Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Esfenvalerate

MRID #: 415030-01

Matrix: Soil/Water

Analysis: GC/ECD

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MRID 41520301

Du Pont Report No. AMR-1555-89

Du Pont Study No. AMR-750-87

MODESTO METHOD SERIES
UNSHARED

MMS-R-581-1
February, 1986

SHELL DEVELOPMENT COMPANY
BIOLOGICAL SCIENCES RESEARCH CENTER
MODESTO, CALIFORNIA

(ESFENVALERATE)

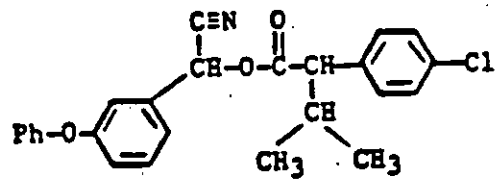
DETERMINATION OF ASANA™ INSECTICIDE RESIDUES IN CROPS, ANIMAL TISSUES, SOIL AND WATER

Electron-Capture Gas Chromatographic Method

Hazardous materials used in this method are designated "(*CAUTION*)" in the REAGENTS Section. A Material Safety Data Sheet for each material so designated may be found in the PRECAUTIONARY INFORMATION Section at the end of the method.

SCOPE

1. A GLC method, using the electron-capture (EC) detector, is described for determining residues of the pyrethroid insecticide MO 70616 (ASANA™ Insecticide) in crops, animal tissues, soil and water. The minimum detectable concentration (MDC) is about 0.01 milligram per kilogram (0.01 ppm) for crops, animal tissues and soil, and about 0.05 micrograms per kilogram (0.05 ppb) for water.

ANALYTE *	CHEMICAL NAME	STRUCTURE
ASANA™ Insecticide (MO 70616)	Benzeneacetic acid, 4-chloro--alpha-(1-methylethyl)-,cyano (3-phenoxyphenyl)methyl ester	

METHOD SUMMARY

2. Crops and lean animal tissues are extracted with hexane/isopropanol (3:1) and the isopropanol removed by water partitioning. The hexane extracts of animal tissues and oily crops are partitioned with acetonitrile to separate lipids from the MO 70616. The acetonitrile is exchanged back to hexane and cleaned up by liquid-solid chromatography through a BOND ELUT Si clean-up column. Hexane extracts of non-oily crops are cleaned up directly without acetonitrile partitioning.

Soil samples are extracted by high frequency vibration in the presence of acetone/hexane (1:1), the solvent is exchanged to hexane, and an aliquot subjected to liquid-solid chromatography using a BOND ELUT Si clean-up column (if necessary).

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* ASANA is made up of four enantiomers which elute as two discrete peaks under the GLC conditions referenced in this method.

Water samples are extracted by partitioning with hexane. Clean-up is achieved by liquid-solid chromatography using BOND ELUT Si disposable clean-up columns (if necessary).

MO 70616 is quantitated by GLC-EC.

SPECIAL APPARATUS

3. (a) Steam table. A flat-topped steam bath with several holes equipped with concentric rings.

(b) Evaporation manifold designed to direct streams of clean, dry air or nitrogen downward into vessels.

(c) Waring blender, explosion resistant drive unit with stainless steel jars. Available from Thomas Scientific, Swedesboro, NJ 08085.

(d) Ultrasonic probe, Braun-Sonic 2000. Available from Sargent-Welch, Skokie, IL 60077.

(e) Lab mill, Wiley Model 4, with assorted sieves. Available from Thomas Scientific.

(f) Hobart food chopper, Model 8142 or equivalent.

(g) Gas liquid chromatograph equipped with an inlet splitter designed for capillary column and electron-capture detector.

(h) Fused silica capillary column, 30M x 0.32mm (ID), coated with a cross-linked, non-polar stationary phase, such as DB-1, available from J & W Scientific, Inc., Rancho Cordova, CA 95670.

(i) Homogenizing mill for organic and aqueous media, equipped with a 30mm, stainless steel generator, Super Dispax or equivalent. Available from Sargent-Welch, Skokie, IL 60077.

(j) Multiport solvent valve equipped with a female Luer connector and male fitting designed for disposable clean-up columns such as the Baker MISER, available from J. T. Baker Chemical Company, Phillipsburg, NJ 08865.

