Hydrolysis of a Chloro-*s*-triazine Herbicide

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Chloro-*s*-triazine herbicides undergo hydrolysis in the environment to nonphytotoxic hydroxy analogues. Transitional forms of hydroxyatrazine [2-hydroxy-4-(ethylamino)-6-(isopropylamino)-*s*-triazine] were identified at different pH values through the use of infrared spectroscopy. The infrared spectra suggest the existence of enol, keto, and protonated-keto forms of hydroxy-*s*-triazines. The following sequential molecular transformations are correlated with changes in pH: (a) an anionic species at pH >11.5, (b) an enol form between pH 11.5 and 3.3, and (c) a keto or protonated-keto species at pH <3.3.

Atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine] may persist in soils for more than one growing season when applied at rates of 2 to 4 lb per acre for control of grasses and broadleaf weeds (Herbicide Handbook of the Weed Science Society of America, 1974). Temperature, moisture, and soil organic matter content optimal for microbial activities generally correlate with the detoxification of atrazine (McCormick and Hiltbold, 1966). Since hydroxyatrazine [2-hydroxy-4-(ethylamino)-6-(isopropylamino)-*s*-triazine], which is nonphytotoxic, 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 the soil environment (Armstrong et al., 1967; Harris, 1967; Skipper et al., 1967; Skipper and Volk, 1972). Direct evidence of chemical hydrolysis of chloro-*s*-triazines was ascertained by allowing atrazine to react with acidic montmorillonitic clays. The subsequent appearance of a carbonyl band in infrared spectra indicated the presence of hydroxyatrazine (Russell et al., 1968).

The research herein was conducted to assign infrared bands to functional groups in atrazine–hydroxyatrazine systems. With the assignment of functional groups for chloro-*s*-triazine compounds and their hydrolytic products, the contribution of hydrolysis to the dissipation of these herbicides and the identification of their degradation products could be determined by infrared spectroscopy.

MATERIALS AND METHODS

Atrazine was hydrolyzed in 20 ml of 6 *N* HCl plus 20 ml of ethanol at room temperature for 24 hr. The hydrolytic products were dried at 65°C and subsequently recrystallized twice from ethanol–water and water. Atrazine was also hydrolyzed by refluxing in 9 *N* HCl for 2 hr and titrating with sodium hydroxide to pH 3.3, 7.0, 8.4, or 10.0. Each precipitate was washed with 50% methanol and dried at 65°C. Additional hydroxyatrazine compounds were obtained upon reaction of technical hydroxyatrazine with equal volumes of ethanol and 1 or 6 *N* HCl solutions. These recrystallized products were not further washed. Since preliminary experiments had indicated that protonated hydroxyatrazine was metastable with respect to heat, subsamples of the acidic hydroxyatrazine preparations were heated at 100°C for 24–48 hr.

Infrared spectra of deuterated hydroxyatrazine were obtained following hydrolysis of technical atrazine in deuterated 1 *N* HCl and recrystallization in the acidic environment and after titration with NaOD to pH 10.2.

All hydrolysis treatments and infrared absorption patterns were replicated. Since the hydrolytic analogues have limited solubilities in organic solvents and water, infrared spectra were recorded in KBr pellets, Nujol, and Fluorocarbon (KEL-F10) mulls between Irtran-2 (ZnS) disks with a Beckman IR-7 spectrophotometer.

RESULTS AND DISCUSSION

Atrazine hydrolyzes to form enol, keto, and protonated-keto forms of hydroxyatrazine with an increase in acidity (Figure 1). Infrared spectra and observed solubility at pH <3.3 and pH >11.5 suggest the following forms: (a) an anionic species at pH values above 11.5, (b) predominately an enol form between pH 11.5 and 3.3, and (c) a keto or protonated-keto (H^+-keto) form at pH values below 3.3.

