Gas-Liquid Radiochromatography of C^{14} Isopropyl 4,4′-Dibromobenzilate Residues in Field-Weathered Soil

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Gas-liquid radiochromatography has been successfully applied in the quantitative determination of residues of C^{14}-isopropyl 4,4′-dibromobenzilate in field-weathered soil. Chloroform extracts of soil samples without cleanup were injected directly into a Barber-Colman radioactive monitoring (ram) gas chromatograph. The response for standard C^{14} isopropyl 4,4′-dibromobenzilate was directly compared to fortified check and treated, field-weathered sample extracts. Only the parent compound peak was observed in the field-treated and fortified extracts. The total radioactivity in soil 14 months after field application was determined by liquid scintillation. Greater than 95% of the residual radioactivity was identified by gas-liquid radiochromatography as the unchanged parent compound.

Acarol miticide, isopropyl 4,4′-dibromobenzilate (Geigy GS-19851) (Figure 1) has been developed by the Geigy Chemical Corporation for use on citrus and pome fruits. In a metabolic study of this compound on apples, C^{14}-labeled Acarol miticide was applied to apple trees, and minimal degradation of the compound was observed (Cassidy *et al.*, 1968). Only the parent compound was found on the apples, although there was some evidence indicating minor metabolic breakdown on the leaves. Part of the study included periodic sampling of the soil under these trees to define dissipation rates and metabolic activity. The final results of this study will be reported elsewhere, and only those techniques relating to gas-liquid radiochromatography will be discussed here. The data presented here are only for samples taken 14 months after field application.

Gas-liquid radiochromatography provides an ideal analytical system for the determination of radioactive residues of isopropyl 4,4′-dibromobenzilate in soil, since a direct comparison of the results with this technique can be made with the total radioactivity determined by liquid scintillation. Rigorous clean-up procedures are not necessary, and the resultant simplified procedure minimizes the possible formation of artifacts that might otherwise occur.

The effluent gas from samples injected for gas-liquid chromatographic separation passes through a quartz combustion tube at 630° C filled with copper oxide and iron wool. Organic compounds are quantitatively converted to water and carbon dioxide, and the hot iron strips oxygen from the water, subsequently liberating hydrogen. Residual water is removed by passing the gas mixture through a magnesium perchlorate trap. The gas is cooled to room temperature, mixed with propane, and passes through the proportional counter. The ionization yield from the bombardment of Argon atoms by β particles is converted into a dc signal which is proportional to the amount of radiation, producing an output signal in the ratemeter. The signal is amplified and subsequently recorded.

With an accurate accounting for all radioactivity in the sample, a complete and final evaluation can be made. Statistical treatment of the results of gas-liquid radiochromatography by the method of least squares analysis achieves unusual accuracy and precision for pesticide residue analysis.

EXPERIMENTAL

Apparatus. Gas-liquid radiochromatographic analyses were performed with a Barber-Colman series 5000 gas chromatograph, equipped with a radioactive monitoring (ram) System and a 0–5 mV recorder. Total radioactivity in sample extracts was determined using a Beckman LS-133 liquid scintillation system.

Procedure. A 50-g air-dried soil sample is Soxhlet extracted for 18 hr with 250 ml of a methanol : 2-propanol (1 to 1) solvent system, according to the method described earlier (Cassidy *et al.*, 1968). The extract is evaporated to near dryness on a steam bath under a stream of dry air, and the residue dissolved in chloroform. The resulting solution is washed through sodium sulfate and then evaporated to a volume of 1.0 ml using a 40° C water bath and a stream of dry air. A 50 μl aliquot is taken for liquid scintillation to determine the precise level of residual radioactivity in the extract, and the remainder is retained for identification and quantitation with the radioactive monitoring (ram) gas-liquid chromatographic system. At this point the extract may be directly analyzed by the ram system, provided there is sufficient radioactivity. Ram-glc analysis is most consistent above a level of 200 dpm per μl. A 3 to 6 μl aliquot is then injected into the gas chromatograph. If there is insufficient radioactivity in the extract for analysis, a 500 μl aliquot of the remaining 950 μl is transferred to a 2 dram vial and evaporated to dryness in a 40° C water bath with a stream of dry air. The residue is then redissolved in an appropriate volume of chloroform, and a 3 to 6 μl aliquot injected into a gas chromatograph equipped with the Barber-Colman ram system, operating at the following conditions:

Injection port temperature, 225° C

Column, 2′ × ¼″ o.d. Pyrex tubing (Corning No. 7740) packed with 3% FFAP on 60/80 mesh Gas Chrom Q

