1.0 INTRODUCTION AND SUMMARY

1.1 SCOPE AND SOURCE OF THE METHOD

1.1.1 Scope

This method is for determining residues of BAS 514 H (Quinclorac) and its metabolites BH 514-2-OH and BH 514-ME in soil. Metabolism studies show that in addition to parent Quinclorac, BH 514 2-OH and BH 514-ME are minor soil metabolites. Also, shown in metabolism studies (Reference 1), due to the acidic nature of Quinclorac a solution of sodium hydroxide is needed to free the Quinclorac from the soil. For sandy or sandy loam soils, extraction is accomplished by mechanically shaking the soil in 0.1 N NaOH for 1 hour. For soils with a clay content, it is best to use a more exhaustive extraction using a reflux of 0.1 N NaOH for 1 hour. The samples are centrifuged, then an aliquot is acidified and partitioned with 8:2 methylene chloride/ethyl acetate. The extract is taken to dryness by rotary evaporation, and then reconstituted into an appropriate volume of the mobile phase. The final quantitative determination of BAS 514 H and BH 514-2-OH is made by LC/MS/MS using multiple reaction monitoring. The methyl ester metabolite of Quinclorac, BH 514-ME, is a very minor metabolism product. When refluxing the soil in 0.1 N NaOH, this minor product is converted and analyzed as parent Quinclorac. The shake method is not sufficient to hydrolyze the ester. Therefore a separate extraction had been developed to analyze for BH 514-ME for the use in conjunction with the shake method. For this determination of BH 514-ME, soil samples are extracted in a mixture of methylene chloride , ethyl acetate, and methanol. The extract, first separated from the soil by centrifugation, is taken to dryness by a nitrogen stream and reconsituted in an appropriate volume of mobile phase. The final quantitative determination of BH 514-ME is made by LC/MS/MS

1.1.2 Source

This method was developed at BASF Corporation, 26 Davis Drive, Research Triangle Park, North Carolina 27709 and ALTA Analytical, 5070 Robert J. Matthews Parkway, El Dorado Hills, CA 95630. This method for Quinclorac determination is a variation of BASF Method A8903 "HPLC Method for the Determination of Quinclorac (3-7-dichloro-8-quinolinecarboxylic acid) and its Metabolite BH 514-1 (3-chloro-8-quinolinecarboxylic acid) in Soil" (Reference 2) This method also extracts with sodium hydroxide and partitions with methylene chloride. Final determination of the free acid is by HPLC using column switching. The limit of quanitation of this method is 0.05 ppm. To ensure a lower limit of quantitation of at least 0.01 ppm, the method of analysis was modified to use the high sensitivity of the LC/MS/MS.

This study was initiated on August 21, 1995.

1.2 REFERENCE SUBSTANCES

Common Name:	Quinclorac		
BASF Code:	BAS 514 H		
Chemical Name:	3,7-dichloro-8-quinolinecarboxylic acid		
Empirical Formula:	$C_{10}H_5Cl_2NO_2$		
Molecular Weight:	242		
Melting Point:	Above 237°C		
Appearance:	White, Solid		
Odor:	Odorless		
Solubility - at 20°C:	(g/100 g solvent, 20°C)		
Water:	6.2×10^{-3}	Toluene:	<0.1
Acetone	0.2	Ethyl acetate	0.1
Acetonitrile	<0.1	n-Hexane	<0.1
Dichloromethane	<0.1	NaOH	(0.1 N) Readily Soluble
Ethanol	0.2		

Common Name:	2-HydroxyQuinclorac
BASF Code:	BH 514-2-OH
Chemical Name:	3,7-dichloro-2-hydroxy-8-quinolinecarboxylic acid
Empirical Formula:	$C_{10}H_6Cl_2NO_4$
Molecular Weight:	258

Common Name:	Quinclorac methyl ester
BASF Code:	BH 514-ME
Chemical Name:	3,7-dichloro-2-hydroxy-8-quinolinecarboxyate
Empirical Formula:	$C_{11}H_8Cl_2NO_4$
Molecular Weight:	256

