Cover Sheet for

# ENVIRONMENTAL CHEMISTRY METHOD

***Pestcide Name:*** Triclopyr

***MRID #:*** 417143-08

***Matrix:*** Water

***Analysis:*** GC/ECD

Triclopyr
BAS 1309

# EPA

# MRID NUMBER

41743-08

417143-C8

# DETERMINATION OF TRICLOPYR AND 3,5,6-TRICHLORO-2-PYRIDINOL IN WATER BY GAS CHROMATOGRAPHY

Research Report ACR 84.3

**Guideline Reference:**

171-4(c) Residue Analytical Method

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## 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 Section 10 (d)(1)(A),(B), or (C).

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**Date:** Nov 19, 1990

Good Laboratory Practice Statement

All phases of this study were conducted prior to the effective date of the final rule on 40 CFR Part 160, EPA FIFRA Good Laboratory Practice Standard.

Submitter of Study: Date: Nov 14, 1990

Sponsor: Date: Nov 14, 1990

Study Director Date: Nov 14, 1990

## Study Title

## DETERMINATION OF TRICLOPYR AND 3,5,6-TRICHLORO-2-PYRIDINOL IN WATER BY GAS CHROMATOGRAPHY

Data Requirements

171-4(c) Residue Analytical Method

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Study Completed On

February 10, 1984

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# DETERMINATION OF TRICLOPYR AND 3,5,6-TRICHLORO-2-PYRIDINOL IN WATER BY GAS CHROMATOGRAPHY

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## 1. Scope

This method is applicable for the quantitative determination of triclopyr (3,5,6-trichloro-2-pyridyloxyacetic acid) and 3,5,6-trichloro-2-pyridinol in water down to 0.01 ppm for triclopyr and 0.05 ppm for 3,5,6-trichloro-2-pyridinol.

## 2. Principle

### a. Triclopyr

Samples of known volume are acidified with concentrated hydrochloric acid and saturated with sodium chloride. The acidified solution is then partitioned with diethyl ether. An aliquot of the diethyl ether solution is reduced in volume on a hot plate and methylated by adding diazomethane reagent. Benzene is then added to the remaining diethyl ether solution and the diethyl ether is removed by evaporation on a hot plate. The benzene solution is then diluted to volume. An aliquot is analyzed for triclopyr methyl ester by gas chromatography.

### b. 3,5,6-Trichloro-2-pyridinol

Samples of known volume are acidified with concentrated hydrochloric acid and saturated with sodium chloride. The acidified solution is then partitioned with benzene. An aliquot of the benzene solution is treated with N,O-bis (trimethylsilyl)acetamide (BSA) to form the pyridinol trimethylsilyl derivative which is determined by gas chromatography.

## 3. Safety Precaution

a. Each analyst should be acquainted with potential hazards of the reagents, products, and solvents before commencing laboratory work. SOURCES OF INFORMATION INCLUDE: MATERIAL SAFETY DATA SHEETS, LITERATURE, AND OTHER RELATED DATA. Safety information on non-Dow products should be requested from the supplier. Disposal of reagents, reactants, and solvents must be in compliance with local, state, and federal laws and regulations.

b. Exercise normal laboratory precautions with acids and bases. Benzene, diethyl ether and methanol are flammable and should be used in well ventilated areas away from ignition sources. Diazomethane is toxic, potentially explosive and should be used in small quantities in a well ventilated hood. For reagent preparation, avoid using etched glassware, ground glass joints or glass tubing with sharp edges. Make all connections with rubber stoppers. The BSA should be handled with regard to its high reactivity.

