

FINAL ANALYTICAL REPORT

STUDY TITLE

Magnitude of the Residue of Oryzalin 4™ in or on Selected Crops
Grown in California
Cherry Trials

DATA REQUIREMENT

California Department of Pesticide Registration (CDPR)

EPA Guidelines OPPTS No. 860.1500
EPA 40 CFR 158

SPONSOR

Nations Ag II, LLC
2901-12 Rivendell
Knoxville, TN 37922

STUDY DIRECTOR

Daniel L. Galt, Ph.D.
Hulst Research Farm Services, Inc.

PERFORMING LABORATORY

Morse Laboratories, Inc.
1525 Fulton Avenue
Sacramento, California 95825
(916) 481-3141

STUDY IDENTIFICATION

HRFS Protocol No.: NA0201
Morse Project No.: ML02-1020-NAG

AUTHOR

Susan Clark

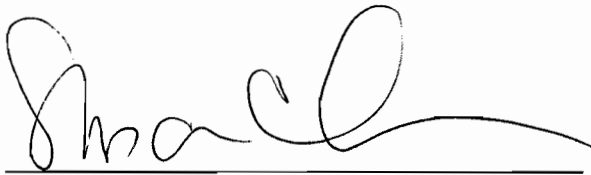
REPORT DATE

June 27, 2002

GLP STATEMENT OF COMPLIANCE
Cherry Trials

All aspects of this study carried out by **MORSE LABORATORIES, INC.** were conducted in accordance with the FIFRA Good Laboratory Practice Standards (40 CFR 160), as applicable, with the following exception:

The oryzalin analytical standard used for this study was not characterized according to the provisions of 40 CFR 160.105(a). The purity provided by the manufacturer was used.



Susan Clark
MORSE LABORATORIES, INC.
Principal Analytical Investigator

June 27, 2002
Date



Gary L. Westberg
MORSE LABORATORIES, INC.
Laboratory Director

June 27, 2002
Date

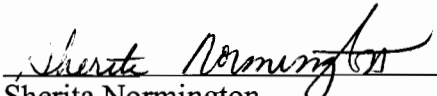
QUALITY ASSURANCE STATEMENT
Cherry Trials

Morse Laboratories, Inc. Project Number: ML02-1020-NAG

Protocol Number: NA0201

Quality Assurance inspections were carried out during the execution of the Study by Quality Assurance personnel according to §40 CFR 160.35 of the EPA Good Laboratory Practice Standards to ensure the integrity of the data. The final analytical report, as submitted to the Study Director, has been determined to be accurate.

<u>Phase Inspected</u>	<u>Dates of Inspection</u>	<u>Date Findings Reported to the Study Director/Management</u>
Protocol Review	06/13/02	06/26/02
In Process	06/17/02, 06/20/02	06/26/02
Raw Data	06/25/02, 06/26/02	06/27/02
Final Report	06/25/02, 06/26/02, 06/27/02	06/27/02



Sherita Normington
Quality Assurance Coordinator

June 27, 2002

ANALYTICAL PHASE IDENTIFICATION

Title: Magnitude of the Residue of Oryzalin 4™ in or on Selected Crops Grown in California

Study No.: NA0201

Sponsor: Nations Ag II, LLC
2901-12 Rivendell
Knoxville, TN 37922

Sponsor Monitor: Jimmy S. Lovell
Nations Ag II, LLC
4680 Monticello Avenue, Suite 18i-174
Williamsburg, VA 23188

Study Director and Testing Facility: Daniel L. Galt, Ph.D.
Hulst Research Farm Services, Inc.
4449 Tully Road
Hughson, CA 95326

Principal Analytical Investigator and Performing Laboratory: Susan Clark
Morse Laboratories, Inc.
1525 Fulton Ave.
Sacramento, CA 95825

Date Study Protocol was signed by the Study Director: March 31, 2002

Experimental Analysis Initiation Date: June 6, 2002 (first field samples pitted)

Experimental Analysis Completion Date: June 24, 2002 (last data calculated)

Date Final Analytical Report was issued: June 27, 2002

AUTHENTICATION

This report is an accurate and authentic representation of the conditions and results of the analytical phase of this study.



Susan Clark
Principal Analytical Investigator

June 27, 2002
Date



Gary L. Westberg
Laboratory Director

June 27, 2002
Date

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Zweig, G., "Analytical Methods for Pesticides and Plant Growth Regulators," Volume VIII, Chapter 28 – Oryzalin, Pages 438 through 441

Morse Laboratories, Inc. Method Modifications, dated June 19, 2002, to Zweig, G., "Analytical Methods for Pesticides and Plant Growth Regulators," Volume VIII, Chapter 28 – Oryzalin, Pages 438 through 441

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1 SUMMARY

The purpose of this study was to determine the magnitude of residues of oryzalin in or on cherry fruit following two pre-harvest, CO₂ backpack sprayer applications of Oryzalin 4™. A total of 6 samples were received from the field test sites on June 6, 2002 and analyzed for residues of oryzalin.

Analytical method performance was monitored through concurrent analysis of freshly fortified control samples along with field samples. Overall oryzalin procedural recoveries yielded a mean of 98% (n = 2) and ranged from 93% to 104%.

The residue of oryzalin found in each cherry sample was determined. Residue values were not corrected for procedural recovery results.

No residues >0.05 ppm (LOQ) of oryzalin were found in any untreated control samples or treated field samples.

2 INTRODUCTION

The purpose of this study was to determine the magnitude of residues of oryzalin in or on cherries following two pre-harvest, CO₂ backpack sprayer applications of Oryzalin 4™. The analytical portion of this study was conducted by Morse Laboratories, Inc. under Morse Project No. ML02-1020-NAG in accordance with HRFS Protocol No. NA0201, entitled "Magnitude of the Residue of Oryzalin 4™ in or on Selected Crops Grown in California" and Protocol Amendments 1, 2, 3, and 4. This report contains the following: information on reference material, experimental details, a summary of the analytical method, calculations, results and discussion, conclusions, residue data, procedural recovery data and representative chromatograms.

3 SAMPLE RECEIPT, LOGGING AND STORAGE

Cherry samples were received frozen in good condition at Morse Laboratories, Inc. on June 6, 2002. One cherry sample was purchased, at ambient temperature, from a local grocery store (Corti Brothers, 5810 Folsom Blvd., Sacramento, CA) on May 28, 2002 for method validation purposes.

Upon receipt, all samples were transferred to a limited-access freezer (FR-16-96) for storage where they remained until they were pitted/ground for analysis. All samples were logged in according to Morse Laboratories, Inc. SOPs using the

original sample numbers assigned to them. Freezer storage temperatures were monitored on a daily basis and were at -20 ± 5 °C.

4 MATERIALS

4.1 Sample Matrix

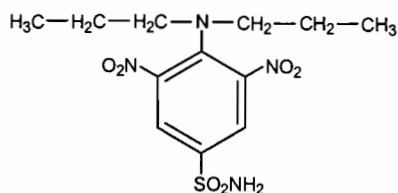
The sample matrix investigated in this study was pitted cherry fruit.

4.2 Reference Material

The analytical standard used for this study was:

Oryzalin

Common name: Oryzalin
Chemical name: 3,5-dinitro-N4, N4-dipropylsulfanilamide
Molecular formula: $C_{12}H_{18}N_4O_6S$
Structural formula:



Oryzalin

CAS no.: 19044-88-3
Physical state: Solid
Molecular weight: 346.40
Source: Chem Service, Inc.
Lot no.: 270-142A
Purity: 98%
Date received: May 17, 2002
Expiration date: September, 2004
Storage: ~1 to 8 °C

5 PREPARATION OF STANDARD SOLUTIONS

5.1 Stock Standard Solution

Twenty-five (25.0) mg (corrected for purity) of oryzalin analytical standard were accurately weighed and quantitatively transferred to a 25 mL volumetric flask and brought to volume with benzene. The resulting concentration was 1000 $\mu\text{g/mL}$. This solution was stored in the dark at approximately 1 to 8 °C when not in use.

5.2 Laboratory (Procedural) Fortification Standard Solutions

The following concentrations of oryzalin were prepared. All solutions were stored in the dark at approximately 1 to 8 °C when not in use.

100 $\mu\text{g/mL}$: 2.5 mL of a 1000 $\mu\text{g/mL}$ standard solution were transferred to a 25 mL volumetric flask. The contents were brought to volume with benzene. The solution was mixed well.

10 $\mu\text{g/mL}$: 250 μL of a 1000 $\mu\text{g/mL}$ standard solution were transferred to a 25 mL volumetric flask. The contents were brought to volume with benzene. The solution was mixed well.

5.3 Oryzalin Dimethyl Derivative

50 $\mu\text{g/mL}$: 250 μL of a 1000 $\mu\text{g/mL}$ oryzalin standard was added to a 125 mL evaporation flask. The standard was manually blown to dryness with nitrogen and dissolved in 20 mL methanol. 0.5 g anhydrous sodium carbonate and 3 mL methyl iodide were added and incubated overnight at 50 °C. Forty (40) mL water was added and partitioned two times with 20 mL dichloromethane. The solvent was evaporated at ~50 °C and the standard brought to 5.0 mL with benzene.

5.4 Instrumentation (Calibration) Standard Solutions

0.03 $\mu\text{g/mL}$: 300 μL of a 0.50 $\mu\text{g/mL}$ oryzalin (as dimethyl derivative) standard solution were transferred to a 5.0 mL volumetric flask. The contents were brought to volume with benzene. The solution was mixed well.

0.10 $\mu\text{g/mL}$: 1000 μL of a 0.50 $\mu\text{g/mL}$ oryzalin (as dimethyl derivative) standard solution were transferred to a 5.0 mL volumetric flask.

The contents were brought to volume with benzene. The solution was mixed well.

0.25 $\mu\text{g}/\text{mL}$: 25 μL of a 50 $\mu\text{g}/\text{mL}$ oryzalin (as dimethyl derivative) standard solution were transferred to a 5.0 mL volumetric flask. The contents were brought to volume with benzene. The solution was mixed well.

0.50 $\mu\text{g}/\text{mL}$: 100 μL of a 50 $\mu\text{g}/\text{mL}$ oryzalin (as dimethyl derivative) standard solution were transferred to a 5.0 mL volumetric flask. The contents were brought to volume with benzene. The solution was mixed well.

