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ARTICLE

Indirect Photodegradation of Clethodim in Aqueous Media. Byproduct Identification by Quadrupole Time-of-Flight Mass Spectrometry

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Aqueous photolysis of clethodim herbicide in the presence of natural substances such as humic acids (HA), nitrate, and Fe(III) ions has been investigated. The photodegradation rate of clethodim was retarded in the presence of HA compared to ultrapure water, while nitrate ions had no effect. On the other hand, water containing different concentrations of Fe(III) ions enhanced degradation of this herbicide. Clethodim transformation gave rise to the formation of nine byproducts, some of them, to the best of our knowledge, described for the first time in this work. The identification of these photoproducts has been accomplished by coupling liquid chromatography to quadrupole time-of-flight mass spectrometry. The main transformation reactions observed for clethodim were photoisomerization to the *Z*-isomer, S-oxidation of *E*- and *Z*-clethodim isomers giving rise to sulfoxide diastereoisomers, reduction of the oxime moiety to yield clethodim imine, oxidative cleavage of the C−S bond, and S-oxidation of clethodim imine leading to the formation of imine ketone and imine sulfoxide.

KEYWORDS: Photolysis; clethodim herbicide; natural substances; QTOF; photodegradation products

INTRODUCTION

Public concern about contamination of water has increased, mainly because a large number of herbicides have been detected in this environmental compartment (*1*−*3*). In the past several decades, the agrochemical industry has concentrated its efforts on developing new herbicides with short half-lives and effectiveness at a low application rate. Many of these herbicides are highly polar and could reach the aquatic compartments easily. The persistence of herbicides depends on transport, degradation, and retention processes. To predict their fate in the natural environment and to assess their risk, it is necessary to improve our knowledge on their chemical reactions under environmental conditions. Moreover, a large number of byproducts can be expected as a consequence of the different transformation processes that can suffer the parent compound.

Cyclohexanedione compounds have emerged as a new class of easily degradable herbicides applied at relatively low dosages (< 300 g/ha) to control both annual and perennial grasses in dicotyledonous crops (*4*). Clethodim, a postemergence systemic herbicide, belongs to this cyclohexanedione oxime family of compounds. It is effective against several graminaceous weeds in broadleaf crops such as soybean, cotton, tobacco, sugar beet, etc. This herbicide interferes with fatty acid biosynthesis via inhibition of enzyme acetyl CoA carboxylase (*4*).

After their application, herbicides can be degraded by biotic and/or abiotic processes in the environment to yield different degradation products. In this sense, photochemical transformation is one of the main abiotic degradation pathways occurring in natural waters. It is worth noting that natural substances present in aquatic systems [dissolved organic matter (DOM), nitrate and metal ions, etc.] may influence the photochemical behavior of organic compounds (*5*−*7*). In a first approach to study the photochemical behavior of organic compounds in different matrices, it is common to conduct the degradation under controlled conditions. Generally, the use of xenon arc lamps is preferred for modeling environmental conditions. These light sources are appropriate for photolysis studies because their characteristic emission spectra are very close to those of natural sunlight.

Although little attention has been paid to degradation products in the past, byproducts need to be considered to gain a complete understanding of the environmental impact of pesticides; otherwise, herbicides' fate could be substantially underestimated.

In many cases, the parent compound and transformation byproduct possess different physicochemical properties. The higher polarity and hence solubility in water of some degradation products increases the risk of contamination of the aquatic media. Water analyses show that the concentrations of degradation products are sometimes higher than those of the parent compounds (*8*). Besides, some of them can present similar or even higher toxicity and/or persistence than the parent compound. In this sense, water for human consumption has to meet the minimum requirements set out in the European legislation (*9*) that establish a maximum residue level for pesticides and their relevant metabolites and degradation and reaction products.

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However, determination of transformation products is sometimes difficult for a number of reasons. Many of them have never been identified or characterized before; analytical standards are scarce, and the concentration level in environmental samples is usually lower than that of the parent compound.

Physicochemical properties of transformation products compared to their parent compound (often more polar and less volatile and thermolabile) make liquid chromatography (LC) the most suitable technique for their determination. This is the case for cyclohexanedione oxime herbicides, where the active substances and the degradation products identified by our group (*7*, *10*) and others (*11*) present these features.

