UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

ENVIRONMENTAL CHEMISTRY SECTION
BUILDING 1105—JOHN C. STENNIS SPACE CENTER
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JUN 18 1996

MEMORANDUM

SUBJECT: ECM Evaluation Report for Hexazinone in Soil
(ECM 0080S1)

FROM: Aubry E. Dupuy, Jr., Section Chief
BEAD/ACB/Environmental Chemistry Section

THRU: Donald A. Marlow, Branch Chief
BEAD/Analytical Chemistry Branch

TO: Henry M. Jacoby, Branch Chief (7507C)
EFED/Environmental Fate and Groundwater Branch

The EFED has requested environmental chemistry method evaluations (ECMEs) for a number of compounds on the various FIFRA priority lists. The enclosed ECM report, "Method Evaluation for the Determination of Hexazinone in Soil (ECM 0080S1)", is submitted in response to a request by EFGWB for this evaluation.

The Registrant's method was evaluated by our contractor. An early review of the method showed the five metabolites to be very poorly recovered with RSDs well above 30 percent. We informed Dr. Leovey of these poor and highly variable data, and she agreed to our performing the method evaluation on the parent hexazinone only. Initial poor results and ambiguity in the method required a second laboratory evaluation. With the exception of low recoveries at the LOQ, the performance data at both the LOQ and 10 x LOQ are acceptable and were generated without major difficulty.

If you have questions, please call me at (601) 688-3212 or Bob Maxey at (601) 688-1225.

Attachment

cc: Bob Maxey/ECS

MEMORANDUM

SUBJECT: Method Validation for Hexazinone in a Soil Matrix.

FROM: Gail Maske, Chemist
Chemical Review Section 1
Environmental Fate and Ground Water Branch
Environmental Fate and Effects Division (H7507C)

THRU: Henry Jacoby, Branch Chief
Environmental Fate and Ground Water Branch
Environmental Fate and Effects Division (H7507C)

Paul Mastradone, Section Chief
Chemistry Review Section 1
Environmental Fate and Ground Water Branch
Environmental Fate and Effects Division (H7507C)

TO: Donald R. Marlow, Branch Chief
Analytical Chemistry Branch
BEAD (7503W)

EFGWB is requesting validation of the analytical method for hexazinone in soil. The following study was submitted to support registration of hexazinone for use on fruit trees, pine forest, fir forest, grasses (e.g. pastures), spruces, transportation rights-of-way, and utility rights-of-way (some indoor uses, as well).

> Roby, M. METHOD EVALUATION FOR THE DETERMINATION OF HEXAZINONE IN SOIL (ECM 0080S1). Submitted and Prepared by Science Application International Corporation under Work Assignment 4-18 and Contract No. 68-D2-0183; Study completed on 12 June 1996; Received by EPA on 20 June 1996; MRID No. 42379201.
>
> The final reported data indicated that the GC/MS method for detection of parent hexazinone (metabolites not to be evaluated/phone conversation with E. Leovey-meno 18 June 1996 from Aubry E. Dupuy, Jr.) in 1:1 acetone:0.1M KH_2PO_4 buffer (v/v) soil extracts has a MDL of 0.01 $\mu g/g$ and LOQ of 0.03 $\mu g/g$.

METHOD EVALUATION FOR THE DETERMINATION OF HEXAZINONE IN SOIL (ECM 0080S1)

FINAL REPORT

Prepared By:
Science Applications International Corporation

Submitted To:
U.S. EPA Office of Pesticide Programs
Stennis Space Center

Work Assignment 4-18
Contract No. 68-D2-0183
June 12, 1996

_____
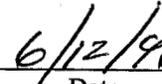Mark Roby
SAIC Task Manager/Study Director

_____
6/12/96
Date

Table of Contents

1.0   SUMMARY AND CONCLUSIONS

This report describes testing of a Registrant's method (MRID# 423792-01) for the determination of hexazinone in soil samples. The laboratory evaluation, major difficulties, experimental conclusions and comments are presented in this section.

1.1   Laboratory Evaluation

The method gave hexazinone recoveries ranging from 46.3% to 60.8% with a mean recovery of 53.4% and a relative standard deviation (RSD) of 11.4% for samples fortified at 0.03 µg/g (LOQ). For samples fortified at 0.30 µg/g (10xLOQ), the recoveries ranged from 92.3% to 117.2% with a mean recovery of 110.2% and an RSD of 10.9%.

The instrument response to hexazinone in samples fortified at the method detection limit (MDL) exceeded the noise level by a factor greater than three (S/N = 14.5). This signal-to-noise ratio suggests that even lower MDLs should be reasonably achievable. For soil samples, the MDL was 0.010 µg/g. A peak was found within the hexazinone retention time window in both the reagent blank and the matrix blank. However, the peak did not exceed one-half the target analyte response in samples fortified at the MDL and, therefore, does not constitute a formal interferent.

