Appendix IV (continued)

ANALYTICAL PROCEDURE FOR THE DETERMINATION OF PROPAMOCARB IN POTATOES

ANALYTICAL METHOD: XAM-34

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APPENDICES:

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1.0 INTRODUCTION

Propamocarb is a systemic fungicide when applied to soil but it can also be used as a foliar spray. Propamocarb is specific against *Phycomycetes* and is recommended as a preventative treatment.

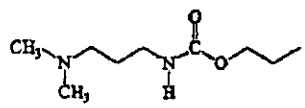
Analytical procedures for fruit, vegetable and sandy loam soils have been reported. These procedures generally used gas chromatography (GC) with a nitrogen/ phosphorus specific detector. A procedure using mass spectrometry (MS) in the electron impact (EI) mode for quantitation has also been reported (Chambers and Charters, 1994). The analytical procedure described in this report utilizes both a thermionic selective detector (TSD) and an ion trap MS for quantitation. The latter operates in the Mass Instability Chemical Ionization (MICI) mode

This analytical method is suitable for the determination of extractable residues of the fungicide propamocarb in potato The limit of quantitation (LOQ) for this procedure is 0 05 ppm.

2.0 REFERENCE COMPOUND

2.1 Hoe 074189: Propamocarb

C.A. Name: propyl [3-(dimethylamino)propyl]carbamate
IUPAC Name: propyl (3-dimethylaminopropyl)carbamate
Structural formula.



Empirical formula: $C_9H_{20}N_2O_2$
Molecular weight: 188.2

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3.0 PRINCIPLE OF THE METHOD

Extractable residues of propamocarb are removed from potatoes by homogenizing with acidified methanol. The extract is filtered and the methanol is removed using a rotary evaporator. The acidic extract is partitioned with dichloromethane and diisopropyl ether. The aqueous extract is basified by addition of 10 N NaOH and the solution is applied to a Chem-Elut column. Propamocarb is eluted from the Chem-Elut, with diisopropyl ether. The eluate is evaporated using a rotary evaporator. The residue is made up to final volume with diisopropyl ether and the extract is analyzed by GC/TSD or GC/Ion Trap MS operating in the SECI mode. The latter uses the189 m/z for quantitation of the propamocarb residues.

A flow diagram of the analytical method is presented in Appendix I.

4.0 EQUIPMENT

Unless otherwise indicated, the equipment listed below may be substituted with functionally equivalent equipment as may be available.

- Balance: Mettler model BB-2440, for samples
- Balance: Mettler model AE 240, for standards
- Commercial Food Processor: Robo-Coupe
- Microliter syringes: 100, 250, 500, 1000 microliter, Hamilton
- Volumetric pipettes, glass: 1.0-mL, 5.0-mL and 10.0-mL
- Polytron Homogenizer, equipped with PT-35 generator
- Beakers, glass: 250-mL
- Büchner Funnels, porcelain
- Adapter, glass: to filter directly into round bottom flask
- Filter papers: glass fiber 934-AH
- Round bottom flask, glass: 500-mL, 24/40 joint
- Separatory funnel, glass: 125-mL
- Round bottom flask, glass: 250-mL
- Rotary Evaporator equipped with water bath
- Disposable Pasteur pipettes, glass: 22.5 cm x 0.7 mm O.D.
- Graduated cylinder, glass: 10-mL, 25-mL 100-mL
- Volumetric flasks, glass: 5-mL, 10-mL, 25-mL, 50-mL
- pH paper

Appendix IV (continued)

5.0 CHEMICALS/REAGENTS

Alternate suppliers/brands of reagents having comparable specifications may be used
- Methanol: (pesticide quality)
- Diisopropyl ether: ACS
- Water: distilled
- Sodium hydroxide: (Reagent Grade)
- Hydrochloric acid: concentrated
- Chem-Elut Cartridge, CE 1020: Analytichem/Varian
- Celite 545

6.0 PREPARATION OF STANDARD SOLUTIONS

The analytical standard is supplied by the sponsor: AgrEvo USA Company or AgrEvo Canada Inc.. It should be stored in a secured freezer when not in use, at about -15°C.

6.1 Propamocarb Fortification Solutions

Solution A
Weigh 50 mg (± 0.1 mg) of Hoe 074189 and quantitatively transfer into a 50-mL volumetric flask. The volume is made up with acidified methanol (1 mL of 1N HCl added to 100 mL of methanol) to give a concentration of 1.0 mg/mL of propamocarb. This stock solution will be used to prepare fortification solutions. The solution is stored in a freezer and must be prepared anew every six months.

Solution B:
Transfer 500 µL of primary stock solution A to a 50-mL volumetric flask. The volume is made up with methanol to give a concentration of 10.0 µg/mL of Propamocarb. This solution is stored in a freezer and is prepared anew every three months.

Solution C:
Transfer 5.0 mL of solution B to a 50-mL volumetric flask. The volume is made up with methanol to give a concentration of 1.0 µg/mL of Propamocarb. This solution is stored in a freezer and is prepared anew every three months. Additional fortification solutions may be prepared if necessary.

6.2 Propamocarb Calibration Solutions

Solution D
Weigh 50 mg (± 1 mg) of Hoe 074189 and quantitatively transfer into a 50-mL volumetric flask. The volume is made up with diisopropyl ether to give a concentration of 1.0 mg/mL of propamocarb. This stock solution will be used to prepare GC calibration standards. The solution is stored in a freezer and must be prepared anew every six months.

Appendix IV (continued)

6.3 **Propamocarb Calibration Solutions, continued**

Solution E
Transfer 500 µL of solution D to a 50-mL volumetric flask. The volume is made up with diisopropyl ether to give a concentration of 10.0 µg/mL of Propamocarb. This solution is stored in a freezer and is prepared anew every month.

The calibration solutions are prepared by dilution of solution E. Typical dilutions are shown below:

Final concentration (ng/µL)	Final volume (mL)	Volume of solution E used (µL)
0.10	50.0	500
0.15	50.0	750
0.25	50.0	1250
0.50	50.0	2500
0.75	50.0	3750
1.0	50.0	5000
1.5	50.0	7500

The calibration standard solutions are prepared anew every month. They are kept in a refrigerator (about 4°C) when not in use.

7.0 **ANALYTICAL PROCEDURE**

7.1 **Sample Preparation:**

The potato sample is ground using a food processor (Robo-Coupe) and a representative analytical sample obtained.

7.2 **Extraction:**

A representative analytical sample (20 ± 0.1 g) is weighed into a 250-mL glass beaker. HCl (1 N, 1.0 mL) is added, followed by methanol (100 mL). The mixture is homogenized, using the Polytron at speed 4, for one minute. The extract is filtered though a glass fiber filter covered with a thin layer of Celite 545 (approximately 1.5 g) into a 500-mL round bottom flask. The beaker is rinsed with 50 mL of methanol which is poured through the filter pad and collected into the same round bottom flask. The methanol is rotary evaporated with a water bath temperature set at approximately 45 °C, leaving only the acidic aqueous extract (approximately 5 mL).

Appendix IV (continued)

7.3 **Liquid/Liquid Partition:**

Additional HCl (5mL, 1N) is added to the aqueous extract The pH is verified at this stage, using pH paper to assure that it is acidic. The flask is swirled to dissolve the residue and the mixture is transferred to a 125-mL separatory funnel. Dichloromethane (20 mL) is added to the round bottom flask and the flask is swirled to dislodge any residue adhering to the walls of the flask. The dichloromethane is poured into the separatory funnel and the mixture is shaken for approximately 30 seconds. The phases are allowed to separate and the dichloromethane layer is discarded. The dichloromethane partition is repeated with an additional portion of 20 mL, which is also used to rinse the round bottom flask before adding to the separatory funnel. The aqueous extract is then partitioned with diisopropyl ether (20 mL) and the aqueous layer is drained into the round bottom flask and set on the rotary evaporator, with the water bath at approximately 45 °C, to remove any traces of organic solvent. Only a few minutes are necessary to accomplish this.

7.4 **Chem-Elut Clean-Up:**

The acidic extract from 7.3 is basified by adding NaOH (10 mL of 10 N) The basified extract is poured into a 25-mL graduated cylinder and the volume is adjusted to 18-20 mL with distilled water The solution is mixed and poured into the Chem-Elut column. After the solution has completely drained, the column is left to equilibrate for 5 minutes. The Chem-Elut column is eluted with 100 mL of diisopropyl ether (DIPE), collecting the eluate into a 250-mL round bottom flask. The diisopropyl ether is evaporated down to approximately 2 mL, using a rotary evaporator with a water bath set at approximately 40°C. The solution is transferred to a 5-mL volumetric flask with small portions of diisopropyl ether. The volume is adjusted to 5 mL with DIPE.
An aliquot of this solution is transferred to an autosampler vial for analysis by GC/TSD or GC/Ion Trap MS.

Appendix IV (continued)

8.0 QUANTITATION GC/MS

8.1 Instrumentation:

The Varian Saturn 3 Ion Trap Mass Spectrometer system consists of a model 3400 GC and a model 8200 autosampler. The MS was operated in the SECI mode.

8.2 Gas Chromatographic Conditions:

GC Column:	DB-5MS 30 m x 25 mm ID, 0.25 µm film
Carrier Gas Flow:	approximately 50 cm/s at 260 °C
Temperatures:	
Inlet:	Split/Splitless injector, 275 °C. Split Ratio 10.1
Column:	Initial: 70 °C, hold: 1.0 min. 70-200 °C at 10 °C/min., hold: 0 min. 200-300 °C at 50 °C/min, hold. 8.3 min
Transfer Line:	300 °C
Mass Spectrometer Interface:	direct capillary
Injection Volume:	3.0 µL
Injection Rate:	5 µL/sec.
Retention Time:	approximately 10 minutes
Limit of Quantitation:	0 025 ppm
Limit of Detection:	0.3 ng (S/N ≥20)

Representative chromatograms and calibration plot are included in Appendix II.

