Journal of Chromatography, 270 (1983) 396–401
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,090

Note

High-performance liquid chromatographic determination of hexazinone residues in soil and water*

D. C. BOUCHARD* and T. L. LAVY

Altheimer Laboratory, Department of Agronomy, University of Arkansas, Route 11, Box 83, Fayetteville, AR 72701 (U.S.A.)

(First received April 20th, 1983; revised manuscript received June 28th, 1983)

The triazine herbicides are used for weed control on a vast acreage of land. Hexazinone-[3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1H,3H)-dione], is a relatively new triazine used primarily to control undesirable hardwood trees in pine stands. Hexazinone has also been used for weed control in other non-crop areas and is currently being evaluated for weed control efficacy in sugar cane.

In comparison to other triazine herbicides, hexazinone is very water soluble[1] and has been shown to move from the site of application into surface water[2–4]. Since the banning of use of (2,4,5-trichlorophenoxy)acetic acid (2,4,5-T) in forests, the use of hexazinone has expanded rapidly. The increasing amount of hexazinone used in forests coupled with hexazinone's mobility in the environment has created a need for a rapid method of extraction and analysis for determining trace levels of hexazinone in soil and water.

A gas chromatographic (GC) method[5] for analysis of hexazinone and its metabolites from a variety of matrices and a high-performance liquid chromatographic (HPLC) method[6] for analysis of hexazinone purified standards have been reported. Our objectives were to develop a rapid HPLC method for analysis of hexazinone in soil and water and to determine the effects of sample freezing and soil autoclaving on hexazinone extraction efficiency. These effects were examined because soil and water samples are often stored at low temperature until time of analysis and autoclaving is sometimes used in pesticide degradation studies to reduce microbial activity in soil and water. HPLC separation of hexazinone from five other representative triazines is also reported.

EXPERIMENTAL

Materials and apparatus

Hexazinone (99+%) was obtained from DuPont (Wilmington, DE, U.S.A.) and metribuzin (99+%) [4-amino-6-*tert.*-butyl-3-(methylthio)-*as*-triazin-5(4H)-one], from Mobay (Kansas City, MO, U.S.A.). Atrazine (98+%) (2-chloro-4-

* Published with permission of the Director of the Arkansas Agricultural Experimental Station, University of Arkansas, Fayetteville, AR, U.S.A.

0021-9673/83/$03.00

ethylamino-6-isopropylamino-*s*-triazine); propazine (99+%), [2-chloro-4,6-bis(isopropylamino)-*s*-triazine]; prometryn (99+%) [2,4-bis(isopropylamino)-6-(methylthio)-*s*-triazine]; and terbutryn (99+%) [2-(*tert.*-butylamino)-4-(ethylamino)-6-(methylthio)-*s*-triazine] were obtained from Ciba-Geigy (Greensboro, NC, U.S.A.). The acetone used was reagent grade and the methanol and acetonitrile UV grade. The soils used were a Taloka silt loam and a Mountainburg fine sandy loam with organic carbon contents of 0.6 and 2.3%, respectively. The water used was from a pristine mountain stream. The extraction vessels were 175-ml linear polyethylene Nalgene® bottles with polypropylene caps. A wrist-action shaker was used for sample agitation, an N-Evap (Organomation Assoc., Northborough, MA, U.S.A.) for solvent evaporation, and 13-mm diameter 0.22-μm Millipore filters in a Swinney adaptor fitted to a 10-ml syringe for final sample filtration. A Hitachi Model 200 spectrophotometer was used to determine the analytical wavelength of hexazinone. The HPLC system consisted of a Waters Assoc. (Milford, MA, U.S.A.) Model 6000A solvent delivery system, Model 710A WISP, Data Module, Model 440 UV detector fixed at 254 nm, and a radial compression module with a 5-mm I.D. Radial-Pak 10-μm C_8 cartridge and C_{18} guard-pak.