4. Equipment

a. Gas chromatograph, Micro Tek, Model MT-220 or equivalent, equipped with a $^{63}$Ni electron capture detector and linearizer. Tracor Inc., Austin, Texas 78721.

b. Strip chart recorder, Linear, Model 555, or equivalent, 1 mV input.

c. Gas chromatographic column, u-shaped borosilicate glass, 6' x 3 mm i.d.

d. Automatic sampler, Hewlett-Packard, Model 7671A. Hewlett-Packard customer service, 23855 Research Drive, Farmington, Michigan 48024.

e. Mechanical shaker, Burrell, wrist action model, Burrell Corp., Pittsburgh, Pennsylvania.

f. Vial, clear glass, 12 dram with Poly-Seal caps.

g. Pipets, volumetric, 1, 2, 4 and 10 mL.

h. Flasks, volumetric, 10 and 25 mL.

i. Syringes, 10 µL (Hamilton No. 701 RN), and 100 µL (Hamilton No. 710 N).

j. Hot plate, Thermo-Stir model, Peninsular Mfg. Co. Inc., Orlando, Florida.

5. Reagents and Materials

a. Argon-methane (95/5) carrier gas. Saginaw Welding, 5350 Davis Road, Saginaw, Michigan 48604.

b. Column packing, 3% OV-1 (w/w) on 100/120 mesh Gas Chrom Q. Applied Science Laboratories Inc., P.O. Box 440, State College, Pennsylvania 16801.

c. Hydrochloric acid, concentrated, ACS. J. T. Baker Chemical Co., Phillipsburg, New Jersey 08865.

d. Sodium chloride, ACS. J. T. Baker Chemical Co., Phillipsburg, New Jersey 08865.

7 e. Benzene, methanol glass distilled. Mallinckrodt Chemical Works, St. Louis, Missouri 63160.

f. Diethyl ether, anhydrous, ACS. VWR Scientific Inc., San Francisco, California 94119.

g. N,O-bis(trimethylsilyl)acetamide (BSA). Supelco, Inc. Bellefonte, Pennsylvania 16823 (Note 12. a., b.).

h. 2-(2-Ethoxy-ethoxy)ethanol. J. T. Baker Chemical Co. Phillipsburg, New Jersey 08865.

i. Diazomethane reagent, approximately 18 mg/mL in diethyl ether. Note safety precautions in Section 3.

Set up a 125-mL long-necked distilling flask equipped with a magnetic stirrer, a dropping funnel and a delivery tube connected to an efficient water condenser and place the flask above a water bath on a hot plate magnetic stirrer. The condenser is connected to a 250-mL flask (collector) and a 50-mL flask (scrubber) in series and cooled in crushed ice. Place 25 mL of ether in the 50-mL scrubber flask and adjust the inlet tubing so it is below the surface of the ether. A solution of 6 g of potassium hydroxide in 10 mL of distilled water is placed in the distilling flask and 35 mL of 2-(2-ethoxy-ethoxy)ethanol and 10 mL of ether are added to the flask. In the dropping funnel, place a solution of 21.5 g of N-methyl-N-nitroso-p-toluene-sulfonamide (Aldrich Chemical Company, Milwaukee, Wisconsin) dissolved in 140 mL of ether. The distilling flask is immersed in the water bath which is maintained at 50-60°C while the N-methyl-N-nitroso-p-toluene-sulfonamide is added from the dropping funnel during a 20 minute period. The distilling flask contents are magnetically stirred throughout the distillation. Interrupt the distillation when the distillate is nearly colorless. Combine the contents of the two receivers and store in culture tubes with foil-lined screw caps. The reagent is stable for several weeks if kept in a freezer in full, closed tubes.

j. **Triclopyr, analytical standard[a].** Dissolve 0.100 g in 100 mL of methanol to obtain a stock solution.

k. **3,5,6-Trichloro-2-pyridinol, analytical standard[a].** Dissolve 0.100 g in 100 mL of benzene to obtain a stock solution.

l. **Triclopyr methyl ester, analytical standard[a].** Dissolve 0.1055 g (acid equivalent) in 100 mL of benzene to obtain a stock solution.

m. **Fortification Standards**

Triclopyr: use stock solution j. above and methanol to make serial dilutions. See table below.

