Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Tepraloxydim

MRID #: 444672-44

Matrix: Soil

Analysis: LC/MS/MS

This method is provided to you by the Environmental Protection Agency's (EPA) Environmental Chemistry Laboratory (ECL). This method *is not* an EPA method but one which was submitted to EPA by the pesticide manufacturer to support product registration. EPA recognizes that the methods may be of some utility to state, tribal, and local authorities, but makes no claim of validity by posting these methods. Although the Agency reviews *all* Environmental Chemistry Methods submitted in support of pesticide registration, the ECL evaluates only about 30% of the currently available methods. Most methods perform satisfactorily but some, particularly the older methods, have deficiencies. Moreover, the print quality of the methods varies considerably because the methods originate from different sources. Therefore, the methods offered represent the best available copies.

If you have difficulties in downloading the method, or further questions concerning the methods, you may contact Elizabeth Flynt at 228-688-2410 or via e-mail at flynt.elizabeth@epa.gov.

444672-44

TEPRALOXYDIM

BASF CORPORATION
AGRICULTURAL PRODUCTS GROUP
AGRICULTURAL PRODUCTS CENTER
P.O. Box 13528
Research Triangle Park, NC 27709-3528

Study Title:

VALIDATION OF BASF METHOD No. D9606: Analytical Method Validation for the Determination of Residues of BAS 620 H and its Metabolites (DP-6 and GP) in Soil using LC-MS/MS

Method No. D9606
Study No. 96084

Data Requirement:

Guideline 164-1 Terrestrial Field Dissipation

Study Completion Date:

March 14, 1997

Author:

Manasi Saha

Performing Laboratory:

BASF CORPORATION
AGRICULTURAL PRODUCTS GROUP
P.O. Box 13528
Research Triangle Park, NC 27709

BASF Registration Document No. 97/5062

This report consists of 85 pages.

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d)(1)(A), (B), or (C).

Company: BASF Corporation
Agricultural Products

Company Agent: Karen E. Warkentien

Title: Senior Registration Specialist

Signature: _____

Date: 21 OCT 97

GOOD LABORATORY PRACTICES COMPLIANCE STATEMENT

This study meets the requirements for 40 CFR 160, Good Laboratory Practices, with the exceptions of the following: not all data entered in the study notebook was signed and dated on the day of entry.

Study Director: Manasi G. Saha 3/14/97

Sponsor: [signature] 3/12/97

Submitter: [signature] 21 OCT 97

BASF CORPORATION
AGRICULTURAL PRODUCTS GROUP
AGRICULTURAL PRODUCTS CENTER
P.O. Box 13528
Research Triangle Park, NC 27709-3528

VALIDATION OF BASF METHOD No. D9606: Analytical Method Validation for the Determination of Residues of BAS 620 H and its Metabolites (DP-6 and GP) in Soil using LC-MS/MS

AUTHOR: Manasi G. Saha

STUDY DIRECTOR: Manasi G. Saha (919) 547-2232

SUPERVISORY PERSONNEL: Arno Krotzky (919) 547-2213

WORK DONE BY: Leonard Collins, Nelson Delgado, Susan Switzer, Bryant Harrison and Charles Marshall
BASF Corporation Agricultural Product Center
P. O. Box 13528
Research Triangle Park, NC 27709

Method No. D9606 **Report Date: March 14, 1997**

ABSTRACT:

Analytical Method No. D9606 was developed to determine trace residues of (RS-EZ)-2-[1-(3-chloro-(2E)-propenyloximino)propyl]-3-hydroxy-5-(terahydropyran-4-yl)cyclohex-2-en-1-one [BAS 620 H], 3-Hydroxy-2-propionyl-5-(tetrahydropyran-4-yl)cyclohex-2-en-1-one [DP-6] and 3-(3,4,5,6-tetrahydro-2H-pyran-4-yl)glutaric acid [GP] in soil. The purpose of the study is to determine recovery efficiency of the above analytes in soil. BAS 620 H is the active ingredient and DP-6 and GP are two major metabolites found in several environmental fate studies (Reference 1). Method development was conducted at BASF Corporation. BAS 620 H and DP-6 were extracted from soil samples by shaking with dichloromethane and were determined by LC-MS/MS. GP was extracted from soil by shaking with 1N NaOH. These extracts were then partitioned into organic solvent by ChemElut® and were determined by LC-MS/MS. This study has shown that Analytical Method No. D9606 is suitable for measuring residues of BAS 620 H, DP-6 and GP down to levels of 0.01 ppm in soil. Over a fortification span from 0.01 to 1.0 ppm, recoveries ranged from 45 to 121% for BAS 620 H with a mean recovery of 99 ± 14% (N = 24), recoveries ranged from 69 to 115% for DP-6 with a mean recovery of 92 ± 10% (N = 24) and recoveries ranged from 63 to 105% for GP with a mean recovery of 83 ± 12% (N = 24).

PAGES OF REPORT: 85

Study Initiation Date:	**July 8, 1996**
Experimental Initiation Date:	**July 8, 1996**
Experimental Completion Date:	**December 16, 1996**

BASF CORPORATION AGRICULTURAL PRODUCTS GROUP

Agricultural Research Center, Research Triangle Park, N.C. 27709

STATEMENT OF THE QUALITY ASSURANCE UNIT

Method Number: D9606

BASF Study Number: 96084

Name/Number of Test Substance:

RS-EZ)-2-[1-(3-chloro-(2E)-propenyloximino)propyl]-3-hydroxy-5-(terahydropyran-4-yl)cyclohex-2-en-1-one (BAS 620 H)
Lot number 691-25-1, 99. 9%

3-Hydroxy-2-propionyl-5-(tetrahydropyran-4-yl)cyclohex-2-en-1-one (DP-6)
Lot number 41-180, 97.9% purity.

3-(3,4,5,6-Tetrahydro-2H-pyran-4-yl)glutaric acid (GP)
Lot number 31-2139-MH, 99.9%

Type of Study: Method Validation

Study Initiation Date: July 8, 1996

The quality assurance unit of the testing facility at the APC has audited this study, the raw data, and the final report and reported any findings to the study director and to management.

Date of Inspection	Report to Study Director and to Management
July 8 , 1996	July 8 , 1996
July 11 , 1996	July 11 , 1996
July 15 , 1996	July 15 , 1996
July 16 , 1996	July 16 , 1996
July 17 , 1996	July 17 , 1996
March 5 , 1997	March 5 , 1997

Judy Fersch
Signature of QAU

TABLE OF CONTENTS

TABLE OF CONTENTS, Continued

TABLE OF CONTENTS, Continued

APPENDICES

1. INTRODUCTION AND SUMMARY

1.1 Scope and Source of the Method

1.1.1 Scope

This method is used to determine residues of (RS-EZ)-2-[1-(3-chloro-(2E)-propenyloximino)propyl]-3-hydroxy-5-(terahydropyran-4-yl)cyclohex-2-en-1-one [BAS 620 H], 3-hydroxy-2-propionyl-5-(tetrahydropyran-4-yl)cyclohex-2-en-1-one [DP-6] and 3-(3,4,5,6-tetrahydro-2H-pyran-4-yl)glutaric acid [GP] in soil. BAS 620 H is the active ingredient and DP-6 and GP are two major metabolites found in several environmental fate studies (Reference 1).

1.1.2 Source

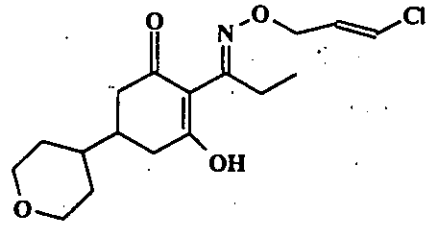
This method was developed at BASF Corporation, RTP, NC.

