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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-methyl-
propionitrile), and cyprazine (2-chloro-4-cyclopropylamino-6-isopropyl-
amino-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 extrac-
tions 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 spectro-
photometry and mass spectrometry. Spectra obtained from 10-25 µg of
trapped eluates are presented and interpreted. Although mass spectro-
metry 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 chloro-

form and methylene chloride were the best solvents for s-triazines.

Birk and Roadhouse (1964) also used chloroform and obtained a mean re-

covery 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°, 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]			Atrazine	Benfield and Chillwell (1964)
H_2SO_4/$CHCl_3$:NaOH				
	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	Cyanazine (95%)	Beynon (1972)
		1/1 EtAc/Pet. spirit	Cyanazine (95%)	
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.

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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°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 with-

out 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 iden-

tifications based on the evidence from a single gas chromatogram. Se-

condly, 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 resi-

due 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-methyl-

propionitrile), and cyprazine (2-chloro-4-cyclopropylamino-6-isopropyl-

amino-s-triazine) were extracted with aqueous methanol using an ultra-

sonic 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 com-

parable 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 (%)			CEC[c]
					Sand	Silt	Clay	
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, ultra-
sonic cleaner (Branson Instruments Co., Stamford, Conn.). The water
level in the ultrasonic tank was adjusted to equal the methanol extrac-
tion 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% dimethyldi-
chlorosilane 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 concen-
tration were injected two to five times. Chromatographic peaks were mea-
sured using the height x width at half-height method. Results were eval-
uated 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.

Recoveries of atrazine, cyanazine, and cyprazine from the fortified soils are shown in Table 7. Two 15 minute ultrasonic extractions were as effective as a 24 hr Soxhlet extraction for atrazine on loamy sand.

Table 7. Recovery of s-Triazines from Fortified Soils

s-Triazine	Soil no.	Extraction method	Mean % recovery ± standard deviation[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 minute sonications per sample.

Using two 30 minute sonications per sample (77.8 ± 3.1% recovery) did not increase extraction efficiency over two 15 minute sonications 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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SECTION 2

CONFIRMATION OF GAS CHROMATOGRAPHIC PEAKS

OBSERVED FOR S-TRIAZINE HERBICIDES

ABSTRACT

The identity of gas chromatographic peaks observed for atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine), cyanazine (2-[(4-chloro-6-ethylamino-2-triazin-2-yl)amino]-2-methyl propionitrile), and cyprazine (2-chloro-4-cyclopropylamino-6-isopropylamino-s-triazine) 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.

INTRODUCTION

Gas chromatography (GC) is often used to identify pesticide residues in various substrates. One limitation is that positive identifications cannot be made solely on the basis of the retention times observed on a single GC column. Egan (1967), Schechter (1968), and McCully (1969) emphasized the importance of confirming GC results, and suggested that the most conclusive confirmatory techniques were infrared (IR) spectrophotometry and mass spectrometry (MS). Chen (1965), Blinn (1965), and Blinn (1971) have described the use of IR spectrophotometry to confirm pesticide identities. The applications of MS and GC-MS to pesticide

residue analysis have been reviewed by Biros (1971).

The purpose of this work was to confirm the identity of the GC peaks observed for the s-triazine herbicides, atrazine, cyanazine, and cyprazine. Although standard herbicide solutions were used, the feasibility of applying the techniques developed to confirm "unknown" extracted samples is discussed.

EXPERIMENTAL SECTION

Gas Chromatography and Trapping Procedure. The GC system and operating conditions used have been previously described (Hill and Stobbe, 1973). A 50/50 splitter was installed to facilitate the trapping of the appropriate GC column effluent. Samples for IR spectrophotometry were trapped directly into Pasteur disposable pipets. The use of this simple and efficient trapping device has been previously described (Biros, 1971). Eluates to be analyzed by MS were trapped directly into glass capillary tubes designed for solid probe sample introduction. The exit port of the splitter was maintained at the detector temperature of $225\text{-}230^{\circ}$. The collection devices, which were slipped over the exit port, had a temperature gradient along them from $225\text{-}230^{\circ}$ to ambient temperature. It was observed that the eluates of the s-triazine herbicides readily condensed in an area part way down the collection devices.

The effluent from 5 μl injections of 1 μg herbicide/μl methanol was monitored by the GC detector until 75 μg eluate for IR spectrophotometry, and 10-12 μg eluate for MS was collected.

Infrared Spectrophotometry. A Beckman model 20A grating IR spectrophotometer with a scan time of 10 minutes was used. Original herbicide compounds were run as macro discs (diameter 1.3 cm) using 0.5 mg of dry compound in 125 mg of spectrograde KBr (dried at 200°). The macro discs were prepared by pressing the ground and mixed sample in an evacuated dye for 3 minutes at 23,000 psi. When aligned in the IR beam, macro discs prepared in this manner normally gave 85% transmittance.

Micro discs (diameter 1.5 mm) containing approximately 25 μg of trapped eluate in 5 mg of KBr were prepared using the method described by Blinn (1965). The trapped eluates were rinsed out of the collection device with pesticide-grade chloroform into a hot (100°) agate mortar containing the KBr. The chloroform was allowed to evaporate before the sample was ground, mixed, and pressed in a micro dye. Using a beam condenser to increase the energy transmitted through the micro discs, only 34-40% transmittance was normally observed and thus attenuation to about 90% transmittance was required. For each herbicide, the spectrum of the original compound was compared to that of its corresponding eluted material.

Mass Spectrometry. A Finnigan model 1015 GC-MS was used with ionization energy 70-80 electron volts. Samples, contained in glass capillary tubes, were introduced directly into the ion source using a probe, heated (30-40°) independently of the source. For each herbicide, the mass spectrum of the original compound was compared to that of its corresponding eluted material.

RESULTS AND DISCUSSION

The results of IR spectrophotometry are presented in Figures 1-6.

For each herbicide, the spectrum of its trapped eluate matched that of

the corresponding original compound. The micro discs readily picked up

atmospheric moisture causing unavoidable water absorptions (3300-3600 cm^{-1})

in the spectra of the trapped eluates. The spectra obtained for atrazine

agreed with previously published spectra (Chen, 1967; Gore et al., 1971).

Due to slight differences in the structure of atrazine, cyanazine, and

cyprazine, there were characteristic IR absorption frequencies observed

for each herbicide. These frequencies are given in Table 8. Suggested

groups causing these absorptions are also included and were assigned using

information contained in Nakanishi (1962) and Dyer (1965).

For each herbicide, the mass spectrum of its trapped eluate was iden-

tical to that of the corresponding original compound, and thus only the

spectra of the trapped eluates are presented in Figures 7-9. The major

ions (>5% relative intensity) in the upper mass ranges are shown, with

ion intensities expressed as percent of the highest or base peak observed

in each spectrum. The base peaks (not shown) were at m/e 58 for atrazine,

m/e 44 for cyanazine, and m/e 41 for cyprazine.

