Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Molinate (Hexamethylenemine)

MRID #: 414218-03

Matrix: Water

Analysis: GC/NPD

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ICI Americas Inc.
Western Research Center
1200 South 47th Street
Box Number 4023
Richmond, California 94804-0023

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000217

DETERMINATION OF HEXAMETHYLENEIMINE RESIDUES IN WATER BY CAPILLARY GAS CHROMATOGRAPHY

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WRITTEN BY:

K. K. Curry
Principal Research Chemist

APPROVED BY:

R. J. Bussey
Supervisor,
Environmental Chemistry Section

WORK PERFORMED BY:

C. L. Eckstein
Research Technician III

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DETERMINATION OF HEXAMETHYLENEIMINE RESIDUES IN WATER BY CAPILLARY GAS CHROMATOGRAPHY

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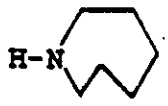
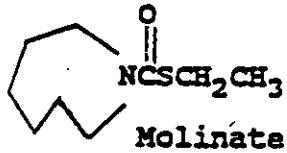
I. SUMMARY/INTRODUCTION

This method is intended for determining hexamethyleneimine (HMI), a metabolite of ORDRAM® Selective Herbicide, in water at levels of 0.01 ppm to 0.1 ppm. Its structure is:



Hexamethyleneimine



Molinate

To extract HMI from water, a sample is treated with NaCl and base to pH 13. Toluene is added and the mixture is shaken. An aliquot of the toluene extract is derivatized with acetic anhydride. The resulting derivative is quantitated by capillary gas chromatography with Nitrogen-Phosphorous detection.

II. MATERIALS/METHODS

A. Apparatus

The equipment and reagents described below were used to generate the data and chromatograms presented in this report. Equipment with equivalent performance specifications and reagents of comparable purity can be used.

1. Gas Chromatograph. Hewlett-Packard Model 5880A, equipped with capillary splitless inlet, Hewlett-Packard Model 7673A automatic sampler, nitrogen-phosphorus detector, and electronic integrator or data acquisition system. Any chromatograph giving equivalent performance may be used.

2. Injection Port Insert. Splitless insert, 2 mm i.d. by 77 mm, Hewlett-Packard Part No. 18740-80220.

3. Chromatographic Column. J & W DB-5, 30m x 0.25mm i.d. x 0.1um film thickness, or equivalent.

4. Glass Bottles. One-ounce narrow mouth bottles with poly seal lined lids.

5. Syringe. 10, 100, and 500 microliter capacities, Hamilton 701N, 710N, 750N or equivalent.

6. Reciprocating Shaker. Eberbach Corporation, model 6010 or equivalent.

B. Reagents

1. Toluene. Nanograde or equivalent.

2. Methanol. Nanograde or equivalent.

3. Sodium Hydroxide. Reagent Grade 50%.

4. Sodium Chloride. Reagent Grade.

5. Acetic Anhydride. Reagent Grade.

6. Sodium Sulfate. Anhydrous, Reagent Grade.

7. Hexamethyleneimine. Analytical reference standard. Available from ICI Americas Inc., 1200 So. 47th Street, Box 4023, Richmond, CA 94804-0023, Attention: Environmental Sciences Department Manager.

8. Calibration and Fortification Solutions

To prepare a stock solution of HMI, weigh to the 4th decimal place a convenient quantity, e.g. approximately 50 mg, of primary standard of known purity into a suitably sized bottle. Calculate the weight of solvent to add, based on the weight of primary standard taken, the purity of the primary standard, the density of the solvent, and the desired solution concentration, typically 1000 µg/mL, as follows:

$$S = \frac{W \times P \times D}{A}$$

where S = the weight of solvent to add (g),

W = the weight of primary standard taken (mg std),

P = the purity of the primary standard (mg a.i./mg std),

D = the density of the solvent (g/mL),

and A = desired solution concentration (mg a.i./mL solvent).

Add the calculated weight of the appropriate solvent to the bottle, close the bottle with a polyseal cap, and mix thoroughly to dissolve the primary standard. Use toluene (D = 0.867 g/mL) for calibration solutions, and methanol (D = 0.7928 g/mL) for fortification solutions.

To prepare working calibration solutions, dilute the stock calibration solution by weight with toluene to give 1.0, 0.1, and 0.01 µg/mL solutions or other concentrations as required.

Dilute the stock fortification solution by weight with methanol to give a 10 µg/mL solution, or other concentrations as required.

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C. Analytical Procedure

1. Extraction

Transfer a 10-mL aliquot of sample to a 1-oz. narrow-mouth bottle containing 3 g of NaCl. Add 0.5 mL of NaOH and check that the pH is ≥13 with pH paper, adding more NaOH if necessary. Add 10 mL of toluene, cap the bottle with a poly-seal lined cap and shake it on the reciprocating shaker for 10 minutes. Allow the phases to separate. Transfer approximately 5 mL of the toluene (upper phase) to a 1-oz narrow-mouth bottle containing about a 0.5-cm layer of sodium sulfate.