1.2 Description of Test and Reference Substances

Active Ingredient and Fortification Compound:

BASF code: BAS 620 H
Chemical name: (RS-EZ)-2-[1-(3-chloro-(2E)-propenyloximino)propyl]-3-hydroxy-5-(terahydropyran-4-yl)cyclohex-2-en-1-one

Structural formula:



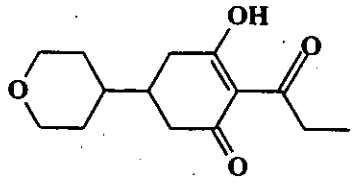
Empirical formula: $C_{17}H_{24}ClNO_4$
Molecular weight: 341.84
Appearance: White solid

1. **INTRODUCTION AND SUMMARY**, continued

Metabolites and Fortification Compounds:

BASF Code: .DP-6
Chemical name: 3-Hydroxy-2-propionyl-5-(tetrahydropyran-4-yl) cyclohex-2-en-1-one

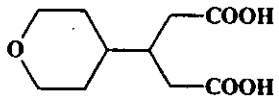
Structural formula:



Empirical formula: $C_{14}H_{20}NO_3$
Molecular weight: 249.31
Appearance: White solid

BASF Code: GP
Chemical name: 3-(3,4,5,6-tetrahydro-2H-pyran-4-yl)glutaric acid

Structural formula:



Empirical formula: $C_{10}H_{16}O_5$
Molecular weight: 216.24
Appearance: White solid

1.3 Principle of the Method

Residues of BAS 620 H and DP-6 are extracted from soil by shaking with dichloromethane and subsequent concentration. The concentrated residues are then determined by LC-MS/MS. Residues of GP are extracted from soil by shaking with 1N NaOH and are then isolated from the alkaline extract into organic solvent by ChemElut® partition and determined by LC-MS/MS. See Figure 1 and Figure 2 for the flow charts of these analytical methods. The limit of quantitation is 0.01 ppm for each analyte.

2. MATERIALS AND METHODS

2.1 Equipment

Equipment	Suggested Sizes/Manufacturer
Centrifuge	IEC Refrigerated Centrifuge Model PR 7000 Beckmann Refrigerated Centrifuge Model CS-6KR
Centrifuge Bottles, Teflon-lined screw cap	Fisher Scientific Co. 150 mL
Laboratory Shaker	Janke and Kunkel Model HS501-D
Gelman Nylon acrodisc (Membrane disc 0.45 um)	VWR Scientific Co. Cat. No 28143-948
Vortex mixer	Fisher Scientific Co.
Rotary evaporator	Büchi Rotovapor Model RE 111, 114
Standard taper Flat-bottom flasks	VWR Scientific Co. 500 mL, 300 and 125 mL
Standard funnels	Fisher Scientific Co.
Syringes, plastic & disposable	Fisher Scientific Co. 1 and 5 mL
Ultrasonic Bath	Fisher Scientific Co., Model FS-14
Vacubrand vacuum pump/controller	Elnik Systems, Inc.

Other general laboratory glassware and supplies as needed.

NOTE: The equipment listed in this section was used in the development of this method. Equipment with equivalent performance may be used, as required.

2.2 Reagents and Chemicals

Reagents and Chemicals	Source/Preparation
Acetonitrile, CAS 75-05-8	Baxter Healthcare Corporation, B & J Brand
Ammonium acetate	Fluka

2. **MATERIALS AND METHODS**, continued

ChemElut®, 50 mL capacity	Varian, Cat No. A1-121980-09
Dichloromethane, CAS 75-09-2	Baxter Healthcare Corporation, B & J Brand
Formic acid (88%), CAS 64-18-6	Aldrich Chemical Co. ACS Cat. No. 39, 938-8
	Fluka (for LC-MS/MS analysis)
Isopropanol, CAS 67-63-0	Baxter Healthcare Corporation, B & J Brand
Sodium hydroxide 1 N NaOH	Fisher Scientific, ACS Reagent Dissolve 40 grams of sodium hydroxide in 800 mL of water. Cool solution and dilute to 1.0 liter.
Sodium chloride	Fisher Scientific, ACS Reagent
Sodium sulfate	Fisher Scientific, ACS Reagent
Water, CAS 7732-18-5	Baxter Healthcare Corporation, B & J Brand
Water for LC-MS/MS analysis	Purified water prepared from Hydol system

NOTE: Equivalent reagents and chemicals from other suppliers may be substituted.

2. **MATERIALS AND METHODS**, continued

2.3 Standard Substances and Solutions

The standard compounds shown in the table below were used for method development and validation.

Compound	Code	Lot Number	Purity
(RS-EZ)-2-[1-(3-Chloro-(2E)-propenyloximino)propyl]-3-hydroxy-5-(terahydropyran-4-yl)cyclohex-2-en-1-one	BAS 620 H	691-25-1	99. 9%
3-Hydroxy-2-propionyl-5-(tetrahydropyran-4-yl) cyclohex-2-en-1-one	DP-6	41-180	97.9%
3-(3,4,5,6-Tetrahydro-2H pyran-4-yl)glutaric acid	GP	31-2139-MH	99.9%

Standard supplied by:

Dr. Rita Laschober
BASF Aktiengesellschaft, APS/UP
Agricultural Research Center
D-67114 Limburgerhof, West Germany
Telephone: 06236/68/2103

(RS-EZ)-2-[1-(3-Chloro-(2E)-propenyloximino)propyl]-3-hydroxy-5-(terahydropyran-4-yl)cyclohex-2-en-1-one (BAS 620 H), 3-Hydroxy-2-propionyl-5-(tetrahydropyran-4-yl) cyclohex-2-en-1-one (DP-6) and 3-(3,4,5,6-Tetrahydro-2H pyran-4-yl)glutaric acid (GP) were maintained frozen (<-5°C) until their use in this study. These substances were characterized as required by 40 CFR part 160, FIFRA Good Laboratory Practices. Data on the synthesis and subsequent characterization of these substances are available to BASF and are located at Landwirtschaftliche Versuchsstation der BASF, Limburgerhof, Germany.

Solutions of BAS 620 H, DP-6 and GP were refrigerated (+4°C) during their use in this study. Stock solutions (1 mg/mL) of BAS 620 H, DP-6 and GP were made fresh every three months and dilutions of the stock solution were made monthly.

Solution Stability Note: Methanol solutions (1 mg/mL) of BAS 620 H, DP-6 and GP are kept in the dark at 4°C were stable for at least three months. A statement concerning the stabilities of solutions used in the method validation study for this report is given in Section 5.2.

2. **MATERIALS AND METHODS**, continued

2.3.1 Standard Solutions for Fortifications and LC-MS/MS Determination

> NOTE: These standard concentrations are suggested. A different standard concentration scheme may be used and additional standards may be prepared as needed.

2.3.1.1 (RS-EZ)-2-[1-(3-Chloro-(2E)-propenyloximino)propyl]-3-hydroxy-5-(terahydropyran-4-yl)cyclohex-2-en-1-one (BAS 620 H): 1.0 mg/mL in methanol

Prepare a 1.0 mg/mL stock solution by weighing an appropriate amount of BAS 620 H into a volumetric flask and dissolving it with an appropriate amount of methanol. For example, to prepare a 10 mL stock solution, dissolve 10.0 mg of BAS 620 H in 10 mL of methanol into 10 mL volumetric flask.

2.3.1.2 3-Hydroxy-2-propionyl-5-(tetrahydropyran-4-yl)cyclohex-2-en-1-one (DP-6): 1.0 mg/mL in methanol

Prepare a 1.0 mg/mL stock solution by weighing an appropriate amount of DP-6 into a volumetric flask and dissolving it with an appropriate amount of methanol. For example, to prepare a 10 mL stock solution, dissolve 10.0 mg of DP-6 in 10 mL of methanol into a 10 mL volumetric flask.

2.3.1.3 3-(3,4,5,6-Tetrahydro-2H pyran-4-yl)glutaric acid (GP): 1.0 mg/mL in methanol

Prepare a 1.0 mg/mL stock solution by weighing an appropriate amount of GP into a volumetric flask and dissolving it with an appropriate amount of methanol. For example, to prepare a 10 mL stock solution, dissolve 10.0 mg of GP in 10 mL of methanol into a 10 mL volumetric flask.

2.3.1.4 Mix Standard Solutions of (RS-EZ)-2-[1-(3-chloro-(2E)-propenyloximino)propyl]-3-hydroxy-5-(terahydropyran-4-yl)cyclohex-2-en-1-one (BAS 620 H) and 3-Hydroxy-2-propionyl-5-(tetrahydropyran-4-yl)cyclohex-2-en-1-one (DP-6): 25.0; 2.5 and 0.25 µg/mL in methanol.

Prepare a 25 µg/mL of mixed standard solution by transferring an appropriate amount of each of the 1.0 mg/mL

2. **MATERIALS AND METHODS,** continued

stock solutions (2.3.1.1 and 2.3.1.2) with a volumetric pipette into a volumetric flask (typically 2.5 mL of each of the 1.0 mg/mL stock solutions in a 100 mL volumetric flask). Dilute to the mark with methanol.

Prepare a 2.5 µg/mL of mixed standard solution by transferring an appropriate amount of the 25.0 µg/mL mixed standard solutions with a volumetric pipette into a volumetric flask (typically 1.0 mL of the 25.0 µg/mL standard solutions in a 10 mL volumetric flask). Dilute to the mark with methanol.