3,5,6-Trichloro-2-pyridinol: use stock solution k. above and methanol to make serial dilutions. See table below.

| 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|
| Conc. of Initial Solution (µg/mL) | Aliquot (mL) | Final Volume (mL) | Final Conc. (µg/mL) | Fortification Level (ppm)[1] |
| 1000 | 10 | 100 | 100 | 10 |
| 100 | 10 | 100 | 10 | 1 |
| 10 | 10 | 100 | 1 | 0.10 |
| 10 | 5 | 100 | 0.5 | 0.05 |
| 1 | 10 | 100 | 0.1 | 0.01 |

[1] Use 1 mL of the appropriate concentration of solution in Column 4 per 10-mL sample to obtain level of fortification in Column 5.

n. **Gas Chromatography Standards**

Triclopyr: use stock solution l. above and benzene to make serial dilutions. See table below.

3,5,6-Trichloro-2-pyridinol: use stock solution k. above and benzene to make serial dilutions. See table below.

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[a] Obtain from Sampling Coordinator, Agricultural Products Department, The Dow Chemical Company, P.O. Box 1706, Midland, Michigan 48640.

| 1 | 2 | 3 | 4 |
|---|---|---|---|
| Conc. of Solution (µg/mL) | Aliquot Taken (mL) | Dilution Volume (mL) | Dilution Conc. (µg/mL) |
| 1000 | 4 | 100 | 40 |
| 40 | 1 | 100 | 0.40* |
| 0.40 | 25 | 50 | 0.20* |
| 0.20 | 25 | 50 | 0.10* |
| 0.40 | 1 | 10 | 0.04* |

*Note: For triclopyr: dilute 1 mL of each asterisked concentration in Column 4 to 10 mL with benzene.

For 3,5,6-trichloro-2-pyridinol: prepare the 3,5,6-trichloro-2-pyridinol trimethylsilyl derivative as follows. Cut a bulb from an eye dropper in half across the width. Snap the prescored top off of a 1-mL ampule of BSA. Place the upper half of the cut bulb over the opening of the ampule, this serves as an air tight septum. Pipet 1 mL of the asterisked dilution conc. standard (Column 4) into a 10-mL volumetric flask. Add 4 mL of benzene to the flask. With a 100-µL syringe, transfer 10 µL of N,O-bis(trimethylsilyl) acetamide (BSA) to the 10-mL flask. Mix well and dilute to 10 mL with benzene.

6. Gas Chromatography

a. Column

Fill the column with the packing described in Section 5. b. and tap on a solid surface while filling until no further settling occurs. The packing in the inlet side should be three inches below the injection septum. Insert a small glass wool plug about 3 mm in length in the effluent end of the column but not in the inlet side. Condition the column at 200°C overnight with an argon/methane flow of 60 mL/min and the column disconnected from the detector.

b. Operating Conditions

1. Column Temperature

a. Triclopyr methyl ester: 165°C.

b. 3,5,6-Trichloro-2-pyridinol (silyl derivative): 150°C.

2. Injector block temperature, 220°C.

3. Detector bath temperature, 350°C.

4. Carrier gas: argon/methane, 95/5 at 60 mL/min (60 psi inlet pressure), passed through a 4Å molecular sieve filter.

5. Recorder: 1 mv.

6. Electrometer

   a. Triclopyr methyl ester; sensitivity - 8x.

   b. 3,5,6-Trichloro-2-pyridinol; sensitivity - 2x.

7. Chart speed, 15 in/hr or 30 cm/hr.

c. Injection Technique

   1. Automatic injector, Hewlett-Packard, Model 7671A.

## 7. Preparation of Standard Curve

Inject 4-μL aliquots of the methyl ester of triclopyr or the silyl derivative of 3,5,6-trichloro-2-pyridinol, covering the concentration range from 0.004 to 0.040 μg/mL, into the gas chromatograph and record the resulting peak height. Plot peak heights on the ordinate as percent full scale deflection versus concentrations of the derivatized compound on the abscissa.

## 8. Recovery of Triclopyr from Fortified Samples

a. Measure 10-mL portions of water into a series of 12-dram vials to be used as control samples.

b. Measure 10-mL portions of the control water into a series of 12-dram vials and fortify by adding appropriate aliquots of the fortification standard solution to obtain a minimum concentration of 0.01 ppm. Prepare each sample in duplicate.

