Method of Determining Bromacil in Soils and Plant Tissues

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A method for the determination of bromacil in agricultural soils and crops using gas-liquid chromatography with an electron-capture detector is described. Several complete analyses can be performed per day because of its simplicity. The specificity is good, with the sensitivity ranging from 0.01 p.p.m. for soil residues to 0.1 p.p.m. for crop residues.

Bromacil (5-bromo-3-*sec*-butyl-6-methyluracil) is a substituted uracil herbicide that inhibits photosynthesis in sensitive plant species (*2*, *4*, *5*). It is being used at present for long-term, noncrop weed control, and has potential utility as a selective herbicide in certain tree crops (*3*).

Pease (*6*) developed a method for the determination of bromacil, making use of temperature-programmed microcoulometric gas chromatography. A relatively faster method, maintaining sensitivity and reproducibility, was desired for determining soil and crop residues and for research purposes. Making use of the electron-capture detector and its ability to detect as little as 0.1 ng. (nanogram) of bromacil, a method was developed. The technique described has been applied successfully to soils and to plant tissues.

Apparatus and Reagents

Aerograph 662 Moduline gas chromatograph equipped with an electron-capture detector, to which was attached an Aerograph 630 voltage control unit.

Sargent SR recorder operated at 1 mv. and $^1/_2$ inc per minute, modified with a filter.

Rinco rotary evaporator with a sparkless induction motor.

Bromacil, standard reference material obtained from E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.

Acetone, Nanograde (Mallinckrodt).

Ethyl acetate, analytical reagent grade (Mallinckrodt).

Method

Gas Chromatography of Bromacil. A $^1/_8$-inch stainless steel column coated with hexamethyldisilazane (HMDS), 13 inches long, packed with 5% neopentyl glycol adipate on Chromosorb P-HMDS (60- to 80-mesh) is used. One nanogram (1×10^{-9} gram) gave a 10% recorder scale deflection with the gas chromatograph operating under the following conditions: injector, 225° C.; column, 195° C.; detector, 210° C.; nitrogen flow rate, 120 ml. per minute; detector voltage, 70 volts; attenuation, 2; gain, 10. The elution time for bromacil is 3 minutes. A calibration curve for bromacil is shown in Figure 1. There is a linear detector response for bromacil up to 7 ng.

Bromacil Solubilities. The relative solubility of bromacil in a number of solvents was tested (Table I). This information was used to calculate partition gradients and to design extraction and cleanup procedures.

Procedure for Soil. Place a 100-gram sample of soil in a 300-ml. glass-stoppered Erlenmeyer flask. Add 10 grams of $(NH_4)_2SO_4$ and 150 ml. of 1.5% NaOH solution. Shake well for 5 minutes and vacuum-

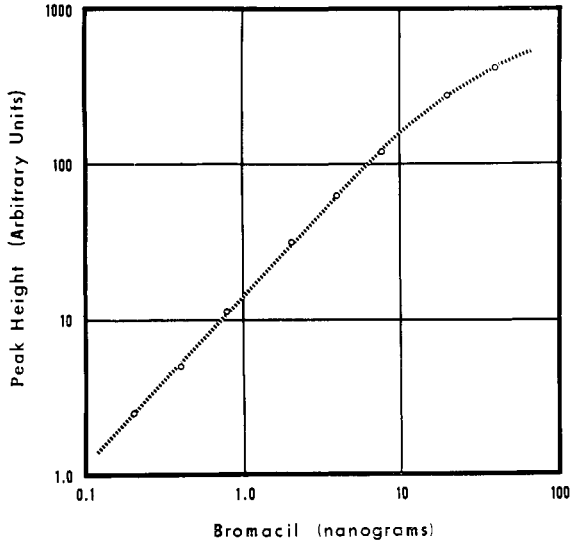

Figure 1. Linearity curve for bromacil

Table I. Approximate Solubilities of Bromacil in Various Solvents at Room Temperature

Solvent	Solubility, P.P.M.
Water, acidified	<10
Hexane	<10
Water, tap	815
Sodium hydroxide, 1.5%	>20,000
Ethyl acetate	>35,000
Chloroform	>>100,000

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filter. Return the soil to the flask and strip as before with 100 ml. of water. Filter and combine the filtrates in a 500-ml. separatory funnel having a Teflon stopcock.

Add 50 ml. of hexane, shake for 1 minute, and, upon separation of the phases, discard the hexane fraction. Acidify the basic aqueous fraction with 5 ml. of concentrated HCl and return to the separatory funnel. Add 50 ml. of ethyl acetate and shake well for 1 minute. Pass the ethyl acetate fraction through a funnel containing anhydrous Na_2SO_4 into a 200-ml. round-bottomed flask. Repeat this extraction with another 50-ml. portion of ethyl acetate. Take the combined ethyl acetate extracts just to dryness, using a vacuum evaporator and a 50° to 60° C. water bath.