6 METHOD

6.1 Sample Identification

Identification established in the field was used to uniquely identify each sample in this study.

6.2 Sample Preparation

Untreated control and treated samples were processed on May 28, 2002, June 6, 2002, and June 7, 2002 following Morse SOP# SP-1, revision #4. Samples were pitted while frozen then ground frozen in a Hobart Grinder with dry ice to a homogeneous consistency, then immediately placed in frozen storage.

6.3 Summary of Analytical Method

The analytical method used for the determination of oryzalin in cherries was Zweig, G., "Analytical Methods for Pesticides and Plant Growth Regulators," Volume VIII, Chapter 28 – Oryzalin, Pages 438 through 441 with Morse Laboratories, Inc. Method Modifications, dated June 19, 2002, to Zweig, G., "Analytical Methods for Pesticides and Plant Growth Regulators," Volume VIII, Chapter 28 – Oryzalin, Pages 438 through 441.

Oryzalin residues were extracted from cherries by blending with methanol. An aliquot of the methanol extract was taken, a salt solution was added, and the extract was partitioned with hexane. The pH of the aqueous methanol extract was adjusted to 8.5 and extracted using dichloromethane. Oryzalin was derivatized to yield oryzalin dimethyl derivative, partitioned into dichloromethane and then purified using an alumina column.

Detection and quantitation were conducted using a gas chromatograph utilizing nitrogen phosphorus detection. The LOQ (Limit of Quantitation) was 0.05 ppm.

6.4 Instrumentation

All samples were analyzed by gas chromatography employing nitrogen phosphorus detection. Typical conditions were as follows:

- **Operating Conditions**

Instrument: Hewlett Packard (HP) Model 5890E Gas Chromatograph equipped with a nitrogen phosphorus detector, electronic pressure controlled inlet (packed, purged), an HP6890 autosampler, and an HP G2070AA ChemStation

Analytical Column: 30 m × 0.53 mm i.d., 1.0 μm film thickness Rtx-200

Guard Column: 1.0 m × 0.53 mm i.d., non-polar dimethyl deactivated fused silica

Gas Carrier: Helium

Flow Rate: Column: 10 mL/min.
Makeup: 15 mL/min.

Temperatures: Injector: 240 °C
Detector: 290 °C
Column: 280 °C isothermal

Injection Volume: 2 μL

Note: The column and conditions stated above were satisfactory for the matrix being analyzed. The specific column packing/coating, carrier gas, column temperature and flow rate listed were typical conditions for this analysis. Specific conditions used in this study are noted on each chromatographic run and are not otherwise documented.

- **Calibration/Sample Analysis**

A four-point standard curve was prepared by injecting constant volumes of standard solutions. Constant volume injections were used for sample extracts as well. Sample responses greater than those produced by the highest concentration

of applicable standard in the standard curve required dilution and reinjection. A curve check standard was typically injected every 3-4 sample injections.

6.5 Calculations

6.5.1 Equations

Calculations for oryzalin were conducted using a validated software application to create a standard curve based on linear regression. The regression functions were used to calculate a best fit line (from a set of standard concentrations in $\mu\text{g/mL}$ versus peak response) and to determine concentrations of the analyte found during sample analysis from the calculated best fit line.

The equation used for the least squares fit was:

$$y = mx + b$$

where:

y = peak response

x = $\mu\text{g/mL}$ found for peak of interest

m = slope

b = y-intercept

1. The amount of analyte (in ppm) found in the sample was calculated using the following equation:

$$\text{ppm} = \mu\text{g/mL found} \times \frac{\text{final vol. (mL)}}{\text{sample wt. (g)}} \times \frac{\text{mL solvent.}}{\text{mL aliquot}} \times \text{GC dil. fact.}$$

where:

$\mu\text{g/mL found}$ = $\mu\text{g/mL}$ of oryzalin (as oryzalin dimethyl derivative) found from the chromatogram

final vol. (mL) = final volume of sample extract submitted to instrumentation

sample wt. (g)	=	gram weight of sample extracted
mL solvent	=	mL of extraction solvent
mL aliquot	=	mL aliquot taken from the extraction solvent and carried through the remainder of procedure
GC dil. fact.	=	the magnitude of dilution required to bracket the response of the sample within the standard curve responses. When the sample requires no dilution, the GC dilution factor = 1

- The percent recovery for fortified control samples was calculated using the following equation:

$$\% \text{ Rec.} = \frac{\text{ppm found in fortified control} - \text{ppm found in control}}{\text{fortification level (ppm) added}} \times 100$$

6.5.2 Example Calculations

- ML ticket #80289, Oryzalin, Cherries, Set #2, 41-CH1-UTC, **Control 3**:

0 peak response (area) → 0.0000 µg/mL

$$\text{ppm} = 0.0000 \mu\text{g/mL} \times \frac{1.0 \text{ mL}}{25.0 \text{ g}} \times \frac{200 \text{ mL}}{8.0 \text{ mL}} \times 1$$

$$\text{ppm} = 0$$

Reported = < 0.05 ppm

- ML ticket #80289, Oryzalin, Cherries, Set #2, 41-CH1-UTC, **Fortified Control 7 @ 0.05 ppm**:

3441 peak response (area) → 0.0464 µg/mL

$$ppm = 0.0464 \mu\text{g/mL} \times \frac{1.0 \text{ mL}}{25.0 \text{ g}} \times \frac{200 \text{ mL}}{8.0 \text{ mL}} \times 1$$

$$ppm = 0.0464$$

$$\text{Reported} = 0.0464 \text{ ppm}$$

$$\begin{aligned} \% \text{ Recovery} &= \frac{0.0464 \text{ ppm} - 0.000 \text{ ppm}}{0.05 \text{ ppm}} \times 100 \\ &= 93\% \end{aligned}$$

3. ML ticket #80289, Oryzalin, Cherries, Set #2,
41-CH1-TRT-A, **Field Sample:**

$$327 \text{ peak response (area)} \rightarrow 0.0034 \mu\text{g/mL}$$

$$ppm = 0.0034 \mu\text{g/mL} \times \frac{1.0 \text{ mL}}{25.0 \text{ g}} \times \frac{200 \text{ mL}}{8.0 \text{ mL}} \times 1$$

$$ppm = 0.0034$$

$$\text{Reported} = < 0.05 \text{ ppm}$$

6.6 Sample Analysis

The samples were analyzed in a group referred to as an "analytical set". The set consisted of one control sample, two fortified control samples and five field samples.

6.7 Representative Chromatography

Example chromatograms of GC standards and the associated calibration curve graph from analytical set #2 are presented in this report as Figures 1 through 5. Example chromatograms of the remaining samples from set #2, one unfortified control, two fortified controls (procedural recovery) and five field samples, are presented as Figures 6 through 13. Additional representative example chromatograms, one unfortified control and one fortified control (procedural

recovery) are presented as figures 14 and 15. An example spreadsheet for analytical set #2 for oryzalin is provided as Figure 16.

6.8 Statistics

Statistical methods used were limited to calculations of the mean, range and standard deviation. A validated software program, SYSTAT, version 8.0, was employed to develop all statistical data.

7 RESULTS AND DISCUSSION

Data resulting from the analyses in this study are summarized in Tables 1, 2, 3, and 4.

7.1 Method Validation Results

The analytical method used to quantitate residues of oryzalin was validated on cherries at 0.05 ppm and 2.5 ppm. Method validation procedural recoveries for oryzalin yielded a mean and standard deviation of $102\% \pm 8.0$ ($n = 6$) and ranged from 86% to 108%.

Oryzalin method validation procedural recovery results are provided in Table 2.

7.2 Control and Sample Residue Results

No residues >0.05 ppm (LOQ) of oryzalin were found in any untreated control samples or treated field samples.

Information regarding the sample and sample extract storage intervals from laboratory sample receipt to GC analysis is presented in Table 1.

Oryzalin residue results from field samples are provided in Table 3.

7.3 Procedural Recoveries

Freshly-fortified control samples were analyzed with the analytical set to monitor the procedural recovery of oryzalin. One procedural control sample and two procedural recovery (fortified control) samples were analyzed. Procedural recovery results were corrected for any detectable control contribution.

Overall oryzalin procedural recoveries yielded a mean of 98% ($n = 2$) and ranged from 93% to 104%.

7.4 Protocol/SOP Deviations

No protocol or SOP deviations were generated for this study.

8 CONCLUSION

The overall recovery range and mean for the procedural recoveries analyzed and described in this report indicate that the samples were successfully analyzed.

9 DISPOSITION OF SAMPLES AND RAW DATA

Samples not consumed in this study, which are judged by the Sponsor to be preservable under frozen storage conditions, will be kept in storage at Morse Laboratories, Inc. until Sponsor approval is obtained for their disposal.

All original study-related raw data (with the exception of Morse Laboratories, Inc. tickets and chain of custody documents) have been transferred to the Study Director. Original facility-related data, as well as authentic copies of the raw data and Final Analytical Report, will be maintained in the archive facilities of Morse Laboratories, Inc. (1525 Fulton Avenue, Sacramento, CA 95825).