8.3 Mass Spectrometer Conditions:

8.3.1 Mass Spectrometer Acquisition Segments:

A/M Amplitude:	4 V	Low Mass:	187 m/z
High Mass:	191 m/z	Scan Rate.	1000 ms
Segment Time:	11.4 min.	Peak Threshold:	0 counts
Filament Delay:	7.5 min.	Mass Defect:	50 mu/100 u
Background Mass:	100 amu	Calibration Gas:	no
Scan Mode:	SECI	Ionization Control:	Automatic
Tune File:	PROPTUNE	Emission Current:	20 µA
CI-Gas	Isobutane		

Appendix IV (continued)

8.4 GC/MS Calibration:

The GC/MS response (peak area) is determined for a series of calibration standards. The 189 *m/z* ion is used to generate extracted ion profiles for propamocarb. Detector response is non-linear and can be described by a logarithmic function of the form shown in Equation 1. For each analytical set, the calibration data was used to perform a power regression analysis. The natural logarithm of the amount injected (ng) was taken as the X-axis and the natural logarithm of the detector response (peak area) was taken as the Y-axis to give Equation 2.

$$\ln y = \ln xm + b \qquad \text{[Eq. 1]}$$

where:
- y = peak area response for analyte in injected sample/standard
- m = slope of the regression line
- x = amount (ng) of analyte found in the sample/standard
- b = intercept of the regression line

$$\ln(\text{peak area}) = \ln(\text{ng in the sample/standard})m + b \qquad \text{[Eq. 2]}$$

8.5 Sample Analysis:

For sample extract of unknown propamocarb content, the amount (ng) found may be calculated, from the observed peak area, using Equation 3.

$$\text{ng (x) in sample} = e^{(1/m)(\ln(\text{peak area}) - b)/m)} \qquad \text{[Eq. 3]}$$

Both samples and standards must be analyzed under the same GC conditions and within the same analytical sequence.

9.0 QUANTITATION GC/TSD

9.1 Instrumentation:

The gas chromatographic system consisted of a Varian 3400 GC equipped with a thermionic specific detector and an 8200 autosampler.

Appendix IV (continued)

9.2 Gas Chromatographic Conditions:

The gas chromatographic system consists of a Varian 3400 GC equipped with a thermionic specific detector (TSD) and an 8200 autosampler

GC Column:	DB-1, 15 m x 0.53 mm ID, 1.5 or 3.0 µm film
Carrier Gas Flow:	4.5 mL/min
Temperatures,	
Inlet:	275 °C
Column:	Initial: 100 °C, hold. 0.2 min 100-140 °C at 5 °C/min., hold: 0 min. 140-230 °C at 30 °C/min., hold: 13.8 min
Detector:	300 °C
Injection Volume:	2.0 µL, on-column
Injection Rate:	1.0 µL/sec
Retention Time:	10.2 minutes
Limit of Quantitation:	0.025 ppm
Limit of Detection:	0.2 ng (S/N ≥3)

Representative chromatograms and calibration plot are included in Appendix III.

9.3 TSD Calibration:

The GC/TSD response (peak area) is determined for a series of calibration standards. The detector response is linear and can be described by a function of the form shown in Equation 1. For each analytical set, the calibration data was used to perform a linear regression analysis. The amount injected (ng) was taken as the X-axis and the detector response (peak area) was taken as the Y-axis to give Equation 2.

$$y = xm + b \qquad \text{[Eq. 1]}$$

where: y = peak area response for analyte in injected sample/standard
m = slope of the regression line
x = amount (ng) of analyte found in the sample/standard
b = intercept of the regression line

$$\text{peak area} = (\text{ng in the sample/standard})m + b \qquad \text{[Eq. 2]}$$

Appendix IV (continued)

9.4 **Sample Analysis:**

The peak area response for Hoe 074189 is computed using the Varian Star Workstation. The amount of material is determined from the corresponding calibration plot. For sample extracts of unknown propamocarb content, the amount found (in ng) may be calculated, from the observed peak area, using Equation 3

$$\text{ng (x) in sample} = \frac{\text{peak area} - b}{m} \qquad \text{[Eq. 3]}$$

Both samples and standards must be analyzed under the same GC conditions and within the same analytical sequence.

10.0 CALCULATION OF RESIDUES

10.1 **Calculation:**

The amount of residues in a sample are expressed in parts per million. They are calculated using the following equation:

$$\text{ppm} = \frac{\text{Amt}}{\text{B}}$$

where: Amt = ng (x) analyte found from the standard curve

B = mg of sample injected

$$\text{B} = \frac{\text{Ws (g)} * \text{Vinj (}\mu\text{L)}}{\text{Vf (mL)}}$$

where: Ws = weight of sample (g)
Vf = final volume of extract (mL)
Vinj = sample volume injected (µL)

$$\text{\% Recovery} = \frac{\text{ppm found} * 100}{\text{ppm added}}$$

Appendix IV (continued)

10.2 **Example Calculation of Procedural Recovery-GC/MS:**

The data for sample POT-F2-1, fortified at 0 50 ppm is given in GC data sheet, X9618V04, (Appendix II). The procedural recovery calculation for Hoe 074189 is shown below:

Amt $= x = e^{(11.8729 - 10.2352)/1.3449} = e^{1.0542} = 2.869$ ng

B $= \frac{20\ g * 3.0\ uL}{10\ mL} = 6.0$ mg

ppm found = $\frac{\text{ng found from curve}}{\text{mg-equivalent injected}}$

ppm found = $\frac{2.869\ ng}{6.0\ mg} = 0.478$ ppm as Hoe 074189

Recovery = $\frac{\text{ppm found}}{\text{ppm fortification level}} = \frac{0.478\ ppm * 100}{0.50\ ppm} = 95\ 6\ \%$

10.3 **Example Calculation of Procedural Recovery-GC/TSD:**

The data for sample POT-F1-3, fortified at 0.05 ppm is given in GC data sheet, X9618V01, (Appendix III). The procedural recovery calculation for Hoe 074189 is shown below:

Amt = x = $\frac{4207 - 125 - 217.18}{10473.3} = 0.369$ ng

where. 125 is the peak area found in the control sample extract

B = $\frac{20\ g * 2.0\ uL}{5\ mL} = 8.0$ mg

ppm found = $\frac{\text{ng found (from linear regression)}}{\text{mg-equivalent injected}}$

ppm found = $\frac{0.369\ ng}{8\ mg} = 0.046$ ppm as Hoe 074189

Recovery = $\frac{\text{ppm found}}{\text{ppm fortification level}} = \frac{0.046\ ppm * 100}{0\ 05\ ppm} = 92.3\%$

Appendix IV (continued)

11.0 QUALITY CONTROL PROCEDURES

11.1 Laboratory Fortified Controls:

To assure the quality of the analytical data, laboratory fortified controls are analyzed with each set of samples. These fortified controls should cover the expected range of residues in the samples and be at least 10% of the samples within an analytical set.

11.2 GC Analysis:

To verify the stability of the response, a standard plot is drawn for the levels of interest. Within an analytical set, analytical standards are typically injected after every sample, this serves as an ongoing quality control for detector sensitivity and analyte retention time. Calibration standards are analyzed at the beginning and end of an analytical set.

The lowest level analytical standard should correspond to 50 to 70% of the limit of quantitation. Residue results must not be determined by extrapolation of calibration data outside of the concentration range of the calibration standards (using ±10% tolerance, typically). Samples with residue levels greater than the calibrated range must be diluted and re-injected so that they do fall within the calibrated range.

The recovery of laboratory fortified controls should fall within a range of 70 - 120%. The analyte signal should be ≥ 3 times the background signal, and the intra-laboratory reproducibility as indicated by the relative standard deviation (n ≥3) obtained from replicated analyses should fall within 20% (rel) of the averaged result.

11.3 Sample Storage:

Field samples should be kept frozen until analyzed After obtaining a representative analytical sample, the remaining material should be promptly refrozen and stored until authorization for disposal is received.

12.0 RESULTS

Potato samples were fortified at 0.050 and 0.500 ppm with Propamocarb. Five replicate samples were analyzed for each level and the recoveries determined. For GC/MS, the mean of the recoveries for the two levels of fortification were: 115 ± 11.6 % and 97.2 ± 3.3 % for 0.050 ppm (LOQ) and 0.50 ppm, respectively (Table I). The procedure was validated also using GC/TSD, the mean of the recoveries for the two levels of fortification were: 74.4 ± 13.5 % and 94.4 ± 5.8 % for 0.050 ppm (LOQ) and 0.50 ppm, respectively (Table II).
These data validate an LOQ of 0.050 ppm for potato.

Appendix IV (continued)

Table I: Recoveries of Propamocarb from Fortified Potato - GC/MS

Sample No.	Fortification Level (ppm)	Found (ppm)	Recovery (%)
POT-F1-1	0.050	0.056	112
POT-F1-2	0.050	0.049	98.9
POT-F1-3	0.050	0.065	131
POT-F1-4	0.050	0.057	115
POT-F1-5	0.050	0.059	119
		Mean Recovery ± std dev.	115 ± 11.6%
POT-F2-1	0.50	0.478	95.6
POT-F2-2	0.50	0.500	100
POT-F2-3	0.50	0.489	97.9
POT-F2-4	0.50	0.461	92.3
POT-F2-5	0.50	0.502	100
		Mean Recovery ± std dev.	97.2 ± 3.3 %
		Overall Mean Recovery (n = 10)	106 ± 12.4

Table II: Recoveries of Propamocarb from Fortified Potato - GC/TSD

Sample No.	Fortification Level (ppm)	Found (ppm)	Recovery (%)
POT-F1-1	0.050	0.037	73.2
POT-F1-2	0.050	0.039	78.6
POT-F1-3	0.050	0.046	92.3
POT-F1-4	0.050	0.027	54.7
POT-F1-5	0.050	0.037	73.2
		Mean Recovery ± std dev.	74.4 ± 13.5
POT-F2-1	0.50	0.478	95.7
POT-F2-2	0.50	0.487	97.4
POT-F2-3	0.50	0.480	96.0
POT-F2-4	0.50	0.421	84.2
POT-F2-5	0.50	0.493	98.7
		Mean Recovery ± std dev.	94.4 ± 5.8
		Overall Mean Recovery (n = 10)	84.4 ± 14.4

Appendix IV (continued)

13.0 REFERENCES

1. Analytical Method for the Determination of Residues of Propamocarb x HCl in Soil, J. Moede; Report Number UPSR 54/91 - PA 66 752.5/14 Schering AG,

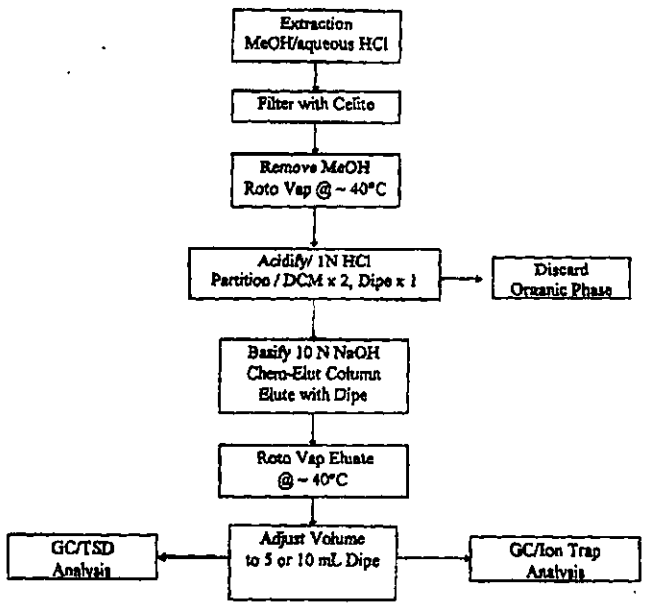
2. Dissipation of Propamocarb X HCl in Soil following application of Banol - USA 1990, A.Wrede-Rucker, Schering report no. UPSR 57/91 - PA 66 752.7/16, July 7, 1992)

3. *Dissipation of Propamocarb HCL in Soil Following Application of Banol* to Bare Plot, USA, 1993, Martin G. Cole; AgrEvo USA Registration reference, Propamocarb HCl/W132 A54951

Appendix IV (continued)

Appendix I

Schematic Flow Diagram XAM-34



Appendix IV (continued)

Appendix II

GC/MS - Representative Ion Chromatograms, Typical Calibration Curve and Chromatographic Data Sheet

Figure 1: Ion Chromatogram (m/z 189) of Hoe 074189 standard at 0.10 ng/µL, 3 µL injected. Reference Data Sheet #: X9618V04.

