STUDY TITLE

A Method for the Determination of Folpet Residues in Avocados and Other Oily Crops

DATA REQUIREMENT

EPA Pesticide Assessment Guidelines, Subdivision O
Series 171-4 Residue Analytical Method

AUTHORS

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STUDY COMPLETION DATE

March 5, 1996

PERFORMING LABORATORY

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PERFORMING LABORATORY I.D.

PTRL Report No. 568W-1
PTRL Project No. 568W

SPONSOR

Makhteshim-Agan of North America Inc.
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New York, NY 10176

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study identified by: "A Method for the Determination of Folpet Residues in Avocados and Other Oily Crops", PTRL Project Number 568W, on the basis of its falling within the scope of FIFRA § 10(d) (1) (A), (B), or (C).

Company: Makhteshim-Agan of North America Inc.

Company Agent: Robert C. Everich, Ph.D.

Title: Study Monitor

Signature:

Date: Apr. 18, 1996

These data are the property of Makhteshim-Agan of North America Inc. and as such, are considered to be confidential for all purposes other than compliance with FIFRA § 10. Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality which may exist under any other statute or in any other country.

STUDY COMPLIANCE STATEMENT

In accordance with PTRL West's intent that all analytical tests conducted by the facility follow good laboratory practices, PTRL's study director confirms that the study was conducted in compliance with the applicable EPA Good Laboratory Practice Standards (40 CFR 160).

Study Director: _____ Date: 3-5-96
Leo T. Nishioka, B.A.
Study Director
PTRL West, Inc.

Sponsor Representative: _____ Date: April 8, 1996
Robert C. Everich, Ph.D.
Study Monitor
Makhteshim-Agan of North America Inc.

Submitter: _____ Date: _____
Andy Eimanis
Manager, Regulatory Affairs
Makhteshim-Agan of North America Inc.

PTRL WEST, INC. QUALITY ASSURANCE UNIT STATEMENT

Compound: Folpet

Title: A Method for the Determination of Folpet Residues in Avocados and Other Oily Crops

GLP QUALITY ASSURANCE INSPECTION:

Date of GLP Inspections:	Date Reported to Study Director:	Date Reported to Management:	Phases of the Study Which Received a GLP Inspection by the Quality Assurance Unit:
11/3/95	11/4/95	11/6/95	Draft Protocol
12/11/95	12/11/95	12/12/95	Matrix Fortification
12/12/95	12/12/95	12/12/95	GC Analysis
2/21-23/96	2/23/96	3/5/96	Raw Data/Draft Final Report

QUALITY ASSURANCE STATEMENT:

The Quality Assurance Unit has reviewed the final study report and has determined that the report accurately reflects the raw data generated during the conduct of this study.

ARCHIVING:

The original project specific data files are located in the archives of PTRL West, Inc., Richmond, California. The above materials may be transferred to the Sponsor's designated archive facility upon their authorization. Facility records, such as Compound Control logs, equipment use, standardization/calibration, and maintenance logs, etc. and a copy of the final report will be maintained in the archives of PTRL West, Inc.

Specimens will be retained by PTRL West, Inc. Following Sponsor's authorization, specimens may be sent to the Sponsor or disposed of, in accordance with PTRL West, Inc. standard operating procedures, after quality assurance verification.

Herbert S. Cervantes

Herbert S. Cervantes
PTRL West, Inc., Quality Assurance

3/5/96
Date

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PTRL West, Inc.
4123-B Lakeside Drive
Richmond, California

CERTIFICATION OF AUTHENTICITY

TITLE: A Method for the Determination of Folpet in Avocados and Other Oily Crops.

PROJECT NO.: PTRL 568W

STUDY INITIATION DATE: November 16, 1995

DATE ANALYTICAL WORK INITIATED: November 16, 1995

DATE ANALYTICAL WORK COMPLETED: December 13, 1995

DATE REPORT ISSUED: March 5, 1996

Director: _____ Date: 3/5/96
Luis O. Ruzo, Ph.D.
PTRL West, Inc.

Study Director: _____ Date: 3-5-96
Leo T. Nishioka, B.A.
PTRL West, Inc.

Report Writer: _____ Date: 3-5-96
Janine E. Rose, Ph.D.
PTRL West, Inc.

Sponsor: Makhteshim-Agan of North America Inc.
551 Fifth Avenue, Suite 1100
New York, NY 10176

SPONSOR CERTIFICATION

This is the Final Report received from the following contract laboratory:

PTRL West, Inc.
4123-B Lakeside Drive
Richmond, CA 94806

Makhteshim-Agan of North America Inc.
Sponsor Representative:

Signature: _____ Date: April 8, 1996

Robert C. Everich, Ph.D
Study Monitor

ABSTRACT

The analytical method for the extraction and quantitation of the residues of Folpet on avocados was developed at PTRL West, Inc., Richmond, CA. The compound of interest was extracted from avocado matrix by blending with phosphoric acid, ethyl acetate, and anhydrous sodium sulfate in a Waring blender. After blending, the extract was filtered through a bed of sodium sulfate. The extraction and filtration steps were repeated with ethyl acetate. The volume of the combined ethyl acetate extracts was measured and a portion of the extract equivalent to 5.0 grams of matrix was removed and rotoevaporated to dryness at 35°C. The residue was resuspended in acetonitrile, using a small quantity of sodium sulfate to help remove the residue from the walls of the flask. The acetonitrile mixture was then partitioned with hexane (three times). The hexane fractions were combined and backwashed with acetonitrile. The acetonitrile extracts were combined and concentrated to dryness *in vacuo*. The resultant residue was dissolved in dichloromethane:acetone (3:7, v/v) and purified by gel permeation column chromatography. Fractions were collected according to the elution profile determined with [^{14}C]Folpet calibration curve. The samples were concentrated to dryness and the residue samples were resuspended in hexane for analysis by gas chromatography equipped with electron capture detection.