1.3 PRINCIPLE OF THE METHOD

For BAS 514 H (Quinclorac) and BH 514-2-OH, soil samples are extracted in 0.1 N NaOH, either on a mechanical shaker or refluxed, centrifuged and decanted. The basic extract is then acidified and partitioned with 8:2 methylene chloride/ethyl acetate. The methylene chloride extract is taken to dryness by rotary evaporation using vacuum. For BH 514-ME, soil samples are extracted in a mixture of methylene chloride, ethyl acetate, and methanol. The extract, first separated from the soil by centrifugation, is taken to dryness by a nitrogen stream and reconsituted in an appropriate volume of mobile phase. The final quantitative determination of BAS 514, BH 514-2-OH, and BH 514-ME is made by LC/MS/MS. The limit of quantitation for each metabolite is 0.01 ppm.

2.0 MATERIALS AND METHODS

2.1

Equipment	Suggested Sizes/Manufacturer
Rotary evaporator:	Buchi Rotovapor or equivalent
N-Evap (Nitrogen Stream	Organomation Assoc. or equivalent
Centrifuge:	IPC PR 700 Refrigrated or Equivalent
Mechanical shaker:	Janke & Kunkel HS 501D
Mini Centrifuge:	Capsule HF-120 Millipore or equivalent 1.5ml/0.45 micron nylon
Centrifuge filter for mini-centrifuge:	MSI or equivalent
Separatory funnels:	250 mL, 125 mL
Flat bottom flasks:	300 mL
Centrifuge glass tubes with teflon lined screw top flasks	50 Fisher Scientific
Glass Bottles	50 mL
Volumetric flasks:	10, 25, 50, 100 mL
Volumetric pipettes:	0.5, 1, 5,10, 25 mL
Graduate pipettes:	1 mL
Long stem funnels:	75 mm id
Conical inserts:	200 µL Varian or equivalent
Autosampler vials:	1.5 mL Varian or equivalent
Phase Separator Paper:	15.0 cm 1 PS
Magnetic stirring bars	2.5 cm
Reflux condenser	Standard joint
Stirring hot plate	Corning PC-351
Ultrasonic Bath	Branson 1200
Top loading balance	
Other general laboratory glassware and supplies.	

Preparations for Mobile phases:

Mobile phase A: Add 100 mL 50mM ammonium acetate and 1 mL of formic acid to 900 mL of water

Mobile Phase B: Add 100 mL 50mM ammonium acetate and 1 mL of formic acid to 900 mL of methanol

2.2. Reagents and Chemicals	Suggested Source/Preparation
Methylene Chloride	Baxter Healthcare Corporation, B&J Brand CAS 75-09-2
Acetonitrile	Baxter Healthcare Corporation, B&J Brand CAS 67-56-1
Methanol	Baxter Healthcare Corporation, B&J Brand CAS 67-56-1
Water	Baxter Healthcare Corporation, B&J Brand CAS 7732-18-5
Ethyl Acetate,	Baxter Healthcare Corporation, B&J Brand CAS 111-90-0
Formic Acid 95-97%	Aldrich Chemical cat#10,652-6 CAS 64-18-6
Hydrochloric Acid	Aldrich Chemical cat#25,814-8, CAS 7647-01-0
Ammonium Formate	Aldrich Chemical cat#15,626-4 CAS 540-69-2
Ammonium Acetate	Aldrich Chemical cat#37,233-1 CAS 631-61-8
Sodium Hydroxide	Aldrich Chemical cat#31,951-1 CAS 1310-73-2

2.3 Standard Substances and Solutions

Standards Used:

BASF Code	Lot Number	Purity
BAS 514 H	39/182-1	99.0%
BH 514-ME	L33/215	99.9
BH 514-2-OH	00820-63	98.6%

Standards supplied by:

BASF Corporation
Agricultural Products Group
P. O. Box 13528
Research Triangle Park, NC 27709

Standards are to be maintained frozen (<-5°C) until its use. Standards were characterized as required by 40 CFR part 160, FIFRA Good Laboratory Practices. Information on the synthesis and subsequent characterization of these substances is available to BASF and is located at BASF Corporation, Agricultural Products Group, Research Triangle Park, North Carolina.

