plant uptake so that the 3% equilibrium concentration does not result in a phytotoxic soil solution for one or more species, control of seed weed species will presumably be lost. The remaining herbicide will no longer be effective, especially under the semitropical conditions of continuous seed germination. The soil-water ratio is very important. In fact "adsorption" is not a static property characteristic of a given soil, but is a condition of equilibrium which exists among soil, water, and herbicide and depends on the relative ratios of each component. The "adsorptive capacity" of a soil for a herbicide may vary from essentially 100% to essentially zero, depending on the amount of water present and the herbicide concentration in the water.

Leaching of monuron was not studied in detail. A single increment of 4 inches of water through a soil column, after monuron had been applied at 11.7 pounds per acre as a solution, resulted in about 90% retention in the top 2 inches of the soil column; about 70% was retained in the top inch and 20% in the second inch. In a different experiment with monuron, about 50% was retained in the top inch after five increments of 1 inch each of water had been added.

Further evidence of the difference between monuron and diuron desorption in several soils was obtained by a static serial-dilution technique. Recovery of the two herbicides is shown in Figure 4 as a semilog function of the soil-solution ratio from 1:4 to 1:20 in dilution steps of 1:8, 1:12, 1:17, and 1:20. We do not know the reason for the reversal of desorption in the one subsoil in which diuron was desorbed more easily than monuron.

The effect of application rate and use of powdered herbicide rather than solutions was studied with diuron at 5, 10, 20, and 40 pounds per acre. The effect of dissolution from the powder was particularly noticeable. After 16 increments of 1 acre-inch each of water, 80.4% of the 5-pound rate was retained in the top inch; 55% was retained in a comparison column using diuron solution at an equivalent 5 pounds per acre. As Table IV indicates, there was no diuron in the percolates below 4 inches except at the 40-pound rate. The ratio of diuron retained in the top inch to that in the second inch decreased from 4 to 1 at 5 pounds per acre to 1.06 to 1 at 40 pounds per acre. The percentage of diuron that appeared below the first inch increased from 20% at 5 pounds per acre to 62.6% at 40 pounds per acre. The percentage increase was roughly linear with the amount of herbicide. The percentage that appeared below the second inch was zero at 5 pounds, 0.7 at 10 pounds, 2.0 at 20 pounds, and 10.8 at 40 pounds. The percolate analyses indicated tha the 40-pound rate was still showing leaching below 4 inches after 16 inche of percolating water.

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Received for review April 26, 1965. Accepted September 27, 1965. Published with the approval of the Director as Paper 154 of the Journal Series, Experiment Station, Hawaiian Sugar Planters' Association.

HERBICIDE RESIDUES

Determination of 3-Amino-1,2,4-triazole Residues in Sugarcane

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3-Amino-1,2,4-triazole applied as an interline herbicide spray solution on young sugarcane at rates up to 40 pounds per acre leaves residues of less than 0.002 p.p.m. by the time of harvesting at the end of 2 years. Detailed improvements in the analytical method, especially in instrumentation, permit measurement of less than 0.1 μg. of amitrole in sugarcane or in concentrated samples of sugarcane juice.

Amitrole (3-amino-1,2,4-triazole) has been found useful for the control of certain perennial grass and broadleaf weeds common in sugarcane fields and on irrigation ditch banks. The control of certain strains of Bermuda grass (*Cynodon dactylon*), wing-leaved passion vine (*Passiflora pulchella*), and *Panicum* and *Digitaria* spp. was of special interest.

Application of directed sprays in sugarcane produced a moderate amount of chlorosis in the leaves, which persisted for 3 to 4 weeks without appearing to affect yields or subsequent growth. A study was designed to determine the residual amitrole in sugarcane after several applications at excessive rates over the period of the crop cycle (2 years) or until disappearance of the amitrole could be assured. One application of 5 pounds (active) per acre with one possible repeat treatment at the same rate constituted the optimum practical and economic use; the study was based on 5, 10, and 20 pounds per acre with one repeat treatment at the same rate after 8 weeks, making total treatments of 10, 20, and 40 pounds per acre. Two tests were conducted, one at the Kilauea Sugar Co. on the island of Kauai under surface, furrow-irrigated, in an area of 50 inches of rainfall per year, and one at the Hilo Sugar Co. on the island of Hawaii under non-irrigated conditions, with rainfall of about 150 inches per year.

