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DETERMINATION OF S-TRIAZINE HERBICIDES IN MANITOBA SOILS

by

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ABSTRACT

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine), cyanazine (2-[(4-chloro-6-ethylamino-s-triazin-2-yl)amino]-2-methylpropionitrile), and cyprazine (2-chloro-4-cyclopropylamino-6-isopropylamino-s-triazine) were ultrasonically extracted from soils with aqueous methanol. Cleanup consisted of chloroform partitioning and column chromatography on deactivated basic alumina. Extracts were determined by gas chromatography with alkali flame ionization detection.

The recoveries of bound s-triazine residues from soils fortified at 1 ppm ranged from 81.6 to 94.5%. Two 15 minute ultrasonic extractions were comparable to 24 hours of Soxhlet extraction for atrazine. Sensitivity is placed at 2 ng of s-triazine in the injected sample and the least determinable concentration is estimated at 0.02 ppm s-triazine in soil. The method developed is thought to be applicable to weathered s-triazine residues in field soils.

The identity of the gas chromatographic peaks observed for atrazine, cyanazine, and cyprazine standards was confirmed using infrared spectrophotometry and mass spectrometry. Spectra obtained from 10-25 μg of trapped eluates are presented and interpreted. Although mass spectrometry was preferred, both confirmatory techniques could be used to identify these s-triazines in "unknown" extracted samples.

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INTRODUCTION

Atrazine, cyanazine, and cyprazine are s-triazine herbicides used for weed control primarily in corn. Under Manitoba conditions these herbicides tend to persist in the soil because of their adsorptive nature, and often cause serious injury to susceptible crops grown the following year.

Soil residues of s-triazines may occur in either a bound or unbound state with a dynamic equilibrium existing between the two states. Unbound soil residues which are available to the plants have been studied using bioassay techniques (Elliott, 1972). Since bound residues may become desorbed from the soil and cause plant injury, it is desirable to be able to estimate both bound and unbound residues in the soil. Future study of the relative amounts of bound and unbound residues found under different conditions should give a valuable insight into the particular s-triazine residue problem in Manitoba.

Experiments were conducted to develop a suitable analytical method for measuring total s-triazine residues in Manitoba soils. Particular emphasis was placed on developing techniques which could be used to confirm residue identities.

LITERATURE REVIEW

Introduction

The major steps in pesticide residue analysis are: sampling, storage of samples, extraction, cleanup, detection and determination, and confirmation of identity (Schechter and Getz, 1967; McCully, 1969). The nature of these steps and the analytical techniques involved have been previously discussed (Van Middelem, 1963; Egan, 1967; Schechter and Getz, 1967; McCully, 1969; Gunther, 1969; Mattson et al., 1970; Blinn, 1971a). In developing and evaluating a residue analysis method fortified samples are used to determine pesticide recoveries (Schechter and Getz, 1967).

Fortification of Soil

Freshe (1971) pointed out that there was a difference between recovering a compound from a fortified soil and extracting "true" residues from weathered soil. Nevertheless, he felt that fortified residues were the best possible approximation to "true" residues. Mattson et al. (1970) stated that good recoveries from soil extracted immediately after fortification did validate the general analytical procedure.

Johnsen and Starr (1967) found a considerable decrease in pesticide recovery from soil extracted 1 month compared to 1 day after fortification. These decreases were attributed to the pesticide having become more tightly bound to the soil. Beynon (1972) stressed the importance

of using bound residues to obtain meaningful pesticide recoveries for a residue analysis method.

Extraction

A number of procedures have been described for extracting s-triazine herbicides from soil. Chilwell and Hughes (1962) concluded that chloroform and methylene chloride were the best solvents for s-triazines. Birk and Roadhouse (1964) also used chloroform and obtained a mean recovery of 86.2% for atrazine extractions.

Benfield and Chilwell (1964) extracted s-triazines by shaking the soil with a mixture of methanol and dichloromethane in the presence of excess ammonia. Abbott et al. (1965) used diethyl ether and ammonia. Recovery data was not reported by either group.

Quantitative recoveries of s-triazines from soil were reported by Henkel and Ebing (1964). They used acetone and 30 minute shakings at room temperature to achieve 88-110% extraction efficiencies.