Solutions of standards are to be refrigerated (+4° C) during their use in this study. Stock solutions (1 mg/mL) are to be made fresh every three months. Dilutions of the stock solution are to be made monthly.

2.3.1 Standard Solutions for Fortifications

NOTE: Storage containers for standard solutions are amber bottles with Teflon®-lined screw caps. These concentrations are suggested. Different preparation and concentration schemes may be used and additional standard concentrations may be prepared and used as needed.

Stock Solution Preparation

Prepare a 1.0 mg/mL BAS 514, BH 514-ME, and BH 514-2-OH (powder) stock solution by separately weighing an appropriate amount into separate volumetric flasks. Use methanol as the solvent. For example, weigh 25.0 mg of Quinclorac into a 25.0 mL volumetric flask. Dissolve with methanol and dilute to the mark.

NOTE:

The solubility of BH 514-2-OH is best achieved by adding 100 µg of 1 N NaOH. After addition of base dilute to the mark with methanol.

Prepare a 100.0 µg/mL combined standard solution by transferring an appropriate amount of each of the 1.0 mg/mL stock solution with a volumetric pipet into a volumetric flask (typically 5 mL of 1.00 mg/mL stock solution into a 50 mL volumetric flask). Dilute to the mark with methanol.

Prepare the 10.0 and 1.0 µg/mL standard solutions by making dilution of the 100.0 µg/mL solution with methanol.

Prepare 0.1 µg/mL standard solutions by making dilution of the 1.0 µg/mL solution.

2.3.2 Standard Solutions for LC/MS/MS Analysis

Prepare the 50, 20, 10 and 5 pg/µl standard solutions by making dilution of the 2.0 µg/mL solution with 50:50 methanol/50 mM Ammonium Acetate solution. For example, to prepare 50 pg/µl solution pipet 2.5 ml of 2.0 µg/ml solution into a 100.0 mL volumetric flask. Dilute to the mark with 50:50 methanol:water.

3.0 ANALYTICAL PROCEDURES-

3.1. QUINCLORAC AND BH 514-2-OH EXTRACTION

Samples are homogenized first by grinding with an appropriate mill with dry ice to maintain the integrity of the samples

3.1.1 Weigh 10.0 g of a soil sample using a top loading balance into a 150 mL glass centrifuge bottle. FOR SOILS WITH A HIGHER CLAY CONTENT A REFLUX EXTRACTION IS NEEDED: Weigh 10.0 g of soil into a 250 mL Erlenmeyer flask equipped with groud glass neck. Add a teflon encased stirrer appropriate for the size flask. As required, fortify control samples with appropriate volumes of fortification solutions. Go to Step 3.2.1

NOTE Fortifications for Procedural Recoveries

It is recommended that at least two fortifications and one untreated sample (control) are run with each set of samples to monitor method efficiency. Typically, one fortification sample at the limit of quantitation is run along with one fortification sample at a higher level. For each fortified sample, an appropriate volume of standard solution is added to a control soil sample by volumetric pipet. For example, for a 0.01 ppm fortification, pipet 1.0 mL of the 0.1 µg/mL standard solution into the control sample. Fortifications are made into the sample before the 0.1 N NaOH is added to the sample for the initial extraction.

3.2 EXTRACTION OF RESIDUE

FOR SHAKE EXTRACTION

3.2.1 Add 50.0 mL of 0.1 N NaOH and shake for 1 hour using the mechanical shaker. Centrifuge the sample for 15 minutes at 3500 rpm, or until the soil is completely separated from the extract and the supernatent is clear, then carefully pipette a 25 ml aliquot into a 250 ml separatory funnel. The centrifugation step is very important! If the supernatent is not clear and free of particulates, repeat. Go to Step 3.2.2