Prepare a 0.25 µg/mL of mixed standard solution by transferring an appropriate amount of the 2.5 µg/mL mixed standard solution with a volumetric pipette into a volumetric flask (typically 1.0 mL of the 2.5 µg/mL standard solution in a 10 mL volumetric flask). Dilute to the mark with methanol.

2.3.1.5 Standard Solutions of (3-(3,4,5,6-tetrahydro-2H-pyran-4-yl)glutaric acid (GP): 25.0; 2.5 and 0.25 µg/mL in methanol.

Prepare a 25.0 µg/mL standard solution by transferring an appropriate amount of the 1.0 mg/mL stock solution (2.3.1.3) with a volumetric pipette into a volumetric flask (typically 2.5 mL of 1.0 mg/mL stock solution in a 100 mL volumetric flask). Dilute to the mark with methanol.

Prepare a 2.5 and 0.25 µg/mL standard solution from 25.0 µg/mL standard solution by appropriate serial dilution in methanol.

Transfer each stock and standard solution to an amber bottle fitted with a Teflon-lined screw cap and store in the refrigerator. Replace stock solution 90 days after preparation. Replace standard solutions 30 days after preparation. Use the standard solutions in methanol for fotification.

2.3.1.6 Preparation of Mixed Solvents and Injection Standard Solutions of BAS 620 H, DP-6 & GP for LC-MS/MS analysis

2.3.1.6.1 Preparation of Mixed Solvents

Solvent mixture I: Water-acetonitrile; 20:80, v/v

2. **MATERIALS AND METHODS**, continued

Solvent mixture II: Water-acetonitrile; 50:50, v/v containing 0.1 % formic acid

Solvent mixture III: Water-acetonitrile; 70:30, v/v containing 0.1 % formic acid and 5 mM ammonium aceate

Solvent mixture IV: MeOH-Solvent mixture III; 1:90, v/v

2.3.1.6.2 Preparation of Injection Standard Solutions of BAS 620 H and DP-6 for LC-MS/MS analysis: 250, 200, 100, 50.0, 25.0, 20.0, 10.0, and 5.0 pg/µL

Prepare a **250 pg/µL** injection standard solution by transferring an appropriate amount of the 2.5 µg/mL mixed standard solution with a volumetric pipette into a volumetric flask. Typically add 2.5 mL of the 2.5 µg/mL standard solution into a 25 mL volumetric flask and then dilute to the mark with **solvent mixture I**.

Prepare a **200 pg/µL** injection standard solution by transferring an appropriate amount of the 2.5 µg/mL mixed standard solution with a volumetric pipette into a volumetric flask. Typically add 2.0 mL of the 2.5 µg/mL standard solution into a 25 mL volumetric flask and then dilute to the mark with **solvent mixture I**.

Prepare a **100 pg/µL** injection standard solution by transferring an appropriate amount of the 200 pg/µL mixed standard solution with a volumetric pipette into a volumetric flask. Typically add 5.0 mL of the 200 pg/µL mixed standard solution into a 10 mL volumetric flask and then dilute to the mark with **solvent mixture I**.

Prepare a **50.0 pg/µL** injection standard solution by transferring an appropriate amount of the 100 pg/µL mixed standard solution with a volumetric pipette into a volumetric flask. Typically add 5.0 mL of the 100 pg/µL mixed standard solution into a 10 mL volumetric flask and then dilute to the mark with **solvent mixture I**.

Prepare a **25.0 pg/µL** injection standard solution by transferring an appropriate amount of the 250 pg/µL mixed standard solution with a volumetric pipette into a volumetric flask. Typically add 1.0 mL of the 250 pg/µL

2. **MATERIALS AND METHODS**, continued

mixed standardsolution into a 10 mL volumetric flask and then dilute to the mark with **solvent mixture II**.

Prepare a **20.0 pg/µL** injection standard solution by transferring an appropriate amount of the 200 pg/µL mixed standard solution with a volumetric pipette into a volumetric flask. Typically add 1.0 mL of the 200 pg/µL mixed standard solution into a 10 mL volumetric flask and then dilute to the mark with **solvent mixture II**.

Prepare a **10.0 pg/µL** injection standard solution by transferring an appropriate amount of the 100 pg/µL mixed standard solution with a volumetric pipette into a volumetric flask. Typically add 1.0 mL of the 100 pg/µL mixed standard solution into a 10 mL volumetric flask and then dilute to the mark with **solvent mixture II**.

Prepare a **5.0 pg/µL** injection standard solution by transferring an appropriate amount of the 50.0 pg/µL mixed standard solution with a volumetric pipette into a volumetric flask. Typically add 1.0 mL of the 50.0 pg/µL mixed standard solution into a 10 mL volumetric flask and then dilute to the mark with **solvent mixture II**.

2.3.1.6.3 Preparation of Injection Standard Solutions of GP for LC-MS/MS analysis: 500, 50, 25.0, 10.0, 5.0, and 2.5 pg/µL

Prepare a **500.0 pg/µL** injection standard solution by transferring an appropriate amount of the 2.5 µg/mL mixed standard solution (2.3.1.5) with a volumetric pipette into a volumetric flask (typically add 2.0 mL of the 2.5 µg/mL standard solution into a 10 mL volumetric flask). Dilute to the mark with **solvent mixture IV**.

Prepare a **50.0 pg/µL** injection standard solution by transferring an appropriate amount of the 500.0 pg/µL mixed standard solution with a volumetric pipette into a volumetric flask (typically add 1.0 mL of the 500.0 pg/µL standard solution into a 10 mL volumetric flask). Dilute to the mark with **solvent mixture IV**.

Prepare a **25.0 pg/µl** injection standard solution by transferring an appropriate amount of the 500.0 pg/µL mixed standard solution with a volumetric pipette into a volumetric flask (typically add 0.5 mL of the 500.0 pg/µL

2. **MATERIALS AND METHODS**, continued

standard solution into a 10 mL volumetric flask). Dilute to the mark with **solvent mixture IV**.

Prepare a **10.0 pg/µL** injection standard solution by transferring an appropriate amount of the 50.0 pg/µL mixed standard solution with a volumetric pipette into a volumetric flask (typically add 2.0 mL of the 50.0 pg/µL standard solution into a 10 mL volumetric flask). Dilute to the mark with **solvent mixture IV**.

Prepare a **5.0 pg/µL** injection standard solution by transferring an appropriate amount of the 50.0 pg/µL mixed standard solution with a volumetric pipette into a volumetric flask (typically add 1.0 mL of the 50.0 pg/µL standard solution into a 10 mL volumetric flask). Dilute to the mark with **solvent mixture IV**.

Prepare a **2.5 pg/µL** injection standard solution by transferring an appropriate amount of the 25.0 pg/µL mixed standard solution with a volumetric pipette into a volumetric flask (typically add 1.0 mL of the 25.0 pg/µL standard solution into a 10 mL volumetric flask). Dilute to the mark with **solvent mixture IV**.

For LC-MS/MS analysis, inject 10.0 µL of the mixed BAS 620 H and DP-6 standard solutions of concentrations 25.0, 20.0, 10.0 and 5.0 pg/µL to construct a standard curve. Standard curve of BAS 620 H is constructed by adding peak areas for Z and E isomer. Similarly inject 10.0 µL of the GP standard solutions of concentrations 25.0, 10.0, 5.0 and 2.5 pg/µL to construct a standard curve. This is a suggested calibration scheme and may be altered as needed.

3. **ANALYTICAL PROCEDURE** (See Figures 1A and 1B, Flow chart for Analytical Method D9606)

3.1 Parent and Metabolite Isolation and Cleanup

3.1.1 Sample Preparation

Bulk soil samples received from the field are homogenized using a blender or mill. Homogenized soil samples are stored frozen (<-5°C) before analysis. Weigh a 50 g or to the nearest tenth of a gram aliquot of the soil sample into a 150 mL centrifuge bottle.

3.1.2 Fortification of Procedural Recovery Sample

3. **ANALYTICAL PROCEDURE**, continued

It is recommended to analyze at least two procedural recovery samples and one untreated sample (control) with each analysis set to monitor method efficiency. Typically, run one procedural recovery sample at the limit of quantitation (0.01 ppm) along with one procedural recovery sample at the expected residue level.

For each fortification, pipette an appropriate amount of mixed standard BAS 620 H and DP-6 fortification solution prepared in 2.3.1 to control soil samples for the analysis of BAS 620 H and DP-6. Similarly for each fortification, pipette an appropriate amount of standard GP fortification solution prepared in 2.3.1 to control soil samples for the analysis of GP. For example, 1.0 mL of the 2.5 µg/mL standard added to 25 g soil results in a fortification level of 0.1 ppm.

