this pH range, glutamate is about 94% in the R_3 form at pH 5.5, close to 100% at pH 7, and about 97% at pH 8 (Figure 1). Further support to the theory that the R_3 form, $-OOC-R-COO^-$, may be
NH_3^+
largely responsible for "glutability" is furnished by Galvin (*4*), who reported that solutions containing 0.5% glutamate and 1% salt, and adjusted to pH values from 3.3 to 8.0 all had essentially the same taste at pH 6, 7, and 8. At pH 5.0, the normal glutamate taste was noticeably reduced to approximately 80% of the original intensity. Figure 1 indicates that 85% of the glutamate is present in the R_3 form, which is in good agreement. Galvin stated that normal glutamate taste was further reduced with a decrease in pH, which is also in agreement with the theory.

If the R_3 form is responsible for "glutability," then in order to obtain the same flavor level at pH 5.0 as at 6.0, more total glutamate would be required—that is, 1.00/0.85 or 1.17 times as much glutamate would be necessary to obtain the same concentration of the R_3 form at pH 5.0 as at pH 6.0. Similarly, at pH 4.5, 1.0/0.64 or 1.56 as much glutamate would be required to obtain the same concentration of R_3 as at pH 6.0.

There appears to be a lower limit of pH (about 4.0) below which it would become impracticable to add glutamate, because of the rapidly decreasing equilibrium concentrations of the R_3 form.

Acknowledgment

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HERBICIDE RESIDUES

Determination of 3-(*p*-Chlorophenyl)-1,1-dimethylurea In Soils and Plant Tissue

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The use of 3-(*p*-chlorophenyl)-1,1-dimethylurea as a herbicide required the development of a simple, accurate method for detecting and determining residues in treated soils and plant tissues. The sample being analyzed is disintegrated by digestion in strong alkali, and the 3-(*p*-chlorophenyl)-1,1-dimethylurea is quantitatively hydrolyzed to *p*-chloroaniline. The resulting aromatic amine is automatically removed and concentrated into a minimum volume of organic solvent. It is then extracted with dilute acid and determined colorimetrically. The method is applicable for determining microgram quantities of 3-(*p*-chlorophenyl)-1,1-dimethylurea in all types of soils examined and in a wide variety of crops The procedures are capable of detecting as little as a few parts per billion of 3-(*p*-chlorophenyl)-1,1-dimethylurea in refined cane sugar and a few parts per 10,000,000 in nearly all plant tissues and soils.

RESIDUAL QUANTITIES of the herbicide, 3-(*p*-chlorophenyl)-1,1-dimethylurea (formerly known as CMU, the active ingredient in Du Pont Karmex W herbicide for agricultural use and Du Pont Telvar W weed killer for industrial use) have been determined in soil by a method which involved extraction with a mixture of acetonitrile and acetic acid, hydrolysis of the recovered active ingredient, and colorimetric determination of the resulting *p*-chloroaniline by diazotizing and coupling (*2*). However, this procedure proved unsuitable for determining residual 3-(*p*-chlorophenyl)-1,1-dimethylurea in plant tissues and in certain highly absorptive types of soil. A more general method has been perfected, based on the caustic hydrolysis principles described in the closing paragraphs of the earlier paper and utilized by Young and Gortner (*4*) for determining 3-(*p*-chlorophenyl)-1,1-dimethylurea in pineapple tissue.

In this improved procedure, the residual 3-(*p*-chlorophenyl)-1,1-dimethylurea is quantitatively hydrolyzed to *p*-chloroaniline by refluxing the sample with a concentrated sodium hydroxide solution. The resulting *p*-chloroaniline is recovered from the caustic digestate by a combination of continuous steam distillation and extraction or by liquid-liquid extraction, following which it is determined colorimetrically. Complete recovery of residual 3-(*p*-chlorophenyl)-1,1-dimethylurea has been obtained from all plant tissues and soils examined. Although elapsed time for a single analysis is essentially the same as in the original extraction method, the caustic hydrolysis

procedure requires substantially less operator time, and many more analyses can be conducted in a given period.