It is desirable to check if the observed molecular ions and fragmen-

tation patterns are compatible with the structure assigned to each spec-

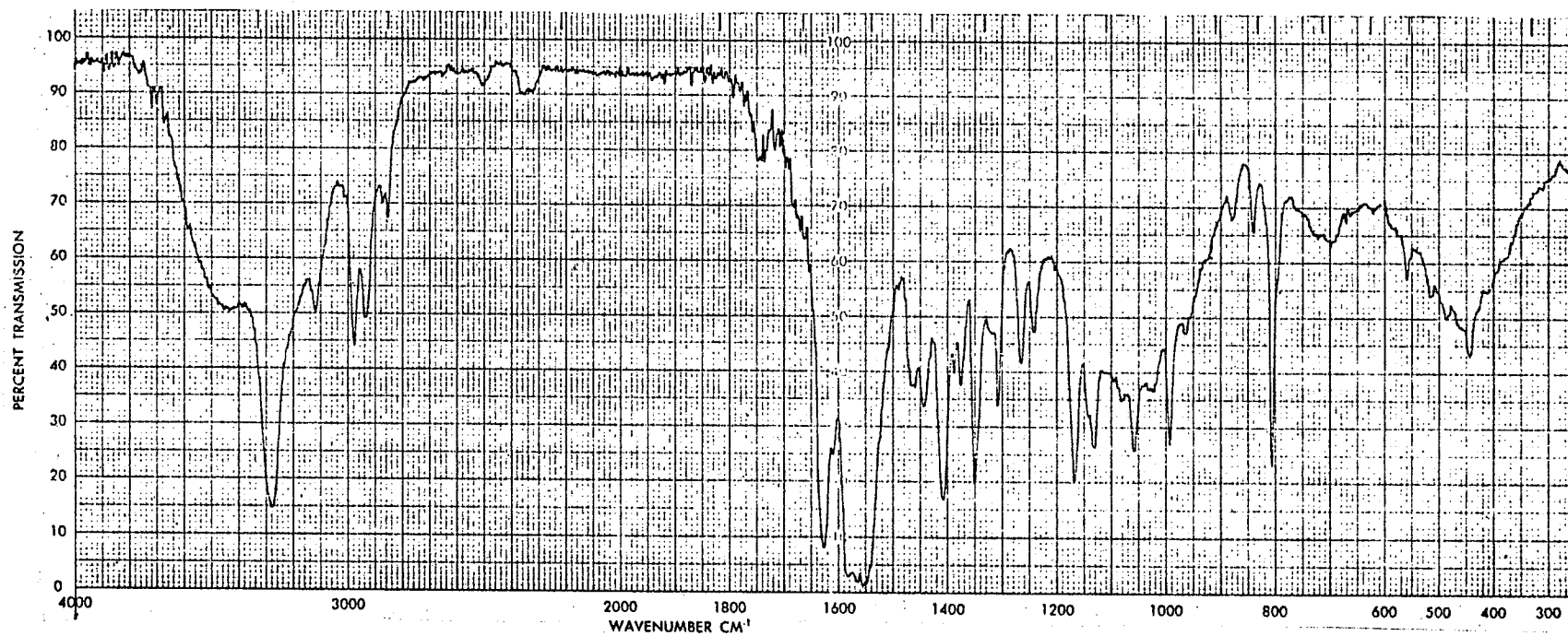trum. The expected molecular ions (M^+) are m/e 215 for atrazine, m/e 240

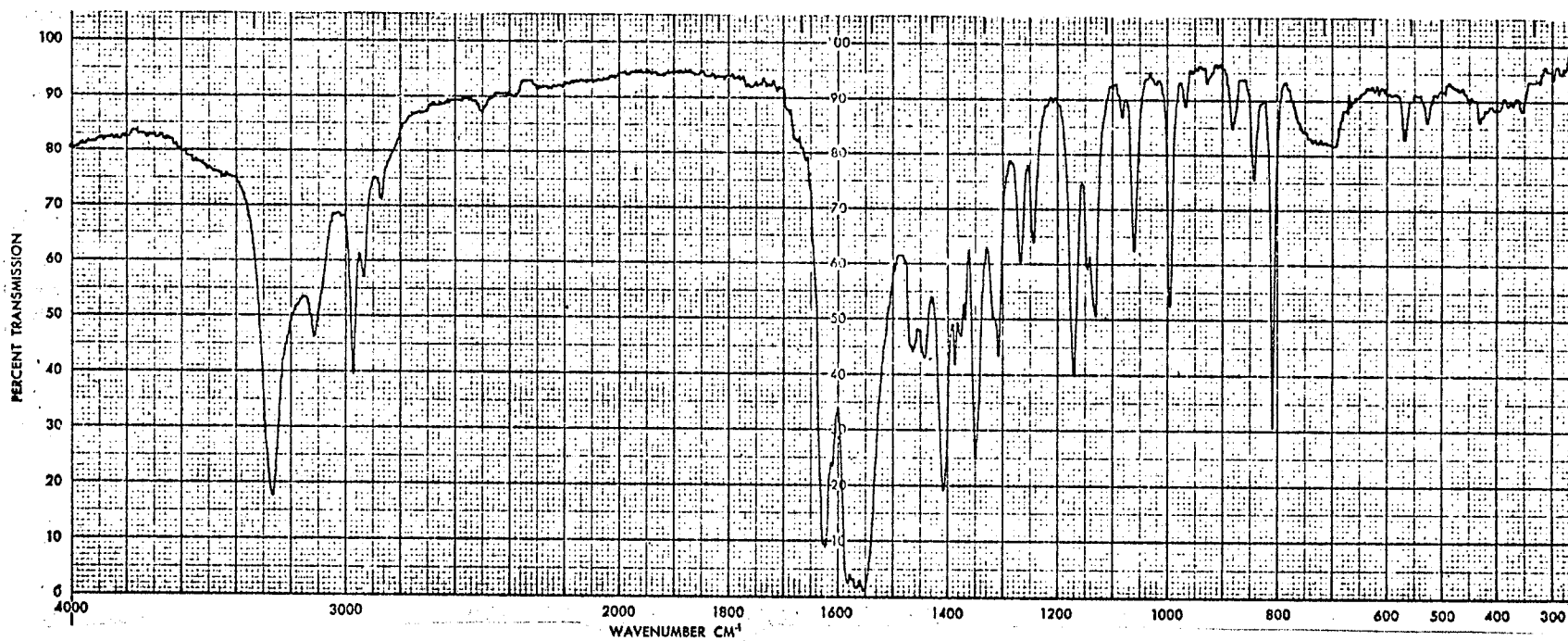Figure 1. IR spectrum of the trapped eluate of atrazine.

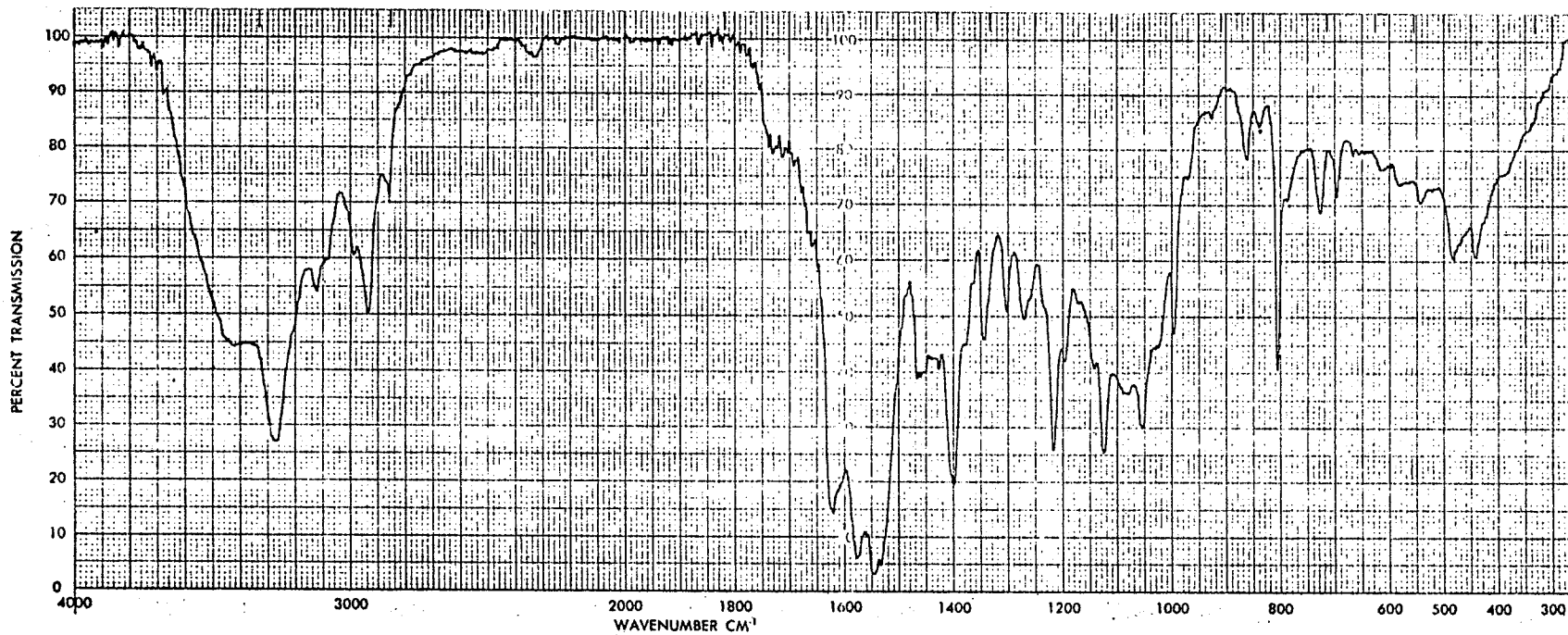Figure 2. IR spectrum of the original compound atrazine.