Strongly alkaline conditions are known to degrade substituted uracils (*1*). An experiment was performed to determine the effect of exposure to 1.5% NaOH solution on bromacil during stripping, with 30 minutes being a typical exposure time; no measurable loss could be found for up to 2 hours (Table II).

So that many samples could be gas-chromatographed on the same day it would be convenient to hold and accumulate them after they had been through the entire procedure, but just prior to the final solvent pickup. Stability of bromacil in samples from two different soils carried through the procedure to this point and stored for up to 4 days was checked. There was no loss of bromacil within this period (Table III). However, in another experiment, storage for over one week led to low recoveries and appearance of a second peak during chromatography. This was probably due to failure to neutralize the alkaline solution and its subsequent carry-over with the ethyl acetate. Neutralization must be complete not only for improving storage ability, but also for enhancing the partitioning of bromacil into the ethyl acetate in the following step (Table I).

The possibility of loss due to high temperature during solvent evaporations was determined on extracts from fortified soils. Samples were dried under vacuum in a water bath at 52° to 83° C. These temperature differences were found to have no effect (Table IV).

Procedure for Leaves and Fruit. Macerate the sample in a blender for several minutes, using either surface-washed leaves or slices of clean, whole citrus fruit. Weigh 25 grams of leaf or 50 grams of fruit macerate into a 500-ml. glass-stoppered Erlenmeyer flask. Add 10 grams of $(NH_4)_2SO_4$, 5 grams of animal charcoal, and 150 ml. of chloroform for leaf or 200 ml. for fruit samples. Shake mechanically for 30 minutes.

Vacuum-filter this material with the aid of a layer of Celite 545. Place a 100-ml. aliquot of sodium sulfate–dried filtrate, representing 16.7 grams of leaf or 25.0 grams of fruit tissue, in a 200-ml. round-bottomed flask and, under vacuum at 50° to 60° C., take to near dryness (only a small amount of oily residue remains).

Transfer the flask contents to a 250-ml. separatory funnel, using 50 ml. of hexane followed by two 50-ml. portions of 1.5% NaOH. Continue from this point as with the soil cleanup procedure at the hexane step. However, only 3.5 ml. of concentrated HCl is needed for the acidification step.

Table II. Effect of Time in 1.5% NaOH Solutions upon Bromacil Recovery

Time, Min.		Found,[a] P.P.M.	Recovery, %
35		0.20	100
35		0.18	90
	Av.	0.19	95
120		0.19	95
125		0.19	95
	Av.	0.19	95

[a] Fortified at 0.20 p.p.m.

Table III. Effect of Time after Soil Processing and Drying upon Bromacil Recovery

	Soil 52		Soil 53	
Days before Analysis	Found,[a] p.p.m.	Recovery, %	Found,[a] p.p.m.	Recovery, %
0	0.19	95	0.20	100
2	0.21	105	0.22	110
4	0.21	105	0.21	105
Av.	0.20	100	0.21	105

[a] Fortified at 0.20 p.p.m.

Table IV. Effect of Bath Temperature during Solvent Evaporation upon Bromacil Recovery

(Soil 53)

Temp., ±2° C.		Found,[a] P.P.M.	Recovery, %
52		0.20	100
62		0.19	95
73		0.18	90
83		0.19	95
	Av.	0.19	95

[a] Fortified at 0.20 p.p.m.

Table V. Bromacil Adsorption on Some California Soils and Breakdown with Time

		Stripped Bromacil, P.P.M.[a]		
Soil Classification	Soil No.	3 days	6 months	Loss, %
Quartz sand	Q.S.	0.87	0.62	29
Superstition stony sand	25	0.75	0.32	57
Handford sandy loam	52	0.72	0.40	44
Vista sandy loam	51	0.67	0.46	31
Ramona loam	53	0.63	0.35	44
Yolo sandy loam	4	0.63	0.22	65
Chino silt loam	50	0.39	0.22	44

[a] All soils fortified at 1.0 p.p.m. with bromacil.