The initial calibration and all calibration check standards met project QC criteria. The initial calibration was linear over the range of 0.30 to 5.0 µg/mL (mean calibration factor RSD = 8.2%). The calibration factors of the calibration check standards run with the samples gave relative percent differences of -15.3% and -1.8%.

Retention times for hexazinone initial calibration, calibration check standards and samples at all fortification levels ranged from 13.31 to 13.33 minutes and were within the retention time window of 13.29 to 13.35 minutes.

1.2   Major Difficulties

There are discrepancies in the procedure as it appears in two separate locations in the MRID, pages 77 and 94. Initial testing of the method was completed using the procedure on page 94. Low recoveries were obtained at the LOQ (0.03 µg/g), 30% mean recovery and 8% RSD, and at 10xLOQ (0.30 µg/g), 42% mean recovery and 14% RSD. All associated calibration data were acceptable. Limited troubleshooting activities indicated that low recoveries were occurring in the initial extraction step. The method was re-tested using the protocol on page 77 which uses a higher extraction solvent volume. Considerably better recoveries were obtained in the re-test than in the initial testing, especially for the 10xLOQ samples (110% mean recovery and 10.9% RSD). However, the mean recovery at the LOQ was still below acceptance criteria (53.4% mean recovery and 11.4% RSD). Again, all calibration data were acceptable. All data in this report reflect those of the method re-test following the protocol on page 77 of the MRID.

SAIC also observed apparent carry-over of hexazinone from one injection to the next. The instrument blank injected immediately after the initial calibration standards showed a small peak falling within the retention time window for hexazinone at m/z 171. A small peak within the hexazinone retention time window was also found in the reagent and matrix blanks. As discussed in the previous section of this report, the response of this peak was insufficient to constitute a formal interferent. The Registrant also observed a "ghosting effect" as discussed on page 22 and 86 of the MRID.

1.3   Conclusions

Method performance met project data quality objective (DQO) criteria for soil samples at the 10xLOQ fortification level. However, the mean recovery value obtained by SAIC for hexazinone in an ECM program soil fortified at the LOQ was low. The individual LOQ recovery values were also low compared to those reported by the Registrant. At the LOQ, the Registrant reported a mean recovery of 109% with an RPD of 11.0% and n = 2.

1.4   Comments

The time required for completing one set of 12 samples (4 replicates each of MDL, LOQ, and 10xLOQ), 5 calibration standards, and associated QC samples (matrix blank, reagent blank, instrument blanks, and calibration check) was approximately 4 working days. Sample preparation (including sample cleanup and extraction); 3 days, GC/MS analysis; 8 hours. Samples can be analyzed overnight and data reduced the following day.

2.0   ANALYTICAL RESULTS

This section presents results from the method re-test. Summary tables are presented along with individual results for each sample at the LOQ and 10xLOQ spiking levels. The mean, standard deviation, and relative standard deviation are calculated in terms of percent recovery and in terms of measured concentration.

2.1   Summary of Recovery and Precision Data

Spike Level ($\mu g/g$)	Recovery Data			Concentration Data	
	Mean	SD[1]	RSD[2]	Mean Conc	SD[1] Conc
LOQ (0.03)	53.4	6.1	11.4	0.016	0.00
10xLOQ (0.30)	110.2	12.0	10.9	0.331	0.04

[1] SD = Standard Deviation
[2] RSD = Relative Standard Deviation

2.2   Analytical Spike Information

Spike Level	Concentration of Spiking Solution ($\mu g/mL$)	Spiked Amount (mL)	Concentration in Sample ($\mu g/g$)	Sample W_i[1] (g)	Sample V_i[1] (mL)
MDL	1.0	0.3	0.01	30	1.0
LOQ	1.0	1.0	0.03	30	1.0
10xLOQ	10.0	1.0	0.30	30	4.0

[1] W_i = Initial Sample Weight
[2] V_f = Final Sample Volume

2.3   Individual Results for Soil Samples Fortified at 0.03 µg/g (LOQ)

Sample Number	Retention Time (min)	Concentration Found (µg/g)	Conc. Fortified Sample (µg/g)	Percent Recovery
1 Hexazinone	13.33	0.018	0.03	60.8
2 Hexazinone	13.31	0.014	0.03	46.3
3 Hexazinone	13.31	0.016	0.03	54.8
4 Hexazinone	13.31	0.016	0.03	51.8

2.4   Individual Results for Soil Samples Fortified at 0.30 µg/g (10xLOQ)

Sample Number	Retention Time (min)	Concentration Found (µg/g)	Conc. Fortified Sample (µg/g)	Percent Recovery
1 Hexazinone	13.33	0.352	0.30	117.2
2 Hexazinone	13.31	0.277	0.30	92.3
3 Hexazinone	13.31	0.345	0.30	115.0
4 Hexazinone	13.31	0.350	0.30	116.5

3.0   EXPERIMENTAL DETAILS

A brief method summary of the analytical method as performed by SAIC, notes on analytical procedure/accomodations to variables, and example calculations are presented in this section.