The recovery efficiency of Folpet from avocado fortifications ranged from 88% to 112%. The minimum limit of quantitation of 0.05 µg/g was achieved by this GC/ECD method .

INTRODUCTION

Folpet is a foliar fungicide registered for use on avocados in the U.S. and various crops in other countries. Makhteshim-Agan of North America Inc. contracted PTRL West to develop an extraction method and validate a gas chromatography with electron capture detection method for the analysis of Folpet in avocados to meet EPA registration criteria for products containing Folpet as the active ingredient (Appendix A). This method complements that published in "A Method for the Determination of Folpan and Phthalimide Residues in Non-Oily Crops," MRID 43630001 (Appendix A) developed for the analysis of Folpet residues in lettuce, grapes, apples squash, tomatoes, potatoes, melons, strawberries and grapes. The study was carried out in support of the data requirements described in Subdivision O, Section 171-4: Residue Analytical Method.

STANDARD REFERENCE SUBSTANCES

The reference standard of Folpet was provided by ChemService with a purity check provided by PTRL West, Inc. (Appendix B). A stock solution was prepared at 500 µg/mL in acetone. Standard fortification solutions of 5 µg/mL and 50 µg/mL were prepared in acetone and a 5 µg/mL linearity solution was prepared in hexane. These were stored frozen (< 0°C) in amber bottles with Teflon lined caps until used for sample fortification and calibration preparation. Volumetric pipettes, microliter syringes, and volumetric flasks were utilized for the preparation of the fortification and calibration standards. Standards were vortexed to ensure homogeneity. Standard solutions were allowed to return to room temperature prior to use. The primary reference standard was stored at room temperature. All reference standards were concluded to be stable throughout the conduct of the study based on the comparison of the gas chromatography chromatograms of the first and last analysis.

Summarized information for the chemical used throughout the conduct of this study are outlined below:

Compound:	**Folpet**
Purity:	99.9%
Molecular Weight:	296.58 g/mole
Lot Number:	141 - 92A
Source:	ChemService
Date Received:	September 18, 1995
Storage Condition:	Room Temperature

-Purity Check

Purity:	97.08%
Molecular Weight:	296.58 g/mole
Lot Number:	141 - 92A
Source:	PTRL West, Inc. Log No. P568W-001A
Date of Analysis:	October 23, 1995
Analysis Reference:	PTRL West, Inc. Project No. 577W
Storage Condition:	Room Temperature

MATRICES

Untreated avocados utilized for the method development and validation were purchased from Hilltop Mall Lucky Supermarket, Richmond, CA. The samples were stored frozen (<0°C).

VALIDATION

Control samples of avocado were fortified in duplicate with 0.0, 0.05, 1.0, and 10.0 µg/g of Folpet for evaluation of method recoveries. All fortifications were accomplished prior to the initial addition of solvent for the extraction procedure. Samples were extracted as described in "Analytical Method Summary" of this report. Average and individual recoveries are shown in Table I.

MATERIALS AND METHODS

Reagents and Materials

-Solvents

Hexane, Optima grade, Fisher Scientific
Ethyl Acetate, Optima grade, Fisher Scientific
Acetone, Optima grade, Fisher Scientific
Dichloromethane, Optima grade, Fisher Scientific
Acetonitrile, Optima grade, Fisher Scientific

O-Phosphoric Acid, Concentrated, Reagent ACS, Fisher Scientific

Sodium Sulfate, anhydrous, ACS grade, Fisher Scientific

BioBeads S-X3, 200-400 Mesh, BioRad, Richmond, CA

Glassware and Miscellaneous Equipment

Balance, Fisher XT Toploading
Balance, Mettler Analytical AT 261
Gel Permeation Chromatograph, Model 1002A, with a 25 mm x 600 mm column, ABC Laboratories, Columbia, MO
Gelman Acrodisc 13 CR PTFE filters, 0.45 µm, HPLC Certified, Gelman Scientific, Ann Arbor, MI

Touch-mixer, Fisher brand, Fisher Scientific, San Francisco, CA, model no. 12-810
Vacuum evaporator, Buchi Model RE 111, with temperature controlled waterbath, Brinkmann Instruments, Inc., Burlingame, CA.
Waring blender with 1 quart stainless steel blending cup
Amber bottles with Teflon lined caps
Boiling flask, round bottom, 125 mL, 250 mL, 500 mL
Cylinder, graduated, 50 mL, 100 mL, 250 mL
Funnel, glass, 10 cm diameter
Glass wool
Knife, stainless steel
Microliter syringe, 1000 µL, 500 µL, 250 µL, 100 µL
Pasteur pipettes, 5 inch, 9 inch
Separatory funnel, 125 mL, 250 mL
Syringe, glass 10 cc with detachable needle
Vials, glass, with Teflon lined cap
Vials, Amber glass (2-mL capacity) with Teflon-lined crimp caps, Chromacol, Inc., Trumbull, CT
Volumetric pipettes of various volumes
Volumetric flasks, 10-mL, 50-mL, 100 mL
Weighing dish, plastic

ANALYTICAL METHOD SUMMARY

Preparation of Standards

A 500 µg/mL stock standard of Folpet was prepared using formula as described under calculation in this report.

Fortification solutions were prepared as follows:

50 µg/mL - 5.0 mL of the 500 µg/mL was diluted with acetone in a 50 mL volumetric flask

5 µg/mL - 5.0 mL of the 50 µg/mL was diluted with acetone in a 50 mL volumetric flask

A stock linearity solution (5.0 µg/mL) was prepared by diluting 1.0 mL of the 500 µg/mL stock standard in 100 mL volumetric flask with hexane.

Preparation of External Standards

Using the 5.0 µg/mL standard in hexane, the following external standards were prepared: 0.025, 0.050, 0.100, 0.200, 0.300, 0.500 µg/mL Folpet. All dilutions were made in hexane, with volumetric flasks, volumetric pipettes, and microliter syringes.

