layer was then refluxed with 10% KOH and oxamyl estimated as already described.

The recovery study results obtained at different levels of fortification on replicate analyses are given in Table I. Except in saline sodic soil the recovery yields were over 80% for fortifications of 2 to 10 ppm after a contact of 12 h. A greater loss in the case of sodic soil was due to alkalinity which had a tendency to decompose oxamyl. Even in this case the recovery was 75% and above. These results indicated essentially satisfactory recoveries and the applicability of the method for estimation of oxamyl residues in various types of soils, water, and plant materials.

Interference Effects. The results of an investigation of the effect of inorganic and organic compounds in solutions containing an average of 50.3 μg of oxamyl are shown in Table II. Analyses of aqueous solutions of oxamyl containing diverse inorganic ions and organic compounds showed that Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, NO_3^-, SO_4^{2-}, nemagon, telone, pyridine, diphenylamine, and trimethylamine did not interfere. Other ions such as Ni^{2+}, Co^{2+}, Zn^{2+}, Fe^{3+}, and Al^{3+} and organic compounds such as dimercon, acetic acid, benzoic acid, methyl alcohol, carbon tetrachloride, and acetaldehyde in the amounts added did interfere. The tolerance limit was considered as that giving a deviation less than three standard deviations.

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Gas Chromatographic Determination of Sencor and Metabolites in Crops and Soil

John S. Thornton* and Charles W. Stanley

A gas chromatographic procedure is described for the analysis of residues of Sencor herbicide and its metabolites in a variety of crops. Conjugated residues are released by refluxing the sample as a part of the initial extraction. Following this, Sencor and metabolites are separated by liquid–liquid partition and the two fractions cleaned up individually for electron-capture gas chromatographic analysis. A separate extraction scheme is described for analysis of soil. Considerable effort was made to ensure maximum recovery of residue from field-weathered samples. Recovery of Sencor is generally in the 80–100% range with metabolite recoveries averaging somewhat less. The sensitivity limit of the method is 0.01 ppm for Sencor and all metabolites.

Sencor [4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4*H*)-one], also known as metribuzin and Bay 94337, is an *as*-triazinone herbicide (Eue et al., 1969) which has shown effective control of a large number of grass and broadleaf weeds infesting agricultural crops such as potatoes, soybeans, sugarcane, and tomatoes.

Metabolism studies have been conducted by a number of workers (Gronberg et al., 1971; Church and Flint, 1972; Hargroder and Rogers, 1974; Hilton et al., 1974). In all cases, the metabolism in plants follows a stepwise pathway as shown in Figure 1, resulting in three identified metabolites: deaminated Sencor (DA), deaminated diketo-Sencor (DADK), and diketo-Sencor (DK). Any of these metabolites could conceivably be found as residues in crops. However, the metabolism studies show it is unlikely that the DK or DA metabolite would ever be present in mature crops grown above the ground. Residues which have been found have been mainly Sencor with minor amounts of DADK. This is in contrast to root crops which have shown residues of Sencor and the DK metabolite and, to a limited extent, DA. The DA metabolite was also shown to be the major photodegradation product in water solutions (Pape and Zabik, 1972).

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Residues of Sencor were first analyzed by Stanley and Schumann (1969). The method employed a single blender extraction, followed by Florisil column cleanup and electron-capture gas chromatographic analysis using a 5% OV-101 column. A modification of that method, reported by von Stryk (1971), employed flame photometric detection in the sulfur mode. Thornton and Schumann (1971) reported an electron-capture gas chromatographic method for Sencor and its DADK metabolite in soybeans. Later, Thornton et al. (1972) reported a procedure for the analysis of Sencor and all three metabolites in sugarcane with electron-capture detection of the compounds after separation on a column of 5% OV-225 on Gas-Chrom Q. Webster et al. (1975) also reported separation of Sencor and metabolites on 5% OV-225 on Chromosorb W (HP).

The method of Thornton et al. (1972) has been refined and extended to additional crops and soil and is reported in detail in this paper. Briefly, the crop procedure involves a refluxing step to release Sencor and any metabolite that may be conjugated. Sencor and metabolite residues are separated from the solids by filtration and the filtrate solution evaporated until only water remains. Following this, Sencor and metabolites are separated from each other by liquid–liquid partition and cleaned up on liquid chromatography columns prior to individual gas chromatographic analysis on a 5% OV-225 column with electron-capture detection. A summary of the analysis

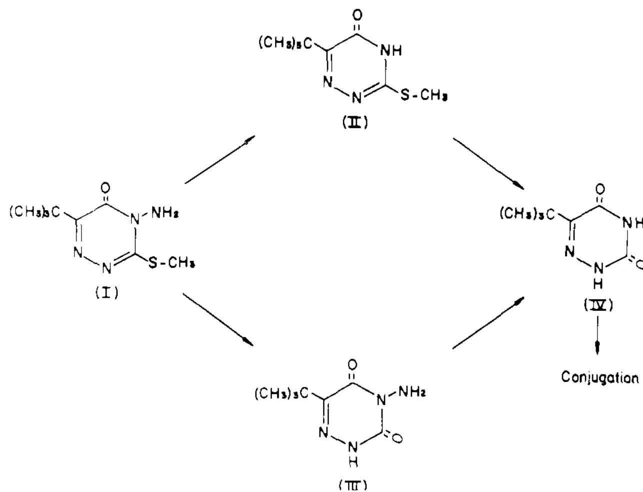

Figure 1. Sencor metabolic pathway: (I) Sencor; (II) deaminated Sencor metabolite (DA); (III) diketo-Sencor metabolite (DK); (IV) deaminated diketo-Sencor metabolite (DADK).

scheme is shown in Figure 2.