3.1   Method Summary

A soil matrix was fortified with hexazinone at three different concentrations corresponding to the MDL, the LOQ, and ten times the LOQ (10xLOQ). The fortification levels were: 0.01 µg/g, 0.03 µg/g, and 0.30 µg/g. Four replicates of each concentration were prepared and analyzed according to the specified procedure. Sample concentrations were calculated using a mean calibration factor determined from a five-point standard curve. The concentration of the five calibration standards were: 0.30, 0.50, 1.0, 3.0 and 5.0 µg/mL. The method protocol is described in brief below.

3.1.1   Extraction

A 30 g soil sample was weighed into a centrifuge bottle and shaken for 30 minutes with 80 mL of 1:1 acetone:0.1$\underline{M}$ KH_2PO_4 buffer (v/v), on a shaker table at 400 rpm. The sample was then centrifuged for 10 minutes at 3700 rpm, and the extract decanted into a 500-mL separatory funnel. The extraction step was repeated twice with another 80 mL of 1:1 acetone:0.1$\underline{M}$ KH_2PO_4 buffer (v/v). The extracts were combined in the separatory funnel. 80 mL of 25% K_2CO_3 in water was added to the separatory funnel and partitioned with 150 mL chloroform for 2 minutes. The organic layer was dried through anhydrous sodium sulfate into a 500-mL round-bottom flask. The extraction was repeated with 70 mL of 1:1 chloroform:acetonitrile, followed by 80 mL of ethyl acetate and combining the extracts in the round-bottom flask. The extract was concentrated to near dryness using a rotary-evaporator at 42°C. The

residue was transferred to a 25-mL concentrator tube using small fractions of a 77.5:20:0.5:2 methylene chloride/ethyl acetate/caffine/triethylamine solution. Using a gentle stream of nitrogen, the final volume was adjusted to 1.0 mL for MDL and LOQ samples and, 4 mL for the 10xLOQ level. A portion of the extract was transferred to a GC vial for GC/MSD analysis.

3.1.2   Analysis

The samples were analyzed by gas chromatography / mass spectrometry, using a Varian 3400 gas chromatograph with a Varian Saturn 3 ion trap mass spectrometer. Chromatographic conditions are listed below. Deviations from the Registrant's conditions are indicated by noting the Registrant's conditions in parenthesis.

Column:	0.25-mm x 30-m DB-1701 (0.25-mm x 25-m DB-1701)
Injection Volume:	1 µL, splitless (3 µL, splitless)
Injector Temperature:	280°C (270°C split)
Oven Temperature:	150°C for 0.4 min. then 15°C/min. to 280°C, hold for 20.94 min.
	(150°C for 0 min. then 25°C/min. to 280°C, hold for 20 min.)
Flow Rate (He):	1 mL/min
Retention Time:	13.31 min
Ions Monitored:	m/z 171

3.1.3   Standard Information:

Hexazinone
Crescent Chemical Company
Lot No. 4046A
Neat, 99% pure
Received on 11/10/95
Opened on 12/14/95

3.1.4   Matrix Information:

Provided by OPP
Iowa Batch # 1 (10/26/95)
Received 2/6/96

3.2   Procedural Notes and Accommodations to Variables

The samples were shaken for 30 minutes on a shaker table at 400 rpm, rather than being shaken for 20 minutes and sonicating for 3 minutes as called for in the Registrant's method.

The samples were centrifuged for 10 minutes at 3700 rpm and left undisturbed for 30 minutes rather than centrifuging for 5 minutes at 5000 rpm, as specified by the Registrant.

The final volumes were 1.0 mL for MDL and LOQ samples and, 4.0 mL for 10xLOQ samples.

The Registrant used a Hewlett-Packard GC/MSD for sample analysis, SAIC used an ion trap GC/MS. The Registrant used the sum of the ions at m/z 128 and m/z 171 for quantitation of hexazinone. SAIC found the ion at m/z 128 to contribute more noise than signal intensity and, therefore, used only the ion at m/z 171 for quantitation.

The Registrant's method is also written for five metabolites. However, SAIC was instructed by OPP to only test the method for the parent compound, hexazinone.

3.3   Calculations

Example calculations are presented for calibration factor, mean calibration factor, extract concentration, and sample concentration. The formula used to calculate RSD is also given.