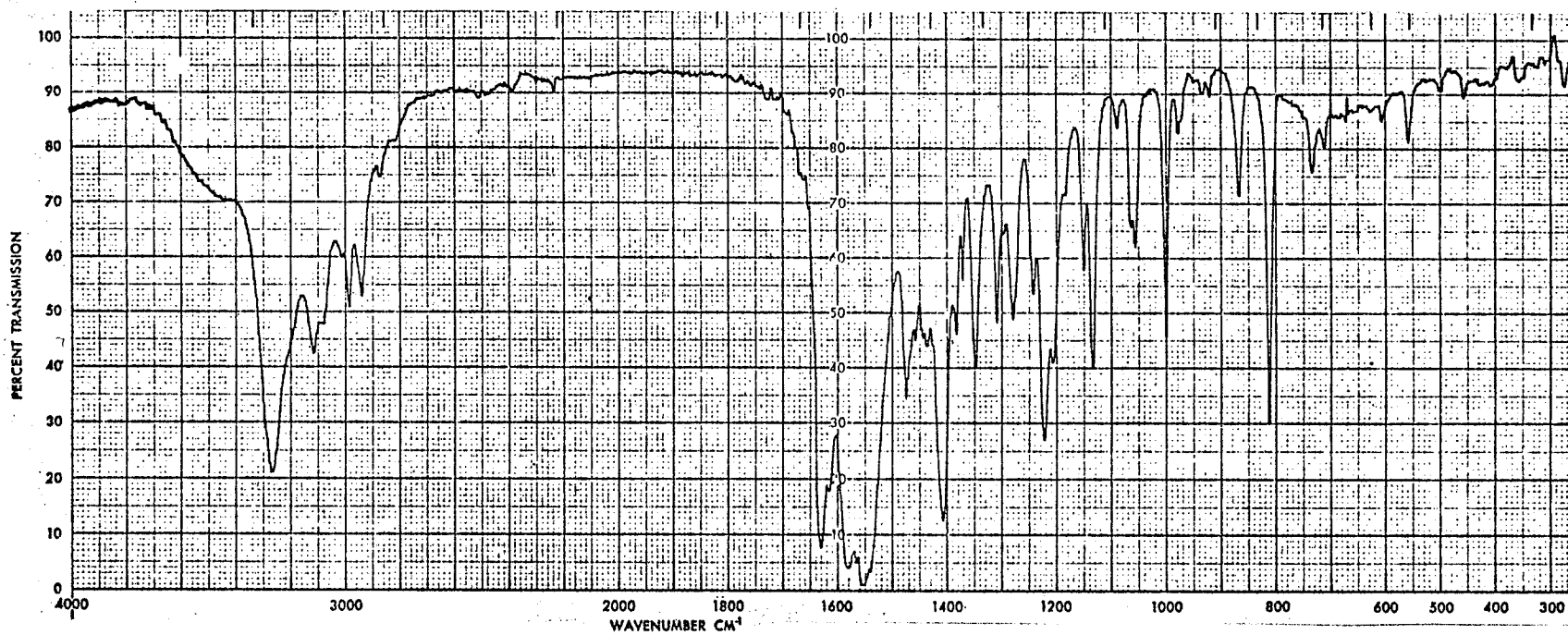Figure 3. IR spectrum of the trapped eluate of cyanazine.

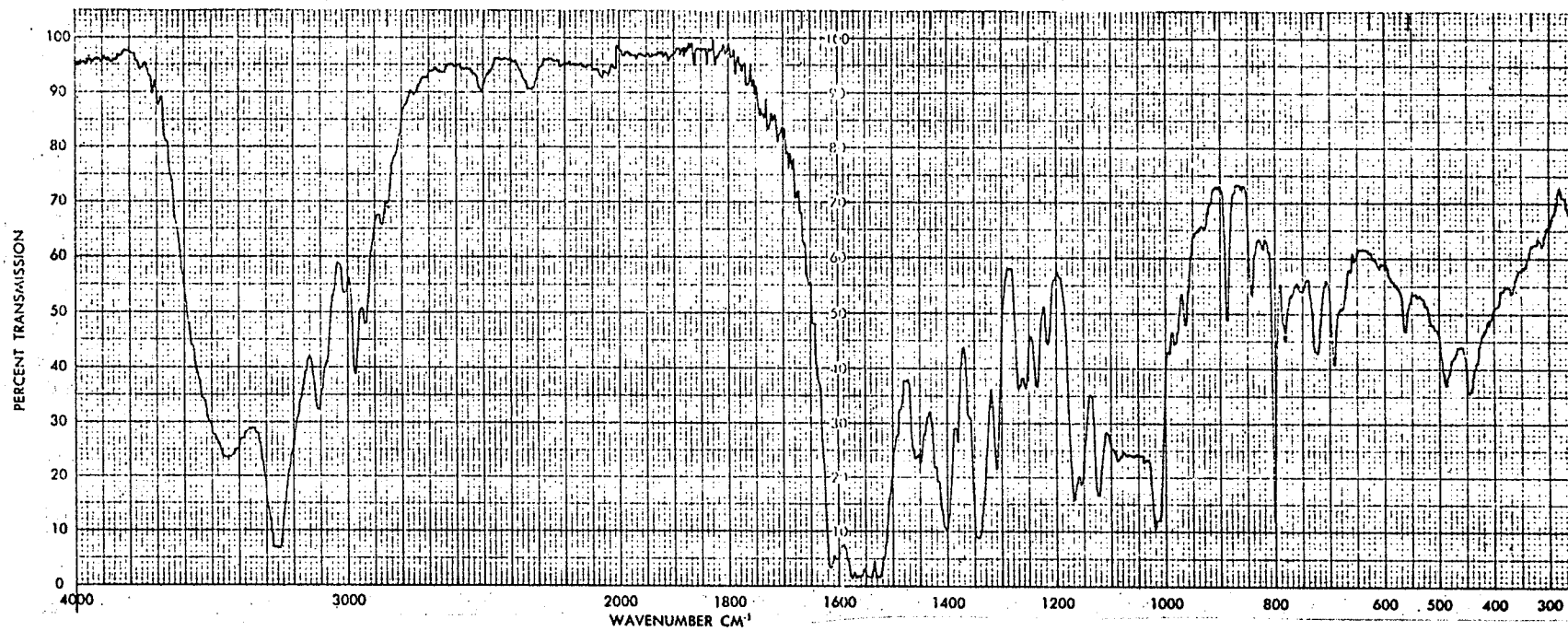Figure 4. IR spectrum of the original compound cyanazine.

Figure 5. IR spectrum of the trapped eluate of cyprazine.
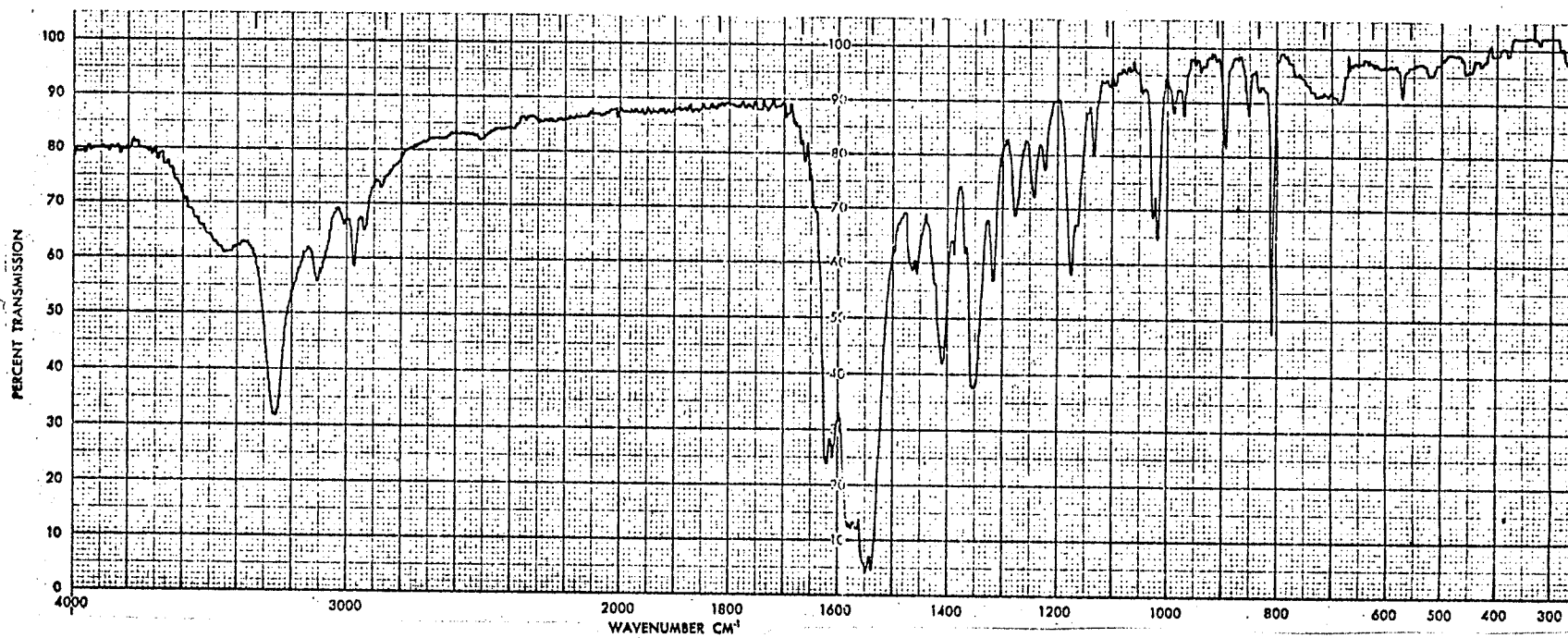
Figure 6. IR spectrum of the original compound cyprazine.

