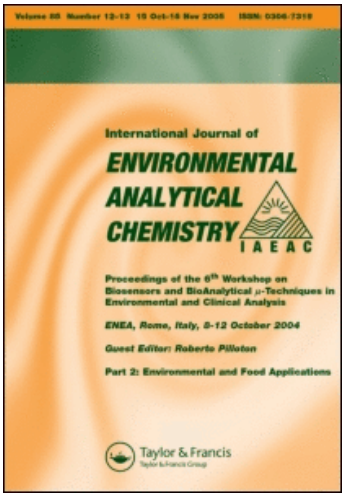

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Determination of Residues of Mesurol and its Toxic Metabolites in Plant and Animal Tissues

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KEY WORDS: Mesurol, carbamate, residue, analysis, gas chromatography.

A specific gas chromatographic procedure is described for the determination of residues of Mesurol [4-(methylthio)-3,5-xylyl methylcarbamate] and its sulfoxide and sulfone metabolites in plant and animal tissues. After initial extraction and precipitation cleanup steps, the extract is oxidized with potassium permanganate to convert Mesurol and its sulfoxide to the sulfone. Final detection of the sulfone is by the sulfur-specific flame photometric detector, thereby allowing little interference from tissue co-extractives. Recovery data from experiments run on a large variety of crops and tissues by adding known amounts of Mesurol and metabolites at the blending step were generally in the 70 to 120% range. The method is sensitive to the 0.03-ppm level.

Mesurol, 4-(methylthio)-3,5-xylyl methylcarbamate, also known as BAY 37344, is a carbamate insecticide being developed for use on a wide variety of field, vegetable and fruit crops. Extensive field testing has been done with apples, pears, cherries and corn. Mesurol also provides excellent control of garden slugs and snails. In addition, it has been used successfully as a seed coating on sugar beet seeds to control pigmy mangold beetle and on seed corn to repel pheasants.

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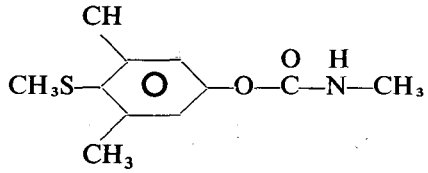
The structural formula for the parent compound is:



Metabolism studies with plants [1,2] showed that Mesurol is primarily converted to the sulfoxide, and, to a lesser degree, to the sulfone. Insect metabolism studies[3] and experiments with rat liver microsomes[4] also indicate that Mesurol is converted to the sulfoxide and sulfone. Rats, orally treated with Mesurol, also metabolized the compound in an oxidative manner with sulfoxide and sulfone compounds being formed.[5] Thus, the primary objective in developing a suitable residue method was to account for the parent compound and its oxidative metabolites with adequate sensitivity.

Various investigators have separated Mesurol from other carbamate insecticides by TLC.[6–8] El-Dib described a TLC method for detecting Mesurol in water samples.[9] A bioassay procedure for detecting residues of Mesurol in fruit and vegetables has also been reported.[10]

Other workers have reported gas chromatographic detection of residue amounts of Mesurol.[11,12] Van Middelem *et al.* prepared the brominated derivative of Mesurol for electron capture detection in plants at the 2-ppm level[13] and later Bache and Lisk used the microwave emission detector to record the sulfur response of hydrolyzed Mesurol phenol in fortified extracts.[14] A procedure for separate characterization of residues of Mesurol, Mesurol sulfoxide, Mesurol sulfone and their respective phenols in apples, pears and corn[15] is the most specific procedure reported to date but is somewhat lengthy for a monitoring procedure. Separate analysis for several compounds in the same sample solution also has the disadvantage of increased possibility of interference from crop extractives or other pesticides.