Some modifications of the crop method are possible when analyzing soil to yield a shorter and somewhat more versatile method. Soil samples are also refluxed to release any adsorbed or conjugated residues. Free residues are separated from the solids by filtration. Following this, Sencor may be cleaned up on a Florisil column for rapid gas chromatographic analysis. Where the metabolite analysis is desired, Sencor and metabolites are first separated from each other on a silica gel column with the Sencor fraction further cleaned up on a Florisil column. Residues are measured by electron-capture gas chromatography at the same conditions used for crops. A schematic diagram of the procedure for soil is shown in Figure 3.

EXPERIMENTAL SECTION

Apparatus and Reagents. The instrument used was a Hewlett-Packard Model 5750B equipped with a high-temperature ^{63}Ni electron-capture detector. The gas chromatographic column was 1 m × 3 mm o.d. borosilicate glass tubing packed with 5% OV-225 solution coated (Applied Science Labs, Inc., 1967) on 80–100 mesh Chromosorb W (HP). Immediately after packing, the column was purged of air with helium and "no flow" conditioned for 8 h at 250 °C before flow conditioning at operating conditions for 16 h. Florisil, PR Grade, 60–100 mesh (Floridin Co., Pittsburgh, Pa.), was activated at 130 °C for 24 h, stoppered, cooled, and deactivated by adding 2.5% water (2.5 ml of water + 97.5 g of dried Florisil). After deactivation, it was allowed to equilibrate 24 h before use. Silica gel (Fisher S-679) was activated at 130 °C for 24 h, stoppered, cooled, and deactivated by adding 7% water. It was also equilibrated 24 h before use. Sencor, DK, and DA metabolites were dissolved in benzene. The DADK metabolite must be dissolved in acetonitrile. Secondary dilution of all standards may be made in benzene. All other solvents were "pesticide grade" or A.R.