0.500 µg/mL standard = 1000 µL of 5.00 µg/mL diluted to 10.0 mL

0.300 µg/mL standard = 600 µL of 5.00 µg/mL diluted to 10.0 mL

0.200 µg/mL standard = 400 µL of 5.00 µg/mL diluted to 10.0 mL

0.100 µg/mL standard = 200 µL of 5.00 µg/mL diluted to 10.0 mL

0.050 µg/mL standard = 1000 µL of 0.500 µg/mL diluted to 10.0 mL

0.025 µg/mL standard = 500 µL of 0.500 µg/mL diluted to 10.0 mL

A calibration curve was generated with each sample set to determine linearity and to quantitate Folpet residues. See Calculations.

Fortification

Fortification Level	Volume	Concentration
0.00 µg/g (Control)	0 µL	0.0 µg/mL
0.05 µg/g	250 µL	5.0 µg/mL
1.00 µg/g	500 µL	50.0µg/mL
10.00 µg/g	500 µL	500.0 µg/mL

Sample Processing and Extraction

Since Folpet is labile in macerated plant systems, the residue method combined the maceration and extraction procedure into one step.

1. Each semi-frozen sample was sampled by incising three or more representative avocados into ~6 gram portions (with seed extracted) and weighing ~25 grams into a plastic weighing dish.

2. Weighed sample was placed into Waring blender and the matrix was fortified at the appropriate level, allowing solvent (acetone) to evaporate.

3. The matrix was immediately spiked with 85% phosphoric acid equivalent to 2 mL per 25 gram of matrix, then ethyl acetate equivalent to 150 mL per 25 grams of matrix and anhydrous sodium sulfate equivalent to 100 grams per 25 grams of matrix was added. Note: The immediate incorporation of phosphoric acid aids in the stability of Folpet.

4. Sample was blended for 2 minutes, then filtered into a 500 mL round bottom flask through a bed of anhydrous sodium sulfate contained in a glass filter funnel fitted with a glass wool plug. The extraction and filtration steps were repeated two more times with 50 mL of ethyl acetate.

5. The bed of sodium sulfate was rinsed with 25 mL of ethyl acetate.

6. Ethyl acetate extracts were combined and the volume measured.

7. A portion of the ethyl acetate extract (equivalent to that from 5 grams of matrix) was removed, placed into a 125 mL round bottom flask and rotoevaporated to dryness at ~35°C.

8. The residue was redissolved in 25 mL of acetonitrile and 100 mL of hexane with the mechanical aid of a small quantity of anhydrous sodium sulfate. The organic phases were transferred to a 250 mL separatory funnel. After swirling for approximately 1 minute, the phases were allowed to separate. The acetonitrile phase was placed in a 125 mL separatory funnel and extracted with hexane (50 mL x 2).

9. The combined hexane extract was washed with acetonitrile (25 mL x 2). The acetonitrile extracts were combined and rotoevaporated to dryness at ~35°C.

10. Gel Permeation Chromatography (GPC) Purification

The GPC column was prepared by swelling ~40g of BioRad BioBeads S-X3 (200-400 mesh) overnight in methylene chloride:acetone (3:7, v/v). The column was packed and purged with eluting solvent (methylene chloride:acetone, 3:7, v/v) at 5 mL/minute until no more bubbles of air were observed. The length of the

column was adjusted and all sample loops were purged with eluting solvent to eliminate air from the system.

Calibration of GPC Column with a 5 mL Sample Loop: A volume (7.5 mL) of [^{14}C]Folpet in solution (representing a ~1.0 ppm fortification level) was loaded, using a glass syringe, onto the GPC containing a 5 mL loop. The sample was eluted with methylene chloride:acetone (3:7, v/v) at 5 mL/min. and 10 mL fractions were collected. Aliquots of each fraction (3 x 1 mL) were taken for liquid scintillation analysis (LSC). For this GPC column (260 mm in length), the radioelution profile (Figure 1) allowed for 14 minutes of waste elution, 15 minutes of sample collection followed by 10 minutes of wash.

Sample Chromatography: The residue (Step 9) was dissolved in 7.5 mL of methylene chloride:acetone (3:7, v/v). Anhydrous sodium sulfate was added, in some cases, to aid in the removal of the solid material from the wall of the flask. The extract was filtered through a Gelman Acrodisc 13CR PTFE filter into the 5 mL sample loop. Each collection tube corresponding to the sample loop was placed into an individual 250 mL round bottom flask. The profile of 14 minutes of waste elution, 15 minutes of sample collection and 10 minutes of wash was performed. The sample was rotoevaporated to dryness at ~35°C.

11. Samples were resuspended in 5 mL of hexane and placed into amber vials with Teflon lined caps.

12. Samples were diluted so that analysis would be within the linearity range.

13. Placed samples into GC vials for analysis.

Chromatography

Instrumentation: Model No. 5890 Hewlett Packard Gas Chromatograph

Column: J & W Scientific DB-1 Fused Silica Capillary Column
30 m x 0.53mm i.d. x 1.5 µm film thickness
(J & W Scientific, Folsom, CA)

Flow: Carrier Gas = 5 mL / minute; Helium
Make-up Gas = 18 mL/ minute; 5% Methane/ 95% Argon (P5)

Injector Temperature: 210°C

Detector Temperature: 300°C

Oven Temperature:

Initial Temperature: 200°C for 15 minutes
Initial Ramp: 200°C to 280°C at 15°C/min
Final Hold 280°C for 5 minutes

Injection Volume: 1 µL; by Hewlett Packard 7673A autosampler (Hewlett Packard Company, Pleasanton, CA)

Retention Time: 11-14 minutes

Separation of the analyte was achieved by capillary column gas chromatography. Due to the variability of the retention time, the analyte was identified by the coincidence of its retention time with the bracketing reference standards, and quantitated by integration of the peak area.

