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.

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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-

tory. Two minutes after completion of the injection begin programming the column temperature at 10° per minute. Program to 300° C. and hold. The retention time from start of programming for bromacil is about 17 minutes.

Calculate the conversion efficiency for each chromatographic run as follows:

1. Determine the amount of bromacil measured coulometrically as the corresponding halide.

For recorder sensitivity of 1 mv. per inch and a chart speed of 1/2 inch per minute,

$$\text{micrograms of bromacil} = \frac{A \times 4.42 \times 10^3}{R \times 31}$$

where A = area, square inches
R = recorder input resistance, ohms

2. Divide the micrograms of bromacil measured by the micrograms injected to determine the conversion efficiency for each run.

3. Calculate an average conversion efficiency to be used in subsequent determinations.

In this laboratory, conversion efficiencies of 40 to 50% have been obtained when determining the bromo-substituted uracil. The apparently low conversion efficiency is due to the fact that elemental bromine rather than hydrogen bromide is the primary combustion product. Bromine reacts with water in the following manner to yield only 50% titratable bromide (*4*, *5*):

$$Br_2 + H_2O \rightleftarrows HBrO + HBr$$

Conversion efficiency should be checked frequently.

Sample Preparation

The preliminary handling of a sample will depend upon the nature of the material to be analyzed.

If the sample is a grain or seed, weigh 25 grams in a 400-ml. tall beaker, add 100 ml. of 1% NaOH solution, and grind with a Sargent wet mill at the minimum spacing for 30 minutes.

If the sample is a fruit, vegetable, or animal tissue, place 25 grams of the chopped material in a Waring Blendor jar, add 100 ml. of 1% NaOH solution, cover, and blend at high speed for 3 to 5 minutes.

If the sample is a soil, place 25 grams of the material in a 250-ml. glass-stoppered Erlenmeyer flask, add 100 ml. of 1% NaOH solution, and shake on a wrist action shaker for 15 minutes.

Procedure

Transfer the blended sample quantitatively to a 250-ml. centrifuge bottle, using several small portions of the 1% NaOH solution as a wash. Centrifuge at 2000 r.p.m. for 5 to 10 minutes and carefully decant the aqueous phase through glass wool into a 500-ml. separatory funnel. Add 100 ml. of the NaOH solution to the substrate remaining in the centrifuge bottle, stopper with a cork plug, and shake vigorously for 2 to 3 minutes. Centrifuge as before and combine the aqueous phase with the contents of the separatory funnel. Acidify the combined extracts with 10 ml. of 10.N H_2SO_4 and add 50 ml. of chloroform, reagent grade. Shake for 2 minutes and allow the phases to separate. (It may be necessary to centrifuge at this stage to get a clean separation.) Collect the chloroform in a 150-ml. beaker. Repeat the extraction twice, each time using 50 ml. of chloroform and combining the solvent extracts in the 150-ml. beaker. Discard the aqueous phase.

Evaporate the solvent to dryness in a hood at room temperature. Take up the residue with 50 ml. of 1.N NaOH solution and transfer to a 125-ml. separatory funnel. Continue the transfer using 50 ml. of commercial grade, redistilled *n*-hexane. Shake for 2 minutes and allow the phases to separate. Discard the hexane layer.

Add 50 ml. of ethyl acetate, reagent grade, to the separatory funnel and shake for 2 minutes. After a clear separation of the layers, collect the ethyl acetate in a 100-ml. beaker. Repeat the extraction using a second 50-ml. portion of ethyl acetate. Combine the solvent extracts and evaporate to dryness. Dissolve the residue with 25 ml. of reagent grade nitromethane and transfer to a 60-ml. separatory funnel.

Table I. Volume and Injection Times

Concn. of Standard Solution, µg./Ml.	Volume Injected, µl.	Injection Time, Min.	Sensitivity Range, Ohms
1	500	2	256
5	500	2	256
25	100	1	256
50	50	30 sec.	256
100	25	15 sec.	256

Table II. Summary of Bromacil Recovery Data

	Residue Level, P.P.M.	No. of Detns.	Recovery, % Av.	Recovery, % Range
Pineapple				
Fruit	0.04–1.4	7	102	91–116
Leaves	0.04–1.4	7	96	83–109
Citrus				
Oranges	0.05–0.92	3	104	92–113
Grapefruit	0.05–4.8	7	95	81–110
Lemons	0.23–0.46	2	85	81–89
Sugar cane	0.10–4.8	5	89	80–93
Alfalfa	0.15–2.3	5	91	76–109
Urine	0.22–5.6	7	98	91–110
Feces	0.22–5.6	7	99	79–118
Tissue				
Kidney	0.08–0.90	4	97	84–107
Liver	0.08–0.45	4	102	90–110
Muscle	0.04–0.90	4	97	84–120
Subcutaneous fat	0.04–0.22	3	115	107–130
Blood	0.04–1.2	2	94	92–96
Soil (Keyport)	0.10–4.8	12	99	84–110

