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Note

Determination of residues of methazole and its metabolites, 1-(3,4-dichlorophenyl)-3-methylurea and 1-(3,4-dichlorophenyl)urea in soil by high-performance liquid chromatography

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The herbicide methazole, 2-(3,4-dichlorophenyl)-4-methyl-1,2,4-oxadiazolidine-3,5-dione, is rapidly degraded in soil[1,2]. The major metabolite is 1-(3,4-dichlorophenyl)-3-methylurea (DCPMU) which further degrades to 1-(3,4-dichlorophenyl)-urea (DCPU). It has been suggested that the major phytotoxic agent is DCPMU[3]. Bond and Roberts[4] have shown that there is little loss of activity of residues over winter and that caution should be taken in choice and timing of following crops, hence it is important to be able to measure residues of both the parent compound and its metabolites. Previously reported methods have measured methazole by gas chromatography and its metabolites by derivatization with heptafluorobutyrylimidazole prior to gas chromatography[5]. High-performance liquid chromatography (HPLC) should allow the separation and determination of methazole, DCPMU and DCPU without derivatization or clean-up and the work described here explores this possibility.

Methanol was selected to extract the compounds because of the high recovery of ^{14}C-labelled methazole obtained by Walker and Roberts[1] and because the experience in this laboratory is that methanol is a generally effective solvent but usually extracts less extraneous materials that may interfere with chromatography than other solvents. Walker and Roberts also reported that DCPMU was not completely extracted from soil and that the difficulty of extraction increased with time after application. It is not clear from their paper whether they extracted wet or dry soil. Therefore a further experiment was included to assess the effect of water on the extraction efficiency of methanol although routinely in this laboratory soils are usually extracted without drying.

MATERIALS AND METHODS

Soils

Soils from two locations were used. Table I gives some details of their composition. They were air dried and passed through a 3-mm sieve prior to fortification.

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TABLE I

SOME PROPERTIES OF THE SOILS USED

	Soil	
	1	*2*
Organic Carbon (%)	4.1	1.6
pH	5.1	7.0
Clay (%)	16	16
Silt (%)	16	11
Sand (%)	68	73
Field capacity (% moisture)	27	16.6

Soil fortification

Aqueous dispersions of methazole, DCPMU and DCPU were prepared from methanolic solutions containing 1 mg ml^{-1} herbicide or metabolite. The concentration of the solutions was such that, when sufficient solution was added to dry soil to achieve 75% field capacity, the concentration was 1.0, 0.5 or 0.1 ppm herbicide or metabolite. All samples were prepared in triplicate and allowed to stand for 48 h before extraction.

Further samples of soil 2 were fortified at 1 ppm with a 1 mg ml^{-1} methanolic solution of DCPMU. Subsamples of this dry soil were extracted immediately and at intervals up to 120 h after fortification.

Extraction

Wet soil: 25 g of soil was shaken with 50 ml of methanol for 1 h using a wrist action shaker. After shaking, the slurry was filtered through a Whatman No. 42 filter paper. A 25-ml aliquot of the filtrate was concentrated to about 1 ml by evaporation under reduced pressure in a water bath at 50°C. The remaining solvent was removed by gentle blowing with air. The residue was redissolved in 1 ml of the eluent used for chromatography.

Dry soil: samples taken at each time interval up to 120 h after fortification were extracted as above. An additional set of three subsamples taken at 120 h was extracted using 50 ml of methanol–water (4:1, v/v).

Chromatography

Reversed-phase isocratic high-performance liquid chromatography was used[7]. A constant-flow pump was connected to a 100 × 5 mm I.D. stainless-steel column packed with Hypersil-ODS (Shandon Southern, Cheshire, Great Britain). Injections were made using a Rheodyne valve. Methazole and its metabolites were measured using a Cecil 212 variable-wavelength UV monitor at 250 nm and 0.1. a.u.f.s.d. Methanol–water (1:1, v/v) was used as the eluent at a flow-rate of 0.5 ml min^{-1}. Peak area was evaluated using a Perkin-Elmer Sigma 10 Chromatography Data Station and was found to be proportional to the concentration in the range 2.5 ng/5 μl to 100 ng/5 μl injection. The optimum wavelength for methazole, DCPMU and DCPU was determined by scanning methanolic solutions between 200 and 300 nm prior to chromatography. Using these conditions retention times for methazole,