When tissue samples or small samples of soil are being analyzed, *p*-chloroaniline can be most successfully recovered from the hydrolysis mixture by continuous steam distillation and extraction, using an automatic apparatus originally described by Vogel (*3*). With soil samples larger than 50 grams, however, liquid-liquid extraction is often a more successful method for recovering the *p*-chloroaniline from the caustic hydrolyzate because of bumping and foaming difficulties encountered in the continuous steam-distillation apparatus. The liquid-liquid extraction of soil slurries may be carried out successfully in a modified estrogen extractor fitted with a magnetic stirring bar to maintain the caustic-soil slurries in suspension.

Procedure

Reagents and Special Apparatus Apparatus for continuous extraction of steam-volatile substances (Figure 1).

Estrogen extractor (available from the Scientific Glass Co., Bloomfield, N. J.) modified with a metal percolation tube and stirring mechanism (Figure 2).

Magnetic stirring motor.

Ball mill with quart jars.

Silicon carbide grains (Grit 6), available from the Carborundum Co., Niagara Falls, N. Y.

Dow Corning Antifoam A.

Lorol No. 5 antifoaming agent.

Hexane, acid-washed.

Diethyl ether, redistilled, peroxide-free.

3-(*p*-Chlorophenyl)-1,1-dimethylurea, recrystallized from methanol.

p-Chloroaniline, recrystallized from ethanol.

Sodium nitrite, reagent grade, 2% solution prepared fresh daily.

Sulfamic acid, 10% solution prepared fresh every 3 days.

N-(1-Naphthyl)ethylenediamine dihydrochloride, 2% solution prepared fresh daily from Eimer & Amend Reagent N-21 or its equivalent.

Hydrochloric acid, 1*N* solution prepared from reagent grade acid.

Sodium hydroxide solutions, 15 and 50% prepared from reagent grade chemicals.

Calibration A standard solution is prepared by dissolving 0.2 gram of recrystallized *p*-chloroaniline in 100 ml. of 1*N* hydrochloric acid and diluting this solution a hundredfold with acid. Appropriate aliquots containing from 5 to 80 γ of *p*-chloroaniline are transferred to 50-ml. volumetric flasks and, diluted to approximately 40 ml. with 1*N* hydrochloric acid and a 1-ml. portion of 2% sodium nitrite is added to each. After a 15-minute diazotization period, a 1-ml. portion of 10% sulfamic acid is added, the contents are shaken vigorously, and 10 minutes are allowed to ensure complete destruction of excess nitrite. Two milliliters of 2% (*N*-(1-naphthyl)ethylenediamine dihydrochloride are added, and the contents are diluted to 50 ml. with 1*N* hydrochloric acid. After 15 minutes, the intensity of the magenta color formed is determined on a photoelectric colorimeter or a spectrophotometer at 560 mμ, using distilled water in the reference cell.

A plot of absorbance versus the *p*-chloroaniline concentration in micrograms per 50 ml. is constructed from these calibration data and used directly in subsequent determinations.

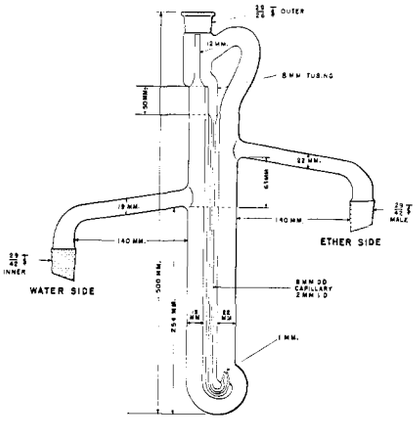

Figure 1. Steam distillation-extraction head

Determination If the continuous digestion-extraction apparatus (Figure 1) is employed, approximately 250 grams of representative tissue or 50 grams of soil are placed in a 2-liter digestion flask. Approximately 500 ml. of distilled water, a few drops of Dow-Corning Antifoam A, 2 or 3 boiling chips, and 190 to 200 ml. of 50% sodium hydroxide solution are added; and the flask is attached to the "distillation-extraction head." The arms of the U-tube are filled with sufficient water to prevent organic solvent from returning to the digestion flask. Approximately 500 ml. of solvent (hexane or ether) and 2 to 3 Carborundum chips are placed in a 1-liter flask, which is then attached to the extraction head. After a 24-inch condenser has been positioned, heat is applied to both still pots at such a rate that condensed vapors of solvent and water pass through the capillary in the form of small sausages, and digestion is continued for 9 hours (during which time all plant tissues investigated to date have completely disintegrated).