3.3.1   Calibration Factor (chromatogram # 3016A036)

$$Calibration\ Factor\ (CF) = \frac{area\ counts}{concentration}$$

For the 0.30-µg/mL calibration standard, area counts = 1072 and concentration = 0.30 µg/mL:

$$Calibration\ Factor\ (CF) = \frac{1072}{0.30} = 3573$$

3.3.2   Mean Calibration Factor (chromatograms # 3016A036 - 3016A040)

$$CF_{mean} = \frac{(CF_1 + CF_2 + CF_3 + CF_4 + \ldots + CF_n)}{n}$$

$$where\ n = number\ of\ calibration\ points$$

The five-point calibration data:

n	Concentration (µg/mL)	Area Counts	CF
1	0.30	1072	3573
2	0.50	1682	3364
3	1.0	3155	3155
4	3.0	11555	3852
5	5.0	16155	3231

Therefore:

$$CF_{mean} = \frac{(3573 + 3364 + 3155 + 3852 + 3231)}{5} = 3435$$

3.3.3   Extract Concentration (chromatogram # 3016A053)

$$Concentration_{extract} = \frac{Area\ Counts}{CF_{mean}}$$

For replicate 1 LOQ, area counts = 1881 and CF_{mean} = 3435:

$$Concentration_{extract} = \frac{1881}{3435} = 0.548\ \mu g/mL$$

3.3.4   Sample Concentration (chromatogram # 3016A053):

$$Concentration_{sample} = \frac{Concentration_{extract} \times Final\ Volume_{extract}}{Sample\ Weight}$$

For replicate 1 LOQ, concentration$_{extract}$ = 0.548 µg/mL, final volume$_{extract}$ = 1.0 mL and Sample Weight = 30 g:

$$Concentration_{sample} = \frac{0.548\ \mu g/mL \times 1.0\ mL}{30\ g} = 0.0183\ \mu g/g$$
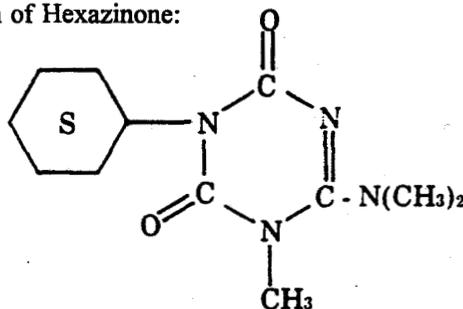
3.3.5   RSD is calculated by dividing the standard deviation of the measured concentration of the analytes by the mean concentration and multiplying by 100%.

$$Mean = \overline{x} = \frac{\Sigma\ x_i}{n}$$

$$std.dev. = s = \sqrt{\frac{\Sigma(\ \overline{x} - x_i)^2}{n - 1}}$$

$$Precision\ as\ RSD = \frac{s}{\overline{x}} \times 100\ \%$$

3.4   Chemical Structure Diagram of Hexazinone:

Initial Calibration Data:

Retention Time (min)	Area Counts	Concentration (μg/mL)	Calibration Factor
13.31	1072	0.30	3573
13.31	1682	0.50	3364
13.31	3155	1.0	3155
13.33	11555	3.0	3852
13.31	16155	5.0	3231

Mean Calibration Factor = 3435
Standard Deviation = 280
Relative Standard Deviation = 8.2

Calibration Check Data:

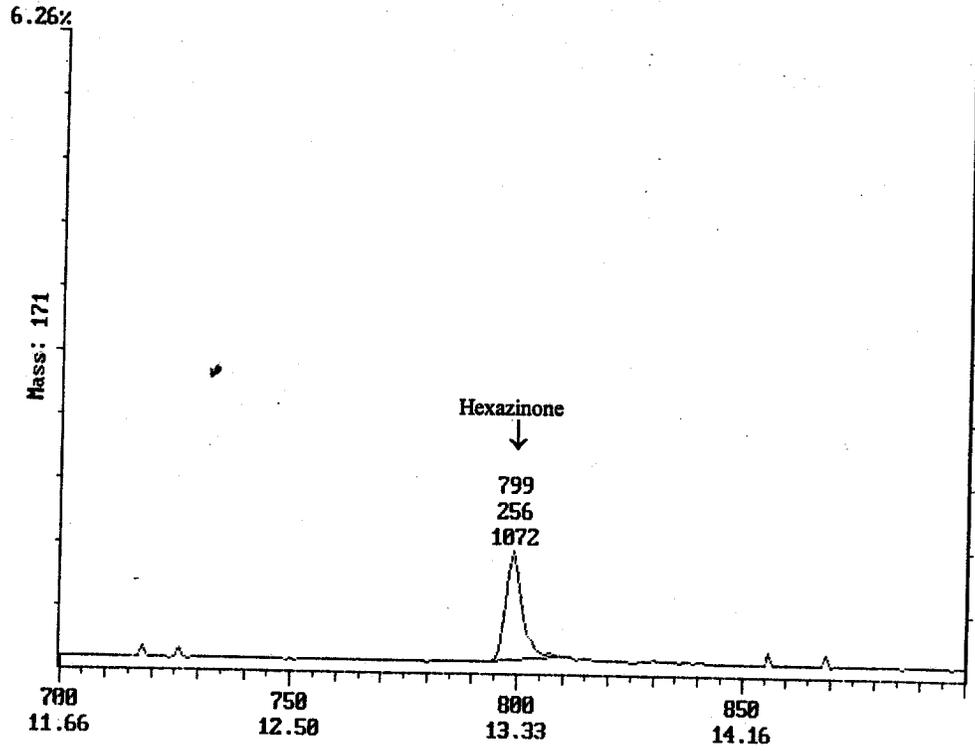| Calibration Check	Retention Time (min)	Area Counts	Concentration (μg/mL)	Calibration Factor	Percent Difference[1]
1	13.33	873	0.30	2910	- 15.3 %
2	13.31	10120	3.0	3373	- 1.8 %