Sheets and Kearney (1964) extracted sandy clay loam 1 week after fortification. Shaking with chloroform, carbon tetrachloride, or n-hexane for 1.5 hours generally gave recoveries of less than 70% for atrazine. They found extraction with chloroform/8M urea or chloroform/0.5M ammonium sulfate to be more effective.

Talbert and Fletchall (1965) found that ethanol/water gave leaching recoveries of 90-100% for atrazine from several soils. They used 1 hour extractions at 66^{o}, and observed that longer extraction times were

necessary for good recoveries at room temperatures.

Using absolute methanol and 3 hour Soxhlet extractions, Sikka (1966) obtained recoveries of 90-98% for atrazine. Soils were fortified at levels of 0.5-5 ppm and were allowed to equilibrate for 5 days before extraction.

McGlamery et al. (1967) compared the effectiveness of 2 extraction methods and 12 solvent systems for recovering atrazine from a clay loam soil. The soil was air-dried for 2 days after fortification at the 1 ppm level. Soxhlet extractions were found to be more effective than shaking. Methanol, which was chosen as the preferred solvent, gave 86.0% recovery using a 2 hour Soxhlet extraction.

The use of a Goldfisch apparatus to extract s-triazines from silty loam soil was reported by Tindle et al. (1968). Using 16 hour chloroform extractions, a mean recovery of 93.2 $\pm$ 2.6% was obtained from soil fortified at 1, 10, and 100 ppm. They noted that Soxhlet extractions took about four times as long to achieve similar recoveries.

Eberle and Hormann (1968) adopted a method using methanol and 12 hour shakings to extract atrazine; whereas Shell Development Co. (1969) recommended shaking for 1 hour with methanol/chloroform to extract cyanazine.

In their review of the chemical determination of s-triazine herbicides in soils, Mattson et al. (1970) presented data comparing extraction procedures for atrazine. They used a silty clay loam soil containing weathered residue levels of 0.08 ppm and 1.9 ppm. A 2 hour water/

acetonitrile reflux extraction was comparable to a 24 hour water/methanol Soxhlet method. Recoveries ranged from 63-103%. A procedure using methanol and 30 minutes of mechanical shaking gave poorer results at the 1.9 ppm residue level. Young and Chu (1973) also used a reflux procedure to extract soils fortified at 0.6-1.6 ppm. Using 30 minute extractions with methanol/ethyl acetate, they obtained recoveries of 84-112% for atrazine.

Ott et al. (1971) described a completely mechanized extraction method for atrazine soil residues. Soil was manually introduced into a Solidprep sampler followed by homogenization with warm acetonitrile/water. Using samples fortified at 0.05-1 ppm levels, 71-89% recovery was obtained. Recoveries from field-treated soil were 86-90% of those obtained by an independent refluxing procedure. It was noted that although this mechanized system lacked precision at lower residue levels, it could process samples every 10.5 minutes and thus would be valuable as a rapid screening method.

Beynon (1972) extracted cyanazine and some of its degradation products from soil using 2 hours of end-over-end tumbling with water/methanol. Recoveries ranged from 88-96% for cyanazine applied to soil at 0.05-2.0 ppm prior to extraction. Analysis for bound residues accounted for 76-90% of the (^{14}C) cyanazine applied to various soil types.

The use of ultrasonic energy to extract organochlorine insecticides from various soils was investigated by Johnsen and Starr (1967, 1970, 1972). They reported that 30 second ultrasonic extractions generally

gave 90-100% pesticide recovery. These results were comparable to those obtained from 8 hours of Soxhlet extraction.

Cleanup

Several authors have reported using liquid-liquid partitioning and/or adsorption column chromatography to cleanup s-triazine soil extracts (Table 1). Benfield and Chilwell (1964) used an internal standard to compensate for the incomplete recovery of atrazine from their cleanup procedure. McGlamery et al. (1967) also found that polyethylene coated alumina columns were useful if soil extracts contained high amounts of pigments. Ott et al. (1971) used calcium chloride to flocculate the soil colloidal particles in their extracts, and allowed them to settle before partitioning the aqueous supernatant.