10 STUDY PERSONNEL

The following personnel were responsible for the conduct of the study:

Job Title

Sample Preparation:

Mike Garrett
Matt Witter

Sample Manager
Laboratory Assistant

Extractions:

Yuk Wong
Kathy Nguyen

Laboratory Technician II
Laboratory Technician II

Instrumentation:

Kevin Clark

Chief GC Chemist

Calculations:

Lesley Davidson

Data Specialist

Principal Analytical Investigator:

Susan Clark

Analytical Project Coordinator

Report Author:

Susan Clark

Analytical Project Coordinator

11 REFERENCES

HRFS Protocol No. NA0201, entitled "Magnitude of the Residue of Oryzalin 4TM in or on Selected Crops Grown in California"

TABLE OF ABBREVIATIONS

Abbreviations used in Tables 1, 2, 3, and 4 are defined below:

Fort.	=	fortification
Fort. Cont.	=	fortified control
GC	=	gas chromatographic
N/A	=	Not applicable
No.	=	Number

TABLE 1

Sampling-to-GC Analysis Interval Data

Sample Identification	Matrix	Sample Type	Sampling Date	Morse Lab Receipt Date	Date Sample Prepared	Extraction Date	GC Injection Date	Sampling-to-Extraction Interval (days)
41-CH1-UTC	Cherries	control	06/04/02	06/06/02	06/06/02, 06/07/02	06/20/02	06/24/02	16
41-CH1-TRT-A	Cherries	treated	06/04/02	06/06/02	06/06/02, 06/07/02	06/20/02	06/24/02	16
41-CH1-TRT-B	Cherries	treated	06/04/02	06/06/02	06/06/02, 06/07/02	06/20/02	06/24/02	16
42-CH2-UTC	Cherries	control	06/04/02	06/06/02	06/06/02, 06/07/02	06/20/02	06/24/02	16
42-CH2-TRT-A	Cherries	treated	06/04/02	06/06/02	06/06/02, 06/07/02	06/20/02	06/24/02	16
42-CH2-TRT-B	Cherries	treated	06/04/02	06/06/02	06/06/02, 06/07/02	06/20/02	06/24/02	16

TABLE 2

Oryzalin Cherry Method Validation Procedural Recovery Data

Sample Identification	Matrix	Set No.	Date Extracted	Analysis Date	Fort. level (ppm)	RESULTS (ppm)	% Recovery
						Oryzalin	Oryzalin
1020A Control 4	Cherries	3	06/19/02	06/21/02	N/A	<0.05	N/A
1020A Control 5	Cherries	3	06/19/02	06/21/02	N/A	<0.05	N/A
1020A Fort. Cont. 9	Cherries	3	06/19/02	06/21/02	0.05	0.0540	108
1020A Fort. Cont. 10	Cherries	3	06/19/02	06/21/02	0.05	0.0519	104
1020A Fort. Cont. 11	Cherries	3	06/19/02	06/21/02	0.05	0.0533	107
1020A Fort. Cont. 12	Cherries	3	06/19/02	06/21/02	2.5	2.57	103
1020A Fort. Cont. 13	Cherries	3	06/19/02	06/21/02	2.5	2.15	86
1020A Fort. Cont. 14	Cherries	3	06/19/02	06/21/02	2.5	2.58	103

Statistics:

Mean: 102%
 Standard Deviation: ± 8.0
 Range: 86% to 108%
 (n = 6)

TABLE 3

Oryzalin Residues Found in Field Samples

Sample Identification	Matrix	Set No.	Sample Type	Results ppm Found
				Oryzalin
41-CH1-UTC	cherries	2	control	<0.05
41-CH1-TRT-A	cherries	2	treated	<0.05
41-CH1-TRT-B	cherries	2	treated	<0.05
42-CH2-UTC	cherries	2	control	<0.05
42-CH2-TRT-A	cherries	2	treated	<0.05
42-CH2-TRT-B	cherries	2	treated	<0.05

TABLE 4

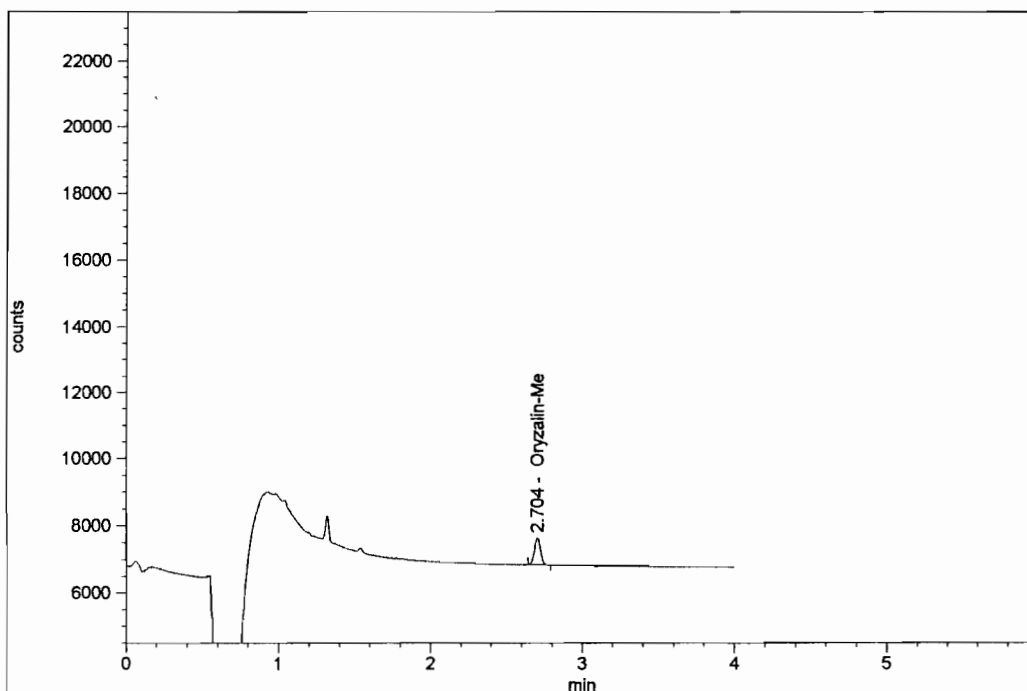
Oryzalin Laboratory Fortification (Procedural) Recovery Data

Sample Identification	Matrix	Set No	Date Extracted	Analysis Date	Fort. level (ppm)	RESULTS (ppm)	% Recovery
						Oryzalin	Oryzalin
41-CH1-UTC Control 3	cherries	2	06/20/02	06/24/02	N/A	<0.05	N/A
41-CH1-UTC Fort. Cont. 7	cherries	2	06/20/02	06/24/02	0.05	0.0464	93
41-CH1-UTC Fort. Cont. 8	cherries	2	06/20/02	06/24/02	2.5	2.60	104

Statistics:

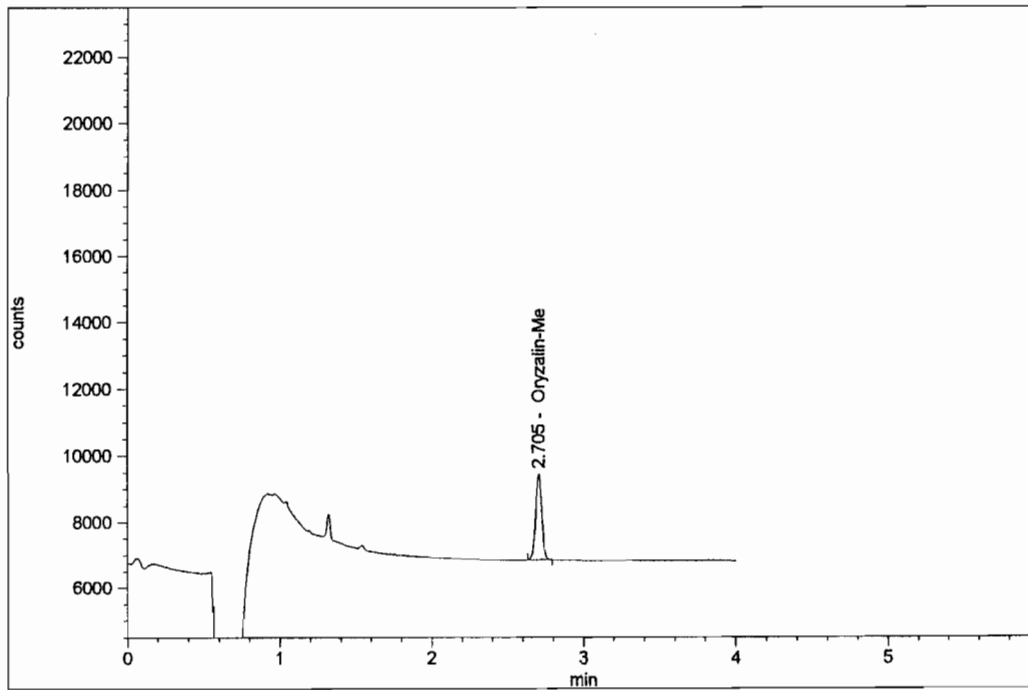
Mean: 98%
 Range: 93% to 104%
 (n = 2)

FIGURE 1
TYPICAL GC STANDARD
Set #2



Oryzalin (as dimethyl derivative) 0.03 $\mu\text{g}/\text{mL}$ Standard
Peak response (area): 2199

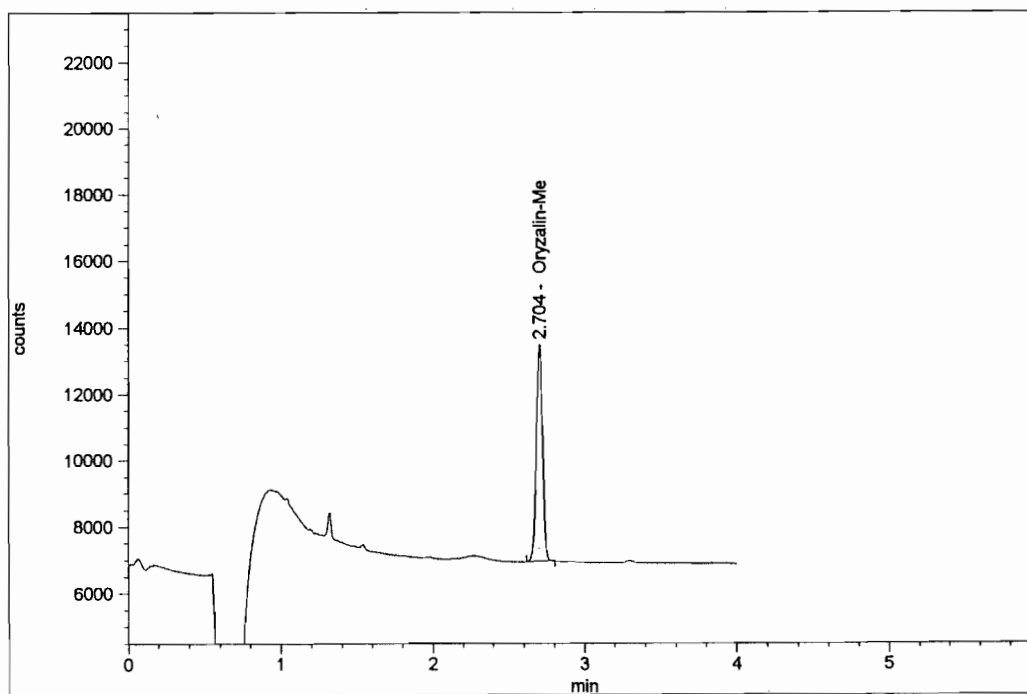
FIGURE 2
TYPICAL GC STANDARD
SET #2



Oryzalin (as dimethyl derivative) 0.10 $\mu\text{g/mL}$ Standard
Peak response (area): 7318