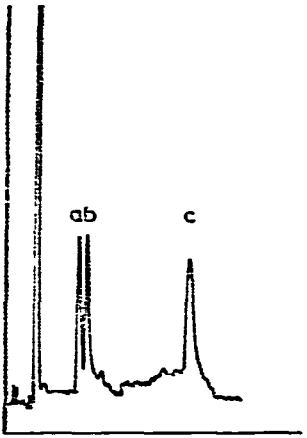

Fig. 1. Typical chromatogram of 10 ng of (a) DCPMU (b) DCPU and (c) methazole.

DCPMU and DCPU were 19.4, 6.7 and 7.5 min, respectively. Fig. 1 shows a chromatogram of 10 ng of DCPMU, DCPU and methazole.

RESULTS AND DISCUSSION

Table II shows that the recovery of methazole, DCPMU and DCPU from wet soil using methanol was satisfactory. Table III shows the comparison between DCPMU extracted from dry soil with methanol and that from wet soil, both fortified at 1 ppm. The results for dry soil show close agreement with those obtained by Walker and Roberts with DCPMU becoming less extractable with time, whereas those for wet soil are essentially constant for the period of the experiment. If the dry soil was extracted after 120 h with methanol–water (4:1, v/v) then the recovery was 93.4%, comparable with that from wet soil. It seems likely therefore either that the presence of water helps to breakdown the soil structure allowing the extractant to work on a greater surface area or that aqueous methanol is simply a better solvent for DCPMU.

TABLE II

RECOVERY (%) OF METHAZOLE, DCPNU AND DCPU FROM SOIL

Figures in parenthesis are standard deviations.

Fortification (ppm)	*Methazole*	*DCPMU*	*DCPU*
Soil 1			
0.1	101.5 (2.4)	92.3 (13.6)	92.2 (5.9)
0.5	95.8 (4.2)	88.8 (3.2)	95.0 (4.5)
1.0	88.3 (4.8)	91.4 (4.0)	100.7 (6.1)
Soil 2			
0.1	98.2 (5.2)	97.4 (8.4)	97.2 (3.1)
0.5	93.2 (0.8)	89.2 (3.9)	96.2 (5.7)
1.0	92.2 (4.8)	89.2 (3.9)	98.6 (6.9)

TABLE III

RECOVERY (%) OF DCPMU FROM WET AND DRY SOIL

Time (h)	*Dry soil*	*Wet soil*
0	91.4	95.9
1	97.1	100.4
2	91.4	101.9
4	71.4	98.9
24	68.6	95.9
48	68.6	96.4
120	70.6	98.9

Walker[8] found that DCPU never accounted for more than 1% of the initial herbicide so it is unlikely that DCPU will be present in the soil in sufficient quantities to determine after normal field application rates. In this case the analysis time can be shortened by using methanol–water (7:3, v/v) as the eluent when retention times for DCPMU + DCPU and methazole become 3.95 and 7.43 min, respectively. The practical limit of detection for this method based on the smallest detectable peak being twice the background signal is about 0.04 ppm for each compound.

This method is not suitable for the less phytotoxic degradation product of DCPMU, 3,4-dichloroaniline as methanol is not an effective extractant.

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REFERENCES

1 A. Walker and M. G. Roberts, *Pestic. Sci.*, 9 (1978) 333.
2 E. R. Butts and C. L. Foy, *Pestic. Biochem. Physicol.*, 4 (1974) 44.
3 F. E. Brockman and W. B. Duke, *Weed Sci.*, 25 (1977) 304.
4 W. Bond and H. A. Roberts, *Weed Res.*, 16 (1976) 23.
5 P. Maini, A. Collina, C. Mellagni and B. Guiati, *Proc. 8th International Velsicol Symposium, 1974*, Velsical Chemical Corp., Chicago, 1974, 9 pp.
6 E. G. Cotterill, *Pestic. Sci.*, 11 (1980) 23.
7 T. H. Byast, *J. Chromatogr.*, 134 (1977) 216.
8 A. Walker, *Pestic. Sci.*, 9 (1978) 326.