Experimental

The two tests were begun in December 1961 in sugarcane $2^1/_2$ months old on four replicate, $^1/_{100}$-acre plots, with additional 20 × 40 foot replicate plots

for harvesting for yield at 8 months of age. The tests included additional 40 × 40 foot plots for harvesting at 24 months if the treatments decreased yields significantly at 8 months. Table I shows the pertinent field data.

Samples for residue analysis consisted of all cane from 10-foot line segments taken at 0 (same day just after spraying), 2, 4, 8, 12, 16, 32, 52 (Kilauea and Hilo), and 96 weeks (Hilo only). Untreated plots were kept free of weeds for the test period to provide comparative yield data and samples for analysis and reference, and for recovery studies.

Analyses were performed according to the Storherr and Burke modification, Division of Food, Food and Drug Administration, U. S. Department of Health, Education, and Welfare (*3*), with a few minor changes. The cation resin containing the amitrole must be washed with 10 × 50 ml. portions of warm water to remove sugar completely or later concentration will lead to charring and development of undesirable color. The activated Nuchar-C190N decolorizing carbon was reduced from 1.0 gram to 500 mg. to minimize amitrole loss; 700 mg. were necessary for concentrated cane juice. The cation resin IR-120(H) containing the amitrole was not heated with ammonia but was shaken 30 minutes at room temperature. For samples of high amitrole content requiring dilution of the extract before color development, the exact acidity of the original solution must be maintained in the diluent. For very small amounts of amitrole (less than 0.03 p.p.m. or 6 μg.), the ordinary colorimeter is not sensitive enough.

The recording spectrophotometric method described below is sensitive to about 0.001 p.p.m. or 0.2 μg. in 200-gram sugarcane samples or about 0.002 p.p.m. or 0.2 μg. in concentrated juice containing 100 grams of solids.

Sugarcane samplings from the field were first coarsely chopped with a silage cutter, then finely chopped and subsampled in a Buffalo food blender to make 200-gram samples. These were blended in a Waring blender with 20 grams of Celite and 400 ml. of 95% ethanol (*3*). A cation resin adsorbed the amitrole from the solution, which was desorbed with ammonia, evaporated with acetonitrile, digested with sulfuric acid, and decolorized with carbon, and the color was developed, after proper volume and acidity adjustment and diazotization, with *N*-1-naphthylethylenediamine (NED).

Analyses of concentrated juice were begun at 8 months because processing to sugar and molasses might concentrate a small chemical residue. Approximately 1-liter samples of cane juice were taken, representing 100 grams of total dissolved solids or about 5 pounds of sugarcane. After refractometric determination of solids, the juice was heated and limed with $Ca(OH)_2$ solution to pH 7.0 to 7.5 as in the normal mill procedure. The flocculated solids (mainly protein and lime salts) were filtered and discarded and the juice was analyzed by the same procedure as cane samples.

Table I. Field Data for Amitrole Residue Study

	Kilauea Sugar Co. (Kauai)	*Hilo Sugar Co. (Hawaii)*
Field No.	231	46
Variety	37-1933	49-5
Crop	Plant	Plant (2 reps.) Ratoon (2 reps.)
Culture	Irrigated	Nonirrigated
Date planted	Oct. 3, 1961	Sept. 20, 1961
First application	Dec. 8, 1961	Dec. 11, 1961
Second application	Feb. 2, 1962	Feb. 6, 1962