Earlier a procedure was reported which involved oxidation to convert Mesurol and its sulfoxide to Mesurol sulfone for ultimate measurement by infrared spectroscopy.[16] With the advent of the sulfur-specific flame photometric detector for gas chromatography, this earlier procedure has now been modified. Advantage is still taken of the permanganate oxidation to convert any residues to the common sulfone derivative, thus simplifying the analysis while still accounting for Mesurol and all toxic metabolites. Sensitivity is also enhanced because all compounds are concentrated into a single peak. The sulfone is then suitably cleaned up and measured gas chromatographically, using flame photometric detection in the sulfur mode. Initial extractions are included for crops and also animal tissues and milk, since portions of plants other than the fruit are often used as animal feed.

ANALYTICAL METHOD

Apparatus

A Hewlett–Packard Model 5750 gas chromatograph equipped with a Melpar flame photometric detector was operated in the sulfur mode (394-nm filter).

Explosion-proof blender motors were used to minimize the fire hazard from volatile organic solvents.

A Büchler Instruments Rotary Tube Evapo-Mix was used to concentrate extracts in centrifuge tubes.

Reagents

Florisil (PR grade, 60–100 mesh) was heated in an oven at 130°C for 24 hr to remove moisture. It was then deactivated by adding 2.5% water (2.5 ml H_2O + 97.5 g dried Florisil) and allowed to equilibrate for 24 hr in a tightly stoppered bottle before use.

Benzene:acetonitrile mixture (95:5, v/v) was used to elute the Florisil column.

The precipitating solution was prepared from 1.25 g NH_4Cl added to 25 ml H_3PO_4 (85%) and diluted to 1 l with distilled water.

Regisil [bis(trimethylsilyl)trifluoroacetamide] was obtained from the Regis Chemical Company, Chicago, Illinois.

Sample preparation

Grind wet crops and animal tissues in a Hobart food chopper in the presence of dry ice and place the samples in frozen storage overnight to allow the dry ice to sublime. Grind dry samples in a Wiley mill to pass a 3-mm screen.

Sample Extraction

Extraction of plant tissues Weigh a 100-g portion of the sample into a blender jar marked at the 500-ml level. (Smaller portions of dry, bulky crops may be used.) Add 350 ml acetone and blend for 2 min. Dilute to the 500-ml mark with 0.05N HCl and blend for one additional minute. Filter through 32-cm Whatman No. 2V fluted filter paper and collect 250 ml of filtrate in a graduated cylinder. In case of slow filtration with some crops, add 5–10 g Hyflo Super-Cel to the fluted filter paper before filtration. Cover the filter funnel with a clean folded cloth towel to minimize evaporation during filtration. Transfer the filtrate to a 500-ml separatory funnel and extract successively with 250-, 100- and 100-ml portions of chloroform. Combine the chloroform extracts in a 1000-ml round-bottomed flask and evaporate just to dryness on a rotary vacuum evaporator at 40°C. Proceed to "Precipitation."

Extraction of animal tissues Weigh a 50-g portion of finely chopped animal

tissue into a 1-quart blender jar. Add 200 ml acetonitrile and blend at high speed for 1 min, then add 50 g powdered anhydrous sodium sulfate and blend for two additional minutes. Filter with vacuum through Whatman No. 541 filter paper covered with a 1/4-in. layer of Hyflo Super-Cel in a Size 3 Büchner funnel. Reblend the filter cake with 200 ml hexane for 2 min and filter as before, omitting the pad of Hyflo Super-Cel. (For animal fat, reverse the order of blending with acetonitrile and hexane, i.e., blend first with hexane.) Rinse the blender jar with 100 ml fresh hexane and use this to wash the filter cake. Transfer the combined filtrate to a 500-ml separatory funnel. Shake the separatory funnel for 30 sec. Allow the phases to separate and drain the lower phase into a second 500-ml separatory funnel containing 250 ml fresh hexane. Shake the second separatory funnel, allow the phases to separate and drain the lower phase into a 500-ml round-bottomed flask. Repeat the two-stage extraction using 50-ml fresh acetonitrile and combine with the first acetonitrile extract. Evaporate the combined acetonitrile extracts just to dryness using a rotary vacuum evaporator at 40°C. Add about 10 ml benzene and evaporate the sample again to dryness to eliminate any traces of acetonitrile which interferes with oxidation. Proceed to "Oxidation."