3.1.3 Extraction

3.1.3.1 Extraction of BAS 620 H and DP-6 in Soil:

Add 50 mL dichloromethane to the centrifuge bottle containing the soil (25 g) and shake at 300 RPM for one hour. Centrifuge at 3000 rpm for 10 min. Attach a glass funnel plugged with glass wool into a 500 mL flat bottom flask, transfer the supernatant by decantation through the funnel and collect.

Add 50 mL dichloromethane to the soil marc, sonicate and vortex to loosen the soil and allow to mix to consistency. Repeat the extraction step above for 30 min. Centrifuge at 3000 rpm for 10 min. and transfer the supernatant into the above 500 mL flat bottom flask by decantation through the funnel.

Add a third aliquot of 50 mL dichloromethane to the soil marc, sonicate and vortex to loosen the soil and allow to mix to consistency. Repeat the extraction step above for 30 min. Centrifuge at 3000 rpm for 10 min. and transfer the supernatant into the above 500 mL flat bottom flask by decantation through the funnel.

Evaporate the combined extract to dryness using a rotary evaporator with the water bath temperature set at approximately 60°C (set vacuum initially at about 650 mbar until removal of all dichloromethane and then gradually decrease the vacuum to about 35 to 45 mbar). **Proceed as indicated in section 3.1.5.1.**

4

3. **ANALYTICAL PROCEDURE**, continued

3.1.3.2 Extraction of GP in Soil

3.1.3.2.1 Extraction

Add 30 mL 1N NaOH to the soil and shake for one hr. Centrifuge at 3000 rpm for 10 min (or speed/time requires to separate the marc from supernatant could vary for different types of centrifuges).

Decant the supernatant from the marc into a 500 mL flat-bottomed flask and add 25 mL 1N NaOH to the marc. Vortex to homogenize the marc and the solvent. Shake for 15 minutes and centrifuge at 3000 rpm for 10 min.

Decant the supernatant from the marc into the previously mentioned 500 mL flat-bottomed flask and add 25 mL 1N NaOH to the marc. Vortex to homogenize the marc and the solvent. Shake for 15 minutes and centrifuge at 3000 rpm for 10 min.

Decant the supernatant into the flask and **proceed as described in the section 3.1.3.2.2** with combined alkaline extract.

3.1.3.2.2 ChemElut® column partition:

Adjust the pH of the extract obtained from **3.1.3.2.1** to about 2 to 3 using formic acid (88%, approximately 10 mL). Check with pH paper. Connect an anticlimb adopter trap to the flask and concentrate the combined extract to about 15 to 25 mL using a rotary evaporator with the water bath temperature set at approximately 60°C (vacuum initially set at about 200 mbar until frothing subsides and then gradually decreased to about 35 to 40 mbar).

NOTE: Do not concentrate the extract to less than 10 mL (a brown ring began to form around the flask at this point). This will cause precipitation of humic acids and low recovery of the analyte. Care should be taken not to lose any solution due to excessive frothing. If it is necessary, evaporate the extract in portions before ChemElut® partition.

Add about 5 to 10 g of solid sodium chloride to the extract. Swirl and sonicate (at least for 2 minutes) to facilitate the dissolution of the sample from the side of the flask and to dissolve sodium chloride to saturate the extract .

Attach a ChemElut® column to the top of a 500 mL flat bottom flask (secure the position of column with tape). Swirl and pour the

3. **ANALYTICAL PROCEDURE,** Continued

extracts with the salts onto the top of the ChemElut® column at once (hold the flask as long as it takes to transfer the majority amount of the extracts & salts). If necessary apply gentle vacuum (2-5 KPa) at the end of the column to allow the solution to absorb. Rinse the flask 3 times with 5 mL of water added to the column each time. Rinse additional times with a small amount of water if color remains in the flask. Rinse the top of the column with about 2 mL of water [DO NOT EXCEED A TOTAL VOLUME OF 20 mL OF WATER TO RINSE]. Again if necessary apply vacuum to absorb the solution into the column surface.

Allow the column to stand for 30 min.

NOTE: It is recommended to wait at least 30 minutes before elution. **This is a good stopping point for this method.**

Add 200 mL of a solvent mixture consisting of dichloromethane:isopropanol:formic acid (90:10:2, v/v/v) in portion using a graduated cylinder to elute the residues. Do not allow the column to run dry. Elution should be done by gravity. Evaporate the eluate to 30 - 40 mL on a rotary evaporator first with a bath temperature maintained at about 60°C (vacuum at 650 mbar) and then set vacuum at about 35 to 45 mbar to continue evaporation to complete dryness with a bath temperature maintained at about 60°C (vacuum at about 35 to 45 mbar)and **proceed as described in section 3.1.4.2.**

3.1.4 Preparation for Sample Analysis

3.1.4.1 Sample preparation for BAS 620 H and DP-6 Analysis

For LC-MS/MS determination, dissolve each sample with acetonitrile-water, 80:20, v/v (**solvent mixture I**), and dilute to 1:10 with appropriate amount of mixed solvent containing acetonitrile-water, 1:1,v/v, 0.1 % formic acid (**solvent mixture II**) just before the analysis. Filter the solution through a membrane syringe filter (a 0.45 micron membrane disc fitted to an 1.0 mL disposable plastic syringe) to the injection vial. Typically the following procedures are used to prepare the samples for analysis:

For control and 0.01 ppm fortifications, add 2.5 mL of **solvent mixture I**. Sonicate and vortex to ensure complete dissolution of residues from the side of the flask. Take 1 ml and dilute to 10 mL with **solvent mixture II**. Sonicate and vortex to ensure complete dissolution of residues.

3. **ANALYTICAL PROCEDURE**, continued

For 0.1 ppm fortifications, add 25.0 mL of the **solvent mixture I**. Sonicate and vortex to ensure complete dissolution. Sonicate and vortex to ensure complete dissolution of residues from the side of the flask. Take 1 mL and dilute to 10 mL with **solvent mixture II**. Sonicate and vortex to ensure complete dissolution of residues.

For 1.0 ppm fortifications, add 250.0 mL of **solvent mixture I** [initially add about 10 mL **solvent mixture I** and sonicate and vortex to ensure complete dissolution of residues from the side of the flask. Transfer the sample solution to a 250 mL volumetric flask. Rinse the flask thoroughly with **solvent mixture I** to ensure complete transfer of the residues solution and then dilute to the mark with **solvent mixture I**. Sonicate and vortex to ensure a homogeneous solution]. Take 1 mL and dilute to 10 mL with **solvent mixture II**. Sonicate and vortex to ensure a homogeneous solution.

3.1.4.2 Sample preparation for GP Analysis

For LC-MS/MS determination, dissolve each sample with an appropriate amount of **solvent mixture IV** [methanol (1 mL) and **solvent mixture III** {acetonitrile-water {3:7, v/v, 0.1 % formic acid and 5 mM ammonium acetate}, **90 mL**}] just before the analysis. Filter the solution through a membrane syringe filter (a 0.45 micron membrane disc fitted to an 1.0 mL disposable plastic syringe) to the injection vial. Typically the following procedures are used to prepare the samples for analysis:

For control and 0.01 ppm fortifications, add 25 mL of **solvent mixture IV**. Sonicate and vortex to ensure complete dissolution of residues.

For 0.1 ppm fortifications, add 250 mL of **solvent mixture IV** [initially add about 10 mL **solvent mixture IV** and sonicate and vortex to ensure complete dissolution of residues from the side of the flask. Transfer the sample solution to a 250 mL volumetric flask. Rinse the flask thoroughly with **solvent mixture IV** to ensure complete transfer of the residues solution and then dilute to the mark with **solvent mixture IV**]. Sonicate and vortex to ensure a homogeneous solution.

For 1.0 ppm fortifications, add 2500 mL of **solvent mixture IV** [initially add about 10 mL **solvent mixture IV** and sonicate and vortex to ensure complete dissolution of residues from the side of the flask. Transfer the sample solution to a 250 mL volumetric flask. Rinse the flask thoroughly with **solvent mixture IV** to ensure complete transfer of the residues solution and then dilute

3. **ANALYTICAL PROCEDURE**, continued

to the mark with **solvent mixture IV**. Take 1 ml and dilute to 10 mL with **solvent mixture IV**]. Sonicate and vortex to ensure a homogeneous solution.

3.1.5 Moisture Determination

Soil analysis results are reported on a "dry weight" basis. Therefore soil sample weights must be corrected for moisture content by any method the laboratory customarily uses. See section 11, Note 2 for an example of a moisture determination procedure.