Treat each of the samples as follows:

c. Add 1 mL of conc. hydrochloric acid, 5 g of sodium chloride and 10 mL of diethyl ether.

d. Cap, shake 5 minutes then centrifuge at 2400 rpm for 1 min.

e. Transfer a 4-mL aliquot of the diethyl ether to a 10-mL volumetric flask and add a boiling chip.

f. Place on hot plate and reduce the volume to about 2 mL. Add 0.25 to 0.50 mL of diazomethane and further reduce the volume to 1 mL (Note 12. c.).

g. Add 1 mL of benzene and remove the diethyl ether by evaporation on the hot plate. Cool and dilute to volume with benzene.

h. Chromatograph 4-µL aliquots of the benzene solution for the determination of triclopyr.

i. Measure the heights of the peaks obtained for the methyl ester of triclopyr in terms of % full scale deflection and determine the residue found in µg/mL by reference to a standard curve derived on the same day.

In the analytical procedures described, the final 10 mL of benzene solution for the determination of the triclopyr represents 0.40 g of the original sample; therefore, 2.5 times the concentration of the final solution expressed in µg/mL is equivalent to parts per million residue in the sample.

j. Correct for blank, if any, by subtracting ppm of the control sample from that of the fortified sample.

k. Calculate the percent recovery of the compound from each of the fortified samples and determine the mean and 95% confidence limit of the mean.

The calculated mean and 95% confidence limit of the mean was 96±6% for the recovery of triclopyr at 0.01-1.0 ppm (Table I). Typical chromatograms of an analytical standard, control and recovery samples are shown in Figure 1.

9. <u>Determination of Triclopyr Residues in Water</u>

a. Analyze 10 mL of water from the control and treated samples as described above in Section 8.

b. Record the peak heights for each sample and using the appropriate standard curve determine the µg/mL concentration.

c. Determine ppm of each sample as follows:

<u>Gross ppm</u> = µg/mL in solution injected x any additional dilution factor x 2.5

<u>Net ppm</u> = Gross ppm in sample - gross ppm in control

<u>Corrected ppm</u> = $\frac{\text{Net ppm}}{\text{Percent Recovery}}$ x 100

10. <u>Recovery of 3,5,6-Trichloro-2-pyridinol from Fortified Samples</u>

a. Measure 10-mL portions of water into a series of 12-dram vials to be used as control samples.

b. Measure 10-mL portions of the control water into a series of 12-dram vials and fortify by adding appropriate aliquots of the fortification standard solution to obtain a minimum concentration of 0.05 ppm. Prepare each sample in duplicate.

Treat each of the samples as follows:

c. Add 1 mL of concentrated hydrochloric acid and 5 g of sodium chloride.

d. Add 10 mL of benzene.

e. Cap, shake 5 minutes then centrifuge at 2400 rpm for 1 min.

f. Pipet 2 mL of the benzene solution into a 25-mL volumetric flask. Add 10 µL of BSA. Mix well and dilute to volume with benzene.

g. Chromatograph 4-µL aliquots of this benzene solution for the determination of the 3,5,6-trichloro-2-pyridinol.

h. Measure the heights of the peaks obtained for the silyl derivative of the 3,5,6-trichloro-2-pyridinol in terms of the % full scale deflection and determine residue found in µg/mL by reference to a standard curve derived on the same day.

In the analytical procedures described, the final 25 mL of benzene solution for the determination of the 3,5,6-trichloro-2-pyridinol represents 0.08 g of the original sample; therefore, 12.5 times the concentration of the final solution expressed in µg/mL is equivalent to parts per million residue in the sample.

i. Correct for blank, if any, by subtracting ppm of apparent compound in the control sample from that in the fortified sample (Note 12. d.).

j. Calculate the percent recovery from each of the fortified samples and determine the mean and 95% confidence limit of the mean.

The calculated mean and 95% confidence limit of the mean was 98±4% for the recovery of 3,5,6-trichloro-2-pyridinol in water at 0.05-1.0 ppm (Table II).