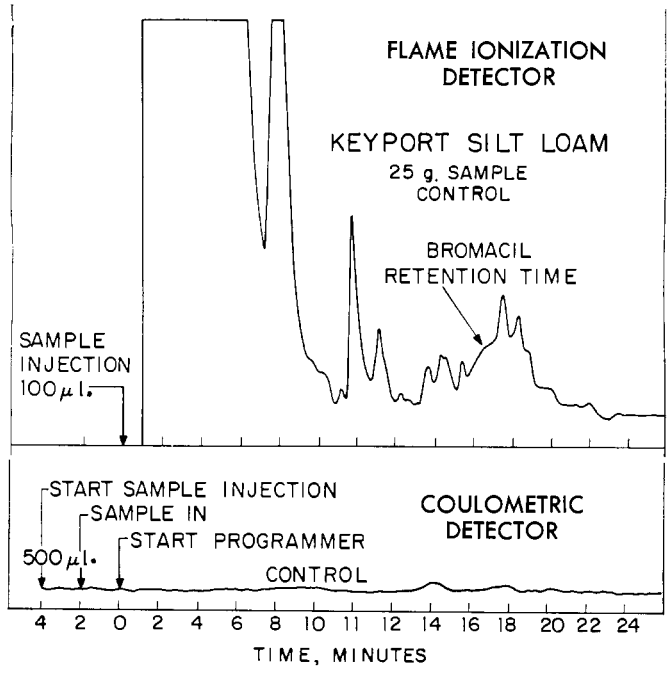

Figure 1. Background on soil extracts

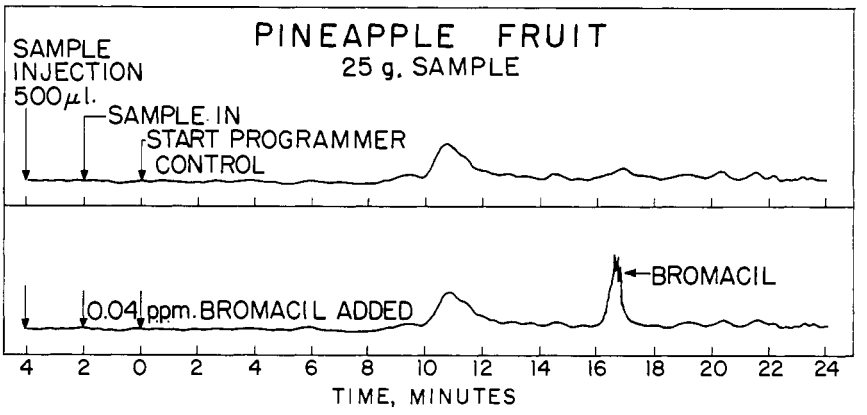

Figure 2. Chromatograms of extracts from pineapple fruit

Complete the transfer using 25 ml. of redistilled *n*-hexane. Shake for 2 minutes and allow the phases to separate. Discard the hexane layer and wash once more with 25 ml. of *n*-hexane. Collect the nitromethane in a 30-ml. beaker and evaporate to about 0.5 ml. Transfer the concentrated extract to a 1-ml. volumetric flask using a finely drawn-out dropper. Assure quantitative transfer by washing with several small portions of nitromethane. Dilute to volume with nitromethane and mix well.

Equilibrate the GC instrument and inject an aliquot of the sample as described under "Calibration." Calculate the micrograms of bromacil using the equations given under "Calibration" and correct for aliquot factor, conversion efficiency, and recovery factor to obtain the micrograms of bromacil in the unknown. Obtain the residue in parts per million by dividing by the sample weight in grams.

Results and Discussion

The applicability of the gas chromatographic method for determining bromacil residues was tested by analyzing a variety of untreated substrates to which known quantities of bromacil were added. Results of recovery studies for bromacil added to untreated soil, crops, and tissue are summarized in Table II. Recovery over the range of 0.04 to 5.6 p.p.m. averaged 98%.

Programmed temperature operation is used in this analysis to permit the injection of sample aliquots up to 500 μl. (*2*) and thereby obtain the reported sensitivity. Use of the selective microcoulometric detector eliminated nearly all background and gave no interference from untreated control samples. Figure 1 compares the background on soil extracts using selective coulometric detection with that obtained using a flame ionization detector. Illustrative chromatograms of extracts from control pineapple fruit and pineapple fruit fortified with bromacil at the 0.04-p.p.m. level using the coulometric detector are shown in Figure 2. Preliminary studies using the electron affinity detector were inconsistent and erratic because column temperatures required to chromatograph bromacil by the techniques herein described are about 50° C. higher than the upper operating limit of tritium foil detectors. Higher backgrounds arising from other electron-capturing species also appear to complicate this approach.

To obtain consistent results, it is necessary to maintain a low furnace temperature and a low oxygen supply when chromatographing extracts containing bromacil. Response to bromine in the coulometric detector is less than theoretical because the elemental bromine formed in the gas stream hydrolyzes in the acetic acid electrolyte to give some hypobromous acid along with the desired hydrobromic acid. Only the latter is titratable. Under the conditions described, approximately equivalent amounts of these two acids are formed, and it is necessary to control both the combustion temperature and the oxygen content of the combustion gas to maintain this ratio. When maintaining these combustion conditions, difficultly combustible materials may pass through the system, causing an increase and/or irregularities in the base line. For example, an unknown component was shown in some of the control pineapple fruit at about 11 minutes' retention time. However, this peak does not interfere with the determination of bromacil, which has a retention time of about 17 minutes.

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