[1] Compared to the mean calibration factor

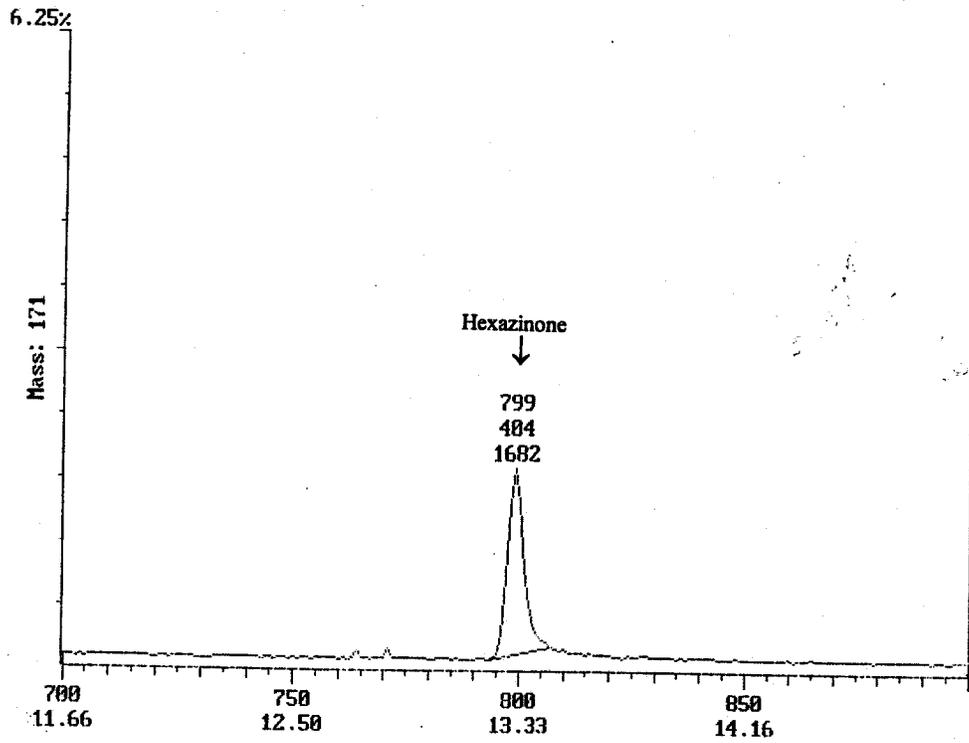## Appendix B - Representative Chromatograms

This section contains representative chromatograms of calibration standards, blanks, and fortified soil samples at each fortification level in the following order:

- Calibration Standards (0.30, 0.50, 1.0, 3.0, and 5.0 µg/mL)
- Reagent Blank, Soil
- Matrix Blank, Soil
- Soil at MDL (0.01 µg/g)
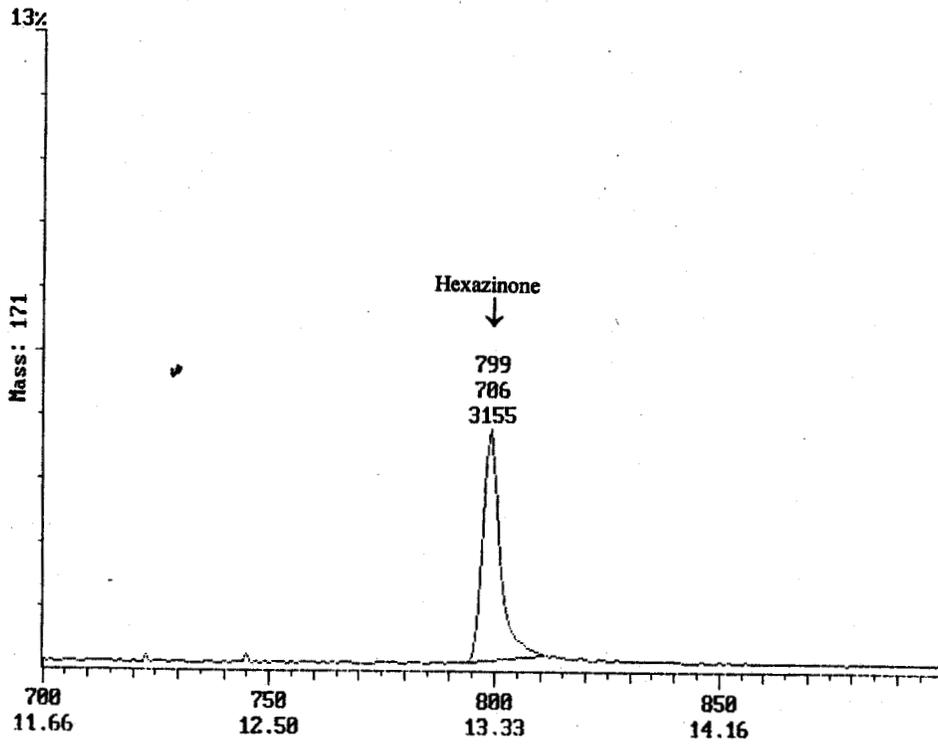- Soil at LOQ (0.03 µg/g)
- Soil at 10xLOQ (0.30 µg/g)

