

Cover Sheet for

**ENVIRONMENTAL CHEMISTRY METHOD**

***Pesticide Name:*** Linuron

***MRID #:*** 424228-01

***Matrix:*** Soil

***Analysis:*** HPLC/UV

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ORGANICS LABORATORY  
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Method No.	Edition	Revision
L35011	1	
Subject: Determination of Linuron in Soil using High Performance Liquid Chromatography		
Submitted By: Steven C. Anderson		
Approved By: Kim Sjogren		
References: J. Ag. Food Chem., 1980, 28, 974-978		

1.0 SCOPE

This method describes the procedure for extracting and analyzing soil samples for Linuron residue. The screening limit for this residue is 0.01 ppm in soil.

2.0 PRINCIPLE

Linuron is extracted from soil by shaking with methanol and water. The extract is then concentrated by rotary evaporation until near dryness. The remaining extract is diluted to 5ml with 45/55 acetonitrile/water and filtered through a 45 micron filter. The extract is then analyzed by HPLC.

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- 3.0 CHEMICALS AND SOLUTIONS
- Acetonitrile, J.T. Baker HPLC grade, or equivalent
  - Linuron Standard Reference Material
  - Methanol, J.T. Baker reagent grade, or equivalent
  - Water, deionized

4.0 APPARATUS

4.1 Equipment

- 4.1.1 Centrifuge, capable of holding 250ml bottles and capable of 1500rpm
- 4.1.2 Centrifuge bottle, 250ml polypropylene, w/lid
- 4.1.3 Cotton, absorbent
- 4.1.4 Filter, 45 micron, for 4.1.13
- 4.1.5 Funnel, 65mm
- 4.1.6 Graduated cylinder, 10ml
- 4.1.7 Graduated cylinder, 100ml
- 4.1.8 Rotary Film Evaporator
- 4.1.9 Ultrasonic bath
- 4.1.10 Shaker, Reciprocating, capable of 200rpm
- 4.1.11 Syringe, 2.5-5ml capacity disposable

4.2 Glassware

- 4.2.1 Boiling Flask, 500ml
- 4.2.2 Pipettes, Disposable Pastuer
- 4.2.3 Vial, Auto Sampler (4ml), w/screw cap and 7mm thin Teflon seal
- 4.2.4 Vial, Solvent Saver (7ml), w/screw cap
- 4.2.5 Volumetric Flask, 5ml, w/lid

4.3 HPLC

- 4.3.1 Column: Dupont Zorbax ODS, number 880952.702, 4.6mm X 25cm, or equivalent.
- 4.3.2 Detector: Shimadzu SPO-6J variable wavelength UV, operable at 254nm, or equivalent.
- 4.3.3 Injector: Shimadzu SIL-6B, capable of reproducibly injecting 20uL.
- 4.3.4 Pump: Waters model 510 or equivalent, capable of operating at pressures up to 2500psi with a solvent flow rate of 1.5 ml/minute.
- 4.3.5 Recorder: Shimadzu C-RJA Chromatopac, capable of reporting peak height, area and retention time, or equivalent.

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5.0 ANALYTICAL PROCEDURE

5.1 Extraction

- 5.1.1 Weigh 50 grams (to 0.005 g) of well homogenized, air dried soil into a 250 ml polypropylene centrifuge bottle fitted with a rubber ringed screw cap.
- 5.1.2 Add 10 ml of deionized water and 100 ml of methanol to the soil sample and shake for one hour at 200rpm on the reciprocating shaker.
- 5.1.3 Centrifuge for fifteen minutes at 1500 rpm. Place a small plug of absorbent cotton in a 65mm funnel and decant the solution through the cotton into a 500ml boiling flask. Keep the funnels and flasks together and set aside.
- 5.1.4 Add 100 ml of methanol to the 250 ml centrifuge bottle, cap and shake to break up soil then return to shaker for one hour.
- 5.1.5 Centrifuge again for fifteen minutes at 1500rpm. Decant the solution again through the cotton into the 500 ml boiling flask, combining this decantant with the decantant from step 5.1.3.
- 5.1.6 Concentrate the combined solutions on a rotary evaporator at 40 degrees C., until about 1ml of solution remains.
- 5.1.7 Using a disposable pastuer pipette, quantitatively transfer the remaining solution to a 5ml volumetric flask, then using about 1ml aliquotes of acetonitrile/water (45/55) and the sonic cleaner, remove as much of the remaining residue from the boiling flask as possible and add it to the volumetric flask. Bring the sample to 5ml with the acetonitrile/water.
- 5.1.8 Filter the resulting solution from 5.1.7 through a 0.45 micron filter, using a disposable syringe, into a 7 ml saver vial. Transfer 2ml of the filtered solution to an auto-sampler vial for analysis and archive the remaining extract.