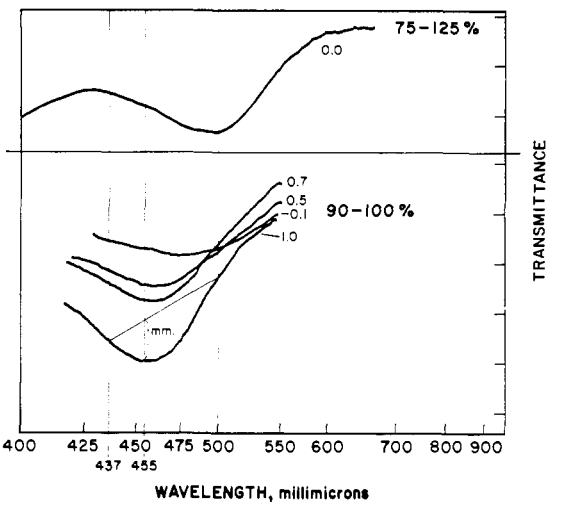

Figure 1. Standard transmittance curves for amitrole on 200 grams of sugarcane

Upper. 0.0 μg. of amitrole
Lower. 0.1, 0.5, 0.7, and 1.0 μg. of amitrole (more expanded scale)
Curve for 0.00 shows naturally occurring dye-coupling product
Beckman DK-2 instrument

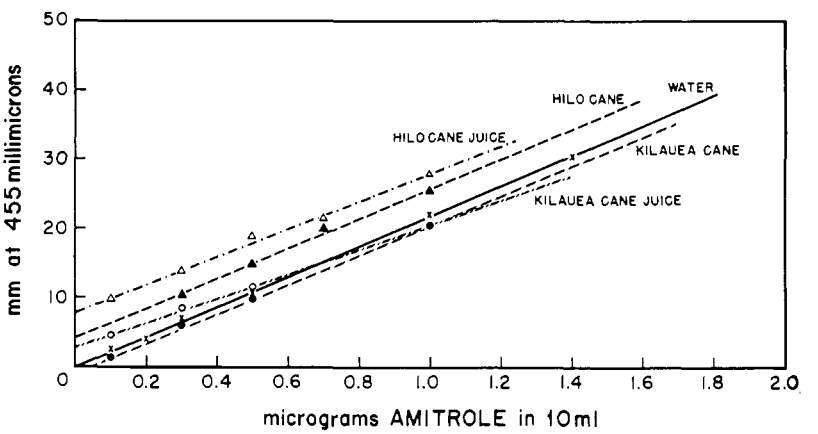

Figure 2. Standard plots for amitrole in sugarcane or sugarcane juice from transmittance curves, compared with amitrole in water

Up to and including the 32-week analyses, color intensity was determined in a Klett-Summerson colorimeter Model 3350, filter 42 (400 to 500 mμ), against reagent blanks. However, all sugarcane samples gave a pinkish color with the dye reagent which was not due to amitrole and had to be compensated for, and it was realized that more sensitive detection would be needed for small quantities of amitrole. Recorded traces were made of the visible spectra of the processed cane extracts, with added NED dye reagent, using a processed cane extract without dye coupler as a reference standard. The major portion of the curve is shown in Figure 1 (zero amitrole), representing the naturally occurring dye-coupled product with a minimum transmittance point (maximum absorbance) at 490 to 500 mμ. This natural interference is not due to solvent or reagent blank. It is not *o*-aminoacetophenone, as suggested by others (*2*), since an authentic sample at 10 to 500 μg. gave a red-violet color with a

Table II. Sugarcane Yields at 8 Months of Age from Amitrole Applications

	Mean Squares, Total Weight	
	Kilauea Sugar Co.	Hilo Sugar Co.
Analysis of Variance		
General average	325.8	718
Coeff. of variation, %	16.2	9.2
No significant effects of treatments		
Summary of Average Measures		
I. Treatment (n = 4)		
Lb. Amitrole per Acre		
X None	345.5	757
A 5 + 5	314.0	720
B 10 + 10	347.3	694
C 20 + 20	296.5	702
HSD[a] (0.05)	Ns	Ns

II. Treatment × Location (n = 2)

Lb. Amitrole per Acre		Plant Field	Ratoon Field
X None	...	821	694
A 5 + 5	...	714	726
B 10 + 10	...	652	736
C 20 + 20	...	728	677
HSD (0.05)	...	Interaction not significant	

[a] HSD. Standard deviation.

