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Title: <u>HYDROLYSIS AND BIOLOGICAL DEGRADATION</u>

<u>OF ATRAZINE IN SOILS</u>

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Detoxification of atrazine in soils results from both chemical

hydrolysis and microbial degradation. Infrared analysis was used

to study the hydrolysis of atrazine upon interaction with soil colloids

and to ascertain the existence of enol, keto, and protonated-keto

forms of hydroxyatrazine. Evolution of $^{14}CO_2$ from ^{14}C-atrazine

and ^{14}C-hydroxyatrazine was indicative of microbial degradation in

soils.

Objectives of this investigation were: 1) to determine the tran-

sitional forms of hydroxyatrazine in different pH environments; 2) to

establish the interactions of atrazine on H^+, Al^{3+}, Ca^{2+}, or Cu^{2+}-

saturated surfaces of "allophane, " montmorillonite, or "natural

montmorillonitic clay"; and 3) to ascertain the contribution of microb-

ial degradation and chemical hydrolysis to atrazine detoxification in

three Oregon soils.

Infrared spectra provide evidence for the existence of enol,

keto, and protonated-keto forms of hydroxy-<u>s</u>-triazines. The

following transition is correlated with changes in pH: 1) an anionic species at pH > 11.5, 2) an enol form between pH 11.5 and 3.3, 3) a keto form at pH < 3.3, and 4) a protonated-keto species at pH < 0. Asymmetrical side chains (ethyl and isopropyl) of hydroxyatrazine apparently induced a doublet at 3400 and 3520 cm^{-1} (protonated-ring ν NH), whereas the symmetrical side chains (ethyl) of hydroxysimazine yielded a single band at 3330 cm^{-1} in the protonated-keto forms. Hydroxypropazine was not protonated in these experiments.

Acidic cations (H^+ and Al^{3+}) on the exchange complex of montmorillonite and Coker soil clay promoted the hydrolysis of atrazine as evidenced by a strong hydroxyatrazine carbonyl band at 1745 cm^{-1} in infrared spectra. Reaction of atrazine with Ca- or Cu-montmorillonite did not produce a 1745 cm^{-1} band, whereas a small degree of hydrolysis of atrazine was indicated in Cu-Coker clay by a weak band at 1745 cm^{-1}. Dehydration increased the hydrolysis of atrazine as evidenced by a more intense band at 1745 cm^{-1} in the reaction product of Ca- or Cu-Coker soil clay plus atrazine, whereas the infrared spectra of Ca- or Cu-montmorillonite plus atrazine were not affected by dehydration. An "allophanic" colloid did not catalyze the hydrolysis of atrazine when the exchange complex was saturated with H^+, Al^{3+}, Ca^{2+}, or Cu^{2+}. "Al-allophane" was not sufficiently acidic to protonate added hydroxyatrazine as a carbonyl band was not observed in the reaction product. Thus under acidic field conditions, one

might expect the smectites to enhance the chemical hydrolysis of atrazine while "allophanic" colloids and perhaps other amorphous materials would be relatively inert.

Respired $^{14}CO_2$ from the ^{14}C-ethyl side-chain component of atrazine represented approximately 10% of the input ^{14}C-activity in Parkdale-A and Woodburn soils and 4.5% in Parkdale-C and Coker soils after 28 days of incubation. The isopropyl side-chain and the ring constituent of atrazine were subject to minimal attack by soil microorganisms. The hydroxyatrazine ring was attacked more readily than the atrazine ring. Hydroxyatrazine accounted for approximately 10% of the extracted ^{14}C-activity from ^{14}C-atrazine-treated Parkdale-A, Parkdale-C, and Coker soils, and 40% from the Woodburn soil. Hydrolysis is considered the dominant pathway of detoxification in the Woodburn soil, whereas detoxification of atrazine in Parkdale-A, Parkdale-C, and Coker soils is a combination of microbial and chemical activity.

Hydrolysis and Biological Degradation
of Atrazine in Soils

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DEDICATED TO

SHAW

Since words cannot adequately express my appreci-
ation for her moral and physical support, her patience
and understanding, and her devotion as a wife and mother
throughout my graduate program, I dedicate this thesis to
Shaw as a token of thanks for everything.

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INTRODUCTION

Selective herbicides to remove undesirable plant species in crop production have contributed to increased crop yields. These same herbicides, however, may contribute residues which can cause serious damage to sensitive crops grown the season(s) following application of the herbicide. Climatic factors, such as temperature, light, and moisture, affect the detoxification of herbicides. Organic matter content, cation exchange capacity, pH, clay minerals, moisture content, cation saturation, and microorganisms are important soil properties which may govern the residual life of herbicides. The plant species and plant density represent vegetative characteristics which may modify herbicidal persistence. Thus, the residual life of herbicides is a function of numerous climatic, vegetative, and soil factors and their interactions are complex.

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-$\underline{s}$-triazine) is used for pre- and post-emergence control of grasses and small broadleaf weeds in crop production. At field rates of two to four pounds per acre, atrazine may persist in soils for more than one growing season.

Previous investigations indicated that temperature, moisture, and organic matter content optimal for microbial activities were

correlated with the detoxification of atrazine. Since hydroxyatrazine (2-hydroxy-4-ethylamino-6-isopropylamino-$\underline{s}$-triazine) which is non-phytotoxic has been extracted from atrazine-treated soils, chemical hydrolysis has more recently received strong endorsement as a major pathway in the detoxification of atrazine in soils. More direct evidence of hydrolysis was demonstrated in the infrared spectra of the reaction products of atrazine and montmorillonite by a carbonyl band which indicates the presence of hydroxyatrazine. Additional infrared spectra suggested the hydrolytic degradation product was adsorbed by montmorillonite as the positively-charged protonated-keto form rather than the enol form.

To elucidate the factors which affect the chemical and/or microbial detoxification of atrazine, the following objectives were undertaken: 1) to determine the relationship between pH and the enol, keto, and protonated-keto forms of hydroxyatrazine; 2) to establish the interactions of atrazine on "allophane," montmorillonite, and "natural montmorillonitic clay" surfaces as affected by dehydration and H^+, Al^{3+}, Ca^{2+}, or Cu^{2+}-saturation; and 3) to ascertain the contribution of microbial degradation and/or chemical hydrolysis to the residual life of atrazine in three Oregon soils.

HYDROLYTIC PRODUCTS OF
CL-S-TRIAZINE HERBICIDES

Infrared Spectra Provide Evidence for the Existence of
Enol, Keto, and Protonated-Keto Forms of Hydroxy-s-
Triazines.

Atrazine, simazine, and propazine (alkylamino-s-triazines)

are herbicides which may persist in phytotoxic concentrations in soils

from six to 12 months. Microbial degradation involves slow dealkyla-

tion of the side-chain constituents, whereas chemical hydrolysis in

soils yields the hydroxy analogs which are nonphytotoxic.

Literature Review

Acid hydrolysis of cyanuric chloride to yield cyanuric acid

(2, 4, 6-trihydroxy-s-triazine) involves protonation of the triazine

ring and subsequent hydrolysis, by water, of the protonated mole-

cule (15). The carbonyl form of cyanuric acid has been reported to

be the most stable form in both neutral aqueous solution and in the

solid state (18). Hirt and Schmitt (14) used ultraviolet spectroscopy

to describe a sequence of forms for cyanuric acid with increased

acidity as follows: 1) a doubly-ionized species at pH 12-14, 2) a

singly-ionized species at pH 8-9, and 3) a keto species at pH 1-4.5.

The sequence given for ammeline (2, hydroxy-4, 6-diamino-s-triazine)

was: 1) a singly-ionized species at pH 11-13, 2) a neutral molecule

at pH 6-8, and 3) a mono-protonated molecule at pH 1-3. Boitsov

and Finkel'shtein (4) reported similar transitional schemes for cyanur-

ic acid and ammeline with the exception that a diprotonated molecule

of ammeline occurred below pH 1.

The increased solubility of hydroxypropazine [2-OH-4, 6-bis(iso-

propylamino)s-triazine] with increasing acidity has been explained by

tautomerization and protonation (32). Chen (6) suggested an enol:keto

tautomerism for the hydroxy analogs of three Cl-s-triazine herbicides.

Basing his interpretation on infrared spectra, he indicated the hydroxy

derivatives may exist as dimers in the solid phase (KBr) by H-bonding

of the enol to the keto tautomers in equilibrium.

Chemical hydrolysis of Cl-s-triazines on montmorillonite clay

occurs as a result of protonation at the colloidal surface (7, 27). Both

groups suggested that the pH at the clay surface would be sufficiently

low to shift hydroxy-s-triazines from the enol form to a protonated-

keto form based on the appearance of a strong carbonyl band (in the

hydrolytic degradation product) at 1740 cm^{-1} which was not present

in the infrared spectra of technical hydroxy-s-triazines. A similar

band was reported by Russell et al. (27) for hydroxyatrazine pre-

pared by treatment with 6N HCl (protonated-keto form). Nuclear

magnetic resonance studies indicated that protonation of the hydroxy

analog occurred on the heterocyclic ring nitrogen (27).

A reduction in the intensity of the carbonyl band of humic acids

after reaction with atrazine suggested that atrazine was adsorbed through the triazine amino groups to the carbonyl groups of humic acids (30). However, Good (11) did not detect H-bonding of imino hydrogens in propazine to the carbonyl group in acetone. Skipper et al.[1] reported the absence of a 1745 cm^{-1} frequency after reaction of atrazine with Ca- or Cu- montmorillonite, whereas the presence of a 1745 cm^{-1} band after reaction with H- or Al-montmorillonite is indicative of the protonated-keto form of the hydrolytic product. Thus, interpretation of the triazine-colloid interactions has relied largely on the presence or absence of the carbonyl group of the hydrolytic products.

Infrared data presented suggest that the carbonyl band of hydroxyatrazine appears between 1775 and 1750 cm^{-1} either as a single frequency or as a doublet dependent upon the environment. Infrared band assignments are also made for other constituents of the triazine molecule (NH, CN, CH, OH). Frequency shifts of infrared bands correlate with the transition of enol → keto → protonated-keto forms of the hydroxy analogs of Cl-s-triazine herbicides.

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[1]Skipper, H. D., V. V. Volk, M. M. Mortland and K. V. Raman. Hydrolysis of atrazine on soil colloids. 1969. (manuscript in preparation)

Materials and Methods

<u>Preparation of Hydrolytic Analogs</u>

The Cl-<u>s</u>-triazines [atrazine, 2-chloro-4-ethylamino-6-iso-
propylamino-s-triazine; simazine, 2-chloro-4,6-bis(ethylamino)-<u>s</u>-
triazine; propazine, 2-chloro-4,6-bis(isopropylamino)-<u>s</u>-triazine]
were hydrolyzed in 20 ml of 6N HCl plus 20 ml of ethanol at room
temperature for 24 hours and dried at 65° C. The analogs were sub-
sequently recrystallized twice from ethanol-water, water and dried
at 65° C. To study the effect of pH on the infrared spectra of hydroxy-
atrazine, atrazine was refluxed (hydrolyzed) in 9N HCl for two hours
and the solution was titrated with NaOH to pH 3.3, 5.0, 7.0, 8.4, or
10.0. The precipitate at each pH was washed once with 50% methanol
and dried at 65° C. Additional spectra were obtained after recrystal-
lization of technical hydroxyatrazine from 1N HCl, 6N HCl, or an
aqueous solution adjusted to pH 5. Since preliminary experiments had
indicated that hydroxyatrazine was metastable with respect to heat,
several samples were heated at 100° C for 24-48 hours. Deuterated
spectra of hydroxyatrazine were obtained following hydrolysis of tech-
nical atrazine in 6N HCl (D_2O). Infrared spectra were determined
after recrystallization in the acidic environment and also after titra-
tion with NaOD to pH 10.2.

Infrared Analysis

Since the hydrolytic analogs have limited solubilities in organic solvents and water, infrared spectra were recorded in KBr, Nujol, and Fluorocarbon (KEL-F 10). The mulls were pressed between two Irtran-2 windows and a Beckman IR-7 spectrophotometer was used to record the spectra between 600-4000 cm^{-1} for KBr disks, 700-4000 cm^{-1} for Nujol, and 1350-4000 cm^{-1} for Fluorocarbon.

Results and Discussion

Tautomeric Forms of Hydroxyatrazine

The work of the previous investigators suggest that hydroxy-atrazine undergoes a transition from an enol to a protonated-keto form as the pH is decreased. Results of this investigation support the existence of enol, keto, and protonated-keto forms of hydroxy-atrazine with increased acidity (Figure 1) as evidenced by shifts in infrared spectra. The infrared evidence and the observed solubility of hydroxyatrazine at pH < 3.3 and pH > 11.5 suggest the following tautomeric forms: 1) an anionic species at pH > 11.5, 2) the enol form between pH 11.5 and 3.3, 3) the keto form at pH < 3.3, and 4) a protonated-keto species at pH < 0 (6N HCl). The infrared spectra of the precipitates from pH 3.3-10 were very similar (Figure 2) which supports the existence of a single species (enol). The enol

O⁻
|
C
2
N1 — — 3N
H   H
(C₃H₇)ᵢ-N-C6 \ — / 4C-N-C₂H₅
(R₂)   5   (R₁)
N

(a) Anionic

$\xrightarrow{\text{H}^+}$
$\xleftarrow{\text{pH 11.5}}$

OH
|
C
N   N
R₂-C   C-R₁
N

(b) Enol

$\xrightarrow{\text{H}^+}$
$\xleftarrow{\text{pH 3.3}}$

O
‖
C
N   NH
R₂-C   C-R₁
N

(c) Keto

O
‖
C
HN⊕   NH
R₂-C   C-R₁
N

$\longleftrightarrow$

O
‖
C
HN   NH
H
(C₃H₇)ᵢ-N=C⊕   C-R₁
N

(d) Protonated-keto

O
‖
C
N   NH
R₂-C   C-R₁
N

(c) Keto

$\xrightarrow{\text{pH} < 0}$

O
‖
C
HN   ⊕NH
R₂-C   C-R₁
N

$\longleftrightarrow$

O
‖
C
HN   NH
H
R₂-C   C=N-C₂H₅
N   ⊖
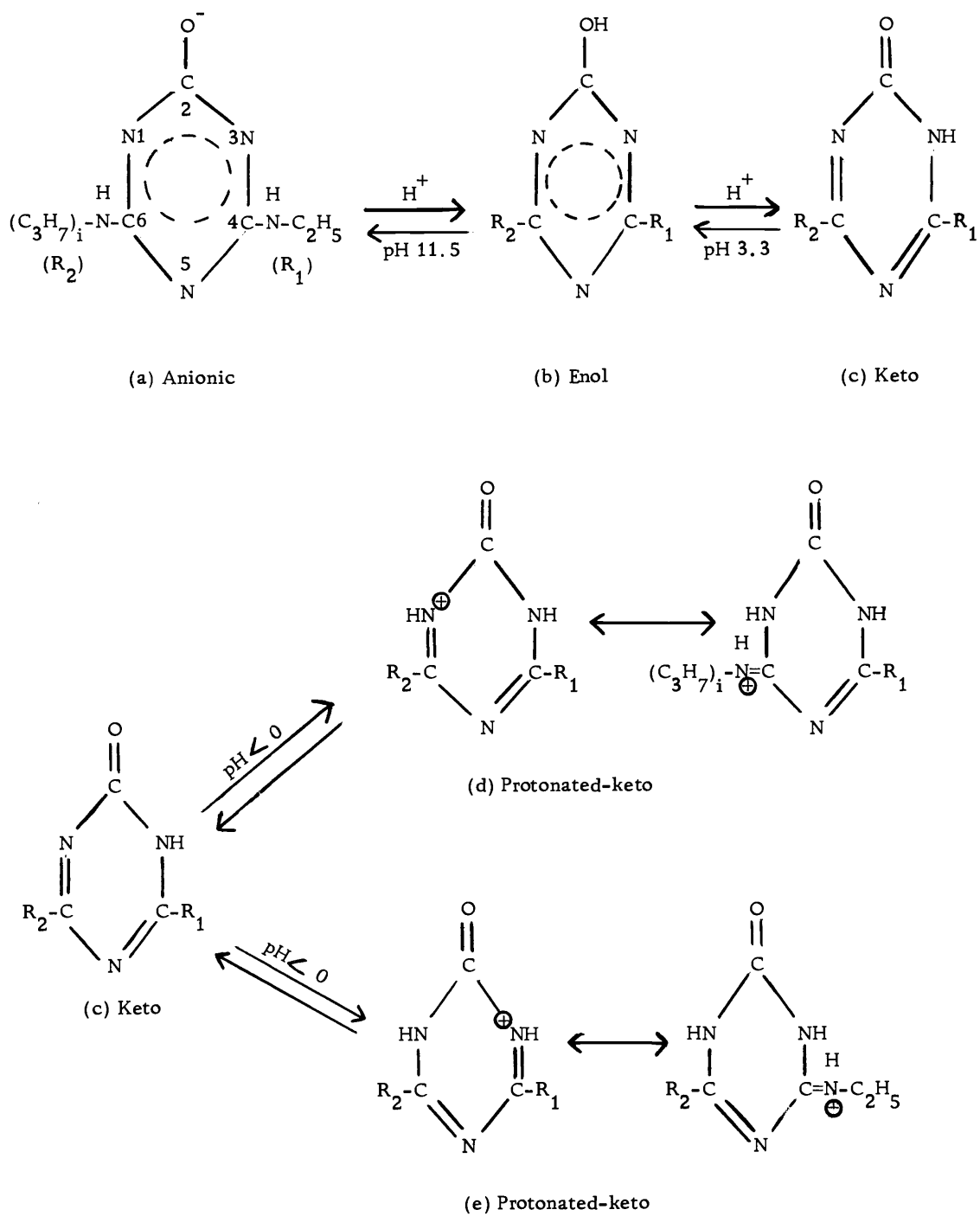
(e) Protonated-keto