Detection by Gas Chromatography (GC)

Bostwick and Giuffrida (1968) investigated several efficiency parameters of GC columns used in pesticide residue analysis. They recommended using glass columns, 6-12 feet x 4 mm i.d., packed with 4-10% liquid phase on 80/100 or 100/120 mesh solid support. A representative list of the columns used for the GC of s-triazines is given in Table 2. Although aluminum and stainless steel columns have been used, most columns were made of the more inert glass tubing. Even with glass columns, Purkayastha and Cochrane (1973) reported on-column decomposition of cyanazine when Reoplex 400 and Carbowax 20M liquid phases were used. Silanized solid support (Supina et al., 1966) and silanized glass

Table 1. Cleanup Methods Used for s-Triazine Soil Extracts

Liquid/liquid partitioning system	Column chromatography solid adsorbent	Eluting solvent	s-Triazine recovered (cleanup efficiency)	Literature reference
CH_2Cl_2:MeOH/H_2SO_4[a] H_2SO_4/$CHCl_3$:NaOH			Atrazine	Benfield and Chillwell (1964)
	Basic Alumina IV	1/20 Ether/CCl_4	Atrazine (85-95%)	McGlamery et al. (1967)
MeOH:H_2O/$CHCl_3$	Basic Alumina V	2/1 Hexane/Ether	Atrazine	Eberle and Hormann (1968)
MeOH:H_2O/Ether			Cyanazine	Shell Development Co. (1969)
CH_3CN:H_2O/CH_2Cl_2	Basic Alumina V	1/20 Ether/CH_2Cl_2	Atrazine	Mattson et al. (1970)
	Basic Alumina V	3/2 Benzene/Ether	Atrazine (95%)	Zimdahl et al. (1970)
CH_3CN:H_2O/Hexane:Ether			Atrazine	Ott et al. (1971)
MeOH:H_2O/Ether	Basic Alumina[b]	1/1 Ether/Pet. spirit 1/1 EtAc/Pet. spirit	Cyanazine (95%) Cyanazine (95%)	Beynon (1972)
CH_3CN:H_2O/CH_2Cl_2	Basic Alumina[c]	6% Ether/CCl_4	Atrazine	Purkayastha and Cochrane (1973)
	Neutral Alumina I	EtAc	Atrazine	Young and Chu (1973)

[a] Partitioned from CH_2Cl_2:MeOH into H_2SO_4; then from H_2SO_4 into $CHCl_3$:NaOH.

[b] Basic alumina deactivated with 13% H_2O.

[c] Basic alumina deactivated with 7.5% H_2O.

Table 2. Columns Used for the Gas Chromatography of s-Triazines

Liquid phase (percent loading)	Solid support (mesh size)	Column dimensions (length x o.d.)[a]	Literature reference
Versamid 900 (2.5%)	Diatoport S (60/80)	3m x 3.5mm[bc]	Henkel and Ebing (1964)
Carbowax 20M (5%)	Anakrom ABS	5' x 1/4" [c]	Mattson et al. (1965)
Reoplex 400 (10%)	Gas Chrom Z (80/100)	1.9m x 3mm [b]	Tindle et al. (1968)
SE-30 (5%)	Chromosorb WS	1m x 4mm [b]	Eberle and Hormann (1968)
Reoplex 400 (2%)	Chromosorb Q (80/100)	5' x 1/8" [d]	Shell Development Co. (1969)
UCW-98 (5%)	Diatoport S (80/100)	6' x 1/4"	Gulf Res. and Development Co. (1969)
DC-710 (5%)	Gas Chrom Q (100/120)	17" x 1/8" [d]	Schultz (1970)
OV-1 (3%)	Chromosorb W HP(80/100)	6' x 6mm	Cochrane and Wilson (1971)
OV-17 (3%)	Gas Chrom Q (100/120)	3' x 1/4"	Greenhalgh and Cochrane (1972)
OV-225 (3%)	Gas Chrom Q (100/120)	0.9m x 4mm [b]	Greenhalgh and Wilson (1972)
CHDMS (2%)	Gas Chrom Q (80/100)	0.6m x 2.5mm[b]	Beynon (1972)
EGA (0.325%)	Chromosorb G	1.5m x 4mm [b]	Swan (1972)

[a] Columns made with glass tubing, unless otherwise noted.

[b] Column bore quoted as inside diameter.

[c] Aluminum tubing.

[d] Stainless steel tubing.

tubing (Gehrke and Leimer, 1971) have been used to make columns more inert. Hartmann (1969) injected a silanizing agent (Silyl 8) during conditioning to improve column inertness. Thompson et al. (1969) reported that pesticide-loading during conditioning improved column performance.