If the apparatus shown in Figure 2 is employed, 100 to 200 grams of soil are placed in the flask of the special extractor. Five drops of Lorol No. 5 antifoaming agent are added along with 8 to 10 grams of boiling stones and 400 ml. of 15% sodium hydroxide solution. The flask is attached to a 24-inch water-cooled condenser by means of an appropriate adapter, and the mixture is refluxed for 4 hours. At the end of this digestion period, during which the 3-(*p*-chlorophenyl)-1,1-dimethylurea is desorbed and hydrolyzed to *p*-chloroani-

Table I. Recovery of 3-(*p*-Chlorophenyl)-1,1-dimethylurea from Various Soils Using Liquid-Liquid Extraction to Isolate *p*-Chloroaniline

(100-gram samples used throughout)

Soil Type	Location	3-(*p*-Chlorophenyl)-1,1-dimethylurea Added, γ	Apparent 3-(*p*-Chlorophenyl)-1,1-dimethylurea Found, γ	% Recovery[a]
Railroad ballast	Charlotte, N. C.	None	9	...
		369	357	94
		246	207	80
		123	121	90
Clay loam	Manhattan, Kan.	None	37	...
		123	137	81
		246	283	100
		100	139	102
		200	207	85
Leon-Immokalee fine sand	Bradenton, Fla.	None	12	...
		100	88	76
		300	271	86
Keyport silt loam	Newark, Del.	None	11	...
		100	98	87
		300	247	79
Sandy loam	Stoneville, Miss.	None	21	...
		200	195	87
		300	280	86
Clay	College Station, Tex.	None	27	...
		200	214	93
		300	292	88
Lintonia silt loam	Baton Rouge, La.	None	15	...
		100	96	81
		200	197	91
		200	207	96
		300	299	95

[a] All recoveries corrected for blanks.

line, the flask containing the soil slurry is cooled in an ice bath while still attached to the condenser. The condenser is rinsed with 20 ml. of dilute hydrochloric acid; and after the addition of 400 ml. of water to minimize the solvent holdup volume, the pot is transferred to the special liquid-liquid extractor. A flask containing 800 ml. of acid-washed hexane is connected to the solvent arm of the extractor, and the hexane is refluxed at such a rate that a steady stream of condensate is delivered to the percolation tube. The liquid-liquid extraction process is continued for 16 hours (overnight) with the magnetic stirrer operating as rapidly as possible to maintain the soil in suspension.

At the completion of either of the extraction cycles, the solvent is cooled and re-extracted with 1*N* hydrochloric acid; six 15-ml. portions of acid are used. The combined acid extracts are collected in a 100-ml. volumetric flask and diluted to the mark with additional 1*N* hydrochloric acid solution. A suitable aliquot containing between 5 and

Table II. Recovery of 3-(p-Chlorophenyl)-1,1-dimethylurea from Various Soils Using Steam Distillation to Isolate p-Chloroaniline

(50-gram samples used throughout)

Soil Type	Location	3-(p-Chlorophenyl)-1,1-dimethylurea Added, γ	Apparent 3-(p-Chlorophenyl)-1,1-dimethylurea Found, γ	% Recovery[a]
Cecil sandy loam	North Carolina	...	6.5	...
		132	128	93
Lintonia silt loam	Louisiana	...	14.4	...
		42	53.4	92
Leon-Immokalee fine sand	Florida	...	10.1	...
		339	335	96
Keyport silt loam	Delaware	...	12.8	...
		170	165	90
Clay	Texas	...	6.0	...
		50	50	88

[a] All recoveries corrected for blanks.