The unequivocal identification of these unknown compounds by LC, especially when no standards exist, has not always been achieved because of the scarcity of structural information. However, one of the latest developments in mass spectrometry [quadrupole time-of-flight (QTOF)] has improved the qualitative information for the structural elucidation of unknown compounds. QTOF provides elevated resolution and sensitivity, high mass accuracy for both parent and fragment ions, and the possibility of performing MS/MS acquisitions that will yield more structural information (*12*, *13*). In this way, QTOF allows the assignments of a highly probable empirical formula for unknown compounds.

Despite the scarce data about cyclohexanedione oxime herbicides, some references have been found in the literature concerning the degradation of clethodim. Sandín et al. (*10*) studied the degradation of this herbicide in chlorinated water, and sulfoxides and sulfone were identified as the main transformation products. Falb et al. (*14*, *15*) observed that clethodim is subject to decomposition in aqueous solutions, is acid-labile, and undergoes degradation when exposed to UV light. They observed up to 31 degradation products, but none of them were characterized.

This paper aims at improving our understanding of the photochemical fate of clethodim herbicide in water. To accomplish this objective, we have identified photoproducts of clethodim generated by exposure to simulated sunlight by means of LC-QTOF. Besides, photodegradation kinetics of clethodim in ultrapure water under simulated sunlight provided by a xenon arc lamp have been determined. In addition, we analyzed the influence of the major factors that may affect its environmental fate in natural waters such as HA (one of the main constituents of DOM), nitrate, and Fe(III) ions.

EXPERIMENTAL SECTION

Reagents and Chemicals. All chemical products were used without further purification. Clethodim (2-{(*E*)-1-[(*E*)-3-chloroallyloxyimino]propyl}-5-[2-(ethylthio)propyl]-3-hydroxycyclohex-2-enone) (98% purity) was obtained from ChemService (West Chester, PA). Humic acid sodium salt (technical grade) and iron(III) perchlorate hexahydrate (reagent grade) were purchased from Aldrich (Steinheim, Germany) and Alfa Aesar GmbH (Karlsruhe, Germany), respectively. Potassium nitrate (suprapur) and formic acid (pa) were purchased from Merck (Damstadt, Germany).

Acetonitrile (HPLC far UV grade) was obtained from Labscan (Stillorgan, Co. Dublin, Ireland). The water used for the LC mobile phase and aqueous solutions was purified with a Millipore system (Milli-Q-50, 18 mΩ).

Because of the low solubility of clethodim in water (3 mg/L), stock solutions (50 mg/L) were prepared by dissolving clethodim in the minimum amount of acetonitrile followed by addition of ultrapure water up to volume. These solutions were stored at 4 °C in the dark, though it was necessary to prepare them daily to avoid isomerization. Standard solutions of clethodim (5 mg/L) were prepared by dilution of aliquots of the stock solutions in ultrapure water.

LC Analysis. Photodegradation kinetics were performed with a LC system (series 1100, Agilent Technologies, Palo Alto, CA) coupled to a photodiode array detector (DAD). The analytical column used was a Waters Nova-Pak C_{18} column (4 μm particle size, 3.9 mm × 150 mm) with an ODS precolumn maintained at 25 °C. The mobile phase was a mixture of ultrapure water acidified with 0.1% formic acid (A) and acetonitrile (B).

Two different chromatographic methods were employed. The first one was an isocratic method, with a mobile phase of 80% B, developed to minimize the time of analysis of the replicates and to calculate kinetic parameters. The second one was a gradient method to study byproduct formation during photodegradation experiments, and the percentage of mobile phase B was changed linearly as follows: 10% at 0 min, 50% at 26 min, and 60% at 43 min. The separation was complete at 45 min.

In both chromatographic methods, the flow rate was 1 mL/min and the injection volume was 20 μL.

For the identification of byproducts, mass spectrometry experiments were performed by coupling the LC system (series 1100, Agilent Technologies) to a hybrid QTOF mass spectrometer (QStar Pulsar I, Applied Biosystems). Just before the separation, an external calibration in the mass spectrometer was performed with a mixture of phosphazenes. The experiments were performed in positive ion mode. The instrumental parameters were set as follows: mass range analyzed, 50−1200; ion spray voltage (IS), 5000 V; ion source gas pressure (GS1), 65 psi; ion source gas pressure 2 (GS2), 65 psi; curtain gas pressure (Cur), 20 psi; declustering potential (DP), 70 V; focusing potential (FP), 250 V; declustering potential 2, 15 V.