Table 8. Characteristic IR Absorption Frequencies (cm^{-1}) for Atrazine, Cyanazine, and Cyprazine[a]

Suggested group	Atrazine	Cyanazine	Cyprazine
$-C{\equiv}N$		2235 (V)	
CH_3 | $-C-$ | CH_3		1223 (M) 1207 (Sh)	
CH_3 $\quad\,$\CH$-$ CH_3	1170 (M)		1174 (M)
$-CH_2CH_3$	1060 (M) 996 (M)	1056 (M) 1000 (M)	
CH_2 |$\quad\,$\CH$-$ CH_2			1017 (M) 1027 (M)

[a] Frequencies approximated from spectra of original compounds.

V, variable; M, medium; Sh, shoulder.

Figure 7. Mass spectrum of the trapped eluate of atrazine.

Figure 8. Mass spectrum of the trapped eluate of cyanazine.

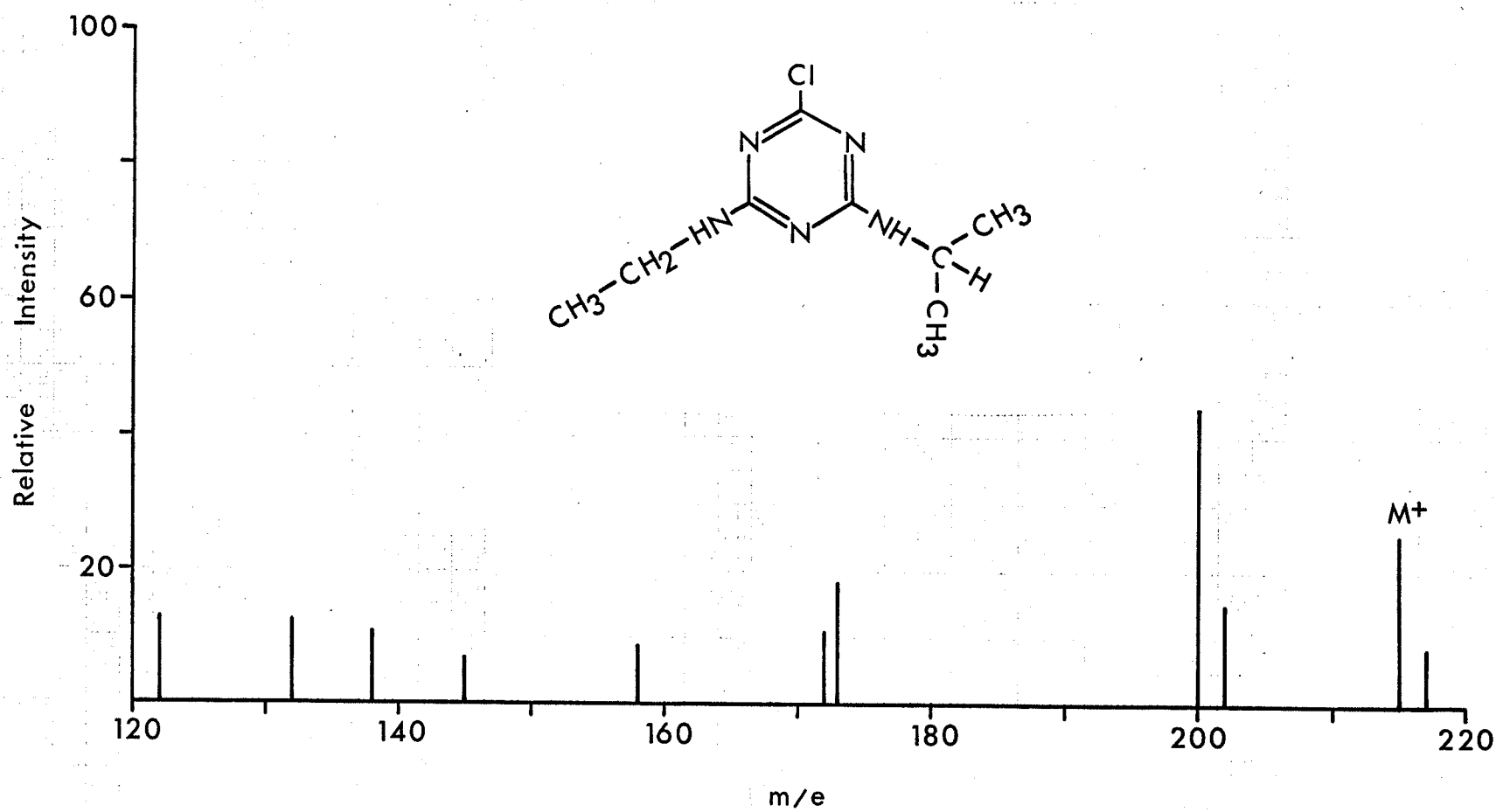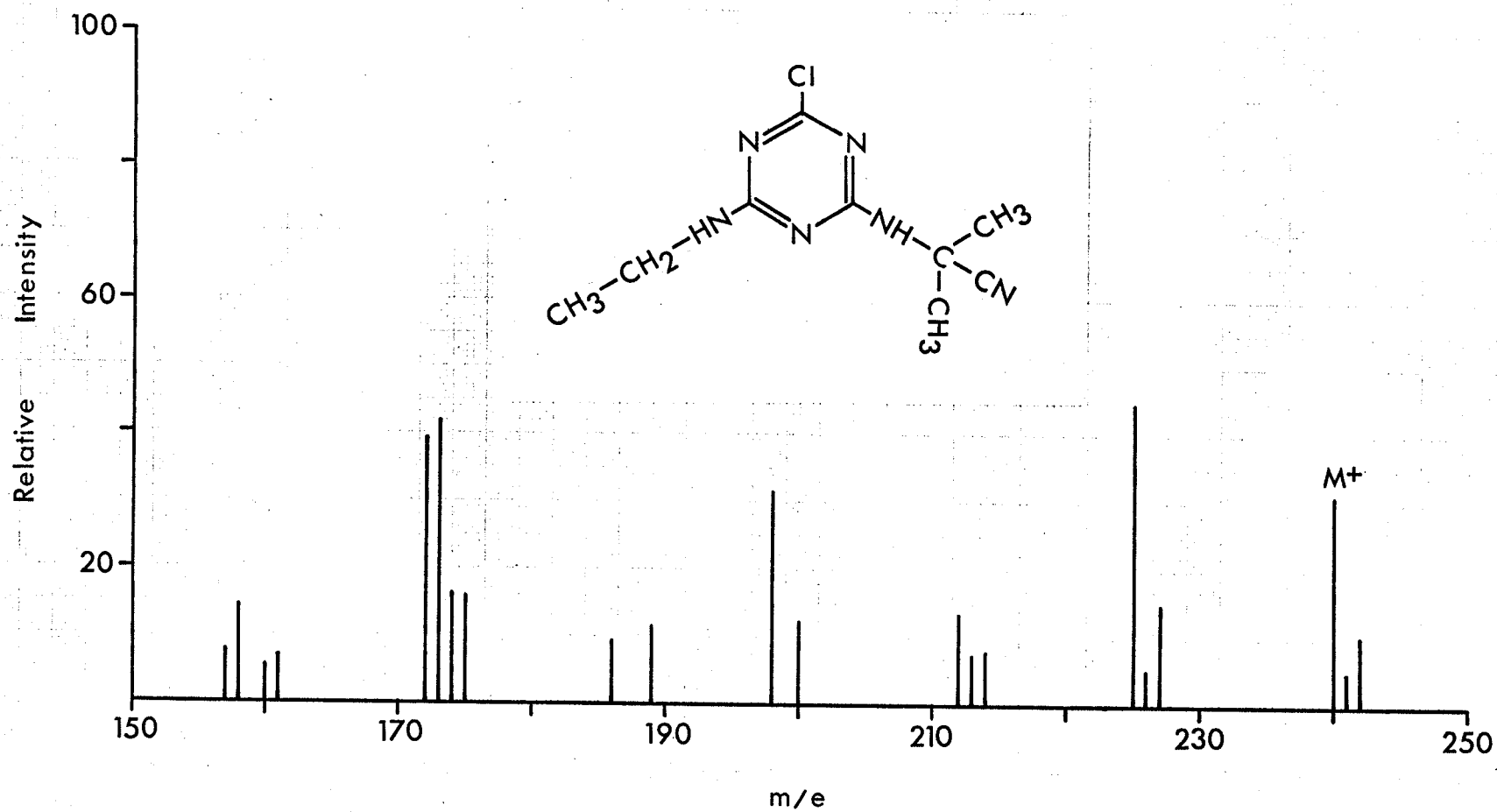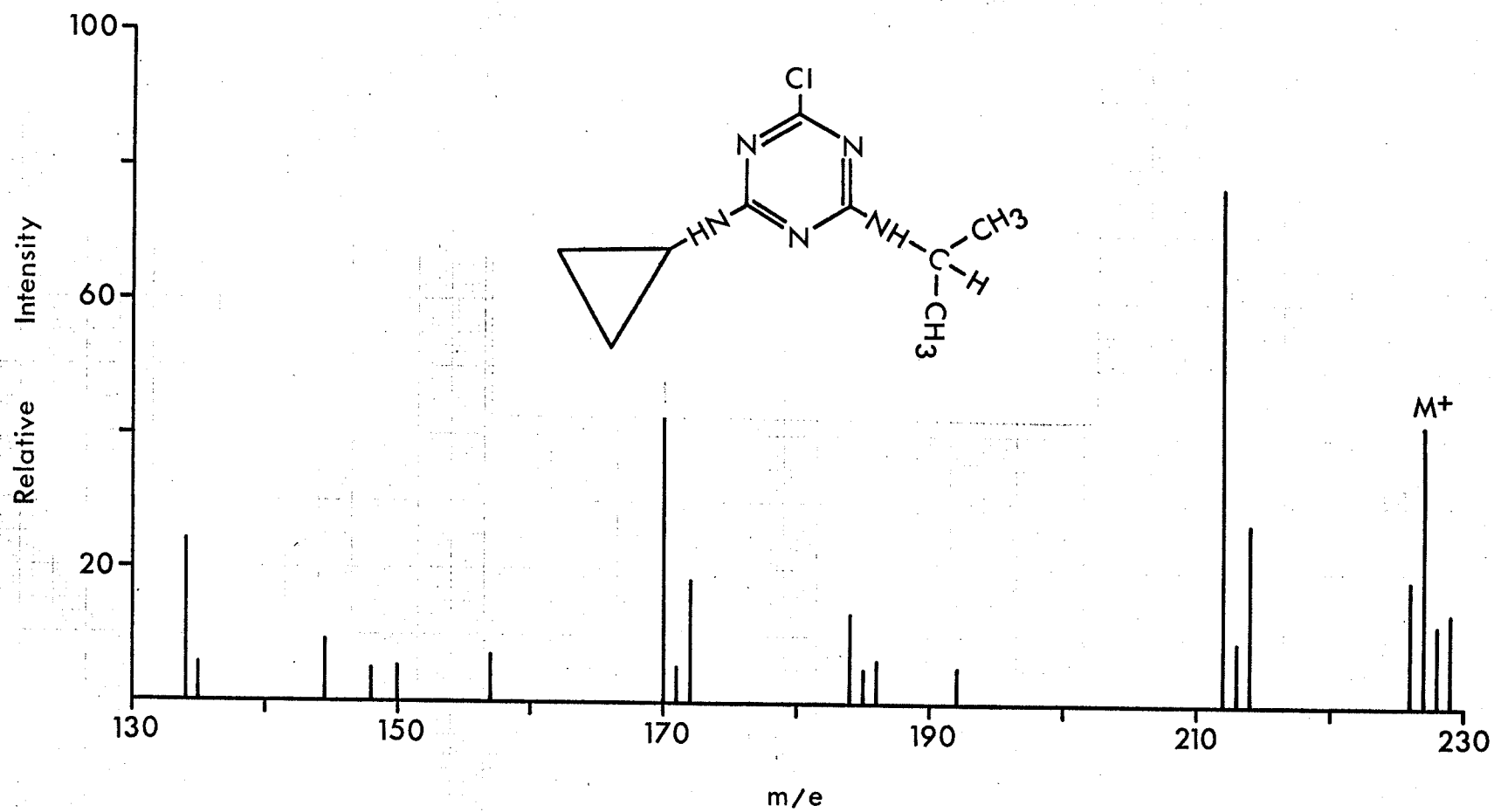Figure 9. Mass spectrum of the trapped eluate of cyprazine.