A typical injection sequence was: hexane solvent blank, 0.025 µg/mL standard, control (x2), 0.050 µg/mL standard, sample (x2), 0.100 µg/mL standard, sample (x2), 0.200 µg/mL standard, sample (x2), 0.300 µg/mL standard, sample (x2), etc.

CALCULATIONS

-Preparation of Stock Folpet Standard

$$\text{Volume of solvent (mL)} = \frac{(W)x(P)}{(FC)}$$

where W = Milligrams of neat standard
P = Purity of neat standard
FC = Final Concentration (mg/mL)

-Recoveries

The recoveries of Folpet from fortified samples were calculated as follows:

Linear regression formula from calibration curve $y = mx + b$

$$\mu g/g \text{ (ppm)} = \frac{(y - b) \times \frac{7.5^*}{5}}{m} \times D$$

where y = Sample peak area minus control peak area

b = Calibration intercept

m = Slope value of the calibration curve

D = dilution/concentration factor

* = GPC factor: - 7.5 mL sample in 5 mL injection loop

$$\text{Percent Recovery} = \frac{\text{Concentration of Fortified Sample } (\mu g / g)}{\text{Concentration Fortified } (\mu g / g)} \times 100$$

To demonstrate validity of the analytical method for acceptable recovery (70 - 120%) of the test substance from avocados, avocado samples were fortified with standards at the following three fortification levels in duplicate:

1) 0.05 µg/g Folpet

2) 1.00 µg/g Folpet

3) 10.00 µg/g Folpet

After spiking the samples at the desired concentration, the samples were extracted and assayed as previously described in the analytical method section. Folpet standards were prepared using the 5.0 µg/mL standard in hexane and were analyzed simultaneously by GC/ECD. A calibration curve (ranging from 0.025 µg/mL to 0.500 µg/mL Folpet) was generated with each sample set. The equation of the line based on the peak area of the mixed standard versus concentration in µg/mL was generated by least squares linear regression calculated by the computer program, Cricketgraph™, version 1.3.2. The correlation coefficient (r^2) calculated for each set of standards could not be less than 0.95

for the data to be considered acceptable. A representative chromatogram of a Folpet standard is shown in Figure 2 and a representative calibration curve is shown in Figure 3. Representative chromatograms of a control untreated avocado sample and two fortified avocado samples are given in Figure 4, 5 and 6, respectively.

LIMIT OF DETERMINATION

The limit of determination for the method is defined by the lowest concentration standard or 0.05 µg/mL to generate at least three times the signal to noise ratio. Based on the dilution factors and sample size, the equivalent ppm in matrix is 0.05 ppm and is considered the limit of determination for the method.

RESULTS AND DISCUSSION

Fortified avocado samples have been validated for residues of Folpet at 0.05, 1.00 and 10.0 µg/g with the limit of determination being 0.05 µg/mL. Results from the method recovery experiments are presented in Table I and Appendix B. The analytical method developed for this study proved to be linear within the calibration range of 0.025 µg/mL to 0.500 µg/mL for Folpet.

The percent recovery of Folpet from samples was 104% at a fortification level of 0.05 ppm, 110% at 1.00 ppm and 89% at 10.0 ppm.

CONCLUSION

An extraction and analysis method has been developed and validated for residues of Folpet on avocado at 0.05, 1.00 and 10.0 ppm. This method validation study yielded highly reproducible percent recoveries within the required range for each of the fortification levels.

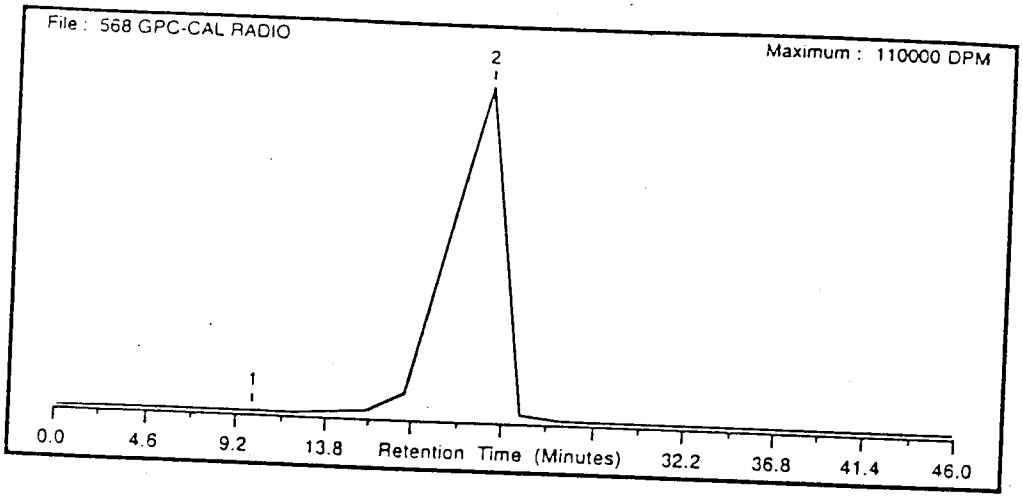
TABLE I

Recovery of Folpet on Avocados

Matrix	Fortification Level Folpet (ppm)	Folpet (%)
Avocados	0.05	**104** (105, 103)
	1.00	**110** (112, 109)
	10.0	**89** (90, 88)

Information presented in Table I was derived from spreadsheet in Appendix B.

Figure 1. Radioelution Profile for [^{14}C]Folpet by Gel Permeation Chromatography.



********** Integration Summary **********

Peak #	Fractions	Peak Area	Area Percent
1	9.00 Min.	83.93	0.05
2	21.00 Min.	174627.75	99.95

Background = 36.83 ± 5.15 DPM.
Total Peak Area = 174711.68 DPM.
Total Fractions Accounted For = 23.