2. Derivative Formation

Add 0.25 mL acetic anhydride to the toluene subsample. Let the derivatized extract sit for at least 1 hour prior to analysis. Treat a 5-mL portion of the calibration standard(s) in exactly the same manner.

3. Fortification

Analyze unfortified and fortified control samples with each set of treated samples to demonstrate method recovery according to the Quality Assurance SOP. For example, for 10-mL samples, transfer a 10 mL aliquot of untreated control water into a 1-oz narrow-mouth bottle containing 3 g of NaCl. Add 0.010 mL of 10 ug/mL methanol fortification solution to produce a fortification level of 0.01 ppm, or add 0.010 mL of a 100-ug/mL methanol fortification solution to produce a fortification level of 0.1 ppm. Add 0.5 mL of NaOH, check pH, add 10 mL of toluene and extract as above.

D. Instrumentation

1. Operating Conditions

Follow the manufacturer's instructions for operation of the gas chromatograph and nitrogen-selective detector. Use these parameters for the analyses or other operating conditions that achieve equivalent sensitivity, reproducibility, and resolution.

Inlet	Splitless insert, purge activated at 0.5 min.
Oven initial temp.	90°C
Initial time	0.5 min
Temp. programming rate	20°C/min
Oven final time	10 min
Oven final temperature	230°C
Injector temperature	220°C
Detector temperature	300°C
Carrier gas	Helium

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Carrier gas pressure	26.5 psi
Carrier gas flow	2.5 mL/min through column, 52 mL/min vented
Injection size	2.0 µL
Quantitation	Peak height (external standard)

Under the above conditions the elution time of HMI is 3.2 minutes. See Appendix A for typical chromatograms.

2. Calibration

The gas chromatograph is calibrated using the analyte calibration solutions specified in section II.B.8. Chromatographic sensitivity is established by analysis of the 0.01 µg/mL calibration solution.

Quantitation of residues at levels above the detection limit is done by an external standard procedure in which peak heights or areas of analyte peaks in sample extracts are compared to corresponding peak heights or areas of analyte peaks in calibration solutions. See Section G below for details of calculational methods.

3. Analysis of Extracts

Inject the sample extracts using the same conditions used for calibration. The identity of the analyte peak in the sample chromatogram is assigned based upon the coincidence of retention times (within 0.03 minutes) with those of the calibration chromatograms. If the response of a peak identified as an analyte exceeds that of the highest calibration solution, dilute the sample extract until its response is within the calibrated range. Reinject the calibration solution after every two to four sample injections and recalibrate as needed. Reinject the calibration solution at completion of the sample analysis.

E. Interferences

No clean-up is required when this procedure is utilized as described. However, extractives from water may occasionally contribute peaks with retention times near that of HMI. Satisfactory resolution can usually be achieved with appropriate oven temperature manipulations or column choice. Appendix A shows typical chromatograms. Analyze extracts of samples from untreated plots to demonstrate the absence of interferences from sample matrices, solvents, or labware.

F. Confirmatory Techniques

Unexpected positive results, as in untreated control or pre-application samples, should be confirmed by other means, preferably by GC/MS, mass selective detection, or use of a second capillary column of different polarity.

G. Calculations

Calculations are done in one of two ways. If the response is linear, a factor can be calculated as described in 1 below. If the response is non-linear, or if the analyst prefers, the analyte responses over a range of calibration solution concentrations can be fit to a linear or an exponential curve, and a factor can then be calculated as in 2 below for each point on the curve that corresponds to an analyte response in an injection of sample extract.

1. Linear Response, Direct Calculation of Factor

a. Calibration Factors for Linear Response

F = the response factor for the analyte (ppm per electronic unit), calculated as follows:

$$F = \frac{C}{P \times S}$$

where C = the concentration of analyte in the calibration solution (µg/mL)

S = the amount of initial sample represented by each milliliter of final extract solution injected (g/mL)

P = the peak area or height (electronic units) of the analyte peak in the chromatogram of the calibration solution

Averaged response factors for multiple injections of calibration solutions and for more than one concentration of calibration solution can be used as appropriate in the calculation of the concentration of the analyte in the sample, as described below.

b. Analyte in Sample

The concentration of the analyte in the original sample is calculated using an external standard method as follows:

$$ppm = F \times R$$

where ppm = the amount of analyte in the soil in parts per million

R = the peak area or height (electronic units) of the analyte peak in the chromatogram of the sample extract

and F = the response factor for the analyte (ppm per electronic unit), calculated as described above.

Note for the above external standard calculations, equal volumes of both the extract and the calibration solutions are injected.