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6.0 High Performance Liquid Chromatographic Analysis

The final determination of Linuron is performed on a HPLC equipped with a reverse phase column and a UV detector at 254nm, a pump flow rate of 1.5ml/minute of mobile phase (45/55 acetonitrile/water) and a chart speed of 1cm/minute.

6.1 Standardization

- 6.1.1 Make up standard solutions by serially diluting a known amount of the reference standard in mobile phase.
- 6.1.2 Inject constant volumes (20uL) of known amounts of standard on the HPLC.
- 6.1.3 Measure the standard peak areas.
- 6.1.4 Plotting peak area vs. concentration, calculate the best fit line using linear regression.

6.2 Residue Determination

- 6.2.1 Inject the solution from 5.1.8 at the same volume used for the standards (20uL).
- 6.2.2 Measure the peak area.
- 6.2.3 Determine the concentration (ppm) of Linuron in the sample aliquot injected by inserting the peak area into the equation of the line obtained in 6.1.4.
- 6.2.4 Calculate the residue as follows:

$$\text{ppm(sample)} = \frac{\text{(extraction (dilution ml) (dilution conc ug/mL) (dilution factor))}}{\text{(grams of sample)}}$$

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**APPENDIX II  
FORTIFICATION LEVELS**



## APPENDIX II FORTIFICATION LEVELS

Sample Description	Reference Study	Fortification/ Extraction Date	Fortification Level ( $\mu\text{g}$ )	Fortification Level (ppm)	Analysis Date
0-month - aged*	AMR 1269-88	-02 JUL 89	49.9	0.998	—
0-month - fresh	AMR 1269-88	-02 JUL 89	49.9	0.998	—
0-month - aged	AMR 1677-90	21 MAY 90	53.85	1.077	31 MAY 90
0-month - fresh	AMR 1677-90	21 MAY 90	53.85	1.077	31 MAY 90
1 month - aged	AMR 1677-90	21 MAY 90	53.85	1.077	26 JUN 90
1 month - fresh	AMR 1677-90	21 JUN 90	53.85	1.077	26 JUN 90
3 month - aged	AMR 1677-90	21 MAY 90	53.85	1.077	24 AUG 90
3 month - fresh	AMR 1677-90	21 AUG 90	54.35	1.087	24 AUG 90
6 month - aged	AMR 1677-90	21 MAY 90	53.85	1.077	27 NOV 90
6 month - fresh	AMR 1677-90	26 NOV 90	54.35	1.087	27 NOV 90
12 month - aged	AMR 1269-88	-02 JUL 89	49.9	0.998	13 JUL 90
12 month - fresh	AMR 1269-88	02 JUL 90	53.85	1.077	13 JUL 90
12 month - aged	AMR 1677-90	21 MAY 90	53.85	1.077	24 JUN 91
12 month - fresh	AMR 1677-90	28 MAY 91	54.35	1.087	24 JUN 91
18 month - aged	AMR 1269-88	-02 JUL 89	49.9	0.998	06 FEB 91
18 month - fresh	AMR 1269-88	23 JAN 91	54.35	1.087	06 FEB 91
24 month - aged	AMR 1269-88	-02 JUL 89	49.9	0.998	18 JUL 91
24 month - fresh	AMR 1269-88	09 JUL 91	54.35	1.087	18 JUL 91
26 month - aged	AMR 1269-88	-02 JUL 89	49.9	0.998	15 SEP 91
26 month - fresh	AMR 1269-88	30 AUG 91	54.35	1.087	15 SEP 91
30 month - aged	AMR 1269-88	-02 JUL 89	49.9	0.9006, 0.9121 <sup>†</sup>	26 DEC 91
30 month - fresh	AMR 1269-88	23 DEC 91	54.35	0.9882	26 DEC 91
		23 DEC 91	—	—	27 DEC 91
		27 DEC 91	—	—	30 DEC 91

