

The Use of Ultrasonic Extraction in the Determination of Some *s*-Triazine Herbicides in Soils

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine), cyanazine (2-(4-chloro-6-ethylamino-*s*-triazin-2-ylamino)-2-methylpropionitrile), and cyprazine (2-chloro-4-cyclopropylamino-6-isopropylamino-*s*-triazine) were extracted from soils with aqueous methanol using an ultrasonic cleaner. Cleanup consisted of chloroform partitioning and column chromatography on deacti-

vated basic alumina. Extracts were determined by gas chromatography with alkali flame ionization detection. After allowing *s*-triazine adsorption, recoveries from soils fortified at 1 ppm ranged from 81.6 to 94.5%. Two 15-min ultrasonic extractions were comparable to 24 hr of Soxhlet extraction for atrazine.

The *s*-triazines atrazine, cyanazine, and cyprazine are used primarily for weed control in corn. Due to their adsorptive nature, residues of these herbicides tend to persist in soil. In soil residue analysis, it is important to use an extraction procedure capable of desorbing the bound residues of these compounds.

A number of methods for extracting *s*-triazine residues from soil have been reported. McGlamery *et al.* (1967) found that a 2-hr Soxhlet procedure using methanol was the most effective method of extracting fortified atrazine residues from a clay loam soil. Tindle *et al.* (1968) used 16-hr Goldfisch extractions with chloroform and reported good recoveries of fortified *s*-triazine residues from a silty loam soil. Mattson *et al.* (1970) found that a 2-hr water-acetonitrile reflux procedure was comparable to a 24-hr water-methanol Soxhlet method for extracting weathered atrazine residues from a silty clay loam soil. Beynon (1972) extracted bound cyanazine residues from various soils using a 2-hr water-methanol tumbling procedure.

The use of ultrasonic energy to extract organochlorine insecticides from various soils was investigated by Johnsen and Starr (1967, 1970, 1972). They reported that ultrasonic extraction was faster, more reliable, and more quantitative than conventional extraction methods.

The purpose of this study was to determine if an ultrasonic method would give satisfactory extraction recoveries for atrazine, cyanazine, and cyprazine after allowing these herbicides to adsorb to the soil. The ultrasonic method used was compared to a 24-hr Soxhlet extraction.

EXPERIMENTAL SECTION

Fortification of Soil Samples. The characteristics of the soils used are given in Table I. Soils were air-dried, ground, and sieved through a 20-mesh screen prior to use. Soil samples (50.0 g each, oven-dried basis) were fortified individually in square quart bottles by pipeting 20 ml of herbicide standard solution (2.5 ppm in methanol) onto the soil surface. Each sample was slurried with excess solvent to mix the treated soil and then air-dried. The resultant herbicide concentration in each sample was 1 ppm on a soil basis. A 3-day equilibration period was allowed before extracting fortified samples unless otherwise indicated.

Ultrasonic Extraction. The fortified soil samples, contained in the quart bottles, were saturated with 50 ml of distilled water and were allowed to equilibrate for 1 hr. Samples were then extracted with 100 ml of methanol using a Sonogen, Model D-50, ultrasonic cleaner (Branson Instruments Co., Stamford, Conn.). The generator of this ultrasonic cleaner has an output of 50 W and is "self-tuning" to operate the transducer at its natural resonant frequency of 40 kHz. The water level in the ultrasonic tank was adjusted to equal the methanol extraction solvent level inside the bottles. Samples were stirred and then sonified for 15 min, unless otherwise indicated, with the sample bottles positioned for maximum cavitation. After

initial sonification, the soil was allowed to sediment before the solvent was decanted and suction filtered into a round-bottomed flask. The remaining sediment was reextracted with another 100 ml of methanol using the same sonification process. The entire contents of the bottles were then suction filtered to give combined sample extracts.

Soxhlet Extraction. Fortified soil samples were placed directly in the Soxhlet chamber between glass wool plugs and were saturated with 50 ml of distilled water. Samples were then extracted for 24 hr using 200 ml of methanol. The extracts were suction filtered prior to cleanup.

Cleanup of Extracts. The sample extract volume was reduced to 5–10 ml by rotary evaporation and then refiltered quantitatively. The extract was then reduced to 5 ml, diluted with 200 ml of saturated NaCl solution and 30 ml of distilled water, and partitioned into three 50-ml portions of chloroform. The chloroform extract was reduced to 5 ml and transferred to a chromatographic column (1 cm i.d.) containing 7.6 cm of freshly prepared basic alumina V. The column was eluted with 75 ml of chloroform and the eluate rotary evaporated to near dryness. A solvent change to methanol was made by adding 50 ml of methanol and again reducing sample volume. Samples were transferred to glass stoppered centrifuge tubes and adjusted to 15-ml final volume in methanol prior to gas chromatographic determination.

Gas Chromatography. A Varian Aerograph Model 1840 gas chromatograph equipped with an Rb₂SO₄ alkali flame ionization detector (AFID) was used. Pyrex columns, 0.83 m × 4 mm i.d. for atrazine and 0.41 m × 4 mm i.d. for cyanazine and cyprazine, were packed with 7% OV-17 on 80–100 mesh Chromosorb W HP. Typical operating conditions were: injection port, 200–220°; column oven, 190–200°; and detector, 225–230°. A nitrogen flow rate of 36–40 ml/min was used. Under these conditions retention times were 7.2, 6.7, and 3.4 min for atrazine, cyanazine, and cyprazine, respectively.