3.2 Instrumentation

3.2.1 Description of Equipment

HPLC Pump:	Thermo Separation Products, P-4000
Autoinjector:	Thermo Separation Products, AS 3000
Data System:	Macintosh 7100/66 Power PC
Column:	Betasil C18 (100 x 2.1 mm) Keystone Scientific, Bellefonte, PA

NOTE: The equipment listed was used for method development. Other equivalent hardware may be used. The use of a guard column is optional.

Mass Spectrometer: PE SCIEX API III

Turbo Ionspray is used to enhance sensitivity, but is not required if adequate sensitivity can be used without the use of Turbo Ionspray. When using Turbo Ionspray, move the needle target a few millimeters further away from the orifice than what is used for normal operation.

This method could conceivably be validated on another instrument manufacturer's platform. If this is the case, instrument parameters and flow splits will be different, however, the basic principles of analysis by LC/MS/MS will remain the same.

3. **ANALYTICAL PROCEDURE,** continued

3.2.2 Typical Operating Conditions

HPLC Operating Conditions:

Mobile Phase A:	Water with 0.1% Formic Acid and 5 mM Ammonium acetate
Mobile Phase B:	Water-acetonitrile, 1:9, v/v with 0.1% Formic Acid and 5 mM Ammonium acetate
Flow:	0.3 mL/min

Isocratic Mobile Phase for the analysis of BAS 620 H and DP-6:	45 % A + 55 % B
Gradient (A/B) for the analysis of GP:	90/10 to 10/90 in 5 minutes, to 90/10 in one minute and re-equilibrate at 5 minutes to 90/10

Injection Volume: 10 µL into 10 µL loop

MS Operating Conditions:

Ionization Mode for BAS 620 H and DP-6:	Positive ion
Ionization Mode for GP:	Negative ion

NOTE: The following recommended instrument parameters were found to be optimal for the instrument used for the method validation. The exact values used must be optimized for each instrument.

Curtain Gas:	Nitrogen @ 1.2 L/min
Nebulizer Gas:	Nitrogen @80 psi
Interface Setpoint:	57 °C
Auxiliary Gas Flow:	Nitrogen @ 7 L/min (~ 1 L/min without Turbo Ionspray)
Collision Gas:	Argon at 290 cgt
Turbo Temperature:	475 °C (not applicable without Turbo)

Acquisition Parameters:

Resolution:	Increase DM1 and DM3 by 0.2 units from unit resolution values for extra sensitivity if needed

3. **ANALYTICAL PROCEDURE**, continued

Settling Mass: 100

Negative Ion Mode (GP):

ISV Voltage:	-4.5 kV
Orifice:	-52 V
Collision Offset (R0/R2):	-30V/-13V
Delay:	3 minutes
Acquire:	10 minutes
Q1/Q3 Masses:	**215/171 ±0.2**
Dwell:	250 msec

Positive Ion Mode (BAS 620 H and DP-6):

ISV Voltage:	5 kV
Orifice:	40 V
Collision Offset (R0/R2):	30V/18V
Delay:	0 minutes
Acquire:	7 min
Q1/Q3 Masses:	**253/197 ±0.2**
	342/250 ±0.2
Dwell:	250 msec

Typical Retention Times:

Analyte	Transition	Retention Time (approx.)
BH 620-GP	215/171	3.5 minutes
BAS 620 H isomer Z	342/250	1.5 minutes
BAS 620 H isomer E	342/250	5.6 minutes
BH 620-DP6	253/197	2.8 minutes

3.2.3 Calibration Procedures

Inject two or more mixed standards of analytes until stable responses are observed. Calculation of results is based on peak area measurements using a calibration curve. The calibration curve is obtained by injecting various amount of the standard solution (e.g. 25.0, 20.0, 10.0 and 5.0 pg/μL for BAS 620 H and DP-6) **concurrently with sample analysis.**

3. **ANALYTICAL PROCEDURE**, continued

Different standard concentrations may be used as appropriate. Plot detector response (peak area) versus weight of standard injected. The standards should give a linear response. The calibration curve for BAS 620 H is constructed by adding the peak areas for the two isomers for each standard injection and plotting (peak area isomer Z + peak area isomer E) versus concentration of standard injected.

3.2.4 Sample Analysis

Inject 10 µL of the calibration standards and samples. Depending on the instrument sensitivity the method may be validated with smaller or larger injection volumes. Directly compare the response (peak area) of unknown samples injected with the standard curve to obtain the weight of BAS 620 H, DP-6 and GP in the sample. For BAS 620 H, the peak area of the two isomers must be added and the total peak area is compared with the calibration curve. The calibration curve from BAS 620 must be constructed by plotting (peak area isomer Z + peak area isomer E) versus the concentration of standard injected.

It is recommended that at least two standards are injected at the beginning and two at the end of the set to ensure the bracketing of samples. To do this, an appropriate injection sequence including standards and samples must be planned. If the peak area of the unknown is larger than the highest standard, dilute the unknown appropriately and reinject.

NOTE: This method could conceivably be validated on another instrument manufacturer's platform. If this is the case, instrument parameters will be different, however, the basic principles of analysis by LC/MS/MS will remain the same. During routine analysis (Reference) a different instrument (API 300) and a different chromatographic system was used to run BAS 620 H and DP-6 analysis. A slightly different chromatographic condition for GP routine analysis was also used. The experimental details are given in special note

3.3 Interference

3.3.1 Sample Matrices

If interfering peaks from the matrix occur in the chromatogram, change the LC-MS operating conditions (see 3.2.2) or use an

3. **ANALYTICAL PROCEDURE,** Continued

alternative HPLC column. It is desirable to clean periodically the chromatographic system by injecting with the solvent.

3.3.2 Other Sources

Other Pesticides:	None known to date.
Solvents:	None known to date.
Labware:	None known to date.

3.4 Confirmatory Techniques

Mass spec determination is a self confirmatory technique. No problems with interference or questionable peak identity have been encountered to date.

3.5 Time Required for Analysis

Analysis of a set of 7 Soil samples requires 1.5 working days for BAS 620 H & DP-6 method and 2 days for GP method respectively including sample work-up and LC-MS/MS analysis.

3.6 Potential Problems

Potential technical problems have been described at the appropriate points in the method.

4. **METHODS OF CALCULATION** (See Figure 2 for an example calculation.)

4.1 Calibration

Construct a linear least squares calibration curve in the form $y = bx+c$ from the standards by plotting peak area versus weight of standard injected for both analytes. The calibration curve for BAS 620 H is constructed by adding the peak areas for the two isomers for each standard injection and plotting (peak area isomer Z + peak area isomer E) versus concentration of standard injected.

4.2 Analyte in Sample

Calculation of results is based on peak area measurements. Using the peak area measurements for

4. **METHODS OF CALCULATION,** continued

BAS 620 H, DP-6 and GP in the samples, the amount of the analyte in ng from the appropriate least squares calibration curve is determined. The peak area for the two isomers of BAS 620 H in each sample must be added and the amount of BAS 620 H in the sample determined from the calibration curve described in 4.1.

Calculate ppm values by the equation below.

$$ppm = \frac{A}{B}$$

where: **A** = ng value interpolated from calibration curve (Obtained automatically or could be calculated using Fig. 3)

$$B = \text{mg Sample Injected} = \frac{\text{Dry Sample Wt. (g)} \times \mu\text{L Injected}}{\text{Final dilution volume (mL)}}$$

The "final dilution volume" includes any dilution which have been made. Using the Dry Sample Weight in the "mg Sample Injected" calculation will yield a ppm value on a "dry weight" basis. The Dry Sample Weight is obtained after determining the moisture content of the original sample (see Section 3.1.9). Moisture correction is not necessary for fortification samples.

4.3 Calculation of Procedural Recoveries

Correct fortification results for residues found in the control sample as follows:

ppm (corrected) = ppm in fortified control - ppm in control

Determine percent recovery from the fortification experiments as follows:

$$\text{\% Recovery} = \frac{\text{ppm (corrected)} \times 100}{\text{ppm BAS 620 H or DP-6 or GP added}}$$

Only results for procedural recovery samples should be corrected for residues in the controls. Do not correct treated sample results for either control residues or recoveries.

5. VALIDATION:

5.1 Description of Protocol

The validation was carried out as BASF Study 96084. Control soil samples were fortified with BAS 620 H, DP-6 and GP at levels ranging from 0.01 to 1.0 ppm. The fortified controls were analyzed and the results converted to recovery values for evaluation. Percent recoveries for BAS 620 H are compiled in Table I. Percent recoveries for DP-6 are compiled in Table II. Percent recoveries for GP are compiled in Table III.

The standard compounds shown in the table below were used for method development and validation.