In MS/MS experiments, the collision energy (CE) for each ion selected was kept at 22 eV. With this equipment, the second chromatographic method previously described was employed. The flow rate of the mobile phase was 0.7 mL/min.

Photodegradation Experiments. Photochemical experiments were conducted in a Suntest CPS+ apparatus from Atlas (Linsengericht, Germany) equipped with a xenon arc lamp (1500 W) and a special glass filter restricting the transmission of wavelengths of < 290 nm. All photodegradation experiments were conducted at an irradiation intensity of 750 W/m. A Suncool chiller was used to maintain a mean internal temperature of 25 ± 1 °C.

The different aqueous solutions (20 mL) of clethodim, prepared at 5 mg/L in ultrapure water, were exposed to simulated solar irradiation in capped cylindrical quartz cuvettes with magnetic stirring.

Direct photodegradation of clethodim was conducted in ultrapure water. To study indirect photodegradation kinetics, experiments were conducted in the same quartz cuvettes and initial concentrations of clethodim in the presence of substances that can be found in natural waters such as HA, nitrate, and Fe(III) ions at various concentrations (0.5−20 mg/L). All these solutions were filtered through 0.2 μm nylon filters (Symta, Madrid, Spain) prior to injection. To evaluate the effect of the different substances added on the photolytic behavior of herbicide, kinetic parameters of direct photolysis were compared with those obtained in indirect experiments.

Meanwhile, control experiments in the dark (dark control) under the same conditions at the initial concentrations of clethodim and natural substances were conducted in parallel for comparison without the application of light. Besides, for all degradation kinetics studies, each experiment was conducted in triplicate until the herbicide disappeared. At selected time intervals, aliquots of 50 μL were collected and quantitatively analyzed directly by LC-DAD for the amount of the compound of interest remaining in solution after irradiation based on external calibration.

For the identification of photoproducts by LC-ESI-QTOF, an 80 mg/L solution of clethodim was irradiated for 4 h.

Analysis of Data. Kinetic parameters of clethodim photolysis were determined assuming the rate of the reaction to be first-order in herbicide concentration, by means of the kinetic equation

$$C_t = C_0 e^{-kt}$$

where C_t represents the concentration at time t, C_0 represents the initial concentration, and k is the rate constant.

Direct plots of herbicide concentration versus irradiation time provide the rate constant. Moreover, half-lives ($t_{1/2}$) of clethodim were determined by the equation $t_{1/2} = \ln 2/k$.

One-way analyses of variance (ANOVA) were conducted to determine differences between intensities and between concentrations of natural substances, at the $p < 0.05$ significance level. Results were analyzed using a statistical procedure (Statgraphics Plus version 4.1).

RESULTS AND DISCUSSION

Photodegradation Kinetics. The spectral characteristics of clethodim are given in **Figure 1**. An overlapping with the solar spectrum in the region of 290−350 nm can be observed, meaning that this herbicide is able to suffer photolysis by direct absorption of solar radiation under natural conditions. European directive EC (94/37/CE) (*16*) indicates that the phototransformation must be taken into account if the molar extinction coefficient is > 10 L mol^{-1} cm^{-1} for $\lambda \geq 290$ nm. In our case, the ε value obtained at $\lambda = 290$ nm for clethodim was 771 L mol^{-1} cm^{-1}, which indicated that photolysis can contribute significantly to its degradation in the environment.

In all cases, first-order reaction kinetics were assumed for the photodegradation rates of clethodim and good correlation coefficients were achieved (> 0.98).

Simultaneously with the irradiation experiments, control experiments in the absence of radiation were performed to rule out other types of dark reactions (hydrolysis, thermolysis, etc.). Quantitative recoveries of clethodim during the entire exposure period to simulated solar irradiation enable us to ignore other transformation processes that are not initiated by radiation.