Table III. Recovery of 3-(p-Chlorophenyl)-1,1-dimethylurea from Various Crops

Crop	Wt. of Sample, Grams	3-(p-Chlorophenyl)-1,1-dimethylurea Added, γ	Apparent 3-(p-Chlorophenyl)-1,1-dimethylurea Found, γ	Net 3-(p-Chlorophenyl)-1,1-dimethylurea Found, γ	% Recovery		Level, P.p.m.
Sugar cane	250	15.6	16.4	15.4	99	at	0.06
		31.2	30.9	29.9	99	at	0.12
		46.8	45.4	44.4	95	at	0.19
		...	1.0	...	...		...
	250	15.6	14.5	14.1	91	at	0.06
		31.2	31.1	29.7	95	at	0.12
		46.8	44.1	43.7	93	at	0.19
		...	0.4	...	...		...
	250	15.6	17.1	15.7	101	at	0.06
		31.2	33.1	31.7	101	at	0.12
		46.8	45.6	44.2	95	at	0.19
		...	1.4	...	...		...
	250	15.6	18.4	16.2	104	at	0.06
		31.2	34.6	32.4	104	at	0.12
		...	2.2	...	...		...
Fresh pineapple	300	...	13.4	...	...		...
		9.0	22.5	9.1	101	at	0.03
		18.0	30.8	17.4	97	at	0.06
		27.0	38.5	25.1	93	at	0.09
		36.0	48.0	34.6	96	at	0.12
Cottonseed	50	21.2	26.0	17.5	83	at	0.42
		106.0	110.5	102.0	96	at	2.12
		...	8.5	...	...		...
	50	10.6	21.5	10.0	94	at	0.21
		31.8	38.0	27.5	86	at	0.64
		42.4	52.3	41.8	99	at	0.85
		...	10.5	...	...		...
Fresh asparagus	200	...	6.6	...	...		...
		19.8	26.2	19.6	99	at	0.10
		39.6	47.6	41.0	104	at	0.20
		59.4	68.2	61.6	104	at	0.30
		42.4	50.8	44.2	104	at	0.21
Frozen asparagus	156	100.0	112.0	101.0	101	at	0.64
	155	...	11.0	...	...		...
Frozen peas	146	...	4.3	...	...		...
	132	100.0	104.0	100.0	100	at	0.69
Onions	196	...	5.5	...	...		...
	197	42.6	46.8	41.3	97	at	0.22
Canned beets	400	...	8.2	...	...		...
		39.6	46.8	38.6	98	at	0.10
Soybeans	100	...	19.1	...	...		...
		39.6	58.5	38.6	98	at	0.40
Frozen corn kernels	300	...	20.7	...	...		...
		39.6	63.6	42.9	108	at	0.13

80γ of *p*-chloroaniline is transferred to a 50-ml. volumetric flask, and the color-development steps described in the calibration section are repeated. After the 15-minute coupling period, the absorbance is determined at 560 mμ, and the *p*-chloroaniline content of the aliquot is determined from the calibration curve. The residue in the original sample is calculated according to the following equation:

$$\text{P.p.m. of 3-(}p\text{-chlorophenyl)-1,1-dimethylurea} = \frac{\text{micrograms of } p\text{-chloroaniline in final aliquot} \times 100 \times 1.56}{\text{volume of final aliquot} \times \text{weight of sample in grams}}$$

Discussion and Experimental Results

Most plant tissues and soils contain small amounts of interfering materials. Therefore, similar determinations must be conducted on representative untreated samples, and the true residual 3-(*p*-chlorophenyl)-1,1-dimethylurea is calculated by deducting the blanks found in the untreated sample from the "apparent 3 - (*p* - chlorophenyl) - 1,1 - dimethylurea" found in the treated modification. As the interfering materials usually exhibit a slow coupling rate when diazotized and made to react with *N*-(1-naphthyl)ethylenediamine dihydrochloride, the level of interference is minimized by determining the color intensities after a 15-minute coupling period, during which time any diazotized *p*-chloroaniline reacts completely.

For operational convenience, the quantity of sample originally charged to the digestion flask should be adjusted to contain approximately 50 γ of total 3-(*p*-chlorophenyl)-1,1-dimethylurea. The size of the digestion-hydrolysis flask of Figure 1 may be increased to 10 liters in order to permit large charges of tissues containing low levels of residue; however, increases in charge size also result in proportional increases in the interferences from naturally occurring materials, and the limit of significant detection is often controlled by the magnitude of this interference rather than by the size of the charge. When unexpectedly high levels of residue are encountered, it usually is not necessary to repeat the hydrolysis and extraction steps, because the procedures and apparatus described have been used successfully to recover the *p*-chloroaniline resulting from as much as 30 mg. of 3-(*p*-chlorophenyl)-1,1-dimethylurea. In these cases, however, the final aliquot selected for diazotizing and coupling should contain no more than 80 γ of *p*-chloroaniline.