\* The soil samples for AMR 1269-88 were weighed, fortified (as 0-month), and extracted approximately July 2, 1989 (see protocol deviations, p 15).

† Fortification levels in ppm were calculated based on reweighed soil contents for the 30-month analysis interval.

**APPENDIX III  
TYPICAL CHROMATOGRAMS**

**REDUCTION FACTOR: 90%**

Retention Time (min)	Peak Label	Retention Time (min)	Peak Label	Retention Time (min)	Peak Label
1.2	CH <sub>4</sub>	1.5	H <sub>2</sub>	2.5	CO <sub>2</sub>
3.5	N <sub>2</sub>	4.5	O <sub>2</sub>	5.5	NO
6.5	CO	7.5	NO <sub>2</sub>	8.5	SO <sub>2</sub>
10.5	HCN	11.5	HCN	12.5	HCN
14.5	HCN	15.5	HCN	16.5	HCN
18.5	HCN	19.5	HCN	20.5	HCN
22.5	HCN	23.5	HCN	24.5	HCN
27.5	HCN	28.5	HCN	29.5	HCN
32.5	HCN	33.5	HCN	34.5	HCN
37.5	HCN	38.5	HCN	39.5	HCN
42.5	HCN	43.5	HCN	44.5	HCN
47.5	HCN	48.5	HCN	49.5	HCN
52.5	HCN	53.5	HCN	54.5	HCN
57.5	HCN	58.5	HCN	59.5	HCN
62.5	HCN	63.5	HCN	64.5	HCN
67.5	HCN	68.5	HCN	69.5	HCN
72.5	HCN	73.5	HCN	74.5	HCN
77.5	HCN	78.5	HCN	79.5	HCN
82.5	HCN	83.5	HCN	84.5	HCN
87.5	HCN	88.5	HCN	89.5	HCN
92.5	HCN	93.5	HCN	94.5	HCN
97.5	HCN	98.5	HCN	99.5	HCN

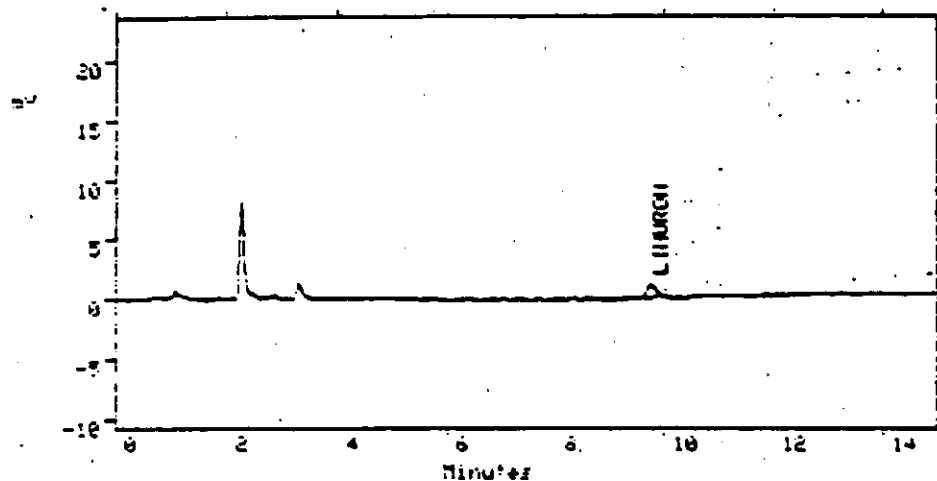
**CHROMATOGRAM OF LINURON STANDARD**

LC Results:

Ret. Name	Ret Time	Area	Height	Int	Amount
LINURON	9.756	13701	1038	58	CAL

Second Plot:

24MSTD1 Manual Injection 1 Ch 1



Linuron Standard  
0.1087 µg/mL

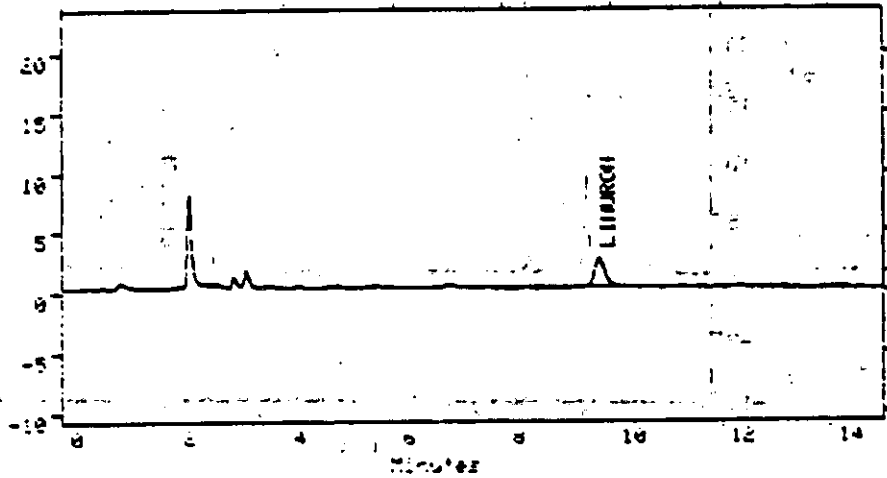
**CHROMATOGRAM OF LINURON STANDARD**

LC RESULTS:

Peak Name	Ret. Time (min)	Area	Height	Int	Amount
LINURON	9.760	34221	2343	86	CAL

Second Plot:

24MSTD2 Manual Injection 1 Ch 1



Linuron Standard  
0.2174 µg/mL

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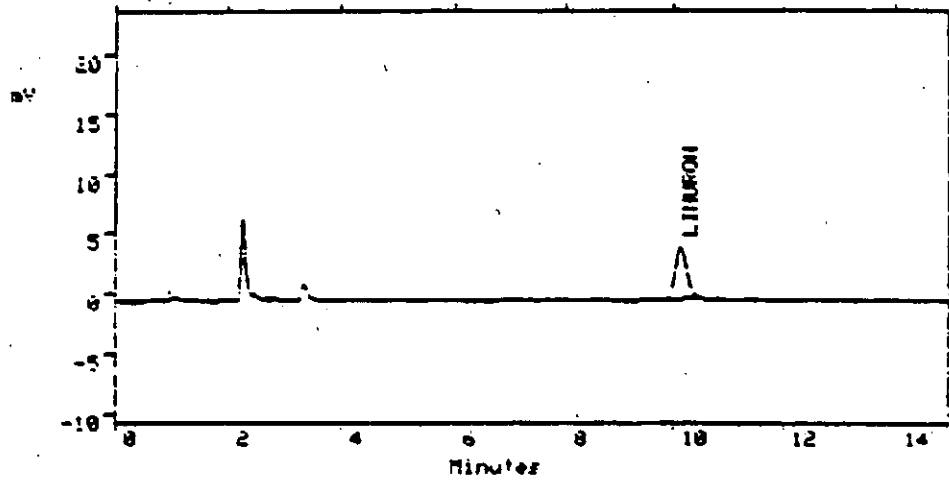
**CHROMATOGRAM OF LINURON STANDARD**

GC Results:

Peak Name	Ret Time	Area	Height	Int	Amount
LINURON	10.120	62421	4272	88	CAL

Second Plot:

24MSTD4 Manual Injection 1 Ch 1



Linuron Standard  
0.4347 µg/mL

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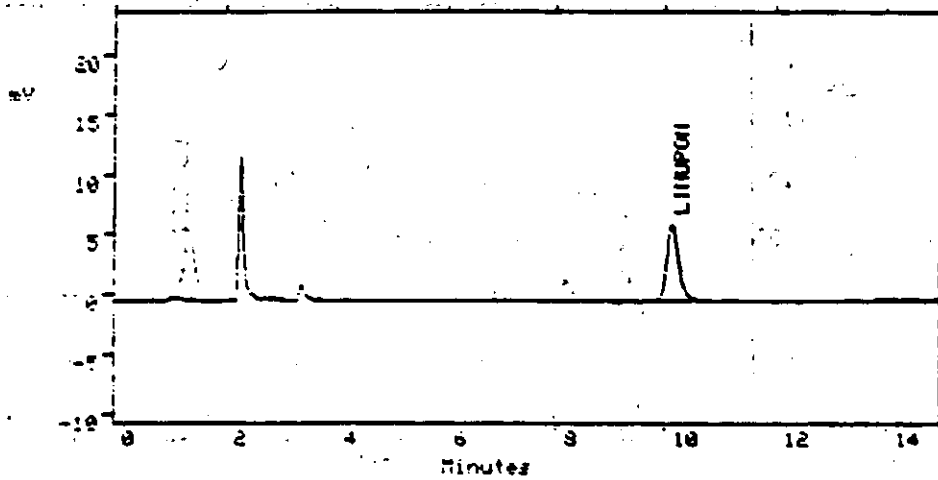
**CHROMATOGRAM OF LINURON STANDARD**

**LC RESULTS:**

Peak Name	Ret Time	Area	Height	Int	Amount
LINURON	10.126	93446	6300	BB	CAL

**Second Plot:**

24MSTD5 Manual Injection: Ch 1



Linuron Standard  
0.6521 µg/mL

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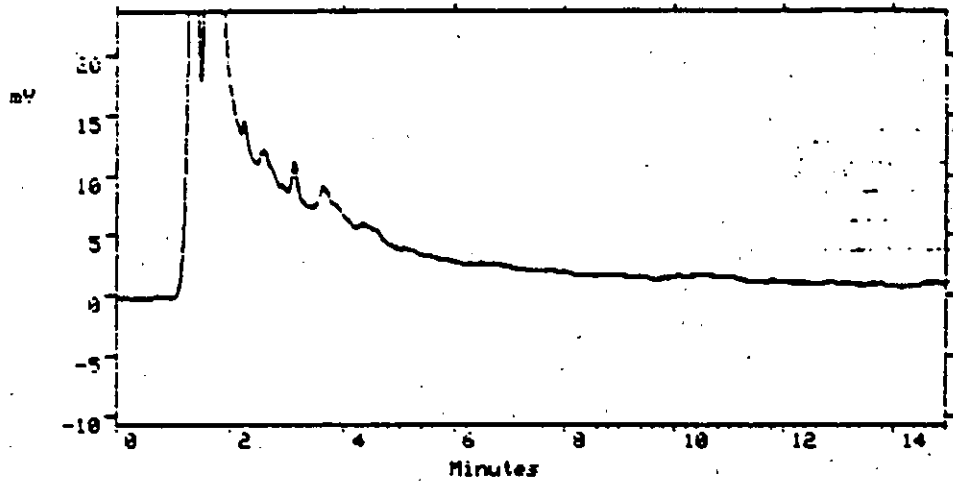
**CHROMATOGRAM OF UNTREATED SOIL SAMPLE**

GC Results:

Peak Name	Ret Time	Area	Height	Int	Amount
LINURON	9.900	-	-	NF	-

Second Plots:

24M2CNTL Manual Injection 1 Ch 1



Untreated Soil Sample  
0.010 ppm linuron

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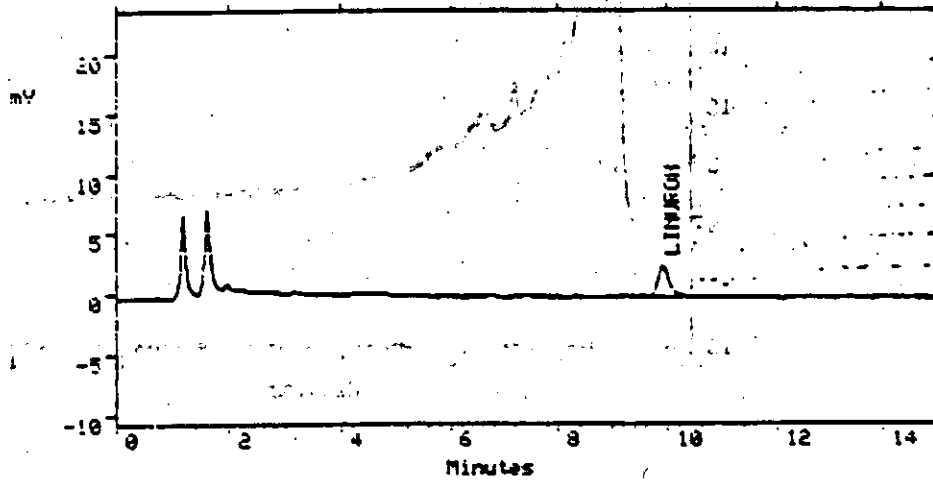
**CHROMATOGRAM OF UNTREATED SOIL SAMPLE FORTIFIED WITH LINURON AND AGED 24 MONTHS**

LC Results:

Peak Name	Ret Time	Area	Height	Int	Amount
LINURON	9.913	33835	2431	BB	0.004

Second Plot:

24MF1 Manual Injection 1 Ch 1



Untreated Soil Sample

Fortified at: 0.998 ppm  
Aged: Frozen 24 months  
Recovery: 61%  
Recovery, normalized: 86%

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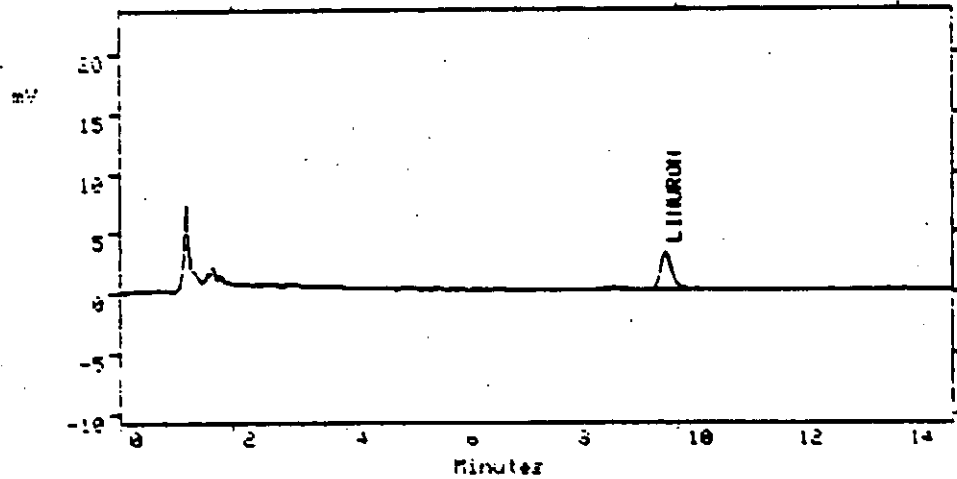
**CHROMATOGRAM OF UNTREATED SOIL SAMPLE FRESHLY FORTIFIED WITH LINURON**

GC Results:

Peak Name	Ret Time	Area	Height	Int	Amount
LINURON	9.774	43390	3050	25	0.005

Second Plot:

2411521V Manual Injection 1 Ch 1



Untreated Soil Sample

Freshly Fortified at: 0.1087 ppm  
Frozen: 24 Months  
Recovery: 71%  
Recovery, normalized: 100%

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**APPENDIX IV  
SUPPORTING RAW DATA**