Compound	Code	Lot Number	Purity
(RS-EZ)-2-[1-(3-Chloro-(2E)-propenyloximino)propyl]-3-hydroxy-5-(terahydropyran-4-yl)cyclohex-2-en-1-one	BAS 620 H	691-25-1	99. 9%
3-Hydroxy-2-propionyl-5-(tetrahydropyran-4-yl) cyclohex-2-en-1-one	DP-6	41-180	97.9%
3-(3,4,5,6-Tetrahydro-2H pyran-4-yl)glutaric acid	GP	31-2139-MH	99.9%

Standard supplied by:

Dr. Rita Laschober
BASF Aktiengesellschaft, APS/UP
Agricultural Research Center
D-67114 Limburgerhof, West Germany
Telephone: 06236/68/2103

The **Test System** consisted of untreated soil samples obtained from BAS 620 H trial sites (BASF Study 95023). Two different soil types were used to validate this method. Soils obtained from California, US site were identified as BASF RCN 95008 and soils obtained from Manitoba site, Canada were identified as BASF RCN 95012. Soil characterization data for these soil samples are summarized in Table IV.

The HPLC column used was manufactured by Keystone and had serial number 0861036D.

5. **VALIDATION**, continued

5.2 Solution Stability

During the course of this study, the stabilities of fortification and LC-MS/MS standard solutions were examined. Solutions were stored in a refrigerator at 4°C. Following table shows the stability of the analytes in various solvent system used within the method.

ANALYTES	SOLUTION	STABILITIES (DAYS)
BAS 620 H and DP-6	**Stock solution in methanol**	150
BAS 620 H and DP-6	**Fortification solutions in methanol**	37
BAS 620 H and DP-6	**Solvent mixture I**	45
BAS 620 H and DP-6	**Solvent mixture II**	21
BAS 620 H and DP-6	**methanol-water (50:50) containing 0.1 % formic acid and 4 mM ammonium formate**	45
GP	**Stock solution in methanol**	121
GP	**Fortification solutions in methanol**	30
GP	**Solvent mixture IV**	14
GP	**Solvent mixture V**	12

As general practice, stock solutions are prepared every three months. Fortification solutions are prepared monthly. Other solutions are prepared according to their stabilty in that particular solution as shown in the above table.

5.3 Protocol Changes

No Changes were made to the validation study protocol.

6. **RESULTS AND DISCUSSION**

6.1 General

The present work describes an analytical method to measure the residues of BAS 620 H and its two major metabolites DP-6 and GP in two types of soil. Recoveries of BAS 620 H ranged from 45 to 121% with a mean recovery of 99 ± 14% (N = 24) and are summarized in Table I. Recoveries of DP-6 ranged from 69 to 115% with a mean recovery of 92 ± 10% (N = 24) and are summarized in Table II. Recoveries of GP ranged 63 to 105% % with a mean recovery of 83 ± 12% (N = 24) and are summarized in Table III. This study has shown that Analytical Method No. D9606 is suitable for measuring residues of BAS 620 H, DP-6 and GP in soil down to 0.01 ppm.

6. **RESULTS AND DISCUSSION,** continued

Two types of soil were used as substrates for the validation study. These soil samples represent the types of soils on which BAS 620 H is customarily used. Soil characterization data are given in Table IV.

Standard curves used to calculate the recoveries shown in Tables I, II and III were generated from standard solutions containing BAS 620 H, DP-6 and GP injected concurrently with the analysis set. Standard injections bracketed the sample injections. Summaries of all the injection standard data used in this study are given in Tables V through VII.

Representative LC-MS/MS chromatograms of BAS 620 H, DP-6 and GP residue analyses of selected control and fortified samples are shown in Figures A.12 through A.27. Typical standard chromatograms are shown in Figures A.1 through A.8. Typical standard curves for BAS 620 H, DP-6 and GP are given in Figures A.9 through A.11.

6.2 Accuracy and Precision

Recoveries of BAS 620 H ranged from 45 to 121% with a mean recovery of 99 ± 14% (N = 24). Recoveries of DP-6 ranged from 69 to 115% with a mean recovery of 92 ± 10% (N = 24). Recoveries of GP ranged 63 to 105% % with a mean recovery of 83 ± 12% (N = 24). No other statistical analysis was performed on the data.

6.3 Determination Limit

The determination limit for BAS 620 H, DP-6 and GP residues in soil was 0.01 mg/kg.

6.4 Ruggedness Testing

Four analysts executed eight sets of extractions and analyses in four different two-day periods. Mean recoveries and estimates of standard deviations for each analysis date were calculated from the data in Tables I, II and III:

Date of Analysis	Analysts	BAS 620 H Recovery	DP-6 Recovery	GP Recovery
7/11/96	Analyst 1	NA	NA	78 ± 11% (N = 6)
7/11/96	Analyst 2	NA	NA	71 ± 7% (N = 6)
7/16/96	Analyst 3	NA	NA	95 ± 8% (N = 6)
7/16/96	Analyst 4	NA	NA	87 ± 5% (N = 6)
7/15/96	Analyst 3	107 ± 4% (N = 6)	100 ± 3% (N = 6)	NA
7/15/96	Analyst 4	96 ± 28% (N = 6)	85 ± 8% (N = 6)	NA
7/16/96	Analyst 1	97 ± 6% (N = 6)	91 ± 4% (N = 6)	NA
7/16/96	Analyst 2	94 ± 7% (N = 6)	91 ± 16% (N = 6)	NA

[1]Six fortified samples and one control sample were analyzed. The means of the analyses for each set were in the range 70-120%. Chromatograms consisted only of peaks of analytes: one peak at the retention time of each analyte.

6.5 Limitations

None known to date.

7. CONCLUSIONS

This study has shown that Analytical Method No. D9606 is suitable for measuring residues of BAS 620 H, DP-6 and GP in soil down to 0.01 ppm.

8. QUALITY ASSURANCE PROCEDURES

The raw data and analytical standards of this study will be stored in the BASF archives at:

BASF Corporation
Agricultural Product Center
26 Davis Drive
Research Triangle Park, NC 27709

9. REFERENCES

1. a) Keller, E., BASF Report No.3633 "Aerobic Soil Metabolism of ^{14}C-BAS 191819 (BAS 620 H)", October, 1994

b) Keller, E., BASF Report No.3729 "Degradation Behavior of Reg. No. of ^{14}C-BAS 191819 (BAS 620 H) in Sterile Soil", December, 1993

c) Yamasaki, R., Nippon Soda Report No. EC-554 "BAS 620 H-anaerobic Aquatic Metabolism Study", December, 1993

9. **REFERENCES**, continued

d) Kanji Ishihara, Nippon Soda Report No. EC-737 "BAS 620 H- Photodegradation Study in water", Study is in progress, planned to be completed in 1996.

e) Shiotani H., NISSO Report No. EC-440 "BAS 620 H- Hydrolysis Study ", Study is in progress, planned to be completed in 1996.

f) Shiotani H., NISSO Report No. EC-518 "BAS 620 H- Photodegradation in Soil Study ", Study is in progress, planned to be completed in 1996.

2. M. Saha; Study No. G95023; "Residue analysis of BAS 620 H and its metabolites (DP-6 and GP) for terrestrial soil dissipation study"; Study is in progress, planned to be completed in 1997.

3. S. Jackson; Study No. 95023; "Soil dissipation of BAS 620 00 H in terrestrial use patterns"; Study is in progress, planned to be completed in 1997.

10. SAFETY AND HEALTH CONSIDERATIONS

10.1 General

Use personal protective equipment such as lab coats, safety glasses and gloves (nitrile/latex gloves are recommended) when performing the operations described in this method. Conduct all transfers, partitions, derivatizations, nitrogen-stream evaporations and SPE procedures in a well-ventilated hood. Guard vacuum equipment such as rotovaps to minimize the possibility of injury caused by flying broken glass. Dispose of hazardous wastes in an environmentally acceptable manner, in compliance with applicable laws and regulations.

10.2 Solvents, Reagents and Standards

It is recommended to review the Material Safety Data Sheets (MSDSs) for all solvents and reagents used in this method. The toxicity of BAS 620 H, DP-6 and GP are unknown.

11. SPECIAL NOTES

1. Sections 3.1.3 to 3.1.4: BASF practice is to complete the method for BAS 620 H and DP-6 from initial extraction through the LC-MS/MS determination step in 1.5 work day. BASF practice is to complete

11. SPECIAL NOTES, continued

the method for GP from initial extraction through the LC-MS/MS determination step in 2 work days.

2. Section 3.1.5: An example procedure for moisture determination is as follows:

Weigh 5 g of wet soil ("Wet weight") accurately into a tarred glass petri dish or other container. Place into a 150°C oven for 16 hours (overnight). Remove the petri dish from the oven and allow to cool in a desiccator. Working quickly, remove the cool petri dish from the desiccator and weigh accurately to obtain "Dry Weight".