To determine the different effects exerted by the composition of media on the photolysis rate, a direct photodegradation assay of clethodim was conducted in ultrapure water. Experimental data showed that herbicide photodegradation was achieved after a short period of time. Clethodim was completely degraded in 2.5 h, and a half-life of 28.9(0.1) min was obtained. This result highlights the efficiency of simulated sunlight in removing this compound in aqueous media. **Figure 2a** shows the kinetics of clethodim in the presence of increasing concentrations of HA. In the presence of HA, an increase in the half-lives of herbicide photolysis was obtained by comparison with direct photolysis [$t_{1/2([HA]=1\ mg/L)}$ = 44.2(0.1)]. **Table 1** lists means of experimental data obtained from 1 to 20 mg/L HA. Analysis of variance showed significant differences between each concentration.

This retarding effect exerted by HA indicates that these substances can act as an "optical filter" absorbing most of the photons emitted and thereby slowing the direct photochemical reaction of clethodim. However, the influence of HA concentration on the photolysis rate was not proportional, since a 20-fold increase in HA concentration resulted in an only 34−81% decrease in the reaction rate. Similar results have been previously reported by other authors (*7*, *17*), and this could be explained as a competition between the HA filter that decreases the rate of clethodim photolysis and the generation of hydroxyl radical that should increase the rate of clethodim photolysis. Nevertheless, none of the nine photoproducts involves a hydroxyl attack. Thus, this second effect could not be involved in the clethodim photolysis.

Hydroxyl radicals can be generated by the photolysis of nitrate ions present in natural waters. These radicals are known to be strong oxidants, which are able to photoinduce the degradation of organic pollutants (*18*). However, in our experiments, the presence of different concentrations of nitrate ions in aqueous solutions (1−20 mg/L) had no effect on the half-life of the studied herbicide (**Table 1** and **Figure 2b**).

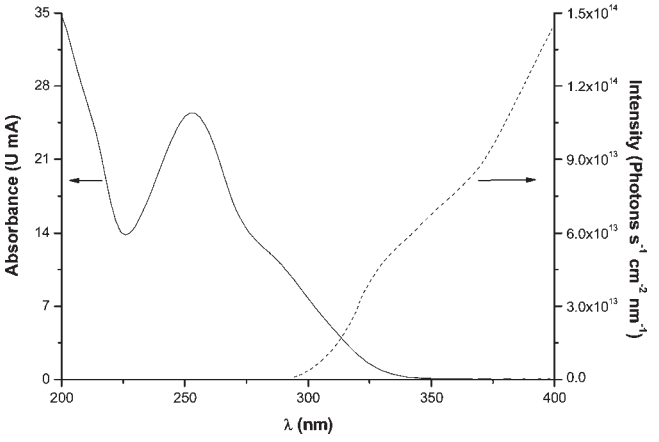


Figure 1. UV absorption spectra of 5 ppm of clethodim in ultrapure water (—) and sunlight emission (· · ·).

Table 1. Kinetic Parameters of Clethodim in the Presence of Various Concentrations of Natural Substances

added substance	concn (mg/L)	k_{obs} [a] ($\times 10^{-3}$ min^{-1})	$t_{1/2}$ (min)	R^2
HA	1	15.7 a ± 0.3	44.1 ± 0.1	0.9992
	5	8.39 b ± 0.29	82.6 ± 0.3	0.9986
	10	6.71 c ± 0.33	103 ± 0.4	0.9974
	15	5.51 d ± 0.15	126 ± 0.3	0.9983
	20	4.70 e ± 0.09	147 ± 0.2	0.9995
NO_3^-	1	21.6 a ± 0.9	32.1 ± 0.2	0.9982
	5	24.1 a ± 1.4	29.0 ± 0.3	0.9971
	10	22.5 a ± 1.2	30.8 ± 0.3	0.9983
	15	22.8 a ± 0.9	30.6 ± 0.2	0.9986
	20	24.0 a ± 1.4	29.0 ± 0.3	0.9972
Fe(III)	1	43.7 a ± 2.4	16.4 ± 0.2	0.9976
	2.5	105 b ± 7	6.60 ± 0.13	0.9968
	5	194 c ± 16	3.57 ± 0.13	0.9944
	10	223 d ± 18	3.11 ± 0.12	0.9950
	20	272 e ± 39	2.55 ± 0.19	0.9869

[a] Different letters show significant differences according to the least significant difference test (LSD) at a significance level of 95%.