3.2.2 Add approximately 1 mL HCl to the 25 mL aliquot to change the pH to < 2.0. Go to Step 3.3.1.

For Reflux Extraction

3.2.1.1 Add 50.0 mL of 0.1 N NaOH to the Erlenmeyer flask and reflux for 1 hour using chilled-water condensers and a hot plate equipped with a stirrer. Stir the soil softly and check the apparatus frequently to prevent the sample foaming and boiling over. After 1 hour, let the sample cool, then carefully pour most of the mixture into a centrifuge tube. It is not necessary to get all of the solution and do not rinse the bottle. Centrifuge the extract for 15 minutes at 3500 rpm, or until the soil is completely separated from the extract and the supernatent is clear, then carefully pipette a 5 ml aliquot into a 125 ml separatory funnel. Save a portion of the solution left in the bottle for re-analysis if necessary. The centrifugation step is very important! If the supernatent is not clear and free of particulates, repeat. Go to Step 3.2.2.1.

3.2.2.1 Add approximately 0.5 mL 88% formic acid to the 5 ml aliquot to change the pH to < 2.0. For both, measure the pH with pH indicator strips. Go to Step 3.3.1.1.

3.3 METHYLENE CHLORIDE PARTITION CLEAN-UP

For Shake Extraction

3.3.1 Extract the aqueous contents (25 mL) of the separatory funnel with a 50 mL portion of dichloromethane: ethyl acetate (8:2). Shake the funnel for 2 min, and allow the layers to separate. Separate the organic layer and pass through phase separation paper into a 250 mL round flat bottom. Do not take emulsions! Repeat twice more for a total of 3 partitions. Rinse the phase separation paper with the methylene chloride:ethyl acetate solvent, and add the rinse to the flask. Go to Step 3.3.2.

NOTE: The centrifuge step 3.2.4 should have removed all particulates that will cause emulsions. Let the samples sit until total separation. If necessary the sample can be centrifuged to aid separation

3.3.2 Concentrate the combined extracts to 5-20 mL using a rotatory evaporator with water bath at 40°±5°C. Transfer the extract to a test tube using methylene chloride as a rinse and nitrogen evaporate to dryness in a water bath at 40°±5°C.

For Reflux Extraction

3.3.1.1 Extract the aqueous (5 mL) of the separatory funnel twice with 20 mL (2 x 20 = 40 total volume) portions of dichloromethane: ethyl acetate (8:2). Shake the funnel for 2 min, and allow the layers to separate. Separate the organic layer and pass through phase separation paper into a 50 mL glass container. Cap the 50 mL glass container and shake. Remove a 20 mL aliquot and transfer to a 50 mL round bottom flask. Go to 3.3.1.2.

3.3.1.2 Concentrate the combined fraction to 5 mL using a rotatory evaporator with water bath at 40°±5°C. Transfer the extract to a test tube using methylene chloride as a rinse, and nitrogen evaporate to dryness in a water bath at 40°±5°C.

3.4 LC/MS/MS ANALYSIS SAMPLE PREPARATION

3.4.1 For the control, 0.01 ppm procedurals and samples, add 0.5 mL methanol/ 50 mM ammonium acetate (1:1) to the dry extract residue, vortex and sonicate for approximate 2 minutes. Dilute other procedural fortifications as needed. Filter through a 0.45 micron nylon filter capsule or centrifuge on the mini centrifuge until all the solution has been filtered.

3.4.2 Place a 200 µL conical insert into a 1.5 mL glass auto sampler vial. Transfer the filtered solution into the 200 µL conical insert in the 1.5 mL glass autosampler vial.

3.5 BH 514-METHYL ESTER - EXTRACTION OF RESIDUE

3.5.1 Weigh 10.0 g of a soil sample using a top loading balance into a 150 mL glass centrifuge bottle. As required, fortify control samples with appropriate volumes of fortification solutions.

3.5.2 Add approximatey 10 g of sodium sulfate to each tube. Add 40 mL of (1:1:1) methanol/methylene chloride/ethyl acetate and shake for 1 hour using the mechanical shaker. Centrifuge the extract for 15 minutes at 3500 rpm, or until the soil is completely separated from the extract and the supernatent is easy to pour, then carefully pipette a 4 ml aliquot into a 16 ml test tube.