for cyanazine, and m/e 227 for cyprazine were obtained. Proposed fragmentation schemes are given in Tables 9-11, and were derived using analogies to the mass spectra reported for simazine (2-chloro-4,6-bis(ethylamino)-s-triazine) and propazine (2-chloro-4,6-bis(isopropylamino)-s-triazine) (Ross and Tweedy, 1970; Jorg et al., 1966). In general, fragment ions due to loss of alkyl groups, alkylamino side chains, chlorine, and eventual ring cleavage are observed for s-triazines.

The information obtained from IR spectrophotometry and MS shows conclusively that the trapped GC peaks for atrazine, cyanazine, and cyprazine were due only to the original herbicide moieties and were not on-column decomposition products. This work also served as a check on the authenticity of the herbicide standards.

Confirmation is an important step in pesticide residue analysis. The techniques used in this study could also be applied to "unknown" extracted samples where the presence of s-triazine residues is indicated by GC. Since most residues found in a substrate occur at levels of 1 ppm or less, one limitation would be the collection of 10-25 μg of eluate. Using 100g samples with residue levels of 1 ppm, a final extract volume of 1 ml would contain approximately 100 ng herbicide/μl. To trap 10 μg of eluate would require 40 injections. This number of injections could be reduced by: (a) using larger injection volumes, (b) using a 50/1 or 100/1 splitter, (c) combining extracts from a number of samples and reducing the combined extract volume. Of the two confirmatory techniques

Table 9. Proposed MS Fragmentation Scheme for
the Trapped Eluate of Atrazine

m/e	Ion composition
215	M^+ (Molecular ion)
200	$(M-CH_3)^+$
173	$(M-CH_3CH=CH_2)^+$
172[a]	$(M-C_2H_5N)^+$
172[b]	$(200-CH_2=CH_2)^+$
158[a]	$(M-C_3H_7N)^+$
158[b]	$(200-CH_3-CH=CH_2)^+$ or $(173-CH_3)^+$
145	$(173-CH_2=CH_2)^+$
138	$(173-Cl)^+$
132	$(158^a-CN)^+$

[a] As reported by Ross and Tweedy (1970).

[b] As reported by Jorg et al. (1966).

Table 10. Proposed MS Fragmentation Scheme for
the Trapped Eluate of Cyanazine

m/e	Ion composition
240	M^+ (Molecular ion)
225	$(M-CH_3)^+$
214	$(M-CN)^+$
213	$(M-HCN)^+$
212	$(M-CH_2=CH_2)^+$
198	$(225-HCN)^+$ or $(213-CH_3)^+$
186	$(214-CH_2=CH_2)^+$ or $(212-CN)^+$
173	$(M-C_4H_5N)^+$ or $(213-CH_2=C=CH_2)^+$
158[a]	$(M-C_4H_6N_2)^+$ or $(213-C_3H_5N)^+$
158[b]	$(225-C_4H_6N)^+$ or $(198-CH_2=C=CH_2)^+$ or $(173-CH_3)^+$

[a] As reported by Ross and Tweedy (1970).

[b] As reported by Jorg et al. (1966).