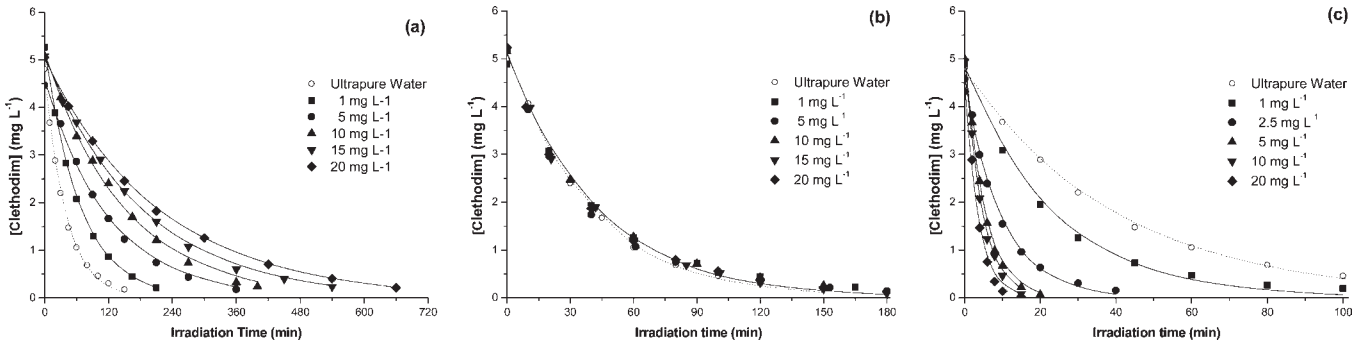


Figure 2. Photodegradation of clethodim in the presence of various concentrations of HA (**a**), nitrate ions (**b**), and Fe(III) ions (**c**) in ultrapure water under simulated solar irradiation.

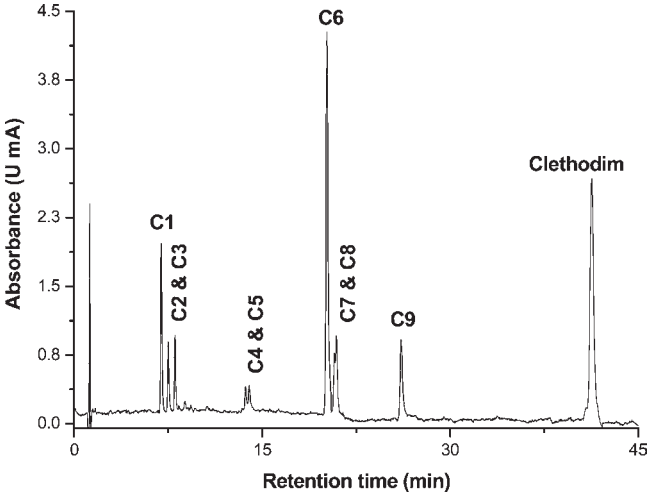


Figure 3. LC-DAD chromatogram of clethodim photodegradation at an irradiation time of 60 min in ultrapure water.

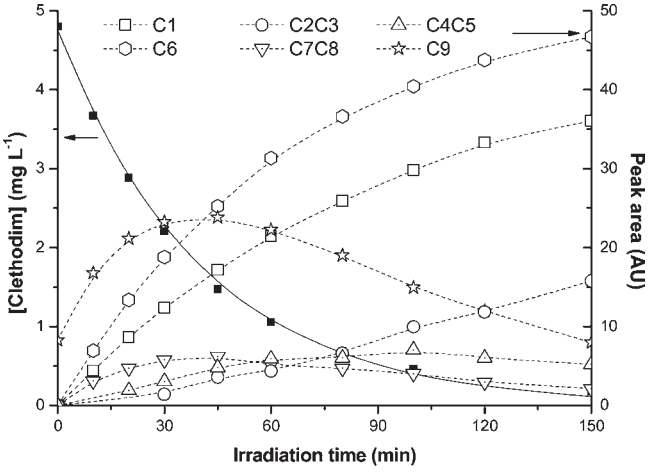


Figure 4. Common evolution of transformation products (empty symbols and dotted lines) formed under the irradiation of clethodim (filled symbols and solid line) in ultrapure water.

Similar results have been previously reported by our group and other authors (*7*, *19*, *20*). Nitrate ion photolysis depends on the irradiating power available in the solution which can be significantly reduced when the substrate itself strongly absorbs in the same UV range. Therefore, clethodim could diminish the amount of radiation absorbed by nitrate ions, reducing their extent of photolysis.