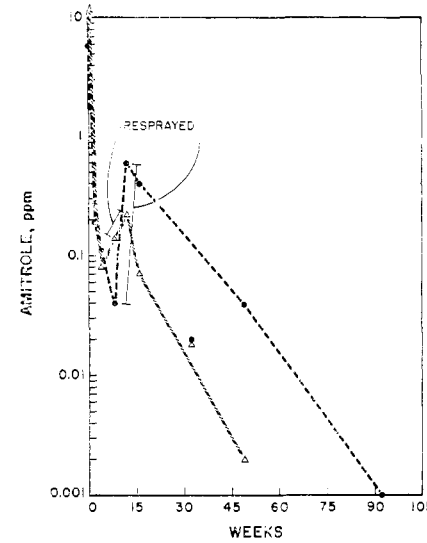

Figure 3. Disappearance of amitrole residues from sugarcane

20 + 20 pounds per acre
△ Kilauea Sugar Co.
● Hilo Sugar Co.

Table III. Amitrole Residue in Sugarcane

(P.p.m. on fresh weight basis)

Sample Interval, Weeks	Treatment: X (No Amitrole) Av.	X Max.	X Min.	A (5 + 5 Lb.) Av.	A Max.	A Min.	B (10 + 10 Lb.) Av.	B Max.	B Min.	C (20 + 20 Lb.) Av.	C Max.	C Min.
Hilo Sugar Co.												
0	0.02	0.03	0.02	0.33	0.53	0.15	1.36	3.46	0.25	5.85	11.00	0.34
2	0.02	0.02	0.01	0.04	0.04	0.03	0.10	0.10	0.09	0.19	0.30	0.08
4	0.02	0.03	0.01	0.04	0.05	0.03	0.03	0.04	0.02	0.11	0.15	0.08
8	0.04	0.05	0.04	0.11	0.14	0.08	0.03	0.06	0.02	0.04	0.06	0.02
12	0.02	0.03	0.02	0.04	0.06	0.02	0.20	0.27	0.06	0.16	0.17	0.14
16	0.02	0.02	0.02	0.04	0.05	0.03	0.07	0.09	0.05	0.14	0.17	0.11
32	0.01	0.01	0.01	0.02	0.02	0.01	0.02	0.02	0.01	0.02	0.03	0.01
52[a]	0.004	0.011	0.000	0.008	0.013	0.002	0.022	0.033	0.012	0.039	0.044	0.033
96[a]	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Kilauea Sugar Co.												
0	0.94[b]	1.83[b]	0.31[b]	4.04	8.05	1.19	16.79	30.75	4.50	57.31	126.25	12.25
2	0.02	0.02	0.02	0.16	0.21	0.09	0.24	0.40	0.12	0.27	0.43	0.11
4	0.02	0.04	0.02	0.07	0.12	0.04	0.21	0.38	0.13	0.08	0.13	0.05
8	0.02	0.03	0.02	0.05	0.08	0.02	0.11	0.26	0.04	0.14	0.45	0.05
12	0.02	0.02	0.01	0.03	0.03	0.03	0.10	0.16	0.04	0.22	0.39	0.07
16	0.01	0.01	0.01	0.02	0.03	0.02	0.04	0.05	0.03	0.07	0.13	0.03
32	0.02	0.02	0.01	0.02	0.02	0.02	0.01	0.02	0.01	0.02	0.02	0.01
52[a]	0.001	0.003	0.000	0.002	0.002	0.002	0.003	0.004	0.000	0.002	0.003	0.001

[a] Beckman DK-2, all others Klett-Summerson.
[b] Wind drift evident in first application.