Table 11. Proposed MS Fragmentation Scheme for
the Trapped Eluate of Cyprazine

m/e	Ion composition
227	M^+ (Molecular ion)
226	$(M-H)^+$
212	$(M-CH_3)^+$
192	$(M-Cl)^+$
186	$(226-CH_2=C=CH_2)^+$
185	$(M-CH_3-CH=CH_2)^+$
184	$(226-CH_3-CH=CH_2)^+$
172[a]	$(M-C_3H_5N)^+$
172[b]	$(212-CH_2=C=CH_2)^+$
170[a]	$(M-C_3H_7N)^+$
150	$(192-CH_3-CH=CH_2)^+$ or $(195-Cl)^+$
144	$(170^a-CN)^+$

[a] As reported by Ross and Tweedy (1970).

[b] As reported by Jorg et al. (1966).

used, MS was preferred. The nature of IR spectrophotometry makes it difficult to distinguish between the nearly identical s-triazines studied, whereas MS allows unequivocal distinction and identification to be made with relative ease.

ACKNOWLEDGMENT

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GENERAL DISCUSSION

Gas Chromatography

GC columns made from materials other than glass tubing were unsatis-
factory. Excessive peak tailing was observed on stainless steel columns.
Teflon and teflon-lined aluminum columns gave poor resolution and a rela-
tively low number of theoretical plates. It is believed that static
charges arising from the teflon prevented high packing densities. One-
piece glass columns were designed to fit the GC, as it was difficult to
eliminate gas leaks at the joint of two-piece columns. GC conditions
were optimized to obtain the maximum number of column theoretical plates
for best resolution and maximum sensitivity. The 0.83 m column had 1000
theoretical plates with respect to atrazine, while the 0.41 m column had
686 theoretical plates with respect to cyanazine. The s-triazines
studied have 5-6 nitrogen atoms per molecule. The nitrogen-specific
Rb_2SO_4 AFID appears to be ideal in terms of selectivity, sensitivity,
and linear response for these compounds. The disadvantage of this detec-
tor was that it required frequent re-optimization of hydrogen and air
flow rates as noted by Tindle et al. (1968). The Rb_2SO_4 salt-tip also
fractured and had to be replaced every three to six months.

Typical chromatograms of soil extracts containing atrazine, cyanazine,
and cyprazine are shown in Figures 10-12. The observed peaks represent
5-7 ng of s-triazine injected. All soil extracts were chromatographed
at least twice. The criterion for acceptable reproducibility was a 5%
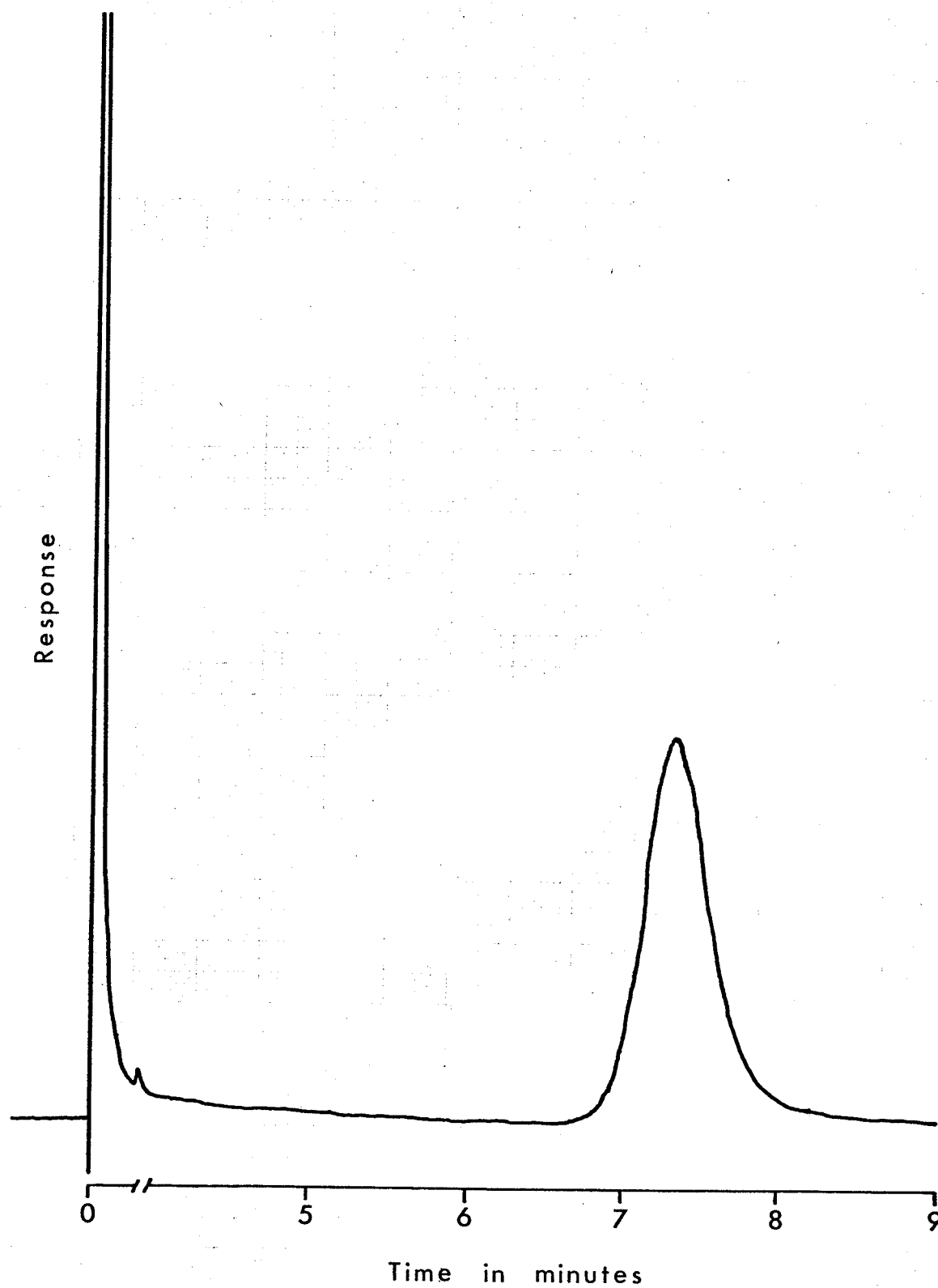
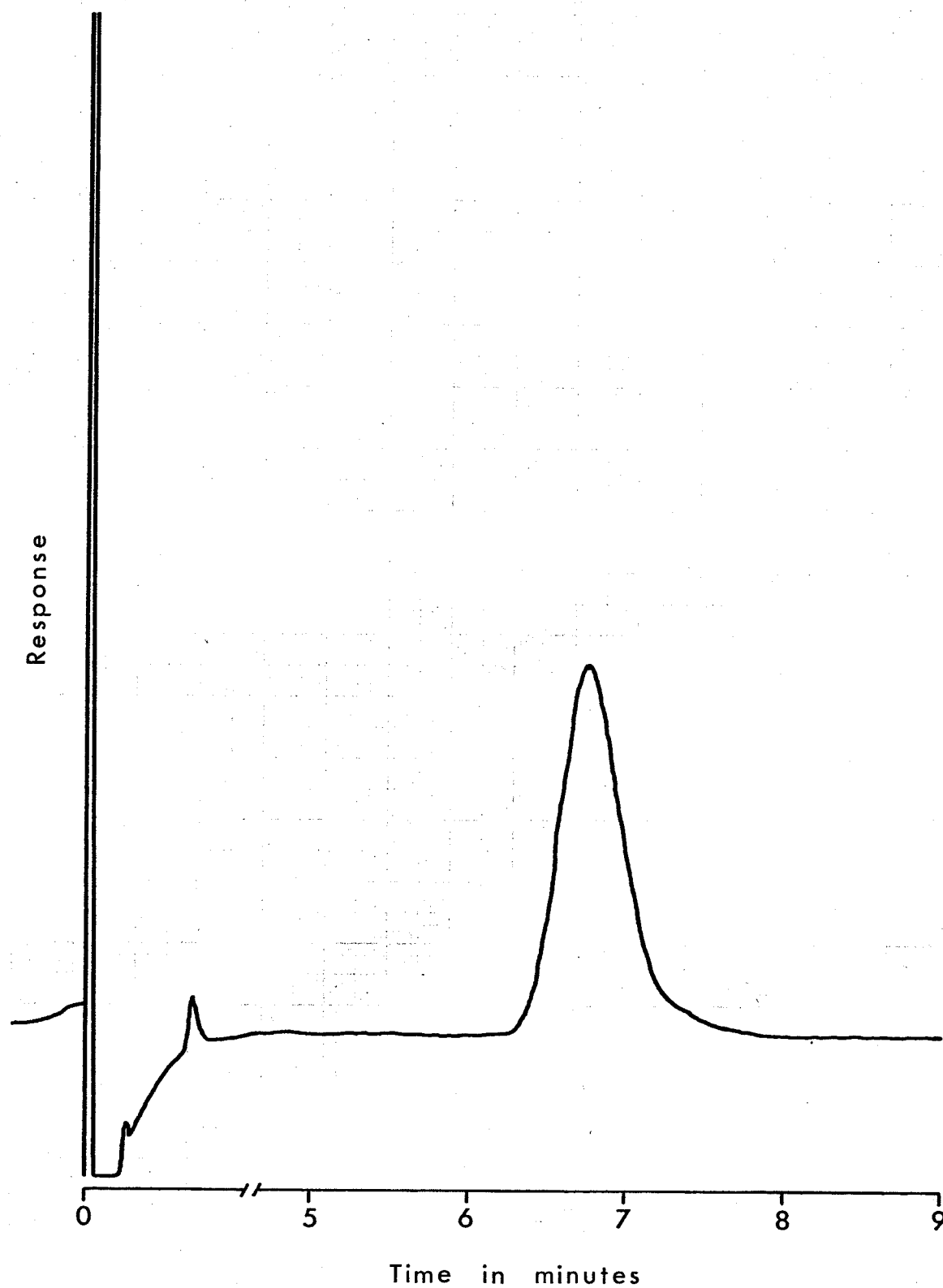Figure 10. Chromatogram of soil extract containing atrazine.

