OV-101 coated on Gas-Chrom Q can be used, but we found that none of these provided adequate resolution with milk samples. Rather than add an additional cleanup step for milk or change columns for different samples, we use the 100 ft × 0.03 in. capillary column for all analyses.

Although whole blood can be analyzed by the glc procedure used for plasma, the chromatograms are more complex and variable, and an abundance of ascorbic acid must be added to prevent heme complexing. In a preliminary test using blood from a lamb dosed with 1 mg/kg of diphenadione, results were poor when whole blood was processed as described for plasma, but when the ascorbic acid was increased from 10 to 100 mg, recovery from whole blood was improved 38% and the residue value determined for whole blood was within 3% of that determined for plasma (corrected for 37.5 hematocrit value). In another test, no diphenadione could be detected in the blood cell fraction removed by centrifugation when it was washed with physiological saline to remove all remaining traces of plasma. Therefore, since the plasma fraction appears to contain essentially all the anticoagulant and residue levels in plasma can be converted by the appropriate hematocrit value to levels in whole blood, we use the simpler plasma analysis.

Figures 1 and 2 illustrate typical experimental uses of the procedure. It has been used routinely in analyzing plasma, liver, kidney, muscle, fat, and milk samples, and satisfactory results have also been obtained with foliage and grain samples from wheat, oats, and corn. The lower limit of detectability for these materials ranges from about 10 ppb for plasma samples down to 0.5 ppb for milk samples.

An additional option is available for plasma samples in which the anticoagulant is known and the samples after preparation and cleanup contain 5 μg or more. We have routinely analyzed such samples by redissolving the dry sample residue from cleanup in 3 ml of acetonitrile, transferring to a quartz cuvette, and analyzing by uv spectrophotometry with a Beckman Model DK-2A recording spectrophotometer at 325 nm. (If the anticoagulant is not detectable by this means, 2 ml can be recovered from the cuvette and analyzed by glc.) Concentrations in unknown samples are determined by substituting the absorbance value into a linear regression equation derived from analysis of fortified plasma samples. Although this method will not distinguish between chlorophacinone and diphenadione or determine concentrations below about 5 ppm, it considerably simplifies the routine analysis of sample series where it is appropriate.

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Gas–Liquid Chromatographic Determination of Sencor (Metribuzin) and Its Major Metabolites and Photoproduct

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A new method has been developed which enables simultaneous glc analysis of the *as*-triazinone herbicide Sencor (metribuzin) (21087-64-9) and its three major metabolites and degradation products. Best results were obtained using 3% Silar 5CP on Chromosorb W (acid washed, DMCS treated). Tritium electron capture detection was more sensitive (0.01 ng of Sencor minimum), but was not selective with respect to soil coextractives. Coulson conductivity detection was less sensitive (1 ng of Sencor minimum), but provided selective quantitation of nitrogen containing compounds; no interfering peaks were observed in soil extracts. An improved method of glc analysis for Sencor alone using the Melpar flame photometric detector is described. Linear response over the range 3 to >150 ng of Sencor was observed. No interfering peaks were observed in soil extracts; the quantifiable minimum was 1 ng of Sencor.

The new herbicide Sencor (BAY 94337, metribuzin) has been registered for several years in Canada for use against broad-leafed and grassy weeds in potatoes, and is now registered in the United States for similar use on soybeans. Use on other crops is being investigated (Saidak, 1974; Duke and Hunt, 1972; Osgood, 1972). The efficacy of Sencor is complemented by its low mammalian toxicity (acute oral LD_{50}^{rat} 1960 mg/kg; Chemagro Corporation, 1971). Sencor has shown no carryover in United States trials, but has shown a tendency, following use in some areas of western Canada, to persist in the soil causing injury to subsequent crops which are not tolerant to Sencor (Stobbe, 1972; Bowden, 1973).

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Sencor (I, 4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4*H*)-one), is known to be metabolized in potatoes to DADK (II, 6-(1,1-dimethylethyl)-1,2,4-triazine-3,5(2*H*,4*H*)-dione), and to DADK and DK (III, 4-amino-6-(1,1-dimethylethyl)-1,2,4-triazine-3,5(2*H*,4*H*)-dione), in soybeans (Church *et al.*, 1972). A further minor metabolite in soybeans, DA (IV, 6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4*H*)-one), has also been shown to be the principal photoproduct of Sencor in aqueous solution (Pape and Zabik, 1972). These three compounds are produced during environmental degradation of Sencor in soil, and a quantitative description of this phenomenon will be the subject of a subsequent paper.