AFID response curves for each herbicide were determined using standard solutions of 0.25–10 ng of herbicide per microliter of methanol. Two microliters of each concentration were injected two to five times. Chromatographic peaks were measured using the height × width at half-height method. Results were evaluated statistically using regression analysis.

The herbicide standard solutions used in fortification were employed as standards when determining extracted samples. Mean response from at least two injections of sample extracts was converted to nanograms using predetermined standard response curves. Any changes in detector sensitivity were monitored by observing response to 5-ng standards injected alternately to sample extracts. A correction factor, the ratio of 5-ng response on the standard curves over the 5-ng response of alternating standard injections, was applied to sample response before using standard curves.

Table I. Physical Characteristics of Soils Used^a

Soil no.	Texture	% soil moisture ^b	pH	% organo- inorganic separates, %			Clay	CEC ^c
				mat-	Sand	Silt		
1	Loamy sand	1.5	7.8	2.6	82.2	7.7	10.1	14.5
2	Silty clay loam	4.2	8.0	2.2	18.3	42.5	39.2	31.6

^a Determined at the University of Manitoba Soil Testing Laboratory. ^b In air-dried soil. ^c Cation exchange capacity in mequiv/100 g.

Table II. Effect of Fortified Soil Equilibration Period on the Recovery of Atrazine^a

Equilibration period before extraction, days	Mean % recovery ^b
3	83.1
6	83.9
10	86.4
25	73.6

^a Ultrasonic extraction from soil no. 1, samples not subjected to cleanup. ^b Mean of two replicate samples.

Table III. Recovery of *s*-Triazines from Fortified Soils

<i>s</i> -Triazine	Soil no.	Extraction method	Mean % recovery ± std dev ^a
Atrazine	1	Soxhlet	82.1 ± 2.9
	1	Ultrasonic	81.6 ± 3.1
	1	Ultrasonic ^b	77.8 ± 3.1
	2	Ultrasonic	83.6 ± 0.5
Cyanazine	1	Ultrasonic	94.5 ± 1.4
Cyprazine	1	Ultrasonic	83.6 ± 0.8

^a Mean of at least five replicate samples. ^b Two 30-min sonifications per sample.

RESULTS AND DISCUSSION

AFID response to atrazine, cyanazine, and cyprazine was linear for 2.0–20 ng of herbicide injected (correlation coefficients ≥ 0.998). The minimum detectable limit (2× noise level) for all three *s*-triazines studied was 0.5 ng, while 5.0 ng injected gave typical responses of 15–20% full-scale deflection. These results agree with the Rb₂SO₄ AFID sensitivity reported by Tindle *et al.* (1968) for atrazine.

The ultrasonic cleaner employed had no built-in power or frequency adjustments for obtaining maximum cavitation. Best cavitation was observed when water bath levels were less than 3 cm and sample bottles were placed in a corner of the ultrasonic cleaner at a slightly tipped angle. Under these conditions, cavitation agitated the soil in a circular motion producing a desirable stirring effect. It was assumed that ultrasonic cavitation did not cause any significant breakdown or alteration of the *s*-triazine herbicides during extraction. Tadic and Ries (1971) found only 1.37% dealkylation when atrazine was suspended in an ultrasonic field for 5 hr.

The cleanup method described was used mainly to remove the humus present in the extracted samples, thus

preventing rapid deterioration of the gas chromatographic column. Injection of crude blank extracts showed no co-extracted interferences at the retention times of the herbicides studied. Comparison of crude and cleaned-up extracts showed that minimal losses of approximately 2.5% atrazine occurred during cleanup. Blank extracts were also devoid of interferences after cleanup.

A 3-day equilibration period was allowed before extracting fortified samples based on the results shown in Table II. There were no apparent differences between extraction recoveries 3, 6, and 10 days after fortification; however, when the soil was extracted 25 days after fortification an unidentified additional peak (retention time 8.6 min compared to atrazine at 7.2 min) was observed. The effect of soil moisture at the time of fortification was also checked. There was no apparent difference in atrazine recovery when air-dried soil was fortified using methanol (84.6%) or 15 ml of water and methanol (84.4%).

Recoveries of atrazine, cyanazine, and cyprazine from the fortified soils are shown in Table III. Two 15-min ultrasonic extractions were as effective as a 24-hr Soxhlet extraction for atrazine on loamy sand. Using two 30-min sonifications per sample (77.8 ± 3.1% recovery) did not increase extraction efficiency over two 15-min sonifications per sample (81.6 ± 3.1% recovery). Although recoveries and standard deviations were in general satisfactory, the best results were obtained for the ultrasonic extraction of cyanazine. This improved extraction may have been due to some solvent effect related to the fact that cyanazine was the most polar of the *s*-triazines studied. Recoveries of atrazine from the silty clay loam soil indicated that the ultrasonic method used was also applicable to this soil type.

Factors such as soil type, fortification levels, and equilibration time before extraction greatly influence recovery. McGlamery *et al.* (1967) used conditions similar to those reported here and obtained 86.0% atrazine recovery using 2-hr Soxhlet extractions. The advantage of ultrasonic extraction is that shorter extraction times can be used.

Although weathered soils were not used in this work, the *s*-triazine herbicides were allowed to adsorb to the soil after fortification. Experiments conducted by Johnsen and Starr (1970, 1972) with organochlorine insecticides in artificially weathered and field-treated soils showed that ultrasonic extraction was usually more effective than other methods.

This study has shown that a simple and relatively inexpensive ultrasonic system can be used for rapid extraction of atrazine, cyanazine, and cyprazine from soils. The method reported here should be applicable to other triazine herbicides.

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