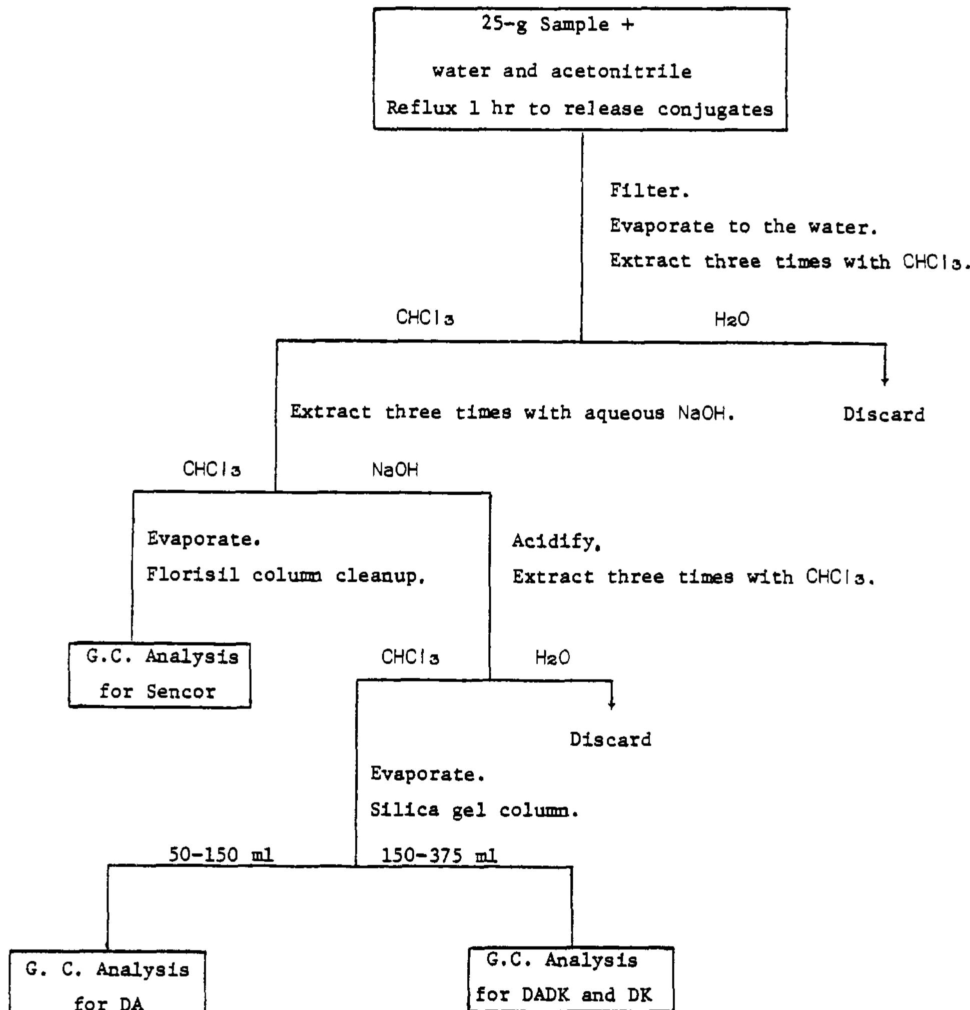

Figure 2. Schematic diagram of Sencor and metabolite analysis in crops.

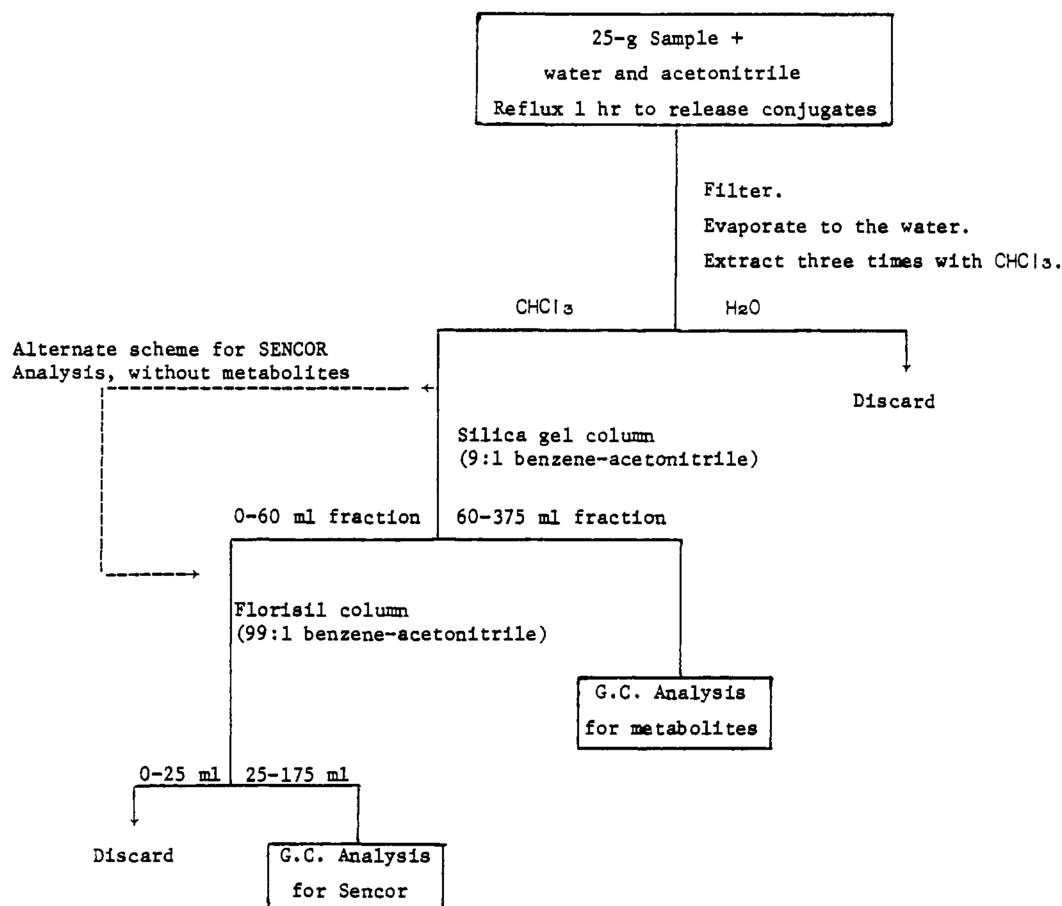

Figure 3. Schematic diagram of Sencor and metabolite analysis in soil.

Extraction of Crops. Grind the entire sample in a food chopper with dry ice. Mix thoroughly and place the samples in frozen storage overnight to allow the dry ice to sublime.

Extraction of Crops, Except Potatoes. Weigh 25 g of the ground sample into a 1-l. boiling flask. Add 50 ml of water and 200 ml of acetonitrile (for bulky samples such as hay or straw, use 75 ml of water and 300 ml of acetonitrile; for extremely bulky samples, reduce sample size to 10 g). Attach the flask to a condenser and heat the sample at reflux for 1 h. Allow the sample to cool and rinse down the condenser with 3–5 ml of acetonitrile. Filter the sample through a 9-cm Whatman GF/A filter paper in a size 2A Büchner funnel. Wash the flask and the solids with 50 ml of acetonitrile (use 100 ml of acetonitrile for bulky samples). For samples with high chlorophyll or wax content, it may be helpful in reducing emulsions if the sample is transferred to a separatory funnel at this point and washed with 200 ml of hexane. Discard the hexane (upper phase).