FIGURE 3

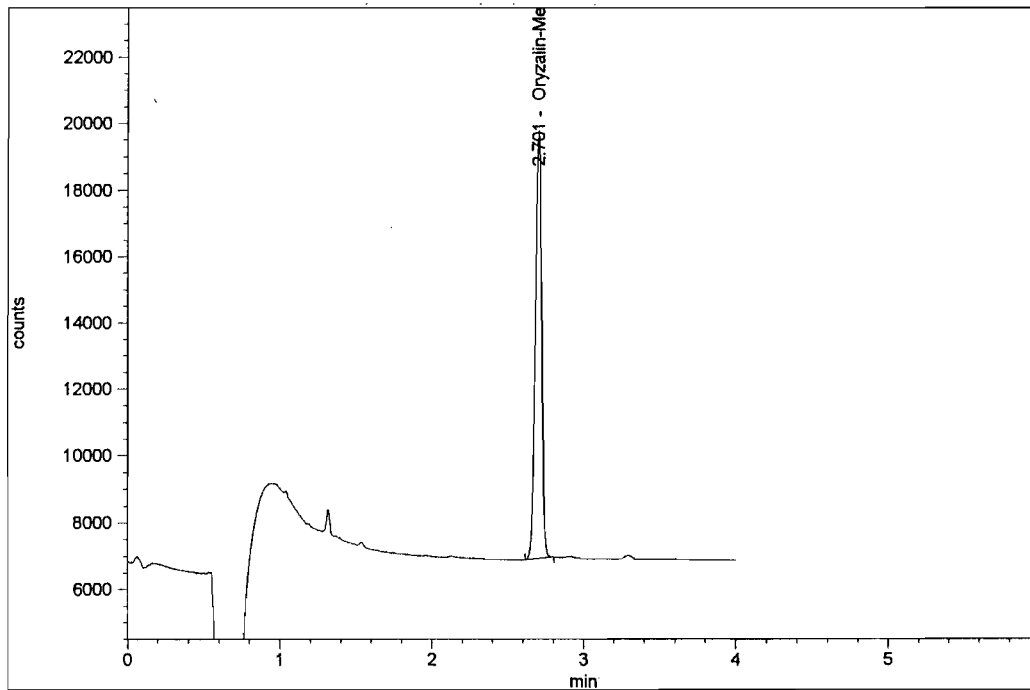
**TYPICAL GC STANDARD
Set #2**



Oryzalin (as dimethyl derivative) 0.25 $\mu\text{g/mL}$ Standard
Peak response (area): 18295

FIGURE 4

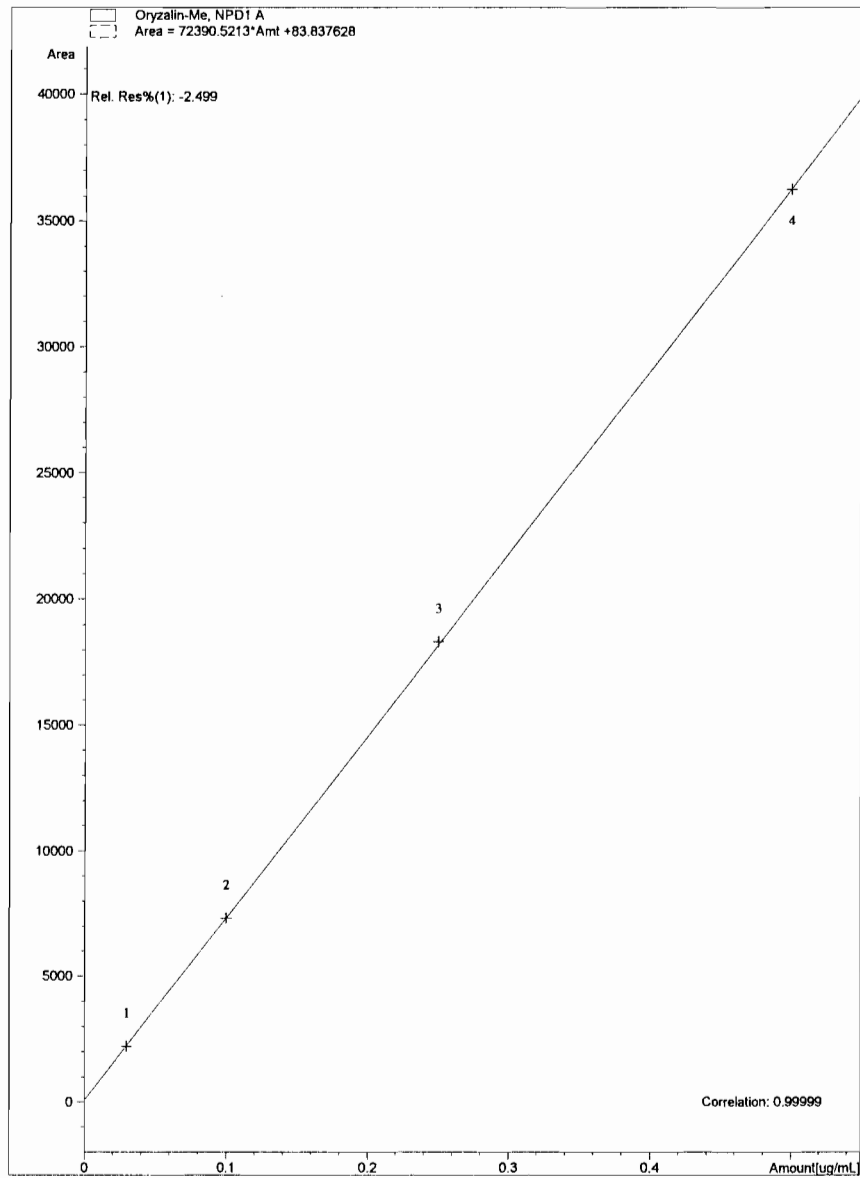
**TYPICAL GC STANDARD
Set #2**



Oryzalin (as dimethyl derivative) 0.50 $\mu\text{g/mL}$ Standard
Peak response (area): 36227