The infrared spectra of the hydrolyzed atrazine precipitates prepared between pH 3.3 and 10 were very similar to each other and also similar to technical hydroxyatrazine, thus supporting the existence of a single molecular species, the enol form. The 1692- and 1645-cm^{-1} frequencies (Figure 2, Table I) decreased upon further acidification with 1 or 6 *N* HCl. These frequencies are assigned predominantly to the skeletal ν(C=N) whose in-plane vibration would change as protons are added to the ring during the formation of the keto and protonated-keto forms of hydroxyatrazine. The triazine rings of the enol, keto, and protonated-keto forms of hydroxyatrazine contain three, two, and less than two double bonds, respectively. Thus, the average bond order of the triazine ring decreases in the above transition and the frequencies of the skeletal ν(C=N) and out-of-plane deformations would decrease. The 1692-cm^{-1} frequency may have a slight contribution from a C⋯O species, since the amide II band (ν(C=N)) of amides has been shown to contain a small percentage of ν(C=O) character (Pimemtel and McClellan, 1960). Chen (1967) previously assigned the 1692-cm^{-1} frequency of hydroxytriazines to ν(C=O) in OH···O=C; however, a covalent ring ν(NH) was not reported for the keto form. Chen (1967) also assigned the 1621-cm^{-1} band in hydroxytriazines to a ν(C=O) in NH···O=C and/or to a C=N skeletal triazine ring in-plane vibration. Good (1961), however, 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 decreased upon protonation.

The spectra from the precipitates prepared at pH 3.3–10 did not reveal a covalent ring ν(NH), characteristic of the keto and protonated-keto forms. A ring ν(NH) did appear at 3220 cm^{-1} in samples prepared from 1 or 6 *N* HCl to support the occurrence of the keto and/or protonated-keto hydroxyatrazine. A 3215-cm^{-1} band (ν(NH)) was also observed in the keto form of cyanuric acid. The absorption bands at 3130 and 3260 cm^{-1} (Table I) were assigned to

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Table I. Infrared Absorption Frequencies of the Enol, Keto, and Protonated-Keto Forms of Hydroxyatrazine

Structural group	Frequency, cm^{-1}, for hydroxyatrazine forms							
	Enol		Keto		Protonated-keto			
Triazine ring								
ν in-plane	1692	1645	1678	1620	1673		1613	
δ out-of-plane	795		765		770			
ν(NH) chain	3260	3130	3270[a]	3130	3240[a]		3100	
ν(ND) chain	2400[a]	2350						
ν(NH) ring			3220		3200	3400	3520	
ν(ND) ring					2350	2520	2600	
ν(ND)/ν(NH)	0.736	0.751			0.734	0.739	0.741	
δ(NH) chain in-plane	1528	1300	1525	1300	1500		1290	
δ(ND) chain in-plane	1130	935			1107		920	
δ(ND)/δ(NH)	0.739	0.719			0.738		0.713	
ν(OH)	2700							
ν(OD)	2100							
ν(OD)/ν(OH)	0.777							
δ(OH) in-plane	1578							
δ(OD) in-plane	1220							
δ(OD)/δ(OH)	0.773							
δ(OH) out-of-plane	885							
ν(C=O)			1775				1750	
ν(CH) chain	3050	2940	3020	2930	3050		2940	
	2980	2885	2980	2880	2980		2900	
δ(Isopropyl) (CH_3 scissors)	1390	1375	1390	1370	1390		1370	

[a] Shoulder.

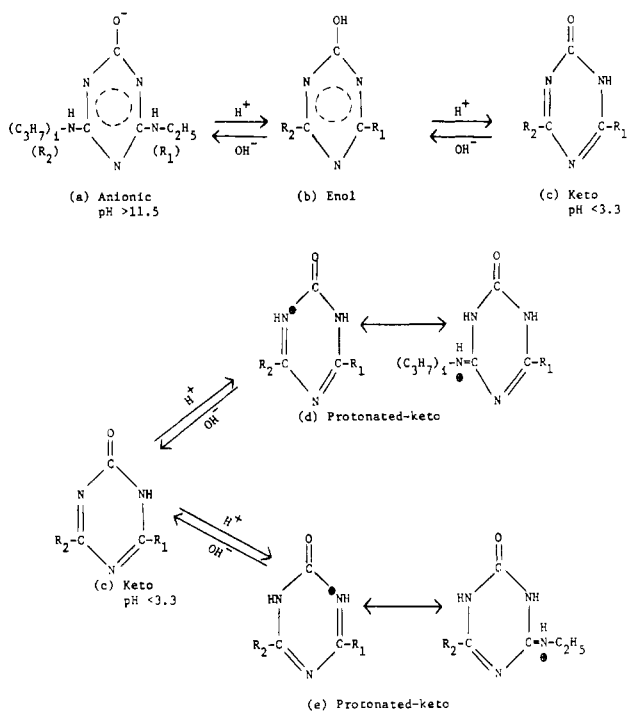

Figure 1. Chemical structures for transitional forms of hydroxyatrazine.

chain ν(NH) vibrations as they were observed in both atrazine and hydroxyatrazine.