Figure 1. Chemical structures for the tautomeric forms of hydroxyatrazine.

and keto forms may be stabilized by several resonance structures, whereas two protonated-keto species (Figure 1d, e) are assumed to exist in a strongly acidic environment (1N HCl or 6N HCl). Since the 5 position is considered to be sterically hindered (32), protonation probably occurs in the 1 or 3 position of the triazine ring. Delocalization of the positive charge in both species may occur by conjugation with the side chains (Figure 1d, e). Spectral evidence for these interpretations will be discussed in the appropriate section regarding the assignment of infrared frequencies.

Infrared Band Assignments

Hydroxyatrazine

The frequencies of the triazine ring vibrations (1692, 1645 cm^{-1}, enol) decrease in the transition from an enol → keto → protonated-keto form of hydroxyatrazine (Figure 2 and Table 1). The existence of a keto species should be correlated with a covalent ring νNH. However, in spectra prepared from solutions of pH 3.3-10, a ring νNH was not detected and appeared at 3220 cm^{-1} only in samples prepared from 1N HCl or 6N HCl (keto and/or protonated-keto form). A 3215 cm^{-1} band was also observed in the keto form of cyanuric acid. Thus, the 1692 cm^{-1} frequency is considered to be predominantly a triazine ring vibration (skeletal νC=N) of the enol form of
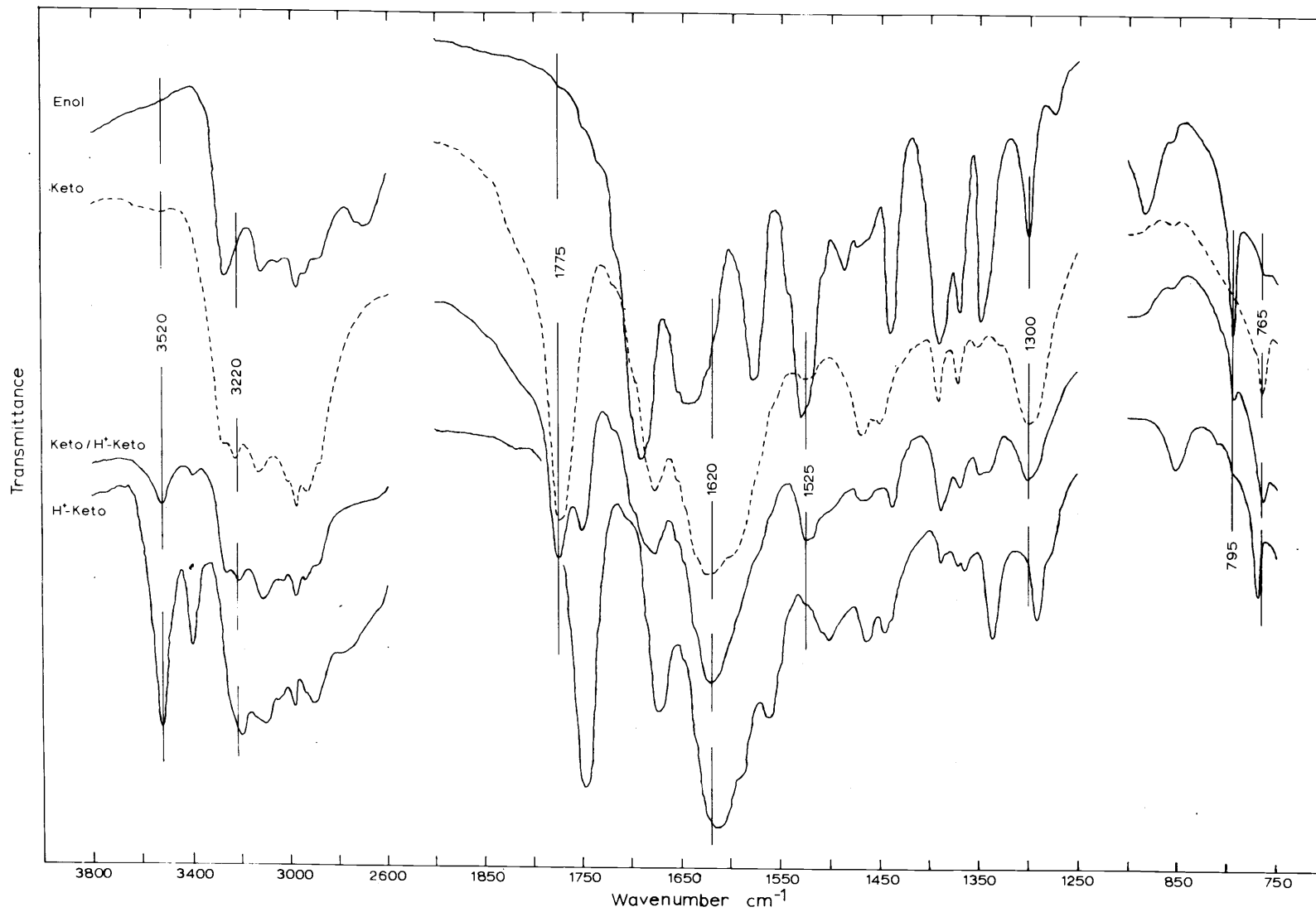
Figure 2. Infrared spectra of the enol, keto, keto/H⁺-keto, and H⁺- keto forms of hydroxyatrazine.

Table 1. Infrared absorption frequencies of the enol, keto, and protonated-keto forms of hydroxy-atrazine.

Structural Group	Hydroxyatrazine Forms					
	Enol		Keto		Protonated-Keto	
	Frequencies, cm^{-1}					
ν Triazine-ring						
in-plane	1692	1645	1678	1620	1673	1613
out-of-plane	795		765		770	
ν NH-chain	3260	3130	3270 (shoulder)	3130	3240 (shoulder)	3100
ν ND-chain	2400 (shoulder)	2350				
D/H ratio	.736	.751				
ν NH-ring			3220		3200	3400 3520
ν ND-ring					2350	2520 2600
D/H ratio					.734	.739 .741
δ NH-chain in-plane	1528	1300	1525	1300	1500	1290
δ ND-chain in-plane	1130	935			1107	920
D/H ratio	.739	.719			.738	.713
ν OH	2700					
ν OD	2100					
D/H ratio	.777					
δ OH in-plane	1578					
δ OD in-plane	1220					
D/H ratio	.773					
δ OH out-of-plane	885					
ν C = O			1775		1750	
ν CH-chain	3050 2980	2940 2885	3020 2980	2930 2880	3050 2980	2940 2900
δ isopropyl (-CH$_3$ scissors)	1390	1375	1390	1370	1390	1370

hydroxyatrazine with perhaps a slight contribution from a C·‥O

species, since the amide II band (νC=N) of amides has been shown

to contain a small percentage of νC = O character (24). Chen (6)

previously assigned the 1692 cm^{-1} frequency of hydroxytriazines to

a νC = O in OH·‥O = C; however, a ring νNH was not reported. The

1645, 1620, and 1613 cm^{-1} frequencies (Table 1) are assigned to tri-

azine ring vibrations in the enol, keto, and protonated-keto forms,

respectively. Chen (6) ascribed a 1621 cm^{-1} band in hydroxytriazines

to a νC = O in NH·‥O = C and/or to a C = N skeletal triazine ring

in-plane vibration; however, Good (11) did not detect H-bonding of

the imino hydrogens in propazine to C = O in acetone. The triazine

ring out-of-plane frequency at 795 cm^{-1} also decreases upon protona-

tion.

In terms of double bonds in the triazine ring, the enol, keto,

and protonated-keto forms have three, two, and less than two by

conjugation with side chains, respectively. Thus, the average bond

order of the triazine ring decreases in the above transition and the

frequencies of the skeletal νC = N and out-of-plane deformations

would be expected to decrease.

Since both Cl-s-triazines (Appendix, Figure 1) and the various

forms of OH-s-triazines exhibited absorption bands at 3130 and 3260

cm^{-1}, these frequencies are assigned to chain νNH vibrations. A

covalent ring νNH at 3220 cm^{-1} was recorded only in samples

prepared from 1N HCl or 6N HCl; however, hydroxyatrazine recrystallized from a pH 5 solution also exhibited a 3220 cm^{-1} frequency (Figure 3). The covalent ring νNH is indicative of a transition to a keto form.

With increasing acidity (1N → 6N HCl), a doublet is observed at 3400 and 3520 cm^{-1}. Since deuterated frequencies (Appendix, Figure 2) of the doublet appeared at 2520 and 2600 cm^{-1} for νND/νNH values of 0.739 and 0.741, respectively, these bands are assigned to a ring νNH of the protonated-keto form (Figures 1d, e and 2) rather than to overtone frequencies of the carbonyl group. The intensity of the deuterated doublet was weak and is considered to result from exchange of the D-protons with atmospheric water. A doublet may be explained on the basis of two protonated-keto species. Protonation at either the 1 or the 3 position would produce a single frequency, whereas a mixture of two protonated species would be expected to give rise to two frequencies (Figure 1d, e).

Spectral evidence also indicates that one of the positions is favored in protonation since the 3520 cm^{-1} frequency appeared first and was the more intense band (Figure 2). Moreover, these high frequency bands are not present in the keto form which has a very strong 1775 cm^{-1} band (Figure 2). The frequencies at 3400 and 3520 cm^{-1} were observed to decrease or disappear upon heating at 100° C for 24-48 hours which indicates the protonated-keto form of

hydroxyatrazine is unstable to heat. A deuterated band at 2350 cm^{-1} is considered to be a mixture of the deuterium analogs of the covalent ring νNH (3220 cm^{-1}) and the chain νNH (3130 cm^{-1}) with νND/νNH ratios of 0.734 and 0.751, respectively.

The stretching frequency of the OH-group (enol form) is assigned to the broad 2700 cm^{-1} band with a deuterated frequency at 2100 cm^{-1} for a νND/νNH value of 0.777. A δOH in-plane frequency is noted at 1578 cm^{-1} with the δOD at 1220 cm^{-1} and the δND/δNH ratio is 0.773. These frequencies are not unexpected since extensive H-bonding has been shown to lower the νOH and increase the δOH frequencies (24).

A carbonyl band (νC=O) is observed in the keto form at 1775 cm^{-1} and at 1750 cm^{-1} in the protonated-keto form. Protonation of the triazine ring lowers the force constant of the νC = O and thus decreases its frequency. A mixture of keto and protonated-keto forms of hydroxyatrazine exhibited a carbonyl doublet at 1775 and 1750 cm^{-1} (Figure 2).

The frequency at 1528 cm^{-1} (enol) may be ascribed to an in-plane deformation of the chain-NH and is observed to decrease to 1500 cm^{-1} in the protonated-keto form. Deuterated analogs were recorded at 1130 and 1107 cm^{-1} for δND/δNH ratios of 0.739 and 0.738, respectively. An absorption band at 1300 cm^{-1} in the enol form is most likely a chain-NH deformation and is noted to decrease

to 1290 cm^{-1} upon protonation. The latter assignment is supported

by ND frequencies at 935 and 920 cm^{-1} and $\delta ND/\delta NH$ ratios of 0.719

and 0.713, respectively.

Infrared absorption frequencies in the 2800-3000 cm^{-1} region

are assigned to the stretching vibrations of chain-CH. Bellamy (3)

has reported a symmetrical CH_3 scissors near 1380 cm^{-1} which

splits into two bands near 1385 and 1375 cm^{-1} in isopropyl $[-CH(CH_3)_2]$

groups. Similar bands are noted in hydroxyatrazine spectra and are

assigned to the symmetrical CH_3 scissors. The bands for νCH and

CH_3 scissors were affected only slightly in the transition from enol →

keto → protonated-keto forms.

The infrared absorption frequencies between 1325-1500 cm^{-1}

are considered to be mixed modes of C = N skeletal vibrations and

chain-NH-CH deformations. Certain similarities to the previous

assignments may be noted: 1) a 1345 cm^{-1} frequency (enol) shifts

to 1333 cm^{-1} upon protonation and may be a chain-NH in-plane defor-

mation or perhaps a CH deformation which has a weak intensity ex-

cept when next to an oxygen or nitrogen atom (3). The 1490 and 1460

cm^{-1} bands probably contain some asymmetric CH_3 scissor vibra-

tions and some contribution from the symmetric CH_2 scissor's defor-

mation. Protonation would be expected to have little effect upon these

frequencies. Since these bands were observed to shift to 1468 and

1448 cm^{-1} upon protonation, they are more likely to be vibrations of

the triazine ring and would correspond to the shifts noted above in
the 1692 and 1645 cm^{-1} bands.

Infrared spectrum containing mixtures of the enol-keto forms
of hydroxyatrazine were observed after recrystallization of hydroxy-
atrazine from an acidic solution (pH 5). The presence of 795 and
1578 cm^{-1} bands, and a broad 2700 cm^{-1} frequency is evidence for
the enol form while the presence of a keto form is supported by the
appearance of 760, 1775, and 3220 cm^{-1} frequencies. The mixture
of the enol-keto species (Figure 3) supports the transition of hydroxy-
atrazine from an enol to a keto form.