FIGURE 5

TYPICAL ORYZALIN (as Dimethyl Derivative) CALIBRATION CURVE
Set #2



+ Peak response

$r = 0.99999$

$m = 72390.5213$

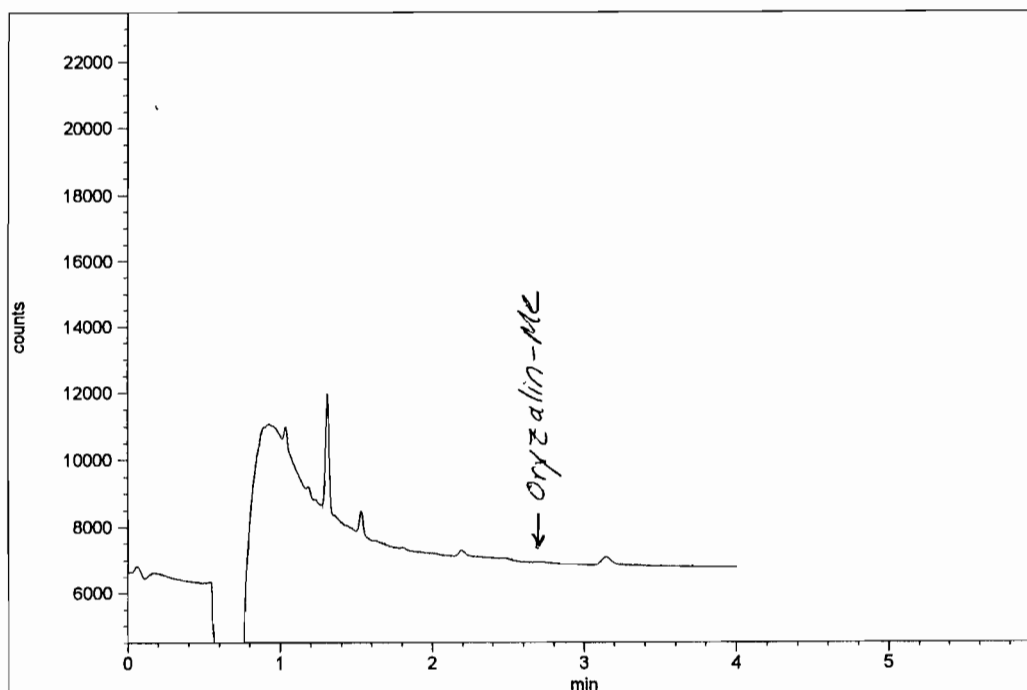
$b = +83.837628$

Equation: $y = mx + b$

Oryzalin (as dimethyl derivative) Calibration Curve

FIGURE 6

**CHERRIES, ORYZALIN, CONTROL SAMPLE
SET #2**



Sample 41-CH1-UTC, Control 3

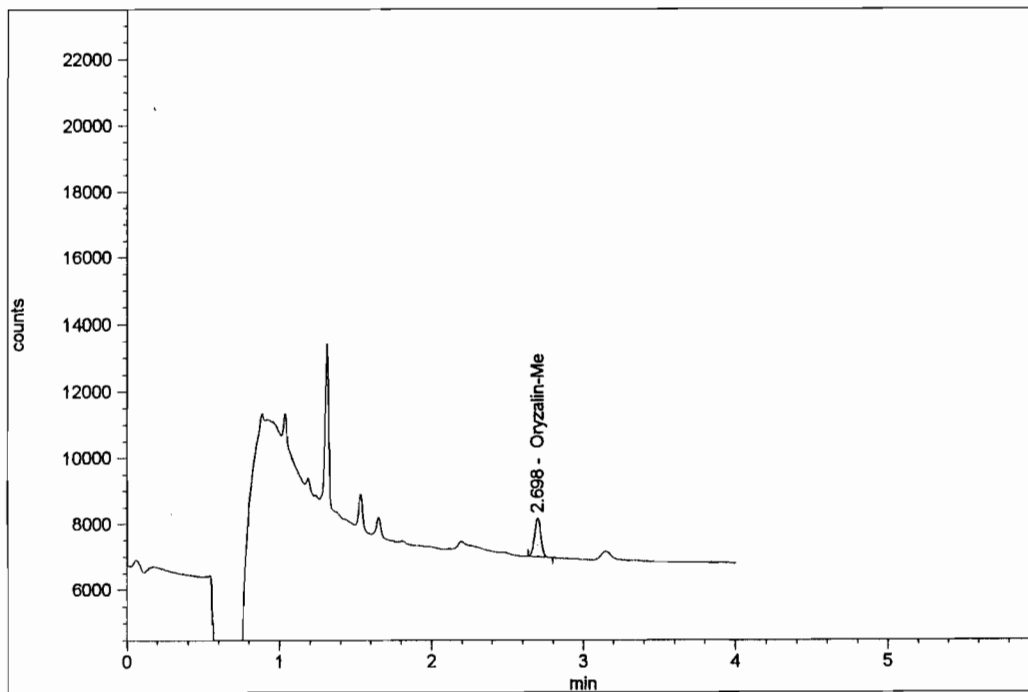
2 μ L

Peak response (area): 0

Oryzalin found: <0.05 ppm

FIGURE 7

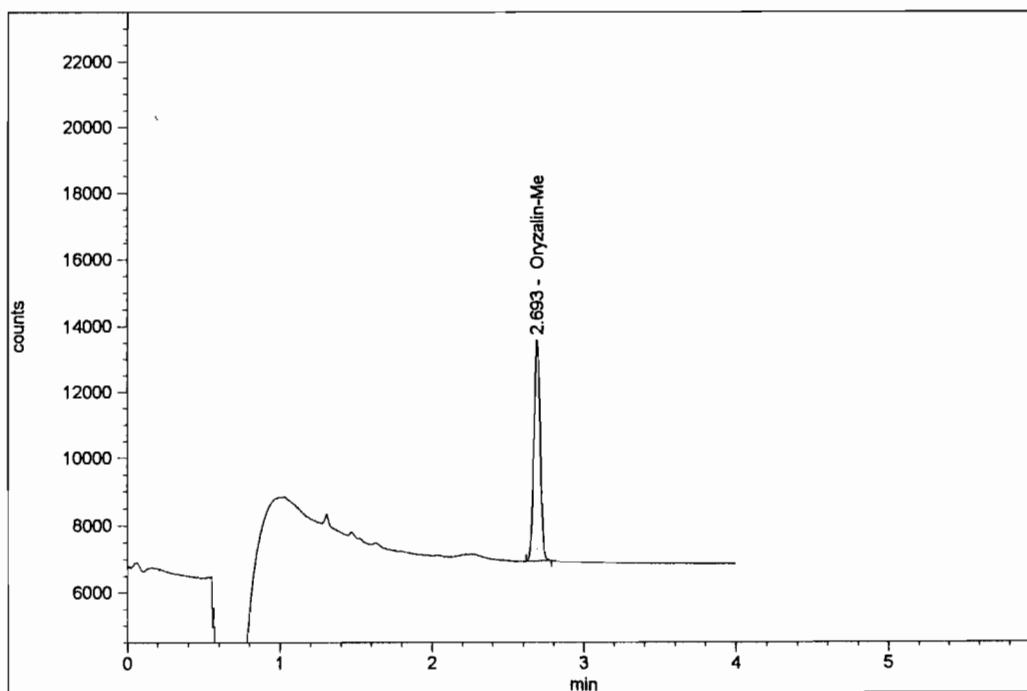
**CHERRIES, ORYZALIN, FORTIFIED CONTROL SAMPLE
Set #2**



Sample 41-CH1-UTC Fortified Control 7
(Oryzalin @ 0.05 ppm)
2 μ L
Peak response (area): 3441
Oryzalin: 93% Recovery

FIGURE 8

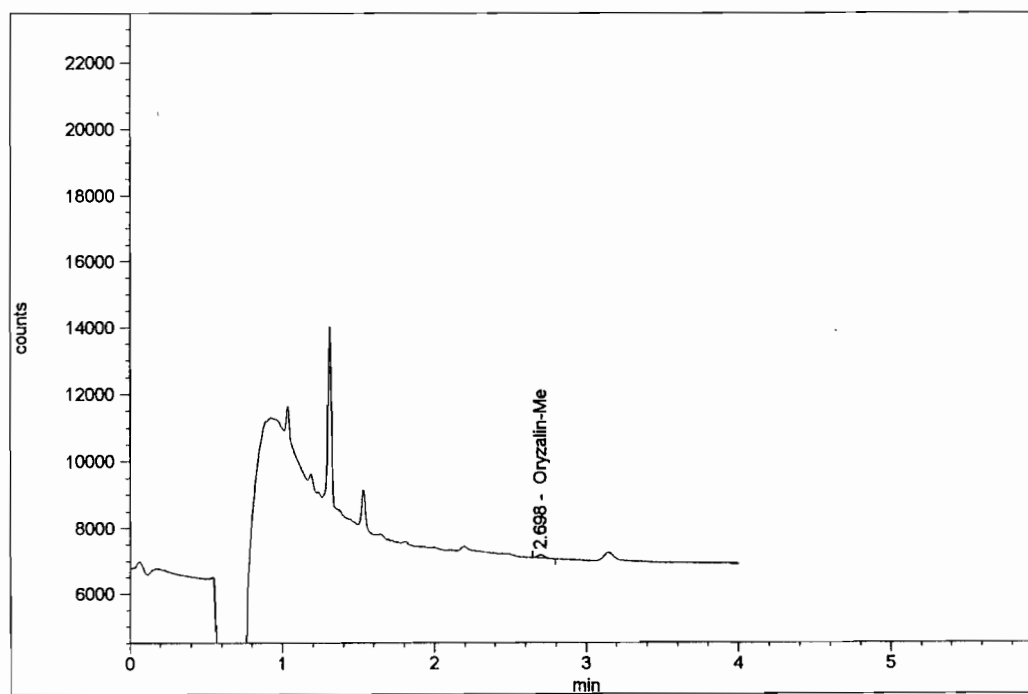
**CHERRIES, ORYZALIN, FORTIFIED CONTROL SAMPLE
Set #2**



Sample 41-CH1-UTC Fortified Control 8
(Oryzalin @ 2.5 ppm)
2 μ L (1 to 10 dilution)
Peak response (area): 18874
Oryzalin: 104% Recovery

FIGURE 9

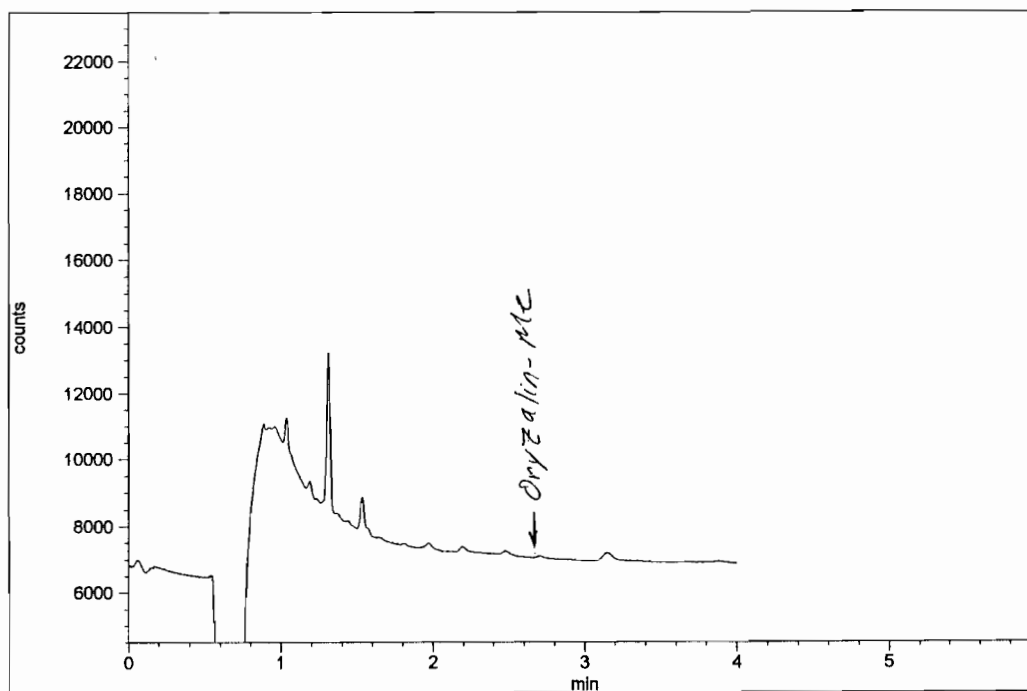
**CHERRIES, ORYZALIN, FIELD SAMPLE
SET #2**



