barium, form insoluble salts when added to solutions of Sesone.

H. Formulations

Sesone is a free-flowing, water-soluble powder containing at least 90% of sodium 2,4-dichlorophenoxyethyl sulfate. An anti-blocking agent is an ingredient in Sesone.

II. ANALYSIS

A. Formulation Analysis—Recommended Method—Determination by Acid Hydrolysis

1. PURPOSE AND LIMITATIONS

The method (Hogsett, unpublished) is not reliable for samples containing more than 10% inorganic salts. For specification grade material, with a minimum purity of 90%, the method yields results reproducible to ±1.0%.

2. PRINCIPLE

Refined Sesone is hydrolyzed with a measured excess of aqueous hydrochloric acid to form an eqnivalent to titratable acidity. The amount of acidity formed, which is determined by titration with standard sodium hydroxide, is a measure of the Sesone originally present.

3. REAGENTS

Hydrochloric acid, standard 0.1 *N*.

Hydrochloric acid, standard 0.5 *N*.

Hydrochloric acid, 6.0% (1.8 *N*). Prepare by diluting 150 ml of conc. HCl hydrochloric acid to 1000 ml with distilled water.

Sodium hydroxide, 0.5 *N*, standardized.

Methyl red indicator, 1.0% aqueous solution.

4. PROCEDURE

a. *Sample Preparation*

From a suitable weighing scoop, introduce 3.5 to 4.0 gm of Sesone into each of the two *heat-resistant* pressure bottles. Weigh the sample to the nearest 0.1 mg. Reserve two additional pressure bottles as blanks. Wash down the sides of each bottle with 25 ml of distilled water. Add five drops of the methyl red indicator and titrate with 0.1 *N* HCl from a yellow or orange color to the first appearance of a red color. Introduce 20 ml of 6.0% HCl into each sample and blank using a suitable transfer pipette.

Stopper the bottles and wrap each securely in a canvas bag. Place the samples and blanks as close together as possible in a steam bath at 98 ± 2°C for 3 hours. Maintain sufficient water in the bath to just cover the liquid in the bottles. Remove the bottles from the bath and allow them to cool in air to room temperature. When the bottles have cooled, loosen the wrappers, uncap to release any pressure, and then remove the wrappers.

To each sample and blank add 50 ml of isopropanol from a graduate, three or four drops of the methyl red indicator, and 50 ml of 0.5 N NaOH from a transfer pipette. Use the same pipette for each addition and allow the same drainage time for each bottle, as this amount is not considered in the final calculation.

Titrate the contents of each bottle with standard 0.5 N NaOH to the first permanent yellow end point.

b. *Calculation*

$$\frac{(A - B)N \times 30.91}{\text{Sample in grams}} = \text{sodium dichlorophenoxyethyl sulfate, \% by weight}$$

where A = N normal NaOH required for the sample, milliliters

B = N normal NaOH required for the blank, average milliliters

B. Residue Analysis—Recommended Method

1. *Purpose and Limitations*

This method has been devised for the determination of Sesone and its hydrolysis products, 2,4-dichlorophenoxyethanol and 2,4-dichlorophenol, in fresh, frozen, and dried plant materials. The procedure is applicable to the analysis of solutions containing 0.003 to 0.220 mg of the herbicide and 0.004 to 0.320 mg of its hydrolysis products. A suitable dilution of the sample must be made for the analysis of solutions of higher concentrations. The practical limit of sensitivity of the method of analysis for the hydrolysis products is dependent upon the reproducibility of the absorbancies obtained for the reagent blank. For example, the absorbance for a reagent blank obtained for a middle cut of redistilled A.C.S.-grade chloroform measured against chloroform as a blank has been established at approximately 0.150 units of absorbance. The reproducibility of each blank determination should be as good as ±0.010 units of absorbance. If a sample produced a net absorbance of ±0.025 units, this would be equivalent to 0.25 p.p.m. using a 75-ml aliquot from a 150-ml extract of a 100-gm sample (equivalent to a net sample weight of 50 gm).

The presence of residues or substances containing other anionic surface-active compounds such as high molecnlar weight alkyl or aryl sulfates will canse erroneous results becanse of their reaction with the methylene blue chloride. However, most of the soil conditioners and insecticides nsed in conjunction with the herbicide; snch as snlfnr, lead arsenate, lime, DDT, and other chlorinated organic compounds, do not interfere. Concentrations of 1000 p.p.m. or more of inorganic chlorides will cause some migration of the methylene blue chloride to the chloroform layer. The presence of residues or substances of eight or more carbon atoms containing one or more hydroxyl groups will cause erroneous results because of their reaction with chlorosulfonic acid to form anionic surface-active compounds.

A blank control extraction is made to correct for any small amount of interfering substances inherent in the plant material itself. Decomposition products caused by the excessive decay or processing of certain plant materials interfere; consequently, the procedure gives reliable results only on relatively fresh materials.

2. PRINCIPLE

Sesone, 2,4-dichlorophenoxyethanol, and 2,4-dichlorophenol are extracted from the plant material with water in a Soxhlet extractor. Half of the extract is washed with redistilled chloroform to remove hydrolysis products of Sesone; the herbicide itself remains in the aqueous phase. The chloroform layer is separated and reserved for the determination of hydrolysis products. Methylene blne chloride solution and chloroform are added to tbe aqueons phase. The colored complex formed by the herbicide, an anionic surface-active agent, and the indicator is qnantitatively extracted into the chloroform layer. The intensity of the color is measured at a wavelength of 650 mμ and the amount of herbicide present is determined hy reference to a previously prepared calibration curve. This method was developed by Hogsett and Funk (1954).

The chloroform solution containing the hydrolysis products is reacted with a dilute solution of chlorosulfonic acid in redistilled chloroform to form the corresponding sulfates. The organic sulfates are re-extracted with 0.5 *N* triethanolamine and added to a two-phase system of chloroform and aqueons methylene blue chloride solution. The intensity of the colored complex in the chloroform layer is measured at a wavelength of 650 mμ, and the amount of the hydrolysis products present is determined as 2,4-dichlorophenoxyethanol by reference to a previously prepared calibration cnrve. Tbe absorbance of low concentrations of the snlfates of 2,4-dichlorophenoxyethanol and 2,4-dichlorophenol when complexed with methylene blue is the same.

3. REAGENTS

Methylene blue chloride solution. Dissolve 0.050 ± 0.005 gm of Eastman Kodak reagent grade methylene blue chloride indicator in 500 ml of water contained in a 1-liter volumetric flask. Carefully add 200 ml of conc. Reagent Grade H_3PO_4, mix the solution, and dilute to 1 liter with additional distilled water.

Chloroform, A.C.S. redistilled; should not exhibit a reagent blank greater than 0.200 absorbance nnits measured on a suitable spectrophotometer at 650 mμ versus pure chloroform as a blank in 1-cm cells. Determine the absorbance for the reagent by following the procedure as outlined below. The absorbance for the reagent blank should be determined on each distillation middle cut. If the chloroform has been properly distilled this value should become a constant and is variable only as a result of the technique used in the procedures.

Chloroform distillation. Introduce 5 gallons of A.C.S. chloroform into a 22-liter kettle attached to a column which has a rectification equivalent to 20 plates. Allow the distillation to attain equilibrium and remove the distillate at a 1:1 reflux ratio until the chloroform-ethanol CBM has been removed (approximately 2 quarts). Collect an additional 25 ml of the distillate, transfer to a 50-ml glass-stoppered separatory funnel, add 5 ml of concentrated nitric acid, and shake vigorously. If a yellow color develops in the acid layer after 5 minutes standing, some ethanol remains and must be removed. Collect a middle cut of the chloroform at a 1:1 reflux ratio, and discontinue the distillation when approximately 6 quarts of the residue remains in the kettle. Store the middle cut in 5-pint brown glass bottles. Reserve the heads and residues for use in Section d, below. Add 3.6 gm of pure hydroquinone to each 5-pint bottle of the chloroform. This stabilizes the chloroform for at least 60 days. The insoluble hydroqninone should be removed by filtration just prior to use and readded to any unused portion.

Nitric acid, concentrated A.C.S. grade.

Phosphoric acid, concentrated A.C.S. grade.

Methanol, A.C.S. grade.

Triethanolamine, 0.5 *N* aqueous solution. Dilute 75 gm of 98% triethanolamine to 1 liter with distilled water.

Sodium 2,4-dichlorophenoxyethyl sulfate, recrystallized from methanol, 98.0% by weight, minimum.

Chlorosulfonic acid, Monsanto designated pure. NOTE: *Chlorosulfonic acid is a hazardous chemical and may cause burns or violent reaction on contact with water. DO NOT POUR CHLOROSULFONIC ACID IN THE SINK.*

Chlorosulfonic acid reagent. Pipette 3 ml of chlorosulfonic acid into

a 100-ml volumetric flask containing a few milliliters of chloroform and dilute to volume with additional redistilled chloroform. Prepare a fresh solution daily. If the reagent turns yellow after several hours, a fresh reagent should be prepared. Check the reagent blank of this chloroform before use.

2,4-*Dichlorophenoxyethanol*, 98.0% minimum hydroxyl value by acetylation with acetic anhydride.

4. EXPERIMENTAL PROCEDURE

a. *Preparation of Sample*

Dice the sample with a suitable cutting instrument so that the pieces will fit into a 250-ml Soxhlet extractor.

If the sample cannot be analyzed immediately, place in a vacuum oven at 50°C and 2-mm vacuum until dried. The dried sample and condensate can then be analyzed at a later date.

b. *Sample Extraction*

Assemble a series of six 250-ml Soxhlet extractors so that the kettles can be conveniently heated by means of strip heaters. Cover the bottom of the extractors with a plug of glass wool. Transfer 150 ml of distilled water to each of the Soxhlet extractor kettles.

Into three of the extractors introduce sufficient treatment plant material to conveniently fill the extractor. For dried plant material, introduce into a Soxhlet thimble 15 to 20 gm of the sample weighed to the nearest 0.10 gm. For fresh materials, use at least 100 gm, weighed to the nearest 1.0 gm.

Introduce approximately the same amount of control or untreated plant material, if available, into the remaining extractors.

Apply heat to the extraction flasks and allow the extractions to continue until three siphonings have occurred. Remove heat and allow the contents of each flask to cool to room temperature.

Filter the contents of each flask through a Whatman No. 1 filter paper using a suitable suction flask. Reserve each filtrate for the analysis procedures. If the extracts cannot be analyzed immediately, store in a suitable cold bath or refrigerator to prevent the formation of mold.

c. *Determination*

i. *Sesone.*

(1) Transfer a 75-ml aliquot of each filtered extract (see the last of Section b, above) into respective 250-ml separatory funnels and add 100 ml of redistilled chloroform from a graduate.

(2) Into an additional separatory funnel introduce 75 ml of distilled water and 100 ml of redistilled chloroform, and reserve as a blank on the reagents.

(3) Shake the contents of the separatory funnels for several seconds and allow to separate into two distinct layers.

(4) Draw off the lower layers and filter through a Whatman No. 1 filter paper into separate wide-mouth pressure bottles and reserve for the hydrolysis products determination as described in Section ii below. Before each analysis, the bottles should be thoroughly washed with cleaning solution and dried with acetone.

(5) From a graduate, add 50 ml of A.C.S.-grade chloroform and 25 ml of methylene blue chloride solution to the remaining aqueous phase.

(6) Shake the contents of each funnel and allow the layers to separate and stand at room temperature for 15 minutes.

(7) Draw off the lower layers into separate 125-ml glass-stoppered Erlenmeyer flasks. Discard the aqueous layers.

(8) Transfer a portion of the blank and sample to the respective cells of a Beckman Model B spectrophotometer and obtain an absorbance reading for the sample at a wavelength of 650 mμ using a reagent blank as reference (see Volume I, Chapter 8 for a discussion of spectrophotometric methods).

(9) From a previously prepared calibration curve, read the mg of Sesone corresponding to the optical absorbance reading.