Table IV. Amitrole Residue in Sugarcane Juice Solids

(P.p.m. on juice solids)

Sample Interval, Weeks	Treatment: X (No Amitrole) Av.	X Max.	X Min.	A (5 + 5 Lb.) Av.	A Max.	A Min.	B (10 + 10 Lb.) Av.	B Max.	B Min.	C (20 + 20 Lb.) Av.	C Max.	C Min.
Hilo Sugar Co.												
32	0.06	0.07	0.06	0.12	0.13	0.09	0.16	0.18	0.13	0.15	0.16	0.14
52[a]	0.006	0.018	0.000	0.054	0.066	0.044	0.115	0.133	0.092	0.116	0.165	0.076
96[a]	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.009	0.002
Kilauea Sugar Co.												
32	0.04	0.05	0.04	0.10	0.14	0.07	0.04	0.04	0.04	0.06	0.10	0.04
52[a]	0.006	0.010	0.003	0.002	0.004	0.001	0.008	0.015	0.004	0.013	0.015	0.011

[a] Beckman DK-2, others Klett-Summerson.

minimum transmittance at 550 mμ. The hydroxylated analog, presumed to arise in the degradation of indole-3-acetic acid or tryptophan (*7*), is unlikely because the interference can be distilled from alkaline solutions. (*o*-Aminoacetophenone has not been processed through the entire series of amitrole cleanup steps, so it is possible that alteration of this contaminant might lead to the observed product.)

The expanded transmittance scale of 90 to 110% (occasionally 75 to 125% for larger quantities of amitrole) of the Beckman Model DK-2 spectrophotometer gave maximum resolution of the peak. Addition of amitrole at 0.1 to 10 μg. and subsequent color development for preparation of a standard curve did not result in two peaks but in a shift of the transmittance minimum from 500 mμ toward the expected amitrole minimum at 455 mμ. At 1.0 to 1.5 μg. of amitrole the peak minimum was at or near 455 mμ. The standard curve was constructed by drawing an artificial base line from the inflection of the curve at 437 mμ to intersect the curve at 500 mμ. At 455 mμ, the distance from this base line to the curve was measured in millimeters (see Figure 1). The standard curve was plotted as millimeters of "peak" height at 455 mμ against the amount of amitrole in micrograms. This plot was linear on rectangular coordinates, at least from 0.1 to 10 μg. of amitrole, when adjustment was made for scale change to 75 to 125% for the larger quantities.

It was necessary to use processed cane extracts with reagents but without the dye coupler as reference solutions, rather than water only for the Hilo samples. Figure 2 shows the deviation among water, cane, and cane juice standard curves; the reasons for the apparent differences are not known, but the curves represent the differences from rather widely varied climates and from cane to concentrated cane juice when a single cleanup technique was used. Standard curves for other cane and cane juice from dry situations on Oahu (not shown) similar to Kilauea on Kauai lie very near the Kilauea lines. Only Hilo cane has so far shown the larger background interference, representing about the extreme of high rainfall conditions.

An attempt was made to evaluate the use of dye-coupled processed cane extracts as reference standards since any deviation from the reference should be amitrole or some other nonnatural reactive substance present in the cane. The work looked promising and with some care could probably be used; however, minor variations in cleanup, age, and variety of cane, etc., produced slight variations which occasionally gave rise to small negative apparent values where amitrole was absent, so this was abandoned in favor of the procedure described.

Table V. Recovery of Amitrole Added to Sugarcane (200 Grams) and Sugarcane Juice Solids (100 Grams)

Sample No.	Amitrole Added μg.	Amitrole Added P.p.m.	Amitrole Recovered μg.	Amitrole Recovered P.p.m.	Recovery, %
			Sugarcane		
1	0.5	0.0025	0.5	0.0025	100
2	0.5	0.0025	0.5	0.0025	100
3	1.5	0.0075	0.65	0.0033	43[a]
4	1.5	0.0075	1.3	0.0065	87
5	2.5	0.0125	2.35	0.0118	94
6	2.5	0.0125	2.35	0.0118	94
7	3.5	0.0175	2.5	0.0125	71
8	3.5	0.0175	2.9	0.0145	83
9	5.0	0.0250	4.05	0.0203	81
10	5.0	0.0250	4.45	0.0223	89
11	0	0	0.0	0.000	...
12	0	0	0.1	0.000	...
26	20	0.10	16.0	0.080	80[b]
21	50	0.25	35.5	0.178	71[b]
27	50	0.25	37.5	0.188	75[b]
22	100	0.50	71.5	0.358	72[b]
28	100	0.50	73.5	0.368	74[b]
20	0	0	5.0	0.025	...[b]
25	0	0	14.0	0.070	...[b]
			Sugarcane Juice		
11	1	0.01	1.00	0.01	100
13	1	0.01	1.25	0.012	125
14	5	0.05	4.25	0.042	85
15	5	0.05	3.75	0.038	75
16	5	0.05	4.60	0.046	92
12	10	0.111	6.35	0.071	64
13	10	0.113	7.00	0.093	70
8	0	0	0	0.000	...
9	0	0	0	0.000	...
10	0	0	0	0.000	...