Extraction of Potatoes. Weigh 50 g of ground potatoes into an 8-oz jar. Add 50 ml of water and cap the jar tightly using two thicknesses of aluminum foil as a liner. Suspend the jar (up to the cap) in a steam bath for 8 h. (Samples may be held at room temperature overnight at this point.) Transfer the contents of the jar to a Waring Blendor. Rinse the jar and foil liner with 200 ml of acetonitrile and add to the blender. Blend the sample for 3 min and filter through 9-cm Whatman GF/A filter paper in a size 2A Büchner funnel. Reblend the filter cake with 200 ml of chloroform and filter as before.

Transfer the filtrate (all samples) to a 1-l. round-bottomed flask and evaporate the sample on a rotary vacuum evaporator at 40 °C until only water remains (some residual acetonitrile can be tolerated). Transfer the water remaining after evaporation to a 500-ml separatory funnel using sufficient additional water (if needed) to make approximately 75 ml. Rinse the flask with 75 ml of chloroform and add the rinse to the separatory funnel. Shake the separatory funnel for 30 s. Drain the lower (chloroform) phase into a second 500-ml separatory funnel. Repeat the partition with two additional 75-ml portions of chloroform. Discard the water phase. Extract the combined chloroform phases with successive 50- and 25-ml portions of 0.1 N NaOH. Drain the chloroform into a 500-ml flask (if chloroform phase is cloudy, drain through a plug of granular anhydrous sodium sulfate and rinse with a few milliliters of fresh chloroform). Evaporate the chloroform to dryness on a rotary vacuum evaporator. Remove the last traces of solvent with a stream of nitrogen. This fraction contains any Sencor residues. Potato samples may be dissolved in benzene and analyzed by gas chromatography at this point. All other crop extracts must be further cleaned up as described under the section Sencor Cleanup.

Combine the two NaOH washes and acidify them with 3 ml of 4 N HCl. Extract the aqueous solution with three 75-ml portions of chloroform and combine the extracts in a 500-ml flask. Add 0.5 ml of decyl alcohol (keeper) to the flask and evaporate the chloroform just to dryness on a rotary evaporator. Remove the last traces of chloroform with a stream of nitrogen. Reserve the sample for the procedure described in the section Metabolite Cleanup.

Sencor Cleanup. Tamp a plug of glass wool into the bottom of a 20 × 400 mm chromatographic tube with integral 300-ml reservoir. Add about 1 cm of granular sodium sulfate. Fill the tube with benzene to the bottom of the reservoir allowing a small amount of benzene to run

out of the bottom of the tube. Tap the tube to dislodge trapped air. Add 10 g of Florisil (2.5% water deactivated) to the chromatographic tube and allow the Florisil to settle. Tap the tube to level the top of the column and dislodge any Florisil from the walls of the tube. Top the column with 5 g of sodium sulfate and drain the benzene to the top of the column. Add 5–10 ml of benzene to the flask containing the Sencor residue. Transfer the benzene to the chromatographic column and rinse the flask with two additional 5-ml portions of benzene. Pass the benzene solution through the column at the rate of 2 drops/s. Rinse the walls of the column with two 5-ml washes of benzene. Allow the benzene to drain to the top of the sodium sulfate. Discard the benzene wash.

Elute the column with 175 ml of benzene–acetonitrile (99:1) at the rate of 2–3 drops per sec. Discard the first 25 ml and collect the remaining 150 ml in a 250-ml round-bottomed flask. Evaporate the eluent just to dryness on a rotary vacuum evaporator in a 40 °C water bath. Remove the last traces of solvent with a stream of nitrogen. Dissolve the residue in 20.0 ml of benzene. Stopper and hold for gas chromatographic analysis.

Metabolite Cleanup; Silica Gel Column. Tamp a plug of glass wool into the bottom of a 20 × 400 mm chromatographic tube with integral 300-ml reservoir. Add about 1 cm of granular sodium sulfate. Fill the tube with benzene–acetonitrile (90:10) to the bottom of the reservoir, allowing a small amount to run out of the bottom of the tube. Tap the tube to dislodge trapped air. Add 10 g of silica gel (7% water deactivated) to the chromatographic tube. Allow the silica gel to settle. Tap the tube to level the top of the column and to dislodge any silica gel from the walls of the tube. Top the column with 5 g of granular sodium sulfate and drain the solvent down to the top of the column. Transfer the metabolite residue (from above) to the column with 4 washes of 5–10 ml of benzene–acetonitrile (90:10). Wash the column with additional benzene–acetonitrile to make 50 ml. Discard this wash. Elute the DA from the column with 100 ml of benzene–acetonitrile (90:10). Change receivers and elute the DADK and DK from the column with an additional 225 ml of benzene–acetonitrile (90:10). Add 0.1 ml of decyl alcohol to the flask containing the DADK–DK and evaporate all column fractions just to dryness on a rotary vacuum evaporator at 40 °C. Remove the last traces of solvent from the flask with a gentle stream of nitrogen. (This will not remove the decyl alcohol from the flask containing the DADK–DK residues.) Transfer the residue in the flasks with benzene to appropriately labeled volumetric tubes or flasks. Dilute to 5.0 ml with benzene. Reserve for gas chromatographic analysis of DA or DADK–DK.