Figure 2: Ion Chromatogram (m/z 189) of Hoe 074189 standard at 0.15 ng/µL, 3 µL injected. Reference Data Sheet #: X9618V04

Figure 3: Ion Chromatogram (m/z 189) of Hoe 074189 standard at 0.25 ng/µL, 3 µL injected. Reference Data Sheet #: X9618V04

Figure 4: Ion Chromatogram (m/z 189) of Hoe 074189 standard at 0.50 ng/µL, 3 µL injected. Reference datasheet #: X9618V04

Figure 5: Ion Chromatogram (m/z 189) of Hoe 074189 standard at 0.75 ng/µL, 3 µL injected. Reference Data Sheet #: X9618V04

Figure 6: Ion Chromatogram (m/z 189) of Hoe 074189 standard at 1.00 ng/µL, 3 µL injected, Reference Data Sheet #: X96180V4

Figure 7: Ion Chromatogram (m/z 189) of Hoe 074189 standard at 1.50 ng/µL, 3 µL injected. Reference Data Sheet #: X96180V4

Figure 8: Ion Chromatogram (m/z 189) of control potato sample, 3 µL of 5 mL injected.
POT-C1, Reference Data Sheet #: X9618V03

Figure 9: Ion Chromatogram (m/z 189) of control potato sample, 3 µL of 10 mL injected.
POT-C2, Reference Data Sheet #: X9618V04

Figure 10: Ion Chromatogram (m/z 189) of fortified control potato sample with Hoe 074189 at 0.050 ppm, 3 µL of 5 mL injected.
POT-F1-2, Reference Data Sheet #: X9618V03

Figure 11: Ion Chromatogram (m/z 189) of fortified control potato sample with Hoe 074189 at 0.50 ppm, 3 µL of 10 mL injected.
POT-F2-2, Reference Data Sheet #: X9618V04

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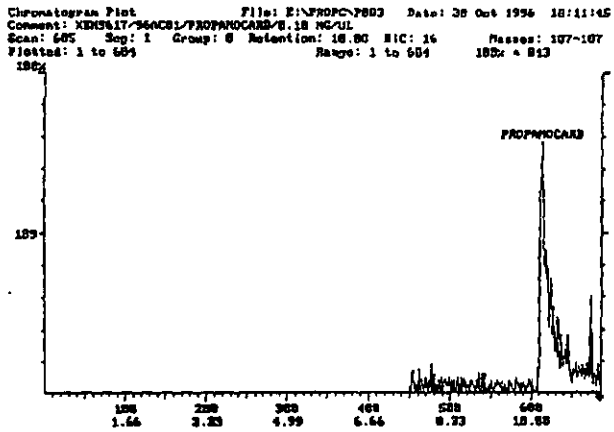
Figure 1: Ion Chromatogram (m/z 189) of Hoe 074189 standard at 0.10 ng/µL, 3 µL injected. Reference Data Sheet #: X9618V04.



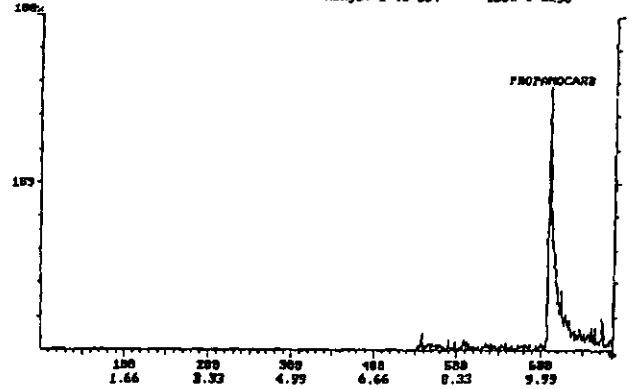
Integration Report Scan File: P003 Cali File: PROP Entries: 1
Comment: XEN9617/96AC01/PROPAMOCARB/0.10 NG/UL
Sorted via: Entry Number ↑ IS Factor: 1.000 Mult: 1.000 Div: 1.000

Name of Compound	S/N Ratio	R Time	Scan#	Pk Height	Peak Area
PROPAMOCARB	24.5	18.21	613	439	4,648

Appendix IV (continued)

Figure 2: Ion Chromatogram (m/z 189) of Hoe 074189 standard at 0.15 ng/µL, 3 µL injected. *Reference Data Sheet #: X9618V04*

Chromatogram Plot File: E:\PROFC\PROS Date: 30 Oct 1996 19:16:23
Comment: XEN9617/96AC81/PROPANOCARB/0.15 NG/UL
Scan: 605 Seg: 1 Group: 0 Retention: 10.80 RIC: 77 Masses: 100-190
Plotted: 1 to 604 Range: 1 to 604 100% = 2230



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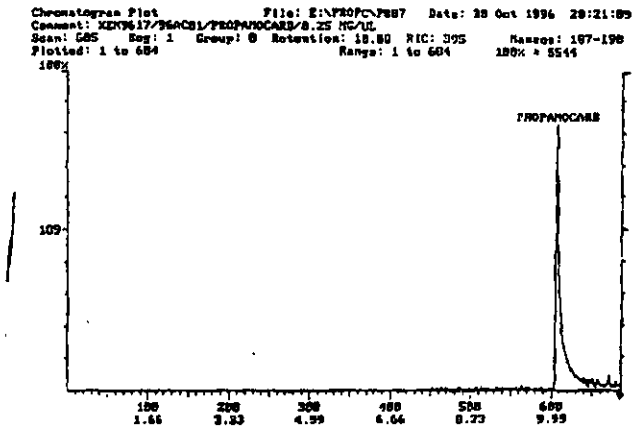
Sorted via: Entry Number ↑ IS Factor: 1.000 Mult: 1.000 Div: 1.000

Name of Compound	M/Z Ratio	R Time	Scan#	Pk Height	Peak Area
PROPANOCARB	58.5	10.14	605	1.720	10,274

Appendix IV (continued)

Figure 3: Ion Chromatogram (m/z 189) of Hoe 074189 standard at 0.25 ng/µL. 3 µL injected. Reference Data Sheet #: X9618V04



Integration Report Quan File: PS07 Call File: PROP Entries: 1
Comment: XEN9617/96AC81/PROPAMOCARB/0.25 NG/UL
Sorted via: Entry Number ↑ IS Factor: 1.000 Mult: 1.000 Div: 1.000

Name of Compound	S/N Ratio	R Time	Scan#	Pk Height	Peak Area
PROPAMOCARB	146.2	18.11	687	4,533	22,695

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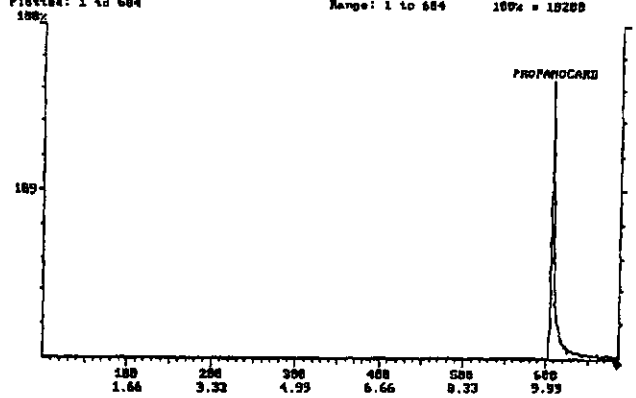
Figure 4: Ion Chromatogram (m/z 189) of Hoe 074189 standard at 0.50 ng/µL, 3 µL Injected. Reference Data Sheet #: X9618V04

Chromatogram Plot File: E:\PROPC\PB89 Date: 30 Oct 1996 21:25:52
Comment: XEN9617/96AC01/PROPANOCARB/0.50 NG/UL
Scan: 685 Seg: 1 Group: 0 Retention: 18.00 RIC: 3737 Masses: 187-191
Plotted: 1 to 684 Range: 1 to 684 100% = 18288



Integration Report Quan File: PB89 Call File: PROP Entries: 1
Comment: XEN9617/96AC01/PROPANOCARB/0.50 NG/UL
Sorted via: Entry Number ↑ IS Factor: 1.000 Mult: 1.000 Div: 1.000

Name of Compound	S/N Ratio	R Time	Scan#	Pk Height	Peak Area
PROPANOCARB	559.1	18.18	685	15,695	55,282

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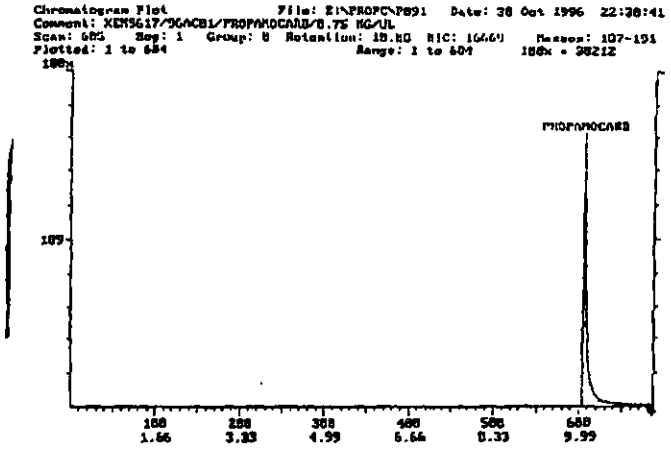
Figure 5: Ion Chromatogram (m/z 189) of Hoe 074189 standard at 0.75 ng/µL, 3 µL injected. Reference Data Sheet #: X9618V04