(10) Calibration curve. Dissolve exactly 0.200 gm of recrystallized Sesone in 100 ml of distilled water in a 1-liter volumetric flask and dilute to volume with additional water. Introduce 1, 2, 5, 10, and 20-ml

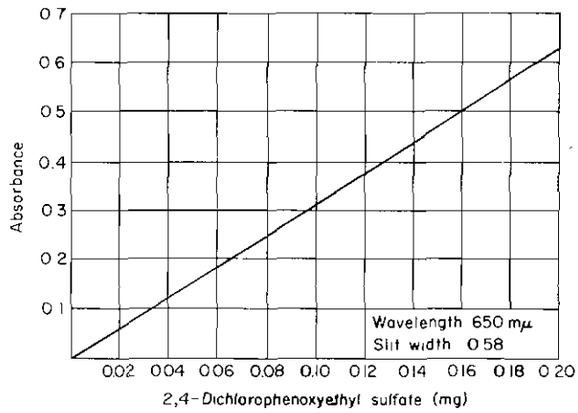


FIG. 1. Calibration curve for Sesone.

aliquots of this solution into 100-ml volumetric flasks and dilute to volume with distilled water.

Obtain the absorbance of each standard, using a 5-ml aliquot as the sample, and 5 ml of distilled water as a blank, following the procedure described in paragraphs (5) to (9) in this section. These amounts correspond to 0.01, 0.02, 0.05, 0.10, and 0.20 mg of Sesone.

Plot a calibration curve of absorbance against milligrams of sulfate using the values obtained above. The calibration adsorption for 0.1 mg is approximately 0.25 units of absorbance. A typical calibration curve is illustrated in Fig. 1.

(11) *Calculation*

$$\text{p.p.m. Sesone} = \frac{A \times 2000}{\text{gm sample}}$$

where A = Sesone obtained from the calibration curve, milligrams. If an absorbance reading is obtained for the control or untreated plant material, an average of the absorbancies for three blank determinations should be subtracted from each sample absorbance.

ii. *Sesone Hydrolysis Products.*

(1) Carefully evaporate each of the chloroform extracts which were set aside during the Sesone procedure to 10 ± 1 ml on a suitable steam bath. Do not evaporate to dryness. *Care must be exercised to prevent moisture from coming into contact with the sample.*

(2) Remove the bottles from the bath and allow the remaining chloroform extract to cool. Into each bottle introduce 10 ml of the chlorosulfonic acid-chloroform reagent, swirl vigorously, and allow to stand for 2 minutes. When the extraction solution is colored, it is imperative that both the sample and the control blank stand for exactly the same length of time.

(3) Add 50 ml of 0.5 N aqueons triethanolamine solution to each bottle, swirl vigorously and transfer the contents of each bottle into separate 250-ml separatory funnels. Wash each bottle with 10 ml of water, collecting the rinsings in the respective funnels.

(4) Stopper each funnel and shake several times, venting the funnel after each agitation. In the case of dark-colored solutions, vigorous agitation will cause an emulsion to form, and consequently a poor separation.

(5) Allow each solution to stand for 10 minutes or until a clear separation of the two layers is affected. Discard the lower or chloroform layer and filter the upper or water layer through a Whatman No. 1 filter paper into a clean separatory funnel containing 25 ml of the methylene

blue chloride solution. Swirl the contents of each separatory funnel, then add 50 ml of A.C.S. grade chloroform.

(6) Shake the contents of each funnel and allow the layers to separate and stand at room temperature for 15 minutes. Draw off the lower layer into a convenient vessel and discard the upper layer.

(7) Transfer a portion of the blank and sample to the respective cells of a Beckman Model B spectrophotometer. Obtain an absorbance reading for the sample at a wavelength of 650 mμ based on a reading of 0 for a blank of the reagents.

(8) From a previously prepared calibration curve, read the total milligrams of 2,4-dichlorophenoxyethanol and 2,4-dichlorophenol as 2,4-dichlorophenoxyethanol corresponding to the optical density.

(9) Calibration curve. Transfer exactly 0.200 gm of pure 2,4-dichlorophenoxyethanol to a 1000-ml volumetric flask containing 500 ml of distilled water. Warm the flask to approximately 60°C and swirl to effect a solution. Dilnte to the mark with additional distilled water.

Introduce 1, 2, 5, 10, and 20-ml aliquots of this solution into 100-ml volumetric flasks and dilute to volume with distilled water.

Obtain the absorbance of each standard using a 10-ml aliquot as the sample. Pipette each sample into a separatory funnel containing 65 ml of distilled water and 100 ml of redistilled chloroform and follow the procedures as outlined in paragraphs (2) throngh (8) under "Sesone procedure" and (1) through (7) under "Sesone hydrolysis products procedure." These amounts correspond to 0.02, 0.04, 0.10, 0.20, and 0.40 mg of pure 2,4-dichlorophenoxyethanol.

Plot a calibration curve of absorbance against milligrams of 2,4-dichlorophenoxyethanol using the values obtained above. The calibration absorption for 0.1 mg is approximately 0.220 absorbance units. A typical calibration curve is illustrated in Fig. 2.

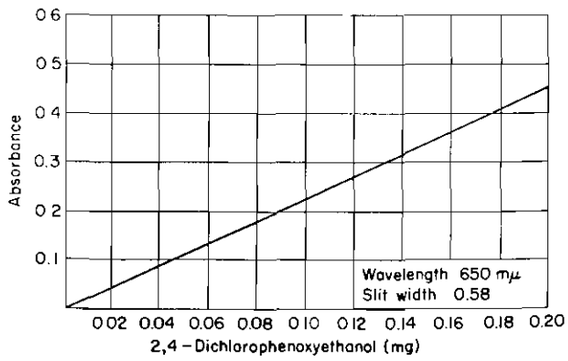


FIG. 2. Calibration curve for 2,4-dichlorophenoxyethanol.

(9) *Calculation*

$$\frac{A \times 200}{\text{gm sample}} = \text{total 2,4-dichlorophenol and 2,4-dichlorophenoxyethanol, p.p.m.}$$

where A = 2,4-dichlorophenoxyethanol, milligrams from calibration curve.

If an absorbance reading is obtained for the control or untreated plant material, an average of the absorbancies for three blank determinations should be subtracted from each sample absorbance.

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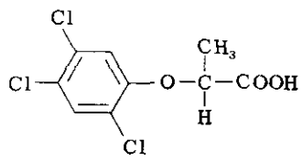
Silvex

R. P. MARQUARDT



2-(2,4,5-Trichlorophenoxy)propionic acid

I. GENERAL

A. Empirical Formula

$C_9H_7O_3Cl_3$ (Mol. wt. 269.51).

B. Source of Analytical Standard

Bioproducts Center, The Dow Chemical Company, Midland, Michigan.

C. Biological Properties

Silvex is a selective herbicide for control of woody plants.

D. History

See Chapter 11 on "2,4-Dichlorophenoxyacetic Acid" in this volume.

E. Physical Properties

Melting point: 177–178°C (uncorr) (Synerholm and Zimmerman, 1945).

Solubilities (grams of silvex/100 gm of solvent at 25°C). Data by Kaufman (1954).