Extraction of Soil. Mix each soil sample so that the entire sample is homogeneous. Allow wet samples to air dry to the point where they can be sufficiently mixed. Weigh 25 g of mixed soil into a 1-l. boiling flask. Weigh a separate 100-g portion of the same soil into a tared beaker and place in an oven at 130 °C overnight for moisture determination.

To the 25-g portion in the boiling flask add 50 ml of water and 250 ml of acetonitrile. Attach the flask to a reflux condenser and heat the sample at reflux for 1 h. Cool the sample sufficiently so it may be handled, rinse down the condenser with 3–5 ml of acetonitrile, and filter with vacuum through 9-cm Whatman No. 541 filter paper in a size 2A Büchner funnel. Rinse the flask with 100 ml of fresh acetonitrile and use this to wash the filter cake.

Transfer the filtrate to a 1-l. round-bottomed flask and evaporate on a rotary vacuum evaporator until only water remains. Transfer the water remaining after evaporation to a 250-ml separatory funnel with sufficient additional water to total approximately 75 ml. Rinse the flask with 75 ml of chloroform and add the rinse to the separatory funnel. Shake the separatory funnel for 30 s. Drain the lower (chloroform) phase into a 300-ml round-bottomed flask. Repeat the partition with two additional 75-ml portions of chloroform. Add 0.5 ml of decyl alcohol to the flask and evaporate the combined chloroform extracts to dryness on a rotary vacuum evaporator at 40 °C. Remove the last traces of solvent with a stream of nitrogen. If analysis for Sencor only is desired, proceed from this point to the procedure described in the section Sencor Cleanup; Florisil Column. If analyzing for Sencor and metabolites, continue as described below.

Silica Gel Column Separation. Prepare the silica gel column exactly as described above in the crop method. Place a clean receiver under the column and transfer the soil residue in the flask to the column with 4 rinses of 5 ml of benzene–acetonitrile (90:10), allowing each rinse to drain into the column before adding the next. Add an additional 355 ml of benzene–acetonitrile (90:10) to the column reservoir and adjust the drip rate to about 2–3 drops/s.

Collect the first 60 ml that elutes. This fraction contains any Sencor present in the sample. Reserve this fraction for Florisil column cleanup. Change receivers and collect the balance of the eluate in a 500-ml round-bottomed flask. This fraction contains the metabolites DA, DADK, and DK. Add 0.5 ml of decyl alcohol to the metabolite fraction and evaporate just to dryness on a rotary vacuum evaporator at 40 °C. Remove the last traces of solvent with a stream of nitrogen. (This will not remove the decyl alcohol keeper in the flask.) Transfer the residue to a volumetric tube or flask with benzene and dilute to 5.0 ml. Stopper and hold for gas chromatographic analysis.

Sencor Cleanup; Florisil Column. Prepare the Florisil column exactly as described above in the crop method. Evaporate the 60 ml of eluate from the silica gel column just to dryness or use the initial soil extract solution if analyzing for Sencor only. Transfer the residue in the flask to the column and elute as described for crops above. Evaporate the eluate just to dryness on a rotary vacuum evaporator using a water bath at 40 °C. Remove the last traces of solvent with a stream of nitrogen. Dissolve the residue in 20 ml of benzene. Stopper and hold for gas chromatographic analysis.

Gas Chromatographic Analysis. The gas chromatograph was operated at the following conditions: injection port, 250 °C; detector, 270 °C; column oven, 200 °C for Sencor analysis and 180 °C for metabolite analyses (separate DA fractions from crop analysis may be injected at 200 °C to shorten the retention time). Carrier gas was 5% methane in argon, 60 ml/min. The detector was operated in the pulsed mode at a pulse interval of 15 μs.

Inject 4 μl of sample or standard solution. Sencor fractions should be injected along with a 0.125 μg/ml Sencor standard. Metabolite fractions should be injected along with a standard containing 2.5 μg each of DA, DADK, and DK in 5.0 ml of benzene. Metabolite standards containing DADK and DK should also contain 0.5 ml of decyl alcohol per 5 ml final volume. Identify Sencor or metabolites by retention times and measure the area or peak height produced on the recorder strip chart. At the operating conditions stated, the retention times for the various compounds are as follows: Sencor, 4.0 min; DADK, 2.8 min; DK, 5.5 min; DA, 7.5 min (200 °C).