A mixture of atrazine and the protonated-keto species of hy-
droxyatrazine and perhaps some enol hydroxyatrazine (Figure 3) was
obtained after recrystallization of atrazine from an acidic solution
(pH 5). The 1750 cm^{-1} band is indicative of the protonated-keto
species. Apparently the latter's concentration was not sufficient to
exhibit the ring νNH bands as seen in Figure 2. Since the C = O
stretching mode has a very large transition moment dipole, it would
be expected to appear at very low concentrations. The presence of
atrazine is demonstrated by infrared frequencies at 805 and 1405
cm^{-1}. An absorption band at 1690 cm^{-1} indicates the presence of
some enol hydroxyatrazine; however, a 2700 cm^{-1} frequency was
not observed. The above mixture would support Horrobin's (15)
mechanism for the acid hydrolysis of s-triazines. Atrazine is
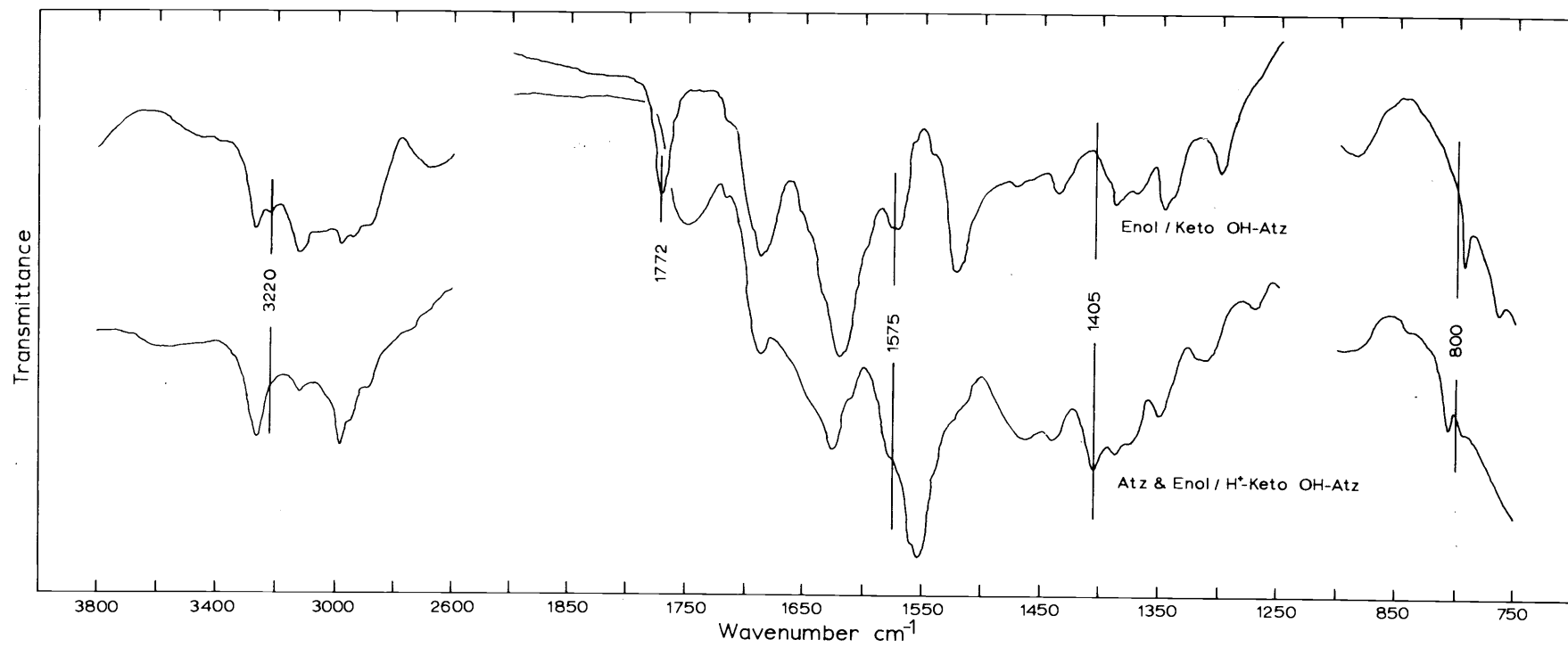
Figure 3. Infrared spectra of enol/keto hydroxyatrazine and atrazine/enol/H^+-keto hydroxyatrazine mixtures.

apparently protonated and hydrolyzed to yield protonated-keto hydroxy-
atrazine with some tautomeric shift to the enol form, whereas a simi-
lar treatment (pH 5, recrystallization) of hydroxyatrazine yields an
enol-keto mixture without the protonated-keto species being detected.
These mixtures would support the transition of enol $\rightarrow$ keto $\rightarrow$ protonat-
ed-keto.

Hydroxysimazine

A number of the assignments for hydroxyatrazine are also appli-
cable to hydroxysimazine and hydroxypropazine (Appendix, Figure 3).
The similarity of the triazine ring out-of-plane deformation (797 $\xrightarrow{H^+}$
760 cm^{-1}) and the appearance of a covalent ring νNH (3220 cm^{-1})
suggest similarities in the chemistry of the three hydroxytriazines.
However, infrared spectra in the regions of the carbonyl stretch and
protonated-keto ring νNH vibrations indicate marked differences.
The hydroxysimazine (pH 8.4) spectrum appears very similar to the
enol form of hydroxyatrazine (Figure 2). Likewise, the hydroxy-
simazine spectrum in a strongly acidic environment (6N HCl) under-
goes a decrease in the triazine ring vibrations (1700 $\rightarrow$ 1670 cm^{-1};
1640 $\rightarrow$ 1620 cm^{-1}). A marked difference between the latter spectrum
and that of protonated-keto hydroxyatrazine is the appearance of a
single high frequency band at 3330 cm^{-1} for protonated-keto hydroxy-
simazine rather than a doublet. The difference may be explained on

the basis of different side-chain constituents. Protonation of hydroxy-simazine (4, 6-bis(ethylamino) at the 1 or 3 position would be expected to produce a single infrared absorption band. Whereas protonation of hydroxyatrazine (4-ethylamino-6-isopropylamino) at the 1 or 3 position may be reasoned to yield two high frequency bands (doublet) because of inductive effects of asymmetric side chains. Ward and Weber (32) suggested that the aqueous solubility process of alkylamino-s-triazines involves protonation of the 1-and/or 3-ring nitrogen since the 1- and 3-endocyclic nitrogen atoms are less sterically hindered than other sites, including the 5-nitrogen group. Since the carbonyl band of hydroxysimazine was observed in a strongly acidic environment at 1750 cm^{-1}, it is assumed to be associated with the protonated-keto form. Additional spectra in less acidic environments (1N HCl or pH > 0) are needed for confirmation.

Hydroxypropazine

Hydroxypropazine exhibited a covalent ring νNH at 3220 cm^{-1} and also a very strong carbonyl band at 1775 cm^{-1} which is suggestive of a keto species. Moreover, high frequency bands of a protonated-keto form (ring νNH) were not observed in hydroxypropazine prepared from 6N HCl. Perhaps protonated-keto hydroxypropazine is very unstable with respect to heat (65° C) or else very difficult to protonate. However, subsequent samples recrystallized from 1N HCl

and 12N HCl at 40° C also failed to produce a high frequency band which could be associated with a vibration of protonated-ring-NH. Thus, hydroxypropazine was apparently not protonated under these conditions.

Conclusions

The decrease in the frequencies of the triazine ring vibrations ($1692 \rightarrow 1678$ cm^{-1}; $1645 \rightarrow 1620$ cm^{-1}) and the appearance of a covalent ring ν NH (3220 cm^{-1}) support the transition from the enol to the keto hydroxyatrazine at pH < 3.3. Additional shifts of the triazine ring vibrations ($1678 \rightarrow 1673$ cm^{-1}; $1620 \rightarrow 1613$ cm^{-1}), a covalent ring νNH (3220 cm^{-1}), and high frequency bands at 3400 and 3520 cm^{-1} (ring ν NH of protonated-keto) support the transition from the keto to the protonated-keto hydroxyatrazine at pH < 0. Limited infrared spectra suggest that hydroxysimazine in an acidic environment (6N HCl) exists as the protonated-keto species with ν C = O at 1750 cm^{-1}, covalent ring ν NH at 3220 cm^{-1}, and a single protonated-ring νNH at 3330 cm^{-1}. Under similar conditions, hydroxypropazine apparently exists as the keto form based on the absence of a high frequency band associated with a protonated-ring νNH and the presence of a covalent ring νNH (3220 cm^{-1}) and a νC = O at 1775 cm^{-1}. These results demonstrate a marked difference in the chemistry of the three hydrolytic analogs of atrazine, simazine, and propazine with

respect to protonation.

As previously demonstrated, infrared spectroscopy serves as an efficient tool to identify the functional groups of unknown compounds. When a Cl-s-triazine molecule hydrolyzes to form an enol, a keto, or a protonated-keto species of hydroxyatrazine, infrared bands characteristic to these compounds appear. Since the atrazine molecule does not contain hydroxyl or carbonyl groups, the presence of an OH-deformation band (1578 cm^{-1}) or a carbonyl band (1725 to 1775 cm^{-1}) denotes the hydrolysis of atrazine.

After developing the relationships between functional groups and infrared bands in the atrazine-hydroxyatrazine systems, one may study the colloid-triazine reactions. Variables such as type of colloid, cation saturation, and moisture content have been shown to influence hydrolytic and adsorption reactions. Since the infrared spectra of the hydrolytic products of atrazine were markedly different, one should be able to introduce the aforementioned variables and thus determine their effects on the hydrolysis of atrazine. With this concept in mind, colloids of montmorillonite, "allophane," and Coker soil clay were saturated with H^+, Al^{3+}, Ca^{2+}, or Cu^{2+}; equilibrated with atrazine or hydroxyatrazine; and the reaction products analyzed by infrared spectroscopy.

HYDROLYSIS OF ATRAZINE ON SOIL COLLOIDS

Introduction

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) has been used extensively for the control of weeds during the past decade and enjoys continued use; nevertheless, its fate in soils and the factors influencing its residual life remain the objectives of numerous research programs. Microbial dissimilation has received considerable emphasis as a factor in the degradation of atrazine; however, chemical hydrolysis, conversion of chloroatrazine to hydroxyatrazine which is nonphytotoxic, has received strong endorsement as a major pathway, perhaps the predominant route, in the detoxification of atrazine in soils (2, 12, 29).

Literature Review

Infrared spectroscopy has been employed to determine the mechanism(s) of bonding and degradation of pesticides on soil colloids. Atrazine was hydrolyzed upon reaction with H-montmorillonite as indicated by the carbonyl band of hydroxyatrazine at 1740 cm^{-1} in the infrared spectrum of the reaction product (27). Furthermore it was shown that hydrolysis of atrazine occurred as a result of protonation at the acidic surface of H-montmorillonite and the

protonated-hydrolytic degradation product (2-hydroxy-4-ethylamino-6-isopropylamino-s-triazine) was adsorbed as an organic cation. The adsorption of another herbicide, 3-aminotriazole, by montmorillonite occurred after protonation of the organic herbicide by the highly polarized water molecules in direct coordination to the Ca, Cu, Ni, and Al cations on the exchange sites of the montmorillonite. The 3-aminotriazolium cation was reported to undergo exchange reactions with other cations (26).

A decrease in the $C = O$ stretching frequency of EPTC (N-di-n-propylthiolcarbamate) in the presence of Li, Na, Ca, Mg, Al, Cu, and Co-saturated montmorillonite indicated coordination (ion-dipole interaction) to the exchangeable metal ion through the oxygen of the carbonyl group (22). An organic cation (pyridinium) on the exchange complex of montmorillonite formed a more stable complex with EPTC than that formed by clay saturated with exchangeable metal ions. Thus, soil organic matter upon interaction with clay may increase and stabilize adsorption of pesticides beyond that observed in inorganic clay systems (20). Ionic bonding (salt linkage) and/or H-bonding have been tentatively proposed as the mechanisms involved in the adsorption of s-triazines by the alcohol-soluble fraction of soil humic acid (30).

The objective of this work was to establish the interactions of atrazine on "allophane," montmorillonite, and "natural

montmorillonitic clay" surfaces. Homoionic colloids saturated with

H, Al, Ca, or Cu were investigated. The effects of dehydration were

also examined.

Materials and Methods

Preparation of Colloids

An "allophanic" colloid was obtained from the C horizon (42-

73 cm) of the Parkdale silt loam soil (Umbric Vitrandepts) after a

hydrogen peroxide treatment and dispersion at pH 2.5. Montmoril-

lonite (Wyoming bentonite, No. 25) and a "natural montmorillonitic

clay, " separated from the Ap horizon (0-10 cm) of the Coker (Chro-

mic Pelloxererts) clay series were water dispersed. Centrifugation

at 750 rpm for 3.9 minutes yielded the $< 2\mu$ fraction of all the above

colloids.

The cation exchange capacity (CEC) of the colloids was deter-

mined by repeated saturation with 0.5N $CaCl_2$, removal of excess

salts with H_2O and 95% ethanol, and displacement of Ca by $MgCl_2$.

The CEC was also determined by saturation with 0.5N KCl and dis-

placement of K with 0.5N NH_4Cl. Calcium was analyzed by atomic

absorption and K was determined by flame emission. Organic mat-

ter was determined in a Leco high frequency induction furnace. After

the removal of iron and amorphous materials, colorimetric analyses

for Si, Al, and Fe (16) were accomplished. X-ray diffractograms of the clay fractions from the Parkdale and Coker soils were prepared: (1) after organic matter removal by H_2O_2 and Fe-extraction by Na-citrate-bicarbonate-dithionite, and (2) after treatment with boiling 0.5N KOH for 2.5 minutes (amorphous removal) and a second Fe-extraction by Na-citrate-bicarbonate-dithionite.

Homoionic colloids of Al^{3+}, Ca^{2+}, or Cu^{2+} were prepared by saturation with the respective 1N chloride salts. Excess salts were removed by wash treatments with H_2O or 50% methanol. H-saturation was accomplished by shaking the colloids with H-saturated IR-120 resin for approximately 30 minutes and subsequent decantation of the H-saturated colloids. The H(Al)-samples were used immediately to reduce the interferences which may arise from the formation of Al-hydroxy interlayers.

Infrared Spectra of Atrazine and Hydroxyatrazine

Technical atrazine (97.6%) and the enol form of hydroxyatrazine (98.7%) were supplied by Geigy Agricultural Chemicals. Keto and protonated-keto forms of hydroxyatrazine were prepared by recrystallization of technical hydroxyatrazine from 1N and 6N HCl, respectively.[1] The infrared spectra of atrazine, keto, and protonated-keto

[1]Skipper, H. D., R. Frech and V. V. Volk. Hydrolytic products of Cl-s-triazine herbicides. 1969. (manuscript in preparation)

hydroxyatrazine were recorded in KBr disks and Nujol and Fluoro-

carbon mulls.

Colloid-Triazine Reaction

Each colloidal system (100 mg) was equilibrated for 24 hours

with 0.5 mmole of atrazine per gram of colloid in 30 ml of 30% meth-

anol. The methanol solvent permitted the higher concentration of

atrazine required for infrared analysis. Aliquots which contained

approximately 7 mg of colloid and 700 μg of atrazine were pipetted

onto Irtran-2 disks and dried at 40° C for 12-24 hours. Irtran-2 is

a ZnS crystal transparent to infrared absorption from 700-4000 cm^{-1}.

The samples were analyzed with a Beckman IR-7 spectrophotometer.

For the hydroxyatrazine experiments, 100 mg of each colloid

were equilibrated for 24 hours with 40 ml of approximately 2.5×10^{-4}

M hydroxyatrazine in 50% methanol. Aliquots which contained approx-

imately 5 mg of colloid and 100 μg of hydroxyatrazine were dried at

40° C on Irtan-2 disks and analyzed.

Results and Discussion

Colloidal Properties

As interpreted from X-ray diffractograms, the clay fraction

of the Coker clay soil contains predominantly montmorillonite which

expanded from 15.8 Å at 54% relative humidity (Ca) to 18.4 Å with

glycerol (Ca). Cation exchange capacities of approximately 85 meq/

100 g after removal of organic matter indicate the presence of im-

purities or perhaps the surface charge density is low to moderate

since the CEC value for montmorillonite is generally given as 80-110

meq/100 g. The clay fraction of the Coker soil also contains 2.1%

organic matter and 14.8% amorphous material (Table 1).