Typical chromatograms of an analytical standard, control, and recovery samples are shown in Figure 2.

11. Determination of 3,5,6-Trichloro-2-pyridinol Residues in Water

a. Analyze 10 mL of water from the control and treated samples as described above in Section 10.

b. Record the peak heights for each sample and using the appropriate standard curve determine the µg/mL concentration.

c. Determine the ppm for each sample as follows:

<u>Gross ppm</u> = µg/mL in solution injected x any additional dilution factor x 12.5

<u>Net ppm</u> = Gross ppm in sample - gross ppm in control

$$\underline{\text{Corrected ppm}} = \frac{\text{Net ppm}}{\text{Percent Recovery}} \times 100$$

12. <u>Notes</u>

a. N,O-bis(trimethylsilyl)acetamide (BSA) is extremely reactive with moisture and rapidly hydrolyses upon contact with aqueous vapors. It must, therefore, be handled under anhydrous conditions and stored under a dry atmosphere.

b. Each vial or bottle of N,O-bis(trimethylsilyl)acetamide must be chromatographed to determine if interfering extraneous peaks are present. Pipet 5 mL of benzene into a 10-mL volumetric flask, add 10 µL of BSA, mix well, dilute to volume with benzene and chromatograph 4-µL aliquots. If interference is present, check other vials of BSA and use one that gives little or no interference.

c. Set temperature of hot plate such that the diethyl ether will just barely evaporate off to minimize volatilization of the compound.

d. Prepare 3,5,6-trichloro-2-pyridinol trimethylsilyl standards at least once a week or daily if possible. Keep flasks containing the standards tightly closed when not in use to protect against moisture as trimethylsilyl derivative will degrade upon contact with moisture.

TABLE I. RECOVERY OF TRICLOPYR FROM FORTIFIED WATER.

| ppm Added | | ppm Found | % Recovery |
|---|---|---|---|
| 0.01 | 10 | 0.0080 | 80 |
| | | 0.0080 | 80 |
| | | 0.0090 | 90 |
| | 30 | 0.0085 | 85 |
| | | 0.0095 | 95 |
| | 790 | | |
| | | 0.0112 | 112 |
| | | 0.0115 | 115 |
| | | 0.0112 | 112 |
| | | 0.0100 | 100 |
| | | 0.0100 | 100 |
| 0.10 | | 0.096 | 96 |
| | | 0.089 | 89 |
| 1.0 | | 0.92 | 92 |
| | | 0.97 | 97 |
| | | | 96±6[a] |

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[a] Mean ± 95% confidence limit of the mean.

TABLE II. RECOVERY OF 3,5,6-TRICHLORO-2-PYRIDINOL FROM FORTIFIED WATER.

| ppm | | |
|---|---|---|
| Added | Found | % Recovery |
| 0.05 | 0.045 | 90 |
| | 0.042 | 84 |
| 50 | 0.049 | 98 |
| 150 | 0.048 | 96 |
| | 0.048 | 96 |
| 1500 | 0.050 | 100 |
| | 0.052 | 104 |
| | 0.051 | 102 |
| | 0.052 | 104 |
| | C.052 | 104 |
| 0.50 | 0.54 | 108 |
| | 0.45 | 90 |
| 1.0 | 0.95 | 95 |
| | 1.06 | 106 |
| | | 98±4[a] |

[a] Mean ± 95% confidence limit of the mean.