Sample 41-CH1-TRT-A
2 μ L
Peak response (area): 327
Oryzalin found: <0.05 ppm

FIGURE 10

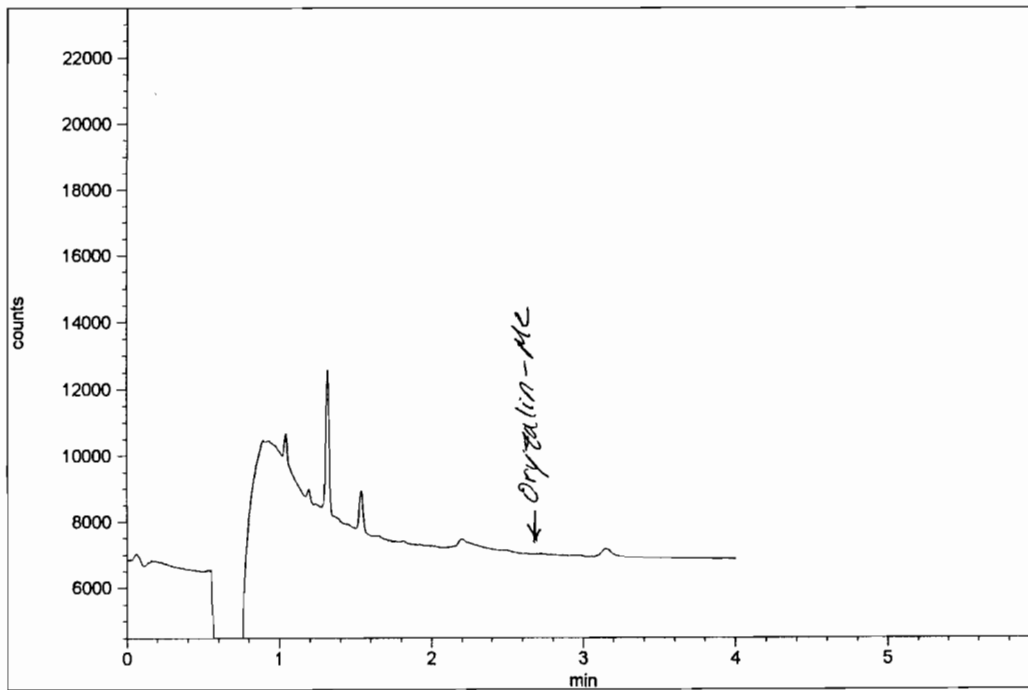
**CHERRIES, ORYZALIN, FIELD SAMPLE
SET #2**



Sample 41-CH1-TRT-B
2 μ L
Peak response (area): 0
Oryzalin found: <0.05 ppm

FIGURE 11

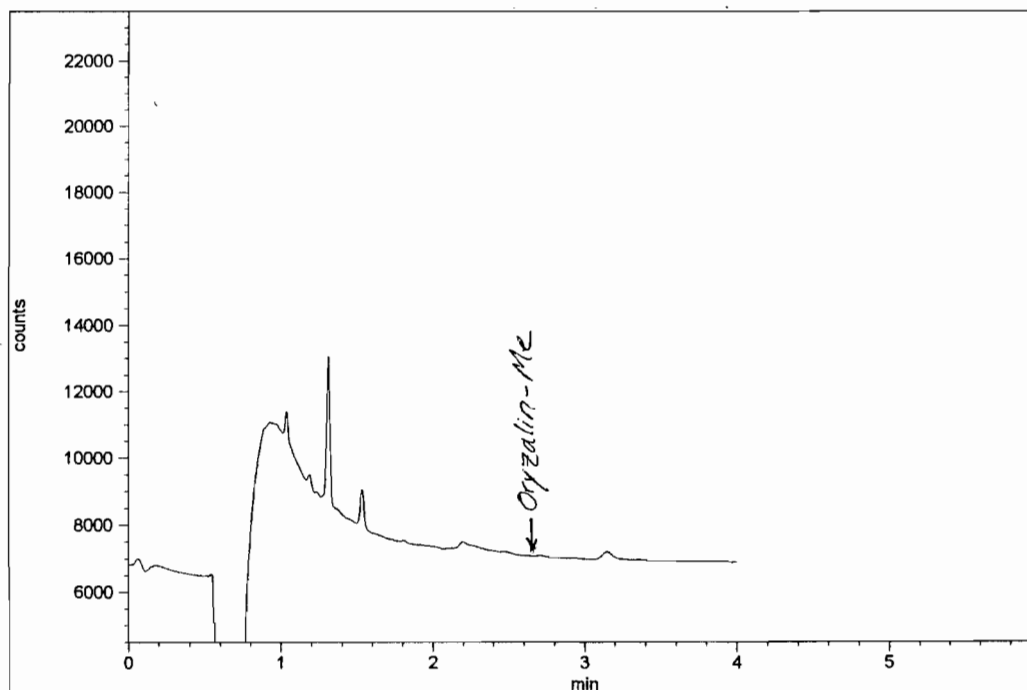
**CHERRIES, ORYZALIN, FIELD SAMPLE
SET #2**



Sample 42-CH2-UTC
2 μ L
Peak response (area): 0
Oryzalin found: <0.05 ppm

FIGURE 12

**CHERRIES, ORYZALIN, FIELD SAMPLE
SET #2**



Sample 42-CH2-TRT-A

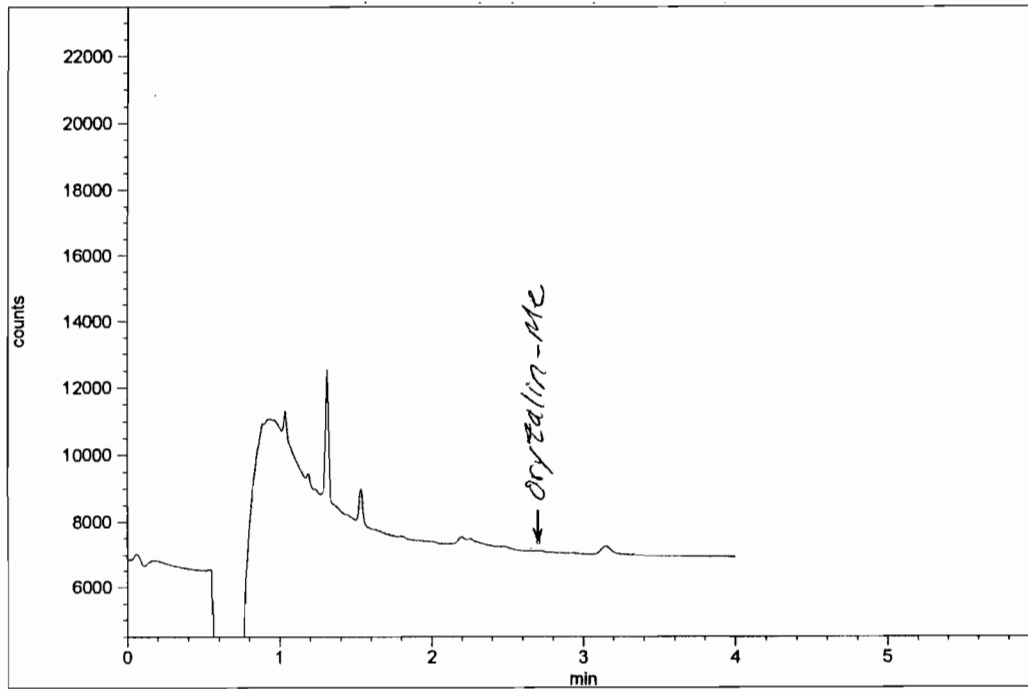
2 μ L

Peak response (area): 0

Oryzalin found: <0.05 ppm

FIGURE 13

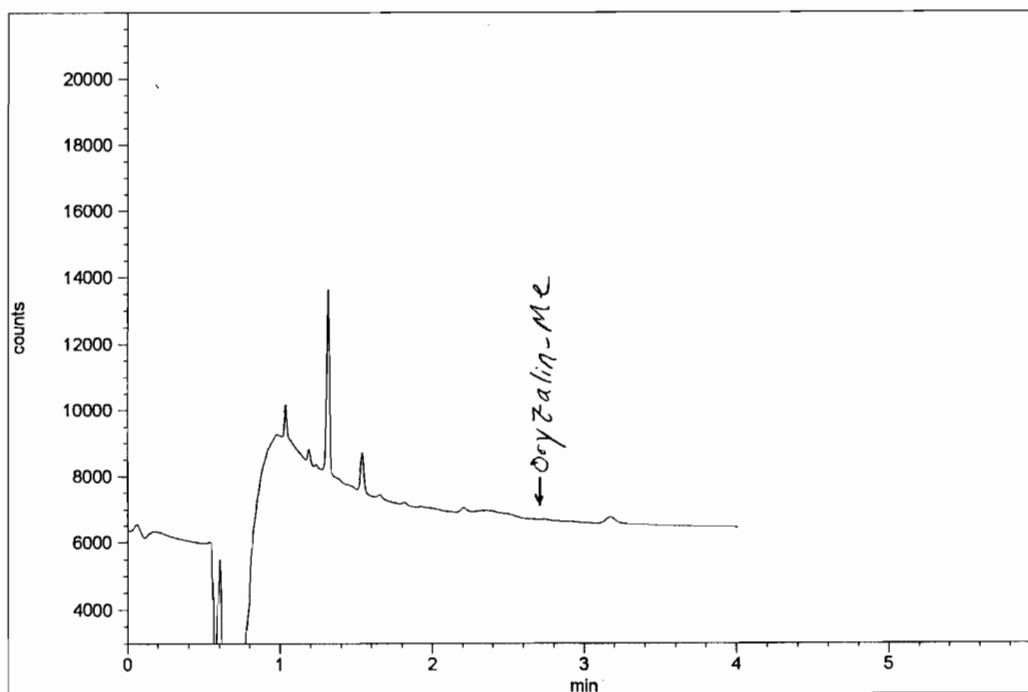
**CHERRIES, ORYZALIN, FIELD SAMPLE
SET #2**