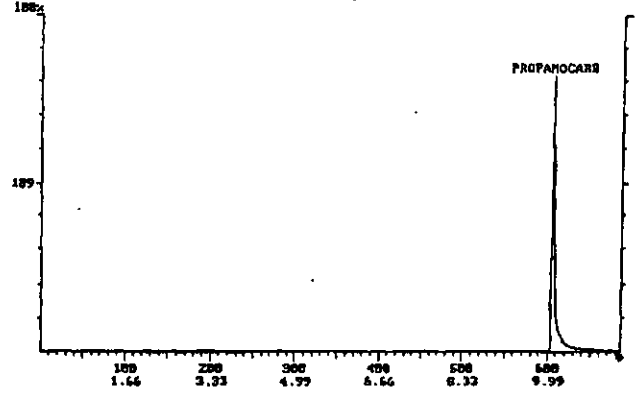
Integration Report Quan File: PB91 Call File: PROP Entries: 1
Comment: XEN9617/96ACB1/PROPAMOCARB/0.75 NG/UL
Sorted via: Entry Number ↑ IS Factor: 1.000 Mult: 1.000 Div: 1.000

Name of Compound	S/N Ratio	R Time	Scan#	Pk Height	Peak Area
PROPAMOCARB	790.9	18.09	606	24,519	82,868

Appendix IV (continued)

Figure 6: Ion Chromatogram (m/z 189) of Hoe 074189 standard at 1.00 ng/µL, 3 µL injected, Reference Data Sheet #: X9618V04

Chromatogram Plot File: E:\PROPC\PB93 Date: 30 Oct 1996 23:25:20
Comment: XEN9617/96ACB1/PROPAMOCARB/1.0 NG/UL
Scan: 685 Seg: 1 Group: 0 Retention: 10.80 RIC: 20059 Masses: 107-191
Plotted: 1 to 684 Range: 1 to 684 188x = 45120

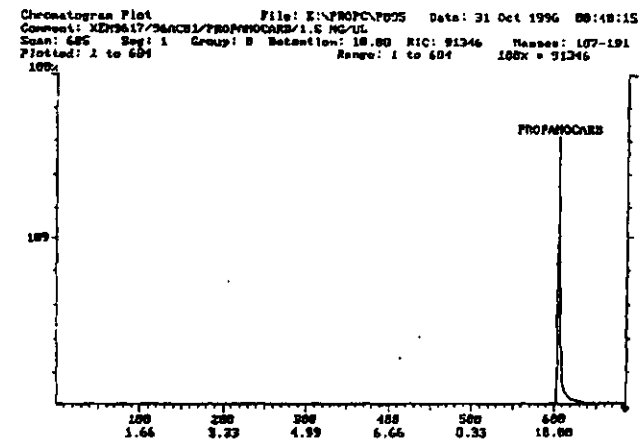


Integration Report Run File: PB93 Cali File: PROP Entries: 1
Comment: XEN9617/96ACB1/PROPAMOCARB/1.0 NG/UL
Sorted via: Entry Number ? IS Factor: 1.000 Mult: 1.000 Div: 1.000

Name of Compound	S/N Ratio	R Time	Scans	Pk Height	Peak Area
PROPAMOCARB	1309.3	10.89	686	36,668	118,093

Appendix IV (continued)

Figure 7: *Ion Chromatogram (m/z 189) of Hoe 074189 standard at 1.50 ng/µL, 3 µL injected. Reference Data Sheet #: X9618V04*

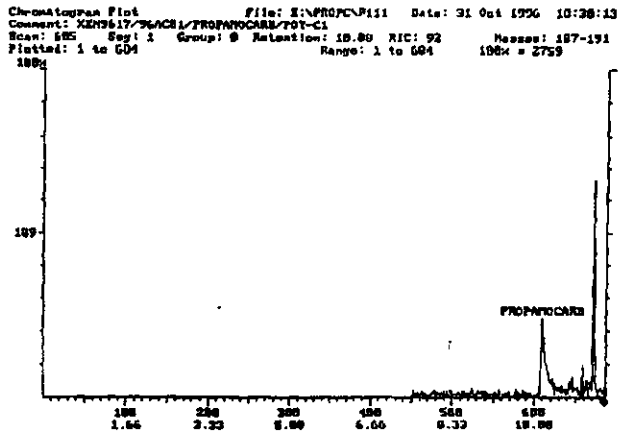


Integration Report Quan File: P095 Call File: PROP Entries: 1
Comment: XEN9617/96ACB1/PROPAMOCARB/1.5 NG/UL
Sorted via: Entry Number ↑ IS Factor: 1.000 Mult: 1.000 Div: 1.000

Name of Compound	S/N Ratio	R Time	Scan#	Pk Height	Peak Area
PROPAMOCARB	2537.0	10.00	685	74,234	199,970

Appendix IV (continued)

Figure 8: Ion Chromatogram (m/z 189) of control potato sample, 3 µL of 5 mL injected.
POT-C1, Reference Data Sheet #: X9618V03

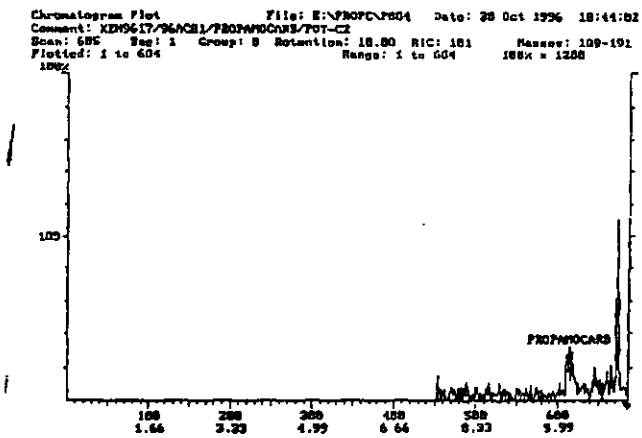


Integration Report Quan File: P111 Call File: PROP Entries: 1
Comment: XEN9617/96ACB1/PROPAMOCARB/POT-C1
Sorted via: Entry Number ↑ IS Factor: 1.000 Mult: 1.000 Div: 1.000

Name of Compound	S/N Ratio	R Time	Scan#	Pk Height	Peak Area
PROPAMOCARB	20.7	10.15	605	643	4.347

Appendix IV (continued)

Figure 9: Ion Chromatogram (m/z 189) of control potato sample, 3 µL of 10 mL injected.
POT-C2, Reference Data Sheet #: X9618V04

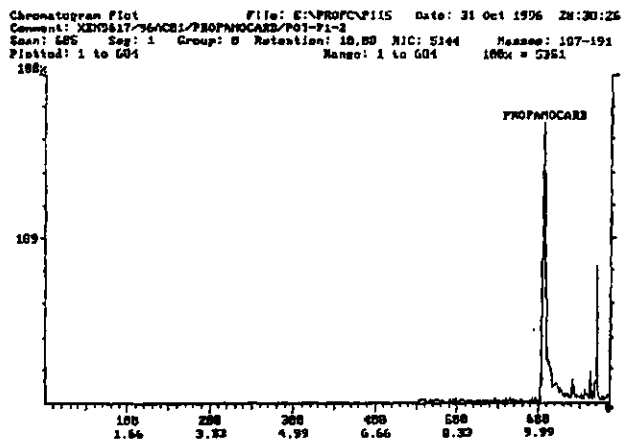


Integration Report Quan File: P004 Call File: PROP Entries: 1
Comment: XEN9617/96ACB1/PROPANOCARB/POT-C2
Sorted via: Entry Number ↑ IS Factor: 1.000 Mult: 1.000 Div: 1.000

Name of Compound	S/N Ratio	R Time	Scan#	Pk Height	Peak Area
PROPANOCARB	5.4	10.23	614	174	731

Appendix IV (continued)

Figure 10: Ion Chromatogram (m/z 189) of fortified control potato sample with Hoe 074189 at 0.05 ppm, 3 µL of 5 mL injected.
POT-F1-2, Reference Data Sheet #: X9618V03

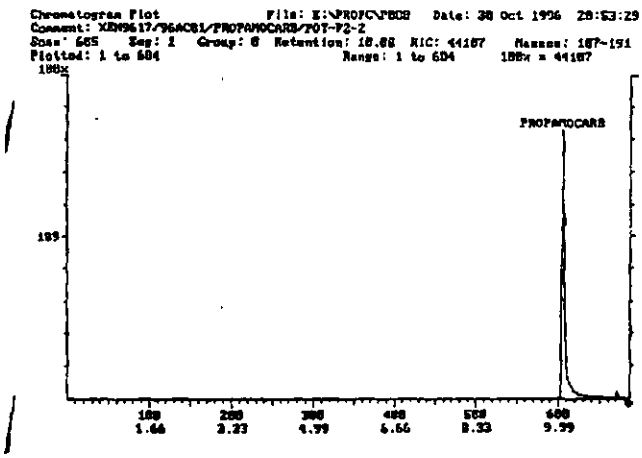


Integration Report Quan File: P115 Call File: PROP Entries: 1
Comment: XEN9617/96ACB1/PROPAMOCARB/POT-F1-2
Sorted via: Entry Number ↑ [S Factor: 1.000 Mult: 1.000 Div: 1.000

Name of Compound	S/N Ratio	R Time	Scan#	Pk Height	Peak Area
PROPAMOCARB	172.7	10.89	686	4,491	19,228

Appendix IV (continued)

Figure 11: Ion Chromatogram (m/z 189) of fortified control potato sample with Hoe 074189 at 0.50 ppm, 3 µL of 10 mL injected.
POT-F2-2, Reference Data Sheet #: X5618V04

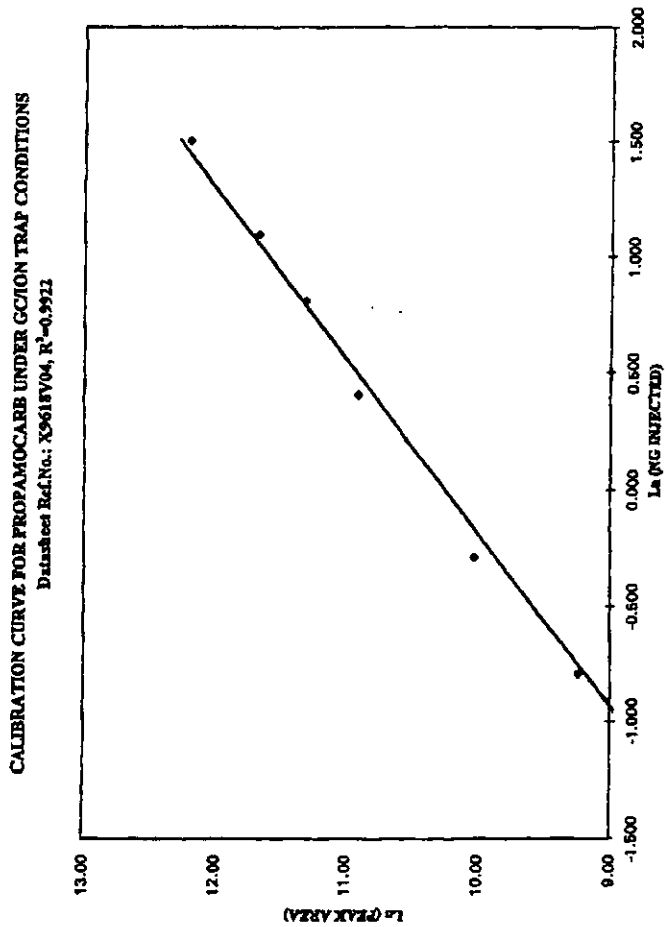


Integration Report Quan File: PROP Call File: PROP Entries: 1
Comment: XEN96I7/96ACB1/PROPAMOCARB/POT-F2-2
Sorted via: Entry Number ↑ IS Factor: 1.000 Mult: 1.000 Div: 1.000