Calibration Standard - 0.30 µg/mL, 1 µL injection volume

6.26%

Mass: 171

Hexazinone
↓
799
256
1072

```
700        750        800        850
11.66      12.50      13.33      14.16
```

Calibration Standard - 0.50 µg/mL, 1 µL injection volume

6.25%

Mass: 171

Hexazinone
↓
799
404
1682

```
700        750        800        850
11.66      12.50      13.33      14.16
```
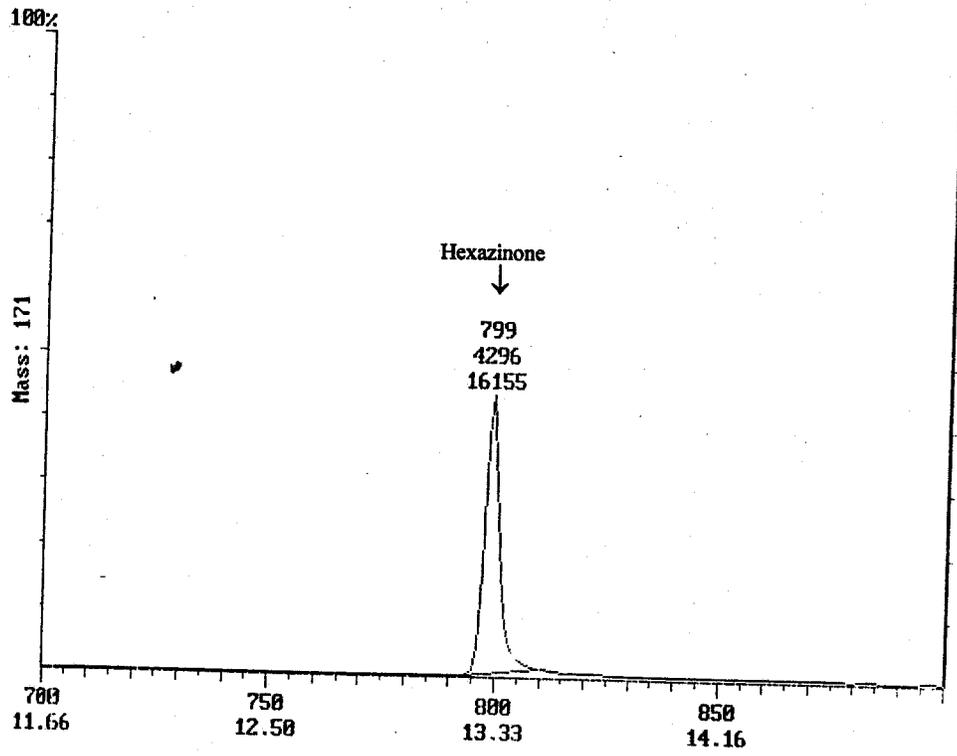
B-2