Extraction of milk Mix the milk sample thoroughly to disperse the cream. Weigh a 200-g portion into a 1-quart blender jar. Add 400 ml acetone and 10 g Hyflo Super-Cel and blend for 3 min. Filter with vacuum through Whatman No. 541 filter paper covered with a 1/4-in. layer of Hyflo Super-Cel in a Size 3 Büchner funnel. Rinse the blender jar with 50 ml fresh acetone and use this to wash the filter cake. Transfer the filtrate to a 1000-ml separatory funnel. Rinse the filter flask with 250 ml chloroform and add to the separatory funnel containing the acetone filtrate. Shake the separatory funnel for 30 sec. Allow the phases to separate and drain the lower phase through 32-cm Whatman No. 2V fluted filter paper into a 1000-ml round-bottomed flask. Repeat the extraction using 100 ml fresh chloroform. Evaporate the combined extracts just to dryness using a rotary vacuum evaporator at 40°C. Dissolve the residue from the previous steps in 50 ml hexane and transfer to a 125-ml separatory funnel. Rinse the flask with 25 ml acetonitrile and add to the separatory funnel. Shake the separatory funnel for 30 sec, allow the phases to separate and drain the lower phase into a second 125-ml separatory funnel containing 25 ml fresh hexane. Shake the second separatory funnel for 30 sec, allow the phases to separate and drain the lower phase into a 100-ml round-bottomed flask. Repeat the two-stage extraction with 20 ml fresh acetonitrile and combine with the first acetonitrile extract. Evaporate the combined acetonitrile extracts just to dryness on a rotary vacuum evaporator at 40°C. Add 5 ml benzene and evaporate again just to dryness to eliminate last traces of acetonitrile. Proceed to "Oxidation."

Precipitation (crop samples only)

Dissolve the residue from the extraction steps in 40 ml acetone. Add 50 ml precipitating reagent. Mix and let stand for 30 min with occasional swirling. Filter with vacuum through Whatman No. 541 filter paper covered with a 1/8-in. layer of Hyflo Super-Cel in a Size O Büchner funnel. Rinse the sides of the round-bottomed flask with 30 ml acetone. To this acetone solution, add 25 ml precipitating solution and use this mixture to wash the filter pad. Repeat the washing step with an additional 20 ml precipitating reagent. Transfer the filtrate to a 250-ml separatory funnel. Rinse the filter flask with 50 ml chloroform and add to the separatory funnel. Shake the separatory funnel for 30 sec, allow the phases to separate and drain the lower phase into a 300-ml round-bottomed flask. Repeat the extraction with two additional 50-ml portions of fresh chloroform. Evaporate the combined chloroform extracts just to dryness on a rotary vacuum evaporator at 40°C.

Oxidation

Place 5-mcg and 20-mcg amounts of Mesurol standard into separate 100-ml round-bottomed flasks in 2–4 ml acetone and carry through the oxidation procedure for use in preparing the standard curve.

Dissolve the sample residue from the previous steps in 2 ml acetone. To samples and standards add 5 ml of 20% $MgSO_4$ and 30 ml of 0.1M $KMnO_4$ solution, washing down the sides of the flask during the additions. Mix and let stand for 10–15 min at room temperature with occasional swirling. (Do not let stand longer than 15 min.) Transfer to a 125-ml separatory funnel using 25 ml chloroform to complete the transfer. Shake the separatory funnel for 30 sec. Centrifuge if necessary and drain the lower phase through a layer of anhydrous sodium sulfate retained in a funnel by a loose plug of glass wool. Collect the filtrate in a 100-ml round-bottomed flask. Repeat the extraction twice more with fresh 25-ml portions of chloroform. Rinse the sodium sulfate with 5–10 ml of chloroform. Evaporate the combined chloroform extracts just to dryness on a rotary vacuum evaporator at 40°C.

Florisil column (necessary for animal tissues and milk only).