REAGENTS

4. (a) Hexane, isopropanol, acetonitrile, acetone, ethyl acetate. Distilled-in-glass solvents from Burdick and Jackson Labs., Muskegon, MI. (*CAUTION*)

(b) Disposable solid phase columns, BOND ELUT 3cc,Si, P/N 601303. Available from Analytichem International, Harbor City, CA 90710.

(c) Analytical standard of ASANA™ Insecticide (MO 70616). Available from Shell Chemical Company, Product Safety and Compliance, HS&E, P. O. Box 4320, Houston, TX. (*CAUTION*)

SAMPLE REDUCTION

5. Homogenize bulk crops and green foliage in a Hobart food chopper prior to sampling or analysis. Grind hays and dried foliage in a Wiley lab mill and homogenize by tumbling in an oversized container. Grind difficult samples (such as leathery animal tissues) in the presence of dry ice or liquid nitrogen in a suitable homogenizer. Allow dry ice or liquid nitrogen to dissipate before sampling for analysis.

EXTRACTION

6. (a) Crops and Animal Tissues (except fat)

Weigh 10-50 grams of representative sample into Waring blender cups. Use 50 grams for watery samples and 10-25 grams for dry samples such as hays, seeds and dried nuts. Total sample volume for dried fibrous samples should never exceed about 150 cc for proper extraction. Most dried nuts (not chestnuts) contain 50-70% oil which contributes volume to the extract. A 25 g sample of dried nuts, will therefore contribute 13-18 mls to the initial extraction solvent. This should be considered when calculating the sample-to-solvent ratio for the sample extract.

Add 200 mls of hexane/isopropanol (75%/25% v/v) to the blender cup containing the sample. Cap blender cup with a tight fitting, vented lid and blend at high speed for one minute. Transfer the extract into a 250 ml separatory funnel through fast flow, fluted filter paper (~32 cm diameter) which was thoroughly washed with acetone. The transfer and filtering should be done as quickly as possible to avoid evaporation/concentration of the solvents. If filter paper plugs up quickly, causing slow filtration, centrifugation may be required. Vacuum filtration is not recommended due to the volatility of the solvents.

Add 100 ml of water to the separatory funnel, shake moderately for ~1 minute and allow phases to separate. Drain and discard lower (aqueous) phase. Wash hexane with two additional 100 ml volumes of water to ensure removal of all isopropanol. The crop-to-solvent ratio, after removal of isopropanol equals the weight (grams) of sample extracted, divided by 150 (mls of hexane). For dry nut meats, the volume of oil contributed by the sample should be considered in the calculations. If 25 grams of peanuts are extracted, the crop-to-solvent ratio would be 25 divided by 163 (150 + 13), or 1 g/6.5 ml. For watery crops and most green foliage proceed with "Clean-up By Liquid-Solid Chromatography" (Section 8). For all other samples proceed with "Clean-up By Liquid Partitioning" (Section 7).

NOTE: While significant amounts of sample extract may be retained by the sample matrix and/or lost in an aqueous emulsion which is discarded, analytical results are not affected since the crop-to-solvent ratio remains constant.

(b) Animal Fat

Weigh 10-20 grams of a representative sample into a Waring blender cup, add 200 ml of hexane, and 20 grams of sodium sulfate. Blend at high speed for 1 minute and decant into a 500 ml Erlenmeyer flask. Repeat the

extraction with an additional 200 ml of hexane and combine extracts in flask. Add three or four boiling chips to the flask and concentrate on a steam table to 50-75 ml. Transfer hexane to a 100 ml graduated cylinder and adjust final volume to 100 ml using hexane to rinse flask and boiling chips. Proceed with "Clean-up By Liquid Partitioning" (Section 7).

(c) Soil

Pass 500 grams or more of representative soil through a No. 8 U.S. Standard sieve. Prior to screening, (1) break up the larger agglomerates into smaller particles, (2) spread soils that are excessively wet onto a flat surface and allow to dry sufficiently enough at room temperature to enable passage through the screen, and (3) discard rocks, plant, and other extraneous material. After screening and blending, determine the soil's water content if necessary.

Weigh 50 grams of a representative sample into a 250 ml Nalgene centrifuge bottle. Add 200 ml of 1:1 acetone/hexane, immerse the probe tip of the Braun-Sonic 2000 about 0.50 to 0.75 inch beneath the surface of the solvent, and apply 300 watts or Peak Envelope Power (PEP) for two minutes. If an ultrasonic generator is unavailable, cap the bottles tightly, secure the bottles on their sides to a reciprocating shaker and shake vigorously for two hours. Balance pairs of the bottles using acetone or water and centrifuge at high speed for about five minutes.