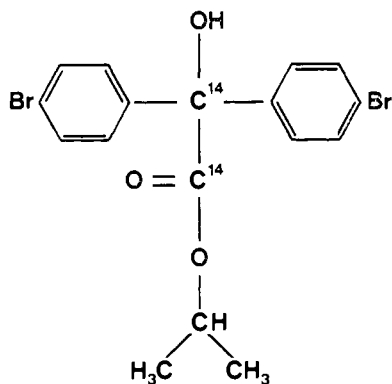Geigy Chemical Corporation, Agricultural Chemicals Division, Ardsley, N.Y. 10502

Figure 1. C^{14} isopropyl 4,4'-dibromobenzilate

Column temperature, 210° C
Transfer line temperature, 260° C
Combustion furnace, 630° C
Carrier gas, Argon, 60 ml per min
Quench gas, propane, 6 ml per min
Detector voltage, 1500-1700V
Sensitivity, 1–3 × 10³
Probable error setting, 15%
Recorder, 0–5 mV
Chart speed, 0.33 in. per min

Calculations. The instrument is calibrated by injections of standard C^{14} isopropyl 4,4'-dibromobenzilate. A solution containing 1.0–1.5 × 10³ dpm standard per μl is prepared in ethanol, and the precise level of radioactivity determined by liquid scintillation. Standards in the range of 1.5–3.0 × 10³ dpm are injected into the chromatograph, and the recorder peak area determined by triangulation. The response factor, (in.)² per dpm standard, is calculated using Equation 1

$$(in.)^2/dpm \text{ standard} = \frac{\text{peak area (in.)}^2 \times \text{sensitivity}}{\text{dpm standard injected}} \quad (1)$$

This response factor can be converted to (in.)² per μg by correcting for the particular specific activity of the standard isopropyl 4,4'-dibromobenzilate as synthesized.

The dpm per μl of parent compound in the original extract of the treated sample prior to concentration is calculated using Equation 2

dpm parent miticide/μl =

$$\frac{\text{peak area (in.)}^2}{(in.)~^2/dpm \text{ standard}} \times \frac{\text{sensitivity}}{\mu l \text{ injected}} \times F \quad (2)$$

where F represents a correction factor for the concentration procedure. The values obtained with Equation 2 can be converted to μg per μl of the miticide in the original sample extract by use of the particular specific activity of the synthesized standard, and ppm may be calculated.

Sample number	Total radioactivity by liquid scintillation (average dpm per μl)	Radioactivity detected as C^{14} Acarol by glc-ram (average dpm per μl)	Average % of total radioactivity detected by glc-ram analysis[a]
1	399	441	111. ± 1%
2	336	342	102. ± 7%
3	317	340	107. ± 3%

[a] Multiple analyses statistically evaluated by least squares analysis.

After a series of multiple analyses, the data are statistically evaluated by least squares analysis to determine the standard deviation.

RESULTS

Table I presents a comparison of four determinations of a single sample on separate days. Despite the wide variation of standard response on each day, no significant effect upon the analytical results can be observed. This variation is dependent upon peak shape, carrier flow, Argon: propane ratio, and detector voltage—factors which will be considered further in the next section.

Since the radioactivity in the soil extracts we obtained was insufficient for glc-ram analysis (< 200 dpm per μl), five-fold concentrations of all extracts were made. Table II compares total radioactivity in the concentrated extracts as determined by liquid scintillation in three field-weathered, treated samples to C^{14} isopropyl 4,4'-dibromobenzilate analyzed by gas-liquid radiochromatography. At least 95% of the total residual radioactivity in all three field-weathered, treated samples 14 months after application is accounted for as parent compound. No radioactive peaks other than the parent compound are present in the treated sample extracts. These results are in agreement with those of thin-layer chromatography and gas chromatography utilizing electron capture detection, as reported previously (Cullen et al., 1968).

Recoveries of untreated soil extracts which had been fortified prior to final evaporation averaged 101.5%, while field-weathered, treated soil averaged 106.3%. Standard deviation by least squares analysis had a maximum value of ± 7%. Wet combustion of the soil residue after extraction showed that 1–3% of the total radioactivity was not extracted from the soil with the methanol:2-propanol Soxhlet procedure. Our later studies have shown that shaking for 30 min in a 10% water in acetone solution is equally as efficient as the 18-hr Soxhlet procedure for extracting the parent compound.

Figure 2 presents typical ramgrams of field-weathered untreated soil, treated soil, and fortification recovery extracts.

Table I. Comparison of Glc-Ram Analyses of Sample No. 3 with Variation in Standard Response

Theoretical dpm sample injected[a]	Standard response (in.)²/dpm	Area (in.)²	Sensitivity (× 10³)	dpm C^{14} isopropyl 4,4'-dibromobenzilate found by ram analysis	% of theoretical radioactivity
1.617 × 10³	0.612	1.017	1	1.662 × 10³	102.8%
1.140 × 10³	1.740	0.720	3	1.240 × 10³	108.8%
1.617 × 10³	1.740	1.008	3	1.740 × 10³	107.6%
1.520 × 10³	0.706	1.175	1	1.658 × 10³	109.2%

[a] Based upon total radioactivity as determined by liquid scintillation.