A number of detectors have been used in the GC determination of s-triazine residues. Chilwell and Hughes (1962) and Henkel and Ebing (1964) used a flame ionization detector and reported minimum detectable concentrations (MDC) of 0.5 ppm s-triazine in soil and 0.1-0.2 ppm s-triazine in soil extracts, respectively.

Several authors used the Dohrmann microcoulometric detector which has a titration cell sensitive to halides (Mattson et al., 1965; Eberle and Hormann, 1968; Zimdahl et al., 1970; Mattson et al., 1970). The MDC reported were 0.01-0.05 ppm s-triazine in crops (Mattson et al., 1965; Eberle and Hormann, 1968), and 0.05 ppm s-triazine in soil (Mattson et al., 1970). The latter showed that detector response was linear (20-60 ng atrazine) and reported a minimum detectable amount (MDA) of 20 ng for atrazine.

Tindle et al. (1968) described the application of a Rb_2SO_4 alkali flame ionization detector (AFID) to s-triazine residue determination. This detector was found to be 1000 times more sensitive to nitrogen-containing organics than to C-H-O compounds. The authors were thus able to obtain a MDC of 0.02 ppm s-triazine in soil without cleanup. They also reported detector linearity and MDA of 0.5 ng for atrazine. It was noted that careful control of flow rates was required to minimize

fluctuations in detector performance. Similar detectors used for s-triazine determinations include: CsBr AFID (Shell Development Co., 1969; Schultz, 1970), RbBr AFID (Schroeder et al., 1972), and RbCl AFID (Swan, 1972; Greenhalgh and Wilson, 1972). The MDA for these AFID were 0.3-1 ng of s-triazine.

Mattson et al. (1970) considered tritium electron capture detectors (^{3}H ECD) to be relatively insensitive to s-triazines, as 100-300 ng were generally required for 50% full-scale deflection (FSD). Similar values were reported by Burke and Holswade (1966). Shell Development Co. (1969) found the ^{3}H ECD to be relatively sensitive to cyanazine as 0.25 ng gave 10% FSD. Beynon (1972) chromatographed cyanazine on a modified ^{3}H ECD and reported a MDA of 0.02 ng and a MDC of 0.01 ppm in soil.

Gulf Research and Development Co. (1969) determined cyprazine residues with a ^{63}Ni ECD. An advantage of this detector was that temperatures higher than the 225^{o}C, ^{3}H ECD limit, could be used. The MDA was approximately 0.5 ng.

Ott et al. (1971) and Laski and Watts (1973) chromatographed atrazine using the Coulson conductivity detector (CCD). They reported sensitivities of 0.05 ppm in soil and 5 ng for 50% FSD, respectively.

Greenhalgh and Cochrane (1972) compared the RbCl AFID and the CCD response to s-triazines. The CCD gave slightly better response to atrazine and cyprazine and was preferred because of its selectivity and ease of operation. AFID response was also more variable.

It has been reported that the ^{63}Ni ECD and the CCD have comparable sensitivities to s-triazines (Cochrane and Wilson, 1971; Purkayastha and Cochrane, 1973; Young and Chu, 1973). For residue determination the CCD was preferred since atrazine soil extracts could be chromatographed without prior cleanup (Purkayastha and Cochrane, 1973; Young and Chu, 1973).

Confirmation

Egan (1967), Schechter (1968), and McCully (1969) pointed out the importance of confirming the identity of pesticides. They described some of the causes of mistaken identities as well as several confirmatory techniques. There were two aspects of confirmation emphasized by all three authors. The first was the unreliability of making pesticide identifications based on the evidence from a single gas chromatogram. Secondly, although no one method could identify an unknown residue with absolute certainty, infrared (IR) spectrophotometry and mass spectrometry (MS), used as ancilliary techniques to GC, gave the most conclusive evidence.

The use of IR spectrophotometry to confirm pesticide identities was described by Chen (1965), Blinn (1965), and Blinn (1971b); while Chen (1967) and Gore et al. (1971) published reference IR spectra of atrazine. Biros (1971) reviewed the applications of MS and GC-MS to pesticide residue analysis. Jorg et al. (1966) and Ross and Tweedy (1970) presented and interpreted the mass spectra of some s-triazines.