Sample preparation

Soil samples (25 g oven-dry basis) were passed through a 0.5-mm sieve and weighed into the polyethylene bottles. The soils were then treated with aqueous hexazinone solutions to give concentrations of 0.04, 0.40, and 4.00 ppm and a 0.3 bar soil moisture content. After 12 h, six replications of each concentration were extracted to determine percent recovery. Additional samples at the 4.00 ppm concentration were frozen at −20°C for extraction at 1, 4, and 12 weeks. Samples untreated with hexazinone were autoclaved at 120°C and 1.4 bar pressure for 1 h and then autoclaved again 48 h later. These samples were then treated to give a hexazinone concentration of 4.00 ppm and were extracted 24 h later. Volumes of 95 ml of water were placed in polyethylene bottles and 5-ml aliquots of aqueous hexazinone solutions were added to give concentrations of 0.001, 0.01, and 0.10 ppm. After being swirled by hand, six replications of each concentration were extracted. Water samples were autoclaved as the soil samples, then were treated to give a hexazinone concentration of 0.01 ppm, and were extracted 24 h later. Additional samples of the 0.01 ppm concentration were frozen at −20°C for extraction at 1, 4, and 12 weeks.

Extraction

To the soils in the Nalgene bottles were added 50 ml of acetone–water (20:80). The bottles were capped and placed on a wrist-action shaker at 180 to 240 oscillations/min for 30 min. The soil slurries were filtered under vacuum through Buchner funnels lined with Whatman No. 42 filter paper. The filters were washed once with 10 ml of acetone–water (20:80), and the filtrates were transferred to 250-ml separatory funnels. Volumes of 25 ml of chloroform were added, and the funnels were shaken vigorously 40 times by hand. The layers were allowed to separate, and the chloroform extracts were collected in 20 × 2.5 cm test tubes. The extractions were repeated, and the two chloroform extracts were combined. The chloroform was evaporated under dry nitrogen in the N-Evap at 55 to 60°C. The test tubes were allowed to cool, and 4 ml of atrazine in methanol were added as internal standard. The concentrations of

the atrazine internal standards (IS) were 1.0, 10.0, and 100 ppm for the 0.04, 0.40, and 4.00 ppm concentrations of hexazinone, respectively. The test tubes were swirled by hand and allowed to sit for 5 min to ensure hexazinone dissolution and mixing. The samples were then filtered through a Millipore filter in a Swinney adaptor and collected in a 4-ml vial.

The water samples were transferred directly to the separatory funnels and extracted as the soil samples. The concentration of atrazine internal standards were 1.0 ppm for the 0.001 and 0.01 ppm water samples and were 10 ppm for the 0.10 ppm water samples.

Chromatography

The injection volume was 50-μl for all samples except the 0.001 ppm water samples for which the injection volume was 200 μl. The samples were eluted isocratically with an acetonitrile–water (50:50) mobile phase at a flow-rate of 1.2 ml/min. The UV detector was operated at the fixed wavelength of 254 nm.

RESULTS AND DISCUSSION

A 50-μl injection of a 1.0 ppm solution of the six triazine herbicides prepared in methanol–water (50:50) yielded the chromatogram contained in Fig. 1. The retention times in minutes of the triazines were hexazinone (4.3), metribuzin (5.3), atrazine

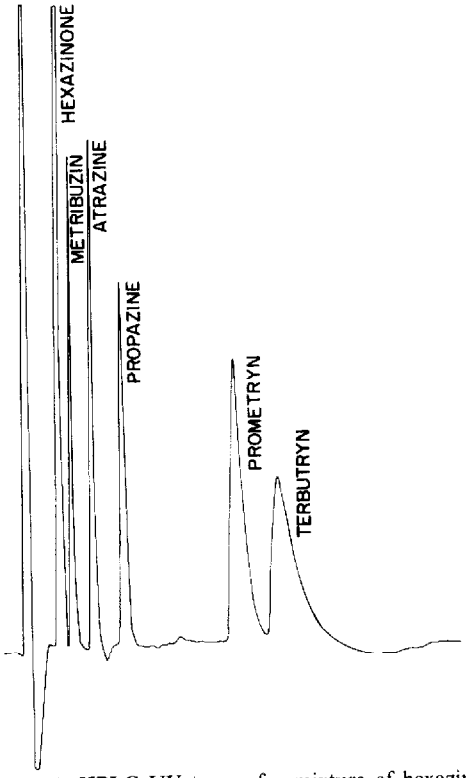

Fig. 1. HPLC–UV trace of a mixture of hexazinone, metribuzin, atrazine, propazine, prometryn, and terbutryn.

