Table II. Effect of Acidity on Development of the Test Color with 34.2 γ of ONCB

Zinc, G.	HCl, Meq.	HCl Excess, Meq., Calcd.[a]	pH	Absorbance	Absorbance/γ/ 25 Ml.[b]
0.2	6	0	1.1	0.441	0.0129
0.1	2.5	0	1.1	0.455	0.0133
0.2	10	3.9	0.7	0.553	0.0162
0.2	10	3.9	0.9	0.561	0.0164

[a] Not directly related to pH, owing to incomplete reaction with zinc.
[b] Obtained on the Evelyn photoelectric colorimeter with filter 540.

Table III. Standard Absorbance Readings Obtained with Filter 540 on the Evelyn Photoelectric Colorimeter

ONCB, γ/25 Ml.	Transmittance, %	Absorbance		A^a/γ
10.7	67	0.174		0.0163
21.4	43¾	0.359		0.0168
32.1	29¾	0.527		0.0164
			Av.	0.0165

[a] A = absorbance.

pH of 0.6 to 1.0 during the coupling reaction in order to achieve maximal color development. Table II gives data on ONCB which confirm this effect. Similar results were obtained on smaller amounts of ONCB. An excess of hydrochloric acid is essential for proper color development.

Adherence to Beer's Law. Absorbance readings obtained on the Evelyn photoelectric colorimeter with filter 540 using matched colorimeter tubes bear a direct linear relationship with varying amounts of ONCB as shown in Table III. The mean absorbance factor, $\frac{\text{Absorbance}}{\gamma}$, is therefore used for the calculations.

Of the various green Evelyn filters available, filter No. 540 is best inasmuch as it transmits light maximally at 540 mμ where maximal absorption of the test color occurs. This is shown by the absorption spectrum in Figure 1 obtained on the Beckman DK-2 spectrophotometer using a 1-cm. cuvette.

Recovery and Sensitivity. Recovery of micro quantities of ONCB added to fresh pineapple fruit tissue and analyzed by the method described above is presented in Table IV. Average recovery of 93% is considered excellent particularly in view of the small amounts of ONCB present.

The method is extremely sensitive—0.01 p.p.m. may be determined. On the Evelyn instrument using the ⅞-inch colorimeter tube, a reading of 96% transmittance is obtained at this concentration.

Literature Cited

(1) Averell, P. R., Norris, M. V., *Anal. Chem.* **20,** 753–6 (1948).
(2) Sherwood, L. V., Monsanto Chemical Co., St. Louis, Mo., private communication, November 8, 1957.
(3) Young, H. Y., Gortner, W. A., *Anal. Chem.* **25,** 800–02 (1953).

Table IV. Recovery of ONCB Added to Pineapple Fruit Tissue[a]

Added, P.P.M.	Recovered, P.P.M.[b]	Recovery, %
0.07	0.06	86
0.07	0.07	100
0.21	0.20	95
0.21	0.19	90
0.36	0.33	92
0.36	0.35	97
	Av.	93

[a] 100-gram sample.
[b] Corrected for a blank value of 0.01 p.p.m. apparent ONCB.

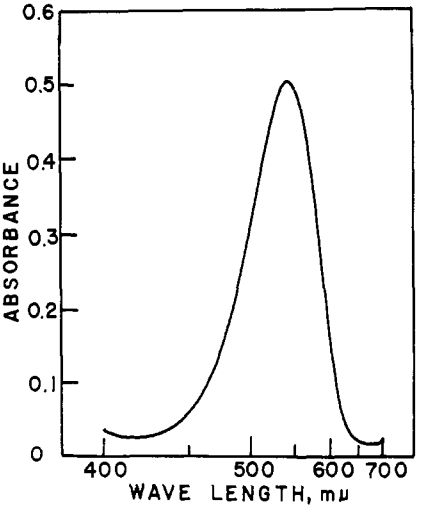

Figure 1. Absorption spectrum of ONCB test color, 55 γ ONCB per 25 ml.

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HERBICIDE RESIDUES

A Colorimetric Method for the Determination of EPTC Residues in Crops and Soils

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ETHYL - *N*,*N* - DI - *n* - PROPYLTHIOLCARBAMATE (EPTC), also known as Eptam, is a promising selective herbicide. A sensitive method which determines the di-*n*-propylamine formed upon hydrolysis of EPTC in concentrated sulfuric acid has been developed for the determination of crop residues. The amine is reacted with carbon disulfide in the presence of ammonia and cupric ion in a two-phase benzene-water system to form, in benzene, the cupric di-*n*-propyldithiocarbamate complex which has an intense absorption peak at 440 mμ. Detection of quantities of EPTC down to 4 γ enables determination of 0.02 p.p.m. of residue by the described procedure.