3.5.3 Concentrate the fraction to dryness using a nitrogen stream with water bath at 40°±5°C. For the control, 0.01 ppm fortification, and samples, dissolve the residue in 2.0 mL of methanol, vortex and sonicate. Add 2.0 mL of 50 mM ammonium acetate and vortex. Dilute other procedural fortifications as needed.

3.5.4 Filter sample through a 0.45 micron nylon filter capsule directly into a autoinjector vial.

3.6 INSTRUMENTATION

3.6.1 Description of Instrument

Instrument:	PE SCIEX API III plus LC/MS/MS SYSTEM triple quadrupole mass spectrometer equipped with Electro Spray Ionization
HPLC:	Shimadzu LC-10AD HPLC pumps (2) with SCL-10A controller and SIL-10A Autoinjector (or equivalent)
Injector:	Thermo Separation Products Spectra System Autosampler 3000
Column:	50 x 2 mm Metachem Inertsil 5 ODS2
Data System:	Apple Macintosh Quadra 900 Data System (PE Sciex Software)

Other equivalent hardware may be used.

INSTRUMENT CONDITIONS ARE SPECIFIC PER EXTRACTION!
PLEASE CHECK CAREFULLY WHICH TO USE!

3.6.2.1: Analysis of Quinclorac and 2-OH-Quinclorac Shake Extraction Samples

HPLC Operating Conditions:

Column:	50 x 2 mm Inertsil 5 ODS2, P/N 0296-050-020 (Metachem Technologies, Torrence, CA (310) 793-2300)
Mobile Phase A:	Water with 0.1% formic acid and 5 mM NH4AOc
Mobile Phase B:	Acetonitrile with 0.1% formic acid
Flow Rate:	0.4 mL/min with ~1:10 post-column split (Valco tee or equivalent)
Gradient (A/B):	90/10 (Hold 1 minute) to 10/90 at 7 minutes Re-equilibrate for 7.1 minutes
Injection Vol.:	25 µL

Note: The total void volume for the system used to develop this method was ~0.6 mL. Gradient times may need to be modified to provide similar chromatography using different LC systems.

API Instrument Parameters:

Note: The following recommended instrument parameters were found to be optimal for the instrument used for the method validation. See Spectra in Figure 3. The exact values used must be optimized for each instrument.

Mode:	Negative Ion Ionspray
ISV Voltage:	-3 kV
Orifice:	-56 V
Curtain Gas:	Nitrogen @ 1L/min
Nebulizer Gas:	Nitrogen @ 80 psi

Auxiliary Gas:	Nitrogen @ 1L/min
Interface Setpoint:	55°C.
Collision Gas:	Argon at ~290 cgt
Collision Offset (R0/R2):	-30V/-23V
Resolution:	Increase DM1 and DM3 by 0.2 units from normal PPG values for extra sensitivity if needed.

Acquisition Parameters:

Delay:	4 minutes
Dwell:	750 msec
Acquire:	3 min
Settling Mass:	100

Analyte	Precursor/Product Ions (m/z)	Retention Time (approx)
BAS 514 H	240/196 ± 0.2	5:10
BAS 514-2-OH	256/212 ± 0.2	4:40

Analysis Of Quinclorac and 2-OH-Quinclorac Reflux Extraction Samples

HPLC Operating Conditions:

Column:	Betasil C18, 100 x 2 mm, P/N 105-701-2-CPF, Keystone Scientific, State College, PA (814) 353-2300
Guard Column:	Betasil, 20 x 2 mm Javelin, P/N 88202-701-P
Mobile Phase A:	Water with 0.1% formic acid and 5 mM NH4AOc
Mobile Phase B:	9:1 Methanol/ 5mM NH4OAc with 0.1% formic acid
Flow Rate:	0.3 mL/min with ~1:3 post-column split (Valco tee or equivalent)
Injection Vol.:	20 µL
Gradient (A/B):	90/10 to 10/90 at 9 minutes Re-equilibrate at 13 minutes

Note: The total void volume for the system used to develop this method was ~0.6 mL. Gradient times may need to be modified to provide similar chromatography using different LC systems.