Name of Compound	S/N Ratio	R Time	Scan#	Pk Height	Peak Area
PROPAMOCARB	1729.0	18.88	685	26,318	125,297

Appendix IV (continued)

Xenos Method XAM-34 29a



Appendix IV (continued)

Xenas Method XAM-34

GAS CHROMATOGRAPHIC DATA SHEET

Xenas Project No.: XEN96-18
Sponsor Study No.: Validation

Extracted : October 30, 1996
Injected: October 30, 1996
Analyst: N. Bertrand *MB*

Compound: Propamocarb
Matrix: Potato

Datasheet Ref. No.: X9618V04
Verified:

GC Run #	Sample	Sample Weight g	Final Vol. mL	Injected uL	Injected mg	Peak Area	Ln(Peak Area)	Found from curve (ng)	Ln(Found from curve (ng))	Found (ppm)	ppm Added	% Recovery
82	DIPE			3	-	ND	ND	ND	ND	ND		
83	0.100 ng/µL			3		4660	8.45	0.300	-1.204			
84	POT-Cl,2 (50 ng/µL)	20.00	10	3	6.0	731	ND	ND	ND	ND		
85	0.150 ng/µL			3		10274	9.24	0.450	-0.799			
86	POT-P2-1	20.00	10	3	6.0	118080	11.67	2.869	1.054	0.478	0.500	95.6
87	0.250 ng/µL			3		22695	10.03	0.750	-0.288			
88	POT-P2-2	20.00	10	3	6.0	125287	11.73	2.999	1.098	0.500	0.500	100
89	0.500 ng/µL			3		55202	10.92	1.500	0.405			
90	POT-P2-3	20.00	10	3	6.0	121803	11.70	2.937	1.077	0.489	0.500	97.9
91	0.750 ng/µL			3		82868	11.33	2.250	0.811			
92	POT-P2-4	20.00	10	3	6.0	112555	11.62	2.768	1.018	0.461	0.500	92.3
93	1.000 ng/µL			3		118093	11.68	3.000	1.099			
94	POT-P2-5	20.00	10	3	6.0	125848	11.74	3.009	1.102	0.502	0.500	100
95	1.50 ng/µL			3		199970	12.21	4.500	1.504			

GC Instrument

Model:	Varian 3400
Serial No.:	7906
Detector:	Ion Trap
Column:	DB-5, 30 m x 0.25 mm
Column No:	5870221
Film thickness:	0.25 µm

MS Conditions

Multiplier:	1800 V
Target:	20000
Emission Current:	20 µA

Temperatures

Column:
initial: 70 °C, hold: 1 min
program 1:
rate: 10.0 °C/min
final: 200 °C, hold: 0 min
program 2:
rate: 50.0 °C/min
final: 300 °C, hold: 5.3 min
Inlet: 275 °C, split ratio 10:1

Regression Output

Propamocarb

R Square	0.992145304
Intercept	10.2552331
Slope	1.344947356
No. Obs.	7

Notes

ND = No peak detected
O/R = peak outside of calibration range
* = subtracted control peak area

Appendix IV (continued)

Appendix III

GC/TSD - Representative Chromatograms, Typical Calibration Curve and Chromatographic Data Sheet

Figure 1: Chromatogram of Hoe 074189 standard at 0.10 ng/µL, 2 µL injected. Reference Data Sheet #: X9618V01

Figure 2: Chromatogram of Hoe 074189 standard at 0.15 ng/µL, 2 µL injected. Reference Data Sheet #: X9618V01

Figure 3: Chromatogram of Hoe 074189 standard at 0.25 ng/µL, 2 µL injected. Reference Data Sheet #: X9618V01

Figure 4: Chromatogram of Hoe 074189 standard at 0.50 ng/µL, 2 µL injected. Reference Data Sheet #: X9618V01

Figure 5: Chromatogram of Hoe 074189 standard at 0.75 ng/µL, 2 µL injected. Reference Data Sheet #: X9618V01

Figure 6: Chromatogram of Hoe 074189 standard at 1.00 ng/µL, 2 µL injected. Reference Data Sheet #: X9618V01

Figure 7: Chromatogram of Hoe 074189 standard at 1.50 ng/µL, 2 µL injected, Reference Data Sheet #: X9618V01

Figure 8: Chromatogram of control potato sample, 2 µL of 5 mL injected. POT-C1, Reference Data Sheet #: X9618V01

Figure 9: Chromatogram of control potato sample, 2 µL of 10 mL injected. POT-C2, Reference Data Sheet #: X9618V02

Figure 10: Chromatogram of fortified control potato sample with Hoe 074189 at 0.05 ppm, 2 µL of 5 mL injected. POT-F1-2, Reference Data Sheet #: X9618V01

Figure 11: Chromatogram of fortified control potato sample with Hoe 074189 at 0.50 ppm, 2 µL of 10 mL injected. POT-F2-2, Reference Data Sheet #: X9618V02

Appendix IV (continued)

Figure 1: Chromatogram of Hoe 074189 standard at 0.10 ng/µL, 2 µL injected. Reference Data Sheet #: X9618V01

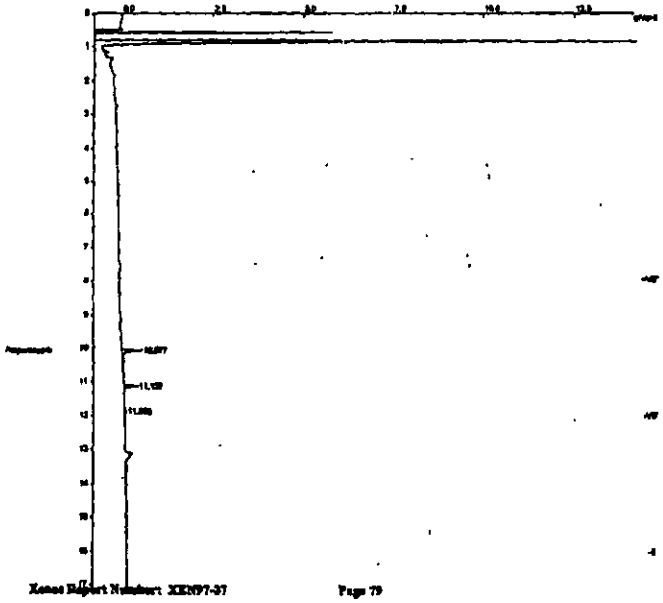
```
Title        : Propamocarb in potato - XEN96-18, 96AC03
Run File     : .c:\star\module18\9618057.run
Method File  : C:\STAR\MODULE18\PROPPOT.MTH
Sample ID    : 0.10 NG/UL

Injection Date: 31-OCT-96 1:12 AM    Calculation Date: 31-OCT-96 1:30 AM

Operator    : Xenos Laboratories     Detector Type: ADCB (1 Volt)
Workstation: MS-DOS 5                Bus Address  : 18
Instrument : GC 3400                 Sample Rate  : 10.00 Hz
Channel     : A = TSD                Run Time     : 18.002 min

********** Star Chromatography Workstation ******* Version 4.5 **************

Chart Speed =    1.00 cm/min   Attenuation = 64            Zero Offset = 5%
Start Time  =   0.000   min     End Time    = 17.200  min   Min / Tick  = 1.00
```



Appendix IV (continued)

Figure 2: **Chromatogram of Hoe 074189 standard at 0.15 ng/µL, 2 µL injected. Reference Data Sheet #: X9618V01**

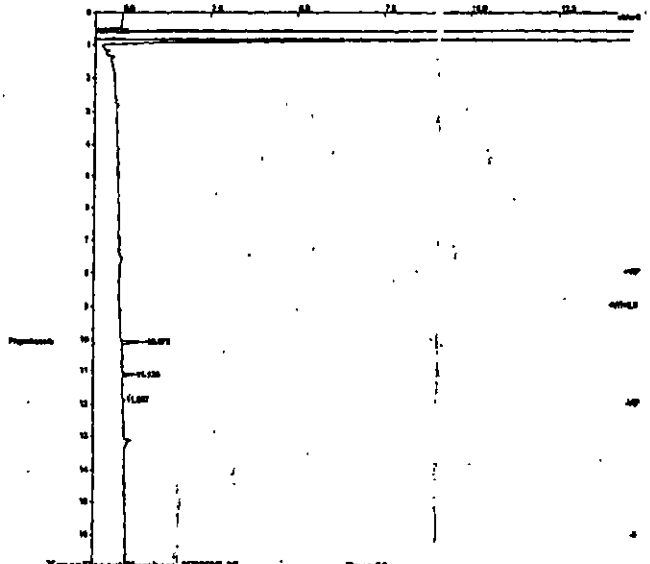
```
Title        : Propamocarb in potato - XEN96-18, 96AC03
Run File     : c:\star\module10\B618089.run
Method File  : C:\STAR\MODULE10\PROPPOT.MTH
Sample ID    : 0.15 NG/UL

Injection Date: 31-OCT-96 2:11 AM    Calculation Date: 31-OCT-96 8:29 AM

Operator     : Xenos Laboratories     Detector Type: ADC5 (: Volt)
Workstation: MS-DOS_5                 Bus Address  : 18
Instrument : GC 3400                  Sample Rate  : 10.00 ;µ
Channel    : A = TSD                  Run Time     : 18.002 min

********** Star Chromatography Workstation ******* Versi n 4.5 ***************

Chart Speed =    1.00 cm/min   Attenuation = 64          Zero Offset = 5%
Start Time  =   0.000   min    End Time    = 17.200  min  Min / Tick  = 1.00
```



Appendix IV (continued)

Figure 3: **Chromatogram of Hoe 074189 standard at 0.25 ng/µL, 2 µL injected. Reference Data Sheet #: X9618V01**