Figure 2. Representative Chromatogram of 0.100 µg/mL Folpet Standard.

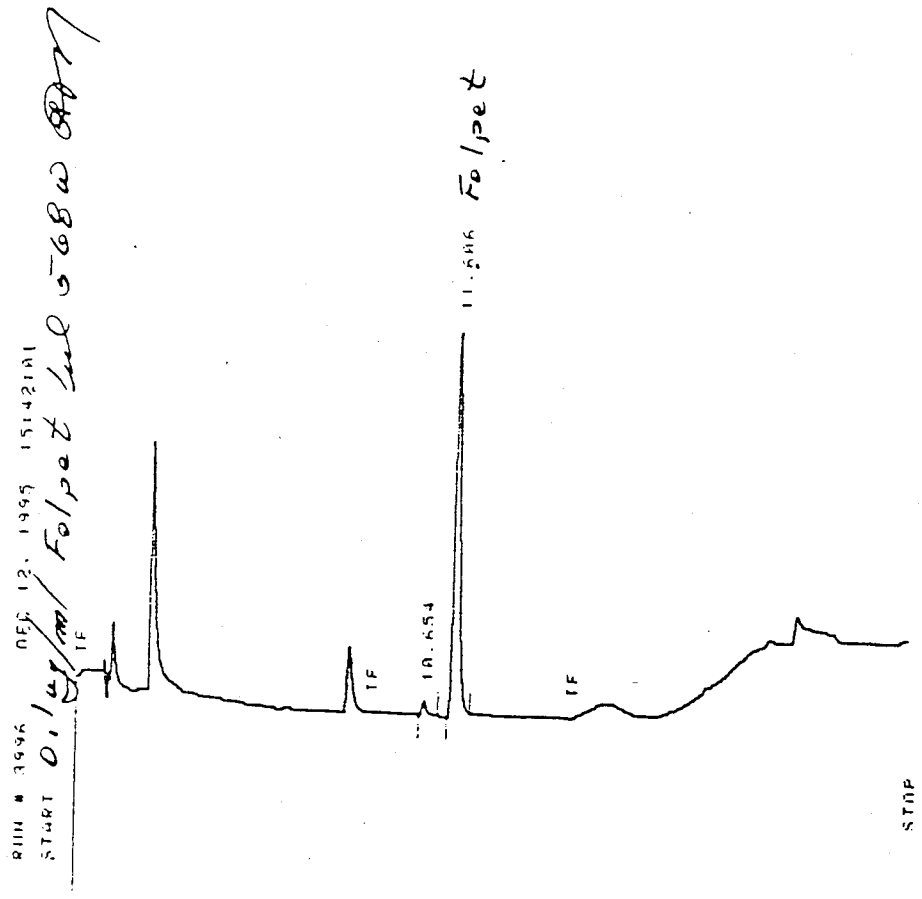


Figure 3. Representative Calibration Curve for Folpet at Concentrations of 0.025 µg/mL, 0.050 µg/mL, 0.100 µg/mL, 0.200 µg/mL, 0.300 µg/mL, and 0.500 µg/mL Solution.

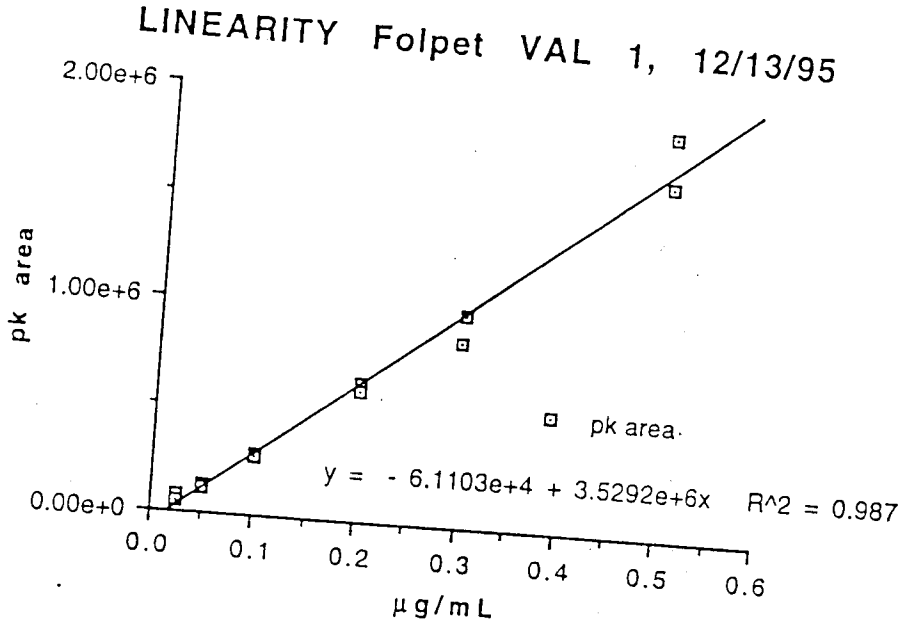


Figure 4. Representative Chromatogram of Untreated Avocados.