(7.5), propazine (9.3), prometryn (17.8), and terbutryn (21.2). These triazines were chosen because they represent the chloro-triazines (atrazine and propazine), the thiomethyl-triazines (terbutryn and prometryn), and the triazines containing a carbonyl group (hexazinone and metribuzin). In contrast to an agricultural field that may receive applications of several pesticides over the course of a growing season and have trace levels of additional pesticides from previous years, herbicide use in the forest is much more restrictive. Currently, there is no significant amount of a triazine herbicide other than hexazinone that is used in the forest, and it is unlikely that environmental samples would contain hexazinone in a mixture with other triazine herbicides.

Confirmation of the identity of the hexazinone peak was performed by another laboratory using GC–mass spectrometry (MS). Identity of the hexazinone peak was provided by comparison of the GC retention time to standards and comparison of the mass spectrum of an actual sample to a hexazinone spectrum from a computer library (Fig. 2).

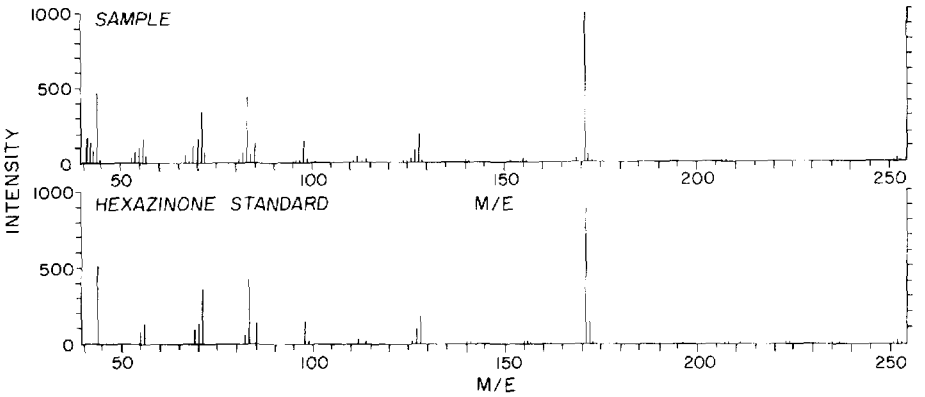

Fig. 2. Mass spectra of actual sample and hexazinone standard.

The analytical wavelength of hexazinone was determined to be 247 nm which resulted in good detector sensitivity with our 254-nm fixed-wavelength detector. Of the six triazines, the detector was most sensitive to hexazinone which gave a detector response 2.0 times that of the next triazine, prometryn.

Chromatograms of blanks and soil and water samples fortified with hexazinone are shown in Fig. 3. The recovery data are given in Table I. The hexazinone recovery from the Mountainburg soil was lower than that from the Taloka soil at 0.40 and 4.00 ppm probably due to the higher organic carbon content of the Mountainburg soil. Soil organic carbon is usually the primary factor in adsorption of pesticides by soil[7]. Studies have shown that autoclaving reduces the adsorptivity[8] and cation-exchange capacity[9] of soil, and therefore would be expected to increase extraction efficiency. However, autoclaving can solubilize soil organic carbon[8] which may interfere with the extraction and detection of pesticides in soil. The decreased hexazinone recovery from autoclaved soil observed in this study may have been due to solubilization of soil organic carbon during autoclaving. The Mountainburg soil with