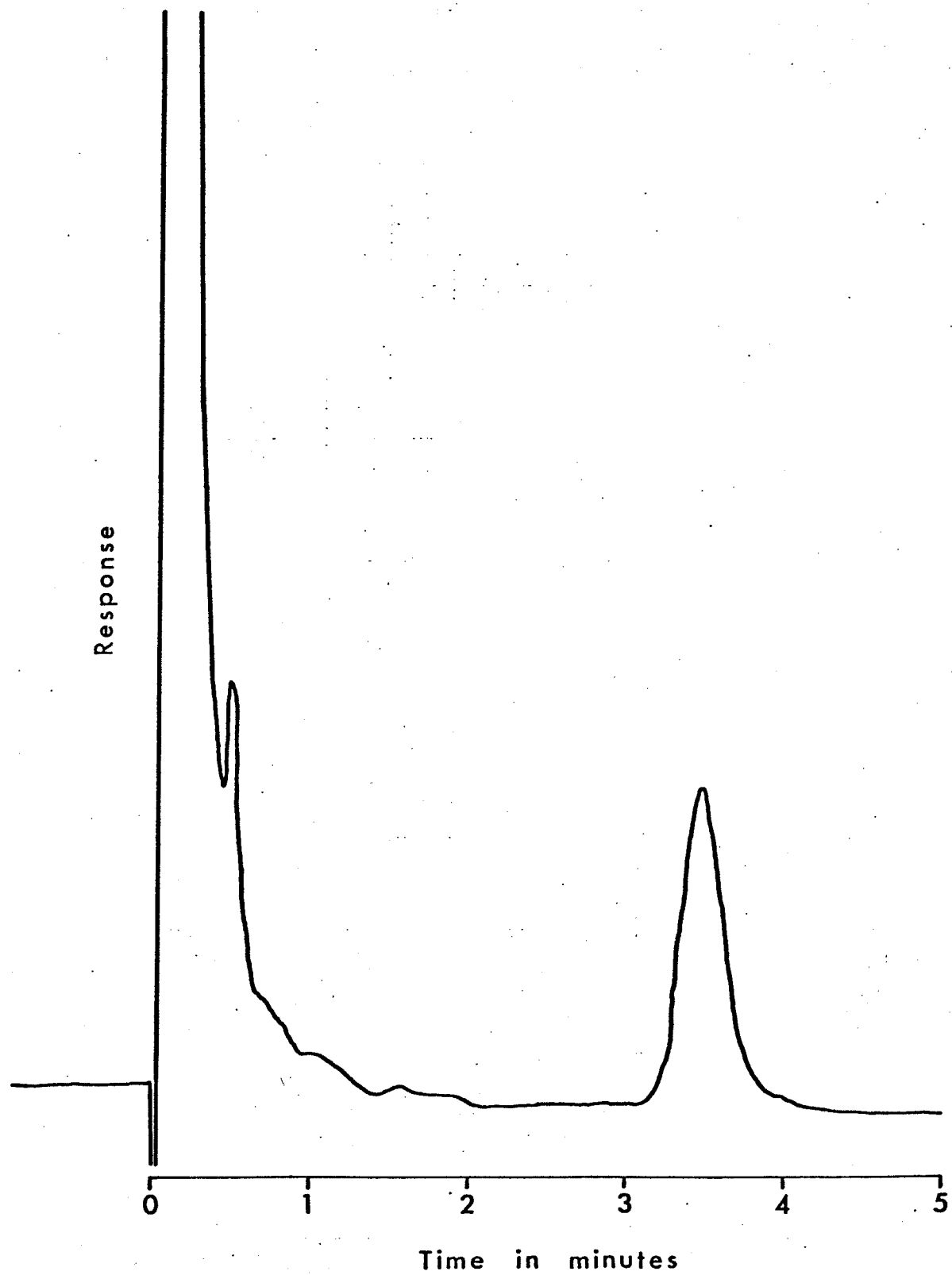Figure 11. Chromatogram of soil extract containing cyanazine.

Figure 12. Chromatogram of soil extract containing cyprazine.

difference in peak heights.

Fortification Procedure

Recoveries of s-triazines from soils are greatly dependent on the fortification procedure used. It is desirable to use a procedure that leaves the fortified residues in a state similar to that of weathered residues in field-treated soils. Fortification conditions should be such that extraction is made as difficult as possible. The following considerations were made when fortifying soils in this study:

1. Soils were not sterilized for fear of altering the soil's nature.

2. Soils were fortified individually in the bottles used for extraction to prevent sub-sampling errors and transfer losses.

3. Soils were meshed to reduce their particle sizes, thereby facilitating faster s-triazine adsorption.

4. Soils were wetted during fortification to expose more sites for s-triazine adsorption in the expanded lattice structure of the clay micelles.

5. The fortified soils were air-dried before extraction, thereby causing contraction of the lattice structure of the clay micelles, and effectively "locking-in" the adsorbed s-triazine residues.

6. The fortified soils were equilibrated for 3 days since s-triazine degradation could occur during longer periods.

Some of these fortification considerations have been previously

reported by Saha et al. (1969a, 1969b).

Evaluation of the Ultrasonic Extraction Method

The ultrasonic extraction method used in this study is compared to other methods of extracting fortified s-triazines from soils in Table 12. In general, the recoveries obtained by ultrasonic extraction are lower than other reported recoveries. A major factor to be considered is the equilibration time allowed between fortification and extraction. Recoveries reported by Tindle et al. (1968), Mattson et al. (1970), and Beynon (1972) show that minimal losses of s-triazine occurred during their procedures, but are not a good indication of the extractability of bound residues. The ultrasonic method extracted only slightly less bound atrazine than did the 2 hr Soxhlet method of McGlamery et al. (1967). The advantage of the ultrasonic method is that shorter extraction times can be used.

Confirmation

The identity of the GC peaks observed for the s-triazine standards was confirmed because two peaks were obtained when cyanazine was injected. The presence of a small "impurity" peak at a retention time of 2.7 minutes, compared to the confirmed cyanazine peak at 6.7 minutes, indicated that either the cyanazine standard contained an impurity or that cyanazine was decomposing during GC.