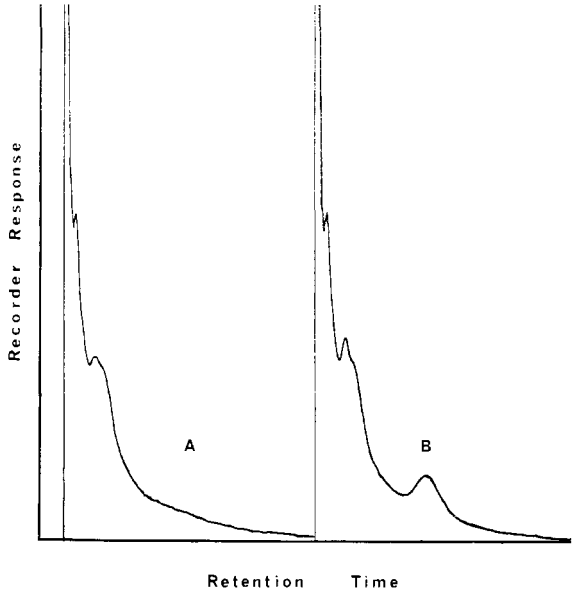

Figure 2. Chromatographs of soil 51

A. Untreated
B. Treated at 0.01 p.p.m. with bromacil
Both chromatographs obtained from 2-μl. injections from 2-ml. final sample volume. Bromacil peak in *B* represents less than 1 ng.

Table VI. Range of Soil Residues on Field Plot Treated with Bromacil at 2 to 6 Pounds per Acre

Interval Since Treatment, Months	Bromacil, P.P.M.	
	0- to 6- inch depth	6- to 12-inch depth
1	0.88–0.19	0.25–0.04
3	0.40–0.17	0.25–0.07
6	0.28–0.03	0.22–0.04

When leaves or fruit are stripped with NaOH, heavy emulsions or gels are formed, particularly with citrus fruit because of their high pectin content. Gel formation is reduced by stripping with chloroform, which has less penetrating ability than a basic aqueous solution, but a far greater partitioning gradient for bromacil (Table I).

Analysis. Dissolve the residue in the round-bottomed flask in acetone to an appropriate volume, so that no more than 7 ng. will be injected in a volume of 2 to 5 μl. To maintain a linear detector response the final solution should be less than 7-p.p.m. bromacil.

Obtain a standard calibration curve for each set of samples, using a 1.0-p.p.m. bromacil solution. Plot peak height against amount of bromacil. The curve is obtained from data points obtained concurrently with values for the unknowns. Samples and standards were injected in an alternate fashion.

Results and Discussion

When bromacil-fortified alkaline solutions were checked for recovery in the absence of soils, 93 to 99% recovery was obtained. If some soils were stripped immediately after fortification with a standard bromacil solution, allowing just enough time for the solvent to dry, good recoveries could be obtained before irreversible binding took place. Recoveries from 90 to 110% for fortified soils are shown in Tables II, III, and IV.

The limit of sensitivity of the method is about 0.01 p.p.m. in soil. There is a small amount of background interference for most soils, and controls should always be included to account for its extent and the making of any necessary corrections. The procedure of determining the standard calibration curve during the actual analysis of the unknowns compensates for the small variable effect on detector response due to instrument drift and any interfering materials. Figure 2 shows typical curves for a soil, untreated and treated at 0.01 p.p.m. Occasionally, in agricultural soils, interference peaks will be found which the cleanup has failed to remove that may complicate or negate the determination.

A preliminary study was made of bromacil breakdown in six California soils, which were placed in Styrofoam cups, fortified with 1.0-p.p.m. bromacil, individually covered, and kept at room temperature. Moisture was maintained at near field capacity by addition of water every 2 weeks. Analyses were made at both 3 days and 6 months. (The initial delay was to permit time for soil binding.) The loss of bromacil in these soils ranged from 31 to 65% during this 6-month interval, whereas the loss from washed quartz sand was only 29% (Table V).

Soil samples from field plots were taken at two depths at three time intervals (Table VI). The concentration of bromacil in the top 6 inches decreased with time, while the concentration in the lower 6 inches remained relatively the same during the 6-month period.

The method is not as accurate for citrus leaves and fruit as it is for soil. The sensitivity on plant materials is about 0.1 p.p.m., with recoveries ranging from 60 to 85%. The difficulty is primarily one of cleanup, as evidenced by varying background and interference peaks.

Analysis of leaves from citrus trees treated at 2 to 6 pounds per acre were usually negative, but occasional values up to 0.3 p.p.m. were found. Bromacil was never detected in the fruit.

This method uses a simplified cleanup procedure, and relies upon gas chromatography and electron-capture detection for specificity and sensitivity. Numerous samples can be analyzed in a single day. Analysis need not be limited to soil and to citrus, since the method has been applied successfully to both asparagus and walnuts.

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Received for review April 11, 1966. Accepted October 10, 1966. Station Paper No. 1742.