To enable quantitative examination of the carryover phenomenon, and to provide means of following the environmental decay of Sencor, a convenient analytical method was required not only for Sencor, but also for the major metabolites and degradation products. A convenient method for glc analysis for Sencor using flame photometric detection has been described by von Stryk (1971); quantitation was performed using a nonlinear plot of log (response) *vs.* log (nanograms of Sencor). Analytical methods have also been described by Thornton and Schumann (1971, 1972) for Sencor, DADK, DK, and DA involving elaborate cleanup, separation of Sencor from DADK and DK, and quantitation of Sencor and metabolites separately using ^{63}Ni electron capture detection. We have developed a rapid glc procedure which permits simultaneous analysis for Sencor DADK, DK, and DA using ^{3}H electron capture and Coulson conductivity detection.

EXPERIMENTAL SECTION

Glc analyses were performed using a Tracor Microtek MT 220 gas chromatograph fitted with a Melpar flame photometric detector (FPD) in the sulfur mode, a Coulson conductivity detector (CCD) in the nitrogen mode, and a Varian ^{3}H foil electron capture detector (ECD) in a modified Solomon configuration (Uthe *et al.*, 1972).

Chromatographic Conditions. For Sencor alone by FPD, a 1.8 m Pyrex, 4 mm i.d., column was packed with 80–100 mesh Chromosorb W HP coated with 3% OV-225: temperatures, inlet, 230°; detector, 220°; column, 215° (isothermal); flow rates, nitrogen carrier, 100 ml/min; oxygen, 25 ml/min; hydrogen, 150 ml/min; air, 20 ml/min; retention time, 2.75 min. An alternate column used successfully was 80–100 mesh Gas-Chrom Q coated with 1% Reoplex 400, column temperature, 180°.

For Sencor, DADK, DK, and DA by ECD and CCD, the columns described in Table I were prepared and used under the conditions recorded in Table II.

Standard Curves. Standards (compounds from Chemagro) were best prepared in ethyl acetate or benzene followed by *n*-hexane. Injection volumes were 1–6 μl (FPD) or 1 μl (ECD and CCD). Peak heights were used to plot log (response) *vs.* log (nanograms of Sencor)^2 for FPD, response *vs.* nanograms of compound for ECD, and log (response) *vs.* log (nanograms of compound) for CCD.

Extraction of Fortified Soil. *Method 1.* Soil samples, 30 g dry weight, were fortified with 1-ml aliquots of an aqueous solution of Sencor, equivalent to 54 μg. The samples were shaken and allowed to stand overnight at 5°. Entire fortified samples were extracted in a Soxhlet apparatus with 20% aqueous methanol (250 ml, 4 hr, 15-min cycle). The methanol was evaporated at 40° under reduced pressure and the Sencor in the aqueous solution remaining was partitioned into chloroform. The chloroform was evaporated at 40° using benzene as a chaser, and the residue in 2 ml of benzene was analyzed by glc: injection volume, 2 μl; 95% recovery.

Method 2. Soil samples, 30 g dry weight, in 6-oz glass cabinet oval medicine bottles with Teflon-lined metal caps, were fortified with 10-μl aliquots of an ethanolic solution containing Sencor, DADK, DK, and DA, equivalent to 40 μg of each. The soil samples were brought to field capacity with distilled water, shaken for 15 min, and allowed to stand overnight at 5°. Entire fortified samples were extracted using four 15-min shakings with 20% aqueous methanol. The extract solution was decanted and filtered and the methanol evaporated at 40°. The Sencor, DADK, DK, and DA in the aqueous solution remaining were partitioned into chloroform. The chloroform was evaporated at 40° using benzene as a chaser, and the residue in 0.20 ml of ethyl acetate was analyzed by glc: injection volume, 1 μl; recovery, 97% (Sencor), 81% (DADK) (clay soil, 24%), 46% (DK), and 94% (DA).

Recovery rates for Sencor using the two extraction methods were essentially equivalent; however, the shaking method was preferred since it is faster and requires less expensive and less fragile apparatus.

Table I. Glc Columns for Sencor, DADK, DK, and DA by ECD and CCD

	Column no.		
	1	2	3
Stationary phase	1% Reoplex 400	3% OV-225	3% Silar 5CP
Solid support[a]	Gas-Chrom Q	Chromosorb W, HP	Chromosorb W, AW DMCS
Column length and i.d.	1.2 m × 2.5 mm	1.8 m × 4 mm	0.6 m × 4 mm

[a] 80–100 mesh for all columns.

Table II. Glc Conditions for Sencor, DADK, DK, and DA by ECD and CCD

	Detector with column no.[a]				
	ECD		CCD		
	1	2	1	2	3
Flow rates, ml/min					
carrier, N_2 or He	50	95	50	85	85
sweep, He			50	28	29
reducing gas, H_2			120	160	160
Temp, °C					
inlet	285	285	285	285	245
column	175	190	175	160	160
transfer line			260	260	250
block			260	260	260
pyrolysis oven			840	840	840
detector	215	215	20	20	20
Voltage	90	90	30	30	30

[a] See Table I for a description of columns.