Determine the Moisture content = "Wet Weight" - "Dry Weight"

Calculate "Percent moisture" = $\frac{\text{Moisture content}}{\text{"Wet Weight"}} \times 100$

Calculate "Dry Sample Weight " = $\frac{\text{"Wet Sample Weight (ppm)"}}{(100 - \text{"Percent moisture"})/100}$

The calculated "Dry Sample Weight" is used in section 4.2 to calculate concentration (ppm) values.

3. During routine analysis (Reference 2) with different soil type it has been observed that method also works with the following modifications:

11.3.1 Analysis of BAS 620 H & DP-6:

Mass Spectrometer:	PE SCIEX API 300
HPLC Pump:	Varian Star 9010
Autoinjector:	PE/AS200
Data System:	Macintosh 8500 Power PC
Column:	Betasil C18 (100 x 2.1 mm) Keystone Scientific, Bellefonte, PA

HPLC Operating Conditions:

Mobile Phase A:	Water with 0.1% formic Acid and 4 mM ammonium formate
Mobile Phase B:	MeOH with 0.1% formic Acid and 4 mM ammonium formate
Flow:	0.3 mL/min

11. **SPECIAL NOTES**, continued

Isocratic Mobile Phase: 80 % B + 20 % A

Injection Volume: 10 µL into 25 µL loop

MS Operating Conditions:

Ionization Mode:	Positive ion
Curtain Gas:	Nitrogen @ 1.2 L/min
Nebulizer Gas:	Nitrogen @80 psInterface
Setpoint:	57 °C
Auxiliary Gas Flow:	Nitrogen @ 7 L/min (~ 1 L/min without Turbo Ionspray)
Collision Gas:	Argon at 290 cgt
Turbo Temperature:	475 °C (not applicable without Turbo).
Acquisition Parameters:	
Resolution:	Increase DM1 and DM3 by 0.2 units from unit mass resolution values for extra sensitivity if needed
Settling Mass:	100
Ionization Mode:	Positive Ion
ISV Voltage:	5 kV
Orifice:	40 V
Collision Offset (R0/R2):	30V/18V
Delay:	0 minutes
Acquire:	7 min
Q1/Q3 Masses:	**253/197 ±0.2** **342/250 ±0.2**
Dwell:	250 msec

11.3.2 Analysis of GP:

Mass Spectrometer:	PE SCIEX API III
HPLC Pump:	Thermo Separation Products, P-4000
Autoinjector:	Thermo Separation Products, AS 3000
Data System:	Macintosh 7100/66 Power PC
Column:	Betasil C18 (100 x 2.1 mm) Keystone Scientific, Bellefonte, PA

11. **SPECIAL NOTES**, continued

HPLC Operating Conditions:

Mobile Phase A:	Water with 0.1% formic Acid and 5 mM ammonium acetate
Mobile Phase B:	Acetonitrile-water (90:10, v/v) containing 5 mM ammonium acetate and 0.1 % formic acid
Flow:	0.3 mL/min
Gradient:	90/10 A/B to 10/90 in 4 min.; Hold at 10/90 to 7 min ; Re-equilibrate to 90/10 at 7.1 min; Inject every 12 min. (approximately)
Injection Volume:	25 µL into 25 µL loop

MS Operating Conditions:

Ionization Mode for GP: Negative ion

Curtain Gas:	Nitrogen @ 1.2 L/min
Nebulizer Gas:	Nitrogen @80 ps Interface
Setpoint:	57 °C
Auxiliary Gas Flow:	Nitrogen @ 7 L/min (~ 1 L/min without Turbo Ionspray)
Collision Gas:	Argon at 290 cgt
Turbo Temperature:	475 °C (not applicable without Turbo)

Acquisition Parameters:

Resolution:	Increase DM1 and DM3 by 0.2 units from unit mass resolution values for extra sensitivity if needed
Settling Mass:	100
Ionization Mode (GP):	Negative Ion
ISV Voltage:	-4.5 kV
Orifice:	-52 V
Collision Offset (R0/R2):	-30V/-13V
Delay:	3 minutes
Acquire:	10 minutes
Q1/Q3 Masses:	**215/171 ±0.2**
Dwell:	250 msec

11. **SPECIAL NOTES**, continued

Typical Retention Times:

Analyte	Transition	Retention Time (approx.)
BH 620-GP	215/171	3.3 minutes
BAS 620 H isomer Z	342/250	1.35 minutes
BH 620-DP6	253/197	2.25 minutes
BAS 620 H isomer E	342/250	2.33 minutes

11.3.3 Injection Standards and Sample preparation for the above methods:

11.3.3.1 Injection Standards and Sample preparation for BAS 620 H and DP-6 analysis

Change **Solvent mixture II** to methanol-water (50:50, v/v) containing 0.1 % formic acid and 4 mM ammonium formate to prepare injection standars (section 2.3.1.6.2) for LC-MS/MS. Typical injection standard concentrations were **50.0, 25.0, 10.0, and 5.0 pg/µL.** Sample extracts in **Solvent mixture I**, were also rediluted (1/10) with methanol-water (50:50, v/v) containing 0.1 % formic acid and 4 mM ammonium formate prior to LC-MS/MS injection (section 3.1.4.1).

11.3.3.2 Injection Standards and Sample preparation for GP analysis

Solvent mixture V: 90:10, v/v, A/B, where as A & B are LC-MS mobile phases for GP analysis

Standards in **Solvent mixture IV** (section 2.3.1.6.3) are diluted with **Solvent mixture V** (1/5) to prepare injection standars for LC-MS/MS analysis. Typical injection standard concentrations were **10.0, 5.0, 2.0, and 1.0 pg/µL.** Sample extracts in **Solvent mixture IV**, were also rediluted with **Solvent mixture V** (1/5) prior to LC-MS/MS injection (section 3.1.4.2).

12. CERTIFICATION

We, the undersigned, hereby declare that this report provides a true and accurate record of the results obtained.

Author: Manasi Saha Date: 3/14/97

Manasi G. Saha

Approved By: Date: 3/12/97

Technical Center Leader,
Residue Environmental Fate-1/MS Lab

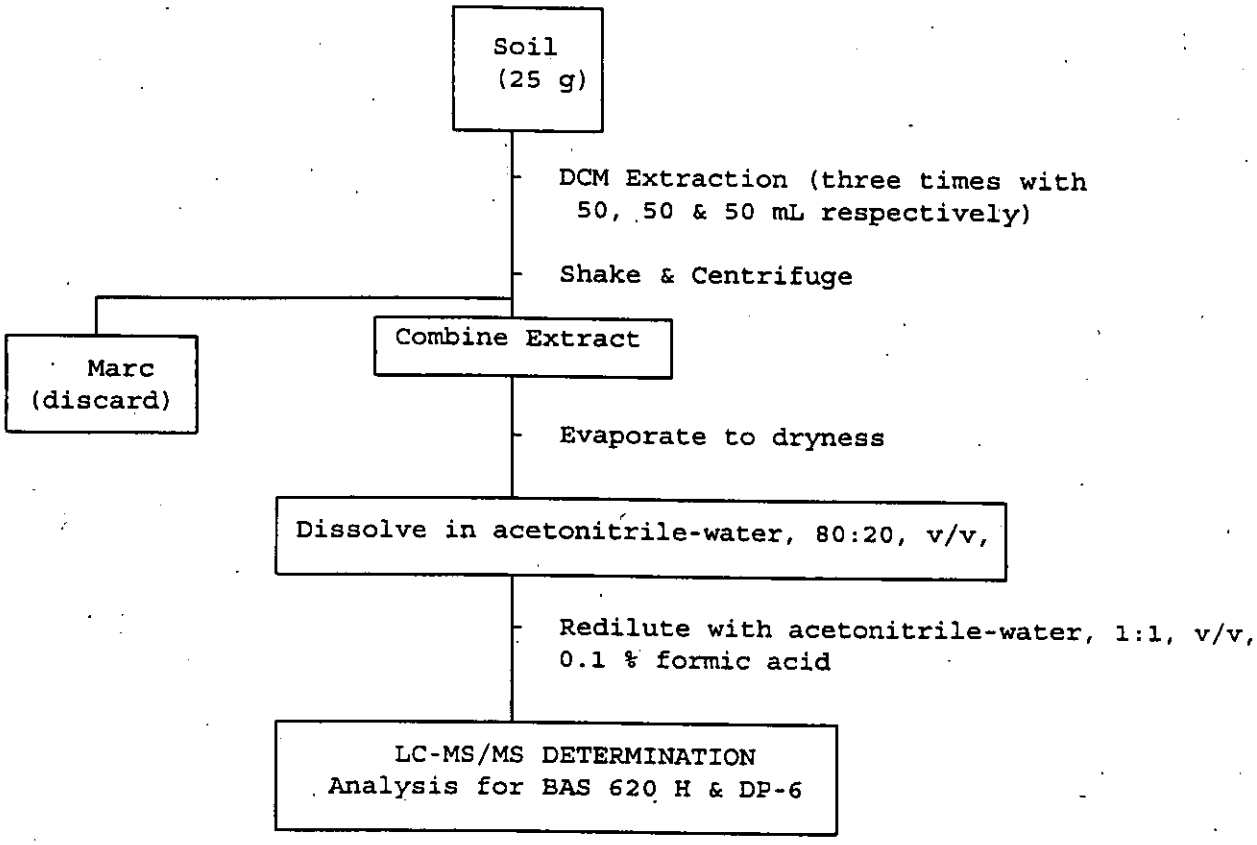


Figure 1A. Flow Chart for Analytical Method D9606 (BAS 620 H & DP-6)

