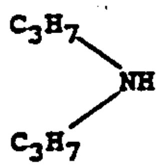
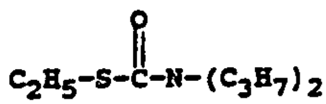
DETERMINATION OF DI-N-PROPYLAMINE, HEXAMETHYLENEIMINE AND ETHYLCYCLOHEXYLAMINE RESIDUES IN SOIL BY DIRECT EXTRACTION AND CAPILLARY GAS CHROMATOGRAPHY

I. SUMMARY/INTRODUCTION

This method is intended for determining di-N-propylamine, hexamethyleneimine and ethylcyclohexylamine in soils at levels of 0.01 ppm to 0.5 ppm. Di-N-propylamine is a metabolite of EPTC, which is the active ingredient in EPTAM Selective Herbicide, manufactured by ICI Americas Inc. Their structures are as follows:

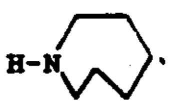


Di-N-Propylamine

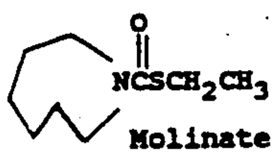


Eptam

Hexamethyleneimine is a metabolite of molinate. Molinate is the active ingredient in ORDRAM Selective Herbicide, manufactured by ICI Americas Inc. Their structures are as follows:

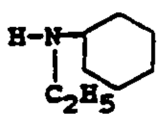


Hexamethyleneimine

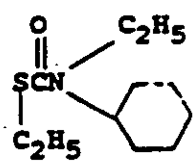


Molinate

N-ethylcyclohexylamine is a metabolite of cycloate. Cycloate is the active ingredient in RONEET Selective Herbicide, manufactured by ICI Americas Inc. Their structures are as follows:



N-Ethylcyclohexylamine



Cycloate

II. MATERIALS/METHODS

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.

A. Apparatus

1. Gas Chromatograph. Hewlett-Packard Model 5880A, equipped with capillary splitless inlet, Hewlett-Packard Model 7672A 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. DB-5 (crosslinked 5% phenylmethyl silicone), 12 m x 0.2 mm x 0.33 um thickness, or equivalent.

4. Glass Bottles. Four-ounce, wide mouth bottles with teflon lined caps, two-ounce narrow mouth bottles with plastic caps, and 4-dram vials with plastic caps.

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

6. Reciprocating Shaker. Eberback Corporation, Model 6010 or equivalent.

7. Centrifuge. IEC International, Model C1582 or equivalent.

B. Reagents

1. Solvents. Toluene, Acetone, Nanograde or equivalent.

2. Di-N-propylamine, hexamethyleneimine, N-ethylcyclohexylamine. Analytical reference-standard available from ICI Americas Inc., 1200 South 47th Street, Box 4023, Richmond, CA 94804-0023, Attention: Environmental Sciences Department Manager.

3. Acetic Anhydride, NaCl, Anhydrous Na_2SO_4, K_2CO_3, 50% NaOH. Reagent grade or equivalent.

4. Calibration and Fortification Solutions

To prepare a stock solution of di-N-propylamine, hexamethyleneimine, or N-ethylcyclohexylamine, 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 acetone (D = 0.792 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 0.02 µg/mL solutions or other concentrations as required.

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

C. Analytical Procedure

1. Extraction

Weigh 40 g of thoroughly mixed soil sample into a 4-oz. wide-mouth bottle. Add 40 mL of distilled water and 10 g of NaCl. Then, add 5 mL of 50% NaOH and 20 mL of toluene. Cap the bottle with a teflon-lined lid and shake it for 2 hours. Centrifuge for 10 to 20 minutes at 2000 rpm to aid in the separation of phases. Remove the top (toluene) phase for derivitization. Alternatively use any convenient weight of soil >20 g, and extract with the same proportions (e.g. 20 g soil: 20 mL water: 5 g NaCl: 2.5 mL NaOH: 10 mL toluene).

2. Derivative Formation

Transfer 15 to 20 mL of the top layer of toluene from the centrifuged sample into a 4-ounce jar and dry it with about 5 g of granular anhydrous Na_2SO_4. Add 1 mL of reagent grade acetic anhydride (99.9%) and let stand, capped, one hour or more at room temperature. Then, transfer approximately 2 mL into a 4-dram vial, add an equivalent volume of 1 M K_2CO_3, cap the vial and shake it vigorously. Let the layers separate for about 5 minutes, transfer the top (toluene) into a separate 4-dram vial, and add about 2 g of anhydrous Na_2SO_4. This extracted and derivatized sample is injected into the G.C.

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 40-g samples, weigh 40 grams of untreated control soil into a 4-oz wide mouth bottle. Add 0.040 mL of 10 µg/mL acetone fortification solution (0.4 µg) to produce a fortification level of 0.01 ppm, or add 0.20 mL of 100 µg/mL acetone fortification solution (20 µg) to produce a fortification level of 0.50 ppm. Add 40 mL of water, 5 g of NaCl, 5 mL of 50% NaOH, 20 mL of toluene and extract and derivatize as above. If a different weight of soil is analyzed, use that weight and adjust the volume or concentration of fortification solution to give the desired analyte concentration. Extract using the same volumes of water and toluene as for the treated samples.

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	1.0 min
Temp. programming rate	15°C/min
Oven final time	10 min
Oven final temperature	220°C
Injector temperature	220°C
Detector temperature	280°C

Carrier gas	Helium
Carrier gas pressure	23 psi
Carrier gas flow	2.4 mL/min through column, 78 mL/min vented
Injection size	1.5 µL
Quantitation	Peak height or area (external standard)

Under the above conditions the elution times of the analytes are: di-N-propylamine, 1.2 minutes; hexamethyleneimine 2.0 minutes; and N-ethylcyclohexylamine 2.8 minutes.

2. Calibration

The gas chromatograph is calibrated using the analyte calibration solutions specified in section II.B.3. Chromatographic sensitivity is established by analysis of the 0.02 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 calibration solution after every two to four sample injections and recalibrate as needed. Reinject 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 soil occasionally contribute peaks with retention times near those of the analytes. 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. The chromatograms in Appendix A demonstrate resolution of di-N-propylamine, hexamethyleneimine and N-ethylcyclohexylamine.

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)

and 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. <u>Exponential non-linear response</u>:

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.