API Instrument Parameters:

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 used for analysis and documented in the raw data.

Mode:	Positive Ion TurboIonSpray
ISV Voltage:	4.5 kV
Orifice:	50 V
Curtain Gas:	Nitrogen @ 1.2 L/min
Nebulizer Gas:	Nitrogen @ 80 psi
Auxiliary Gas:	Nitrogen @ 6 L/min
Turbo Temperature:	470 °C
Interface Setpoint:	65 °C.
Collision Gas:	Argon at ~290 cgt
Collision Offset (R0/R2):	30V/0V

Resolution:	Increase DM1 and DM3 by 0.2 units from normal PPG values for extra sensitivity if needed.

Acquisition Parameters:

Delay:	7 minutes
Dwell:	750 msec
Acquire:	2.5 min
Pause:	0.02msec
Settling Mass:	100

Analyte	Precursor/Product Ions (m/z)	Retention Time (approx)
BAS 514 H	242/161	8:30
BAS 514-2-OH	258/184	7:55

Analysis of Quinclorac Methyl Ester

HPLC Operating Conditions:

Column:	Betasil C18, 100 x 2 mm, P/N 105-701-2-CPF, Keystone Scientific, State College, PA (814) 353-2300
Guard Column:	Betasil, 20 x 2 mm Javelin, P/N 88202-701-P
Mobile Phase A:	water with 5mM NH4OAc and 0.1% formic acid
Mobile Phase B:	9:1 Methanol/ 5mM NH4OAc and 0.1% formic acid
Flow Rate:	0.4 mL/min with ~1:10 post-column split (Valco tee or equivalent)
Injection Vol.:	20 µL
Gradient (A/B)	Isocratic @ 40/60

Note: The total void volume for the system used to develop this method was ~0.6 mL. Gradient times may need to be modified to provide similar chromatography using different LC systems.

API Instrument Parameters:

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 used for analysis and documented in the raw data.

Mode:	Positive Ion Turbo Ionspray
ISV Voltage:	4 kV
Orifice:	65 V
Curtain Gas:	Nitrogen @ 1.2 L/min
Nebulizer Gas:	Nitrogen @ 80 psi
Auxiliary Gas:	Nitrogen @ 6 L/min
Turbo Temperature:	470 °C
Interface Setpoint:	65 °C.
Collision Gas:	Argon at ~290 cgt
Collision Offset (R0/R2):	30V/15V
Resolution:	Increase DM1 and DM3 by 0.2 units from normal PPG values for extra sensitivity if needed.

Acquisition Parameters:

Delay:	3.5
Dwell:	750 msec
Acquire:	2.5 min
Pause:	0.02 msec
Settling Mass:	100

Analyte	Precursor/Product Ions (m/z)	Retention Time (approx)
BAS 514-Me	256/224 ± 0.2	4:10

3.6.3. Calibration Procedures

The standard curve is derived using the area response of the analytes (y) versus the concentration of the native compounds (x) from all standards injected with the analysis set. A weighted linear regression (1/x) standard curve is used for quantitation of all samples.

A five point calibration curve must be used for quantitation of sample extracts. At least two standards at each concentration must be injected in an analysis set.

Instrument analysis must begin and end with the injection of a standard. No more than three samples may be injected between standard injections.

Acceptance of each sample set will be made by evaluation of the correlation coefficient of the standard curve for each analyte. Correlation coefficients must be >0.98.

3.6.4 Sample Analysis

Three separate analytical conditions are described. The first method for the analysis of Quinclorac and 2-OH-Quinclorac by the shake extraction, uses a 50 x 2 mm column and negative ionization. The second method used for the analysis of Quinclorac and 2-OH-Quinclorac by the Reflux Extraction which produces extracts with a high matrix content uses a longer analytical column (100 x 2 mm), different organic modifier, and turbo ionspray to enhance sensitivity. A third method is described for analysis of the methyl ester by positive ionization using turbo ionspray.