[a] Mechanical loss.
[b] Klett-Summerson colorimeter, all others Beckman DK-2, Klett recoveries corrected for zero blank.

Several other attempts to improve the degree of cleanup by eliminating the naturally occurring dye-reactive substance were unsuccessful. The first, according to the procedure of Storherr and Onley (*4*), employed a powdered cellulose chromatographic column and fractionation of the extract with selected solvents. The fraction presumably containing amitrole showed reduced contamination from but not elimination of the natural component.

The second procedure (*2*) has been used to separate the natural component of sugarcane from various chloro-substituted anilines as the colored NED-coupled dyes. This procedure has been used extensively in this laboratory and has the advantage that the components of the mixture are colored and can be seen on the powdered cellulose column. Although the yellow color of the NED-coupled diazotized amitrole was not so distinct as the red or red-violet chloroaniline dyes, the major difficulty was that the yellow dye was extremely mobile and moved off the column ahead of the fairly mobile, pink natural substance. Poor separation of the two components with the acid eluent made the method unpromising and other systems were not investigated to any extent. The procedure might be useful for separating amitrole from the more tightly adsorbed chloroaniline dyes if both were present.

The interfering substance was oxidized and removed with dilute $KMnO_4$, but amitrole was also destroyed.

Results and Discussion

Although there was a moderate bleaching effect on the sugarcane leaves from the interline application of the high rates of amitrole, there was no evidence of permanent injury, newly emerging leaves were green and normal, and total yields taken at 8 months of age showed no significant decrease in weight of cane in the plots compared with untreated weeded checks (Table II). Since these yields were normal, no attempt was made to harvest plots at 24 months of age.

The detailed analytical results are shown in Table III for 200-gram sugarcane samples and in Table IV for concentrated juice with 100 grams of solids. Averages were generally from four, occasionally from two, six, and eight samples.

Recovery of amitrole added to sugarcane and to juice solids is shown in Table V.

Considerable amounts of copper were apparently present in the young cane samples from the Hilo Sugar Co. The ion exchange resin removed the copper from solution; elution of the amitrole with ammonia turned the resin black.

Regeneration of the used resin eluted the intense blue copper-ammonia complex; there seemed to be no interference with the analysis and the effect lessened as the cane matured.

Inspection of the residue data showed that, at 12 months of age, amitrole was not detectably present in the Kilauea sugarcane but small residues appeared in the Hilo tissues. Two possible reasons for this were considered: If the metal chelation properties of amitrole are assumed, the copper in the Hilo soil may have influenced the amount of amitrole uptake from the soil; or, since the rainfall at Hilo is over twice that at Kilauea and the soil evaporation rate consequently higher at Kilauea, soil leaching of amitrole would be more extensive at Hilo and cane root uptake would be greater. The difference was not apparent visually and it is difficult to see how the effect would last over such a time period. The two sugarcanes were of different varieties and the observed residue differences may be fortuitous based on varietal response. In any case, no residue was apparent at 22 months even at the limit of sensitivity of the analytical method. The disappearance of the amitrole applied at 20 pounds per acre, with one repeat treatment of 20 pounds per acre after 8 weeks, is shown in Figure 3.