Water	0.0176
Methanol	13.39
Diethyl ether	9.76
Acetone	18.00
~~~

n-Heptane	0.086
Benzene	0.472
Carbon tetrachloride	0.095

Silvex is a white, crystalline material, stable and nonhygroscopic.

II. ANALYSIS

See Chapter 11 on "2,4-Dichlorophenoxyacetic Acid" in this volume.

References

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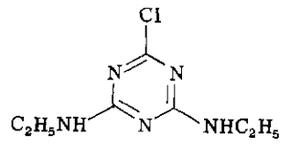
Simazine

E. Knüsli, H. P. Burchfield, and Eleanor E. Storrs[1]



2-Chloro-4,6-bis-ethylamino-*s*-triazine

I. GENERAL

A. Empirical Formula

$C_7H_{12}ClN_5$ (Mol. wt. 201.66).

B. Alternative Names

Trial number G 27692. Trade names for general use: Simazine Geigy; Primatol S. Trade names for selective use: Simazine Geigy; Gesatop. In some countries, a mixture of simazine and aminotriazole is sold. It is named Vorox for special weed problems in the general field and Domatol for the selective field.

C. Source of Analytical Standard

J. R. Geigy S.A., Basel, Switzerland; Geigy Agricultural Chemicals, Ardsley, N.Y.

D. Biological Properties

1. herbicidal activity

Simazine is a pre-emergence herbicide for general and selective use. The actual and potential fields of selective applications are: corn, vine, asparagus, small grains, peas, beans (*Vicia* spp.), alfalfa, sugar cane, pineapple, rubber, tea, coffee, cocoa, sisal, pyrethrum, strawberries, blueberries, cranberries, red currants, boysenberries, raspberries, pip fruit trees, citrus, forestry, ornamentals, sunflowers, special lawn and turf grasses (*Cynodon dactylon, Zoisia* spp.).

[1] Sections I, II,A,B,C,1–3 by E. Knüsli; Section II,C,4 by H. P. Burchfield and E. E. Storrs.

2. TOXICITY

Acute oral toxicity: LD_{50} for mouse, rat, rabbit, chicken, pigeon: more than 5,000 mg/kg.

Chronic toxicity: A series of rats fed for 2 years with a daily oral administration of 2, 20, and 200 p.p.m. (related to food intake) of simazine 50 W were comparable in all respects with the controls.

Cutaneous toxicity: A 10% aqueous suspension of simazine 50 W applied for 5 consecutive days to rats caused no signs of adsorptive toxicity.

Toxicity to fish: No toxic symptoms of saturated aqueous solutions shown by carp and trout.

E. HISTORY

H. Gysin and E. Knüsli are co-inventors (to J. R. Geigy S.A., Basel, Switzerland) of the Swiss Patents 329,227 and 342,784. See also Gast *et al.* (1956).

F. PHYSICAL PROPERTIES

Melting point: 225–227°C.

Solubility:

Water at 0°C	0.0002% (2 p.p.m.)
Water at 20°C	0.0005% (5 p.p.m.)
Water at 85°C	0.0084% (84 p.p.m.)
Methanol at 20°C	0.04% (400 p.p.m.)
n-Pentane at 25°C	0.0003% (3 p.p.m.)
Ethyl ether at 25°C	0.03% (300 p.p.m.)
Chloroform at 20°C	0.09% (900 p.p.m.)

Simazine is colorless, not combustible, not explosive, not corrosive.

pK value in water at 22°C: 1.65.

UV spectra: see Fig. 1.

IR spectra: see Fig. 2.

G. CHEMICAL PROPERTIES

1. METHOD OF SYNTHESIS

To synthesize simazine react cyanuric chloride with two equivalents of ethylamine in the presence of an acid acceptor.

2. PROPERTIES

Simazine sublimates at higher temperatures; it is stable in neutral, slightly acidic or basic media; it hydrolyzes to the herbicidally inactive 2-hydroxy-4,6-bis-ethylamino-*s*-triazine in acidic or basic media, especially

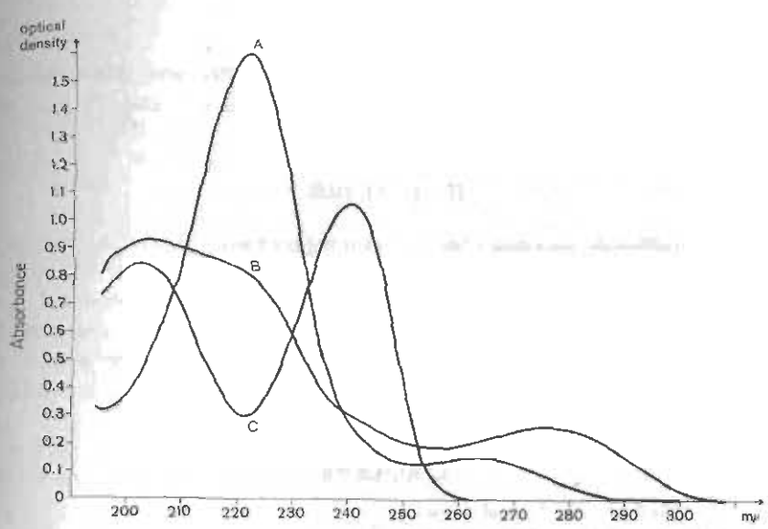


FIG. 1. UV Spectra of: A, simazine in water; B, simazine in 1.3 *N* sulfuric acid (before hydrolysis); C, product of hydrolysis (2-hydroxy-4,6-bis-ethylamino-*s*-triazine) in 1.3 *N* sulfuric acid (cation). Concentration of simazine = 8.2 μg/ml in each solution.

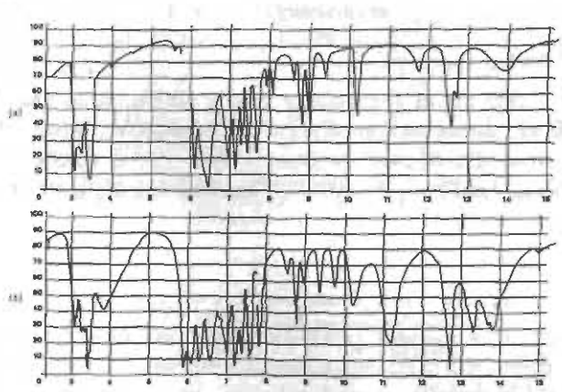


FIG. 2. Infrared spectrum of (a) 2-chloro-4,6-bis-ethylamino-*s*-triazine (simazine) and (b) 2-hydroxy-4,6-bis-ethylamino-*s*-triazine (hydroxysimazine). Technique, Nujol; apparatus, Perkin Elmer, Model 21; prism, NaCl; resolution, 927; response, 1–1; speed, 1 μ/min.

at higher temperatures. Physical data of 2-hydroxy-4,6-bis-ethylamino-*s*-triazine are as follows: melting point, >250°; solubility at 25°C in water, 0.0004%; in methanol, 0.0011%; in chloroform, 0.00003%.

H. Formulations

Simazine is available in the following formulations: 80% wettable powder, 50% wettable powder, 25% paste formulation, and 4% and 8% granules.

II. ANALYSIS

A. Recommended Method for Wettable Powders

1. PRINCIPLE

In this method (Suter, 1955) the chlorine atom of simazine is converted to ionic chloride by reacting simazine with an excess of morpholine and titrating potentiometrically. The potentiometric reading for ionic chloride of other origin is subtracted.

2. REAGENTS

Morpholine, Analytical Grade.
Sulfuric acid, Analytical Grade, 50%.
Silver nitrate, 0.1 *N*.
Ethanol, chlorine-free.

3. EXPERIMENTAL PROCEDURE

a. *Total Chlorine Content*

Weigh 1.500 gm of the sample into a 100-ml round-bottomed flask and add 20 ml of morpholine. Keep for 30 minutes on the boiling water bath and rinse afterwards quantitatively with distilled water into a 400-ml beaker. Acidify with 50% H_2SO_4, cool to room temperature, and titrate potentiometrically with 0.1 *N* $AgNO_3$.

b. *Ionic Chloride Content*

Weigh 1.500 gm of the sample and transfer to a 400-ml beaker. Wet with 10 ml of ethanol, dilute with distilled water to about 300 ml. Acidify slightly with 50% H_2SO_4 and titrate immediately potentiometrically with 0.1 *N* $AgNO_3$.

c. *Discussion of Interferences*

A possible contaminant 2,4-dichloro-6-ethylamino-*s*-triazine simulates a higher content of simazine than is actually present. The method does not differentiate between simazine and analogous 2-chloro-4,6-diamino-*s*-triazines.

d. *Sensitivity*

±0.5%.

e. *Calculation*

$$\% \text{ Simazine} = \frac{(a - b) \times 201.66 \times 100}{w \times 10{,}000} \quad (1)$$

where a = 0.1 N $AgNO_3$ corresponding to total chlorine, milliliters
b = 0.1 N $AgNO_3$ corresponding to ionic chloride, milliliters
w = weight of sample, grams

B. Supplementary Method for Wettable Powders

1. PRINCIPLE

This method (Suter, 1955/1958) is based on the titration of one ethylamino group of simazine with perchloric acid.

2. REAGENTS

Acetonitrile (distilled over phosphorus pentoxide).
Glacial acetic acid (Analytical Grade).
Perchloric acid in glacial acetic acid, 0.1 *N*.

3. EXPERIMENTAL PROCEDURE

a. *Extraction*

Extract an amount of wettable powder (w = weight of the sample in grams), equivalent to about 0.5 gm of simazine exhaustively with acetone. Evaporate the solvent to dryness.

b. *Titration*

Transfer the residue quantitatively with 100 ml of acetonitrile and 20 ml of glacial acetic acid into a 250-ml beaker. Add from a burette 20 ml of 0.1 *N* perchloric acid while stirring. When the material is completely dissolved, titrate potentiometrically with 0.1 *N* perchloric acid (c = total amount of 0.1 *N* perchloric acid consumed, ml). Electrodes: glass/silver.

c. *Discussion of Interferences*

Other *s*-triazine derivatives bearing at least two amino groups are included in the value obtained. 2,4-Dichloro-6-ethylamino-*s*-triazine does not interfere.

d. *Sensitivity*

±0.5%.

e. *Calculation*

$$\% \text{ Simazine} = \frac{c \times 201.66 \times 100}{w \times 10{,}000} \quad (2)$$

4. CALCULATION FOR 2,4-DICHLORO-6-ETHYLAMINO-*s*-TRIAZINE OR 2,4,6-ETHYLAMINO-*s*-TRIAZINE

a. *Calculation*

If the values following Eqs. (1) and (2) are different, either 2,4-dichloro-6-ethylamino-*s*-triazine or 2,4,6-tris-ethylamino-*s*-triazine may be present. The content of these contaminants is calculated as follows:

If the value of Eq. (1) is higher than Eq. (2), then Eq. (2) = content of simazine

$$\frac{[\text{Eq. (1)} - \text{Eq. (2)}] \times 193.04}{201.66 \times 2} = \text{content of 2,4-dichloro-6-ethylamino-}s\text{-triazine} \quad (3)$$

If the value of Eq. (2) is higher than Eq. (1), then Eq. (1) = content of simazine

$$\frac{[\text{Eq. (2)} - \text{Eq. (1)}] \times 210.28}{201.66} = \text{content of 2,4,6-tris-ethylamino-}s\text{-triazine} \quad (4)$$

b. *Accuracy of the Method*

The standard deviation of the two methods (chlorine determination and perchloric acid titration) is ±0.5% for simazine. Therefore, the values calculated for 2,4-dichloro-6-ethylamino-*s*-triazine or 2,4,6-tris-ethylamino-*s*-triazine, respectively, may only be considered significant if they are higher than 0.5%.

c. *Discussion of Interferences*

The analogous 2,4-dichloro-6-amino-*s*-triazines or 2,4,6-tris-amino-*s*-triazines are not differentiated.

C. Residue Analysis

1. REVIEW OF METHODS

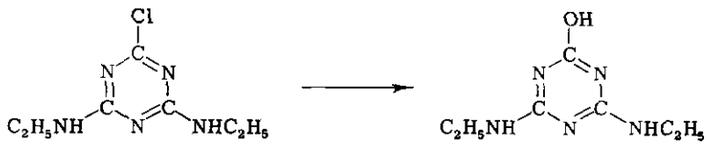
Simazine can be determined by physicochemical as well as biological

methods. Data for spectrometric and colorimetric determinations and for the determination by bioassay are given.

2. RECOMMENDED METHOD—SPECTROMETRIC METHOD

a. *Principle*

In this method (Delley, 1956; Gigger *et al.*, 1961) the residue is extracted with chloroform and washed. The chloroform is re-extracted with simultaneous hydrolysis by shaking with 50% sulfuric acid. In this process, the chlorine of simazine is replaced by a hydroxy group:



The cation of the hydrolysis product shows a maximum absorption at 240 mμ.

b. *Reagents*

Sulfuric acid, Analytical Grade, 50%.

Chloroform, Reagent Grade.

Carbon tetrachloride, Reagent Grade.

Diethyl ether, Reagent Grade.

Eluting solvent. 5% Diethyl ether in carbon tetrachloride v/v.

Aluminum oxide. Woelm, basic, activity grade I (from Alupharm Chemicals, Post Office Box 755, New Orleans, La.).

Aluminum oxide. Woelm, basic, activity grade V. Prepared by mixing 85 gm of Woelm, basic alumina, activity grade I with 15 gm of water. The mixture is set aside for at least 2 hours and preferably overnight, before use.

c. *Analytical Procedure*

i. *Extraction.*

A 200-gm sample of the crop to be analyzed is homogenized with 100 ml of chloroform, 400 ml of chloroform is added, and the slurry is shaken for one-half hour. The solvent is decanted through anhydrous sodium sulfate and the volume of the filtrate is recorded. An aliquot part of the extract, equivalent to 50 gm of crop sample is carefully evaporated to dryness on a water bath in a 250-ml beaker, using a gentle stream of air. The residue is taken up in 10 ml of carbon tetrachloride.

ii. *Column Clean-up.*

A dry packed column is prepared by adding 25 gm of Woelm basic aluminum oxide, activity grade V, to a 1.9-cm column equipped with a fritted disk. The column is tapped gently to eliminate channeling and to achieve uniform packing.

Ten milliliters of CCl_4 containing the crop extract is added to the column surface and the beaker is rinsed with 10 ml of CCl_4. A 250-ml suction flask is attached to the column as a receiver. When the surface of the 20-ml extract has reached the column surface, 80 ml of CCl_4 are added and run through the column. A slight amount of suction may be used to maintain a uniform flow rate.

When the solvent surface again reaches the surface of the column, the 250-ml suction flask is replaced by another one, and at the same time 100 ml of 5% diethyl ether in carbon tetrachloride is added to the column. All of the 100-ml eluate is then collected, heated on a steam bath to remove the ether phase, and transferred to a 250-ml separatory funnel fitted with a Teflon stopcock.

iii. *Insertion of an Additional Column Clean-up.*

An additional clean-up is provided by loading the chloroform extract, as described in Section ii above, on activity grade I acidic aluminum oxide, eluting the simazine with chloroform and evaporating to dryness, taking up the simazine in carbon tetrachloride and proceeding as described above. Hydroxysimazine loaded on activity grade I acid aluminum oxide is not eluted by chloroform, but by absolute methanol.

iv. *Extraction Without Chromatographic Clean-Up.*

Extract the sample to be tested with chloroform. Wash the chloroform extract, which has a volume of about 200–400 ml, twice with 100 ml of 2% NaOH, then three times with 100 ml of 0.5 *N* HCl and finally once with water. Afterward, rinse the washed extract into a clean Erlenmeyer flask. Small drops of water suspended in the chloroform cause a slight turbidity and are removed by filtering through cotton wool. Concentrate the extract to a volume of about 10 to 20 ml.

This procedure may be tried first, and if the interferences are relatively low, the chromatographic clean-up steps (Sections ii and iii above) may be eliminated.

v. *Hydrolysis.*

Transfer the concentrate which contains 0–150 μg of Simazine, to

a clean 100-ml separatory funnel. Rinse the Erlenmeyer flask with a small amount of CCl_4 and add it to the extract in the separatory funnel. The final volume will be between 20 and 25 ml.