Decant the available extract into 500 ml separatory funnels and add 200 ml of water to each. Cap and shake moderately for one minute, with venting shortly after initial shaking. Drain and discard the lower (aqueous) phase and repeat with a second 200 ml aliquot of water, shaking vigorously. Drain and discard any interfacial "cuff" that may have precipitated out during water washing. The sample-to-solvent ratio is now 0.5 g/ml. Drain the hexane extracts into labeled, 8-oz. bottles and cap with foil lined caps. Since most soil sample can be analyzed without any clean-up, proceed to "Sample Analysis" (Section 10). If interferences are encountered during GLC analysis proceed to "Clean-up By Liquid-Solid Chromatography" (Section 8).

(d) Water

MO 70616 is extremely hydrophobic and will quickly sorb onto particulates or containers from aqueous solutions. Small enough water samples should be taken such that the entire sample can be analyzed, since proper sub-sampling is difficult and results can be misleading. Eight or sixteen ounce glass sampling bottles with aluminum foil-lined caps are adequate. The following method assumes 8 oz. glass sample bottles containing 200-250 grams of water for analysis. Glassware, solvent volumes, and technique should be adjusted according to actual sample size, using this method as a guide.

Weigh sample bottle and sample prior to analysis. Decant entire sample (usually 200-250 ml) into a 500 ml separatory funnel leaving as much sediment as possible in the bottle. Add 20 ml of acetone to the bottle, cap with original aluminum foil-lined cap, and shake vigorously. Add 50 ml of hexane to the bottle, cap, and shake again. Transfer the contents of the

bottle to the separatory funnel containing the water sample and shake vigorously for at lease one minute. Reweigh the empty sample bottle to obtain sample weight. Drain and discard lower (aqueous) phase. Concentrate hexane extract to 1 or 2 ml. Proceed with "Clean-up By Liquid-Solid Chromatography" (Section 8).

CLEAN-UP BY LIQUID PARTITIONING

7. Transfer an aliquot of hexane extract, equivalent to 2 grams of sample (1 gram of animal fat or vegetable oil), to a 250 ml separatory funnel and add hexane to make a total volume of 50 ml. Add 50 ml of hexane-saturated acetonitrile and shake vigorously for ca 1 minute. Drain the acetonitrile into a 250 ml Erlenmeyer flask. Add a second aliquot of hexane-saturated acetonitrile to the hexane extract and shake vigorously. Combine the acetonitrile aliquots and add a few boiling chips. Concentrate the acetonitrile on a steam table using 100 ml aliquots of hexane until only hexane remains. Concentrate the hexane to 10-15 mls and transfer, with rinsing, to a 30-50 ml, tapered glass tube. Concentrate the hexane to 1-2 mls using gentle air streams and hot water baths. Proceed with "Clean-up By Liquid-Solid Chromatography" (Section 8).

CLEAN-UP BY LIQUID-SOLID CHROMATOGRAPHY

8. Simple and efficient clean-up for MO 70616 extracts is achieved through the use of small disposable clean-up columns packed with 40-micron Si particles, such as BOND ELUT Si columns. While the following method assumes use of the Baker MISER valve (see Section 3(j)) any system capable of delivering solvent under slight pressure to the column should be adequate.

Place a disposable Si column in a narrow glass tube so that the flange of the column rests on the lip of the tube. Transfer 1-2 mls of extract (equivalent to 1.0 gram for most samples) to the top of the column packing. Use a rubber, disposable pipette bulb to gently force the extract into the packing until 0.1-0.2 ml remains above the packing. Add about 1 ml of hexane and force through the packing until ~0.1 ml remains above the packing. Repeat with two additional 1-ml aliquots of hexane to ensure complete washing of the sample matrix onto the upper packing bed and that the analyte is focused at the very top of the packing. Since hexane does not move MO 70616 on Si, the amount of hexane used does not effect the elution pattern (profile) of the compound.

Fasten the column tightly to the special fitting of the MISER valve and draw ~15 ml of 2% ethyl acetate, 98% hexane into a 20 ml syringe fitted at the top of the valve. Position the valve selector to the disposable column and gently force 4.0 mls of solvent through the column. Discard this first 4 mls. Gently force an additional 8 mls through the column, collecting in a graduated, tapered 10 or 15 ml centrifuge tube. Dilute or concentrate according to anticipated analyte concentration in preparation for GLC analysis. Proceed with next section.