Sample 42-CH2-TRT-B
2 μ L
Peak response (area): 0
Oryzalin found: <0.05 ppm

FIGURE 14

CHERRIES, ORYZALIN, CONTROL SAMPLE
SET #3



Sample 1020A, Control 4

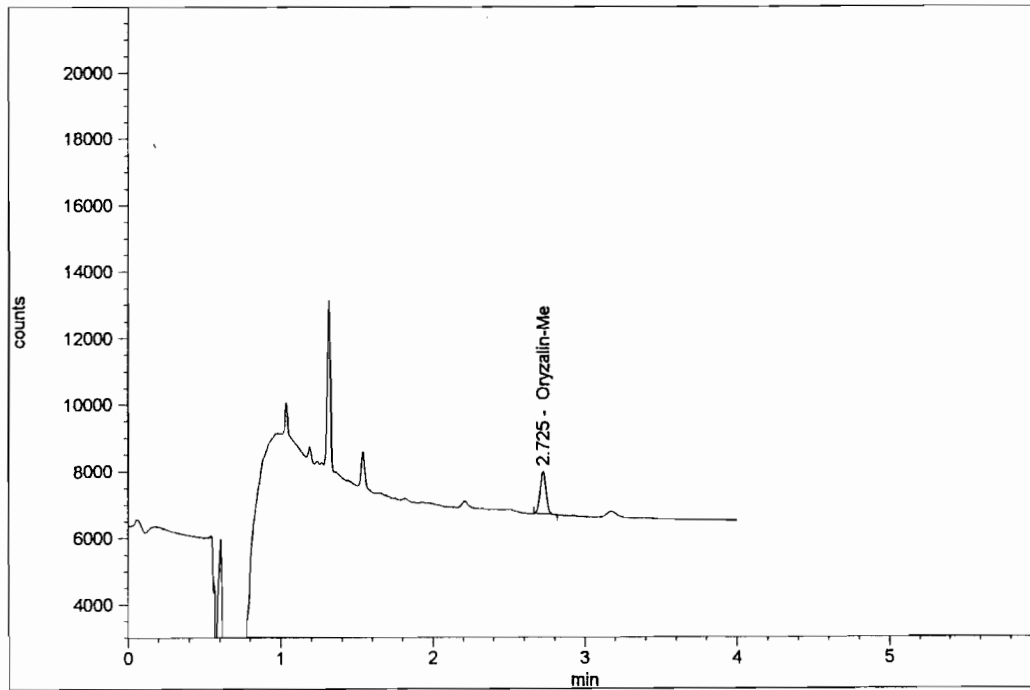
2 μ L

Peak response (area): 0

Oryzalin found: <0.05 ppm

FIGURE 15

**CHERRIES, ORYZALIN, FORTIFIED CONTROL SAMPLE
Set #3**



Sample 1020A Fortified Control 9
(Oryzalin @ 0.05 ppm)
2 μ L
Peak response (area): 3538
Oryzalin: 108% Recovery

Figure 16

Residue results for onyzalin in cherries

Sample Identification	Set No	Samp Wt g	Fort level ppm	Extraction Date	Injection Date	mL solv	mL aliq	Final Vol mL	Inj Vol uL	Dil Fact	Peak Resp	ug/mL found	ppm	reported ppm	% Rec
41-CH1-UTC cont.3	2	25.0		06/20/02	06/24/02	200	8.0	1.0	2	1	0	0.0000	0	<0.05	
41-CH1-UTC fort.cont.7	2	25.0	0.05	06/20/02	06/24/02	200	8.0	1.0	2	1	3441	0.0464	0.0464	0.0464	93
41-CH1-UTC fort.cont.8	2	25.0	2.5	06/20/02	06/24/02	200	8.0	1.0	2	10	18874	0.2596	2.596	2.60	104
41-CH1-TRT-A	2	25.0		06/20/02	06/24/02	200	8.0	1.0	2	1	327	0.0034	0.0034	<0.05	
41-CH1-TRT-B	2	25.0		06/20/02	06/24/02	200	8.0	1.0	2	1	0	0.0000	0	<0.05	
42-CH2-UTC	2	25.0		06/20/02	06/24/02	200	8.0	1.0	2	1	0	0.0000	0	<0.05	
42-CH2-TRT-A	2	25.0		06/20/02	06/24/02	200	8.0	1.0	2	1	0	0.0000	0	<0.05	
42-CH2-TRT-B	2	25.0		06/20/02	06/24/02	200	8.0	1.0	2	1	0	0.0000	0	<0.05	
std.curve 0.25 ug/mL	2				06/24/02						18295				
std.curve 0.50 ug/mL	2				06/24/02						36227				
std.curve 0.03 ug/mL	2				06/24/02						2199				
std.curve 0.10 ug/mL	2				06/24/02						7318				

APPENDIX I

Zweig, G., "Analytical Methods for Pesticides and Plant Growth Regulators," Volume VIII, Chapter 28 – Oryzalin, Pages 438 through 441

Morse Laboratories, Inc. Method Modifications, dated June 19, 2002, to Zweig, G., "Analytical Methods for Pesticides and Plant Growth Regulators," Volume VIII, Chapter 28 – Oryzalin, Pages 438 through 441

Analytical Methods for

PESTICIDES AND
PLANT GROWTH REGULATORS

Edited by

GUNTER ZWEIG

*Office of Pesticide Programs, U.S. Environmental Protection Agency
Washington, D.C.*

Volume VIII

GOVERNMENT REGULATIONS,
PHEROMONE ANALYSIS,
ADDITIONAL PESTICIDES

Edited by

GUNTER ZWEIG *and* JOSEPH SHERMA

*Office of Pesticide Programs
U. S. Environmental
Protection Agency
Washington, D. C.*

*Department of Chemistry
Lafayette College
Easton, Pennsylvania*



ACADEMIC PRESS New York San Francisco London 1976

A SUBSIDIARY OF HARCOURT BRACE JOVANOVICH, PUBLISHERS

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O. D. DECKER AND W. S. JOHNSON

B. Residue Analysis

1. RECOMMENDED METHOD

a. Principle

Oryzalin is quantitatively extracted from plant tissue and soil with methanol. The extract is purified by liquid-liquid partitioning and column chromatography over alumina. Oryzalin is converted to the *N*¹,*N*¹-dimethyl derivative (*N*¹,*N*¹-dimethyl-3,5-dinitro-*N*⁴,*N*⁴-dipropylsulfanilamide) for detection and measurement by gas-liquid chromatography using an electron capture detector.

b. Reagents

Stock Solution. Accurately weigh 10 mg of analytical standard, transfer quantitatively to a 200-ml volumetric flask with benzene, and dilute to volume (50 µg/ml). Store in a refrigerator and prepare fresh every 3 months. Dilutions should be protected from light and prepared fresh weekly.

Methanol, analytical reagent.

Sodium chloride, analytical reagent, 5% w/v in deionized water.

n-Hexane, analytical reagent, redistilled before use.

Carbon tetrachloride, analytical reagent.

Sodium hydroxide, analytical reagent, 1.25 *N*, in deionized water.

Dichloromethane, analytical reagent, redistilled before use.

Sodium sulfate, anhydrous, analytical reagent.

Methyl iodide, analytical reagent, redistilled before use.

-*Alumina*, Alcoa F-20, use as received and standardize as follows:

Place a glass wool plug in the bottom of a 14 mm × 250 mm chromatographic column equipped with a Teflon stopcock and 250-ml reservoir. Add 30 ml benzene to the column. Add 20 gm alumina, and rinse down with benzene.

Remove entrapped air by stirring, and allow the alumina to settle. Gently add 5 ml anhydrous sodium sulfate to the top of the alumina and drain the benzene to the surface of the sodium sulfate. Add 250 µg of the dimethyl derivative of oryzalin to a flask containing 5 ml of benzene. Transfer to the column and start to drain. Rinse the flask with two successive 5-ml portions of benzene, allowing each to go into the column before the next addition. Add an additional 50 ml benzene to the column, allow to drain at a flow rate of 3-5 ml/minute and discard. Add 85 ml of 95:5 benzene-ethyl acetate to the column and observe the movement of the yellow band as the column drains. Note the

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volume of solvent required to move the leading edge of the band to within 1 inch of the bottom of the column and discard this amount of eluate as a forerun. Continue the elution of the yellow band and record the volume required to completely remove the yellow color from the column. This volume, plus an additional 20 ml, will be collected in the column chromatographic procedure to insure complete removal of the compound. Normally, 60–65 ml of 95:5 benzene–ethyl acetate should be collected.

Benzene, analytical reagent, redistilled before use.

Ethyl acetate, analytical reagent.

All reagents should be checked for impurities by electron capture gas chromatography before use in the assay.

c. *Apparatus*

Blender, Omni-mixer (Ivan Sorvall Co.) or equivalent.

Gas chromatograph, Hewlett-Packard model 5713A, with a model 18731A ⁶³Ni linear EC detector, and a model 7123A 1-mV recorder. The chromatographic column is 120 cm × 3 mm i.d. glass tubing packed with 5% XE-60 on 80–100 mesh Chromosorb W HP (Applied Science Laboratories, State College, Pennsylvania). The column is conditioned at 250°C for 16 hours prior to use.

Chromatographic columns, 14 mm i.d. × 250 mm, equipped with a 250-ml reservoir top, and Teflon stopcock.

Rotary vacuum evaporator, Rinco or equivalent.

d. *Experimental Procedure*

i. *Sample Preparation*

(a) *Crops*. Weigh a 25-gm sample of ground and mixed crop tissue into a quart Mason jar. Add sufficient methanol to make 200 ml total liquid with allowance for the water content of the sample. Blend the sample on an Omni-mixer at moderate speed for 5 minutes. Allow the insolubles to settle, and transfer 20 ml of the supernatant liquid to a 250-ml separatory funnel containing 80 ml 5% sodium chloride solution. Add 20 ml additional methanol to the separatory funnel. Wash the aqueous methanol mixture twice with 30-ml portions of hexane and discard the hexane washes. Add 10 ml 1.25 N sodium hydroxide to the aqueous methanol to raise the pH to >12. Wash the aqueous methanol with 30 ml of carbon tetrachloride and discard the wash. Add 20 ml or more of saturated boric acid to the aqueous methanol to give a pH of 8–9. Extract the aqueous methanol twice with 20 ml dichloromethane passing each

extract through anhydrous sodium sulfate into a 125-ml boiling flask. Rinse the sodium sulfate with 10 ml dichloromethane. Remove the dichloromethane by rotary vacuum evaporation using a 50°C water bath. Add 20 ml methanol to the flask and swirl to dissolve. Proceed with the derivatization.

(b) *Soils*. Weigh a 25-gm sample of well-mixed soil into a quart Mason jar. Add 200 ml methanol and blend the sample on an Omnixer at moderate speed for 5 minutes. Allow the insolubles to settle, and transfer 20 ml of the supernatant liquid to a 125-ml boiling flask. Proceed with the derivatization.

ii. *Derivatization*

Add 0.5 gm anhydrous sodium carbonate and 3 ml methyl iodide to the methanol solution of crop or soil extract. Stopper the flask lightly and incubate overnight at 50°C. Following incubation, add 40 ml distilled water to the flask, and transfer the mixture to a 125-ml separatory funnel. Rinse the flask with 20 ml dichloromethane and add to the separatory funnel. Shake the funnel and pass the lower dichloromethane layer through sodium sulfate into a 125-ml boiling flask. Repeat the extraction with an additional 20-ml portion of dichloromethane and combine the extracts. Rinse the sodium sulfate with 10 ml dichloromethane. Remove the solvent by rotary vacuum evaporation using a 50°C water bath.

iii. *Column Chromatography*

Prepare an alumina column using Alcoa F-20 alumina that has been previously standardized. Dissolve the residue in the flask in 5 ml benzene, transfer to the column and start to drain. Rinse the flask with two successive 5 ml portions of benzene, allowing each to go into the column before the next addition. Add an additional 50 ml benzene to the column, allow to drain at a flow rate of 3–5 ml/minute and discard. Add 85 ml 95:5 benzene-ethyl acetate to the column and collect the volume of eluate as determined by the alumina standardization. Remove the solvent by rotary vacuum evaporation using a 50°C water bath.

iv. *Gas Chromatography*

Column temperature: 230°C.