Add 1 ml of about 50% H_2SO_4, shake for 30 seconds and allow to stand for about 30 minutes. Repeat this procedure three times. At the end of the fourth 30-miuute period, the hydrolysis is completed. It is important that the separatory funnel be shaken four times and allowed to staud in between each time for at least 30 minutes; i.e., the hydrolysis takes at least 2 hours or more. Add 9 ml of water and mix gently. After separation of the layers, draw off the bottom layer and discard it. Wash the upper aqueous layer once with chloroform. Pour the washed aqueous layer into another clean 100-ml separatory funnel and shake it with 25 ml of diethyl ether. Unremoved chloroform would interfere with the determination at 225 millimicrons. Transfer an aliquot of the aqueous layer directly into a 1-cm quartz cell for reading.

If the sample contains more than 150 μg of simazine, a larger amount of water may be used for the dilution. For developing the cation approximately 0.001 N acid is sufficient. Such dilution has to be considered in the calculation.

vi. *Spectrophotometric Reading.*

Measure the absorbance at 225, 240, and 255 mμ. For comparison, use a cell filled with water. The readings at 225 and 255 mμ are used for a background correction. Examples of absorbance readings are given in Table I.

TABLE I
SAMPLE ABSORBANCE READINGS

	E^{225} [a]	E^{240}	E^{255}	Δ
Blank value of the reagents	0.048	0.029	0.024	−0.007
100 μg of simazine	0.410	1.10	0.12	0.835
Blank value of an extract from 50 gm of untreated corn leaves	0.327	0.261	0.239	−0.022

[a] E^x = extinction at X mμ.

A spectrophotometer (Beckman DU) with a hydrogen lamp, a photocell, sensitive to blue, and 1-cm quartz cells may be used.

d. *Interferences*

Other 2-chloro-4,6-diamino-*s*-triazines will interfere.

e. *Sensitivity and Recovery*

In absolute terms, quantities of 2 μg may be detected. The limit of detection of the method varies somewhat with different substrates; for corn it is 0.1 p.p.m., for asparagus 0.05 p.p.m. The recoveries in standard determinations with addition of known amounts of simazine are 80–100%.

f. *Calculation*

To determine the simazine content, it is necessary to prepare a calibration curve using known amounts of simazine. The measured absorbances give the difference, Δ, by the following Eq. (5):

$$\Delta = A^{240} - \left(\frac{A^{255} + A^{225}}{2}\right)$$

where A^{λ} = absorbance at wavelength λ.

The difference Δ is corrected by subtracting a blank value obtained for the reagents used. It is convenient also to calculate the instrument factor (F) from the graph; for instance, for the Beckman DU used this factor was 120.

It is necessary to carry out a blank test on an untreated sample, and it is convenient not only to calculate the final value of the simazine content but also to express the difference Δ of the untreated sample. The blank values are often negative so that subtraction of a negative value corresponds to an addition of the representative figure.

g. *Applicability of the Method to Different Crop and Food Materials and Soil*

The method has been successfnlly applied to the determination of residues in corn (leaves, stalks, kernels), asparagns, grapes, apples, pears, citrus, millet, cabbage, milk (see Section h), and soil (Section i).

h. *Modification of the Method for the Determination of Simazine in Milk*

i. *Principle.*

In this method (Delley, 1956) the casein and the milk fat are precipitated, and the simazine extracted from the aqueous solution with chloroform.

ii. *Reagents.*

Acetic acid, 2 *N.*

Sodium acetate buffer solution. 1 part by volume of 2 *N* acetic acid + 1 part by volume of 1 *N* NaOH.

iii. *Precipitation.*

Transfer 200 ml of milk into a 600-ml beaker, and add 200 ml of methanol and mix thoroughly. Introduce, with stirring, 20 ml of the sodium acetate buffer solution in the form of a fine jet. The proteins precipitate in a well filterable form. Now cool in ice, and afterwards pass the mixture through a folded filter paper. Squeeze the residue retained in the filter paper slightly, then rinse it by means of a water-jet into the beaker. To this residue add 200 ml of methanol and adjust also the aqueous filtrate to 200 ml. Add 10 ml of 1 *N* NaOH under constant stirring to get the main part of the residue in solution. Repeat the precipitation with 10 ml of 2 *N* acetic acid, cool, filter again and carefully squeeze the residue in the filter paper.

iv. *Extraction.*

Combine both filtrates in a 1000-ml separatory funnel and extract three times with 60-ml portions of chloroform. The two layers separate after a period of time. Drain off a possibly remaining intermediate emulsion phase together with the chloroform layer. The combined chloroform extracts contain simazine. Now follow procedure as described in Sections c, iv–vi.

The sensitivity of this method is 0.02 p.p.m.

v. *Recovery.*

Values from milk without any simazine added are similar to that of the reagents alone. Some recovery values are shown in Table II. The

TABLE II
RECOVERY OF SIMAZINE FROM FORTIFIED MILK SAMPLES

No.[a]	225 mμ	240 mμ	255 mμ	$\Delta = E^{240} - \frac{E^{225} + E^{255}}{2}$	Simazine found, μg	Yield %
1	0.068	0.048	0.038	−0.005	—	—
2	0.156	0.189	0.076	0.073	8.8	88
3	0.214	0.524	0.076	0.379	46	92
4	0.329	0.885	0.107	0.667	80.5	80

[a] 1 = milk without any additive; 2 = milk + 0.05 p.p.m. simazine; 3 = milk + 0.25 p.p.m. simazine; and 4 = milk + 0.5 p.p.m. simazine.

yield of simazine added to milk is 80–90%. It is recommended that a correction factor be determined by adding simazine to milk. Proceed as follows:

Dissolve the required amount of simazine in 1–2 ml of methanol and pour it into the milk. Simazine dissolved in milk is stable, and even after three days there is no remarkable decrease of the simazine content.

i. *Modification of the Method for the Determination of Simazine in Soil*

Depending on the adsorptive power of the soil, the standard method described before does not always give satisfactory results. An improvement seems to be reached by extracting the soil exhaustively with dichloromethane (Storrs and Burchfield, 1960) or dioxane (Freed, 1961). In any case, strictly comparative tests with added known amounts of the herbicide to the respective soil are highly recommended. For practical information on soil residues, bioassays (see below) are often very useful.

3. RESIDUE ANALYSIS BY BIOASSAY

Bioassays indicate the amount of simazine available to the test plants, but do not give absolute values. However, in many practical cases the only question of interest may be whether a special crop plant is damaged or killed by residues. When absolute results are desired, it is necessary to run strictly comparative tests with known amounts of simazine added to the specific soil type in question. It may also be useful to carry out the determination simultaneously by bioassay and chemical methods. The following extreme case (Gast, 1959) may be cited:

A heavy quantity of charcoal was admixed with soil which had been treated with simazine at a rate normally lethal for a series of different test plants. The plants were unharmed, i.e., the toxic material was not available to the plants because of the strong adsorption by the charcoal. By extraction with chloroform and subsequent analytical determination by the spectrometric method (see above), however, a considerable amount of simazine present could be traced out without difficulty. No example has been found until now where simazine was available to the test plants, but could not be found by extraction and subsequent analytical determination.

a. *Test Plants Proposed*

Barley (Van der Zweep, 1958), oats (Burnside, 1959; Gast, 1958/1959; Slife, 1958), grain sorghum (Gast, 1958/1959; Talbert, 1960/1961), soybeans (Burnside, 1959; Talbert, 1960/1961), and corn (Burnside, 1959) were proposed as indicator plants suitable for the determination of simazine by bioassay.

With oats, the limits of possible determinations were found to be 0.015 p.p.m. in a sandy soil and 0.03 p.p.m. in a black soil (Slife, 1958). In a Waukegan silt loam, Clinteland oats were reported to be

useful for the determination of residues in the range from 0.025 to 1 p.p.m. (Burnside, 1959). For better differentiation, more linear development, better reproducibility under different temperature and light conditions and easier planting and establishment, Flambeau soybeans were preferred to oats. Their useful range lies in 0.5–8 p.p.m. simazine (Burnside, 1959). For the high ranges from 8–256 p.p.m., corn A 502 × A 556 was proposed as indicator plant (Burnside, 1959).

b. *Example of a Determination by Bioassay*

In a Mexican silt loam bioassays were carried out with Clark soybeans in the following way (Talbert, 1960/1961):

i. *Standard Curve.*

Standard amounts of simazine were thoroughly mixed into the soil taken from an area adjacent to the experiment. Rates of 1.5, 1.25, 1.00, 0.75, 0.50, 0.25, and 0.00 p.p.m. of the chemical were used. Six cups of each rate were planted with Clark soybeans and were thinned to six plants each as they emerged. After 30 days of growth, the tops were harvested, dried to constant weight, and weighed. The weights of the plants treated with the different rates of simazine were expressed as per cent of the weights of the untreated control plants. With these values, the standard curve was established.

ii. *Determination of Soil Residues.*

The soil samples from the field to be assayed were allowed to come to room temperature and then thoroughly mixed. Three 500-gm portions were removed from each sample and put into 16-ounce cups. This (with two replications) makes a total of six samples. Ten soybean seeds were planted in each cup and were grown and harvested under the same conditions as used in establishing the standard curve. The values were compared to the standard curve.

iii. *Range of Phytotoxic Effects.*

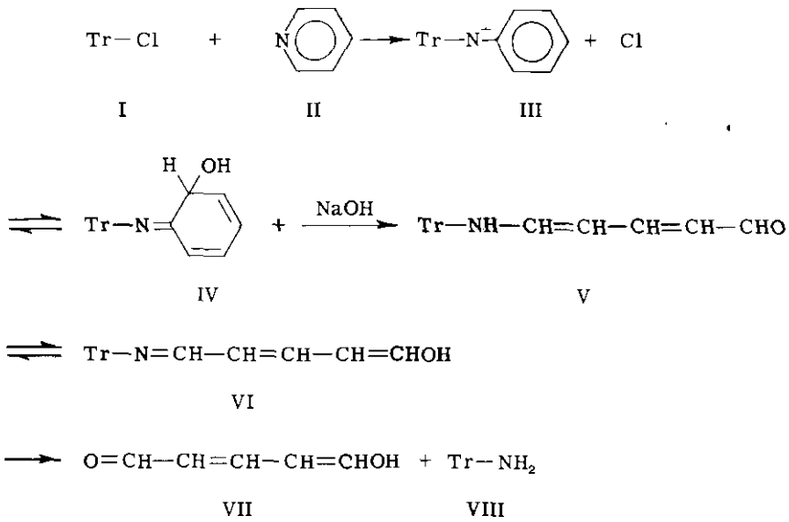
Significantly reduced plant weight was observed from 0.5 p.p.m. upward.

4. COLORIMETRIC METHOD

a. *Principle of Method*

Simazine can be analyzed by a modification of the method developed for dyrene (Burchfield and Storrs, 1956). The mechanism is probably the same as described by Zincke (1904) for the reaction which

takes place between 1-chloro-2,4-dinitrobenzene and these reagents. The successive steps are believed to occur as follows, where the symbol Tr represents the triazine.



Chloro-triazines react readily with pyridine because the halogen atom in the heterocyclic ring is labile. A nucleophilic substitution reaction takes place between the triazine (I) and pyridine (II) to yield a quaternary pyridinium halide (III) which undergoes addition of a hydroxyl group to form the carbinol base (IV). In the presence of alkali, the heterocyclic ring opens to yield an anil of glutaconic dialdehyde (V) which exists in equilibrium with the enol form (VI). The equilibrium mixture of V and VI probably yields the color which is measured. The absorption maximum is at 436 mμ (Fig. 3). The color fades rapidly, probably owing to hydrolysis of the anil (VI) to glutaconic dialdehyde (VII) and an amino triazine (VIII).

Although the reactions leading to color development by simizine and dyrene are probably the same, the conditions nnder which they take place are different. Simazine is less reactive than dyrene, so more drastic treatment must be employed for the nucleophilic substitution between I and II to take place to give the quaternary pyridinium halide (III). Dyrene contains two chlorine atoms substituted in the heterocyclic ring with a single anilino group modifying reactivity, while simazine contains only one chlorine atom attached to the triazine ring which is deactivated by two electron-releasing alkylamino groups. Therefore, it is less electrophilic than dyrene, and substitution does not occur as rapidly.

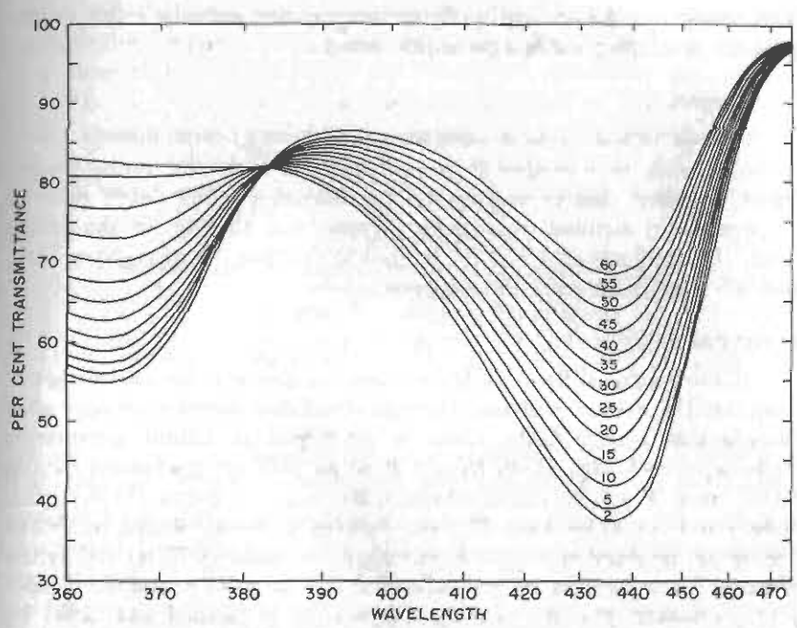


FIG. 3. Absorption spectrum of reaction product of simazine with pyridine and alkali at various time intervals (given in minutes) after addition of the alkali.

Maximum color development is obtained with simazine, by heating it in a boiling water bath with pyridine for 2 hours, in contrast to an optimum reaction period of 20 minutes at room temperature for dyrene. Optimum results are obtained at a final concentration of 1 *N* NaOH and 12% by volume of pyridine. As with dyrene, color intensity is increased by the addition of the amino acid glycine, although the absorption maximum remains the same. Therefore, the pyridine reagent is saturated with glycine to produce the maximum amount of color possible.