Calibration Standard - 1.0 µg/mL, 1 µL injection volume

Calibration Standard - 3.0 µg/mL, 1 µL injection volume

B-3
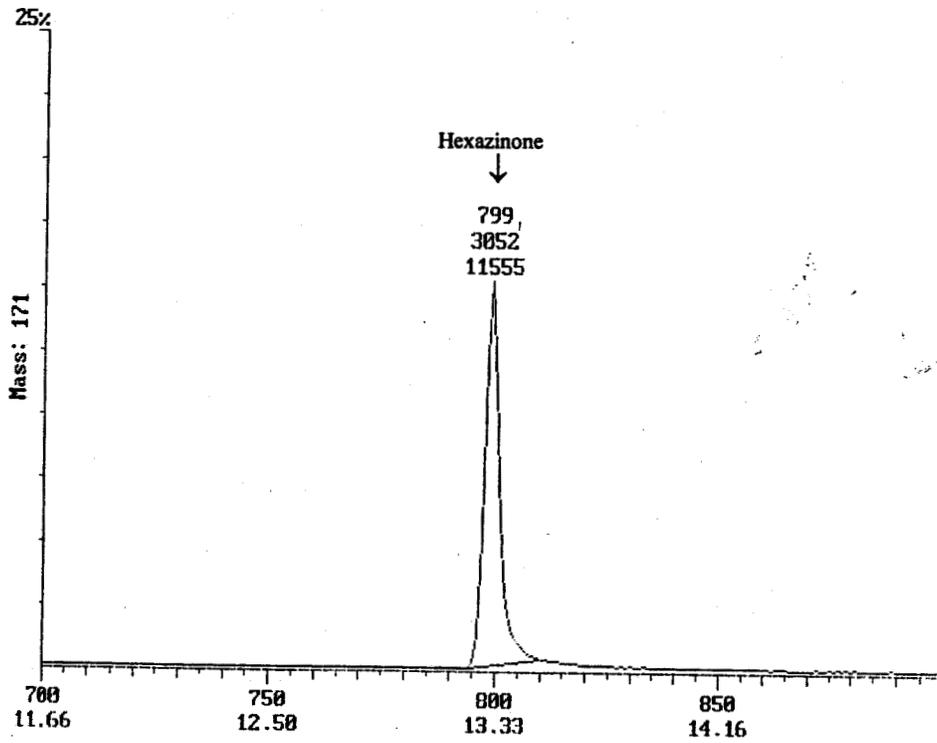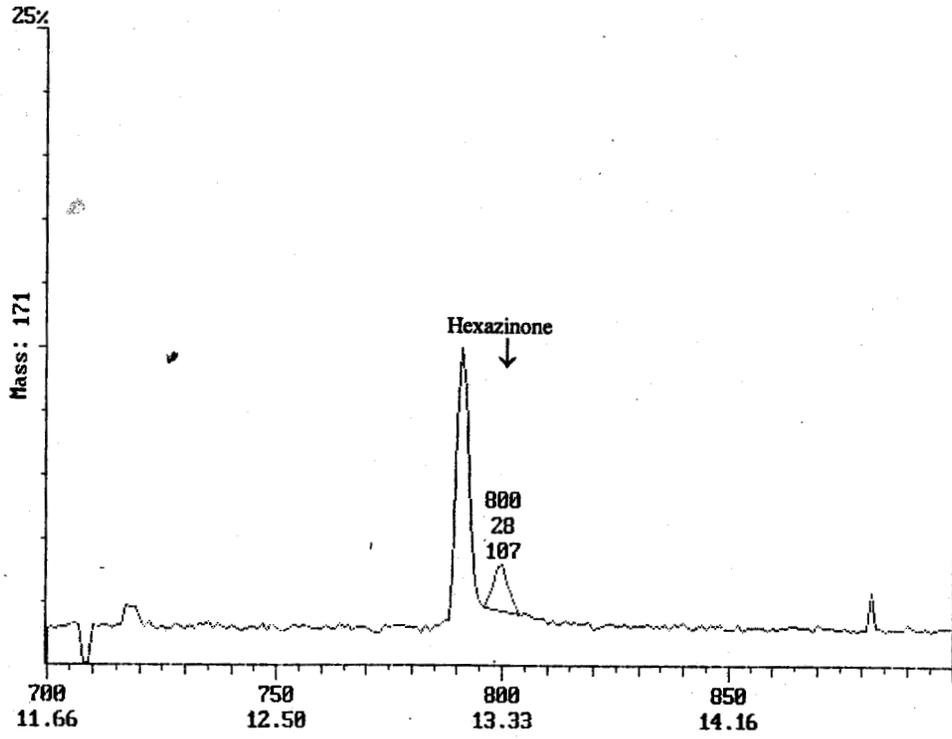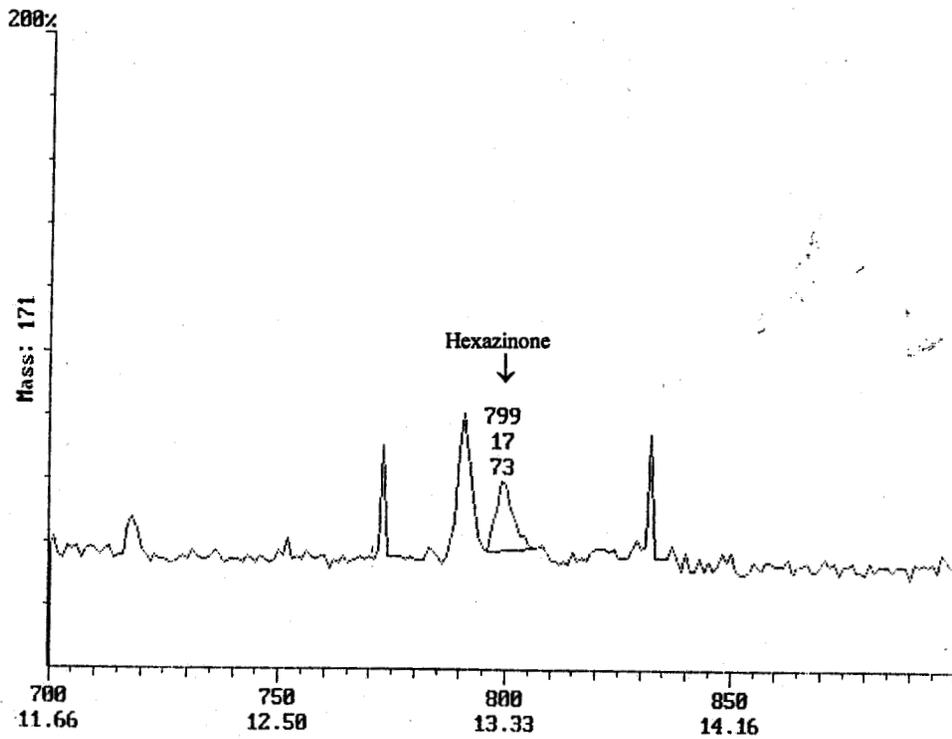
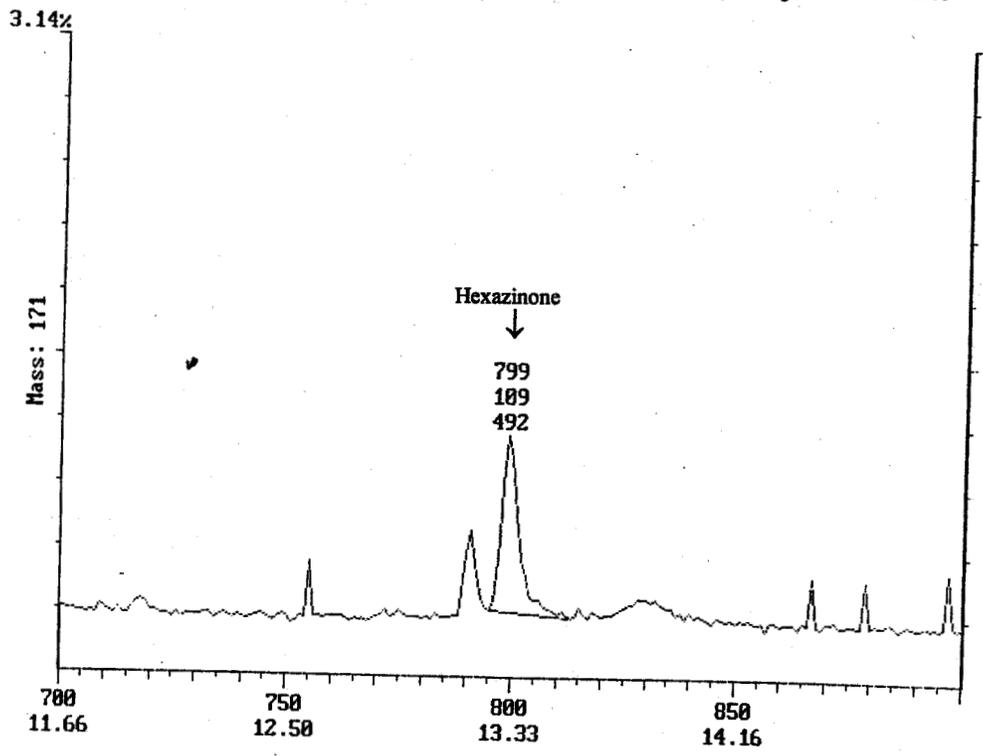## Calibration Standard - 5.0 µg/mL, 1 µL injection volume

Mass: 171

Hexazinone
↓
799
4296
16155

100%

700   750   800   850
11.66 12.50 13.33 14.16

Reagent Blank - 1 mL final extract volume, 1 μL injection volume

25%

Mass: 171

Hexazinone
↓

800
28
107

700   750   800   850
11.66 12.50 13.33 14.16

Matrix Blank - 1 mL final extract volume, 1 μL injection volume

200%

Mass: 171

Hexazinone
↓

799
17
73

700   750   800   850
11.66 12.50 13.33 14.16

B-5

Soil at MDL - 0.01 µg/g, 1 mL final extract volume, 1 µL injection volume

3.14%

Mass: 171

Hexazinone
↓
799
109
492

700   750   800   850
11.66   12.50   13.33   14.16

Soil at LOQ - 0.03 µg/g, 1 mL final extract volume, 1 µL injection volume

6.25%

Mass: 171

Hexazinone
↓
799
438
1881

700   750   800   850
11.66   12.49   13.33   14.16

B-6

Soil at 10xLOQ - 0.30 µg/g, 4 mL final extract volume, 1 µL injection volume

B-7