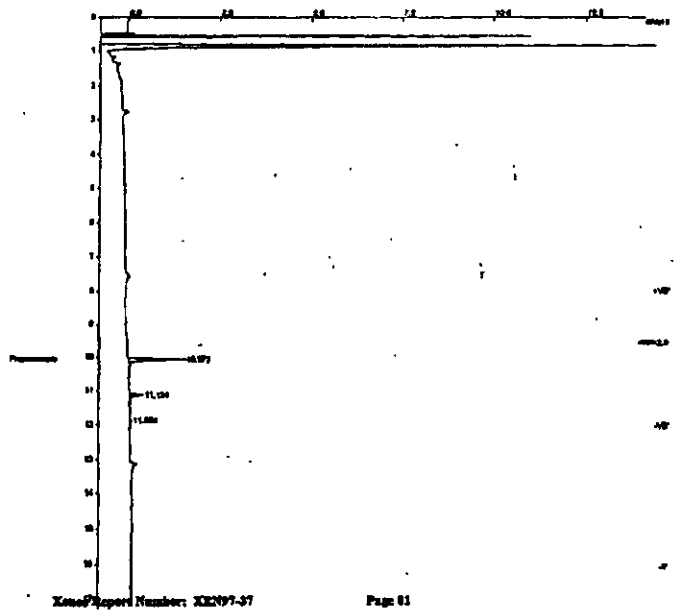
```
Title        : Propamocarb in potato - XEN96-18, 96AC03
Run File     : c:\star\module18\9618061.run
Method File  : C:\STAR\MODULE18\PROPPOT.MTH
Sample ID    : 0.25 NG/UL

Injection Date: 31-OCT-96 3:10 AM     Calculation Date: 31-OCT-96 3:28 AM

Operator     : Xenos Laboratories     Detector Type: ADC8 (1 Volt)
Workstation: MS-DOS 5                 Bus Address  : 18
Instrument : GC 3400                  Sample Rate  : 10.00 Hz
Channel      : A - TSD                Run Time     : 18.002 min

********** Star Chromatography Workstation ******* Version 4.5 **************

Chart Speed =    1.00 cm/min   Attenuation = 64          Zero Offset = 5%
Start Time  =   0.000   min     End Time    = 17.200  min   Min / Tick  = 1.00
```



Appendix IV (continued)

Figure 4: **Chromatogram of Hoe 074189 standard at 0.50 ng/µL, 2 µL injected. Reference Data Sheet #: X9618V01**

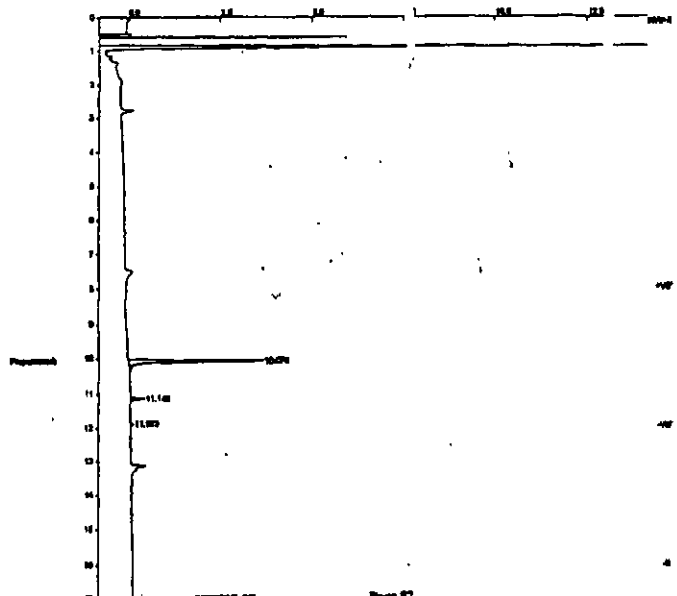
```
Title        : Propamocarb in potato - XEN96-18, 96AA 13
Run File     : c:\star\module18\9618063.run
Method File  : C:\STAR\MODULE18\PROPPOT.MTH
Sample ID    : 0.50 NG/UL

Injection Date: 31-OCT-96 4:09 AM    Calculation Date: 31-OCT-96 4:27 AM

Operator     : Xenos Laboratories    Detector Type: NPD (1 Volt)
Workstation: MS-DOS_5                Bus Address  : 18
Instrument : GC 3400                 Sample Rate  : 10.00 Hz
Channel    : A = TSD                 Run Time     : 13.002 min

********** Star Chromatography Workstation ******* Version 4.5 ********** ****

Chart Speed =    1.00 cm/min   Attenuation = 64          Zero Offset = 54
Start Time  =    0.000   min    End Time    = 17.200  min   Min / Tick  = 1.00
```



Appendix IV (continued)

Figure 5: **Chromatogram of Hoe 074189 standard at 0.75 ng/µL, 2 µL injected. Reference Data Sheet #: X9618V01**

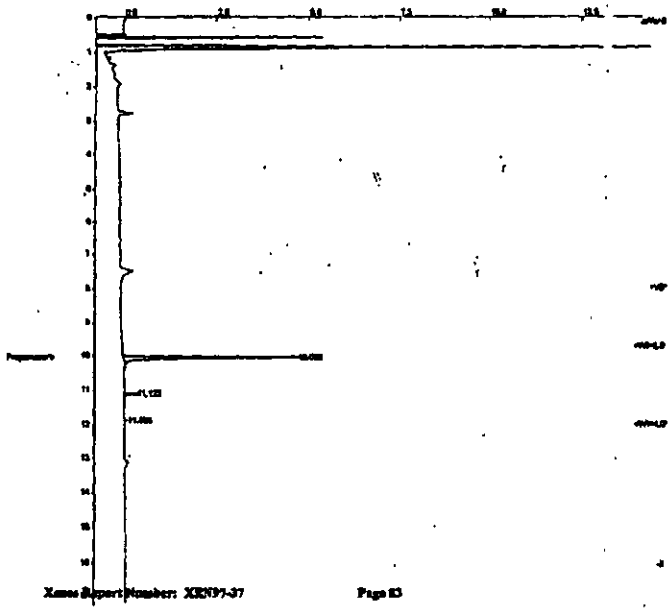
```
Title        : Propamocarb in potato - XEN96-18, 94AC03
Run File     : c:\star\module18\9618065.run
Method File  : C:\STAR\MODULE18\PROPPOT.MTH
Sample ID    : 0.75 NG/UL

Injection Date: 31-OCT-96 5:08 AM    Calculation Date: 31-OCT-96 5:26 AM

Operator    : Xenos Laboratories     Detector Type: ADCB (1 Volt)
Workstation: WS-DOS 5                Bus Address  : 18
Instrument : GC 3400                 Sample Rate  : 10.00 Hz
Channel     : A - TSD                Run Time     : 18.002 min

********** Star Chromatography Workstation ******* Version 4.5 ************

Chart Speed =    1.00 cm/min   Attenuation = 64          Zero Offset = 5%
Start Time  =    0.000  min     End Time    = 17.200  min   Min / Tick  = 1.00
```



Appendix IV (continued)

Figure 6: Chromatogram of Hoe 074189 standard at 1.00 ng/µL, 2 µL injected.
Reference Data Sheet #: X9618V01

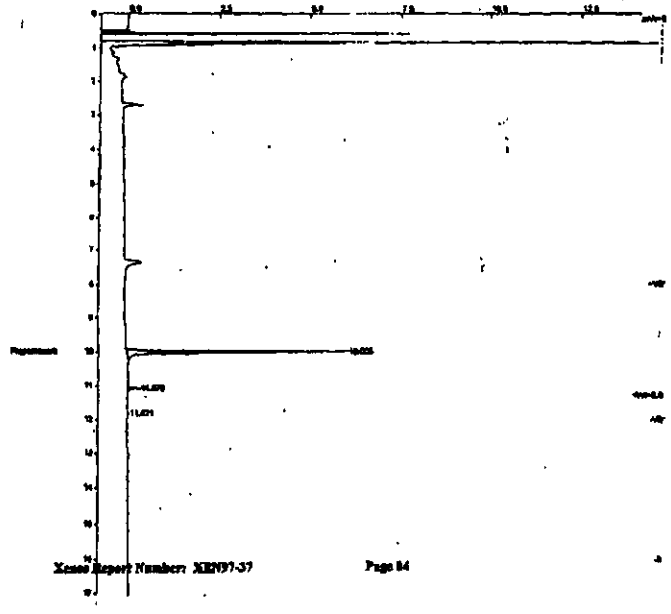
```
Title        : Propamocarb in potato - XEN96-18, 96AC03
Run File     : c:\star\module18\9618067.run
Method File  : C:\STAR\MODULE18\PROPPOT.MTH
Sample ID    : 1.0 NG/UL

Injection Date: 31-OCT-96 6:06 AM    Calculation Date: 31-OCT-96 6:24 AM

Operator    : Xenos Laboratories     Detector Type: ADCB (1 Volt)
Workstation: MS-DOS 5                Bus Address  : 18
Instrument  : GC 3400                Sample Rate  : 10.00 Hz
Channel     : A = TSD                Run Time     : 18.002 min

********** Star Chromatography Workstation ******* Version 4.5 **************

Chart Speed =    1.00 cm/min   Attenuation = 64          Zero Offset = 5%
Start Time  =    0.000   min    End Time    = 17.200  min   Min / Tick  = 1.00
```



Appendix IV (continued)

Figure 7: **Chromatogram of Hos 074189 standard at 1.50 ng/µL, 2 µL injected. Reference Data Sheet #: X9618V01**

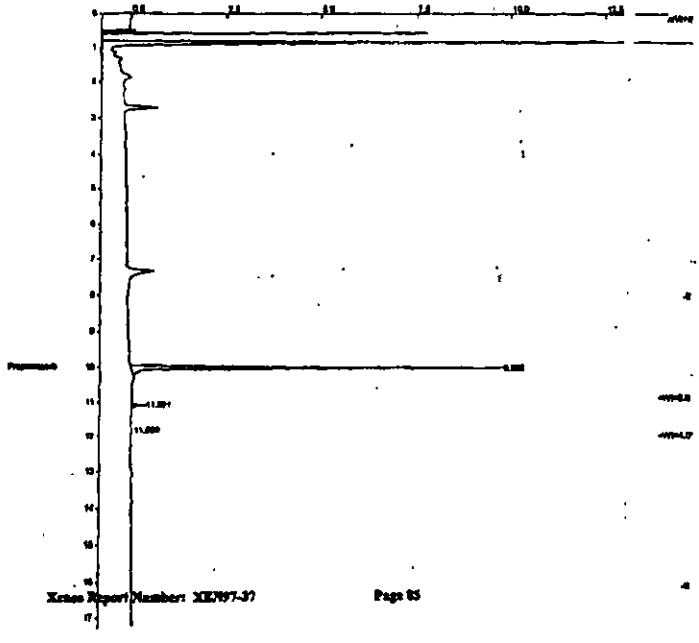
```
Title        : Propamocarb in potato -.XEN96-18, 96AC03
Run File     : c:\star\module18\9618069.run
Method File  : C:\STAR\MODULE18\PROPPOT.MTH
Sample ID    : 1.5 NG/UL

Injection Date: 31-OCT-96 7:05 AM     Calculation Date: 31-OCT-96 7:23 AM

Operator     : Xenos Laboratories     Detector Type: ADCB (1 Volt)
Workstation: MS-DOS_5                 Bus Address  : 18
Instrument : GC 3400                  Sample Rate  : 10.00 Hz
Channel      : A - TSD                Run Time     : 18.002 min

********** Star Chromatography Workstation ******* Version 4.5 ************

Chart Speed =    1.00 cm/min   Attenuation = 64          Zero Offset = 58
Start Time  =    0.000   min    End Time    = 17.200  min   Min / Tick  = 1 00
```