Standards and extracts are analyzed using a reversed phase liquid chromatography analytical column interfaced to a triple stage mass spectrometer using ionspray atmospheric pressure ionization (API). Turbo-ionspray is used to enhance sensitivity in two of the three analysis methods described.

A post column split (~1:10) is used for the analyses. A Valco Tee is connected to the bulkhead fitting on Ionspray flange using a short piece of 0.007" tubing. Connect a piece (~370 mm) of 100 micron fused silica capillary tubing to the other side of the bulkhead fitting, and thread the free end of the fused silica tubing through the ionspray needle to within about 1 mm from the end. Connect the column to the tee using a short piece of 0.007" tubing. Connect an appropriate length of tubing to the tee to provide the desired split. This piece is placed in a waste container during analysis. A good start is to use a 5

foot piece of 0.007" peek tubing for the length of fused silica specified above. Lengthen or shorten the fused silica or waste tube as appropriate to obtain the desired split.

Inject 20 or 25 µl of sample into the LC/MS/MS as described above. Directly compare peak area of unknown samples injected with the standard curve to obtain pg of BAS 514, BH 514-2-OH or BH 514-ME in the sample.

Bracket every 1-3 samples with standards to check for shifts in sensitivity. If the peak area of the unknown is larger than the highest standard, dilute the unknown appropriately and reinject. It is good practice occasionally to inject solvent (methanol) blanks to ensure that BAS 514, BH 514-2-OH, or BH 514-ME are not carried over from run to run.

3.7 INTERFERENCES

3.7.1 Sample Matrices

Ionization suppression has been observed from with extracts generated by the reflux method. The use of methanol as an organic modifier, combined with a longer column and turbo ionspray reduces the suppression effect. Verification that suppression has been controlled can be determined by analysis of matrix fortification samples. If interfering peaks occur in the chromatogram that can not resolved, analyze another aliquot of the extract from 3.2.1.1 by BASF Method A8903 for quinclorac. (Reference 2)

3.7.2 Other Sources

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

3.8 CONFIRMATORY TECHNIQUES

Due to the high specifity of the MS/MS, analyzing product ions, a confirmatory technique is not necessary.

3.9 TIME REQUIRED FOR ANALYSIS

1. Shake Extraction

   Extraction and Analysis of extracts generated by the shake method takes approximately 5 hours and 5 hours, respectively for 17 samples

2. Reflux Extraction

   Extraction and Analysis of extracts generated by reflux extraction takes approximately 6 hours and 8 hours respectively for 17 samples

3. Methyl Ester Method

   Extraction and Analysis of extracts generated for the methyl ester takes approximately 3 hours and 5 hours, respectively for 17 samples.

3.10 POTENTIAL PROBLEMS

Sodium hydroxide extractions of soils are exhaustive and may remove different components of soils which vary from region to region. In Step 3.3. emulsions may form more easily in soils with higher organic contents. If emulsions do form, centrifugation of the extract will aid in partitioning.

4.0 METHODS OF CALCULATION

4.1 CALIBRATION

Construct a linear least squares working curve in the form $y = bx + c$ from the standards by plotting peak area versus weight of standard injected.

4.2 ANALYTE IN SAMPLE

Calculate results based on peak area measurements. Using the peak area measurements for BAS 514 H, BH 514-2-OH, BH 514-ME in the samples, determine the amount of BAS 514 H, BH 514-2-OH, BH 514-ME from the least squares working curve.

Calculate ppm values by the equation below.

$$ppm = \frac{A}{B \times 1000}$$

where A = pg value interpolated from standard curve

B = mg Sample Injected = $\frac{\text{sample wt.(g)} \times \text{nl injected}}{\text{Final dilution volume (mL)}}$

The "final dilution volume" includes any dilutions which have been made.

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:

$$\% \text{ Recovery} = \frac{\text{ppm} \times 100}{\text{ppm BAS 514 and BH 514-2-OH added}}$$

Do not correct treated sample results for either control residues or procedural recoveries.