Recording the deviation from a base line measurement in which the natural dye-reactive component is always present required construction of standard curves for each location with processed cane juice without amitrole present, and, as a check on the variability, for each variety and each age of cane sampled. The preferred procedure has been to prepare the untreated checks and use an aliquot of this plus all reagents except NED as the reference cell solution and to prepare standard curves from further aliquots. Other work had suggested that the color interference is greater in young sugarcane, which contains more leaf and meristematic tissue in relation to stalk weights. This assumption has not been borne out here; the major differences seemed to be between cane from relatively dry and from high rainfall areas.

Acknowledgment

The authors acknowledge the generous financial assistance of the Amchem Products Co. in support of this study, and the helpful suggestions of Hirsh S. Segal of that company. Norman Nomura, Norman Kondo, and David Miyauchi assisted in development of methods and sample preparation.

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Received for review May 10, 1965. Accepted September 27, 1965. Published with the approval of the director as Paper 155 of the Journal Series, Experiment Station, Hawaiian Sugar Planters' Association.

HERBICIDE RESIDUES

Determination of Bromacil Residues

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A method for determining bromacil residues in soil and plant and animal tissues using microcoulometric gas chromatography is based on the gas chromatographic measurement of bromacil after it has been extracted from the tissue with an alkaline solution and subsequently partitioned into an organic solvent. Intermediate cleanup steps are required. The sensitivity of the method is about 0.04 p.p.m. based on a 25-gram sample, with an average recovery of better than 85%.

The bromacil weed killer, Hyvar X (registered trademark of E. I. du Pont de Nemours & Co. for 5-bromo-3-*sec*-butyl-6-methyluracil), was introduced as an industrial herbicide for noncrop uses in 1962 (*1*). An analytical method was developed for determining traces of the active component in soils and in plant and animal tissues, so that reliable information could be obtained on uniformity of application, rate of disappearance, runoff, and other questions relating to the industrial use of this herbicide. It is based on the gas chromatographic measurement of the intact bromacil after it has been extracted from the sample with an alkaline solution and subsequently partitioned into an organic solvent. It is capable of detecting 1 μg. of bromacil in about 25 grams of sample, using a selective microcoulometric detector. Recoveries of better than 85% have been demonstrated for samples of soils, animal tissues, and certain crops.

Apparatus and Reagents

Dohrmann Microcoulometric Gas Chromatograph, modified for programmed temperature operation using F & M Model 240 power proportioning temperature programmer (*3*).

Chromatographic Column, 20% General Electric SE-30 silicone gum plus 0.2% Epon Resin 1001 on 60- to 80-mesh Diatoport S (F & M Scientific Co., Avondale, Pa.), 2-foot, stainless steel, $^1/_4$-inch o.d., $^3/_{16}$-inch i.d.

Bromacil, standard reference material available from Industrial and Biochemicals Department, Biochemicals Sales Division, E. I. du Pont de Nemours & Co., Wilmington, Del.

Agla Micrometer Syringe, Burroughs-Wellcome Co., Tuckahoe, N. Y.

Calibration

Equilibrate the gas chromatograph as follows: vaporizer block temperature, 280° C.; vaporizer block oven, 280° C.; furnace temperature, 730° C.; column temperature, 300° C.; carrier flow, helium, 75 cc. per minute; purge flow, helium, 175 cc. per minute; oxygen flow, 20 cc. per minute.

Before making the chromatographic runs, condition the column by maintaining its temperature at 300° C. for 48 hours and inject three successive 100-μl. aliquots of a 1% solution of bromacil in ethyl acetate, over a 3- to 4-hour period.

Prepare a series of standard solutions containing 1, 5, 25, 50, and 100 μg. per ml. of bromacil in ethyl acetate. Set the column temperature to 100° C. and, using a micrometer syringe, inject 500 μl. of the 1 μg. per ml. standard solution evenly over a 2-minute period. The injection period may be shortened when injecting a volume less than 250 μl.

Table I suggests the volume to be injected and the injection time for the various standard solutions. This may vary, of course, depending upon the sensitivity of the instrument and will have to be established in each labora-