A doublet appeared at 3400 and 3520 cm^{-1} in hydroxyatrazine prepared under more acidic conditions, 1 and 6 *N* HCl. Since deuterium stretching frequencies (Figure 3) of the doublet appeared at 2520 and 2600 cm^{-1} for ν(ND)/ν(NH) values of 0.739 and 0.741, respectively, these bands were assigned to a protonated-keto cyclic ring NH, rather than to overtone frequencies of the carbonyl group. The weak intensity of the doublet from the deuterated protonated-keto compound resulted from exchange of the D protons with atmospheric water. Protonation at either the one or the three position would produce a single frequency, whereas a mixture of two protonated species with asymmetrical side chains (Figure 1d,e) would be expected to give rise to two frequencies to explain the doublet. Spectral evidence also indicates that one of the protonated sites was favored since the 3520-cm^{-1} frequency appeared first and was the more intense band (Figure 2). Moreover, these high frequency bands were not present in the keto compound which had a very strong 1775-cm^{-1} carbonyl band (Figure 2). In contrast, hydroxysimazine [2-hydroxy-4,6-bis(ethylamino)-*s*-triazine], with its symmetrical side chains, exhibited a single high frequency band at 3330 cm^{-1}. Acidic treatments (12 *N* HCl) on hydroxypropazine [2-hydroxy-4,6-bis(isopropylamino)-*s*-triazine] did not produce high frequency absorption bands. Hydroxypropazine was apparently not protonated or the protonated form was very unstable (spectra not presented).

The frequencies of hydroxyatrazine at 3400 and 3520 cm^{-1} decreased or disappeared upon heating at 100°C for 24–48 hr indicating the instability of the protonated-keto form. The deuterated band at 2350 cm^{-1} (Figure 3) was considered a mixture of the deuterium analogues of the covalent ring ν(NH) (3220 cm^{-1}) and the chain ν(NH) (3130 cm^{-1}) with ν(ND)/ν(NH) ratios of 0.734 and 0.751, respectively.

The stretching frequency of the OH group in the enol form was assigned to the broad 2700-cm^{-1} band (Figure 2). When the OH group was deuterated, the frequency decreased to 2100 cm^{-1} for a ν(ND)/ν(NH) value of 0.777. A δ(OH) (bending) in-plane frequency was noted at 1578 cm^{-1} with the deuterated frequency occurring at 1220 cm^{-1} (δ(ND)/δ(NH) ratio of 0.773). These frequencies were not unexpected since extensive hydrogen bonding has been shown to lower the ν(OH) and increase the δ(OH) frequencies (Pimemtel and McClellan, 1960).

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

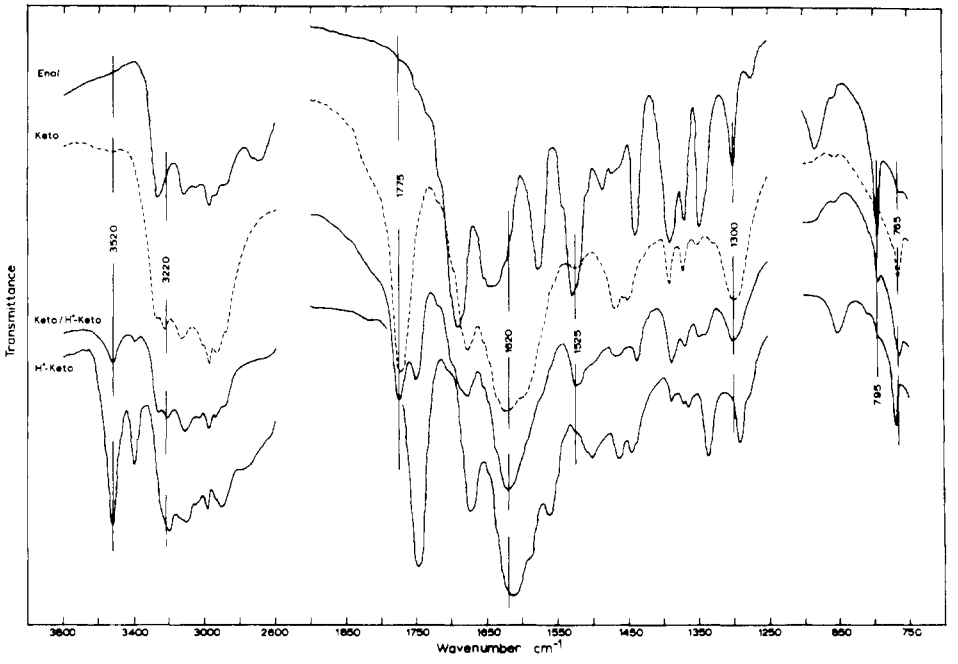

Figure 2. Infrared spectra of the enol, keto, and H^+-keto forms of hydroxyatrazine. Enol spectrum prepared from technical hydroxyatrazine. Spectra of keto and H^+-keto forms prepared from hydroxyatrazine recrystallized in an acidic environment (1 or 6 *N* HCl).