The clay fraction of the Parkdale-C soil was resistant to col-

lapse with K-saturation and heating which indicates that chlorite and

chloritic-intergrades are the predominant clay minerals. Since the

CEC increased from approximately 15 to 60 meq/100 g upon iron re-

moval, iron apparently serves as a cementing and/or coating agent

in the clay fraction. A decrease in CEC upon amorphous removal

(60 to 25 meq/100g) suggest that the "allophanic" constituent (49.1%)

accounts for approximately 50% of the CEC. The Parkdale-C clay

fraction contains 0.8% organic matter even after the initial hydrogen

peroxide treatment required for dispersion.

Triazine Infrared Bands

Infrared absorption bands characteristic of the atrazine mole-

cule as reported by Chen (6) are the triazine ring out-of-plane defor-

mation at 806 cm^{-1} and skeletal $\nu C = N$ bands at 1555-1580 cm^{-1}

(broad) and 1622 cm^{-1} (Figure 1). Infrared assignments have also

Table 1. Properties of the clay fractions from Parkdale-C silt loam and the Coker clay soils.

Colloid	Organic matter	Fe_2O_3		Cation Exchange Capacities			Amorphous Components[2]			
				+ OM[1] + Fe	-OM -Fe	-Amorp. -Fe	SiO_2	Al_2O_3	Fe_2O_3	Total[3]
	------- % --------			------- meq/100 g-------			------------------%-----------------------			
Parkdale-C[4] sil	0.8	14.1	Ca/Mg	17.2	62.2	25.3	22.8	22.7	3.6	49.1
			K/NH4	11.5	57.9	24.1				
Coker c	2.1	3.0	Ca/Mg	102.5	81.3	83.8	7.2	4.2	3.4	14.8
			K/NH4	83.2	79.7	98.1				

[1] (+) = before O.M. removed; (-) = after O.M. removed.

[2] Amorphous removal: boiling 0.5N KOH, 2.5 min.

[3] % Amorphous = $\Sigma(SiO_2 + Al_2O_3 + Fe_2O_3)$

[4] Parkdale-C required an initial H_2O_2 treatment for dispersion.
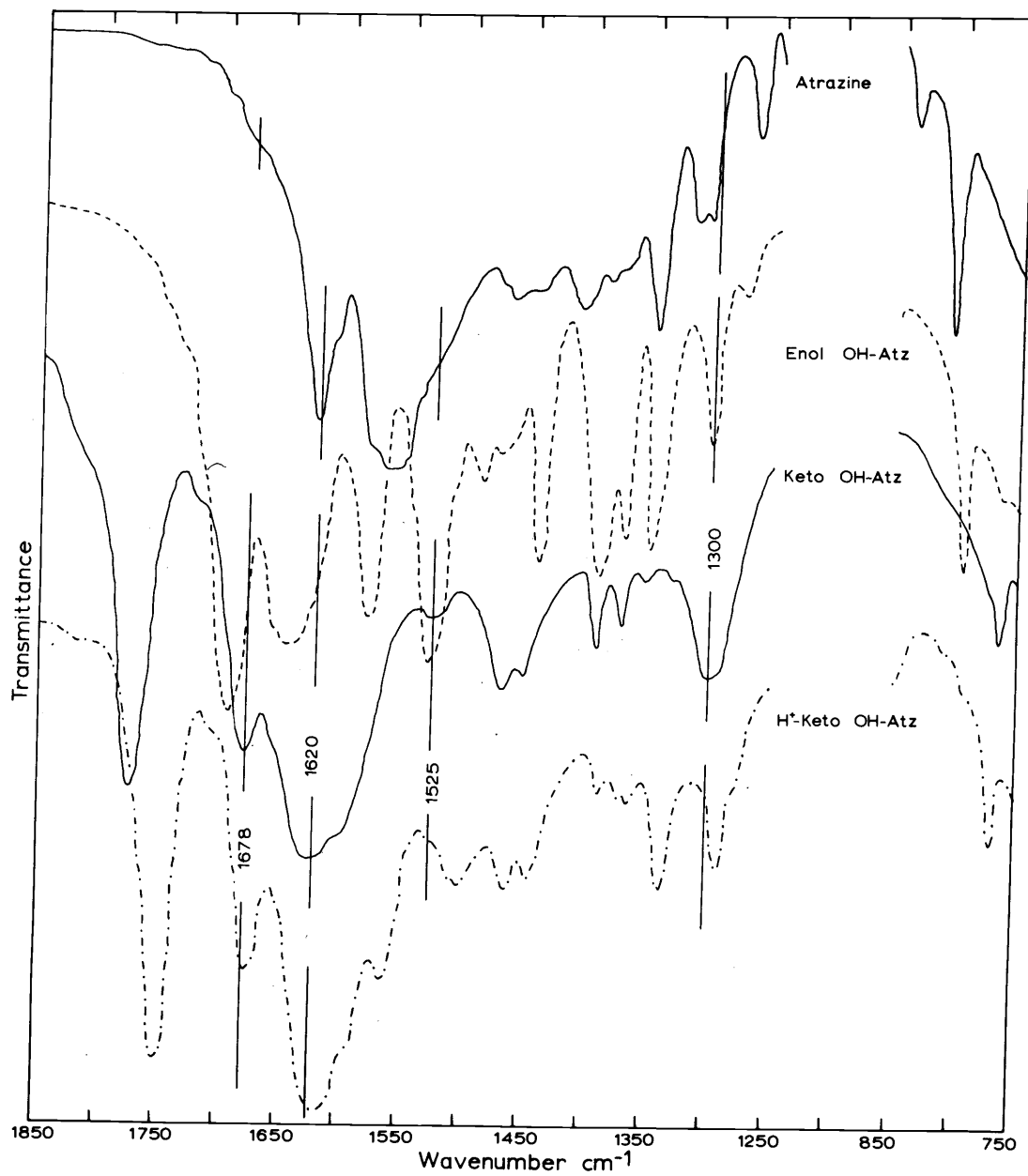
Figure 1. Infrared spectra of atrazine and enol, keto, and H$^+$-keto forms of hydroxyatrazine.

been made for enol, keto, and protonated-keto hydroxyatrazine.[1]

Characteristic infrared bands for enol hydroxyatrazine include the

triazine ring out-of-plane deformation at 797 cm^{-1}, an OH-deforma-

tion at 1578 cm^{-1}, skeletal νC = N bands at 1692 and 1645 cm^{-1},

and chain-NH deformations at 1528, 1345, and 1300 cm^{-1}. The tri-

azine ring vibrations shift to 760, 1670, and 1622 cm^{-1}, respectively

and the OH-deformation (1578 cm^{-1}) is absent in the protonated-keto

species of hydroxyatrazine. The chain-NH deformations shift to

1500, 1333, and 1290 cm^{-1}, respectively in protonated-keto hydroxy-

atrazine (Figure 1). Moreover, a strong carbonyl frequency (1750

cm^{-1}) is observed in the latter species while the nonprotonated-keto

hydroxyatrazine has a carbonyl band at 1775 cm^{-1}. The presence or

absence of the infrared bands for atrazine and its hydrolytic analogs

are indicative of the interactions between atrazine and soil colloids.

Montmorillonite-Triazine Reactions

After interaction of atrazine with H- or Al-montmorillonite, a

strong carbonyl band at 1745 cm^{-1} (Figure 2) indicates the formation

of hydroxyatrazine from the hydrolysis of atrazine. The 1337 and

1290 cm^{-1} bands in the reaction product are similar to those of the

protonated-keto form of hydroxyatrazine (Figure 1). The 1525 cm^{-1}

frequency did not shift to 1500 cm^{-1} as noted for the protonated-keto

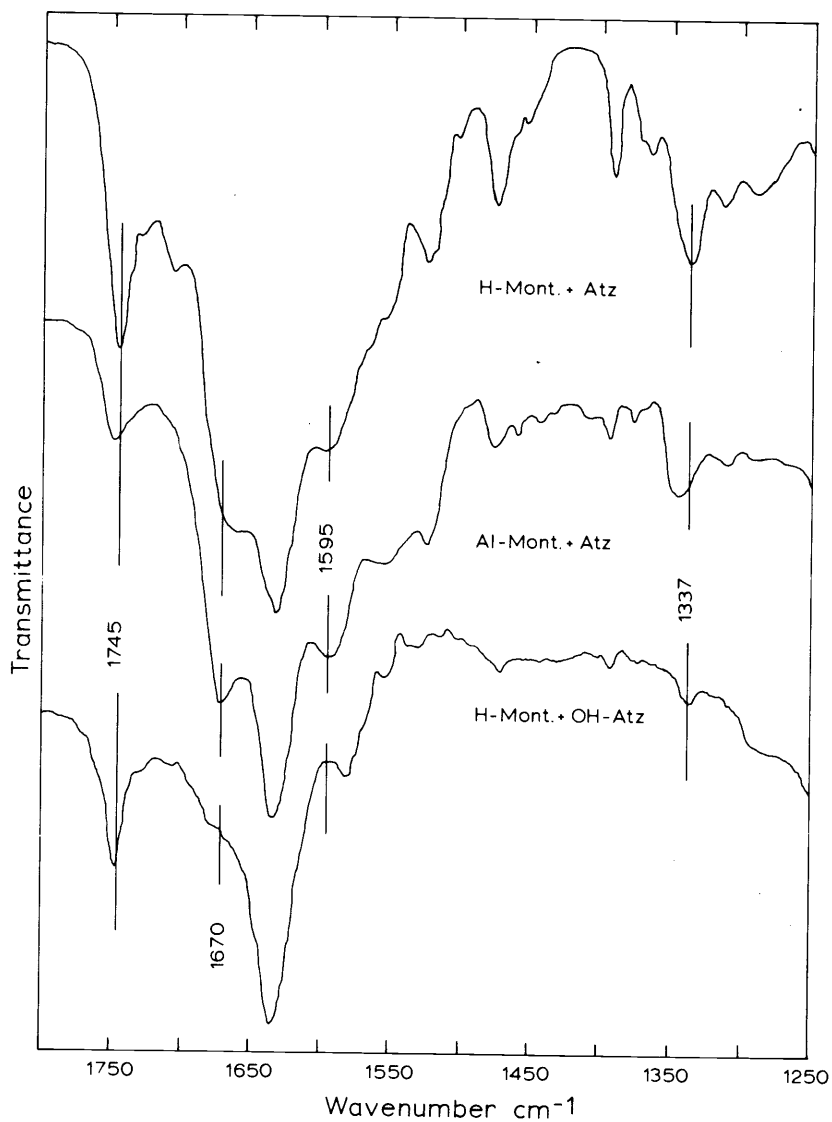hydroxyatrazine and may represent an interaction of the chain-NH

Figure 2. Reaction products of atrazine with H- and Al-
montmorillonite and hydroxyatrazine with H-
montmorillonite.

with the clay system. Moreover, the 1745 and 1337 cm^{-1} bands are

observed in the H-montmorillonite plus hydroxyatrazine spectrum

(Figure 2) which again suggests the hydrolytic product is present as

the protonated-keto form of hydroxyatrazine. However, it is realized

that H-bonding of the carbonyl group of the keto form of hydroxyatraz-

ine or interaction with the surface cations may shift the carbonyl fre-

quency from 1775 to 1745 cm^{-1}. Reaction of hydroxyatrazine with

H-montmorillonite in a strongly acidic solution (2N HCl) produced

the 1745 cm^{-1} carbonyl band of the protonated-keto form[1] (data not

shown) which indicates the carbonyl band of protonated-keto hydroxy-

atrazine was not affected by the clay system. Infrared absorption

bands which represent structural Si-O and Al-O below 1100 cm^{-1}

and H$_2$O and clay hydroxyl groups above 3100 cm^{-1} restricted obser-

vations of triazine frequencies in these regions (Appendix, Figure 4).

Reaction of atrazine with Ca- or Cu-montmorillonite did not

produce a 1745 cm^{-1} band (Figure 3). Moreover, drying at 75° C

for 12-24 hours to increase the surface acidity (21) did not change

the spectra. Possible shifts of the atrazine ring vibrations (1620 →

1665 cm^{-1}; 1570 → 1630 cm^{-1}) may indicate perturbation of the atra-

zine skeletal vibrations by interaction with the cations on the clay

surface (C = N...M^{2+}). If hydrolysis of atrazine had occurred in the

Ca- or Cu-montmorillonite systems, the infrared spectra should be

similar to the spectrum of Cu-montmorillonite plus hydroxyatrazine

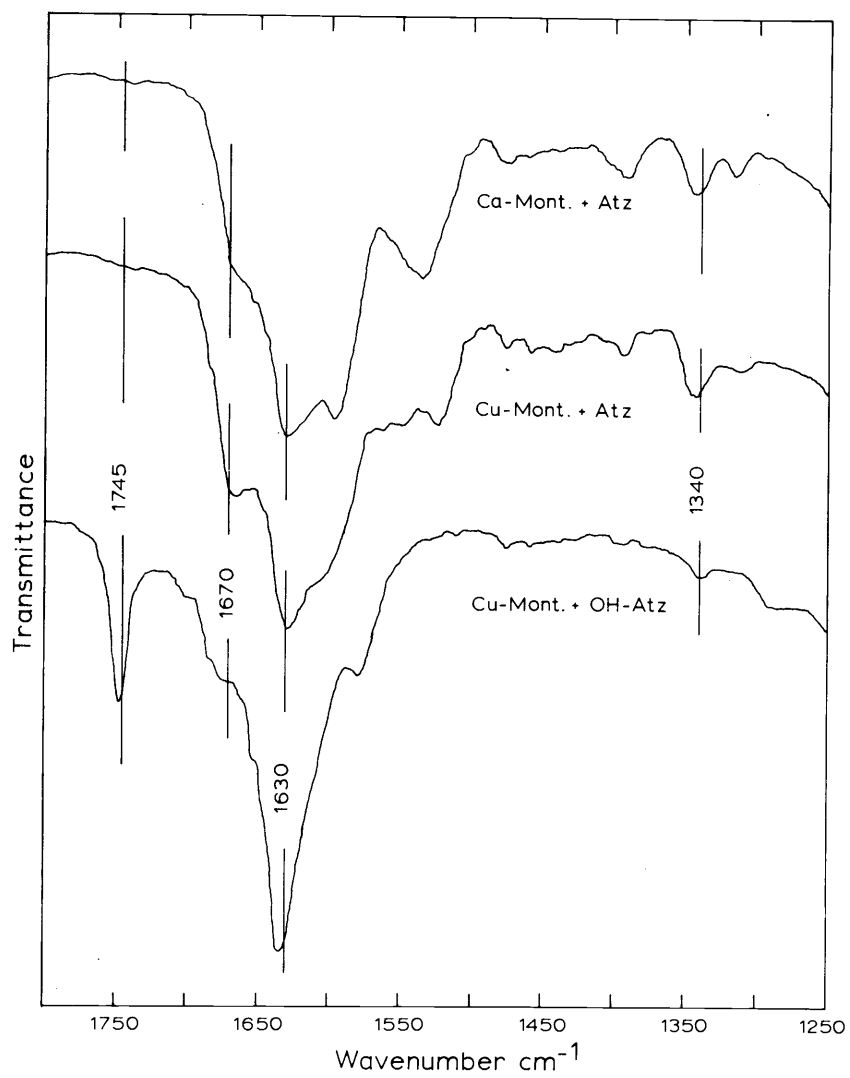Figure 3. Reaction products of atrazine with Ca- and Cu-
montmorillonite and hydroxyatrazine with Cu-
montmorillonite.

Figure 4. Reaction products of atrazine with Ca- and Cu-
Coker, hydroxyatrazine with Cu-Coker, and
atrazine with Ca-Coker (75° C).

increased hydrolysis as a result of increased surface acidity (21).