RESULTS AND DISCUSSION

Sencor Alone by FPD. Quantitative results for Sencor using the Melpar FPD with a flow rate ratio for O_2/H_2 of 0.15 provide a linear standard curve over a 3.0 to >150 ng range when log (response) is plotted *vs.* log (nanograms of Sencor)^2. This plot provides a superior quantitation method to that of von Stryk (1971), and is consistent with the findings of other workers with other sulfur containing compounds (Brody and Chaney, 1966; Bowman and Beroza, 1968; Mizany, 1970). Mizany, in particular, reported linear plots for log (response) *vs.* log (nanograms of S)^2 for diethyl sulfide over the range 2–10 ng of S when the flow rate ratio of O_2/H_2 was 0.15; our linear range, 3.0 to >150 ng of Sencor, corresponds to 0.4 to >23 ng of S.

Detection of Sencor at the 1-ng level (0.03 ppm) was possible in spiked soil samples using method 1, and no interference from coextractives was encountered.

Table III. Retention Times for Sencor, DADK, DK, and DA (Minutes)

	Detector with column no.[a]				
	ECD		CCD		
	1	2	1	2	3
DADK	6.73	2.52	7.05	6.75	4.75
DK	9.30	4.52	10.67	13.00	10.00
Sencor	8.55	6.27	9.25	17.33	14.33
DA	18.03	12.50	21.17	37.00	29.28

[a] See Table I for a description of columns.

Sencor Plus DADK, DK, and DA by ECD and CCD. The chromatographic conditions used by Thornton and Schumann (1971, 1972) (5% Reoplex 400 on Gas-Chrom Q) did not satisfactorily resolve Sencor and DK. In addition, DK and DA tailed to an undesirable extent in spite of extensive attempts to correct the problem. The Reoplex 400 phase tended to bleed off the column at temperatures above 200°; column life was short, and conditioning at 200° for 2 weeks was necessary before ^3H ECD could be conveniently used in conjunction with this column. Resolution was improved on the Reoplex 400 column toward the end of its useful life. Columns with 2% Reoplex 400 and finally 1% Reoplex 400 provided further improved resolution, and tailing of DK and DA was substantially reduced when the inlet temperature was raised to 285°. The new 1% Reoplex 400 column (column 1) resolved Sencor and DK and provided retention data for the four compounds by ECD reported in Table III.

The problem of the short life of the Reoplex 400 column remained. The column described earlier for Sencor analysis by FPD, *i.e.*, 3% OV-225 on Chromosorb W HP, which had previously proved unsuccessful for breakdown product analysis, was treated with a commercial silylating agent (Silyl-8, Pierce Chemical Co.) and conditioned at 235° and a flow rate of 40 ml/min for 16 hr. Injection of a solution containing the four compounds yielded, by ECD, the retention data reported for column 2 in Table III. The Sencor and DK peaks were eluted in reverse order with respect to column 1, but all peaks were completely resolved.

Although ECD is a sensitive method for the quantitation of Sencor, DADK, DK, and DA (minimum detectable amount of Sencor is approximately 0.01 ng), this detector also responds to other compounds. During analyses of soil extracts, for example, large quantities of soil coextractives give interfering peaks. Cleanup procedures to remove these coextractives from extracts containing DADK, DK, and DA are long and tedious, and reduce recovery rates by an appreciable extent. The CCD in the nitrogen mode has proven to be an effective selective detector for nitrogen containing herbicides in soil extracts (Purkayastha and Cochrane, 1973). Combining column 1 or 2 with CCD provided the retention data shown in Table III. The separation was similar to that obtained using ECD; however, the peaks were somewhat broadened, probably because of the relatively large dead volume of the CCD system. Interference from soil coextractives was not observed, a particularly significant result in view of the concentrated nature of the soil extract samples.

Column 3, containing the new Silar 5CP stationary phase, reported to be excellent for the separation of polar compounds of similar structures (Hill, 1973; Gas-Chrom Newsletter, 1973), provided superior resolution to that obtained using column 2. Results appear in Table III.

Table IV. Linear Range and Minimum Detectable Quantities for Sencor, DADK, DK, and DA Using Column 3 and CCD

Compd	Linear range, ng[a]	Min detectable quant., ng[b]
DADK	40–200	2.4
DK	20–400	10
Sencor	5–500	1
	800–2000	
DA	40–200	20

[a] Log (response) *vs.* log (nanograms compound). [b] Signal-to-noise ratio approximately 10:1.

Quantitation of Sencor and breakdown products using column 3 and CCD is especially useful because of the high degree of selectivity and the wide linear response range of this detector (see Table IV). The described procedure is a convenient method for determination of residues of Sencor, DADK, DK, and DA from soil extracts. Whereas ECD requires extensive cleanup to remove interfering coextractives, CCD analysis provides good sensitivity with minimum cleanup.

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