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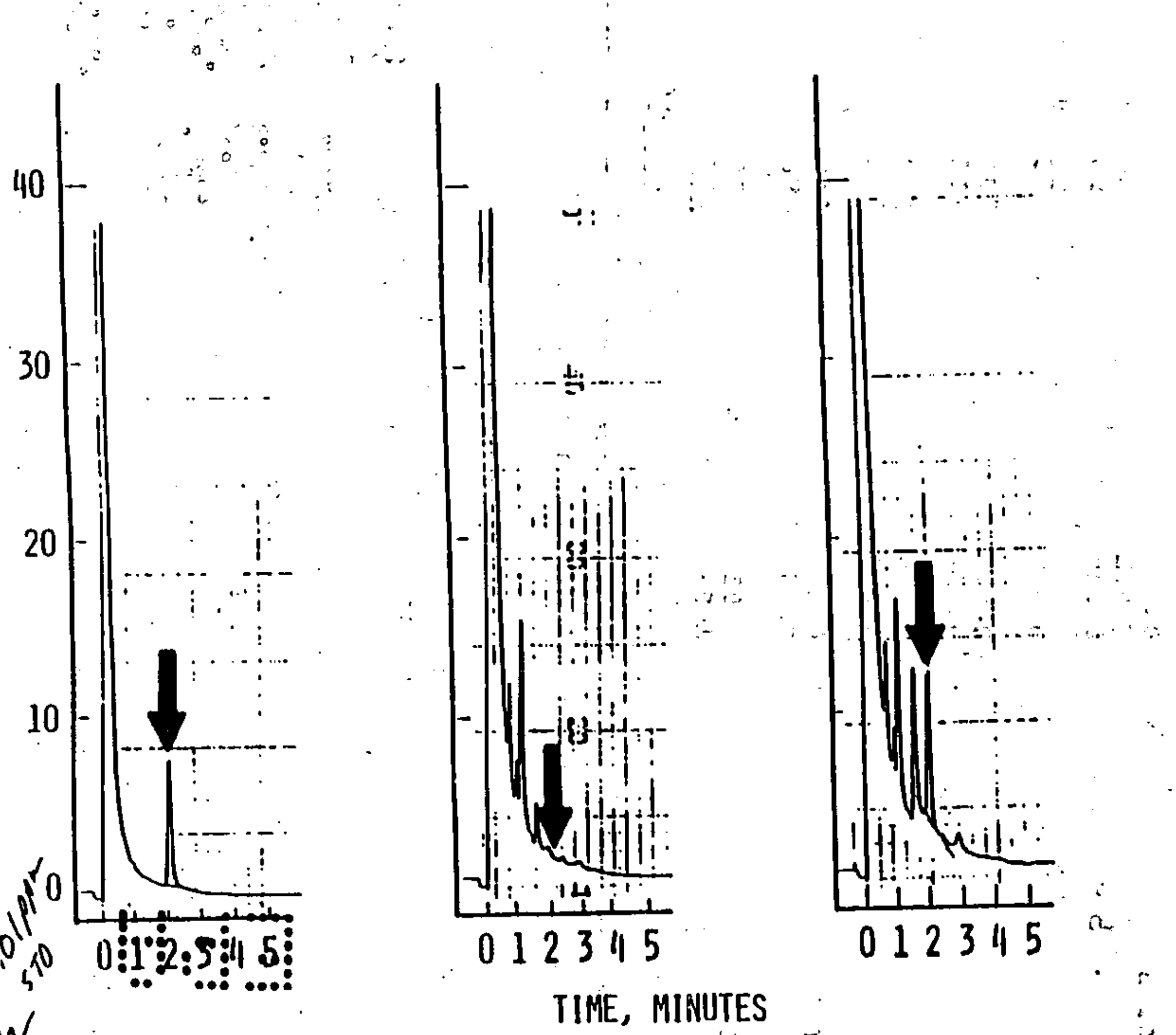