Parkdale-C "Allophanic" Clay-Triazine Reactions

The infrared spectra of the Parkdale-C "allophanic" clay con-
tained oxalate bands at 1300, 1425, and 1718 cm^{-1} (Appendix, Figure
4) which are believed to arise from the hydrogen peroxide treatment
(9) used initially to disperse the colloid. The H-Parkdale-C clay
fraction was subject to more interference by the 1718 cm^{-1} band
than Al-Parkdale-C. Preliminary infrared spectra of Cu-Parkdale-
C and Ca-Parkdale-C clay fractions were similar to H-Parkdale-C
and Al-Parkdale-C, respectively. The effect of cation saturation on
the 1718 cm^{-1} frequency requires that interpretation of the infrared
spectrum of atrazine plus an "allophanic" colloid be made only after
comparison to the appropriate check pattern. Comparison of spectra
of atrazine and colloids saturated with different cations would be mis-
leading.

Weak absorption bands in the 800 cm^{-1} region of homoionic
samples of the "allophanic" Parkdale-C clay fraction (Appendix, Fig-
ure 4) permitted the detection of the atrazine (806 cm^{-1}) and hydroxy-
atrazine (797 cm^{-1}) ring out-of-plane deformations (Figure 5). An
806 cm^{-1} absorption band and the absence of a 1745 cm^{-1} band indi-
cates that atrazine was not hydrolyzed by H, Al, Ca, or Cu-saturated
Parkdale-C colloids (Appendix, Figure 5). The surface acidity of the
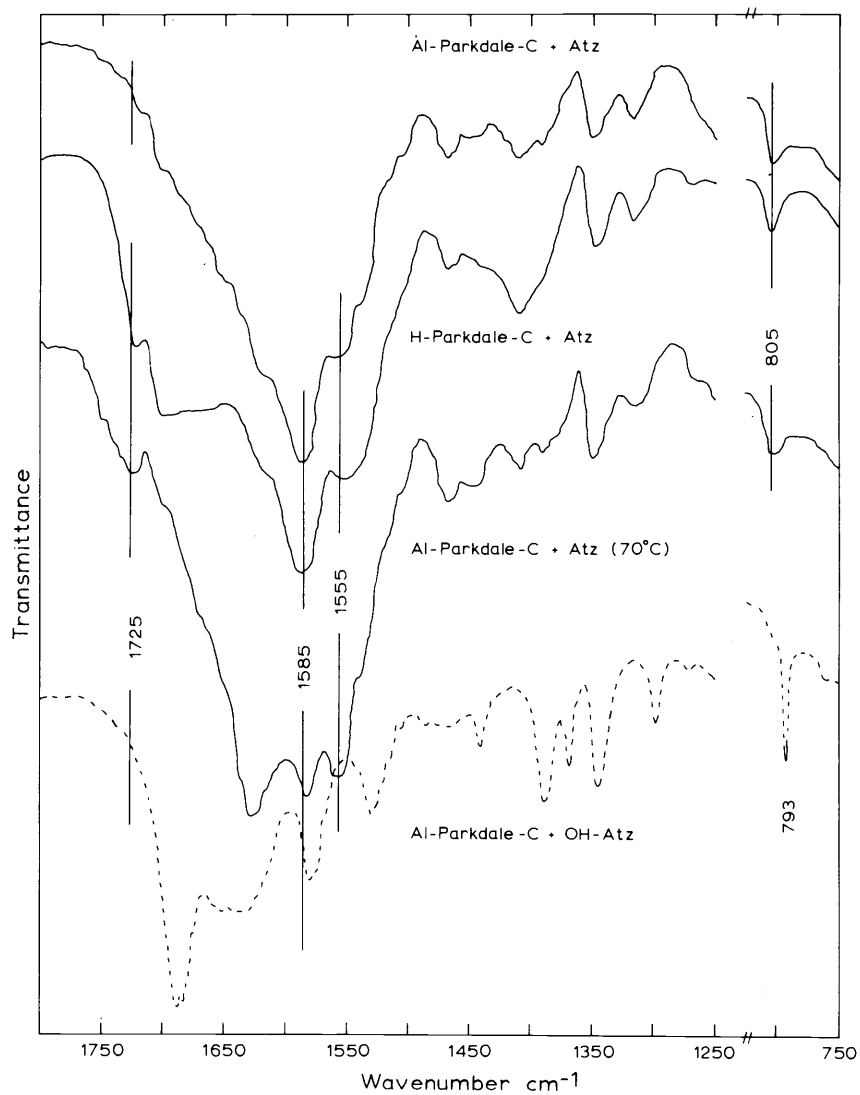
Figure 5. Reaction products of atrazine with Al- and H-
          Parkdale-C, atrazine with Al-Parkdale-C
          (70° C), and Hydroxyatrazine with Al-
          Parkdale-C.

"allophanic" colloid must be much lower than for the previous colloids. A lower CEC (Table 1) may also in part explain the absence of hydrolysis in Parkdale-C. The appearance of a broad 1725 cm^{-1} band upon drying the Al-Parkdale-C plus atrazine reaction product (75° C, 24 hours) suggests that some hydrolysis has occurred since the 1725 cm^{-1} frequency was not present prior to dehydration (Figure 5). The large number of hydroxyl groups that may participate in H-bonding in "allophanic" materials (31) may account for a 1725 cm^{-1} rather than a 1745 cm^{-1} band. The presence of 793 and 1688 cm^{-1} (enol hydroxyatrazine) bands in the Al-Parkdale-C plus hydroxyatrazine reaction provides evidence that the "allophanic" surface is not sufficiently acidic to protonate the hydroxy analog (Figure 5).

Conclusions

The presence of a strong carbonyl band (1745 cm^{-1}) indicates that montmorillonite and a "natural montmorillonitic clay" were active in the hydrolytic degradation of atrazine, especially when the exchange complex was saturated with the more acidic cations, H^+ and Al^{3+}. The atrazine ring vibration at 806 cm^{-1} and the absence of a carbonyl band at 1745 cm^{-1} indicated an "allophanic" colloid saturated with H^+, Al^{3+}, Ca^{2+}, or Cu^{2+} did not promote hydrolysis of atrazine. Moreover, Al-Parkdale-C was not sufficiently acidic to protonate hydroxyatrazine, as a carbonyl band was not observed

in the reaction product. Thus, a major difference exists between "allophanic" colloids and montmorillonite as catalysts in the protonation and hydrolysis of atrazine. Under acidic field conditions one might expect the smectites to enhance chemical hydrolysis of atrazine while the "allophanic" and perhaps other amorphous materials would be relatively inert.

Soils are complex bodies of organic and inorganic constituents and the fate of soil-applied herbicides is affected by various soil components such as organic matter content, clay minerals, moisture content, clay-organo combinations, and biological populations. Elucidation of the mechanisms and agents responsible for detoxification of herbicides is complicated by the numerous variables in soil systems. Consequently, soils are often fractionated to obtain the mineral or organic colloids which serve as "models" in the degradation and adsorption of herbicides.

The previous study demonstrated hydrolysis of atrazine on montmorillonitic surfaces, especially when the exchange complex was saturated with the acidic cations, H^+ and Al^{3+}, whereas very little hydrolysis occurred in Ca^{2+}- or Cu^{2+}-saturated montmorillonitic samples. An "allophanic" colloid did not hydrolyze atrazine when saturated with H^+, Al^{3+}, Ca^{2+}, or Cu^{2+}. A major difference exists between the "allophanic" and montmorillonitic colloids with respect to hydrolysis of atrazine.

The lack of hydrolysis in the "allophanic" and neutral mont-morillonitic systems stimulated an investigation into the mechanism(s) of degradation in Parkdale and Coker soils. Atrazine-^{14}C-ethyl, atrazine-^{14}C-isopropyl, or atrazine-^{14}C-ring were applied to soils and the evolution of $^{14}CO_2$ was measured at three-day intervals. Hydrolysis of atrazine in the incubated soil systems was ascertained by the presence of hydroxyatrazine in methanol extractions (Soxhlet).

DEGRADATION OF ATRAZINE IN THREE OREGON SOILS

Literature Review

Increased temperature, moisture and organic matter content of soils stimulate microbial degradation of s-triazine herbicides. McCormick and Hiltbold (19) and Roeth et al. (25), have reported the degradation rate of atrazine to increase two to three-fold for each 10° C temperature increase from 15 to 35° C. The latter authors also observed up to a six-fold increase in $^{14}CO_2$ evolved with an increase in the moisture content from 40 to 80% of field capacity. A positive correlation between atrazine inactivation and oxidation of soil organic carbon has been reported (19). Kaufman and Kearney (17), in an excellent review of microbial degradation of triazine herbicides, describe three major degradative pathways: 1) hydrolysis at the number 2-carbon, 2) N-dealkylation of side chains, and 3) ring cleavage. Chemical hydrolysis is described as probably the predominant pathway for detoxification of Cl-s-triazines in soils.

The objective of this investigation was to ascertain the importance of microbial degradation and/or chemical hydrolysis of the atrazine molecule in three Oregon soils as influenced by moisture content, organic matter content, air-flow rate, and atrazine concentration. Degradation of ring-labeled hydroxyatrazine was also examined.

Materials and Methods

Soil Properties

The surface horizons of Parkdale loam (Umbric Vitrandepts),

Coker clay (Chromic Pelloxererts), Woodburn silt loam (Aquultic

Argixerolls), and the C-horizon of the Parkdale soil series (Table 1)

were examined for their atrazine degradation potential. Organic

matter (Walkley-Black oxidation) and total N (Kjeldahl) ranged from

1.1 to 4.7% and from 0.06 to 0.13%, respectively. All soils had a

neutral pH of 6.6 to 7.2 (soil: H_2O = 1:1). To determine the mois-

ture content at field capacity, a plexiglass tube (1 1/4 x 5 inches) was

filled with soil. Sufficient water was added to saturate approximately

the upper two to three inches of soil. The excess water was allowed

to drain for 48 hours into the dry soil. Samples were removed from

the upper two inches to determine the moisture content at field capa-

city. Cation exchange capacities (total extractable base) ranged from

12.6 to 52.5 meq/100 g.

[14]C-labeled Substrates

Approximately 1-2 μc (0.1-0.3 ml) of ^{14}C-atrazine dissolved

in methanol or ^{14}C-hydroxyatrazine dissolved in 0.1N HCl were ap-

plied in 5 ml water to 50 g (dry weight) of soil. Sodium hydroxide

Table 1. Properties of Parkdale loam (A and C horizons), Coker clay (Ap) and Woodburn (Ap) silt loam soils.

	Moisture content at field capacity	Organic matter	N	pH	CEC	Dominant clay minerals	Fungi[1]	Bacteria and Actinomycetes[2]
	------------------- -% ----------------			Soil:H_2O 1:1	meq/100 g		---------Viable counts/g -------	
							Ten thousands	Millions
Parkdale-A l	54. 2	3. 1	0. 13	6. 8	21. 1	Chlorite Chloritic intergrades	34	11. 5
Parkdale-C sil	55. 8	1. 1	0.06	7. 2	12. 6	Chlorite Chloritic intergrades	0. 2	0. 6
Coker c	37. 2	4. 7	0. 13	6. 9	52. 5	Montmorillonite	4. 1	32. 5
Woodburn sil	32. 1	3. 1	0. 13	6. 6	18. 9	Vermiculite, mica, some beidellite and kaolinite	24	21

[1] Rose bengal agar + streptomycin   [2] Yeast extract agar

(0. 1 N) was added to the hydroxyatrazine solution to neutralize 90%
of the acidity, prior to the soil application. Location of the ^{14}C-label
and specific activities for the ^{14}C-substrates utilized were: 1) atra-
zine-^{14}C-ring = 7.3 μc/mg; 2) hydroxyatrazine-^{14}C-ring = 10.4 μc/
mg; 3) atrazine-^{14}C-ethyl (NH-^{14}CH$_2$CH$_3$) = 7.7 μc/mg; and 4) atra-
zine-^{14}C-isopropyl [NH-^{14}CH(CH$_3$)$_2$] = 5.5 μc/mg. Concentrations
of ^{14}C-labeled atrazine or hydroxyatrazine were chosen to approxi-
mate field rates of 2 to 3 ppm with additional concentrations at 2X-
field rates.

Incubation and Aeration

Compressed air was passed over the soil-triazine systems
which were incubated in 250-ml Erlenmeyer flasks in a water bath
at 27 ± 1° C. The air-flow rate to all systems was maintained at
approximately 5 cc/min to simulate air exchange under field condi-
tions. Both air-flow rate and composition of the aeration gas have
been shown to influence the microbial decomposition of straw (23).
To evaluate the effect of air-flow rate on ^{14}CO$_2$ evolution, a rate of
10 cc/min was examined in the degradation of atrazine-^{14}C-ethyl in
the Parkdale-A soil.

The CO$_2$ released from the soil during incubation was absorbed
in 25 ml of a 2:1 ethanol:ethanolamine solution (Appendix, Figure 6).
The trapping solutions were changed after 24 hours and every three

days thereafter. Five milliliter aliquots of the trapping solution

were counted to a standard deviation of 1% (29).

Moisture Content

Each soil system was incubated at moisture contents chosen to

approximate optimal conditions for microbial activity. The soils

were moistened 48 hours prior to addition of the triazine compounds.

Upon addition of the 5 ml of ^{14}C-labeled atrazine or hydroxyatrazine

solutions, the Parkdale soils were brought to 80% of field capacity

and the Coker soil to 90% of field capacity. To determine the effect

of moisture content on microbial degradation of atrazine, the Wood-

burn soil was adjusted to 90%, 70%, or 50% of field capacity. Since

the compressed air was bubbled through water prior to passing over

the soils, a decrease of only 1 to 2% was noted in the soil moisture

content after 28 days incubation.

The CO_2 respiration studies were not completed in the same

time period; however, the difference in $^{14}CO_2$ evolved between time

periods for similar treatments was no greater than the difference

between duplicates in the same time period.

Soxhlet Extraction

After 28 days of incubation, during which time the $^{14}CO_2$

evolved was collected, the soils were extracted with methanol for

24 hours in a soxhlet apparatus (methanol:soil=5:1) and the extrac-

tant volume was adjusted to 200 ml with methanol. Two milliliter

aliquots of extractant were partitioned in a mixture of 5 ml chloro-

form, 2 ml H_2O, and 0.5 ml of 0.2N HCl. The aqueous phase which

contained the hydroxyatrazine was washed once with 5 ml chloroform.

The aqueous phase (2.5 ml) plus 0.5 ml of a H_2O wash were trans-

ferred to 10 ml of Bray's scintillation fluor (5) to determine the

^{14}C-hydroxyatrazine activity (1). In preliminary experiments, 85-

90% of ^{14}C-hydroxyatrazine was extracted from aqueous solutions.

Results and Discussion

Atrazine-^{14}C-Isopropyl

Parkdale-A and C soils respired approximately 1.0 and 0.3%,

respectively of the input atrazine-^{14}C-isopropyl side chain as $^{14}CO_2$

in three weeks (Table 2). These results demonstrate a very low rate

of attack by soil microorganisms on the isopropyl side-chain constitu-

ent as compared to the ethyl side-chain component. A slight decrease

in percent $^{14}CO_2$ evolved during three weeks of incubation is noted in

the Parkdale-A soil at the higher concentration. The evolution of

$^{14}CO_2$ was not affected by the 2X-concentration in the Parkdale-C

soil.

Table 2. Evolution of $^{14}CO_2$ from ^{14}C-atrazine and ^{14}C-hydroxyatrazine-treated soils.