Appendix IV (continued)

Figure 8: **Chromatogram of control potato sample, 2 µL of 5 mL injected. POT-C1, Reference Data Sheet #: X9618V01**

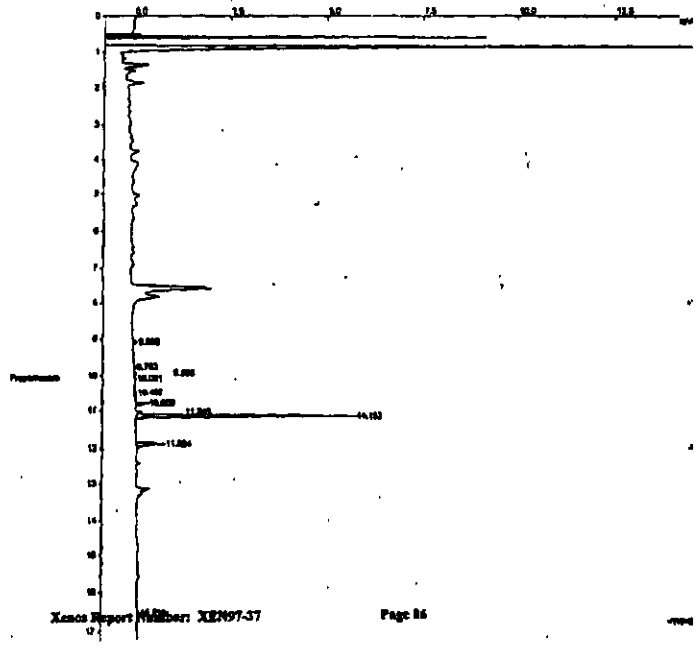
```
Title        : Propamocarb in potato - XEN96-18, 96AC03
Run File     : c:\star\module18\9618038.run
Method File  : C:\STAR\MODULE18\PROPPOT.MTH
Sample ID    : POT-C1

Injection Date: 31-OCT-96 1:42 AM    Calculation Date: 31-OCT-96 2:00 AM

Operator    : Xenos Laboratories     Detector Type: ADCB (1 Volt)
Workstation: MS-DOS_5                Bus Address  : 18
Instrument : GC 3400                 Sample Rate  : 10.00 Hz
Channel     : A - TSD                Run Time     : 18.002 min

*********** Star Chromatography Workstation ******* Version 4.5 ***************

Chart Speed =    1.00 cm/min   Attenuation = 64          Zero Offset = 5%
Start Time  =   0.000   min     End Time    = 17.200  min   Min / Tick  = 1.00
```



Appendix IV (continued)

Figure 9: Chromatogram of control potato sample, 2 µL of 10 mL injected. FOT-C2, Reference Data Sheet #: X9618V02

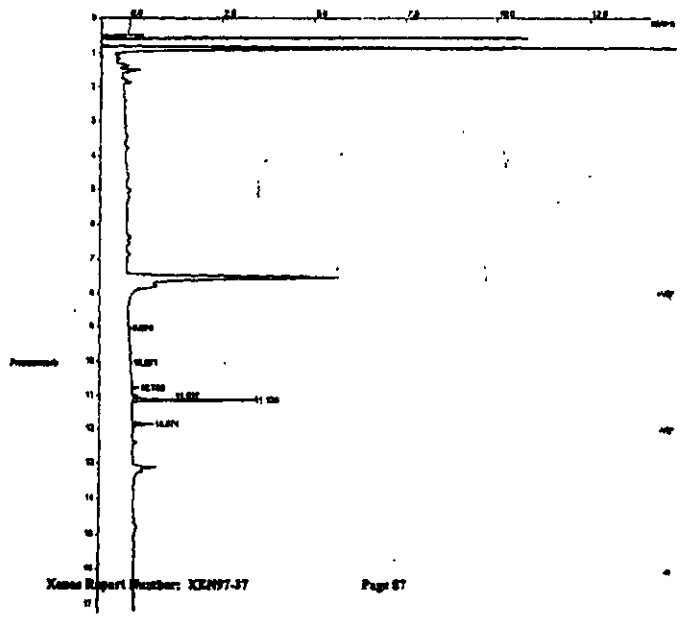
```
Title        : Propamocarb in potato - XE#96-18, 96AC03
Run File     : c:\star\module18\9618044.run
Method File  : C:\STAR\MODULE18\PROPPOT.MTH
Sample ID    : POT-C2

Injection Date: 30-OCT-96 6:50 PM     Calculation Date: 30-OCT-96 7:08 PM

Operator    : Xenos Laboratories      Detector Type: ADCB (1 Volt)
Workstation: MS-DOS 5                 Bus Address  : 18
Instrument : GC 3400                  Sample Rate  : 10.00 Hz
Channel    : A - TSD                  Run Time     : 18.002 min

*********** Star Chromatography Workstation ******* Version 4.5 **************

Chart Speed =    1.00 cm/min   Attenuation = 64          Zero Offset = 58
Start Time  =   0.000   min    End Time    = 17.200  min  Min / Tick  = 1.00
```



Appendix IV (continued)

Figure 10: Chromatogram of fortified control potato sample with Hoe 074189 at 0.05 ppm, 2 µL of 5 mL injected.
POT-F1-2, Reference Data Sheet #: X9618V01

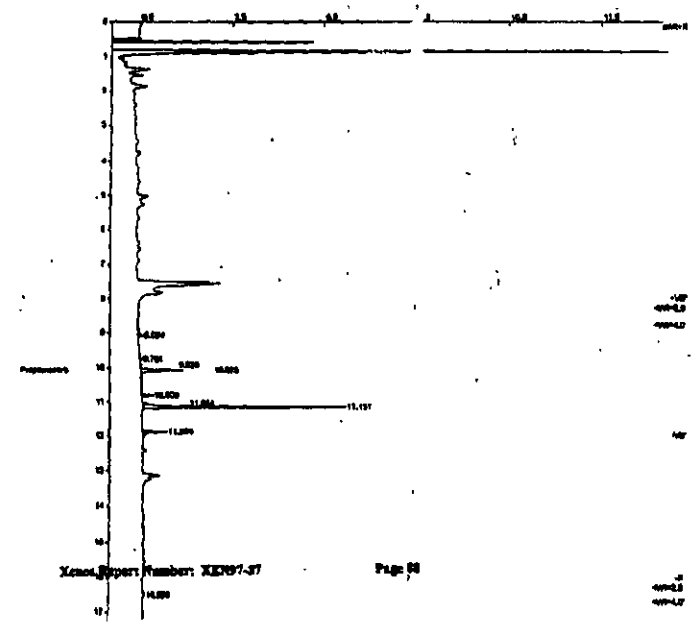
```
Title        : Propamocarb in potato - XEN96-18, 96AC03
Run File     : c:\star\module18\9618062.run
Method File  : C:\STAR\MODULE18\PROPPOT.MTH
Sample ID    : POT-F1-2

Injection Date: 31-OCT-96 3:39 AM    Calculation Date: 31-OCT-96 3:58 AM

Operator     : Xenos Laboratories     Detector Type: ADCB (1 Volt)
Workstation: MS-DOS_5                 Bus Address  : 18
Instrument : GC 3400                  Sample Rate  : 10.00 Hz
Channel    : A = TSD                  Run Time     : 18.002 min

********** Star Chromatography Workstation ******** Version 4.5 **************

Chart Speed =    1.00 cm/min   Attenuation = 64          Zero Offset = 5%
Start Time  =    0.000   min    End Time    = 17.21[illegible] min   Min / Tick  = 1.00
```



Appendix IV (continued)