Simazine can be extracted from some soil samples using dichloromethane. The solvent is evaporated, and the residue is treated with pyridine and sodium hydroxide to develop color. However, a highly colored blank is sometimes obtained from the soil extract, so an alternate procedure must often be used.

In this method, simazine is extracted from soil with carbon tetrachloride. The solvent is evaporated, and the residue treated with pyridine. The pyridine solution is evaporated, and the residue is taken up in 0.1 *N* HCl. This solution is extracted with carbon tetrachloride to remove the interfering colored materials. The aqueous layer containing the

quaternary pyridinium salt (III) is evaporated, and the color is developed by adding sodium hydroxide solution.

b. *Reagents*

Carbon tetrachloride is used for extraction and tetraethylene glycol dimethyl ether as a fixative to prevent losses of herbicide during evaporation. Pyridine reagent is prepared by saturating a 70% (v/v) solution of pyridine in distilled water with glycine, and filtering off the excess solid. Hydrochloric acid (0.1 *N*) is used to dissolve the quaternary base, and 3 *N* NaOH for color development.

c. *Apparatus*

Glass-stoppered flasks of 125-ml capacity are used for extracting soil samples. The extract is filtered through 60-ml fine porosity sintered glass funnels into suction flasks. Color is developed in 125-ml Erlenmeyer flasks equipped with 24/40 female ⅋ necks, and air condensers having 24/40 male ⅋ joints. The condensers are made of 8-mm (O.D.) glass tubing and are 20 cm long. The end venting to the air should be drawn out to an aperture of 2 mm. Extractions are made in 25-ml separatory funnels. A temperature bath regulated at 35° to 40°C is used for evaporating solvents. The air used for evaporation is cleaned and dried by passing it through a trap containing glass wool and calcium chloride. The reaction is carried out in a boiling water bath. Color intensity can be measured on any standard photometer which can be operated at 436 $m\mu$ using 1-cm cells.

d. *Experimental Procedure*

i. *Analytical Method.*

Soil containing 15 to 20% moisture is used. It is sifted through a fine screen before subsampling to eliminate stones and undecayed organic matter.

The soil (10 gm) is placed in a 125-ml glass-stoppered flask, and carbon tetrachloride (20 ml) is added. The mixture is agitated gently on a mechanical shaker for 20 minutes to extract the simazine. The suspension is then filtered through a fine porosity sintered-glass funnel under vacuum, the soil being transferred to the funnel during the process. When the filter cake is dry, the soil is resuspended in fresh carbon tetrachloride (30 ml) and refiltered. The flask and soil are washed with two 15-ml additional portions of solvent. The combined extracts are added to a 100-ml volumetric flask and diluted to volume. An aliquot of the extract (30 ml) is placed in a 125-ml Erlenmeyer flask equipped with an air

condenser. Tetraethyleneglycol dimethyl ether (80 μl) is added, and the solvent is evaporated in a current of clean dry air, keeping the flask in a water bath at 35–40°C.

The residue is dissolved in pyridine reagent (2 ml), and water (10 ml) is added. An air condenser is attached, and the flask is heated on a boiling water bath for the length of time required for the particular triazine being analyzed (Table III). After the reaction is complete, the

TABLE III
REACTION TIME IN PYRIDINE REQUIRED FOR VARIOUS TRIAZINES

Triazine	Time, hours
Simazine	2
Atrazine	2
Propazine	2
Trietazine	4
Ipazine	5

pyridine solution is evaporated to dryness on a water bath; the residue is transferred quantitatively to a 10-ml separatory funnel using carbon tetrachloride (3 ml) and 0.1 *N* HCl (3 ml). The mixture is shaken, and the upper layer reserved for analysis. The Erlenmeyer flask is washed with two additional portions (3 ml) of 0.1 *N* HCl. These are also extracted with carbon tetrachloride, and the aqueous layers combined. The water is then evaporated until the volume is less than 0.5 ml. The remaining liquid is diluted with water (2 ml) and sodium hydroxide reagent (1 ml) is added to develop the color.

The solution is transferred immediately to a 1-cm colorimeter cell. Absorbance measurements are made at 1-minute intervals against a reagent blank at a wavelength of 436 mμ. The value at 2 minutes after the addition of alkali is interpolated. Color fades at a rate of about 1% per minute at 25°C (Fig. 3), so the spectrophotometer settings should be adjusted before adding the sodium hydroxide solution.

Soil samples which do not contain triazines should be run at the same time, and the absorbances obtained on these subtracted from those obtained on the samples.

This procedure is satisfactory for the analysis of soils containing about 10 p.p.m. of simazine. At lower concentrations, the size of the aliquot taken for analysis should be increased proportionately.

ii. *Interferences.*

Extractable colored materials are present in most soils. Therefore, the procedure described above was developed, in which the extract is par-

titioned between carbon tetrachloride and 0.1 *N* HCl after the first step in the analytical procedure has been carried out. The quaternary pyridinium salts which are formed as intermediates are soluble in water, while most of the colored materials from the soil are partitioned preferentially into the organic layer. Since soils vary in the amount of organic matter they contain, it is necessary to determine the background obtained with untreated soil in each case.

iii. *Sensitivity.*

Sensitivity depends on sample size, degree of dilution, quantities of reagents used, size of the absorption cell, and ratio of color intensity to background. The absorbance obtained on a solution containing 1 μg of simazine per milliliter is about 0.2 using a 1 cm light path.

iv. *Recovery.*

Recovery from soil is of the order of 90 to 100% when the soil blank is low enough to permit omission of the partition step. When the partition step must be included, overall recovery is of the order of 75 to 80%, based on the color intensity obtained with pure simazine. However, qnantitative results can be obtained by using a standard curve based on the extraction and analysis of soils containing known amounts of herbicide. Hydrolysis products of simazine and reaction products with metabolites in soils do not yield colored products. Hence, this method will determine only the amount of "active" herbicide present in the soil. However, the decomposition rates of simazine and related compounds are much slower than for dyrene.

v. *Standard Curve.*

A good straight line relationship is obtained when the concentration of triazine in micrograms per milliliter of solution is plotted against absorbance (Fig. 4). However, the color intensity fluctuates somewhat from day to day because of changes in room temperature. Therefore, it is usually advisable to run standards with each set of analyses. Since Beer's law is followed accurately, this can be done at a single concentration and the results calculated algebraically.

vi. *Sample Calculations.*

The amount of simazine in the soil sample is calculated from the following relationship:

$$\mu g/g = \frac{cA_u Vf}{A_s W}$$

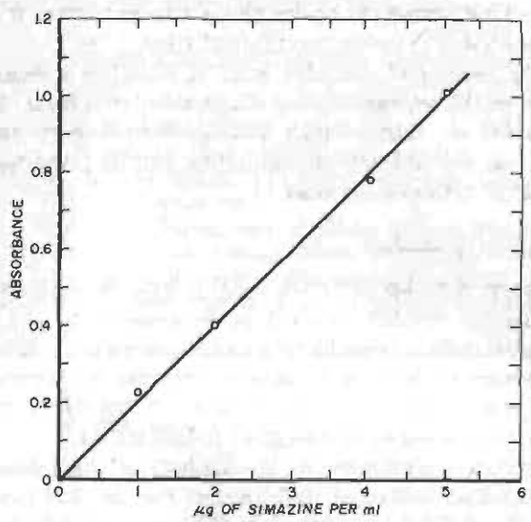


FIG. 4. Relationship between concentration of simazine and absorbance.

where c is the concentration of simazine in the standard solution, A_s the absorbance obtained on the standard solution, V is the total volume, f is a dilution factor which can be varied according to the amount of simazine in the sample, A_u is the absorbance obtained on the unknown, and W is the weight of soil in grams.

e. *Applicability of Recommended Method to Different Crops or Food Materials*

The present method has been used only for the analysis of soils. The analysis of crops or food materials might require additional clean-up steps such as liquid-solid chromatography on alumina (see dyrene) or on a sulfonic-acid type ion exchange resin.

f. *Discussion of Method*

This method has high sensitivity and is comparatively easy to use. The chief drawback is that the color fades rapidly; so the absorbance measurements must be made at a preset time after the addition of alkali. However, the accuracy is satisfactory.

The rate at which the color fades is dependent upon the temperature. Under conditions where the room temperature does not go above 30°C, little difficulty is encountered if standards are run daily. However, at higher temperatures, the color may fade so rapidly that quantitative

results cannot be obtained. Under these circumstances, it is necessary to run the analyses in an air conditioned room.

Tetraethylene glycol dimethyl ether is added as a fixative to retard volatilization of the triazine during evaporation of solvent. This material forms peroxides on aging, which produce very rapidly fading of the color formed on the addition of alkali. This can be prevented by storing the fixative over activated alumina.

g. *Modifications of Method*

If auxiliary clean-up steps are used, it may be possible to omit the step in which the reaction product of simazine with pyridine is partitioned between carbon tetrachloride and aqueous acid. In this case, the reaction mixture obtained by heating the residue with pyridine reagent (2 ml) and water (10 ml) is cooled; and 7 *N* NaOH (2 ml) is added. Absorbances are measured as described in Section d,i.

The method is applicable to the analysis of the related herbicides atrazine, propazine, trietazine, and ipazine. For the first two compounds mentioned, the method is identical to the one used for simazine. However trietazine requires 4 hours heating in a boiling water bath with pyridine-glycine reagent, while ipazine requires 5 hours. Color intensity varies with the nature of the herbicide, but the absorption maxima are identical. Thus if simazine is assigned an absorbance of 1, atrazine gives a relative value of 0.94; propazine, 0.87; trietazine, 0.64; and ipazine, 0.57.

The addition of ethylcyanoacetate, barbituric acid, or 2-thiobarbituric acid to a simazine:pyridine:sodium hydroxide solution gives pink, reddish-violet, and blue colors, respectively. The absorption maxima for these colored products are at 550, 582, and 625 mμ, respectively, and it is suggested that these three modifications may be useful for determining simazine in the presence of yellow-colored plant and soil extracts (Ragab, 1959.)

Reactions with pyridine and alkali are useful for the analysis of many other classes of pesticides containing active halogen or reactive double bonds. Thus methods employing the same general techniques have been developed for dyrene, 1-fluoro-2,4-dinitrobenzene, captan, and 3,4-dichlorotetrahydrothiophene-1,1-dioxide. Positive qualitative tests have been obtained on many other pesticides (Burchfield and Schuldt, 1958.)

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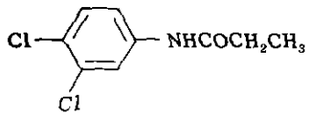
Stam

C. F. Gordon, A. L. Wolfe, and L. D. Haines



3′,4′-Dichloropropionanilide

I. GENERAL

A. Empirical Formula

$C_9H_9ONCl_2$ (Mol. wt. 218.08).

B. Alternative Names

FW-734, *N*-(3,4-dichlorophenyl)propionamide. The name Stam is the registered trademark of the Rohm and Haas Company.

C. Source of Analytical Standard

Samples of Stam suitable for purification may be obtained from Rohm and Haas Company, Agricultural and Sanitary Chemicals Sales Department, Washington Square, Philadelphia 5, Pa.

D. Biological Properties

The acute oral LD_{50} for rats is 1400 mg/kg. Stam is a selective herbicide for the post-emergence control of grasses and broad-leaved weeds among various agronomic crops. It has been proved particularly effective for control of grasses, broad-leaved weeds, and sedges in rice fields.

E. Physical Properties

Melting point: 92-93°C.

Solubility: water, 0.02 gm/100 ml; benzene, 7 gm/100 ml; acetone 170 gm/100 ml; and methanol, very soluble.

Vapor pressure: <0.001 mm at 50°C.

F. Chemical Properties

1. Method of Synthesis

By a reaction of 3,4-dichloroaniline and propionic acid or a derivative thereof, Stam is obtained.

2. Chemical Reactions

Stam hydrolyzes to 3,4-dichloroaniline and propionic acid in the presence of acid or alkali.

G. Formulation

Stam F-34 is an emulsifiable concentrate of 3′,4′-dichloropropionanilide.

II. ANALYSIS

A. Formulation Analysis—Review of Methods

