

This gave an  $R_f$  comparison and also provided a semiquantitative measurement of the amount of bromacil present in a sample. Approximate  $R_f$  values observed were 0.20 on silica gel G and 0.33 on aluminum oxide G. Saturation of the chamber with solvent vapor helped to prevent edge effect, permitting easy comparison of  $R_f$  values.

Four kinds of soil, a light brown loamy sand, a gray-brown loam, a yellowish brown sandy clay loam, and a light gray-brown clay were fortified with bromacil at the 0.1- and 1.0-p.p.m. level. The cleanup method proved adequate for the thin-layer chromatography step for each type of soil.

## The Chromatographic Determination of 4-Trifluoromethyl-2,4'-dinitrodiphenyl Ether Residues in Soybeans

Soybeans are ground to pass a 20-mesh screen and blended with a 1 to 1 mixture of acetone-hexane. The herbicide is partitioned into the hexane portion by the addition of distilled water. The hexane portion is placed on a silica gel column and soybean oil and other interfering compounds are eluted with

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Four-trifluoromethyl-2,4'-dinitrodiphenyl ether is a herbicide recently introduced by the CIBA Corp. (C-6989). It shows considerable promise for use in agronomic crops, especially soybeans. However, no method has been published for residue determinations. The presence of a  $CF_3$  and two  $NO_2$  groups on the herbicide molecule indicated that it could be readily detected using electron capture gas chromatography.

### MATERIALS AND METHODS

Soybean samples were harvested from plots in Alabama, South Carolina, Wisconsin, Kentucky, and Missouri. These plots had been treated pre-emergence with C-6989 at rates of 0, 2, 3, 4, or 6 pounds per acre.

Samples were ground in a Wiley mill to pass a 20-mesh screen. Alcohol, acetone, hexane, benzene, and various combinations of these chemicals were used as extracting solvents. A 1 to 1 mixture of hexane and acetone extracted the herbicide most efficiently. Twenty grams of soybean powder were blended with 50 ml. of the mixture for 5 minutes in a Waring Blendor. After suction filtration, the soybeans were again blended using 50 ml. of the mixture. The blender should be tightly stoppered, as overflow vapors present a possible fire hazard. The filtrates were combined and the herbicide was partitioned into the hexane portion by addition of 50 ml. of water. The water-acetone portion was extracted with a second 50 ml. of hexane. The two hexane extractions were combined and washed with 50 ml. of water.

A silica gel column was prepared by pouring 100- to 200-mesh silica gel into a 1.2-cm. inside diameter buret plugged with glass wool. The column was approximately 30 cm. high and was preconditioned by washing with both hexane and acetone.

The entire hexane extract was poured onto the silica gel column and the column washed with an additional 10

ml. of hexane. The herbicide is eluted with acetone and the concentration determined using electron capture gas chromatography. The method is sensitive to 0.05 p.p.m. with recovery of better than 85%. No residues were found in soybeans treated pre-emergence at herbicidal rates of 2 to 6 pounds per acre.

ml. of hexane. The herbicide was retained on the column, but most of the interfering compounds were eluted with the hexane. The herbicide was eluted from the column with acetone and its concentration determined using electron capture gas chromatography. The herbicide was concentrated in the first 10 ml. of acetone eluted.

Samples were analyzed with a Barber-Colman Model 5360 gas chromatograph equipped with a radium-226 electron capture detector. A 6-foot spiral glass column which had an inside diameter of 5 mm. was packed with 10% DC-200 on 100- to 200-mesh Gas-Chrom Q. The temperatures of the detector, column, and injector were 240°, 210°, and 265° C., respectively. The carrier gas was prepurified nitrogen at a flow rate of approximately 90 ml. per minute.

Recovery percentages and herbicide concentrations in field samples were determined by comparing peak heights produced by the samples to peak heights produced by known quantities of herbicide.

### RESULTS AND DISCUSSION

The recovery of C-6989 from soybeans is shown in Table I. There was a linear relation between peak height and herbicide content over a range of concentrations from 0 to 0.5  $\mu$ g. per ml. This corresponds to concentrations

Table I. Recovery of 4-Trifluoromethyl-2,4'-dinitrodiphenyl Ether from Soybean Seed

Herbicide Added, P.P.M.	Herbicide Found, P.P.M.	% Recovery
0.25	0.220	88.0
0.20	0.172	86.0
0.15	0.127	84.7
0.10	0.092	92.0
0.05	0.048	96.0
0.00	0.000	00.0

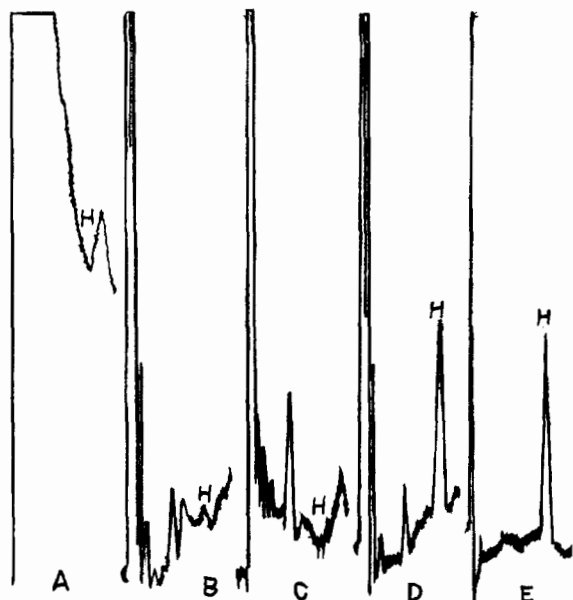


Figure 1. The determination of C-6989 in soybeans

- A. Check sample prior to cleanup with a silica gel column
- B. Sample after cleanup
- C. Field sample treated pre-emergence at 2 pounds/acre
- D. Check sample spiked with 0.1 p.p.m. herbicide
- E. 0.1-p.p.m. standard
- H. Location of the herbicide on the chromatogram

of 0 to 0.25 p.p.m. when 20 grams of soybeans are used. The detection limit of 0.05 p.p.m. was determined in part by interfering compounds and in part by the sensitivity of the instrument. The deviations between the amount

of herbicide added and the amount recovered were 15% or less.

Figure 1 shows chromatograms of various soybean samples. *A* represents a check sample in which the silica gel column was omitted from the cleanup procedure. *B* represents the same sample with the silica gel column included. The column was particularly effective in removing interfering oils from the sample. Frequently 6 to 8 ml. of oil were removed from each 20 grams of soybeans.

Chromatogram *C* is from a sample taken from a field in Alabama which had been treated pre-emergence with 2 pounds per acre of herbicide. Analyses were made on samples from Alabama and Missouri which had received applications of 2 and 4 pounds per acre, from Wisconsin and Kentucky which had received 3 and 6 pounds per acre, and from South Carolina which had received 4 pounds per acre. None of these samples contained detectable quantities of herbicide.

Chromatograms *D* and *E* compare a check soybean sample spiked with 0.1 p.p.m. with an equivalent standard. The spike was prepared by adding the herbicide to the soybean meal. In some samples, extraction was begun immediately after spiking. In others the herbicide and sample were kept in a refrigerator several hours before extracting. Recovery percentages were the same with both methods.

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