Soil[2]	Substrate	Conc. ppm	% of $^{14}CO_2$ evolved/3-day interval, expressed as % of input [1]									
			1	4	7	10	13	Σ 13	16	19	22	Σ 22
Parkdale-A	Atrazine	2.96	0.33	0.59	0.56	1.15	1.15	3.78	1.20	1.10	1.27	7.46
Parkdale-A[3]	^{14}C-ethyl	2.96	0.40	0.52	0.88	1.12	1.12	4.04	1.20	1.10	1.29	7.68
Parkdale-A	Atrazine-^{14}C-isopropyl	2.78	0.06	0.14	0.09	0.14	0.12	0.56	0.16	0.14	0.12	0.99
Parkdale-A		5.20	0.06	0.12	0.10	0.11	0.08	0.47	0.14	0.11	0.14	0.84
Parkdale-C		2.78	0.01	0.06	0.04	0.04	0.04	0.20	0.04	0.03	0.02	0.29
Parkdale-C		5.20	0.01	0.04	0.04	0.06	0.04	0.19	0.04	0.03	0.02	0.28
Parkdale-A	Atrazine-^{14}C-ring	2.58	0.01	0.02	0.01	0.01	0.01	0.06				
Woodburn		2.58	0.01	0.02	0.02	0.03	0.04	0.12				
Parkdale-A	OH-Atrazine-^{14}C-ring	1.59	0.16	1.28	0.63	0.20	0.14	2.40				
Woodburn		1.59	0.59	1.76	0.52	0.13	0.10	3.08				

[1] Average of duplicates; air-flow rate = 5 cc/min

[2] Moisture content as % of field capacity: Parkdale-A and Parkdale-C = 80%; Woodburn = 90%

[3] Air-flow rate = 10 cc/min

Atrazine-^{14}C-Ethyl

Evolution of $^{14}CO_2$ from the ethyl side-chain component of

atrazine was dependent on soil type, concentration, moisture content,

and the air-flow rate which passed over the samples during incubation.

The Parkdale-A (Figure 1) and Woodburn (90% of field capacity, Fig-

ure 2) soils exhibited the highest rates of $^{14}CO_2$ evolution with 9.5%

and 10.1%, respectively being evolved in four weeks. The Parkdale-C

(Figure 1) and Coker (Figure 2) soils respired 4.4% and 4.3%, respec-

tively. The slight difference in concentrations of atrazine (2.55, 2.96,

or 3.26 ppm) would have a small effect on CO_2 evolution as discussed

later. Even though the Coker clay soil contained the highest organic

matter content (4.7%), its degradative potential was no greater than

the C-horizon of the Parkdale silt loam soil (1.1% O. M.). The $^{14}CO_2$

evolved from Parkdale-A (3.1% O.M.) was approximately two-fold

greater than from Parkdale-C soil (Figure 1). Moreover, the microb-

ial population in the Coker clay soil was much greater than in the

Parkdale-C soil (Table 1) which suggests that degradation by soil

microorganisms may be a function of qualitative as well as quanti-

tative differences in the microbial populations. Perhaps adsorption

of atrazine by the neutral Coker clay soil which contained approxi-

mately 65% montmorillonite protected the atrazine molecule from

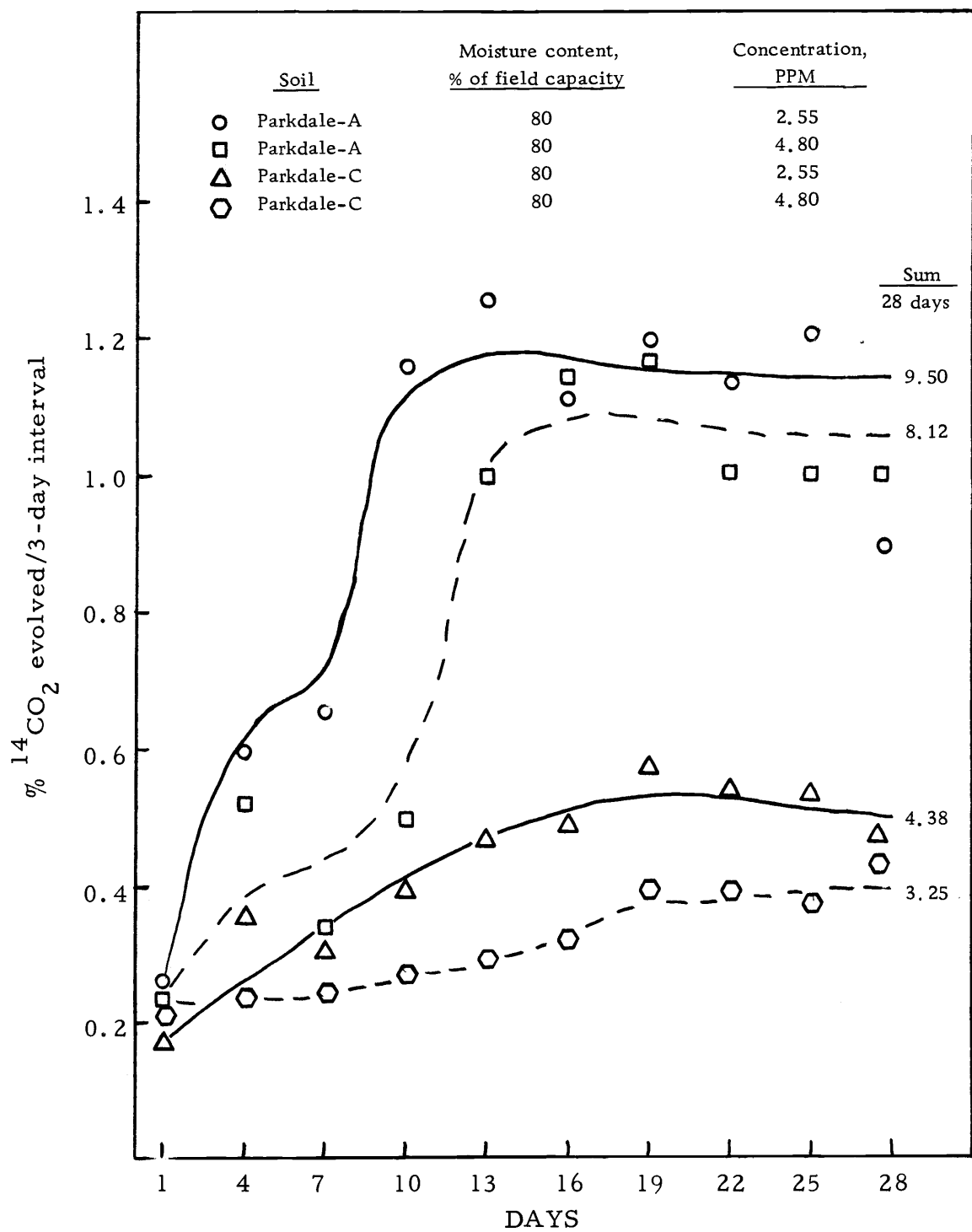microbial attack (12). Weber and Coble (33) have reported a

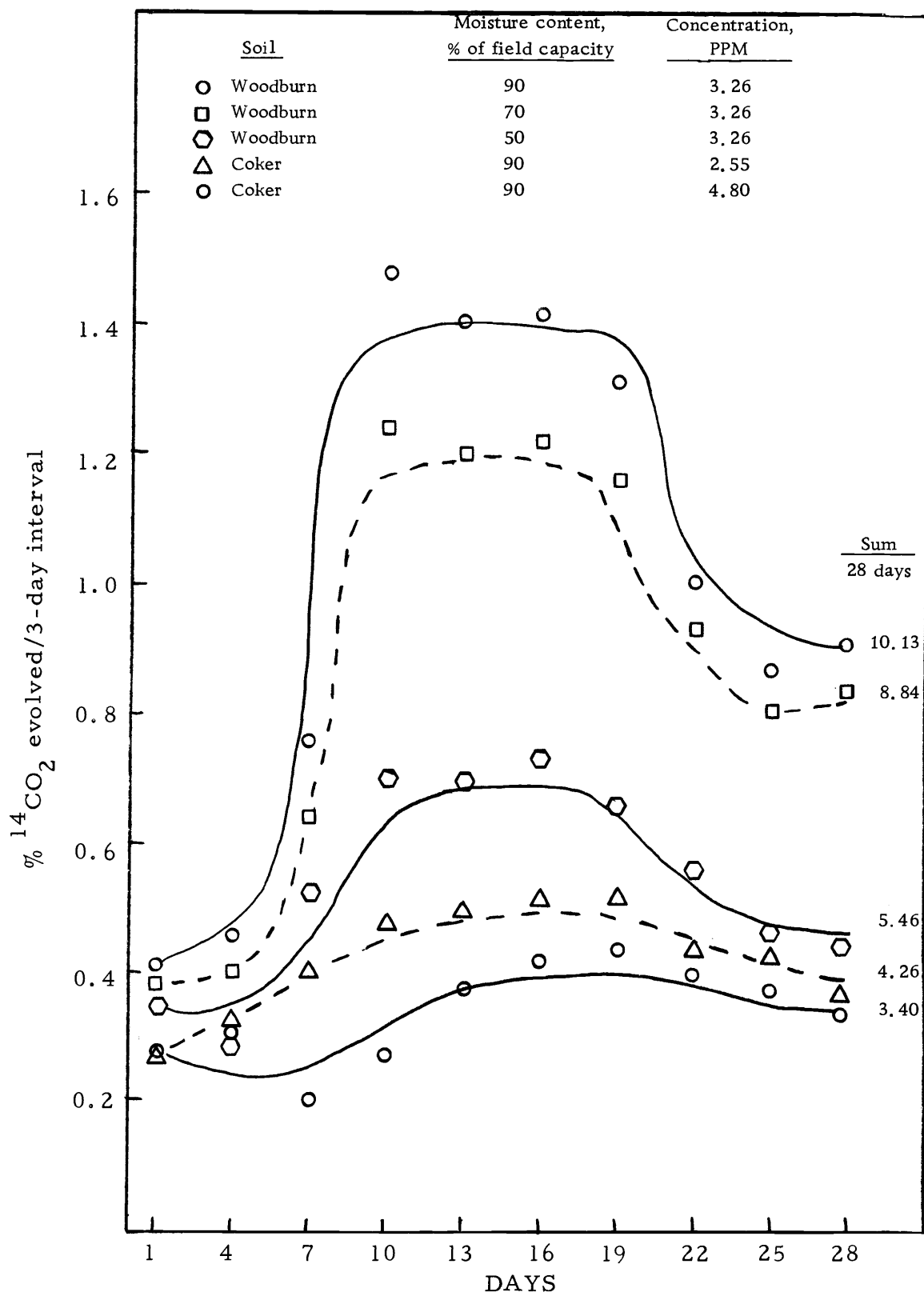Figure 1. Evolution of $^{14}CO_2$ from atrazine-^{14}C-ethyl-treated Parkdale-A and C soils.

Figure 2. Evolution of $^{14}CO_2$ from atrazine-^{14}C-ethyl-treated Woodburn and Coker soils.

reduction in microbial attack on diquat when the herbicide was ad-

sorbed by montmorillonite. Thus, a general correlation between

organic matter content and microbial populations and atrazine degrad-

ation is not operative across these soils, but may be operative be-

tween the two horizons of the Parkdale silt loam soil.

Since the percent $^{14}CO_2$ evolved from atrazine-^{14}C-ethyl was

approximately eight-fold greater than observed for atrazine-^{14}C-

isopropyl, microbial attack appears to be predominantly on the ethyl

side-chain of atrazine. Possible degradative pathways of the side-

chain components of s-triazines include: 1) dealkylation of the ethyl

side-chain by hydroxylation of the carbon atom adjacent to the amino

group, if analogous to free radical systems (17); and 2) dealkylation

and deamination of aromatic molecules by monooxgenases (13). Steric

hinderance may limit the availability of the isopropyl side-chain to

microbial attack.

When atrazine-^{14}C-ethyl was applied at concentrations twice

that of field rates, the percent $^{14}CO_2$ evolved in four weeks decreased

in Parkdale-A from 9.5% to 8.1%, 4.4% to 3.2% in Parkdale-C (Fig-

ure 1), and 4.3% to 3.4% in Coker (Figure 2) soils. Moreover, a

decrease in the percent of $^{14}CO_2$ evolved is noted in Parkdale-A and

Coker soils between the four and seven day intervals, which was

not observed at the lower rates. Thus, caution must be exercised

in the interpretation of CO_2 evolution data when substrates are added

at concentrations in excess of field rates.

Woodburn soil at 50%, 70%, and 90% of field capacity respired 5.5%, 8.5%, and 10.1%, respectively (Figure 2) of the ^{14}C-ethyl component in four weeks. The lower moisture contents may reduce microbial activity, may limit the availability of atrazine, or may cause increased atrazine hydrolysis. Degradation of hydroxyatrazine-^{14}C-ethyl, which may arise from hydrolysis of atrazine-^{14}C-ethyl during incubation, has not been examined.

A lag phase is suggested in the degradation of the ^{14}C-ethyl side chain of atrazine in Parkdale-A and Woodburn soils. A two-fold increase in $^{14}CO_2$ evolution occurred between the seven and ten day intervals in the Parkdale-A soil at field rates (Figure 1) and between the ten and 13 day intervals at the 2X-concentration of atrazine. Approximately two-fold increases in the $^{14}CO_2$ evolution are noted for the field rate in the Woodburn soil at 50%, 70%, and 90% of field capacity between the four and seven and the seven and ten day intervals (Figure 2). The magnitude of the CO_2 released from the Woodburn soil at 50% of field capacity was lower than observed at 70% or 90% of field capacity.

The Parkdale-C (Figure 1) and Coker (Figure 2) soils did not exhibit an "apparent lag phase" in the degradation of atrazine-^{14}C-ethyl. These soils respired a low and relatively constant rate of $^{14}CO_2$ throughout the 28 days incubation. The previously reported

lag phase in microbial degradation of triazine herbicides based on

CO_2 evolution may indicate a lag phase, but could have been compli-

cated by increased respiration of the soil population induced by the

triazine herbicides (8).

An air-flow rate of 10 cc/min stimulated approximately a 1.5-

fold increase in $^{14}CO_2$ evolution from atrazine-^{14}C-ethyl between the

four and seven and between the seven and ten day intervals in Park-

dale-A soil (Table 2). However, the evolved $^{14}CO_2$ was only 0.2%

higher than the 5 cc/min rate in three weeks (Table 2) which is not

considered significant. The displaced lag phase induced by the higher

flow rate may affect short term experiments.

Atrazine-^{14}C-Ring

A low rate of $^{14}CO_2$ was evolved from the heterocyclic triazine

ring as only 0.1% of the added ^{14}C-atrazine was evolved in two weeks.

Thus, the Cl-s-triazine ring was highly resistance to cleavage by the

microbial populations of Parkdale-A and Woodburn soils.

Hydroxyatrazine-^{14}C-Ring

The percent $^{14}CO_2$ evolved from the triazine ring of hydroxy-

atrazine was approximately 25-fold greater than observed for the

atrazine ring (3.08% vs 0.12%) in the Woodburn soil (Table 2) and

40-fold greater in the Parkdale-A soil (2.40% vs 0.06%). Initial

concentrations of atrazine-^{14}C-ring (2.58 ppm) and hydroxyatrazine-^{14}C-ring (1.59 ppm) may account for some of the differences in percent $^{14}CO_2$ evolution in light of the reduced $^{14}CO_2$ evolved from atrazine-^{14}C-ethyl at the 2X-concentration (Figures 1 and 2). Since dihydroxylation is a prerequisite for enzymatic fission of the benzene ring (10), hydrolysis of chloro-s-triazines to hydroxy-s-triazines may also be required for cleavage of the triazine ring. A three-fold increase in $^{14}CO_2$ evolution is expected between the one and four day interval, whereas an explanation for the eight-fold increase in Parkdale-A soil and the rapid decrease after four days in both Parkdale-A and Woodburn soils is not readily apparent (Table 2). Possible factors affecting the $^{14}CO_2$ evolved from hydroxyatrazine-^{14}C-ring include ^{14}C-impurities, greater availability during the initial periods, and perhaps toxic metabolities. A slight difference between the Parkdale-A (2.40%) and the Woodburn (3.08%) soils is noted in their abilities to degrade hydroxyatrazine (Table 2).