The colorimetry is based upon the method of Dowden (*1*) for determination of secondary alkylamines, which previously had been adapted to the determination of octamethylpyrophosphoramide (OMPA) by Hall, Stohlman, and Schechter (*3*). OMPA does not interfere, however, when 100 γ are processed through the steam distillation procedure. Possible interference by free amines is eliminated by acidifying the steam distillate prior to extraction of EPTC in preparation for hydrolysis. A wide

EPTC, ethyl-*N,N*-di-*n*-propylthiolcarbamate, is hydrolyzed in sulfuric acid to di-*n*-propylamine from which is formed the cupric dithiocarbamate complex having an absorption peak at 440 mμ. Crop and soil extraction procedures are presented.

variety of crops have exhibited background values of less than 0.02 p.p.m., excluding turnips which seriously interfere with the colorimetry.

EPTC is extracted from samples with hexane or by direct steam distillation. The latter method was employed originally by Fang (*2*) in radiotracer studies of EPTC residues. Interference with the colorimetry is eliminated effectively by a combination of procedures utilizing steam distillation, benzene extraction of acidified amine solution, and determination of background absorbance prior to color formation.

Special Reagents

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

EPTC Standard Solution. One hundred micrograms of EPTC per ml. of iso-octane.

Sample Extraction and Cleanup

Hexane Extraction Method. Macerate 800 grams of sample with 1600 ml. of hexane in a 1-gallon Waring Blendor until thoroughly blended. A stable emulsion usually forms but can be broken by mixing in approximately 1000 grams of anhydrous sodium sulfate and allowing to stand overnight in a sealed container. Filter 800 to 900 ml. of hexane and reserve for further processing.

Concentrate a 400-ml. aliquot of hexane extract, representing 200 grams of sample, to about 15 ml. by rapid distillation through a three-ball Snyder column. Wash the column with 5 ml. of hexane, cool, and add 100 ml. of water. Replace the Snyder column with a distillation head and condenser, and steam-distill the EPTC and the residual hexane plus about 80 ml. of water into a 125-ml. separatory funnel. Add 5 drops of concentrated hydrochloric acid to the distillate and extract the aqueous phase with the codistilled hexane. Draw off the aqueous phase and carefully transfer the hexane to a dry 40-ml., glass-stoppered, graduated centrifuge tube, avoiding the introduction of any water droplets into the tube. Return the aqueous phase to the separatory funnel, re-extract with an additional 10 ml. of hexane, and transfer the hexane as before to the centrifuge tube. Care must be taken to prevent introducing water droplets into the centrifuge tube because dilution of the sulfuric acid will seriously retard the rate of hydrolysis of EPTC. The combined hexane, representing 200 grams of sample, is now ready for hydrolysis and colorimetry.

Direct Steam Distillation Method. Add 400 grams of crop sample macerated with 1000 ml. of water, or 400 grams of soil sample and 1000 ml. of water, to a 4-liter wide-mouthed Erlenmeyer flask. Add 25 ml. of glacial acetic acid and distill about 400 ml. of distillate into a 500-ml. separatory funnel. Add 5 drops of concentrated hydrochloric acid to the distillate and extract the EPTC from the aqueous phase with two 30-ml. portions of iso-octane. Combine the iso-octane extracts, which represent 400 grams of sample, and store them for subsequent hydrolysis and colorimetry.

Hydrolysis and Colorimetry. Add 1 ml. of 96% sulfuric acid to a 40-ml. centrifuge tube containing either the hexane extract or an aliquot of iso-octane extract representing 200 grams of sample. Residues up to 3.5 p.p.m. can be determined without adjusting the indicated sample size. Shake vigorously for 1 minute to extract the EPTC into the acid phase. Allow the phases to separate and discard the solvent phase by withdrawing it with a suction tube. (Small amounts of residual solvent will not interfere.) Heat the tube in an 85° ± 1° C. glycerol bath for 20 minutes to hydrolyze the EPTC. Twice during the heating period gently roll the tube to rinse the sides with hot acid.

Cool the tube in an ice bath and cautiously dilute the cold acid to 20 ml. with water. Add 1 drop of phenolphthalein solution and neutralize by the dropwise addition of 50% sodium hydroxide while swirling and cooling. Promptly acidify by the dropwise addition of concentrated hydrochloric acid, add 4 extra drops of acid, and dilute to 25 ml. with water. Extract the slightly acidic aqueous solution with two 10-ml. portions of benzene to remove benzene-soluble background color from the amine hydrochloride in the aqueous phase. Withdraw and discard each benzene portion with a suction tube, taking care to remove as much of the benzene as possible after the last extraction.

Add 1 drop of phenolphthalein solution and add 50% sodium hydroxide dropwise until the solution is basic. Add 2 extra drops of sodium hydroxide. Introduce 10.0 ml. of benzene and extract the free amine from the basic solution. Withdraw the aqueous phase and replace it with 15 ml. of water plus 2 drops of 50% sodium hydroxide. Shake vigorously to wash the benzene layer. Allow the phases to separate, transfer about 4 ml. of the benzene layer into a test tube, and dry over 1 gram of granular anhydrous sodium sulfate to remove turbidity. Transfer the clarified benzene 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 copper-ammonia reagent and carbon disulfide. Without delay or interruption shake moderately for 4 minutes. Allow the solution to clear and then dry a portion of the benzene, as before. Read the

Table I. Preparation of Standard Curve for EPTC[a]

EPTC, γ	Net Absorbance, 440 Mμ
0	0.003
4	0.012
10	0.027
20	0.050
50	0.121
100	0.239
200	0.475
400	0.947

[a] EPTC was added in 30 ml. of iso-octane to a 40-ml. centrifuge tube and processed through the colorimetric procedure.