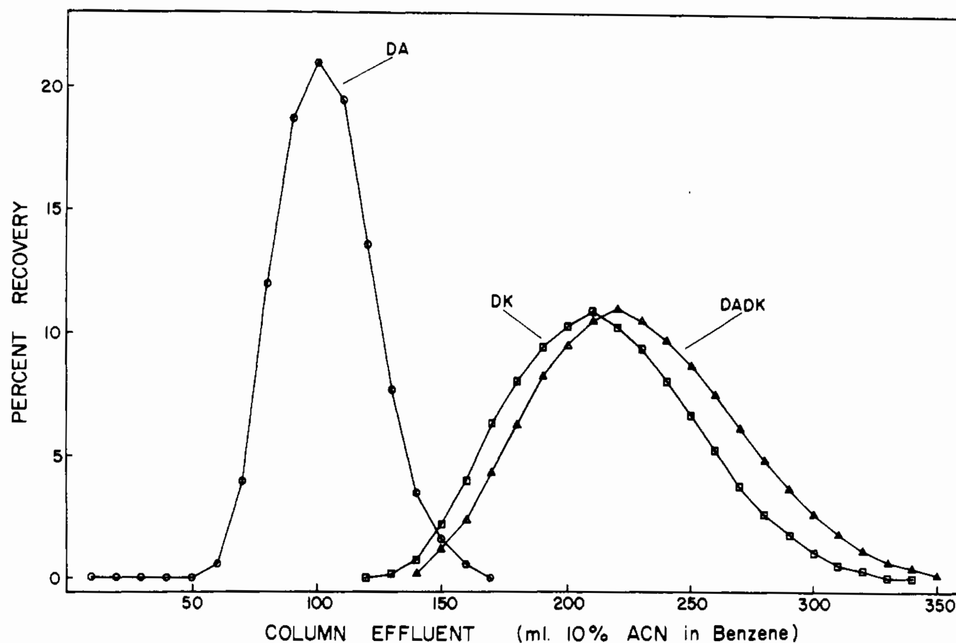

Figure 4. Elution pattern of Sencor metabolites on 7% water-deactivated silica gel.

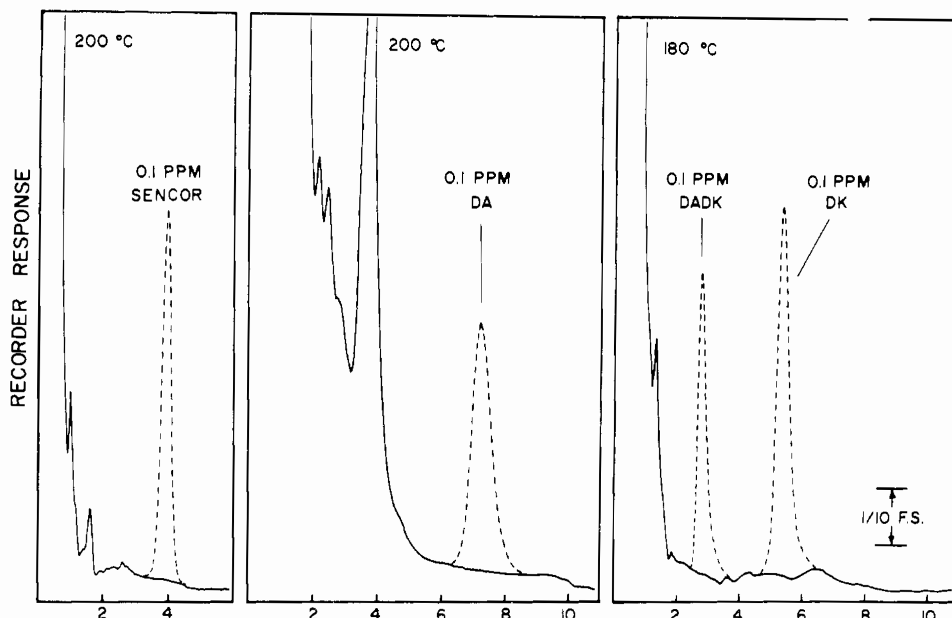

Figure 5. Typical chromatograms showing alfalfa control response and superimposed recovery of 0.1 ppm of Sencor and its three metabolites.

Calculation of Sencor or metabolites in a sample is accomplished by use of the following equation where response for the unknown is compared to the response for a known quantity in a standard solution:

$$\text{ppm} = \frac{\text{sample area}}{\text{std area}} \times \frac{\text{std injection (ng)}}{\text{wt of sample (g)}} \times \frac{\text{final vol (ml)}}{\text{sample injection } (\mu\text{l})}$$

Any residues of metabolites result from conversion of the active ingredient of Sencor by metabolic action. Thus, these residues should be reported in Sencor equivalents, taking into account the molecular weight difference if total residues are to be computed. Multiplication factors (mol wt Sencor/mol wt metabolite) for converting metabolite residues to Sencor equivalents are 1.266, 1.163, and 1.075 for DADK, DK, and DA, respectively.