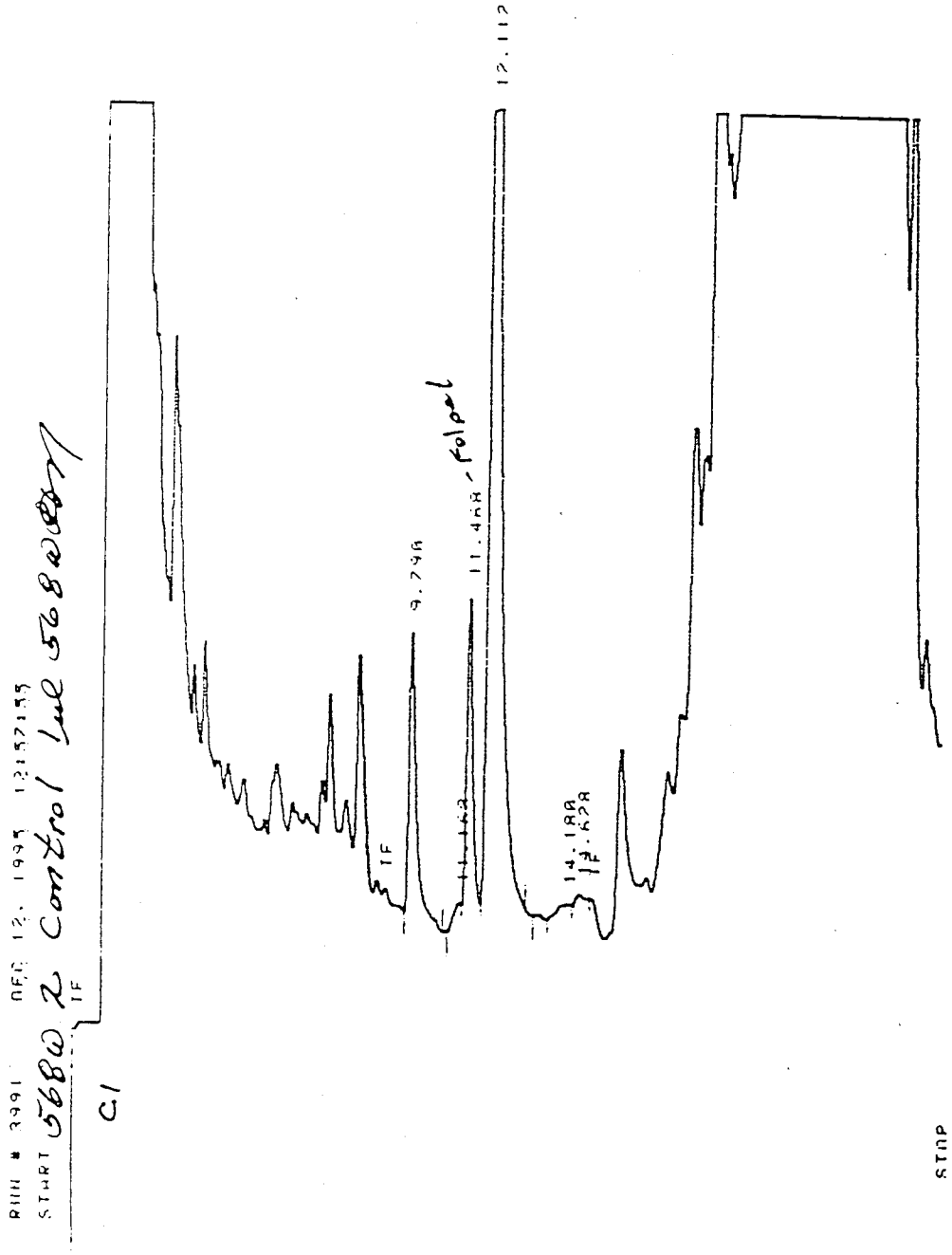


Figure 5. Representative Chromatogram of Untreated Avocados Fortified with 0.05 µg/g Folpet.