A chlorine analysis may be performed in order to determine the amount of the active ingredient in the formulation. Of the common methods for the determination of chlorine, the sodium reduction procedure yields low results, and the sample size restriction in the Parr bomb procedure (Peel *et al.*, 1943; Gunther and Blinn, 1955) leads to poor precision unless extreme care is exercised. The sodium diphenyl procedure (Blinn, 1960) shows considerable promise but is still under evaluation. The reader is also referred to Volume I, Chapter 11 for a theoretical discussion of several total halide analytical procedures.

B. Residue Analysis

1. Review of Methods

Since Stam F-34 has been introduced for commercial application only recently, no residue method for 3′,4′-dichloropropionanilide has been published to-date. A report on this method was made at the 144th meeting of the American Chemical Society (Gordon *et al.*, 1963).

2. Recommended Method

a. *Principle*

The method is based on a direct alkaline hydrolysis of the crop snbstrate to liberate 3,4-dichloroaniline (3,4-DCA) which is then distilled from the hydrolyzate. The 3,4-DCA is then extracted from the

distillate into benzene and cleaned up by Florisil column chromatography. The 3,4-DCA is extracted into sulfuric acid followed by diazotization and coupling with *N*-1-naphthylethylenediamine dihydrochloride, which produces a purple dye solution the concentration of which can be determined spectrophotometrically.

b. *Reagents*

The following reagents have given good results in this method. Other grades or sources of reagents may be used but should first be put through the residue procedure to ascertain that an absorbance of 0.005 or less is obtained. All aqueous solutions are prepared with de-ionized water.

Acetic acid, glacial, Baker & Adamson, Reagent Grade.

Aluminum oxide, Merck, Reagent Grade.

Ammonium sulfamate, Du Pont Technical Grade; a 0.5% aqueous solution is prepared weekly.

Anti-foam 60, General Electric, 20% aqueous solution.

Benzene, Baker & Adamson, Reagent Grade. Two gallons of benzene are chromatographed through a 2 inch diameter column containing 1 pound of aluminum oxide (dry packed). Recycle the first 200 ml of chromatographed benzene. Store each gallon of chromatographed benzene over 100 gm of anhydrous sodium sulfate. Only chromatographed benzene can be used in this procedure.

Florisil, 100/200 mesh, activated at approximately 1050–1100°K by Floridin Co., P.O. Box 989, Tallahassee, Fla. Before preparing a chromatographic column, 3 gm of Florisil are dried at least 16 hours at 130°C with a maximum depth of 2 cm.

Glycerine.

Hydrochloric acid, Baker & Adamson Reagent Grade. Prepare a 1.2 *N* solution.

Methylene dichloride, Merck, Reagent Grade.

N-1-Naphthylethylenediamine dihydrochloride, Eastman, White label (NEDA). A 1.0% aqueous solution is prepared immediately before use. If reagent blanks carried through the procedure result in an absorbance greater than 0.005, the *N*-1-naphthylethylenediamine dihydrochloride should be recrystallized twice from 0.1 *N* HCl.

Potassium nitrite, Baker & Adamson, Reagent Grade. A 0.2% aqueous solution is prepared weekly.

Sand, Ottawa, washed (A. H. Thomas Co., Phila., Pa.)

Sodium chloride, Baker & Adamson, Reagent Grade.

Sodium sulfate, anhydrous, granular-type.

Sodium hydroxide, Baker Analyzed, 50% solution, Reagent Grade. This solution is diluted 1:1 with water and cooled before use.

3′,4′-Dichloropropionanilide, Rohm & Haas Co., purified. Fortification solutions containing 1, 2, 5, 10 μg/ml in methylene dichloride are prepared.

Sulfuric acid, Baker & Adamson, Reagent Grade. An aqueous 8 *N* solution is prepared.

c. *Apparatus*

Chromatographic column, plain, with Kimax Teflon stopcock, 1 cm I.D., 45 cm length. Fisher & Porter, #274-001.

Clamp, ball-joints, #28; #35.

Condenser, Pyrex, 35/25 ball joint at the bottom. This serves as a reflux condenser.

Condenser, Pyrex, 28/12 ball joint at the bottom. This serves as a distillation condenser. The two condensers are joined at the top by ½″ glass tubing so that the condensers are parallel and approximately 7 inches apart, center to center.

28/12 Socket joint extended to 10 inch length with 2 mm capillary tubing. This serves as a distillation condenser extension allowing the distillate to be introduced below the surface of dilute HCl in a separatory funnel.

Separatory funnels, 125 ml, 60 ml, with Teflon stopcock.

Stainless steel flask, silver soldered, 500 ml, 35/25 socket joint (Scientific Glass Co., Bloomfield, N.J., #JF-6350).

Stirring bar, oval, Teflon-covered, ¾ inch diameter, 1⅝ inch length (Chemical Rubber Co., Cleveland 14, Ohio; #22-2390).

Heat shield (made from a 1-quart paint can). A hole is cut in the bottom of the can to permit the neck of a stainless steel flask to extend above the shield. The shield is used around the steel flask during reflux and distillation to permit more efficient heat utilization.

Graduated cylinder, 10-ml.

Erlenmeyer flask, 50-ml.

Glass column, 2 inch diameter × 36 inch length, with Teflon stopcock.

Interval timer, Victor (Fisher Scientific Co., #6-660).

Spectrophotometer, Beckman, Model B, or equivalent.

Wiley cutting mill, Standard Model No. 3, (Fisher Scientific, #8-336-5A). This mill is used with a 1 mm screen when milling dry samples such as rice kernels or straw.

Note: One unit of each of the above items, except the last four, is

required for each assembly. Six to eight assemblies can be operated by one analyst.

d. *Experimental procedure*

i. Sample Preparation.

(a) *Dry samples.* Samples such as rice kernels or rice straw are milled with a Wiley mill immediately before analysis. A 50-gm sample is sufficient for milling.

(b) *Moist samples.* Samples are diced or chopped until the average particle size is about ⅜ inch.

ii. *Method of Analysis.*

(a) *Sample size.* Dry samples: 10 grams; moist samples: 30 grams; samples having little interfering compounds, such as potatoes: 100 gm.

(b) *Hydrolysis.* Transfer a weighed, representative sample to the steel flask and add 325 ml of 25% NaOH. Insert a magnetic stirring bar and swirl the contents so that the solids are suspended in the NaOH. Place the flask on the heater-stirrer and stir at approximately 240 r.p.m. It is important that efficient stirring be maintained throughout the hydrolysis and distillation, otherwise the solids will cake and char leading to low recoveries and high interference in control samples. Add 10 ml benzene and 10 drops of Anti-foam solution, place the heat shield around the flask, put two to three drops glycerine on the steel socket, clamp the flask to the reflux condenser, clamp the condenser extension to the distillation condenser, place the tip of the condenser extension below the surface of 5 ml 2 *N* HCl contained in a 125-ml separatory funnel. Then turn the heater on and reflux for 17 hours.

(c) *Distillation and extraction of 3,4-dichloroaniline.* Distill the benzene and 50 ml of water from the steel flask after draining the water from the reflux condenser and after opening the valve to circulate cold water through the distillation condenser.

The distillate is delivered below the surface of the HCl which had been placed in a separatory funnel upon setting up the apparatus for reflux. Distillation is performed as rapidly as possible, limited only by the extent of foaming which may occur with some samples.

Adjust the distillate to pH 10 by dropwise addition of 50% NaOH. Add 15 gm of NaCl and shake the separatory funnel 3 minutes at a rate of 100 shakes per minute. Allow the separatory funnel to stand for 5 minutes to allow separation of phases. Discard the aqueous phase. Draw the benzene layer into a 50-ml Erlenmeyer flask containing 2 gm

sodium sulfate and shake 100 times and set aside until the Florisil column has been prepared.

(d) *Column chromatography.* Pour 3 gm of dried Florisil into 1-cm chromatographic column and tap the column until the Florisil is packed down to a height of 8 cm. Pour 1 cm of sand on top of the Florisil, open the stopcock and add enough benzene to wet the column.

Pour the benzene extract from the distillate on the column and wash the flask with one 10-ml benzene rinse, allowing the benzene to flow down to the sand. Discard all of the benzene which passes through the column from this rinse.

Elute the column with benzene and collect the next 50 ml coming off the column. Transfer the eluate to a 60-ml separatory funnel and extract twice for 3 minutes with 2 ml 8 *N* H_2SO_4 each time. The separatory funnel should be scrupulously clean so that a maximum amount of the acid phase will be recovered. Allow the separatory funnel to stand for 5 minutes and then drain the acid phase after each extraction into a 10-ml graduated cylinder.

(e) *Color development.* To the H_2SO_4 in the cylinder add 1 ml glacial acetic acid and stir vigorously with a stirring rod. Let stand 2 minutes and then add 0.5 ml of 0.1% potassium nitrite solution, stir, and let stand 2 minutes.

Add 1 ml. 0.5% ammonium sulfamate solution, stir vigorously and let stand for 5 minutes. Add 0.5 ml 1% NEDA solution, stir, and dilute to exactly 7.0 ml with 8 *N* H_2SO_4. Let the solution stand for 15 minutes for maximum color development.

Read the absorbance of the solution in calibrated 1-cm cells at 550 mμ. A reagent blank sample is run concurrently with the crop samples and used in the reference cell when reading the absorbance of the crop samples. Reagent fortifications should be run through the procedure to assure that the method is in constant control.

(f) *Direct color development.* One milliliter of a methylene dichloride solution containing 0.5-10.0 μg 3′,4′-dichloropropionanilide is carefully evaporated to dryness at room temperature under a gentle stream of nitrogen in a 10-ml graduated cylinder. Do not overdry. Then add 4 ml 8 *N* H_2SO_4, hydrolyze in boiling H_2O bath for 15 minutes, cool to room temperature, and continue with the color development as specified in the previous section.

iii. *Interferences.*

Since Stam F-34 is hydrolyzed to 3,4-dichloroaniline, any compound which yields 3,4-dichloroaniline or related compounds upon alkaline hydrolysis will interfere.

iv. *Sensitivity.*

The sensitivity of the method ranges from 0.01 to 0.05 p.p.m. depending on the crop material.

v. *Recovery.*

Recovery has ranged from 75 to 90%.

vi. *Standard curve.*

Ten micrograms of 3′,4′-dichloropropionanilide analyzed by direct color development results in an absorbance of 0.366. Beer's law is obeyed over the range from 0.5 to 20 μg.

3. APPLICABILITY OF RECOMMENDED METHOD TO DIFFERENT CROP OR FOOD MATERIALS

This method can be used for the analysis of residues on a variety of crops, providing that the samples are chopped or milled to a size so that the concentrated NaOH can digest the crop within the allotted reflux time. The method has been applied to rice plants, rice kernels, tomatoes and potatoes.

4. DISCUSSION OF METHOD

By means of carbon-14 tagged 3′,4′-dichloropropionanilide, it was found that common solvents gave poor extraction efficiency of the radioactivity from green rice plants. The radioactive compound could be freed from the crop only by exhaustive alkaline hydrolysis.

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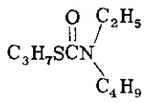
Tillam

G. G. PATCHETT, G. H. BATCHELDER, AND J. J. MENN



n-Propyl ethyl-*n*-butylthiolcarbamate

I. GENERAL

A. EMPIRICAL FORMULA

$C_{10}H_{21}ONS$ (Mol. wt. 203).

B. ALTERNATIVE NAMES

Tillam was formerly coded Stauffer Experimental Compound R-2061. The name Tillam is the registered trademark of the Stauffer Chemical Company.

C. SOURCE OF ANALYTICAL STANDARD

Stauffer Chemical Company, Richmond Research Center, 1200 South 47th Street, Richmond, Calif.

D. BIOLOGICAL PROPERTIES

Tillam is a selective herbicide effective against a number of annual grasses and broadleaf weed species including watergrass (*Echinocloa* spp.), red-root pigweed (*Amaranthus retroflexus*), and lamb's-quarters (*Chenopodium album*). It was chosen for commercial development due to its selectivity on sugar beets (Antognini and Reed, 1960) and tomatoes. Most susceptible weed species are controlled at a dosage of four pounds of active ingredient per acre. Tillam must be incorporated into the soil with a disk, rotary tiller, or other soil incorporation equipment, immediately after application, for optimum results.

The acute oral LD_{50} to rats is 1120 mg/kg and the acute dermal LD_{50} to albino rabbits is greater than 2936 mg/kg.