GLC ANALYSIS

9. GLC Operating Conditions. GLC columns and operating parameters are chosen which achieve optimum balance between sensitivity and degree of

resolution with symmetrical peaks emerging at reasonable retention times. Retention times are relative since variation will occur between system configurations and columns. Due to the number of variables involved, the operating conditions listed below should serve only as a guide.

Instrument	:	Varian GC, Model 3700
Detector	:	EC, ^{63}Ni
Column	:	30M x 0.32 mm ID, fused silica, open tubular, coated with DB-1
Inlet	:	Split type, inlet liner packed with ~1 cm 3% SE-30 as mixing chamber
Split Ratio	:	~10:1
Temperatures, °C		
Column	:	260
Inlet	:	280
Detector	:	340
Carrier Gas	:	He, 30 PSI at inlet
Detector Make-up Gas	:	N_2, ~30 ml/min.

Typical recorder response for 0.06 nanograms of MO 70616		1st Peak	2nd Peak
Percent of full scale	:	12	60
Noise level	:	<1%	<1%
Retention time, min.	:	3.9	4.2

SAMPLE ANALYSIS

10. (a) In addition to treated samples, each sample set should include an untreated check (control) and controls spiked with MO 70616 at anticipated residue levels. Since the anticipated residue level for a control sample would be less than the minimum detectable concentration (usually 0.01 ppm), a sample spiked at 2-5 times the MDC should be included (i.e. 0.02-0.05 ppm).

(b) Weigh 100.0 mg of MO 70616 into 100 ml volumetric flask, dilute to the mark with acetone, and mix thoroughly. This stock solution of 1.0 mg/ml can be used for all subsequent dilutions required for standards. Concentration levels of standards used for spiking vary considerably depending on sample size, expected residues, etc. Calculate concentration levels required for spiking and prepare standards using acetone. Prepare GLC

standards in the range of 0.005 to 0.025 µg/ml MO 70616 using hexane as diluent.

(c) Condition the GLC inlet and column by making a few 5-10 µl injections of the control extract. Inject 3.0 µl aliquots of the GLC standards in order to construct a calibration curve. If several sample extracts are to be run, make 3.0 µl injections of samples with intermittent (every 3-4 samples) injections of standards in order to monitor instrument response.

(d) Construct a calibration curve by plotting ng of standard injected versus peak height (or area). Since MO 70616 gives two GLC peaks, the average of the two peaks should be used for calculations. If, however, the peak ratios are identical for samples and standards, the highest (second) peak may be used alone, to simplify calculations. Calculate the MO 70616 content of the sample by means of the following equation:

$$C = \frac{W}{S}$$

Where:

C = concentration of the compound in milligrams/kilogram of sample (ppm)