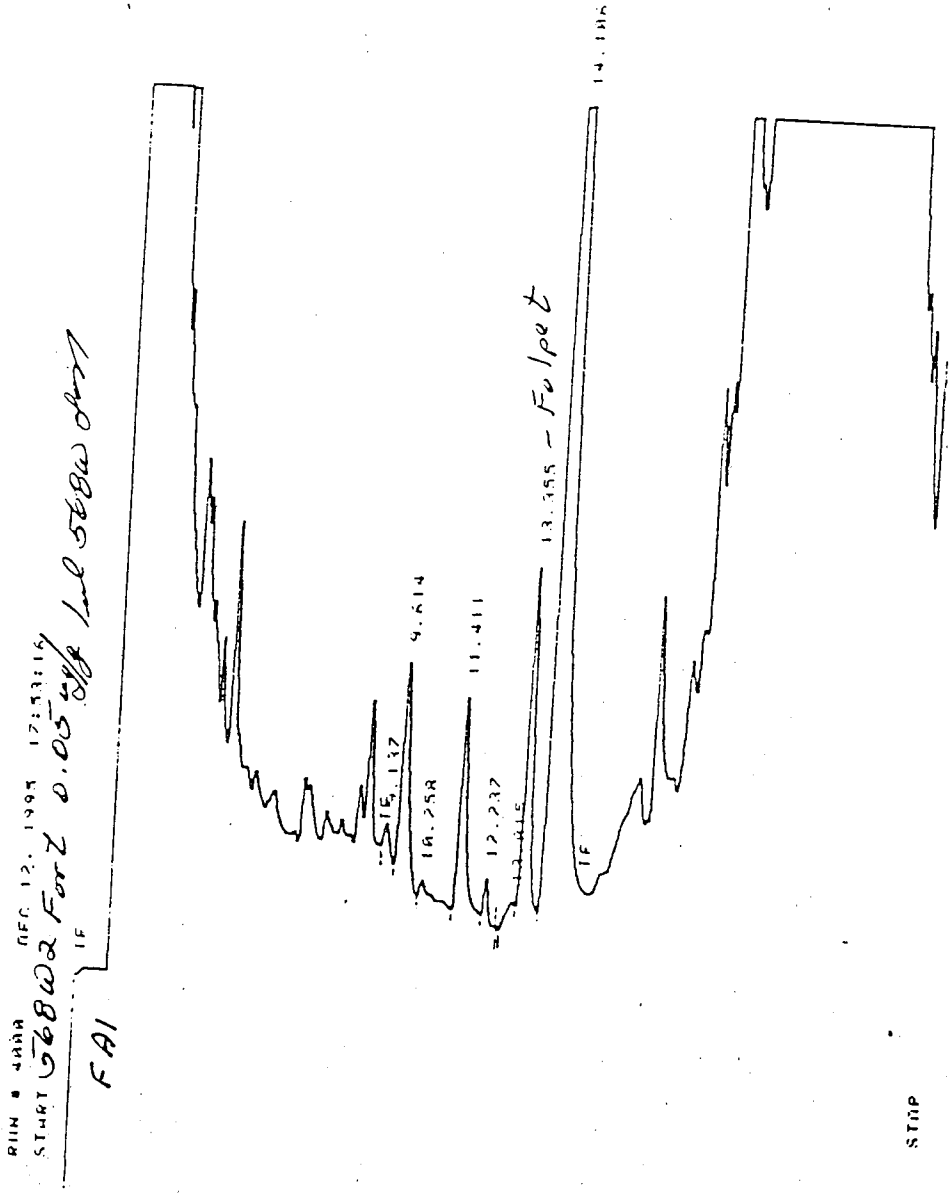
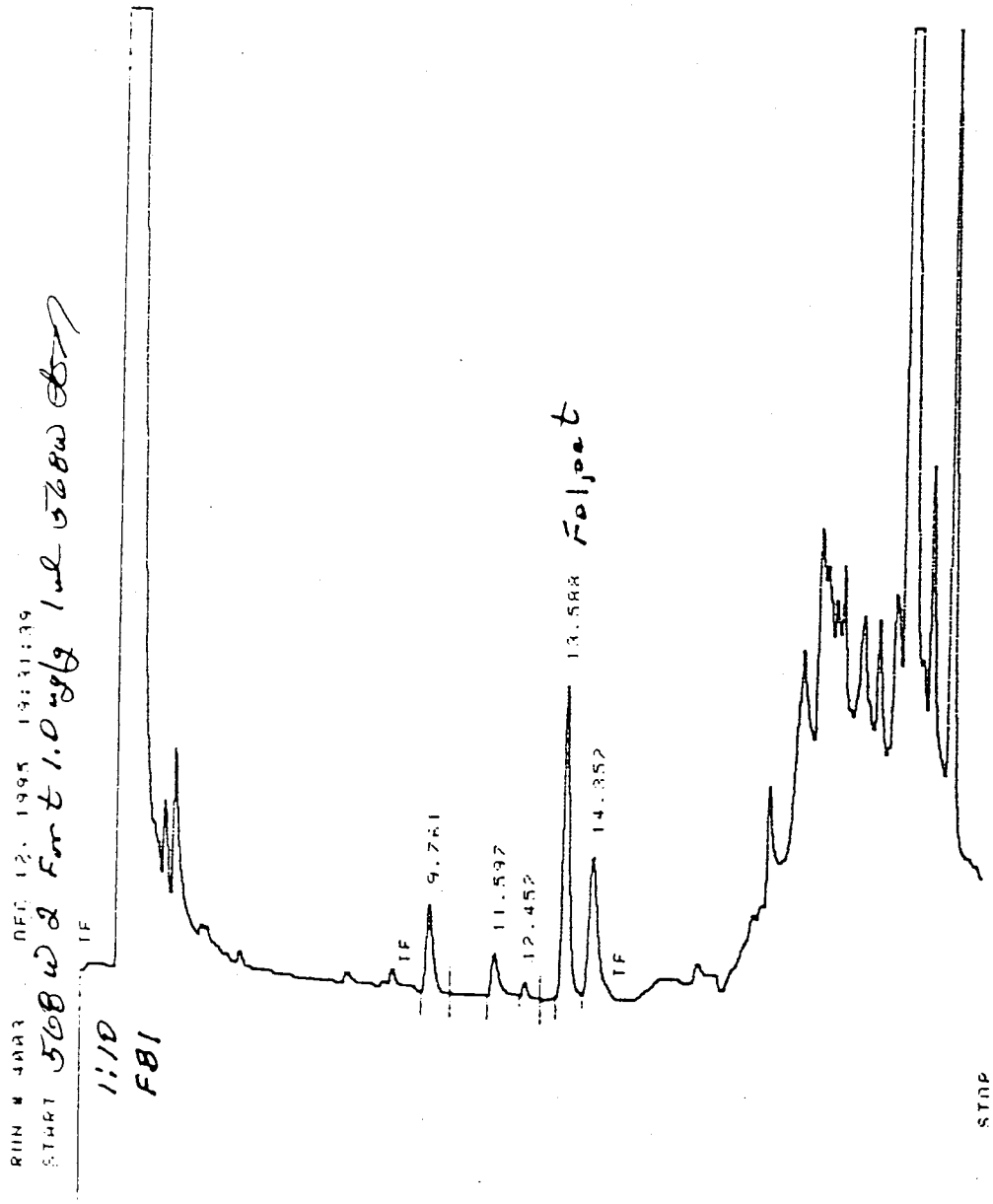


Figure 6. Representative Chromatogram of Untreated Avocados Fortified with 1.0 µg/g Folpet, Dilution Factor 10.



Appendix A. Study Protocol and Protocol Amendment.

PTRL WEST, INC. STUDY PROTOCOL

1.0 Title: Method Development and Validation for Magnitude of the Residue of Folpet in Avocado

1.1 Guideline Number: EPA Pesticide Assessment Guidelines; Subdivision O; 171-4

2.0 PTRL Project Number: 568W

2.1 Study Director: Leo Nishioka, PTRL West, Inc.

3.0 Objective: To develop and validate an analytical method for Folpet in Avocado.

4.0 Testing Facility:
PTRL West, Inc.
4123-B Lakeside Drive
Richmond, CA 94806
Telephone: (510) 222-4163, Extension 236
Fax: (510) 222-4861

5.0 Sponsor:
Makhteshim Agan of North America, Inc.
551 Fifth Avenue, Suite 1100
New York, NY 10176

5.1 Sponsor Representative:
Robert C. Everich, Ph.D.
Telephone: (212) 661-9800
Fax: (212) 661-9038

6.0 Test/Reference Substances:

Test/reference substance for Folpet will be purchased commercially. All unused material will be returned to Sponsor after the study is completed. All calibration and fortification solutions must be prepared only from these characterized analytical standards. Preparation of all standards must be documented and identified as prepared from a particular standard. These must be included in the raw data and transferred to Sponsor upon authorization.