DISCUSSION

The method described in this paper measures Sencor and its metabolites in a wide variety of crops and soils. It is an adaptation of an earlier procedure for potatoes (Thornton and Stanley, 1972) requiring no cleanup of the Sencor portion and only simple silica gel column chromatography of the metabolites with collection in one common fraction. As noted in the above stepwise procedure, this is still sufficient for potato analyses. It was necessary to modify the procedure to obtain sufficient cleanup for other crops, especially chlorophyll-containing crops. For all crops other than potatoes, the DA is separated from DADK and DK and collected in a separate fraction from the silica gel column; see Figures 2 and 4. Most of the interfering crop extractives elute in the DA fraction but are sufficiently separated from the DA by gas chromatography so they do not interfere as shown in Figure 5. If all three metabolites were collected in a

common fraction, the DADK–DK portion of the gas chromatogram would be obscured by peaks for crop extractives. (This is not a problem with soil extracts.) In addition, for green crops the Florisil column is usually necessary for cleanup of Sencor residues.

Metabolism studies with ^{14}C-labeled Sencor utilized acid hydrolysis to release conjugated metabolites from solids or water fractions (Church and Flint, 1972). The steaming procedure used for potatoes was shown to be as effective as the acid hydrolysis in releasing conjugated residues from potatoes and also did not degrade Sencor as did acid hydrolysis. However, with green crops the steaming procedure gave more crop extractives. It also appeared to cause more decomposition of Sencor and yielded lower recovery of the metabolites than did a reflux procedure. One-hour reflux with acetonitrile–water released maximum residues from field-treated sugarcane samples and caused the least amount of decomposition of added metabolites in laboratory-fortified samples. Additional studies with alfalfa that had been treated with ^{14}C-labeled Sencor and sampled 49 days later showed the 1-h acetonitrile–water reflux extraction to be as effective in solubilizing activity as repeated reflux extractions or even acid hydrolysis using 2 N HCl (Stanley, 1974). Thus, a 1-h reflux was adopted for general use.

Sencor, DADK, DK, and DA in a common solution can be separated from each other by gas chromatography using a column of 5% OV-225 on Chromosorb W (HP). However, because of the great difference in chemical characteristics between Sencor and its metabolites, it is preferable to separate them into fractions prior to cleanup. This is accomplished by a chloroform–aqueous NaOH partition or by column chromatography on silica gel. Sencor is then analyzed separately after being cleaned up by Florisil column chromatography. The metabolites are further cleaned up by silica gel column chromatography prior to GC analysis. Interferences from coextractives carried through the cleanup procedures are minimized by analyzing the various fractions at different gas chromatographic temperatures.

In cases where a large number of samples are to be analyzed for residues, the metabolite fraction may be set aside for later analysis after results for Sencor are complete. In this way only those metabolite fractions need be analyzed where the corresponding Sencor fractions show significant residues. This is possible because of the way Sencor is slowly converted to its relatively nonactive metabolites which are then gradually degraded. This is in contrast to some chemicals which are rapidly converted from the parent compound to metabolites which are also active pesticides. After analysis of many hundred field-weathered samples, the pattern is well established of always seeing some Sencor parent compound if metabolites are present.

Recovery experiments were run on many different crops and products by adding Sencor and metabolites before the initial extraction. Recoveries of Sencor, DADK, and DA were generally in the 75–100% range at levels of 0.1 ppm or above but were somewhat more variable at levels below 0.1 ppm. DK recoveries were the least consistent of all four compounds, ranging generally from 50 to 80% in all crops except potatoes where values were higher and more consistent. Low DK recovery is not a particular problem since DK is a minor metabolite in all crops investigated except potatoes. Perhaps the higher DK recovery in potatoes is due to the different initial extraction used. Low recovery of all the compounds is sometimes a problem in samples with a high volume-to-weight ratio such as cereal straw. This problem can be minimized by decreasing sample size and increasing the volumes of solvent used to initially extract the sample, as indicated in the stepwise procedure. Some of the crops which have been successfully analyzed by this procedure are alfalfa, asparagus, barley, corn (cob, kernel, forage), lentils, peas, potatoes, sanfoin, soybeans, sugarcane (and products), tomatoes, and wheat. Some representative values are given in Table I.

Table I. Recovery of Sencor and Metabolites from Various Crops

Crops	Compd added[a]	No. of replicates	% recovery[b]
Alfalfa	Sencor	8	84
	DADK	8	80
	DK	8	50
	DA	8	79
Beans (dry)	Sencor	4	85
	DADK	6	98
	DK	4	67
	DA	4	77
Cereal grain	Sencor	10	87
	DADK	10	75
	DK	3	71
	DA	3	98
Cereal straw	Sencor	6	78
	DADK	4	77
	DK	3	59
	DA	3	71
Potatoes	Sencor	15	85
	DADK	10	90
	DK	15	82
	DA	7	93
Sugarcane	Sencor	6	86
	DADK	6	83
	DK	5	63
	DA	5	82
Tomatoes	Sencor	7	91
	DADK	7	103
	DK	7	88
	DA	7	96
Soil	Sencor	37	93
	DADK	29	88
	DK	2	86
	DA	2	90

[a] Samples were fortified with Sencor or metabolite compounds at levels ranging from 0.05 to 0.1 ppm except straw which was fortified at 0.5 ppm. [b] Average.