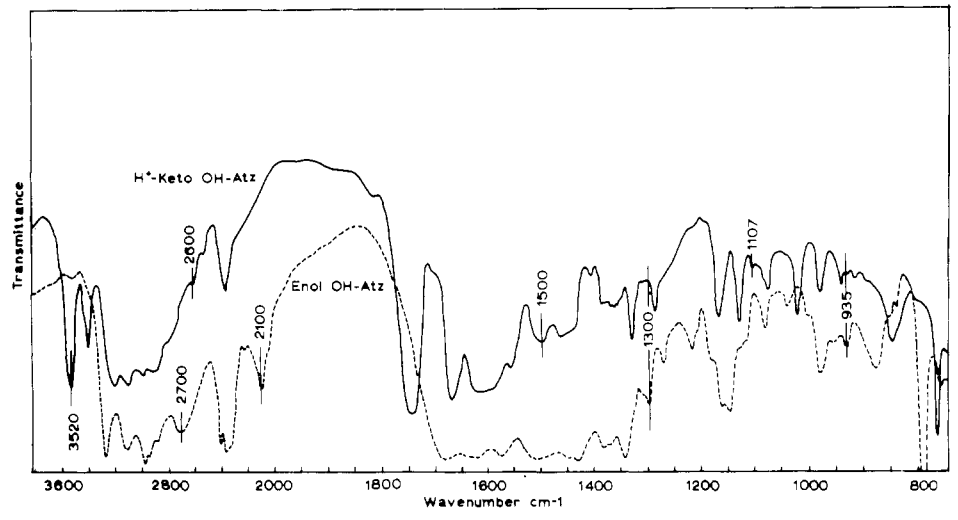

Figure 3. Infrared spectra of deuterated H^+-keto and enol forms of hydroxyatrazine. H^+-keto was recrystallized in an acidic environment (1 *N* $HCl-D_2O$) and enol was titrated with NaOD to pH 10.2.

The enol frequency at 1528 cm^{-1} (Figure 2), ascribed to an in-plane bend of the chain NH, decreased to 1500 cm^{-1} in the protonated-keto form. Analogues prepared from deuterated water exhibited spectral frequencies at 1130 and 1107 cm^{-1} for $\delta(ND)/\delta(NH)$ ratios of 0.739 and 0.738, respectively. The absorption band at 1300 cm^{-1} in the enol form was most likely a chain–NH bend which decreased to 1290 cm^{-1} upon protonation. The latter assignment was supported by frequencies from the deuterated compounds at 935 and 920 cm^{-1} with corresponding $\delta(NH)$ ratios of 0.719 and 0.713, respectively.

Infrared absorption frequencies in the 2800–3000-cm^{-1} region were assigned to the stretching vibrations of chain–CH (Figure 2). Bellamy (1958) has reported a symmetrical CH_3 scissors vibration near 1380 cm^{-1} which splits into two bands near 1385 and 1375 cm^{-1} in isopropyl groups, a situation seen in the hydroxytriazines (Figure 2). The bands for $\nu(CH)$ and CH_3 scissors were affected only slightly in the transition from enol → keto → protonated-keto forms.

The infrared absorption frequencies between 1350 and 1500 cm^{-1} were considered to be mixed modes of C=N skeletal vibrations and chain–NH–CH deformations. The 1345-cm^{-1} frequency (enol) shifted to 1333 cm^{-1} upon protonation, indicating a chain–NH in-plane deformation or perhaps a CH deformation with a weak intensity except when next to an oxygen or nitrogen atom (Bellamy, 1958). The 1490- and 1460-cm^{-1} bands probably contained some asymmetric scissors vibrations and some contribution from the symmetric CH_2 scissors deformation. Protonation may 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 probably include contributions from triazine ring vibrations and would correspond to shifts noted in the 1692- and 1645-cm^{-1} bands.