W = weight of MO 70616 in nanograms found in the aliquot of sample injected

S = amount of the sample in milligrams represented by the aliquot injected

VALIDATION OF THE METHOD

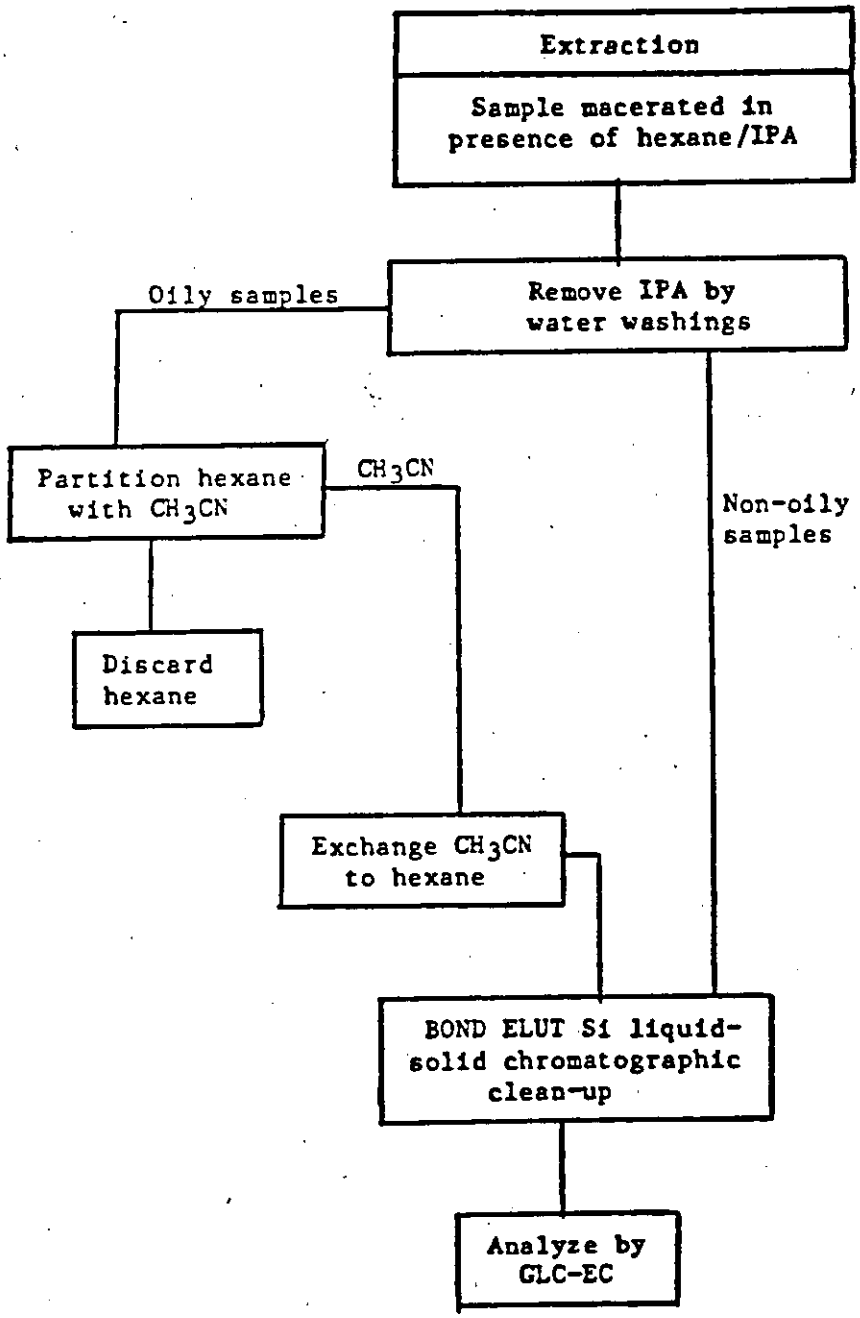
11. Precision and accuracy data are compiled from recovery results that have been determined in this laboratory during routine analysis.

Compound	Samples Analyzed	Fortification Range, ppm	No. of Observations	% Recovery Mean	% Recovery Std. Dev.
MO 70616	Various[1)]	0.05-1.0	71	102	14.2

1) Crops included: sugarcane, tobacco, soybean seeds and hay, sugar beet roots and tops, sunflower seeds, peaches, peanuts, cottonseed, barley grain and straw, cucumbers, grapes, onions, lima beans, lettuce, alfalfa cabbage, sorghum grain and fodder, lemons and pears. Water samples were spiked at about 0.1 ppb.