Tamp a plug of glass wool into the bottom of a 20 × 400 mm chromatographic tube. Cover the glass wool with about 1/2 in. of Superbrite glass beads. Fill the chromatographic tube up to the reservoir with benzene. Slowly sprinkle in 10 g Florisil (2.5% water-deactivated). Allow the Florisil to settle and top the column with about 1 in. of granular sodium sulfate. Drain the level of the solvent down to the top of the sodium sulfate. Dissolve the residue from the oxidation steps in 5 ml benzene and transfer to the column. Rinse the flask with 2 additional 5-ml portions of benzene and add

to the column. Pass the benzene through the column at a rate of 2–3 drops per second. Rinse the walls of the column with an additional 10 ml benzene and drain the benzene down to the top of the sodium sulfate. Discard the benzene. Elute the column with 225 ml benzene-acetonitrile (95:5) at the rate of 2–3 drops per second. (For milk only, discard the first 70 ml of eluate.) Evaporate the column eluate just to dryness on a rotary vacuum evaporator at 40°C.

Silylation

Quantitatively transfer the residue from "Oxidation" (or the "Florisil Column" in the case of animal tissues) to a 13-ml graduated centrifuge tube, using 4–6 ml acetone to complete the transfer. Evaporate the acetone on a rotary tube Evapo-mix to about 1.5 ml. (Evaporate milk samples to just less than 1 ml.) Add 100 mcl of Regisil to the tubes containing the extract (use 50 mcl for milk samples). Dilute milk samples to 1.0 ml and all other samples to 2.0 ml final volume. Mix thoroughly, stopper tightly and let stand overnight.

Gas chromatographic analysis

Inject 8 mcl of derivatized extract (use 10 mcl in the case of milk samples) into the gas chromatograph maintained at the following conditions: Column—2 ft × 4 mm i.d. glass column packed with 5% D.C. 200 solution coated[17] on 80–100 mesh Gas Chrom Q; gas flows—helium carrier gas, 80 ml/min; set other gases to give maximum response and minimum noise as per manufacturer's recommendation; temperatures—column, 170°C, injection port, 180°C, detector, 200°C.

Identify the silylated Mesurol sulfone peak by its retention time and measure the response produced on the recorder strip chart. At the operating conditions employed, the retention time for the derivative is approx. 4.0 min.

Preparation of standard curve Inject varying amounts of the oxidized and silylated Mesurol standards into the gas chromatograph to obtain response values from 5 to 100 ng. Multiply the area obtained for each standard by the electrometer attenuation. Determine the ppm equivalence of each standard in relation to the samples to be calculated by use of the following equation:

$$\text{ppm of standard} = \frac{\text{ng of standard injected}}{\text{sample wt (g)}} \times \frac{\text{final sample vol. (ml)}}{\text{mcl sample injected}}$$

Using log-log paper, plot a standard curve for these values as shown in Figure 1. Values for samples analyzed can then be read from the standard curve directly in ppm. Any samples containing residues high enough that the amount injected exceeds 100 ng should be diluted so that response falls on

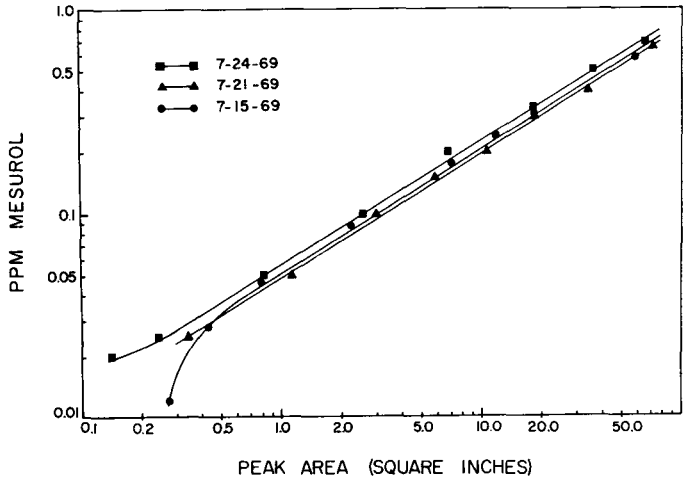


FIGURE 1 Flame photometric standard curves (log-log plot) showing linearity and reproducibility on three separate days.

the plotted curve. For such samples, the ppm value obtained from the curve is multiplied by the dilution factor to obtain ppm in the sample. Crop sample extracts are actually split in half during the initial extraction, thus ppm values must be multiplied by 2. A new standard curve should be prepared for each group of samples on a given day.