Figure 1 Flow Chart for Quinclorac Methods

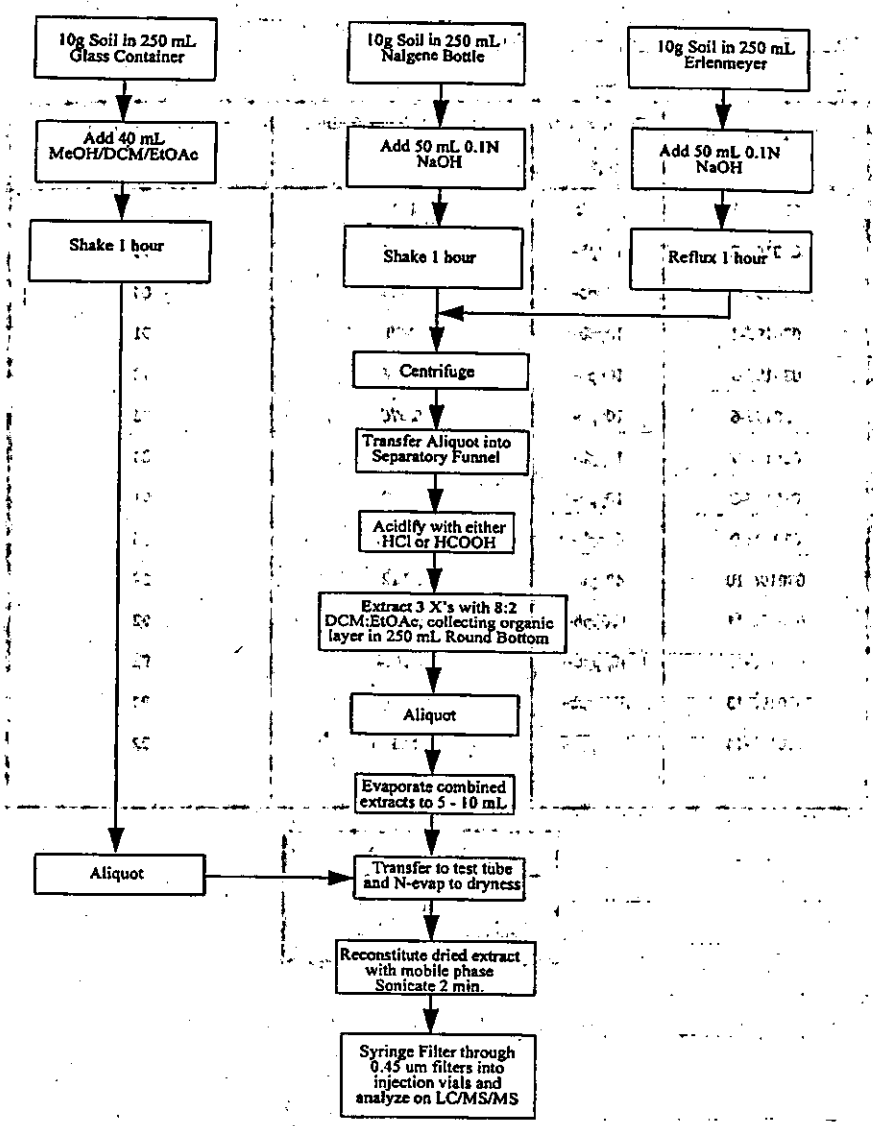
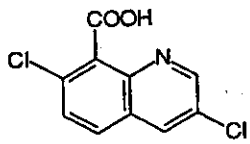
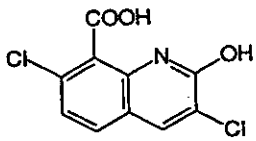


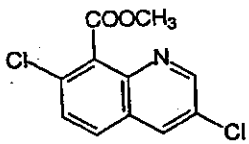
FIGURE 2 QUINCLORAC STRUCTURES



Quinclorac (BAS 514 H)



2-Hydroxyquinclorac (BH 514-2-OH)



Quinclorac methyl ester (BH 514-ME)