METHOD FLOWCHARTS

12. Residue Determination of MO 70616 in Crops and Animal Tissues



GFB/sia
02/18/86

PROJECT NO. : 43531101 DATA ID. : 1

* * * * * * * * * * * ANALYTICAL RESULTS * * * * * * * * * * *

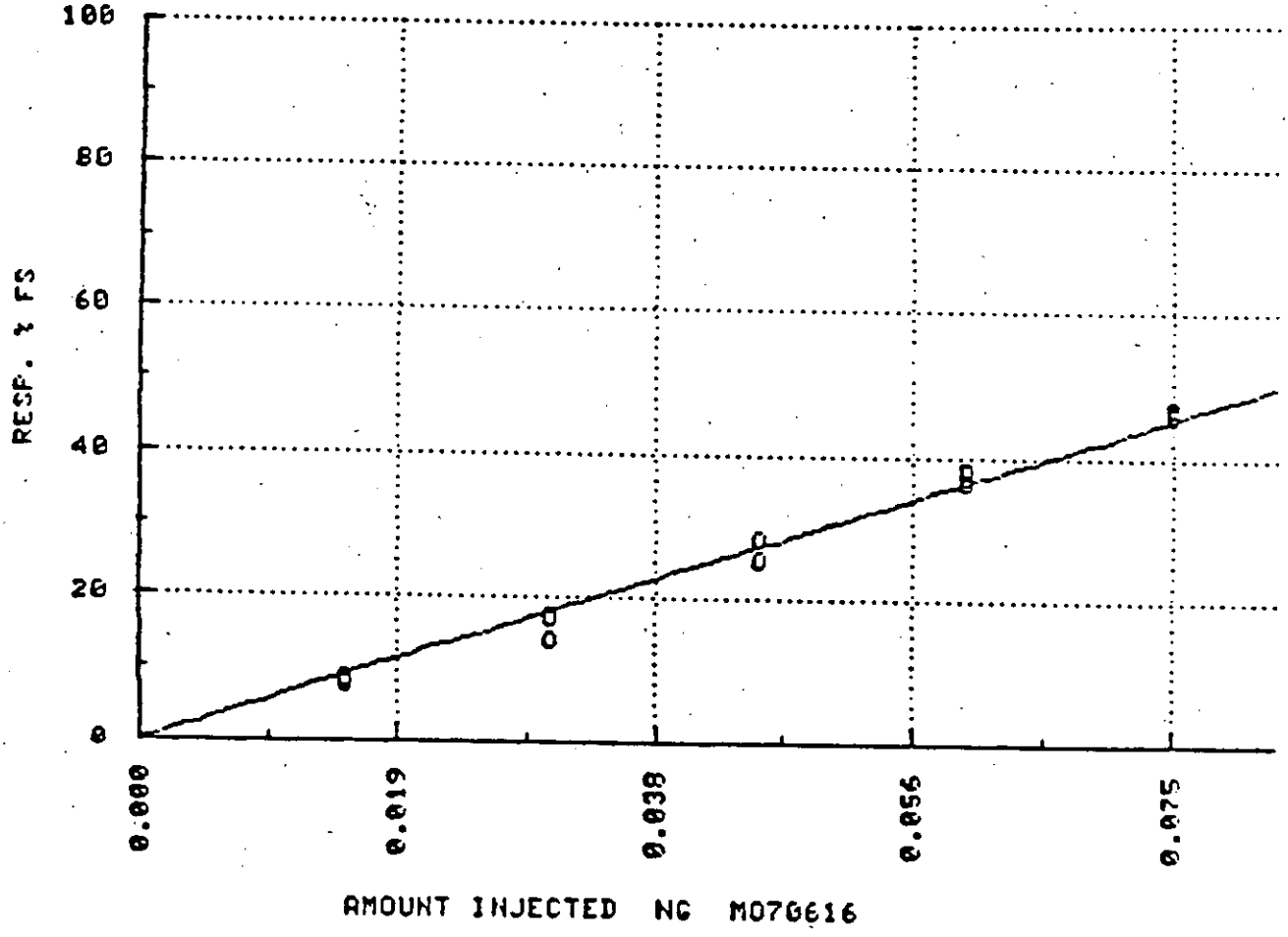
MINIMUM DETECTABLE CONC. (3.6 % FS)..... 0.010 PPM

AVERAGE RETENTION TIME FOR 11 STANDARDS.... 3.40 MIN

AVERAGE DEVIATION FROM THE CALIBRATION CURVE 8.1 %

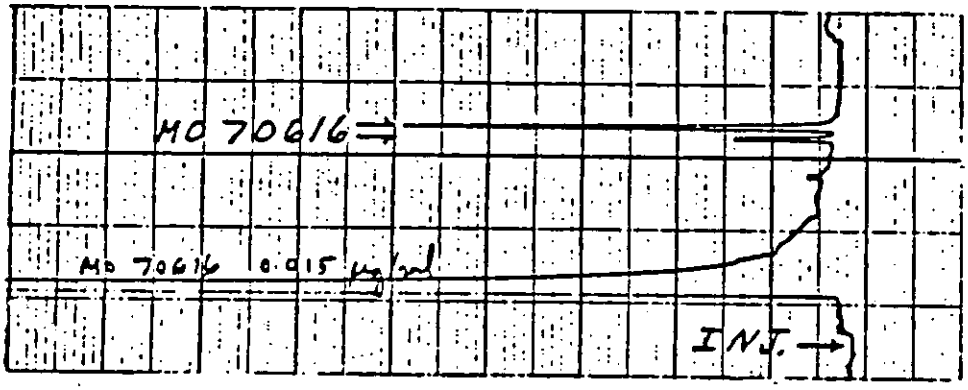
NG EQUIVALENT TO 1 % FULL SCALE..... 0.0017