The decrease in the frequencies of the triazine ring vibrations (1692 → 1678 cm^{-1}; 1645 → 1620 cm^{-1}) and the appearance of a covalent ring $\nu(NH)$ (3220 cm^{-1}) support the transition from enol to keto hydroxyatrazine at a pH of less than 3.3. Additional shifts of the ring vibrations

(1678 → 1673 cm^{-1}; 1620 → 1613 cm^{-1}), a covalent ring ν(NH) at 3220 cm^{-1}, and high frequency bands of 3400 and 3520 cm^{-1} (ring ν(NH)) of protonated-keto support the transition from keto to protonated-keto hydroxyatrazine at more acidic pH values.

The relationships reported herein between functional groups and infrared bands in the atrazine–hydroxyatrazine systems may be used to study the influence of colloids, cation saturation, temperature, pH, and moisture content on the hydrolysis of chloro-*s*-triazines. Infrared spectroscopy would also assist in adsorption–desorption–protonation experiments of chloro-*s*-triazines in aquatic and soil systems.

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Behavior and Fate of Ethylenethiourea in Plants

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When young seedlings and excised leaves of corn, lettuce, pepper, and tomato were pulse-treated with [4,5-^{14}C]ethylenethiourea (ETU), the labeled ETU was readily absorbed by either the roots or the petioles of excised leaves and was translocated primarily via the xylem. After 20 days, only 1 to 2% of the initial dose remained as [^{14}C]ETU, but several degradation and/or metabolic products were present in the methanol-soluble extracts, and part of the ^{14}C was found in the methanol-insoluble residue. Methanol-soluble and -insoluble degradation products were determined in the various tissue sections for a period of 20 days after treatment. Only minor amounts of $^{14}CO_2$ were obtained from treated plants or excised leaves, but a major degradation product was isolated from pepper plants and was tentatively identified by infrared and mass spectroscopy as ethyleneurea (EU), the oxygen analog of ETU.

Ethylenebis(dithiocarbamate) fungicides are used extensively on a number of food crops to control plant pathogenic fungi. These compounds are subject to decomposition, and they yield ethylenethiourea (ETU) as one of the degradation products. Recent reports indicate that ETU is carcinogenic and goitrogenic (Graham and Hansen, 1972; Graham et al., 1973; Innes et al., 1969; Ulland et al., 1972). Analysis of commercial formulations of these fungicides showed that a considerable quantity of ETU was present as a degradation product and an impurity (Bontoyan et al., 1972; Czegledi-Janko and Hollo, 1967; Fishbein and Fawkes, 1965; Lopatecki and Newton, 1952; Ludwig et al., 1954; Petrosini et al., 1963). However, information about the fate of ETU in plants is conflicting, and it has not been clearly established whether ETU is accumulated and persistent (Ross and Ludwig, 1957; Vonk and Sijpesteijn, 1970) or rapidly degraded (Yip et al., 1971).

Because of concern about possible ETU residues in food crops, investigations were undertaken to obtain additional information about the behavior and fate of ETU in plants. Areas studied were ETU uptake and movement, ETU persistence, ETU degradation and/or metabolism, and the isolation and identification of major products of ETU in several mono- and dicotyledonous plants. The results are summarized in this report.

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EXPERIMENTAL SECTION

Chemicals. [4,5-^{14}C]ETU (4.9 mCi/mmol) was purchased from Mallinckrodt (St. Louis, Mo.). Preparative TLC was used to remove minor radioactive impurities from the material before it was used for stock solutions. The nonradioactive ETU and EU used as reference compounds were obtained from Pfaltz and Bauer Chemical, Inc. (Flushing, N.Y.) and recrystallized before use. ETU was recrystallized twice from 95% ethanol (mp 202–203°C), and EU was recrystallized twice from chloroform (mp 130–131°C).

Plant Material. Seeds of bean (*Phaseolus vulgaris* L. var. Black Valentine), corn (*Zea mays* L. var. Northrup King, PX 448), lettuce (*Lactuca sativa* L. var. Great Lakes 659), pepper (*Capsicum frutescens* L. var. Early Calwonder), and tomato (*Lycopersicon esculentum* Mull. var. Sheyenne) were germinated in vermiculite saturated with one-third strength Hoagland's solution. After 15 to 21 days of growth, the seedlings were transplanted into individual containers filled with vermiculite and grown to maturity under greenhouse conditions. Mature plants were used as the source of excised leaves; the fruits of bean, pepper,