The applicability of the direct caustic hydrolysis procedures for determining residues of 3-(*p*-chlorophenyl)-1,1-dimethylurea was tested by analyzing a variety of untreated soils and plant tissues to which known quantities of 3-(*p*-chlorophenyl)-1,1-dimethylurea were added. Typical recovery data (Tables I, II, and III) show that essentially theoretical recoveries of microgram quantities of 3-(*p*-chlorophenyl)-1,1-dimethylurea are obtained. The reproducibility of the procedure was investigated by analyzing a series of aliquots taken from soils treated with 3-(*p*-chlorophenyl)-1,1-dimethylurea. Using dry ball-milling, standard deviations of 18 and 12% relative were obtained on seven replicates taken from soils containing 0.73 and 3.59 p.p.m., respectively. When homogenization was carried out by adding sufficient water to the soil to produce a thin paste and ball-milling the fluid mass, the standard deviations were reduced to 4.8% relative at the 1.23 p.p.m. level and 4.6% at the 2.36 p.p.m. level. These experiments serve to emphasize the importance of proper sample selection and proper subsampling procedures prior to the application of the hydrolysis, isolation, and color-development steps.

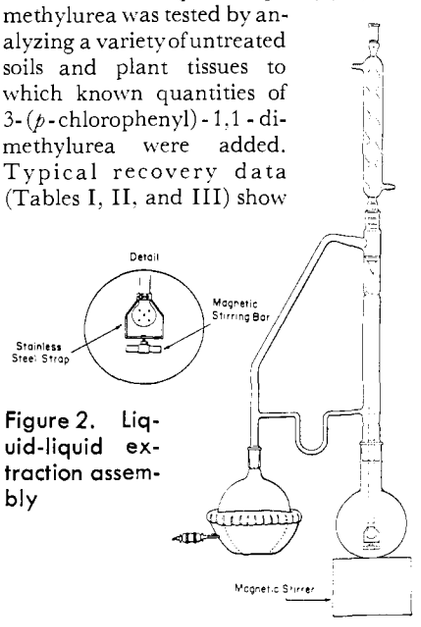

Figure 2. Liquid-liquid extraction assembly

The data presented in Table IV illustrate an actual application of the direct caustic hydrolysis procedure in which the residue levels were determined in various sugar refinery fractions during the processing of sugar cane harvested from plots treated with 3-(*p*-chlorophenyl)-1,1-dimethylurea. The maximum sensitivity realized in this study was obtained in the analysis of refined sugar, which exhibited an interference level of less than one part per billion of apparent 3-(*p*-chlorophenyl)-1,1-dimethylurea.

The chemical identity of the naturally occurring compounds which interfere with the final colorimetric steps of the procedure is discussed in another paper (*1*), and a simple chromatographic procedure is described for separating the dyes formed by diazotizing and coupling mixtures of *p*-chloroaniline and the interfering materials. This separation effectively minimizes interferences from naturally occurring materials, and the limits of significant detection can be lowered appreciably when samples containing relatively high blanks are being analyzed. Although incorporation of the chromatographic isolation step contributes significantly to the operator time required per analysis, it is recommended when extremely small residues of 3-(*p*-chlorophenyl)-1,1-dimethylurea must be determined.

Table IV. Residual 3-(p-Chlorophenyl)-1,1-dimethylurea in Sugar Refinery Fractions

[3 pounds per acre of 3-(p-chlorophenyl)-1,1-dimethylurea applied as preemergence treatment 7 months prior to harvest. Data obtained on cane from plots in Louisiana]

Sample	Treatment, Lb./Acre	Sample, Grams	3-(p-Chlorophenyl)-1,1-dimethylurea Found: Apparent, γ	Net, γ	Net, p.p.m.
Stalks	None	250	3.3	...	...
	3	250	5.2	1.9	0.008
	3	250	5.2	1.9	0.008
Juice	None	1000	10.5	...	...
	3	1000	9.1	...	<0.005
Sirup	None	1000	38.2	...	...
	3	1000	23.4	...	<0.02
Molasses	None	100	7.1	...	...
	3	100	6.2	...	<0.035
Unwashed sugar from first crystallization	None	500	<1.0	...	...
	3	500	<1.0	...	<0.002
	None	1000	<1.0	...	...
	3	1000	<1.0	...	<0.001

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