Table II. Recovery of EPTC Added to Hexane Extracts[a]

Crop	EPTC Added, p.p.m.	EPTC Recovered, %
Alfalfa		
Fresh	0.25	88
Dried	1.00	76
Beans, dried	0.10	100
	0.05	80
Beets	0.20	80
Clover	0.50	84
Corn	0.50	78
	0.10	100
Onions	0.50	72
Peppers	0.50	87
Potatoes	0.20	75
Snap bean plants	0.10	100
Spinach	0.50	88
Strawberries	0.20	85
Sugar beets	0.05	80
Tomatoes	0.50	79

[a] Aliquots of raw hexane extract representing 200 grams of crop were fortified with EPTC and processed through the hexane concentration, steam distillation, and colorimetric determination procedures.

Table III. Recovery of EPTC Added to Macerated Crops

Crop	EPTC Added, p.p.m.	EPTC Recovered, %
Hexane Extraction Method		
Beans, dried	0.20	90
Carrots	0.50	78
Snap bean plants	0.10	100
Sugar beets	0.20	105
Tops	0.20	90
Direct Steam Distillation Extraction Method		
Blackberries	1.0	101
	0.1	100
Cantaloupes	1.0	90
	0.1	80
Onions, dried	1.0	86
Peas	1.0	79
Soil		
Sandy loam	6.0	92
Clay	2.0	99

absorbance at 440 mμ within 10 minutes after the color is formed.

During color formation, 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 also can result in side reactions. The color formed in absence of sample extractives is normally stable after drying, but slow fading has been observed in the presence of crop extractives.

Determine the amount of EPTC in the sample by referring the net absorbance (the difference between absorbance before and after color formation) to a standard curve prepared by processing aliquots of the EPTC standard solution in a similar manner. Typical absorbance values are listed in Table I.

The colorimetry is not significantly affected by the small quantities of sample extractives introduced into the concentrated sulfuric acid. Typical recovery values are presented in Table II for EPTC added to a variety of crop extracts.

Discussion

Thirty consecutive colorimetric determinations of 400 γ of EPTC over a 7-week period of routine analyses by a single operator have resulted in a relative average deviation of ±3.4%.

EPTC is miscible in all proportions with most organic solvents but is only slightly soluble in water (375 ± 15 p.p.m. at 20° C.). It is readily extracted with hexane from a variety of crops (Table III). The hexane extraction procedure has been employed for most crop residue analyses to date. However, the recently adapted direct steam distillation method of extraction is being used currently for all soil and most crop samples. The latter method has the significant advantages of combining the extraction and cleanup steps, and of eliminating the handling of large quantities of hexane. Crops with a high starch content, such as potatoes and dried beans, cannot be extracted conveniently by this method because of excessive foaming and scorching. Some typical recoveries of EPTC added to macerated crops, employing both extraction methods, are given in Table III.

Acknowledgment

The authors are indebted to B. J. Adelson for his invaluable technical assistance.

Literature Cited

(1) Dowden, H. C., *Biochem. J.* **32,** 455–9 (1938).
(2) Fang, S. C., Thiesen, Patricia, J. Agr. Food Chem. **7,** 770–1 (1959).
(3) Hall, S. A., Stohlman, J. W., Schecter, M. S., *Anal. Chem.* **23,** 1866 (1951).

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HERBICIDE RESIDUES

Improved Extraction Procedure for the Determination of EPTC Residues in Potatoes

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An improved method of extracting the herbicide, EPTC, from potatoes is described. Potatoes are blended with isopropyl alcohol and Skellysolve B (*n*-hexane). After filtering, hexane separates and is collected. The aqueous isopropyl alcohol is extracted with hexane. The combined hexane extracts are evaporated and EPTC is determined. Stable emulsions do not form when isopropyl alcohol is used. Recovery of 0.1 p.p.m. of EPTC from potatoes ranged from 93.5 to 99.0%. Analysis of potatoes treated with EPTC for weed control showed no residue.

Ethyl *N*,*N*-di-*n*-propylthiolcarbamate (EPTC, Eptam) is a selective pre-emergence herbicide which will control grassy and certain broadleaf weeds. A method by Batchelder and Patchett (*1*) for determining EPTC residues has been developed. It involves extraction of the herbicide from crop material with Skellysolve B (*n*-hexane) and separation from plant waxes and pigments by steam distillation. EPTC is then hydrolyzed to di-*n*-propylamine. The amine is determined colorimetrically after reaction with carbon disulfide in the presence of cupric ion and ammonia to form the yellow cupric dithiocarbamate.

Extraction of EPTC from potatoes by blending with hexane produces emulsions which are difficult to break. Recovery of EPTC using the above method after hexane extraction is erratic. Extraction with hexane–isopropyl alcohol eliminates the formation of stable emulsions and good recovery of EPTC is obtained.