6.1 Active Ingredient: The chemical used in the validation trials will be Folpet (N-[trichloromethylthio]phthalimide, CAS No. 133-07-3).

6.2 Purity: Certification of analysis of all standards will be provided by the manufacturer and confirmed at PTRL West.

6.3 Safety: Material Safety Data Sheets (when available) will be provided by Sponsor.

6.4 Receipt: Receipt of test substances will be documented as to label identification, date, batch number, person receiving the sample, and amount and conditions of the sample. Use of the chemical will be logged, storage will be as per instructions of Sponsor up to six months after completion of the project.

6.5 Stability: Stability of the analytical reference standard/test substance under contract laboratory storage conditions will be established by analytical comparison prior to the experimental start date and at the experimental termination of the validation study.

6.6 Route of Administration: Known amount of diluted reference standard solution will be spiked on the control matrix with a syringe of appropriate volume.

6.7 Justification for Route of Administration: To assess known quantity of residues introduced to the control matrix and recovered from the analytical method.

7.0 Matrix to Analyze:

Avocados (will be obtained commercially).

7.1 Justification: Avocados are a proposed end use crop.

7.2 Identification: Whole avocado matrices will be identified and tracked by the assignment of unique laboratory numbers by PTRL.

7.3 Storage Conditions: Samples will be stored frozen (< 0 °C) until analyzed.

8.0 Laboratory Study Schedule Data:

8.1 Proposed Experimental Start Date: December 1, 1995

8.2 Proposed Experimental Termination Date: February 1, 1996

8.3 Projected Final Report Date: March 1, 1996

9.0 Method Development:

The initial approach will utilize an existing method provided by the Sponsor and appended to this protocol (Appendix 1) and previous experience developed at PTRL West (PTRL Project 418W) with folpet residue determination in avocados.

The method described in Appendix 1 is based on ethyl acetate extractions from the whole fruit, filtration through flurasil sep-pak and electron capture detection (ECD) by gas chromatographic (GC) separation. However, additional cleanup steps may be necessary to remove lipid co-extractants (PTRL Project 418W). Linearity for ECD response will be established in organic solvent. Avocado samples (10–100 g) will be fortified in duplicate with folpet at levels equivalent to the limit of quantitation (LOQ) 0.05 ppm, the proposed tolerance level and one intermediate concentration. The projected LOQ is that determined for a method applied to strawberries (see Appendix 1). The proposed tolerance level provided by the Sponsor will be 10 ppm.

10.0 Validation Studies:

Validation will be conducted using non-treated avocado samples (pit will be removed) as the matrix of investigation.

10.1 Samples will be analyzed in duplicate at the following three fortification levels: The LOQ, the proposed tolerance level (10 ppm) and one intermediate concentration (1 ppm).

10.2 Method: As developed in Section 9.0. Modifications (if any) will be described in the Final Report.

10.3 Quantitation: Quantitate by peak area integration and comparison to external standard calibration. Residues of analytes will be calculated in µg/g for each sample extract using the linear regression equation generated by the standards injected as specified in the method.

10.4 Linearity Curves: (In organic solvent) To be determined daily during analysis with minimum of four data points as specified in the method.

10.5 Recoveries will generally be considered acceptable when within 70 and 120% of the fortification level.

10.6 Proposed Statistical Methods: The following statistical methods will be applied to the data generated for the validation study: correlation coefficients, standard deviation and averages.

10.7 Method for the control of bias: Control and three fortification levels will be analyzed in duplicate. Average and standard deviations will be calculated.

11.0 Sample Handling/Processing:

Samples will be processed by PTRL West. Individual samples will be evaluated for integrity, assigned unique identification and stored frozen.

12.0 Quality Assurance:

The study will be audited by the PTRL Quality Assurance Unit on a regular basis while in progress to assure compliance with Good Laboratory Practice regulations, adherence to the protocol and PTRL West Standard Operating Procedures. The draft report will be audited by the PTRL West Quality Assurance Unit prior to submission to Sponsor to assure that the final report accurately describes the conduct and the findings of the study.

The study is a GLP regulated study and will be included on the master schedule of PTRL West.

13.0 Records to be Maintained:

All original data records and original final report will be the property of the Sponsor and will be sent to Sponsor upon authorization. An exact copy of the Final Report will be retained in the archives at PTRL West, Inc.

14.0 Specimens:

All specimens will be stored at PTRL West, Inc. until Sponsor's acceptance of the Final Report. No specimens will be disposed of without the Sponsor's permission.

15.0 Reports:

This study will be reported in a Final Report including method validation and representative chromatograms generated by PTRL.

16.0 Protocol Modifications:

Modifications of the protocol may be accomplished during the course of this investigation. However, no changes will be made in the study design without the verbal or written permission of Study Monitor. In the event that Sponsor verbally requests or approves changes in the protocol, such changes will be made by appropriate documentation in the form of protocol amendments. All amendments of the protocol and reasons for the modification will be signed and dated by the Study Director and the Study Monitor.

17.0 Signatures

Study Director: _____ Date: 11-16-95
Leo Nishioka, B.A.
PTRL West, Inc.

Study Monitor: _____ Date: 11-22-95
Robert C. Everich, Ph.D.
Makhteshim Agan of North America, Inc.

Testing Facility Management: _____ Date: 11-16-95
Luis O. Ruzo, Ph.D.
PTRL West, Inc.