2. Curve Fit for Linear or Non-Linear Response

If the instrumental response to injections of calibration solutions is reproducible and either linear or exponentially non-linear, a concentration-response curve can be used for sample quantitation. Any valid curve-fitting program can be used. Input the concentration and response for each injection of calibration solution. The program will generate the formula for the corresponding linear or exponential curve. From the formula, determine the calculated concentration for each injection of calibration solution as described below. The calculated and actual concentrations should agree within 10 % relative; that is, the ratio of the actual to the calculated concentration should be between .9 and 1.1. If the agreement is adequate, calculate the concentration of analyte in the sample, and corresponding response factor as follows:

a. Linear Response:

The formula will be of form $Y = mX + b$, where

Y = the concentration of the analyte, ppm,

X = the analyte response, peak height or area units,

and

m and b = constants calculated by the curve-fit program.

Since the analyte concentration should be zero if the response is zero, the constant b should be zero if there are no systematic errors in the analysis. However, it is not necessary for b to be zero for the calculational method to be valid, as long as calibration solution responses are reproducible and the calculated concentrations of the calibration solutions are within 10 % of the actual concentrations.

For each sample injection, determine Y by using the response, X, in the formula.

Calculate the response factor, F, from the formula:

$$F = Y/X$$

Note that this factor should be the same for any point on a linear curve which passes through the intercept; $b = 0$.

b. Exponential non-linear response:

The curve will be of form $Y = aX^b$, where

Y = the concentration of the analyte, ppm,

X = the analyte response, peak height or area units,

and
a and b = constants calculated by the curve-fit program.

For each sample injection, determine Y by using the response, X, in the formula.
Calculate the response factor, F, from the formula:

$$F = Y/X$$

The response factor will be different for each point on the curve.

III. DISCUSSION

A. Precision and Accuracy

Fortified water samples were prepared as described under C-3 and analyzed according to the method to establish accuracy. As Table 1 shows, recoveries from 8 samples fortified from 0.01 to 0.1 ppm ranged from 79% to 130% with a mean recovery of 107%.

The precision of the method depends on variations in extraction and instrumental analysis. The variations in extraction and instrumental analyses can be evaluated from the data obtained during analyses of fortified samples. Two samples fortified at 0.01 ppm were analyzed in triplicate to provide information on instrumental precision; the mean coefficient of variation for these analyses was 12%. The overall coefficient of variation for 10 recovery determinations was 14%.

B. Limit of Detection

The detection limit of the method is 0.01 ppm as determined by fortifications at the 0.01 ppm level with 1 cm peak heights.

C. Safety Precautions

Personnel untrained in the routine safe-handling of chemicals and good laboratory practices should not attempt to use this procedure. Information on any specific chemical regarding physical properties, hazards, toxicity, and first-aid procedures can be found on the Material Safety Data Sheets accompanying the chemical or available from the chemical supplier. In general, always wear safety glasses, work in a well ventilated area, avoid inhaling vapors, and avoid contact of any chemical with skin and clothing. Flammable solvents should be kept away from potential sources of ignition.

1. Toluene, Methanol, Acetic Anhydride

Flammable.
Use in well-ventilated area; avoid breathing vapor. Avoid contact with skin and clothing.

2. Sodium Hydroxide

Avoid contact with skin and eyes.

3. Hexamethyleneimine

Avoid contact with skin and eyes.
Use in well-ventilated area.
Wash with soap and water after any accidental contact.

IV. CONCLUSIONS

The method is specific for the analysis of HMI in H_2O. Only readily available laboratory equipment and reagents are required. The analysis can be completed by one person in an 8-hour period. Untreated and fortified untreated samples should be extracted and analyzed with each set of samples to demonstrate absence of interferences and adequate recovery. If determination of HMI at a concentration other than 0.01 ppm to 0.1 ppm is required, suitably fortified samples must be analyzed to validate the method at that concentration.

V. CERTIFICATION

This is to certify that this is a complete and unaltered report prepared by the Analytical Department of ICI Americas Inc., Western Research Center.

K. K. Curry

5/24/89

K. K. Curry, Ph.D., Study Director
1200 South 47th Street
Box 4023
Richmond, CA 94804-0023
(415) 231-1000

Date

APPROVALS

Environmental Chemistry Supervisor:

Robert J. Bursey

Robert J. Bursey, Ph.D.

5/24/89

Date

VI. TABLES AND FIGURES

A. Table 1. Recoveries of HMI from Water.

Table 1

Recoveries of Hexamethyleneimine from Fortified Water

Sample ID	Fortification (ppm)	Injection Number	Percent Recovery
F 0.01-1	0.01		107
F 0.01-2	0.01		114
F 0.01-3	0.01		112
F 0.01-4	0.01		135
F 0.01-5	0.01	1	79
		2	101
		3	101
		AVG	94
		CV	13.6
F 0.01-6	0.01	1	88
		2	108
		3	105
		AVG	100
		CV	10.7
F 0.05-1	0.05		90
F 0.05-2	0.05		96
F 0.1-1	0.1		125
F 0.01-2	0.1		130
		Overall mean	107
		CV	14

VIII. REFERENCES

WRC Laboratory Notebook 11733, pages 36-45.

APPENDIX A

Representative Chromatograms

Figure 1. Example chromatograms for the determination of hexamethyleneimine in water; toluene extracts 1 g water per mL toluene.