Standard curves have been run to determine linearity of response for Sencor and metabolites. Response for Sencor was linear over a 30-fold range up to 1.5 ng injected. Response for the three metabolites was linear over a 30-fold range up to approximately 7.5 ng. In all cases, the upper limit amounts are equivalent to about 0.15 ppm if sample size and portions described in the procedure are used. Samples containing residues in excess of this amount should be diluted and reinjected to make sure response is linear.

If 0.1 in.^{2} is considered the smallest area that can be measured with a polar planimeter, the level of detectability for the method is determined by the amount of chemical necessary to produce this area. In general, 0.1 ppm of each of the 4 compounds produced an area on the recorder strip chart of 1 in.^{2} or better, indicating the level of detectability to be approximately 0.01 ppm. Due to changes in sensitivity from day to day and from one instrument to another, this limit will vary. Also, the size of any control peak will vary with the procedure, the crop, and the instrument sensitivity. Electron-capture response for the metabolites is less than for Sencor. This difference in response is

compensated for by dissolving final metabolite residues in smaller volumes. Somewhat better sensitivity may be established if peak measurements are made by peak height or electronic integration and the limit of detection is defined as twice the noise level of the baseline.

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Gas–Liquid Chromatographic/Mass Spectrometric Confirmatory Assay for Thiabendazole and 5-Hydroxythiabendazole

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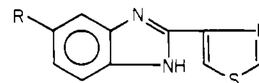
A combined gas–liquid chromatographic/mass spectrometric confirmatory assay for thiabendazole and 5-hydroxythiabendazole at 0.1 ppm in animal tissue isolates has been developed. On-column methylation converts these compounds to their *N*-methyl and *N,O*-dimethyl derivatives, respectively. Identification and quantitation are achieved by selective ion monitoring of the M − 1, M, and M + 1 ions from *N*-methylthiabendazole and the M and M − 15 ions from *N,O*-dimethyl-5-hydroxythiabendazole.

The availability of analytical methods for the determination of possible residues in edible tissues of food animals is a necessity with respect to the use of animal health drugs. In addition, a method ("confirmatory assay") to validate positive findings in the primary routine assay for drug or metabolite is an FDA requirement. A confirmatory assay should be based upon characteristic structural features of the compounds(s) of interest, and it is for this reason that combined GLC–mass spectrometric (MS) techniques are well suited for validation of assay procedures based on other less specific methods.

Obtaining the appropriate mass spectrum at the retention time of the compound of interest is convincing evidence for the presence of that compound in the injected sample. However, a mass spectrum resulting from a single scan is of little value for quantitative purposes. The use of a mass spectrometer as a selective GLC detector is well established (Brooks and Middleditch, 1971; Jenden and Cho, 1973; Watson, 1973). Thus, Cala et al. (1972), Palmer and Kolmodin-Hedman (1972), and Mirocha et al. (1973) have employed GLC–MS techniques to validate other assays for pyrimethamine in chicken tissue, *p,p'*-DDE in human plasma, and diethylstilbestrol in swine feedstuff, respectively.

Thiabendazole, 2-(4-thiazolyl)benzimidazole (TBZ), is a widely used anthelmintic (Brown et al., 1961) and antifungal (Robinson et al., 1964) agent. A fluorescence method is currently employed for the assay of TBZ in animal tissues (U.S. Department of Health, Education and Welfare, 1973). As this drug is polar and does not exhibit satisfactory GLC behavior at the submicrogram level, Jacob et al. (1975) converted it to the trimethylsilyl derivative for GLC–MS analysis in their study on the photolysis of TBZ. More recently Tanaka and Fujimoto (1976) employed methylation [dimethylformamide dimethyl acetal (Thenot et al., 1972; Thenot and Horning, 1972)] to achieve the GLC determination of TBZ in fruits.



R = H (TBZ)
R = HO (HTBZ)

A confirmatory assay for TBZ should be capable of adequate detection at ≤0.1 ppm (based on wet tissue sample), and at such a level derivatization of polar drug-related compounds is desirable so as to obtain reliable GLC results. We have chosen on-column methylation using trimethylanilinium hydroxide in methanol (Brochmann-Hanssen and Oke, 1969), a reagent shown to be useful in the GLC analysis of a number of drugs (Hammer et al., 1971; VandenHeuvel et al., 1975). This approach not only converts TBZ to its *N*-methyl derivative, but also yields the *N,O*-dimethyl derivative of 5-hydroxy-2-(4-thiazolyl)benzimidazole (HTBZ), a known metabolite of TBZ (Tocco et al., 1964).

Mass fragmentography (Hammar et al., 1968) or MF, a selective ion monitoring technique, in combination with

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