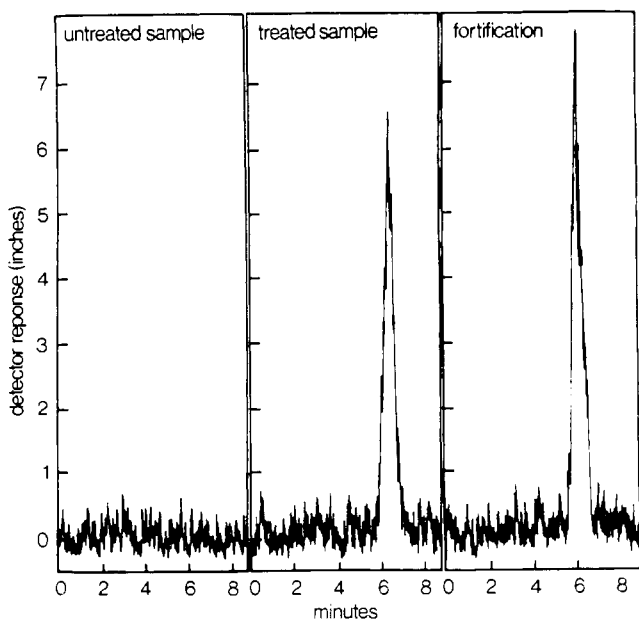**Figure 2. Typical ramgrams of soil extracts**

Left, untreated soil, equivalent to 1.05 g. Middle, treated soil, equivalent to 1.05 g, 1.68×10^3 dpm C^{14} isopropyl 4,4'-dibromobenzilate found. Right, fortification, equivalent to 1.15 g soil, 2.94×10^3 dpm C^{14} isopropyl 4,4'-dibromobenzilate found (102% recovery). Sensitivity: 1×10^3

DISCUSSION

These results indicate good correlation between the amount of radioactivity found by liquid scintillation and gas-liquid radiochromatography. The latter is quite accurate, since it is not subject to chemiluminesence, sample temperature, and quenching effects, while still maintaining a very high counting efficiency. In addition, the ram system provides the ability to resolve organic radioactivity into its component sources. The procedures utilized in this study provide for a relatively fast method of detection for C^{14} 4,4'-dibromobenzilate at the 0.05 ppm quantitative lower limit (approximately 1000 dpm). Semiquantitative results are possible above the 0.005 ppm level. These ppm limits will depend upon the specific activity of the compound synthesized. The specific activity of the material we used was 3.93 μCi per mg.

The ram system provides an excellent means of organic C^{14}

determination, as in the case of C^{14} isopropyl 4,4'-dibromobenzilate, without interference from extractable extraneous substances in the residue, thereby allowing relatively simple cleanup procedures. Since no organic radioactivity other than labeled pesticides and their possible degradation products are present in the soil, no peaks are observed for constituents common to soil. This is in contrast to flame ionization, electron capture, and the microcoulometric detection systems, for which more rigorous cleanup is required. The nonradioactive organic materials in a simple extract of soil are combusted to CO_2 and H_2 in ram system, but since there is no β-particle activity in the CO_2, no radioactive peak is monitored. Therefore, the main consideration is one of sufficient concentration of radioactivity in the extract for detection in the proportional counter.

The most critical factors in the ram system are the propane-argon ratio, and the balance between the gas mixture and detector voltage. Insufficient quench gas results in avalanching of response, subsequent high background, and a general unreliability, while excess quench gas decreases sensitivity. A plot of response *vs.* voltage at a constant gas mixture ratio results in a plateau curve. Conditions should be adjusted so that the voltage utilized falls in the middle of the plateau portion of the curve, thereby insuring reproducibility.

The volume of organic solvent injected should not exceed 5 to 6 μl, since the volume of CO_2 evolved from the combustion of more than 6 μl of organic solvent will alter the balance between high voltage and energy absorption within the counter. Peak shape is also significant in that a peak width of 1.0 to 1.5 min is required for ideal counting accuracy.

Combustion temperature is not quite as critical as the previous factors, but still remains significant. At 750° C organic compounds are combusted to carbon monoxide; at less than 600° C combustion may be incomplete and will result in a loss of sensitivity.

LITERATURE CITED

Cassidy, J. E., Kocvara, H. M., Murphy, R. T., paper presented at the 156th ACS Meeting, Division of Agricultural and Food Chemistry, Abstract No. 62, 1968.
Cullen, T. E., Kahrs, R. A., Mattson, A. M., Murphy, R. T., paper presented at the 156th ACS Meeting, Division of Agricultural and Food Chemistry, Abstract No. 103, 1968.