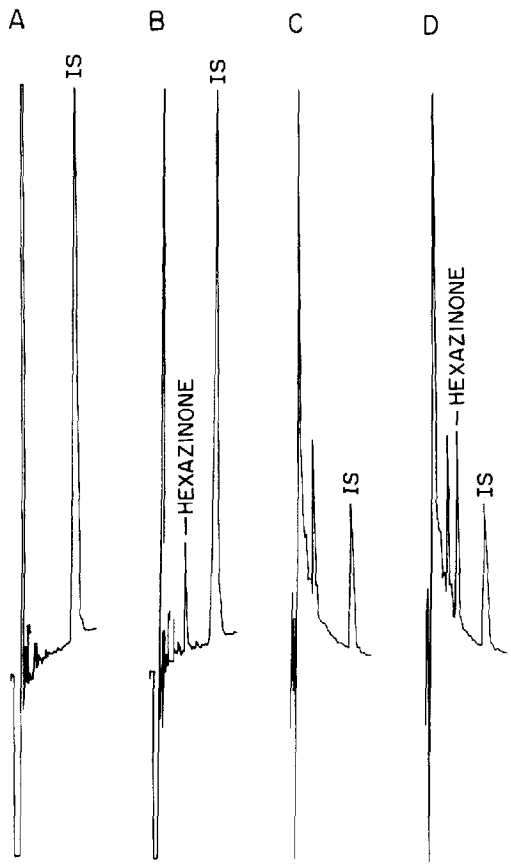

Fig. 3. HPLC–UV trace of soil and water sample fortified with hexazinone. A, water blank; B, 0.001 ppm in water; C, soil blank; D, 0.04 ppm in soil.

TABLE I

RECOVERY OF HEXAZINONE FROM SOIL AND WATER

Six replicate samples were analyzed at each concentration.

Matrix	*ppm*	*Recovery ± S.D. (%)*
Taloka silt loam	0.04	76 ± 3
	0.40	86 ± 2
	4.00	84 ± 2
Mountainburg sandy loam	0.04	75 ± 3
	0.40	76 ± 1
	4.00	73 ± 2
Water	0.001	96 ± 6
	0.010	97 ± 2
	0.100	95 ± 1

the higher organic carbon content showed greater effects of autoclaving on extraction efficiency. It was observed that the acetone–water extracts of the autoclaved soil treatments took longer to filter and formed a persistent emulsion layer when partitioned with chloroform indicating that additional organic material had been extracted from the autoclaved soil. Autoclaving had no effect on the extraction of hexazinone from water. Storage at −20°C had no effect on the extraction efficiency of hexazinone from soil or water (Table II).

TABLE II

EFFECTS OF STORAGE AT −20°C AND AUTOCLAVING ON RECOVERY OF HEXAZINONE FROM SOIL AND WATER

Six replicate samples were analyzed at each concentration.

Matrix	*ppm*	*Recovery ± S.D. (%)*				
		Autoclaved	*Weeks at −20°C*			
			0	*1*	*4*	*12*
Taloka silt loam	4.0	79 ± 2	84 ± 2	83 ± 4	83 ± 1	83 ± 2
Mountainburg sandy loam	4.0	66 ± 2	73 ± 2	73 ± 2	74 ± 1	75 ± 2
Water	0.01	95 ± 2	97 ± 2	94 ± 2	96 ± 1	94 ± 3

The method presented here should be useful for hexazinone residue studies. It allows for rapid analysis of large numbers of samples with limits of detection comparable to, or better than, other published methods for hexazinone analysis.

ACKNOWLEDGEMENTS

We thank R. W. Reiser and W. R. Johnson of DuPont for the GC–MS work.

REFERENCES

1 *Herbicide Handbook*, Weed Science Society of America, Champaign, IL, 1979.
2 D. C. Bouchard and T. L. Lavy, *Abstr. Weed Sci. Soc. Amer.*, (1983) 91.
3 D. G. Neary, P. B. Bush and J. E. Douglas, *Abstr. Weed Sci. Soc. Amer.*, (1981) 45 56.
4 D. G. Neary, J. E. Douglas, P. B. Bush and D. Mayack, *Abstr. Weed Sci. Soc. Amer.*, (1980) 53–54.
5 R. F. Holt, *J. Agr. Food Chem.*, 29 (1981) 165–172.
6 C. E. Parker, C. A. Haney, D. J. Harvan and J. R. Hass, *J. Chromatogr.*, 242 (1982) 77 96.
7 G. W. Bailey and J. L. White, *Residue Rev.*, 32 (1970) 29–92.
8 T. H. Dao, D. B. Marx, T. L. Lavy and J. Dragun, *Soil Sci. Soc. Amer. J.*, 46 (1982) 963–969.
9 D. C. Wolf, T. H. Dao, H. D. Scott and T. L. Lavy, *Soil Sci. Soc. Amer. J.*, (1983) in press.