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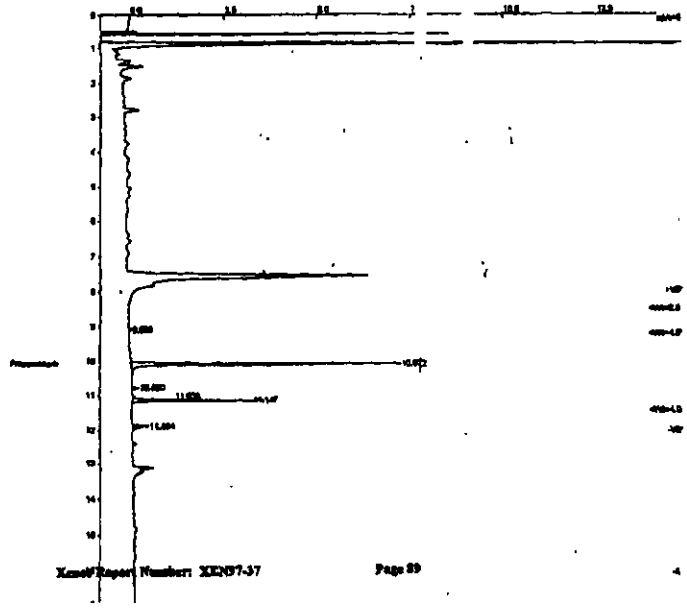
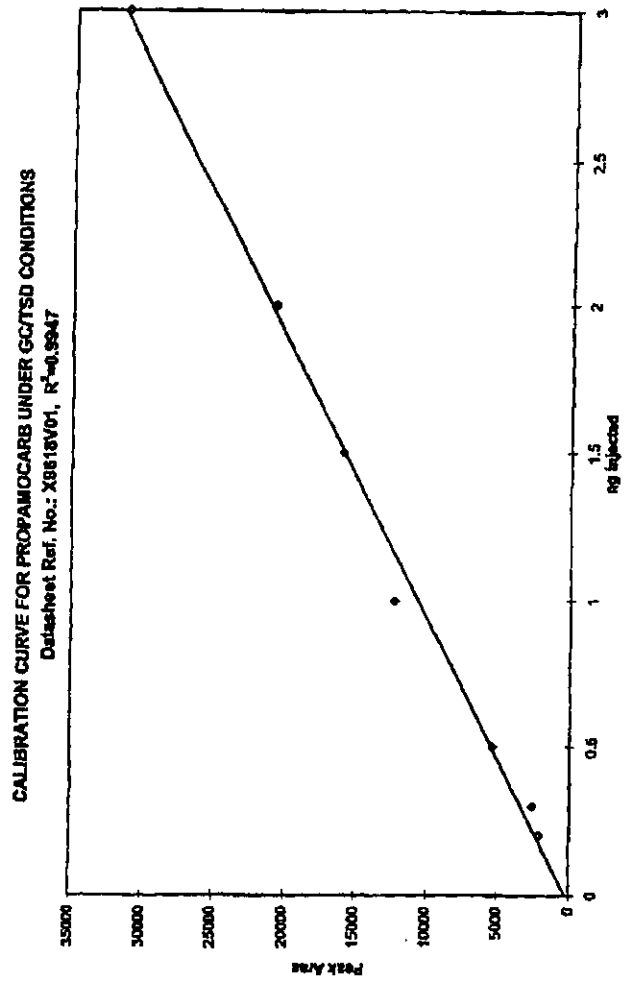
Figure 11: Chromatogram of fortified control potato sample with Hoe 074189 at 0.50 ppm, 2 µL of 10 mL injected.
POT-F2-2, Reference Data Sheet #: X9618V02

```
Title        : Propamocarb in potato - XEN96-18, 96Ac03
Run File     : c:\star\module10\9618040.run
Method File  : C:\STAR\MODULE10\PROPPOT.MTH
Sample ID    : POT-F2-2

Injection Date: 30-OCT-96 8:47 PM    Calculation Dat : 30-OCT-96 9:06 PM

Operator     : Xenos Laboratories    Detector Type: A CB ( Volt)
Workstation: MS-DOS_5                Bus Address  : 1 .
Instrument : GC 3400                 Sample Rate  : 1 .00  s
Channel    : A = TSD                 Run Time     : 1 .002 min

*********** Star Chromatography Workstation *******  ersi : 4.5 ***************

Chart Speed =    1.00 cm/min   Attenuation = 64          Zero Offset = 5%
Start Time  =   0.000   min     End Time    = 17.200  min  Min / Tick  = 1.00
```



Appendix IV (continued)



Appendix IV (continued)

GAS CHROMATOGRAPHIC DATA SHEET

Xenos Project No.: XE96-18
Sponsor Study No.: Validation

Extracted : October 29, 1996
Injected: October 30, 1996
Analyst: N. Bertrand

Compound: Propamocarb
Matrix: Potato

Xenos Ref. No.: X9618V01
Verified:

GC Run #	Sample ID	Sample Weight g	Final Vol. mL	Injected uL	Injected ng	Peak Area	Found from curve/injected (ng)	Found (ppm)	ppm Added	% Recovery
56	DIPE			2		331	ND	ND		
57	0.100 ng/µL			2		2076	0.200			
58	POT-C1	20.00	5	2	8.0	125	-0.009	-0.001		
59	0.150 ng/µL			2		2524	0.300			
60	POT-F1-1	20.00	5	2	8.0	3407	0.293	0.037	0.05	73.2
61	0.250 ng/µL			2		5389	0.500			
62	POT-F1-2	20.00	5	2	8.0	3636	0.314	0.039	0.05	78.6
63	0.500 ng/µL			2		12342	1.000			
64	POT-F1-3	20.00	5	2	8.0	4207	0.369	0.046	0.05	92.3
65	0.750 ng/µL			2		15997	1.500			
66	POT-F1-4	20.00	5	2	8.0	2633	0.219	0.027	0.05	54.7
67	1.00 ng/µL			2		20833	2.000			
68	POT-F1-5	20.00	5	2	8.0	3408	0.293	0.037	0.05	73.2
69	1.50 ng/µL			2		31382	3.000			

GC Instrument
Model: Varian 3400
Serial No.: 8682
Detector: TSD
Column: DB-1, 15 m X 0.53MM
Column No: 6167814A
Film thickness: 5.0 µm

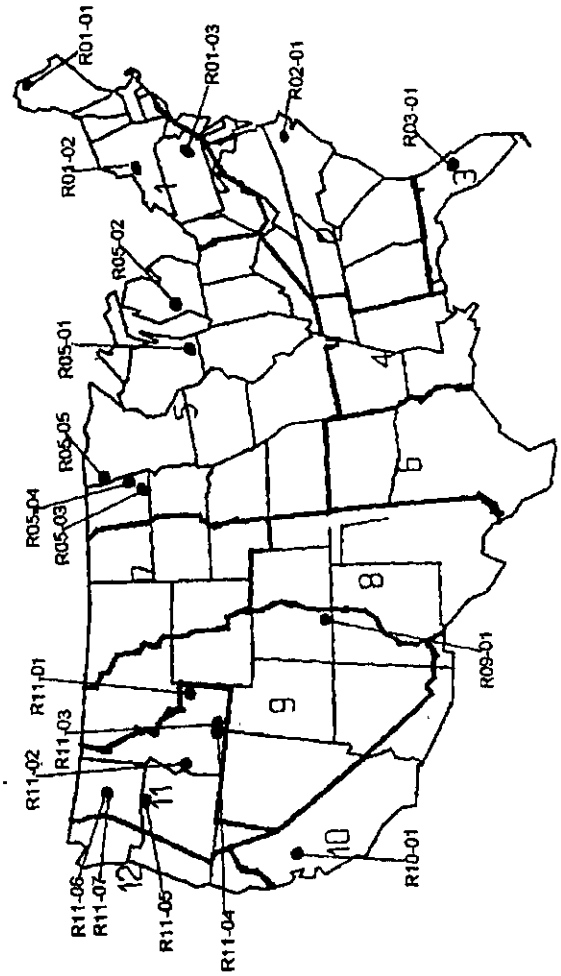
Integrator
Model: Varian Star Workstation
Chart speed: 1.0 cm/min
Attenuation: 64
Range: 13

Temperatures
Column:
initial: 100 °C, hold: 0.2 min
program 1,
rate: 5.0 °C/min
final: 140 °C, hold: 0 min
program 2:
rate: 30.0 °C/min
final: 230 °C, hold: 13.0 min
Inlet: 275 °C
Detector: 300 °C Bead Power: 3.140

Regression Output
Propamocarb
R Square 0.9947327
Intercept 217.18048
Slope 10472.343
No. Obs. 7

Notes
ND = No peak detected
O/R = peak outside of calibration range
* = estimated control peak area

Appendix V Map of Trial Locations



EPA ADDENDUM

PP#6F04707

Propamocarb HCl on whole potatoes and tomatoes

1. ACB used a Hewlett-Packard 6890 *plus* Gas Chromatograph equipped with a nitrogen phosphorus detector (GC-NPD). The detector was interfaced to a TurboChrom (ver 6.1.1) data system. The analytical column was a fused silica DB-1, 15m, 0.53 mm i.d. (Megabore), 1.5 µm film thickness (J&W Scientific). The column temperature was programmed according to the method. A Hewlett-Packard 7683 Automatic Liquid Sampler was used for sample injections. A 4mm splitless straight bore injection liner was used in the injection port. The injection port was maintained at 275 °C. The detector temperature was 300 °C. Helium was used as the carrier gas with a constant flow rate of ~5 mL/min @ 100 °C. The detector gas flows were set at the following: hydrogen @ 3 mL/min, air @ 60 mL/min, and nitrogen (make-up) @ 19 mL/min. A 2 µL injection volume was used and the retention time under these conditions was approximately 8.8 min. The analysis time was 25 min.

2. The TurboVap®LV (Zymark Corporation, Hopkinton, MA) operated at 40 °C under a nitrogen stream was used in place of the vacuum rotary evaporators to concentrate samples during the final step only (the evaporation of 100 mL of DIPE to 2 mL). Initially, ACB used the TurboVap at 45 °C. to evaporated the methanol from the first extraction step rather than the Rotary Vacuum Evaporator set at 45 °C. This reduced the acidic aqueous to 5 mL (as recommended in the method) but took about 3 hours of time/sample. In a teleconference with the petitioner, it was suggested that 3 hours was too long and some loses might be occurring in that step. The recoveries were consistently lower when the TurboVap was used during the first step. Recoveries were improved when the rotary vacuum evaporator was used during the first step of the extraction. In our laboratory, it was difficult to achieve the 5 mL volume as recommended in the method. Our final volumes ranged from 10-12 mL. This created problems in the subsequent steps of the extraction.

3. ACB found, and the petitioner agreed, that the volume of 10N NaOH could be altered from the method (10 mL) and still yield good recoveries. Following the first step from the rotary vacuum evaporator, an additional 5 mL of 1N HCl is added to the extract to ensure a low pH, this acidic aqueous extract should be transferred to the 25 mL graduated cylinder before the base is added (the 20 mL volume cannot be exceeded). ACB found that 2-3 mL of base was enough to partition the propamocarb into the organic phase when the extract was poured onto the Chem Elute™ columns. If less than 2 ml of base was added to the acidic aqueous extract, low or no recoveries resulted. The Chem Elute™ columns are a gravity flow system (propamocarb is a very volatile compound and if a vacuum was applied to the Chem Elute™ columns,

losses of analyte occurred). The 5 min equilibration timing should begin as soon as the meniscus from the 20 mL volume disappears onto the column.

4. The method suggests the use of calibration curves to determine sample concentrations. ACB ran calibration curves to demonstrate linearity over the dynamic range of the fortified samples. ACB determined sample concentrations from a ratio of sample responses to the average of standard responses that bracketed the samples during analysis. The bracketing standards were made to be the same concentration as the expected concentration of the fortified samples if 100% recovery were obtained. ACB observed some sample carry-over between injections. ACB found that it was important to use fresh washing solvent for both vial A and vial B in the autosampler tower at the beginning of a sample set. ACB suggests the use of blank injections of DIPE within a sample set to minimize carry-over. ACB used acetone for both washing solvents A and B, the method did not specify a solvent.

5. ACB determined that a good stopping point was after the first step of the extraction. The acidic aqueous extract could be stoppered and stored in the refrigerator overnight and the clean-up resumed the following day. Once the final extracts were prepared, they were assayed the same day (usually unattended overnight) with bracketing standards. The method did not give information regarding the stability of the final extract nor did they mention possible stopping points.

6. The fortification solutions and the calibration standard solutions are made from individual weighings of analytical reference standards. ACB recommends that both weighings occur under the same laboratory conditions (same day), since one set of solutions is measured against a different set of solutions.