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E. History

Tillam was originally synthesized and developed at the research laboratories of Stauffer Chemical Company. Patents have been applied for.

F. Physical Properties

Boiling point: 187°C (100 mm); 142°C (20 mm); and 126°C (10 mm).

Solubility. It is miscible with most organic solvents including acetone, benzene, toluene, xylene, methanol, isopropanol, and kerosene. Its solubility in water is 92 p.p.m. at 21°C.

Vapor pressure. Approximately 0.01 mm Hg at 25°C (by extrapolation of boiling point curve between 10 and 100 mm Hg).

Refractive index: $n_D^{30} = 1.4752$.

Density: $d_4^{30} = 0.9458$.

G. Chemical Properties

1. method of synthesis

React 1 mole of propyl chlorothiolformate with 1.05 moles of ethyl butylamine and 1.25 moles of aqueous sodium hydroxide. Two other methods of synthesis of 265 thiolcarbamates have been described by Tilles (1959).

2. chemical stability

Tillam is very stable towards hydrolysis in dilute acidic and basic solutions.

H. Formulations

Tillam is formulated as an emulsive liquid containing 6 lb of the active ingredient per gallon and is designated Tillam 6-E and as a granular product containing 5% of the active ingredient which is designated Tillam 5-G. More concentrated granular products may also be used in some areas.

II. ANALYSIS

A. Formulation Analysis

1. review of methods

Two methods are available for technical and formulated Tillam. A specific method based upon determination by gas chromatography is

preferred, but a general method based upon Kjeldahl nitrogen is useful in control analyses where other nitrogen containing materials are absent. Classical Kjeldahl methods are suitable provided techniques are used which minimize Tillam loss during the acid digestion.

2. RECOMMENDED METHOD

Tillam is determined by the gas chromatographic method as described for the analysis of Eptam (Chapter 12 of this volume), the only difference being a higher column temperature due to the longer retention time of Tillam. Tillam is chromatographed at 190°C and the peak area is referred to a standard Tillam sample chromatographed under identical conditions. The retention time for Tillam under these conditions is about 6 minutes and it is easily separated from Eptam which precedes it.

B. Residue Analysis

1. REVIEW OF METHODS

Tillam crop residues are determined by the colorimetric method described for Eptam which is based upon the determination of the secondary amine after hydrolysis. This method will not distinguish between the two herbicides, but in practice no detectable residues of either compound have been observed on crops treated as recommended. If the need arises, Eptam and Tillam residues could be individually determined after steam distillation by employing gas chromatography (Hughes and Freed, 1961). The hydrogen flame detector or microcoulometric detector for snlfur may be used where higher sensitivity or lower background is of interest (see Volume I, Chapter 9).

2. RECOMMENDED METHOD

Use the identical colorimetric method as described for Eptam (Chapter 12 of this volume) (Batchelder and Patchett, 1960).

a. *Recovery*

Typical recovery values are shown in Table I for several crops extracted by tbe steam distillation procedure. Low recoveries may result from a prolonged distillation time and so it is recommended that the distillation be completed in less than an hour whenever possible. The use of antifoam aids in the rapid distillation of many crops.

b. *Standard Curve*

A linear response is effected for Tillam in the range of 4 to 400 μg

TABLE I
RECOVERY OF TILLAM ADDED TO MACERATED CROPS

Crop	Tillam added p.p.m.	Per cent recovered
Broccoli	0.25	75
Cabbage	1.0	89
Corn, fresh kernels	0.20	72
Peppers, green	0.25	78
Rice, unhulled	0.10	72
Sugar beet roots	0.10	77
Sugar beet tops	0.25	75
Sugar beet tops	0.10	61
Tomatoes	1.0	72
Tomatoes	0.25	70
Tomatoes	0.10	79

with typical absorbance values of 0.012 and 0.881, respectively. The reagent background has a typical absorbance value of 0.003.

c. *Sample Calculations*

Determine the amount of Tillam in the sample by referring the net absorbance to a standard curve prepared by processing aliquots of the Tillam standard solution in a similar manner.

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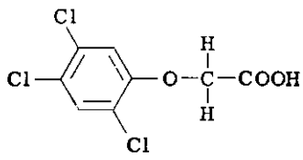
2,4,5-Trichlorophenoxyacetic Acid

R. P. MARQUARDT



2,4,5-Trichlorophenoxyacetic acid

I. GENERAL

A. EMPIRICAL FORMULA

$C_8H_5O_3Cl_3$ (Mol. wt. 255.49).

B. ALTERNATIVE NAMES

2,4,5-T acid.

C. SOURCE OF ANALYTICAL STANDARD

Bioproducts Center, The Dow Chemical Company, Midland, Michigan.

D. BIOLOGICAL PROPERTIES

This compound is a selective herbicide for controlling woody plants.

E. HISTORY

See Chapter 11 on "2,4-Dichlorophenoxyacetic Acid" in this volume.

F. PHYSICAL PROPERTIES

Melting point: 153°C (Pokorny, 1941); and 154–155°C (uncorr.) (Synerholm and Zimmerman, 1945).

Solubilities (grams of 2,4,5-T acid/100 gm solvent at 25°C). Data by Kaufman (1954):

Water	0.0278
Methanol	49.60
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Ethanol	54.82
Diethyl ether	23.43
n-Heptane	0.039
Toluene	0.732
Xylene	0.608

II. ANALYSIS

See Chapter 11 on "2,4-Dichlorophenoxyacetic Acid" in this volume.

References

Kaufman, D. C. (1954). The Dow Chemical Company. Personal communication.
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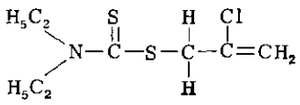
Synerholm, M. E., and Zimmerman, P. W. (1945). *Contribs. Boyce Thompson Inst.* **14,** 91–103.

27

Vegadex

R. A. Conkin and L. S. Gleason



2-Chloroallyl diethyldithiocarbamate (CDEC)

I. GENERAL

A. Empirical formula

$C_8H_{14}NClS_2$ (Mol. wt. 223.79).

B. Alternative Names

CP 4742, CDEC. The name Vegadex is the registered trademark of the Monsanto Chemical Company.

C. Source of Analytical Standard

Monsanto Chemical Company, 800 North Lindbergh Blvd., St. Louis 66, Missouri.

D. Biological Properties

CDEC is a pre-emergence herbicide which controls the germinating seed of the following weeds: annual bluegrass, barnyard grass, bullgrass or goosegrass, careless weed, chickweed, crabgrass, foxtail, henbit or blueweed, pigweed (spiny or redroot), and purslane.

Vegadex has been granted label clearance for use in the following crops: collards, kale, mustard, spinach (2 applications), turnip, broccoli, brussels sprouts, cabbage, cauliflower, celery, Hanover salad, endive, lima beans, sweet corn, cantaloupes, okra, hydrangea, spirea, yews, lettuce, soybeans, chicory, field corn, tomatoes, iris, euonymus, azeleas, privet, snap beans, escarole, field beans (Wyo. only), garden or table beets (E. of Rocky Mountains), gladiolus, transplanted tomatoes, potentilla, and juniper.

CDEC will not control established plants or plants originating from

vegatative plant parts. Neither has this compound been known to control dormant seeds.

The acute oral LD_{50} of Vegadex for rats is 850 mg/kg and it is classified as moderately irritating to skin and eyes.

E. History

Vegadex was discovered by Drs. John D'Amico and Marion W. Harman while working in the Monsanto Research Laboratories at Nitro, West Virginia. It was first granted label clearance for use on 19 crops in 1956. Since this petition, other uses have been granted label clearance.

Biological properties and composition of matter of Vegadex are covered in U. S. Patents 2,854,467 and 2,919,182 issued to Dr. Marion W. Harman and Dr. John J. D'Amico on September 30, 1958 and December 29, 1959 respectively; and assigned to Monsanto Chemical Company.

F. Physical Properties

Boiling point: 128°C at 1 mm Hg.
Vapor pressure: 2.2×10^{-3} mm Hg at 20°C.
Hydrolysis rate (½ life in days): 47 at pH 5; 30 at pH 8.
Melting point: liquid at room temperature.
Refractive index: 1.5822 (N_D^{25}).
Specific gravity: 1.088 at 25/15.6°C.
The solubility of Vegadex is given in the following tabulation:

Solvents	Amount	Solution point °C
Buffer soln. pH 5	0.0092 gm/100 ml	25
Buffer soln. pH 8	0.0106	25
		Cloud point (°C)
Xylene	22.0% w/w	0
	33.3% w/w	2
	50.0% w/w	5
Shell E-407R	20.0% w/w	0
	33.3% w/w	2
	50.0% w/w	5

It is soluble in ether, acetone, benzene, chloroform, ethanol, ethyl acetate, heptane, kerosene, isopropyl alcohol. In the tabulation above cloud point instead of solution point are reported because the solutions could not be frozen.

G. Chemical Properties

1. METHOD OF SYNTHESIS

React sodium diethyldithiocarbamate with 2,3-dichloropropene.

2. CHEMICAL REACTIONS

CDEC will dehydrochlorinate very slowly. It can be decomposed with mineral acids.

H. Commercial Formulations

Vegadex is available as a 4 lb/gallon emulsifiable concentrate; and a 20% concentrate on Attapulgite granules.

II. ANALYSIS

A. Formulation Analysis

1. REVIEW OF METHODS

Liquid or granular Vegadex formulations may be analyzed for their CDEC content by any standard method for the determination of total organic chlorine (see Volume I, Chapter 11). Methods other than the one described below should be thoroughly evaluated with an analytical reference standard.

Technical CDEC may also be analyzed by ultraviolet spectroscopy. The absorption of a dilute solution in methanol is determined at a wavelength of 278 mμ. Concentrations are determined from a calibration curve prepared from a known reference standard.

2. RECOMMENDED METHOD

a. *Principle*

This method is based on the sodium-isobutyl alcohol reduction of organic chlorine to inorganic chloride. The organically bound chlorine is quantitatively liberated as chloride by refluxing with an alcohol-sodium mixture; and is determined by titration with silver nitrate solution.

b. *Reagents*

Isobutyl alcohol, C.P., (low chlorides).
Metallic sodium, C.P.
Hydrogen peroxide, 30% solution.
Silver nitrate, standard solution, 0.1 *N*.
Methanol-water mixture, 50% v/v.

Nitric acid solution, 50% v/v.
Phenolphthalein indicator solution.

c. *Apparatus*

Condenser, Allihn-type.
Round-bottom flask, 100-ml.
Leeds and Northrup pH indicator No. 7664 or equivalent.
Silver:potassium sulfate-mercurous sulfate electrode system or equivalent.
Whatman extraction thimbles, 10 × 50 mm.

d. *Experimental Procedure*

Accurately weigh 0.25–0.45 gm of technical CDEC into a 100-ml round-bottom flask. Immediately add 20 ml of isobutyl alcohol and stopper the flask. Weigh 2.0 gm of metallic sodium, divide into ¼-inch squares and add these to the flask using tweezers. Add two or three boiling chips, connect the flask to a water-cooled condenser and reflux for 45 minutes with an electric heating mantle.

Upon completion of the reflux period, cool the flask by immersing it in a beaker of cold water for 1 minute. (Do not disconnect the flask from the condenser at this time.) Cautiously add 20 ml of 50% methanol through the condenser and heat for 1 minute after reflux to quench the sodium. Cool by immersing in a beaker of cold water for 1 minute.

Cautiously add 10 ml of 30% hydrogen peroxide through the condenser and heat for 10 minutes after reflux to oxidize the sulfur. Cool by immersing in a beaker of cold water for one minute. Wash down the condenser with 5 to 10 ml of acetone.

Remove the flask, acidify using phenolphthalein indicator and 15 ml of 1:1 nitric acid:water. Quantitatively transfer to a 250-ml beaker with acetone and small amounts of water (5–10 ml). The water is used to dissolve any remaining salts in the flask.

Titrate potentiometrically with 0.1 *N* silver nitrate. (The Volhard method may also be used.)

Calculation:

$$\% \text{ Assay of CDEC} = \frac{\text{ml } 0.1\ N\ AgNO_3 \times 2.2379}{\text{Weight of sample}}$$

Vegadex granules may be assayed using the same equipment with a few modifications; i.e., the water-cooled condenser should have a drip-tip so that the condensed solvent can drain into the flask at one point; a glass joint approximately 5 inches long is connected between the condenser and flask.

Prior to weighing a sample, make two pin holes opposite each other approximately 5 mm from the top of the extraction thimble. After weighing the sample, a piece of copper wire approximately 30 mm long is inserted through the holes and bent downward like an inverted "U" to act as a holder for a piece of copper wire which is extended down through the condenser.

Stopper the Whatman extraction thimble with a cork and place in a 30-ml beaker and tare. Add approximately 1.5 gm of CDEC granules and immediately stopper and reweigh.

Add 20 ml of isobutyl alcohol and two or three boiling chips to the round-bottom flask. Weigh 2.0 gm of metallic sodium, divide into ¼ inch squares and add to the flask using tweezers.