* * * * * * * * * * * CALIBRATION CURVE * * * * * * * * * * *

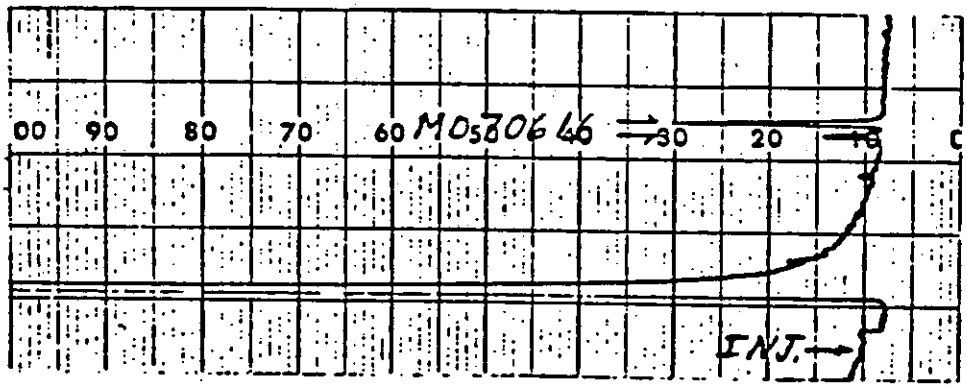


TYPICAL CALIBRATION CURVE FOR M070616 USING AVERAGED PEAK HEIGHTS FOR ISOMERS

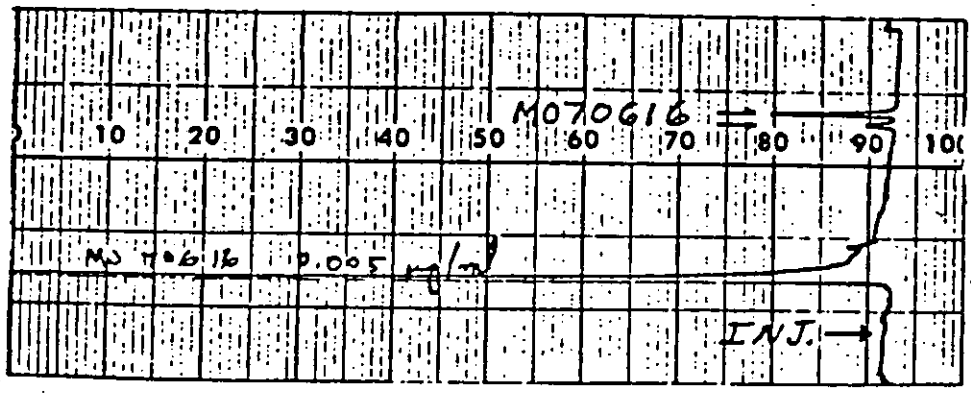
0.06 ng MO70616



0.045 ng MO70616

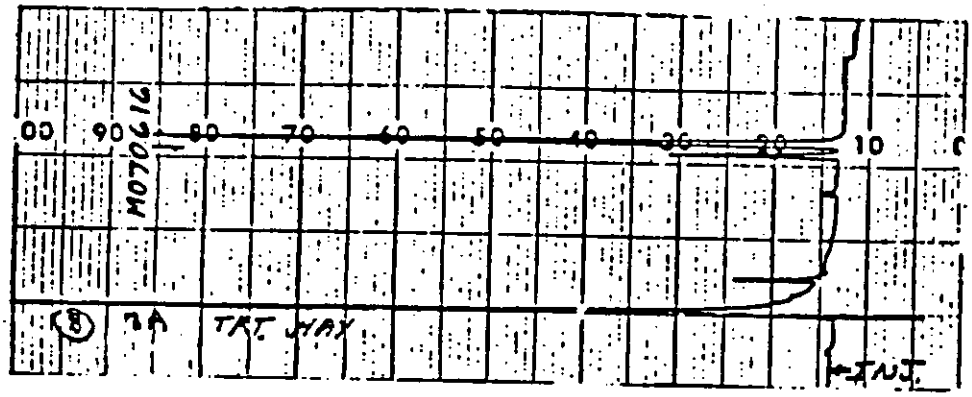


0.03 ng MO70616

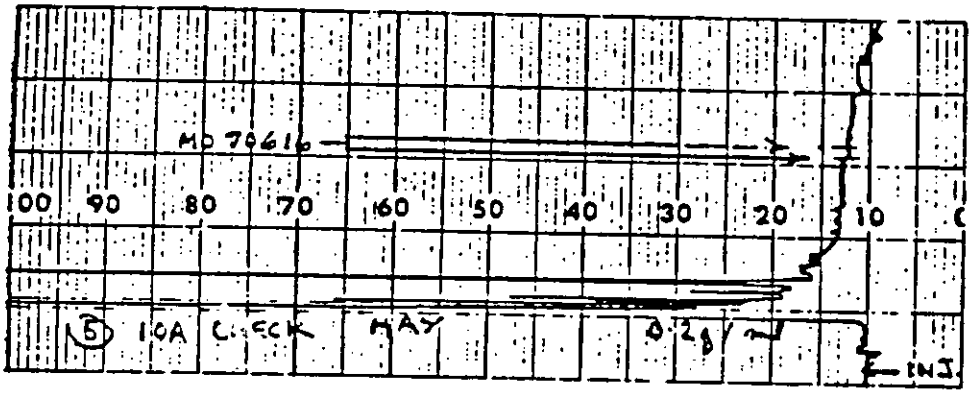


0.015 ng MO70616

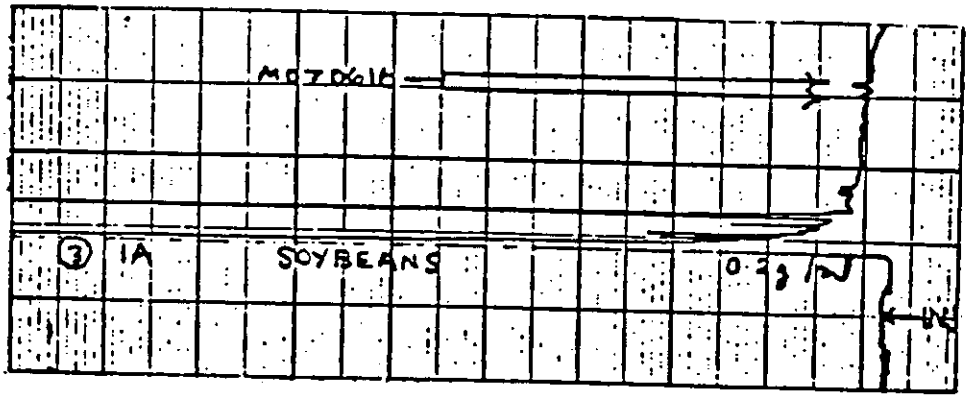
TYPICAL CHROMATOGRAMS OF MO70616 STANDARDS



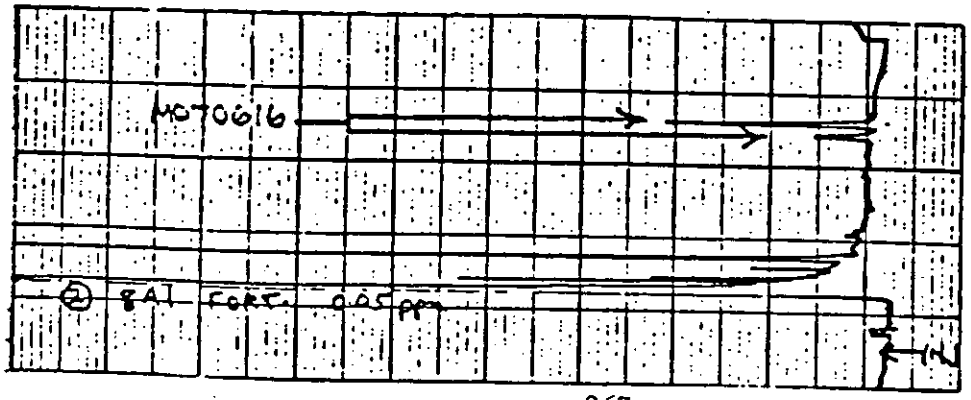
FIELD TREATED
SOYBEAN HAY; 3.1
PPM MO70616
0.024 mg EQUIVALENT
SAMPLE INJECTION



CHECK SOYBEAN
HAY
0.60 mg EQUIVALENT
SAMPLE INJECTION



FIELD TREATED
SOYBEANS; <0.01
PPM MO70616
0.60 mg EQUIVALENT
SAMPLE INJECTION



CHECK SOYBEANS
SPIKED AT 0.05 PPM
MO70616
0.60 mg EQUIVALENT
SAMPLE INJECTION

TYPICAL CHROMATOGRAMS FOR MO70616 IN SOYBEAN EXTRACTS

APPENDIX XV

Shell Report MMS-R-478-1, Determination of SD 43775 Residues in Crops, Animal Tissues, Soil, and Water (EPA MRID No. 0006364)