Chemical Hydrolysis of Atrazine

A range from 65% to 80% of the ^{14}C-activity added to the soils as atrazine was recovered by soxhlet extraction with methanol (Table 3). The percent recovery of ^{14}C-activity from atrazine-^{14}C-ethyl-treated Woodburn soil increased from 59.9% to 69.5% as the moisture content of the samples decreased from 90% to 50% of field capacity.

Table 3. Recovery of atrazine and hydroxyatrazine from atrazine-^{14}C-ethyl-treated soils.[1]

Soil	Moisture content, % of field capacity	^{14}C-atrazine input		Extracted ^{14}C-activity		Hydroxyatrazine in extract	
		ppm	μc	μc	%	μc	%
Parkdale-A[2]	80	4.80	1.849	1.257	68.0	0.135	10.7
Parkdale-C	80	4.80	1.849	1.451	78.5	0.181	12.5
Coker	90	4.80	1.849	1.423	77.0	0.144	10.1
Woodburn	50	3.26	1.255	0.872	69.5	0.327	37.5
Woodburn	70	3.26	1.255	0.831	66.2	0.354	42.6
Woodburn	90	3.26	1.255	0.752	59.9	0.284	37.8

[1] Average of duplicates extracted with methanol after 28 days of incubation.

[2] Extracted after 40 days; $^{14}CO_2$ evolved = 12% of input.

Hydroxyatrazine still accounted for approximately 40% of the extracted ^{14}C-activity at the three moisture contents (Table 3). Hydroxyatra-

zine accounted for approximately 10-12% of the ^{14}C-activity recovered

from the Parkdale-A, Parkdale-C, and Coker soils (Table 3). If one

assumes the extraction efficiency of hydroxyatrazine from each soil

to be equivalent, the four-fold increase in the hydrolytic potential for

the Woodburn soil may arise from a lower soil pH (28), a greater

surface charge density of vermiculite (21), or a more acidic form

of organic matter in the Woodburn soil. In other work the authors

have noted the lack of hydrolysis in the clay fraction of Parkdale-C

and only a trace of hydrolysis for the neutral Coker soil clay fraction.[1]

The 10-12% of hydroxyatrazine extracted by methanol in these studies

probably reflects an incubation time of 28 days rather than 24 hours

which was utilized in the studies on clay-triazine systems.

Conclusions

The atrazine molecule is subject to slow microbial degradation

by N-dealkylation of the ethyl side-chain constituent, while both the

isopropyl side-chain component and the triazine ring undergo minimal

attack. The hydroxyatrazine ring appears more susceptible to

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[1]Skipper, H. D., V. V. Volk, M. M. Mortland, and K. V. Raman. 1969. Hydrolysis of atrazine on soil colloids. (manuscript in preparation)

microbial attack than the atrazine ring based on the $^{14}CO_2$ evolved.

Increased moisture content (50% to 90% of field capacity) resulted in

a two-fold increase in the microbial degradation rate of atrazine-^{14}C-

ethyl. Hydolysis is considered the dominant pathway of detoxification

of atrazine in the Woodburn soil, whereas a combination of chemical

hydrolysis and microbial degradation are responsible for detoxifica-

tion of atrazine in the Parkdale-A, Parkdale-C, and Coker soils.

SUMMARY AND CONCLUSIONS

Infrared and solubility data suggest the following tautomeric forms of hydroxyatrazine with changes in pH: 1) an anionic species at pH > 11.5, 2) an enol form between pH 11.5 and 3.3, 3) a keto form at pH < 3.3 and 4) a protonated-keto species at pH < 0. Characteristic infrared bands for the forms of hydroxyatrazine are: 1) enol, OH deformation at 1578 cm^{-1} and triazine ring stretch at 1692, 1645, and 795 cm^{-1}; 2) keto, carbonyl band at 1775 cm^{-1}, triazine ring stretch at 1678, 1620, and 765 cm^{-1}, and covalent ring-NH stretch at 3220 cm^{-1}; and 3) protonated-keto, carbonyl band at 1750 cm^{-1}, triazine ring stretch at 1672, 1613, and 770 cm^{-1}, covalent ring-NH stretch at 3200 cm^{-1}, and protonated-ring-NH doublet at 3400 and 3520 cm^{-1}. The infrared absorption bands of the triazine ring shifted to lower frequencies as a result of the bond order decrease of hydroxy-atrazine ring with increased acidity.

A number of the infrared band assignments for enol, keto, and protonated-keto hydroxyatrazine are also applicable to hydroxysimazine and hydroxypropazine. However, marked differences between hydroxyatrazine and hydroxysimazine or hydroxypropazine were noted in infrared spectra of the latter two analogs when recrystallized in a strongly acidic environment (6N HCl). A single protonated-ring-NH stretch band at 3330 cm^{-1} was observed in the protonated form of

hydroxysimazine rather than a doublet as noted above in the protonated-keto hydroxyatrazine. Inductive effects of asymmetrical side chains in hydroxyatrazine may account for the doublet at 3400 and 3520 cm^{-1}, whereas symmetrical side chains in hydroxysimazine are correlated with a single band at 3330 cm^{-1} (protonated-ring-NH stretch).

A high frequency band (3200 to 3600 cm^{-1}) which could be associated with a stretch vibration of protonated-ring-NH was not observed in the infrared spectra of hydroxypropazine recrystallized from 1N HCl, 6N HCl, or 12N HCl which indicates hydroxypropazine was apparently not protonated. Thus, a marked difference exist between the three hydrolytic analogs of atrazine, simazine, and propazine with regards to protonation.

The presence of a strong carbonyl band (1745 cm^{-1}) of hydroxy-atrazine indicates that montmorillonite (Wyoming bentonite) and a "natural montmorillonitic clay" from the Coker clay soil hydrolyzed atrazine, especially when the exchange complex was saturated with the acidic cations, H^+ and Al^{3+}. The reaction products of atrazine with Ca- or Cu-montmorillonite did not exhibit a 1745 cm^{-1} band, whereas a weak carbonyl band was observed in the reaction product of atrazine with the Cu-Coker soil clay sample. The presence of the hydrolytic product in the Cu-Coker soil clay may reflect a more acidic surface than exist in the Cu-montmorillonite. Dehydration at 75° C increased the hydrolysis of atrazine in Ca- or Cu-Coker

clay samples as evidenced by a more intense carbonyl band of hydroxyatrazine (1745 cm^{-1}), whereas the reaction was not affected in Ca- or Cu-montmorillonite samples.

An "allophanic" colloid (Parkdale-C) did not catalyze the hydrolysis of atrazine when the exchange complex was saturated with H^+, Al^{3+}, Ca^{2+}, or Cu^{2+}. The infrared spectrum of the Al-saturated "allophanic" colloid treated with atrazine and subsequently heated to 70° C did show a weak carbonyl band at 1725 cm^{-1} which is indicative of some hydrolysis.

The lack of a hydroxyatrazine carbonyl band in the reaction product of atrazine plus the Parkdale-C colloid indicates a major difference between "allophanic" colloids and montmorillonite as catalysts in the protonation and hydrolysis of atrazine. Thus under acidic field conditions one might expect the smectites to enhance chemical hydrolysis of atrazine while the "allophanic" and perhaps other amorphous materials would be relatively inert.

The atrazine molecule is subject to slow microbial degradation by N-dealkylation of the ethyl side-chain component. Approximately 10% of the input atrazine-^{14}C-ethyl was evolved as $^{14}CO_2$ from the Parkdale-A and Woodburn soils while approximately 4.5% was respired from the Parkdale-C and Coker soils in four weeks. Approximately 1% and 0.3% of the atrazine-^{14}C-isopropyl side-chain constituent was evolved from the Parkdale-A and Parkdale-C soils,

respectively in three weeks. The atrazine ring was subject to minimal attack (0.1% $^{14}CO_2$ evolved in two weeks) by the soil microorganisms in Parkdale-A and Woodburn soils. The hydroxyatrazine ring was subject to more degradation than the atrazine ring since approximately 3% of the hydroxyatrazine-^{14}C-ring was evolved as $^{14}CO_2$ in two weeks.

An increase in the moisture content from 50% to 90% of field capacity stimulated a two-fold increase in $^{14}CO_2$ evolved from atrazine-^{14}C-ethyl in the Woodburn soil. An atrazine concentration of 5 ppm (ca. 2X-field rates) reduced the $^{14}CO_2$ evolved from atrazine-^{14}C-ethyl in Parkdale-A soil by 1% (9.5% to 8.1%) and a similar effect of concentration was observed in Parkdale-C and Coker soils.

Hydroxyatrazine accounted for approximately 10% of the ^{14}C-activity (applied as atrazine-^{14}C-ethyl) extracted by methanol from Parkdale-A, Parkdale-C, and Coker soils, and 40% from the Woodburn soil. Hydrolysis is considered the dominant pathway of detoxification of atrazine in the Woodburn soil, whereas a combination of chemical hydrolysis and microbial decomposition participate in the inactivation of atrazine in Parkdale-A, Parkdale-C, and Coker soils.

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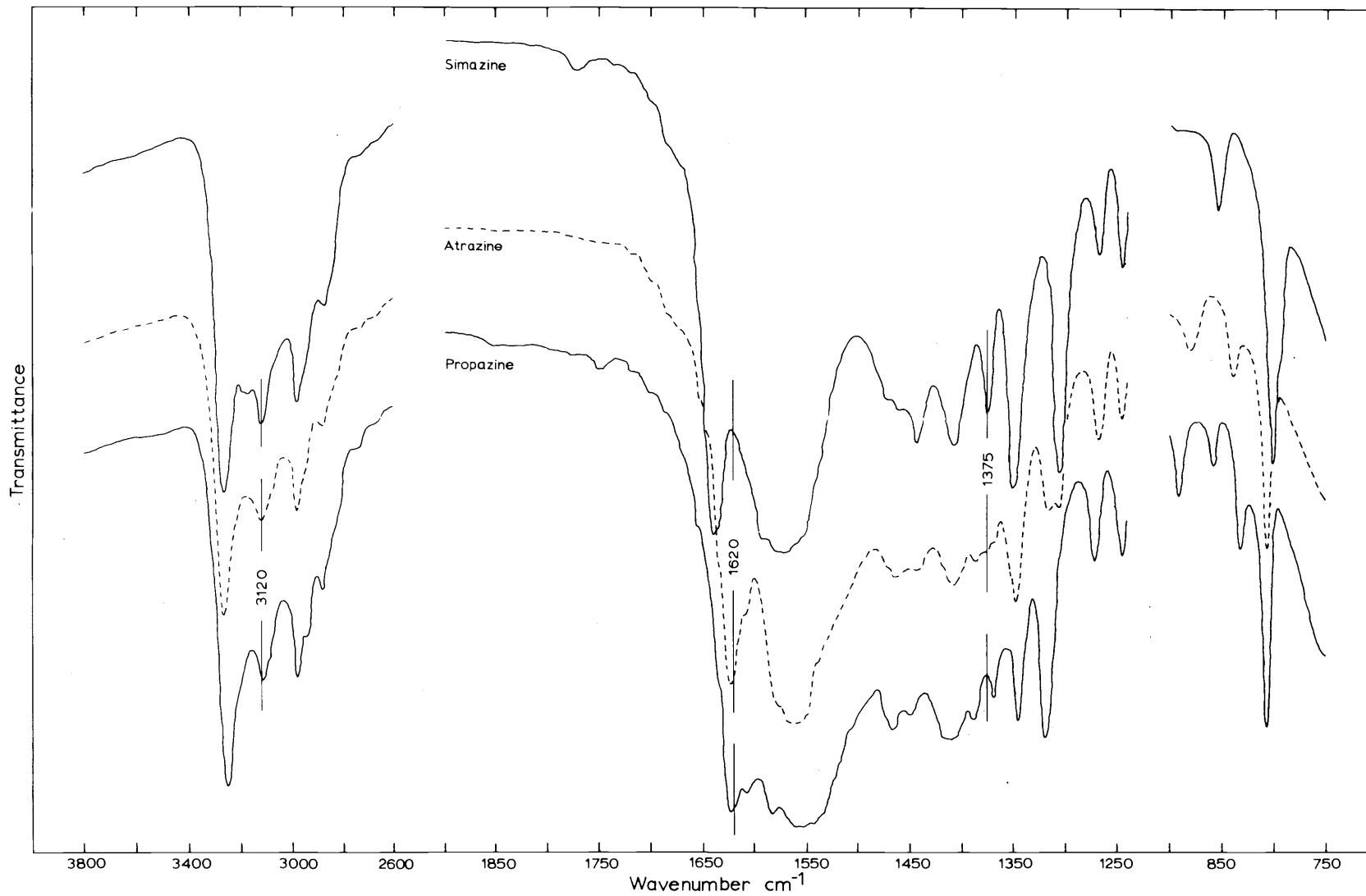
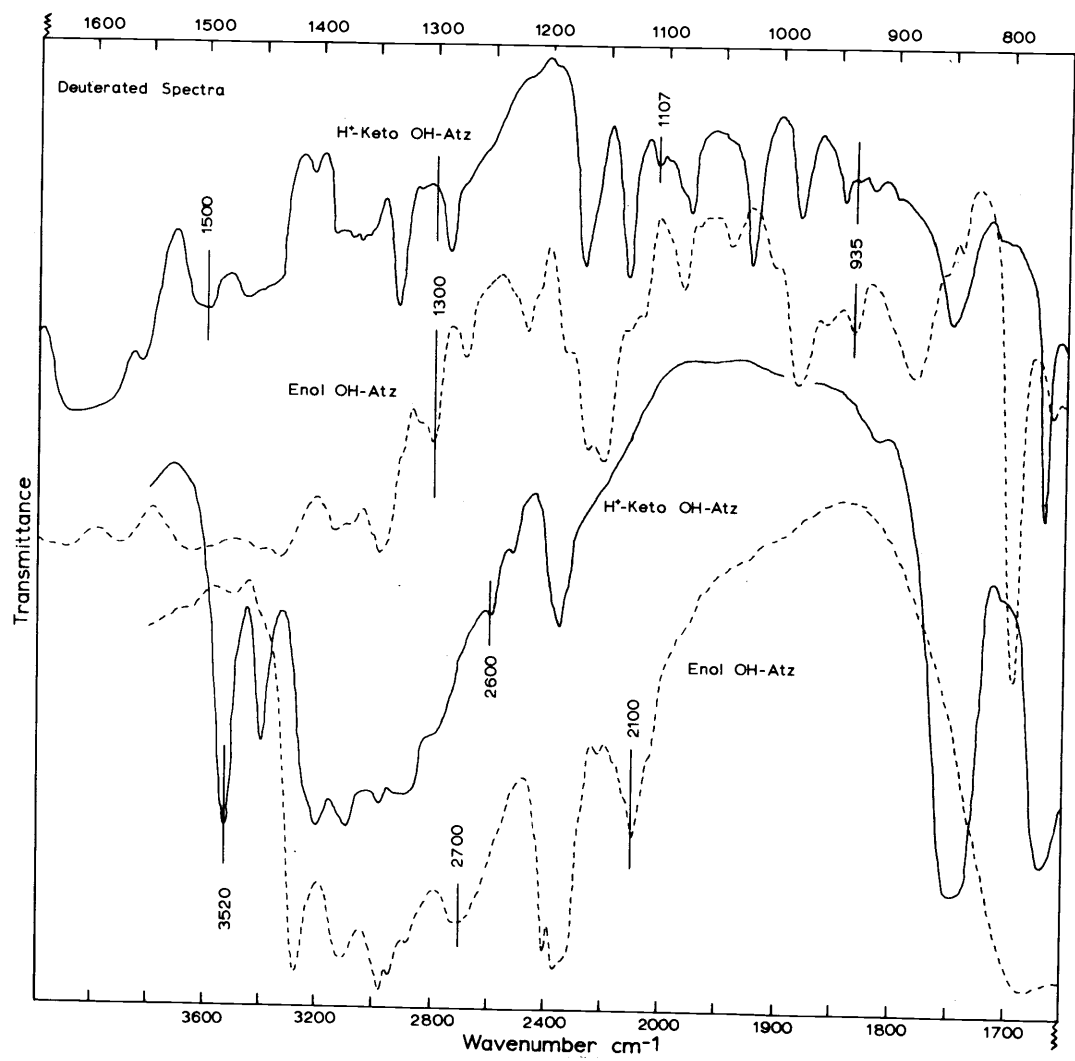Figure 1. Infrared spectra of simazine, atrazine, and propazine.