DISCUSSION

The method described in this report measures not only Mesurol residues, but also its metabolites, the sulfoxide and sulfone. Although all three of these compounds show response at a characteristic retention time when injected into the gas chromatograph, considerations of simplicity and sensitivity make it more desirable to oxidize the parent compound and the sulfoxide to the sulfone with subsequent gas chromatographic analysis of only one peak. Room temperature oxidation to the sulfone is quantitative using 0.1M potassium permanganate for 15 min.[18] These oxidation conditions do not affect the carbamate portion of the molecule. Oxidation also converts most tissue extractives and pigments to a water-soluble form, making them easy to remove.

An alternate initial extraction procedure has also been used with good results for crops such as corn, which contain large amounts of oil. This consists of blending 100 g of sample with 200 ml acetonitrile with subsequent vacuum filtration. The filter cake is reblended with 200 ml fresh acetonitrile

and filtered as before. The combined acetonitrile phases are then shaken with 250 ml hexane to remove non-polar oils and the acetonitrile phase is evaporated and processed further for gas chromatographic analysis starting with the precipitation step. Thus, by removing the lipids at an earlier step in the procedure, fewer difficulties from the co-extractives are experienced at the precipitation and oxidation steps.

In general, initial extractions were patterned after those procedures used in metabolism experiments to remove maximum amounts of radiolabeled Mesurol or metabolites from crops or tissues. Exhaustive extraction experiments on field-weathered samples were not done.

A Florisil column cleanup step is included for animal tissues and milk where additional cleanup is necessary after oxidation, prior to gas chromatographic analysis. If necessary, other samples may be carried through the column steps for further cleanup. In some cases the extract may still appear brown-colored even after Florisil column chromatography. This does not appear to cause any difficulty with the flame photometric detector.

Silylation of the sample residue after oxidation converts the Mesurol sulfone to a compound which is more easily chromatographed and yields better sensitivity in the sulfur detector. It appears that the very reactive Regisil is removing the carbamate group and substituting a trimethylsilyl group in its place. This reaction is not instantaneous and takes varying amounts of time depending on which type of tissue extracts are involved. Thus, the reaction is allowed to proceed overnight to ensure complete conversion.

Gas chromatographic response of the flame photometric detector in the sulfur mode is not linear.[19] Instead, response corresponds approximately to the square of the concentration. Quantitation under these circumstances is almost impossible using a single concentration standard. However, response is reasonably stable for a given amount of standard injected. Therefore, a log-log plot of standard response of the silylated Mesurol sulfone from 5 to 100 ng is used to determine residues in treated and fortified samples. As can be seen in Figure 1, the response varies only slightly from day to day and the slope remains constant.

Recovery experiments were run on a number of different crops and various animal tissues by fortifying the samples with 0.005 to 5 ppm Mesurol or its sulfoxide or sulfone prior to blending. Representative recovery values for all three compounds are recorded in Table I. Control values in each case were generally less than the detectable limits of the procedure. Representative control and recovery chromatograms are shown as Figure 2 for recovery of 0.1 ppm of Mesurol from apple pulp. Chromatograms for other types of samples appeared similar.