Detector temperature: 300°C.

Injection temperature: 280°C.

Electrometer attenuation: Setting to provide 30–40% full scale recorder deflection on injection of 0.3–0.4 ng of the dimethyl derivative of oryzalin. For the gas chromatograph described, the cell current is constant at 10^{-9} A, and the signal measured is the pulse frequency required to maintain the standing current.

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Carrier gas: Argon (90): Methane (10).

Carrier gas flow rate: 75 ml/minute.

A series of concentrations of standard solutions of dimethyl oryzalin (e.g., 0.025–0.25 $\mu\text{g/ml}$) are prepared to determine the response curve for the electron capture detector. To facilitate standard curve interpolations, the GC standards may be prepared in terms of oryzalin equivalents (1 μg oryzalin = 1.08 μg dimethyl derivative). Otherwise, the mass of dimethyl derivative corresponding to a particular point on the curve must be multiplied by 0.925 to correct for differences in molecular weight of the oryzalin and its dimethyl derivative. Inject 3–3.5 μl of each standard solution into the gas chromatograph. Plot peak response versus nanograms injected to obtain the standard response curve. Check the response with each set of samples assayed, and frequently inject a standard dilution (e.g., 0.1 $\mu\text{g/ml}$) during the course of the assay to determine whether detector response has changed.

Dissolve crop extracts in 1.0 ml benzene and soil extracts in 2.0 ml benzene. Inject the same volume of sample extract as used for the response curve. One microliter of crop extract is equivalent to 2.5 mg of crop tissue, and 1 μl of soil extract is equivalent to 1.25 mg of soil.

v. *Recovery*

Standard recovery samples are assayed with each set of experimental samples. Recovery samples are prepared by adding a methanol solution of oryzalin to 25 gm of control crop tissue or soil to provide a fortification level of 0.05 or 0.10 ppm, respectively. The efficiency of the derivatization of oryzalin is monitored by reacting an amount of oryzalin standard equivalent to the amount present in the recovery sample. The methylated oryzalin standard should also be passed over an alumina column prior to gas chromatography.

Standard recovery samples have given values of 70–75% for crops such as soybeans, peaches, plums, prunes, citrus fruits, and nut meats. Recovery from soil is about 92%. The within-day coefficient of variation for the methylation of oryzalin is about 6%.

vi. *Sensitivity*

The test sensitivity is about 0.01 ppm as determined by the recovery of oryzalin from soybean seed.

vii. *Sample Calculation*

The number of nanograms of oryzalin present in the number of milligrams of plant tissue or soil injected into the gas chromatograph may be obtained from the standard curve. The value obtained may be corrected to reflect assay efficiency as determined from the recovery samples.



1525 Fulton Avenue
Sacramento, CA 95825

TEL: (916) 481-3141
FAX: (916) 481-2959
E-Mail: morseslab@morselabs.com

June 19, 2002

Morse Laboratories, Inc. Method Modifications to Zweig, G., "Analytical Methods for Pesticides and Plant Growth Regulators," Volume VIII, Chapter 28 – Oryzalin, Pages 438 through 441

Reason for Modifications:

Clarification of method steps as well as specific modifications for the analysis of fruit crops.

Modifications Made to Applicable Sections:

b. *Reagents*

Stock Solution

Weigh 25.0 mg (corrected for purity) oryzalin analytical standard. Quantitatively transfer to a 25-mL volumetric flask and dilute to volume with benzene for a 1000 $\mu\text{g/mL}$ stock standard solution. Store in the refrigerator when not in use. Prepare a fresh solution every three months.

Fortification Standard Solutions

Dilute appropriate volumes of the stock standard solution with benzene using volumetric flasks. Store all fortification solutions in the refrigerator when not in use.

The following concentrations are typically prepared: 100 $\mu\text{g/mL}$, 10 $\mu\text{g/mL}$, 1.0 $\mu\text{g/mL}$.

Oryzalin dimethyl derivative

Add 250 μL of a 1000 $\mu\text{g/mL}$ oryzalin standard to a 125-mL flat-bottom flask. Manually blow to dryness with N_2 and dissolve in 20 mL methanol. Continue with the derivatization step and subsequent dichloromethane partition and evaporation. Add 5.0 mL of benzene to the standard flask to yield a 50 $\mu\text{g/mL}$ oryzalin derivatized (dimethyl derivative) standard. Store oryzalin dimethyl standard in the refrigerator when not in use. Prepare fresh solutions weekly.

Standardization of Alcoa F-20 Alumina

- ◆ 19 mm × 300 mm chromatographic column equipped with a Teflon stopcock and 250 mL reservoir.
- ◆ Add ~5 g anhydrous sodium sulfate to the top of the alumina.

c. *Apparatus*

Gas Chromatograph: HP5890 Gas Chromatograph equipped with an N/P detector, electronic pressure controlled inlet (packed, purged), an HP7673 autosampler, and an HPG2070AA ChemStation

Column: 30 m × 0.53 mm i.d., 1.0 μm film thickness RTX-200

Guard column: 3 m × 0.53 mm i.d., non-polar dimethyl deactivated fused silica

d. *Experimental Procedure*i. *Sample Preparation*(a) *Crops*

- ◆ Transfer 8 mL of the supernatant liquid to a 250 mL separatory funnel.
- ◆ Add 35 mL 5% sodium chloride solution to the separatory funnel.
- ◆ Add 10 mL additional methanol to the separatory funnel.
- ◆ After addition of hexane, shake aqueous methanol/hexane mixture 30 seconds each time.
- ◆ Omit addition of 10 mL 1.25 N sodium hydroxide.
- ◆ Omit methanol wash using 30 mL carbon tetrachloride.
- ◆ Omit addition of saturated boric acid to aqueous methanol to yield pH 8-9.
- ◆ Add ~0.3 mL of 0.5 N NaOH to yield pH 8.5.
- ◆ Evaporate dichloromethane to ~1 mL using a rotary evaporator at ~50 °C. Manually blow the sample to dryness with N₂.

ii. *Derivatization*

- ◆ Shake the separatory funnel containing the derivatized sample extract and dichloromethane for 30 seconds each time.
- ◆ Drain the dichloromethane through ~5 g of anhydrous sodium sulfate.
- ◆ Evaporate the dichloromethane extract to ~1 mL using a rotary evaporator at ~50 °C. Manually blow the sample to dryness with N₂.

iii. *Column Chromatography*

- ◆ After rinsing the sample flask, wash the column with 20 mL benzene. Discard.
- ◆ Elute the analyte with 30 mL of benzene. Collect into a 125-mL flat-bottom evaporation flask.
- ◆ Evaporate the benzene extract to ~1 mL using a rotary evaporator at ~50 °C.
- ◆ Quantitatively transfer the extract to a 100 × 13 mm test tube calibrated at 1.0 mL using benzene.
- ◆ Evaporate on an N-EVAP at ~50 °C to 1.0 mL. 1 mL = 1.0 g

iv. *Gas Chromatography*

Gas Carrier: Helium

Flow Rate: Column: 10 mL/min.
Makeup: 15 mL/min.

Temperatures: Injector: 240 °C
Detector: 275 °C
Column: 275 °C isothermal

Injection Volume: 2 μ L

- ◆ Dilute appropriate volumes of the dimethyl derivative standards with benzene using volumetric flasks. Store calibration solutions in the refrigerator when not in use.

The following standard concentrations are typically prepared using the dimethyl derivative standards: 0.03 μ g/mL, 0.10 μ g/mL, 0.25 μ g/mL, 0.50 μ g/mL. The concentrations reflect oryzalin (not oryzalin dimethyl derivative) content.

- ◆ Prepare a four-point standard curve by injecting constant volumes of the calibration standard solutions. Use constant volume injections for sample extracts as well. Sample responses not bracketed by the standard curve require dilution and reinjection.

vii. *Sample Calculations*

Calculations for instrumental analysis are conducted using a validated software application to create a standard curve based on linear regression. The regression functions are used to calculate a best fit line (from a set of standard concentrations in μ g/mL versus peak response) and to determine concentrations (in μ g/mL) of the analyte found during sample analysis from the calculated best fit line.

The equation used for the least squares fit is:

$$y = mx + b$$

where:

- y = peak response
- x = $\mu\text{g/mL}$ found for peak of interest
- m = slope
- b = y - intercept

The calculations for ppm found and percent recovery (for fortified samples) are:

1. The amount of oryzalin (ppm) found in the sample is calculated according to the following equation:

$$\text{ppm} = \mu\text{g/mL found} \times \frac{\text{final vol. (mL)}}{\text{sample wt. (g)}} \times \frac{\text{mL solvent}}{\text{mL aliquot}} \times \text{GC dil. fact.}$$

where:

$\mu\text{g/mL found}$ = $\mu\text{g/mL}$ of oryzalin (as oryzalin dimethyl derivative) found from the chromatogram

final vol. (mL) = final volume of sample extract submitted to instrumentation

sample wt. (g) = gram weight of sample extracted

mL solvent = mL of extraction solvent

mL aliquot = mL aliquot taken from the extraction solvent and carried through the remainder of procedure

GC dil. fact. = the magnitude of dilution required to bracket the response of the sample within the standard curve responses. When the sample requires no dilution, the GC dilution factor = 1

2. The percent recovery for fortified control sample is calculated as follows:

$$\% \text{ Recovery} = \frac{\text{ppm found in fortified control} - \text{ppm found in control}}{\text{ppm added}} \times 100$$