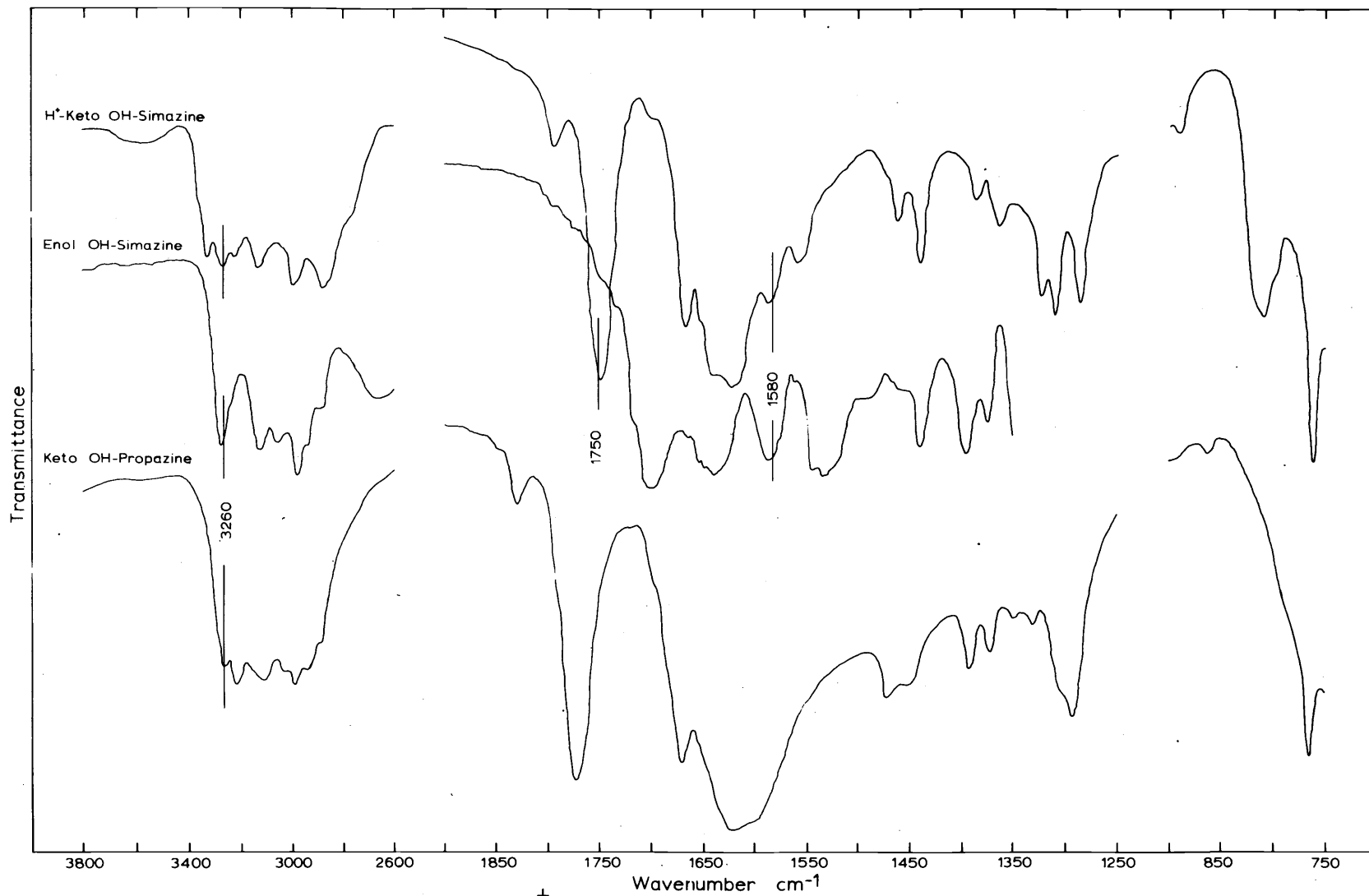Figure 2. Infrared spectra of deuterated H$^+$-keto and enol forms of hydroxyatrazine.

Figure 3. Infrared spectra of H$^+$-keto, enol hydroxysimazine, and keto hydroxypropazine.
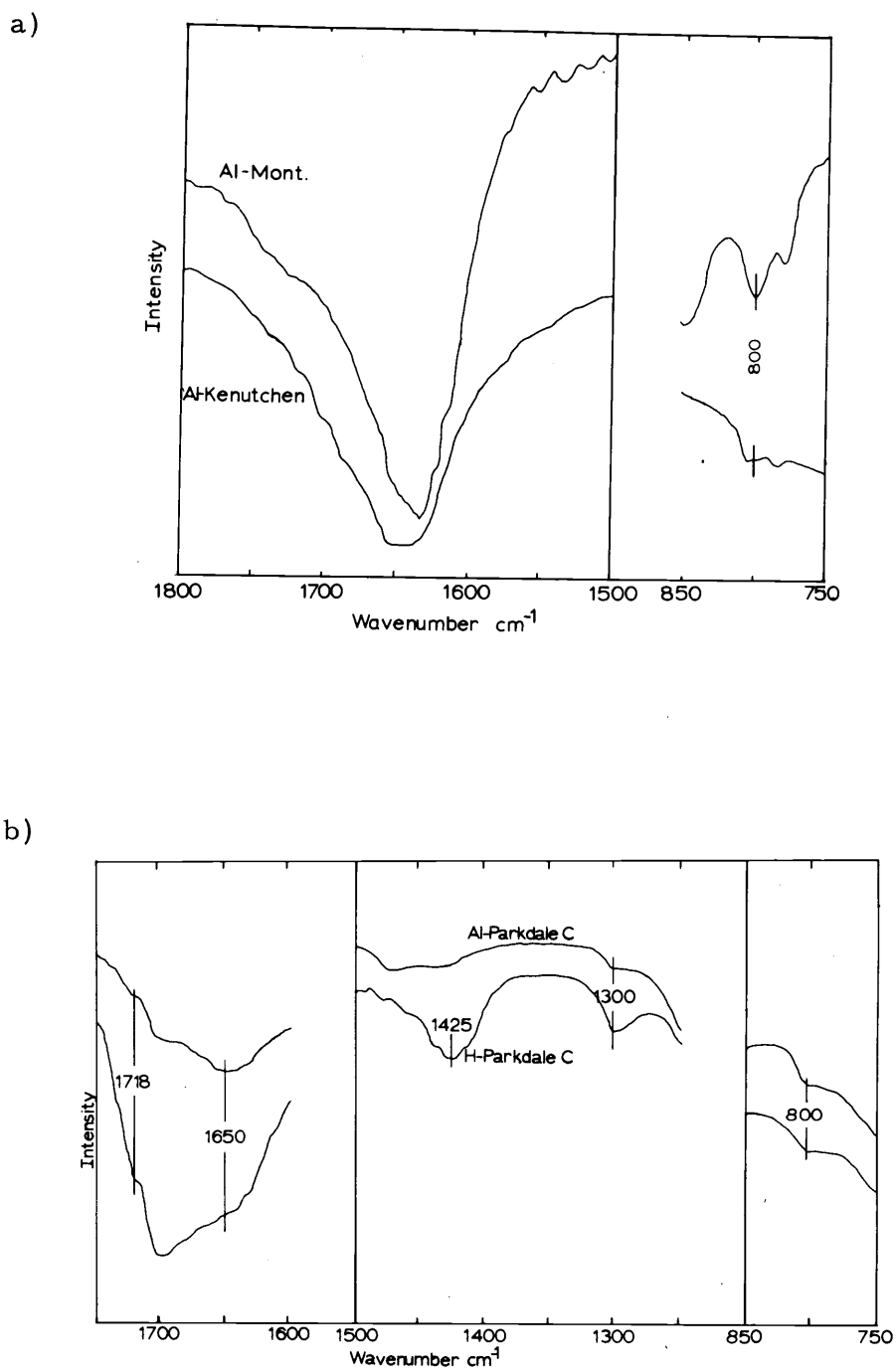
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a)

b)

Figure 4. Infrared spectra of homoionic clays: a) Al-mont-
morillonite and Al-Kenutchen (Coker) and b) Al- and
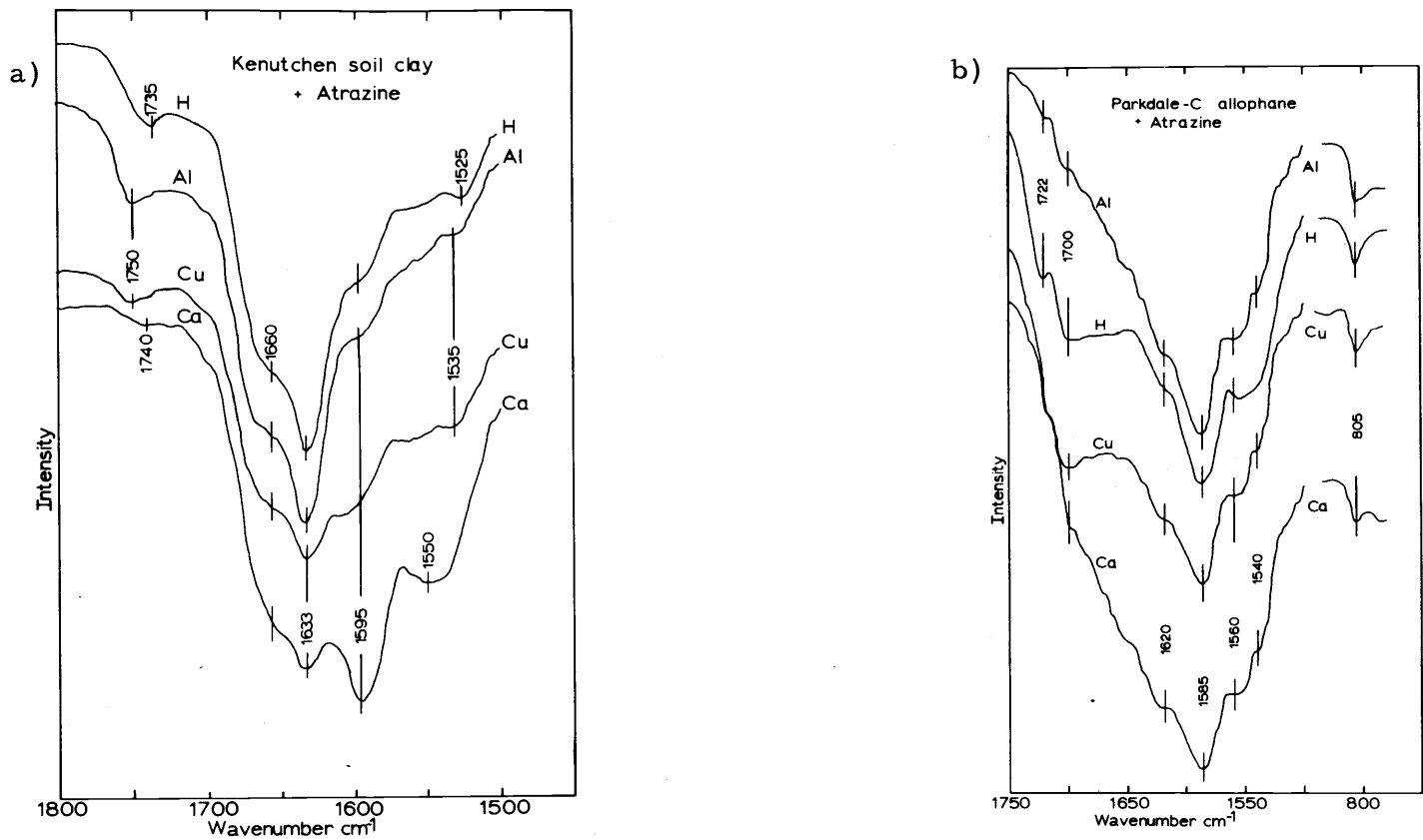H-Parkdale-C.

Figure 5. Infrared spectra of the reaction products of: a) Atrazine plus H-, Al-, Cu-, or Ca-Kenutchen (Coker) soil clay and b) Atrazine plus Al-, H-, Cu-, or Ca-Parkdale-C (allophane) clay.
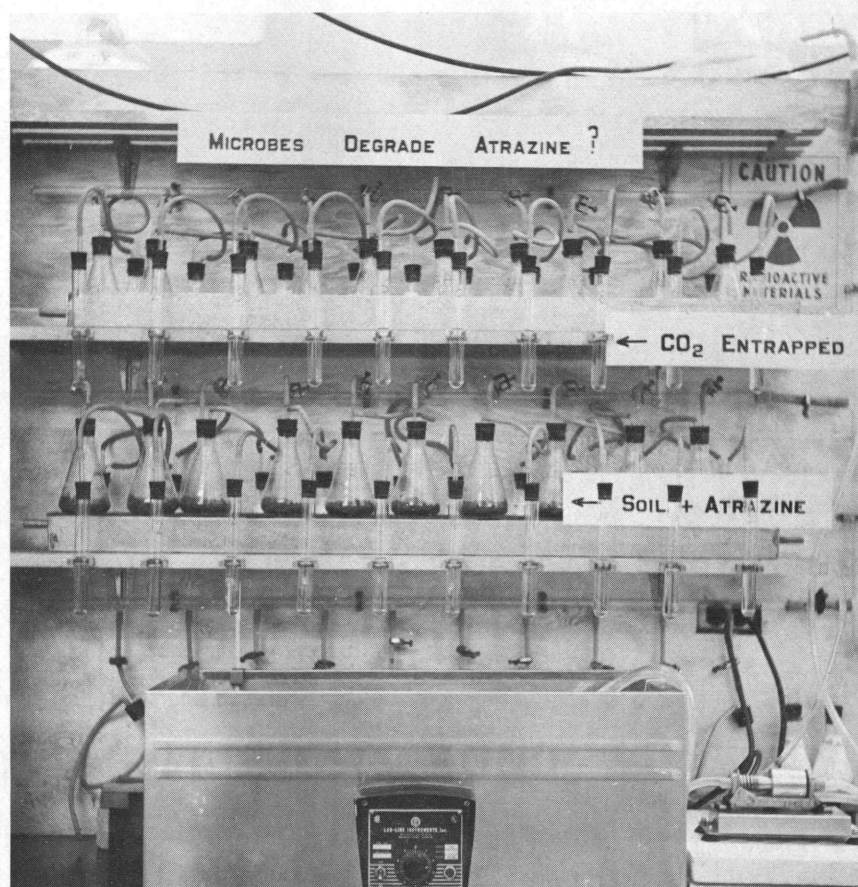
Figure 6. Microbial degradation of ^{14}C-atrazine is measured by evoluation of $^{14}CO_2$.