Linear response (using the log-log plot) can generally be obtained down

TABLE I

Recovery of Mesurol and metabolites from various plants and animal tissues

Sample	ppm added[a]	Recovery(%)[b]		
		Mesurol	Sulfoxide	Sulfone
Apple peel	0.5	88	90	120
	0.1	79	80	96
	0.05	118	86	68
Apple pulp	0.1	117	108	96
	0.05	78, 120	104, 98	82, 120
Pear peel	0.5	100, 92	91, 95	112, 110
Pear pulp	0.1	86	96	115
	0.05	126, 80	137, 82	135, 87
Corn kernel	0.1	120	70	70
	0.05[c]	84, 88, 92	72, 91, 83	71, 73, 72
Corn forage	0.1	80	80	80
	0.05	100	—	—
Sugar beet tops	0.5	102, 99, 104	77, 75, 87	73, 65, 69
	0.05	92, 109, 85	87, 93, 86	97, 103, 91
Sugar beet roots	0.5	86, 92, 95	93, 107, 97	81, 82, 87
	0.05	100, 95, 115	95, 91, 86	117, 111, 107
Fodder beet tops	0.05	81, 92, 84	83, 83, 80	96, 86, 97
Fodder beet roots	0.05	103, 95, 95	111, 103, 96	100, 108, 93
Bovine brain	0.05	114	112	86
Bovine fat	0.05	86	84	118
Bovine heart	0.05	98	110	86
Bovine kidney	0.05	100	100	104
Bovine liver	0.05	86	106	88
Bovine steak	0.05	102	88	112
Bovine milk	0.005	112	104	100

[a] Mesurol, Mesurol sulfoxide, Mesurol sulfone added to separate samples at the level indicated.
[b] Recovery percentages are from separate determinations.
[c] Alternate initial extraction used (see Discussion).

to the 0.03-ppm level. This represents a peak area of approx. 0.1 in.^{2}, which is near the limit of sensitivity of accurate measurement with a polar planimeter. The reliable limit of sensitivity for the method is, therefore, stated to be approximately 0.03 ppm (for the equivalent of 50 g of sample in the final extract) with absolute limit of detectability approaching 0.01 ppm. Sensitivity for milk is a factor of 10 better because of larger initial sample size and smaller final volume.

In the course of developing the method, considerable difficulty was encountered in the oxidation step from diacetone alcohol present in the acetone. Diacetone alcohol was formed by condensation reaction during redistillation of the acetone for purification. The presence of this agent

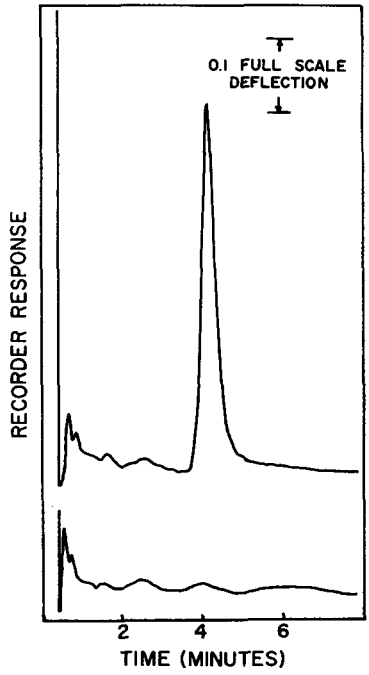


FIGURE 2 Gas chromatograms of apple pulp control (lower curve) and apple pulp fortified with 0.1 ppm of Mesurol (upper curve).

caused the permanganate to be rapidly converted to MnO_2 in large amounts which caused incomplete oxidation and also hampered the chloroform extraction after oxidation. Care should be taken to use acetone which is free from diacetone alcohol.

At early stages in the development of the method, poorly shaped peaks for the Mesurol derivative were sometimes observed. This problem was usually eliminated by changing the glass wool at the column inlet to prevent buildup of oils from repeated injections. Another cause of poorly shaped peaks and low sensitivity was partial hydrolysis of the silyl derivative on standing. In this case, an additional 10 mcl of Regisil added to the sample eliminated the difficulty.

The solution-coating technique used to prepare the gas chromatographic column packing was helpful in producing a column which did not exhibit tailing or adsorption of the Mesurol derivative. In addition, the column was 'no flow' conditioned at 250°C for 2 hr, followed by flow conditioning at operating conditions at least 4 hr before use. Several columns prepared in this manner have given identical results, indicating that the procedure is reproducible.

Mesurol slowly decomposes in solution, so it is necessary to prepare fresh standards every five days.

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