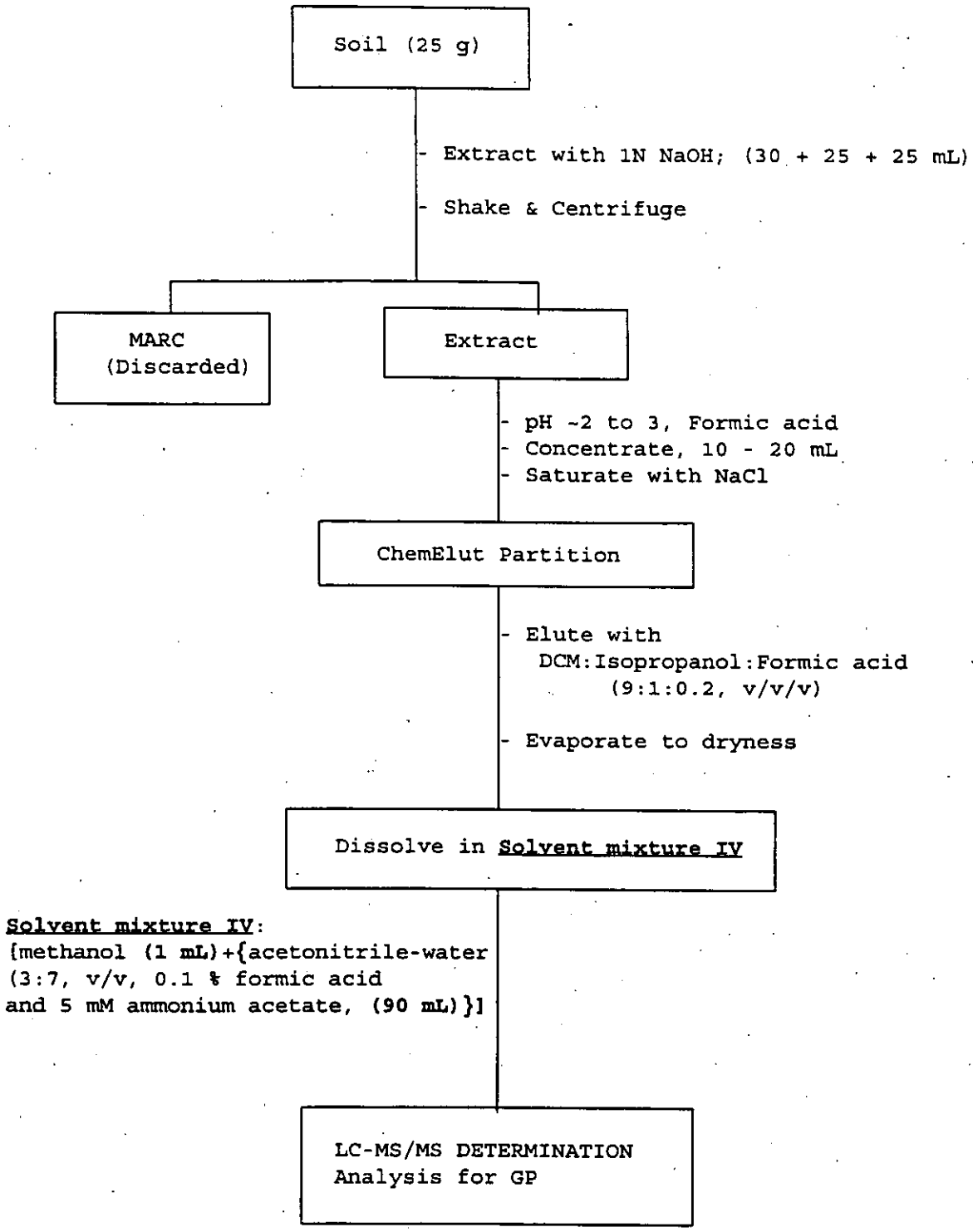


Figure 1B. Flow Chart for Analytical Method D9606 (GP)

BASF Sample Number 96084/1100-02-11. Fortified Soil at 0.01 ppm for BAS 620 H Recovery.

A = ng value calculated from standard curve

Standard curve: ng (BAS 620 H) = $\frac{\text{Peak Area - 7.39e+2}}{\text{1.365534e+2}}$

Total Peak Area (E + Z isomer): 15870

Use full computer/calculator precision in any intermediate calculations. Round only the final value.

A = ng (BAS 620 H) = $\frac{\text{15870 - [- 5.4841827e+11}}{\text{1.365534e+2}}$

= 0.1108 ng

B = mg sample injected = $\frac{\text{Sample weight (g) x } \mu\text{L injected}}{\text{Final dilution volume (mL)}}$

= $\frac{25 \times 10}{25}$ = 10 mg

D = molecular weight conversion factor = 1

ppm = $\frac{A \times D}{B}$ = $\frac{0.1108 \times 1.0}{10}$

= 0.01108 ppm

The corresponding control sample (BASF Sample 96084/1100-02-9) contained 0.00 ppm of BAS 620 H residue.
Net Recovery = 0.01108 - 0.0000 = 0.01108 ppm (111%)

Figure 2. Typical Calculation for the LC-MS/MS Quantitation of BAS 620H Residues in Soil.

BASF Sample Number 96084/1100-02-11. Fortified Soil at 0.01 ppm for DP-6 Recovery.

A = ng value calculated from standard curve

Standard curve: $ng\ (DP\text{-}6) = \frac{\text{Peak Area} - [-5.4841827e+1]}{7.352955}$

Peak Area: 7718

Use full computer/calculator precision in any intermediate calculations. Round only the final value.

$A = ng\ (DP\text{-}6) = \frac{7718 - [-5.4841827e+1]}{7.352955}$

$= 0.1057$ ng

$B = \text{mg sample injected} = \frac{\text{Sample weight (g)} \times \mu\text{L injected}}{\text{Final dilution volume (mL)}}$

$= \frac{25 \times 10}{25} = 10$ mg

D = molecular weight conversion factor = 1

$ppm = \frac{A \times D}{B} = \frac{0.1057 \times 1.0}{10}$

$= 0.01057$ ppm

The corresponding control sample (BASF Sample 96084/1100-02-9) contained 0.00 ppm of DP-6 residue.
Net Recovery = 0.01108 - 0.0000 = 0.1057 ppm (106%)

Figure 3. Typical Calculation for the LC-MS/MS Quantitation of DP-6 Residues in Soil.

BASF Sample Number 96084/1109-15-11. Fortified Soil at 0.01 ppm for GP Recovery.

A = ng value calculated from standard curve

Standard curve: $ng\ (GP) = \frac{\text{Peak Area} - 8.5794403e+11}{1.1947212e+2}$

Peak Area: 8997

Use full computer/calculator precision in any intermediate calculations. Round only the final value.

$A = ng\ (GP) = \frac{8997 - 8.5794403e+11}{1.1947212e+2}$

$= 0.074588$ ng

$B = \text{mg sample injected} = \frac{\text{Sample weight (g) x } \mu\text{L injected}}{\text{Final dilution volume (mL)}}$

$= \frac{25 \times 10}{25} = 10$ mg

D = molecular weight conversion factor = 1

$ppm = \frac{A \times D}{B} = \frac{0.074588 \times 1.0}{10}$

$= 0.074588$ ppm

The corresponding control sample (BASF Sample 96084/1109-15-7) contained 0.00 ppm of GP residue.
Net Recovery = 0.074588 - 0.0000 = 0.074588 ppm (75%)

Figure 4. Typical Calculation for the LC-MS/MS Quantitation of GP Residues in Soil.