Insert the copper "U" shaped holder wire through the thimble and hook on to a wire extended down through the condenser so that the open end of the thimble fits close under the drip-tip of the condenser. Connect the flask to a condenser by means of the 5-inch glass joint and reflux for 45 minutes with an electric heating mantle.

Proceed as given above for technical CDEC.

3. DISCUSSION OF METHOD

Every effort must be made to avoid contamination of the equipment and reagents with chloride. All equipment should be rinsed with dilute nitric acid and then by several rinses of chloride-free distilled water.

The sensitivity of the method depends greatly upon the conditions of application. The use of 0.1 *N* silver nitrate requires the analyst to exercise extreme care in measuring incremental portions during the titration. The final potentiometric end point may be estimated with good precision by computations of inflections.

A correction for possible inorganic chlorides should be made when analyzing either the technical or formnlation material. Weigh a 5.0-gm sample of the technical product or 20.0 gm of granules into a 250-ml beaker. Add 100 ml of acetone (20 ml of distilled water plus 80 ml of acetone for granules), a drop of concentrated sulfuric acid and stir. Titrate with 0.1 *N* silver nitrate under the same electrode system as above.

Calculation:

$$\% \text{ Inorganic chlorine in sample} = \frac{\text{ml } 0.1\ N\ AgNO_3 \times 0.35457}{\text{Wt. of sample}}$$

$$\% \text{ Total chlorine} - \% \text{ inorganic chlorine} = \% \text{ organic chlorine}$$

$$\% \text{ Organic chlorine} \times 6.312 = \% \text{ CDEC}$$

B. Residue Analysis

1. Review of methods

Earlier residue work was accomplished by means of a sensitive ultraviolet absorption method. CDEC in isooctane absorbs very strongly at a wavelength of 278.5 mμ. Although plant extractives absorb strongly in the same region, application of simple but effective clean-up techniques allows a direct measurement of the pesticide (Conkin and Jaworski, 1958).

CDEC can he hydrolyzed in sulfuric acid to give the corresponding amine sulfate. The free amine is distilled from an alkaline medium and then reacted to form the cupric dithiocarbamate complex. Detection of as little as 5 μg of CDEC is possible by this procedure.

2. Recommended method

a. *Principle*

CDEC can be hydrolyzed with dilute sulfuric acid to give the corresponding secondary amine sulfate. The reaction of secondary amines with cupric dithiocarbamate is a classic approach to the determination of traces of the compounds (Dowden, 1938; Feigl, 1956; Hall *et al.*, 1951; Lowen, 1951). The free amine is distilled from an alkaline solution and trapped in dilute hydrochloric acid. Interferences extracted from crop and soil samples are essentially eliminated by washing the solvent extract with dilute hydrochloric acid. A further reduction of the background material is made by a solvent wash of the acidified distillate. This combination of washes effectively removes traces of free amines in the initial solvent extract and colored pigments that may steam distill with the free amines.

b. *Reagents*

Chloroform, USP.
Sulfuric acid, 10% v/v.
Carbon disulfide, A.R.
Ammonium hydroxide, A.R.
Copper sulfate pentahydrate, A.R.
Sodium hydroxide, 50%.
Sodium sulfate, anhydrous, granular, A.R.
Chloroform-carbon disulfide mixture, 1% CS_2 (v/v).
Copper-ammonia reagent. Dissolve 0.5 gm of copper sulfate in 10

ml of distilled water and dilute to 500 ml with concentrated ammonium hydroxide.

Standard amine solution. Using a glass-stoppered dropping bottle or a Victor-Meyer bulb, weigh approximately 0.1000 gm of diethylamine into a 100-ml volumetric flask containing 5 ml of 0.1 *N* HCl. Dilute to volume with distilled water. Pipette an aliquot equivalent to 1000 μg and transfer to a second 100-ml volumetric flask. Dilute to the mark with distilled water. Each milliliter will contain 10 μg of amine.

c. *Apparatus*

See Chapter 20 on "Randox" in this volume.

d. *Experimental Procedure*

i. *Sample Preparation.*

Using a Waring Blendor or similar type apparatus, grind a 500-gm sample of the crop. Transfer the ground mass to a 2000-ml wide-mouth jar. Add a volume of chloroform equal to the sample weight and tumble the sample for 1 hour. Filter and transfer the filtrate to a suitable size separatory funnel. Wash the chloroform extract with three 100-ml portions of 10% HCl. Discard the aqueous layer each time. Bottle and store the chloroform extract in a refrigerator until ready to use.

Measure an aliquot of the chloroform equivalent to 100 gm of the crop sample and transfer to a 250-ml round-bottom flask fitted with a standard-taper neck. Add 100 ml of 10% H_2SO_4, a few carborundum boiling stones and place on a steam bath. Evaporate the chloroform in the presence of the H_2SO_4.

Connect the flask to a vertical condenser and reflux for 15 minutes. Wash down the condenser with distilled water and allow the flask to cool. Connect the flask to a take-over distillation unit equipped with a separatory funnel side-arm arrangement. Immerse the condenser tip in 5 ml of 0.1 *N* HCl contained in a 250-ml separatory funnel.

Introduce 50 ml of 50% NaOH through the side-arm separatory funnel. Swirl or gently agitate the apparatus as the caustic is slowly added to prevent the sudden liberation of excess heat. Distill the liberated amine into the collection funnel, keeping the tip of the condenser beneath the surface of the dilnte acid at all times. Collect 40–60 ml of the distillate, which is usually sufficient for a quantitative recovery of the amine.

Remove the collection funnel and extract the distillate with three 5-ml portions of chloroform. Discard the chloroform extracts. Proceed with the

B. Residue Analysis

1. Review of methods

Earlier residue work was accomplished by means of a sensitive ultraviolet absorption method. CDEC in isooctane absorbs very strongly at a wavelength of 278.5 mμ. Although plant extractives absorb strongly in the same region, application of simple but effective clean-up techniques allows a direct measurement of the pesticide (Conkin and Jawbrski, 1958).

CDEC can be hydrolyzed in sulfuric acid to give the corresponding amine sulfate. The free amine is distilled from an alkaline medium and then reacted to form the cupric dithiocarbamate complex. Detection of as little as 5 μg of CDEC is possible by this procedure.

2. Recommended method

a. *Principle*

CDEC can be hydrolyzed with dilute sulfuric acid to give the corresponding secondary amine sulfate. The reaction of secondary amines with cupric dithiocarbamate is a classic approach to the determination of traces of the compounds (Dowden, 1938; Feigl, 1956; Hall *et al.*, 1951; Lowen, 1951). The free amine is distilled from an alkaline solution and trapped in dilute hydrochloric acid. Interferences extracted from crop and soil samples are essentially eliminated by washing the solvent extract with dilute hydrochloric acid. A further reduction of the background material is made by a solvent wash of the acidified distillate. This combination of washes effectively removes traces of free amines in the initial solvent extract and colored pigments that may steam distill with the free amines.

b. *Reagents*

Chloroform, USP.
Sulfuric acid, 10% v/v.
Carbon disulfide, A.R.
Ammonium hydroxide, A.R.
Copper sulfate pentahydrate, A.R.
Sodium hydroxide, 50%.
Sodium sulfate, anhydrous, granular, A.R.
Chloroform-carbon disulfide mixture, 1% CS_2 (v/v).
Copper-ammonia reagent. Dissolve 0.5 gm of copper sulfate in 10

ml of distilled water and dilute to 500 ml with concentrated ammonium hydroxide.

Standard amine solution. Using a glass-stoppered dropping bottle or a Victor-Meyer bulb, weigh approximately 0.1000 gm of diethylamine into a 100-ml volumetric flask containing 5 ml of 0.1 *N* HCl. Dilute to volume with distilled water. Pipette an aliquot equivalent to 1000 μg and transfer to a second 100-ml volumetric flask. Dilute to the mark with distilled water. Each milliliter will contain 10 μg of amine.

c. *Apparatus*

See Chapter 20 on "Randox" in this volumc.

d. *Experimental Procedure*

i. *Sample Preparation.*

Using a Waring Blendor or similar type apparatus, grind a 500-gm sample of the crop. Transfer the ground mass to a 2000-ml wide-mouth jar. Add a volume of chloroform equal to the sample weight and tumble the sample for 1 hour. Filter and transfer the filtrate to a suitable size separatory fnnnel. Wash the chloroform extract with three 100-ml portions of 10% HCl. Discard the aqueous layer cach time. Bottle and store the chloroform extract in a refrigerator until ready to use.

Measure an aliquot of the chloroform equivalent to 100 gm of the crop sample and transfer to a 250-ml round-bottom flask fitted with a standard-taper neck. Add 100 ml of 10% H_2SO_4, a few carborundum boiling stones and place on a steam bath. Evaporate the chloroform in the presence of the H_2SO_4.

Connect the flask to a vertical condenser and reflux for 15 minutes. Wash down the condenser with distilled water and allow the flask to cool. Connect the flask to a take-over distillation unit equipped with a separatory funnel side-arm arrangemeut. Immerse the condenser tip in 5 ml of 0.1 *N* HCl contained in a 250-ml separatory funnel.

Introduce 50 ml of 50% NaOH through the side-arm separatory funnel. Swirl or gently agitate the apparatus as the caustic is slowly added to prevent the sudden liberation of excess heat. Distill the liberated amine into tbe collection funnel, keeping the tip of the condenser beneath the surface of the dilute acid at all times. Collect 40–60 ml of the distillate, which is usually sufficient for a quantitative recovery of the amine.

Remove the collection funnel and extract the distillate with three 5-ml portions of chloroform. Discard the chloroform extracts. Proceed with the

color formation as outlined below (see "Standard Curve"). Convert the absorbance at 435 mμ to micrograms of amine by use of the standard curve.

ii. *Preparation of Standard Curve for Amines.*

Pipet 1, 4, 7, and 10 ml of the standard amine solution and transfer to 125-ml separatory funnels. Dilute with distilled water to 50 ml. Add 10 ml of chloroform-carbon disulfide mixture, 5 ml of copper-ammonia reagent, and 1 to 2 ml of 50% NaOH. Shake for 3 minutes, allow the layers to separate and draw off the chloroform layer into a 25-ml Erlenmeyer flask. Dry over 1 to 2 gm of sodium sulfate until the colored solution is free of any cloudiness. Occasional swirling may be necessary. Decant a portion of the clear solution into a 1-cm cell and measure the absorbance at 435 mμ with a Beckman DU spectrophotometer (or equivalent) using distilled water as a reference. Prepare a standard curve by plotting absorbance against concentration.

iii. *Calculation.*

$$\text{p.p.m. CDEC} = \frac{\text{Micrograms diethylamine}}{\text{Aliquot sample weight (grams)} \times 0.326}$$

Correct the parts per million of CDEC for per cent recovery as determined from a series of untreated crop samples to which known amounts were added. Recoveries have averaged 80% or better in all applications.

3. APPLICABILITY OF METHOD TO DIFFERENT CROP OR FOOD MATERIALS

The method is applicable to a variety of vegetable crops with no special difficulties.

4. DISCUSSION OF METHOD

The use of chloroform as the extraction solvent often leads to difficulties due to the formation of tight emulsions. The use of anhydrous granular sodium sulfate in amounts up to that equal to the sample weight will greatly aid in breaking up emulsions that may form during the tumbling or shaking step. It is also possible to place the sample in a deep freezer overnight. Filtration is accomplished as the sample warms to room temperature.

Reaction times for the formation of the colored copper complex may vary with the volume and temperature of the distillate. A maximum of 5 minutes is allowed for shaking, using a wrist-action mechanical shaker equipped with a timer clock. The time and volume should be adjusted to give maximum consistent recoveries. Instrumental readings should be

made without delay as the color is subject to fading in a few minutes. Excessive cloudiness in the chloroform layer is normally removed by drying over a gram or two of anhydrous sodium sulfate.

5. MODIFICATION OF METHOD

The validity of results following modification of the method should be verified by the use of an analytical reference sample.

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