There has been little published directly concerning the confirmation

of s-triazine residues in soil. Shell Development Co. (1969) proposed using an AFID to confirm cyanazine found in crops by their EC-GC method. They also studied 17 other common pesticides which could coincide with cyanazine during GC analysis and found no interfering peaks.

SECTION 1

THE USE OF ULTRASONIC EXTRACTION IN THE DETERMINATION OF SOME S-TRIAZINE HERBICIDES IN SOILS

ABSTRACT

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine), cyanazine (2-[(4-chloro-6-ethylamino-s-trazin-2-yl)amino]-2-methylpropionitrile), and cyprazine (2-chloro-4-cyclopropylamino-6-isopropylamino-s-triazine) were extracted with aqueous methanol using an ultrasonic cleaner. Cleanup consisted of chloroform partitioning and column chromatography on deactivated 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.

INTRODUCTION

The chloro-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).

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 3. Soils were air-dried, ground, and sieved through a 20 mesh screen prior to use. Soil samples (50.0g 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.

Table 3. Physical Characteristics of Soils Used[a]

Soil no.	Texture	% Soil moisture[b]	pH	% Organic matter	Inorganic separates (%) Sand	Silt	Clay	CEC[c]
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/100g.

Ultrasonic Extraction. The fortified soil samples, contained in the quart bottles, were saturated with 50 ml of distilled water and were extracted with 100 ml of methanol using a Sonogen, Model D-50, ultrasonic cleaner (Branson Instruments Co., Stamford, Conn.). 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 minutes, 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

re-extracted 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 hours using 200 ml of methanol. The extracts were suction-filtered prior to cleanup.

Cleanup of Extracts. 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 20 ml saturated NaCl solution and 30 ml 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.) packed with freshly prepared basic alumina V to a height of 7.6 cm. 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 a Rb_2SO_4 alkali flame ionization detector (AFID) was used. The gas chromatographic operating conditions used are shown in Table 4. Pyrex columns, 0.83 m x 4 mm i.d. for atrazine, and 0.41 m

x 4 mm i.d. for cyanazine and cyprazine were packed with 7% OV-17 on 80/100 mesh Chromosorb W HP. Prior to packing, both the glass wool and the columns were acid-washed with HCl and silanized using 20% dimethyldichlorosilane in toluene. Both columns were fitted to allow on-column injections. During conditioning, columns were pesticide-loaded and treated with Silyl 8 (Pierce Chemical Company).

Table 4. Gas Chromatographic Operating Conditions[a]

Parameter	Atrazine	Cyanazine	Cyprazine
Detector temperature	230°	225°	225°
Injection port temperature	220°	200°	200°
Column temperature	200°	190°	190°
Nitrogen carrier gas	36 ml/min	40 ml/min	40 ml/min
Retention time[b]	7.2 min	6.7 min	3.4 min

[a] Hydrogen and air flow rates required frequent optimization.

[b] On the appropriate column; shorter column was used for cyanazine and cyprazine to reduce retention times.

AFID response curves for each herbicide were determined using standard solutions of 0.25-10 ng herbicide per μl methanol. Two μl of each concentration were injected two to five times. Chromatographic peaks were measured using the height x 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 pre-determined 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.

RESULTS AND DISCUSSION

AFID response curves as determined by regression analysis are presented in Table 5. AFID response to atrazine was linear over the concentration range used. Response to cyanazine and cyprazine was linear except for the two lowest concentrations which were excluded from regression

Table 5. Standard Response Curves for the s-Triazines Studied

s-Triazine	Regression line	Correlation coefficient	Standard deviation of y at any given x
Atrazine	y = 0.957 x -0.213	0.999	0.261
Cyanazine	y = 0.504 x -0.650	0.998	0.184
Cyprazine	y = 0.388 x -0.240	0.999	0.102

analysis. It was observed that although responses remained linear, exact regression lines varied from day to day, and if uncorrected could cause errors in determining extracted samples. The minimum detectable limit (2 x 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 Rb_2SO_4 AFID sensitivity reported by Tindle et al. (1968) for atrazine. Similar responses have been observed for atrazine, cyanazine, and cyprazine with other types of AFID (CsBr, Schultz, 1970; RbBr, Schroeder et al., 1972; RbCl, Swan, 1972; Greenhalgh and Wilson, 1972).

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 clean-up. 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 6. 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 water and methanol (84.4%).

Table 6. 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.