

ANALYTICAL METHODS

Reference Standards

Reference standards of diuron and DCPMU were obtained from Agricultural Products, E. I. du Pont de Nemours and Company, Wilmington, Del. The diuron standard (IN-14740; Lot #149) was 99.6% chemically pure, and the DCPMU standard (IN-15654; Lot #12) was 99.0% chemically pure. Stock solutions of the reference standards were prepared in methanol; the HPLC analytical standards were prepared from these stock solutions in acetonitrile/water (3:7).

Extraction and Analysis of Soil Samples

The extraction and analysis of soil samples at Enviro-Test Laboratories were performed according to the U.S. EPA Good Laboratory Practice regulations. Storage, preparation, extraction, and analyses were conducted by Enviro-Test personnel.

Portions (ca. 50 g) of air-dried homogenized soil were shaken for ~30 min in methanol with a wrist-action shaker. The mixtures were centrifuged to sediment the solids, and the supernatant fluids were decanted. The extraction was repeated, and the two extracts from each sample were combined and concentrated to near dryness (rotary evaporation, ca. 42°C). The concentrate was quantitatively transferred to a separatory funnel, diluted with water, and extracted two times with methylene chloride. The methylene chloride extracts were dried by passage through Na_2SO_4 and evaporated to dryness. The residues were dissolved in

5 mL of acetonitrile/water (3:7). Aliquots (500 μ L) of these solutions were filtered (Millipore, 0.45 μ m) and analyzed by HPLC.

The chromatographic conditions used were as follows:

Instrument: Varian 5500 Liquid Chromatograph with
Spectra Physics 4290 integrator
or
Waters 600A Liquid Chromatograph with
Hewlett Packard 3388A integrator

Column: Zorbax Rx, 4.6 x 250 mm, 5 μ m
or
Supelco C18, 4.6 x 250 mm, 5 μ m

Temperature: Ambient or 35°C

Typical Mobile Phases: Acetonitrile/water:30/70
or
Acetonitrile/water:32/68

Flow Rate: 1.2 mL/min

Sample Volume: 50 - 100 μ L

Wavelength: 254 nm

Chart Speed: 0.25 cm/min

Retention times: DCPMU: ca. 16 min; diuron: ca. 21 min (the
retention times varied slightly, depending
on the conditions).

Sample chromatograms are presented in Appendix III.

Calculation of Sample Residues

Standard curves based on peak response (in thousands) were constructed by analyzing suitable concentrations of standards containing diuron and DCPMU. The amount of diuron or DCPMU residue was calculated using the following equation:

$$\text{ppm of diuron or DCPMU} = \frac{\text{peak response} \times \text{avg. R.F.}}{\text{injection volume (mL)}} \times \frac{V}{W}$$

where

peak response	=	peak response of analyte in either area or height
avg. R.F.	=	average response factor (the sum of the response factors divided by the number of determinations)
R.F.	=	response factor (the concentration of the standard/peak height of standard)
V	=	total volume (in mL) of sample solution
W	=	sample weight (in g)

Residue values in the control and treated samples were expressed in parts per million (ppm). The quantitation limit of this method is 0.01 ppm for both diuron and DCPMU, based on a 50-g sample.

Protocol Deviations

Additional samples were taken of Day 418 and Day 538 at the Newark, Delaware site and on Day 415 at the Madera, California site.

Ninety-centimeter samples were taken on Days 210, 243, 299, 359, and 418 at Newark, Delaware.