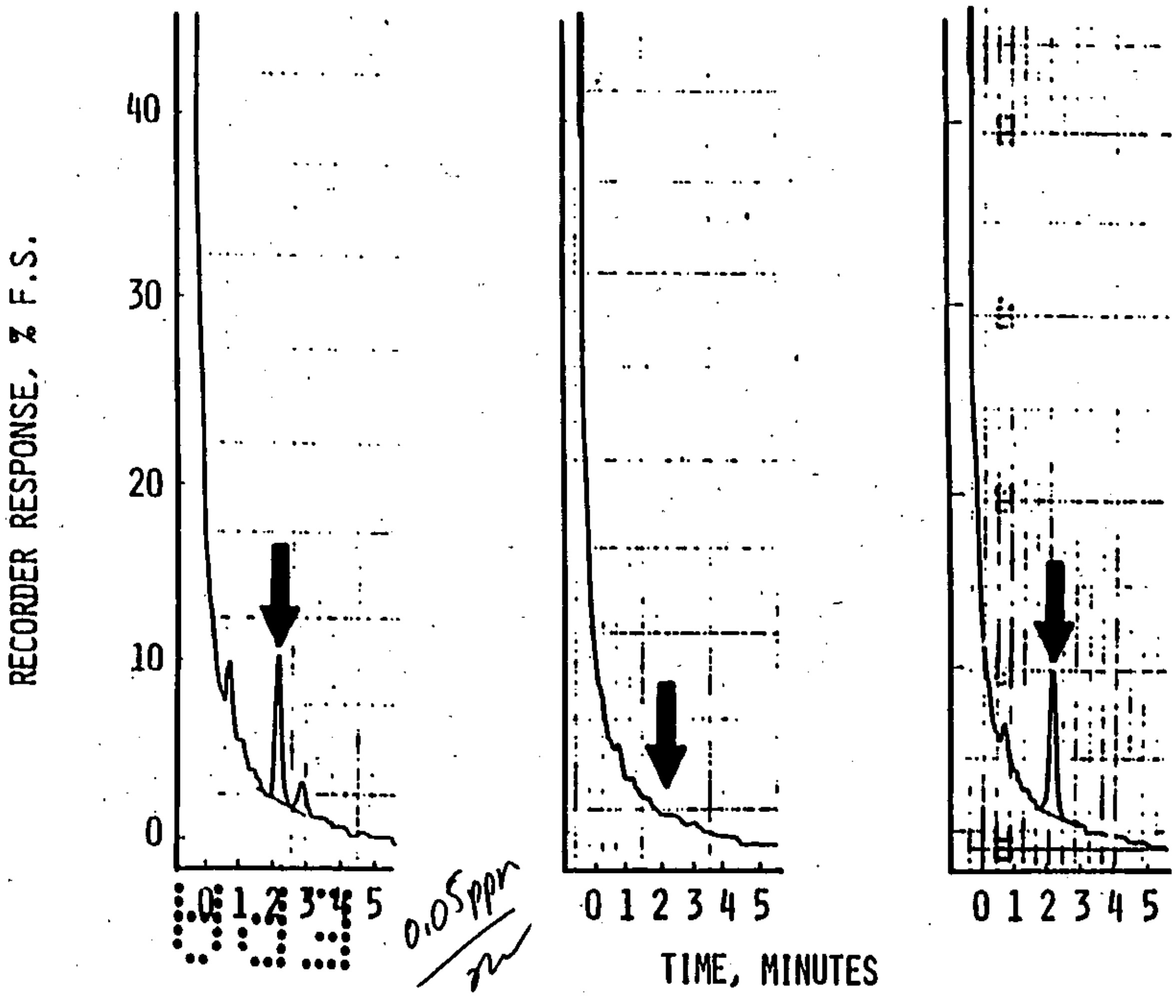
Equivalent-0.01ppm
STO

0.004 µg/ml Std.
Peak Height 7.3%
Equivalent to 0.01 ppm

Control
Peak Height 0%
0.000 Gross ppm

Recovery
Fortified at 0.01 ppm
Peak Height 8.2%
112% Recovery

FIGURE 1. TYPICAL CHROMATOGRAMS OF STANDARD, CONTROL AND RECOVERY SAMPLES FROM THE ANALYSIS OF TRICLOPYR IN WATER.



0.05ppm

0.004 µg/ml Std.
Peak Height 8.2%
Equivalent to 0.05 ppm

Control
Peak Height 0%
0.000 Gross ppm

Recovery
Fortified at 0.05 ppm
Peak Height 8.0%
98% Recovery

FIGURE 2. TYPICAL CHROMATOGRAMS OF STANDARD, CONTROL AND RECOVERY SAMPLES FROM THE ANALYSIS OF 3,5,6-TRICHLORO-2-PYRIDINOL IN WATER.

# END