Purkayastha and Cochrane (1973) reported that on-column decomposition of cyanazine occurred when glass columns containing Reoplex 400 and

Table 12. Comparison of Soil Extraction Methods for Fortified s-Triazines

Extraction method	Solvent used	Fortification level(s)	Soil type	Equilibration time[a]	% s-Triazine recovered	Literature reference
2 hr Soxhlet	MeOH	1 ppm	clay loam	2 days	86.0 (atrazine)	McGlamery et al. (1967)
16 hr Goldfisch	$CHCl_3$	1, 10, 100 ppm	silty clay loam	none reported	93.2 ± 2.6 (atrazine)[b]	Tindle et al. (1968)
2 hr reflux	H_2O/CH_3CN	0.05-2.0 ppm	not reported	none	80 - 114 (atrazine)[c]	Mattson et al. (1970)
2 hr tumbling	$H_2O/MeOH$	0.05-2.0 ppm	various	none	88 - 96 (cyanazine)[c]	Beynon (1972)
2 x 15 min ultrasonic	$H_2O/MeOH$	1 ppm	silty clay loam	3 days	83.6 ± 0.5 (atrazine)	Hill and Stobbe (1973)
			loamy sand	3 days	81.6 ± 3.1 (atrazine) 94.5 ± 1.4 (cyanazine)	

[a] Equilibration time allowed between fortification and extraction.

[b] Overall mean % recovery from all fortification levels.

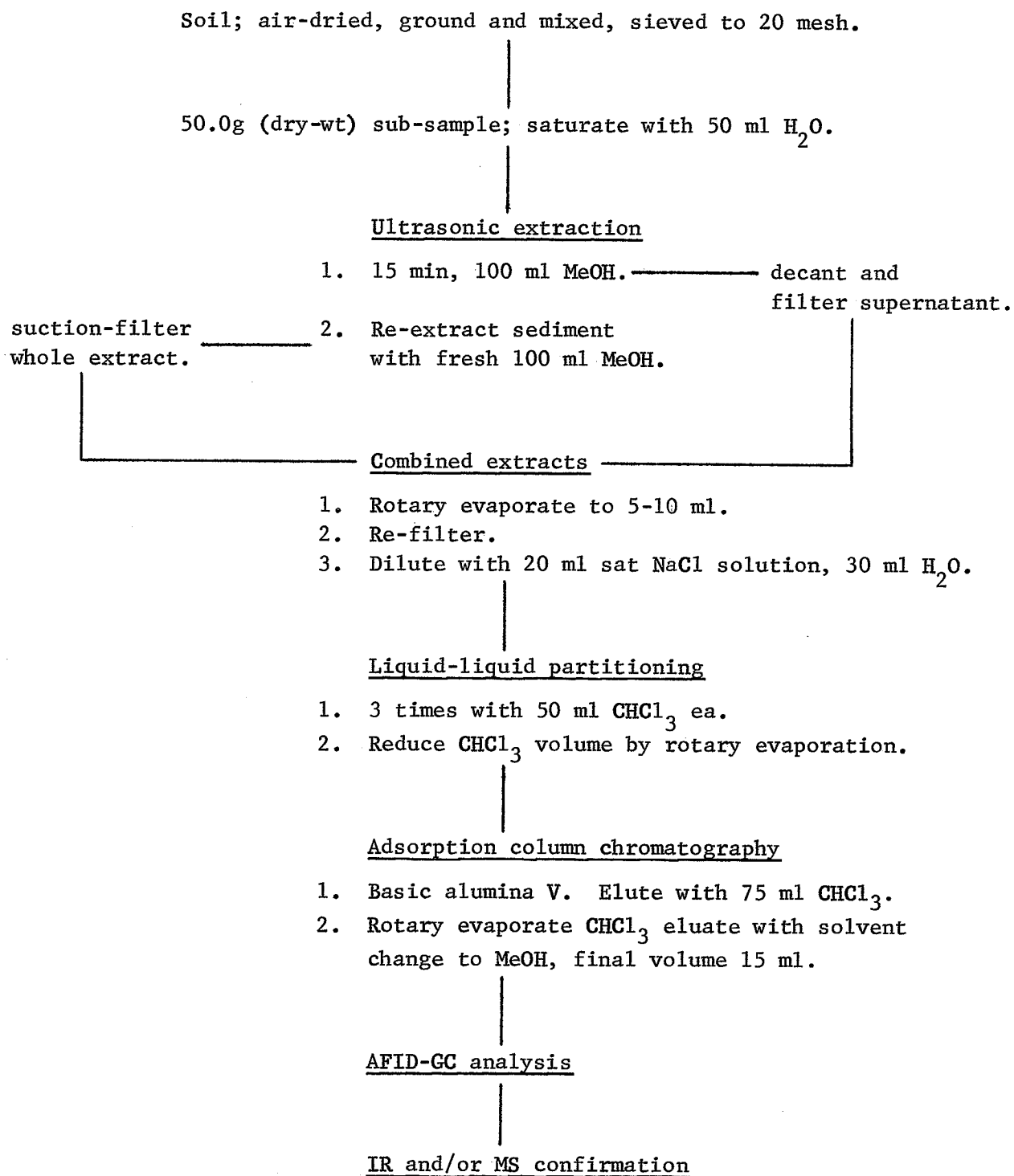[c] Range of recoveries from all fortification levels.

Carbowax 20M liquid phases were used. The retention time of the decom-
position product was much longer than that of cyanazine. Decomposition
of cyanazine was not observed using OV-17 liquid phase. Shell Develop-
ment Co. (1969) used stainless steel columns containing Reoplex 400
liquid phase and reported thermal degradation of cyanazine when column
temperatures exceeded 190°.

In the present study, the size of the "impurity" peak varied some-
what in relation to the cyanazine peak. The "impurity" peak remained
evident when injector and column temperatures were lowered below 190°,
and when freshly prepared cyanazine standard solutions were used. An
impurity was not evident in the mass spectrum of the cyanazine standard.
It is possible that the cyanazine standard contained an impurity in
trace amounts which could be detected by GC but not by MS. The "impurity"
peak was not trapped and analyzed by MS.

From the results obtained, the origin of the "impurity" peak could
not be satisfactorily explained as an impurity in the cyanazine standard
or as a GC decomposition product. It is also possible that cyanazine
was decomposed in the hot stainless steel syringe tip during injection.
Since the "impurity peak" was relatively small and the response to the
confirmed cyanazine peak was linear, extraction recovery results were
not significantly affected.

METHOD SUMMARY

A summary of the method developed in this study for determining s-triazine herbicides in soils is presented in Figure 13. Since the ultimate goal is to apply this method to field samples, the procedures used are outlined as if an "unknown" soil sample was presented for s-triazine residue determination. By reducing the final extract volume from 15 ml to 1 ml, it is estimated that a least determinable concentration of 0.02 ppm s-triazine in the soil could be achieved.

Figure 13. Determination of s-Triazine Herbicides in Soils

Soil; air-dried, ground and mixed, sieved to 20 mesh.

50.0g (dry-wt) sub-sample; saturate with 50 ml H_2O.

Ultrasonic extraction

1. 15 min, 100 ml MeOH. ──────── decant and
                                   filter supernatant.

suction-filter   2. Re-extract sediment
whole extract.   with fresh 100 ml MeOH.

Combined extracts

1. Rotary evaporate to 5-10 ml.
2. Re-filter.
3. Dilute with 20 ml sat NaCl solution, 30 ml H_2O.

Liquid-liquid partitioning

1. 3 times with 50 ml $CHCl_3$ ea.
2. Reduce $CHCl_3$ volume by rotary evaporation.

Adsorption column chromatography

1. Basic alumina V. Elute with 75 ml $CHCl_3$.
2. Rotary evaporate $CHCl_3$ eluate with solvent
    change to MeOH, final volume 15 ml.

AFID-GC analysis

IR and/or MS confirmation

SUGGESTIONS FOR FURTHER WORK

The extraction conditions used could likely be further optimized by studying such factors as: solvent choice, solvent:soil ratios, water: methanol ratios, pH, extraction times, and number of extractions. The use of an overhead stirrer in combination with ultrasonic energy may improve extraction efficiencies.

The p-value approach of Beroza et al. (1969), Suffet and Faust (1972), and Suffet (1973) would aid in the selection of the best liquid-liquid partitioning parameters. Cleanup might be unnecessary if a replaceable pre-column humus trap could be designed for GC. A procedure for re-conditioning used Rb_2SO_4 salt-tips would be valuable.

The method developed for determining s-triazine herbicides in soils should be further evaluated using other soil types, other fortification levels, and field-treated soils. The small field plot method of Smith (1972) could be used to obtain known amounts of weathered s-triazine residues in soil samples.

Trapping and identifying the GC "impurity" peak observed for the cyanazine standard may help to resolve the question of cyanazine on-column decomposition. The possibility of cyanazine decomposing within the hot syringe needle during injection could also be investigated.

The confirmatory techniques developed should be applied to "unknown" extracted samples.

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