Another important constituent of natural waters is Fe(III) ion. Photodegradation of clethodim was performed in the presence of various concentrations of Fe(III). An increasing concentration of Fe(III) resulted in a decrease in half-life compared to that with direct photolysis in ultrapure water (**Table 1** and **Figure 2c**). The analysis of variance showed significant differences among the different concentrations. The increase in the rate constant was up to 6 times higher when 20 mg/L Fe(III) ions were present in the solution. Several authors described this enhanced effect of Fe(III) as a result of the formation of hydroxyl radicals (*5*, *21*). Furthermore, it has also been reported in the literature that the organic molecule can form a complex with the Fe(III) ion and later undergoes a direct photolysis (*22*).

Photoproduct Evolution. During irradiation and until the complete disappearance of clethodim, nine different byproducts were observed. **Figure 3** shows a typical LC-DAD chromatogram of an irradiated solution of clethodim.

The kinetic disappearance of clethodim in ultrapure water with the concomitant appearance and evolution of the nine photoproducts is presented in **Figure 4**. The apparition and evolution of these byproducts have been followed by LC−UV during the degradation process with HA, nitrate, and Fe(III) ions and in ultrapure water. None of these photoproducts are commercially available; hence, they could not be quantified.

Photoproduct C9 was present since the beginning of the photodegradation, and its concentration increased to reach a maximum amount in the first third of the irradiation time and thereafter slowly decreased to trace levels. The same evolution pattern followed compounds C4 and C5, and C7 and C8, though they were formed in smaller amounts. C1 and C6 were the main photoproducts formed, and although a plateau was not reached during the irradiation time, the formation pattern of these byproducts shows a continuous increase as clethodim is degraded. Similar behavior was observed for C2 and C3.

The byproducts detected in the presence of different concentrations of HA, nitrate, and Fe(III) ions were the same as those in direct photolysis; however, differences were observed in the evolution pattern of some photoproducts. As the concentration of HA increased, the main photoproducts changed from C1 and C6 to C2 and C3, and C7 and C8. Moreover, the amount of compound C9 diminished and the amount of C4 and C5 kept approximately constant. In the case of nitrate ions, the evolution pattern and amount formed of all degradation products were the same compared to those in the direct photolysis with ultrapure water.

As in HA, the amounts of photodegradation products vary with the concentration of Fe(III) ions. In the presence of increasing amounts of Fe(III) ions, the amounts of byproducts C1 and C6 diminished to trace levels but the amounts of C7 and C8 increased. Amounts of products C2 and C3, C4 and C5, and C9 reached their maxima at 2.5 mg/L Fe(III) ions and thereafter decreased to a constant concentration.

Identification of Photodegradation Products. Both positive and negative ionization modes were tested in this work. Ion response and fragmentation were higher using the positive mode. By using a QTOF analyzer, it was possible to obtain highly accurate m/z values and/or isolate different precursor ions for the study of their MS/MS fragmentation pattern. For all the compounds, their possible molecular formulas were calculated (**Table 2**).

To determine the most probable molecular formula, various criteria were considered as the rule of the number of nitrogen atoms, the DBE (double bond equivalent), and the error. Accurate mass analysis of the sodium adduct was used for further confirmation of the proposed elemental composition. Besides, chlorine and sulfur isotopic signals for the protonated molecule and for fragment ions allow additional information for the identification of the degradation products to be gained.

The photodegradation experiments resulted in the formation of nine degradation products, all of them more polar than clethodim (**Figure 3**). Byproduct C9 (t_R = 26.09 min) was detected in a small amount (8%) when a freshly prepared stock solution of clethodim was analyzed. This minor peak also appeared at time zero of photodegradation kinetics and remained constant in the dark controls during the irradiation time. However, the concentration of C9 increased under irradiation as previously described.

These photoproducts were identified on the basis of accurate mass measurements of the $[M + H]^+$ ions and their product ions, as shown in **Table 3**.

To obtain useful information for the interpretation of the product ion spectra of the unknown byproduct, we investigated

Table 2. Accurate Mass Measurements for the $[M + H]^+$ Ions of Clethodim and Degradation Products by LC-ESI-QTOF[a]

experimental mass (*m*/*z*)	calculated mass (*m*/*z*)	error (ppm)	DBE	formula
360.1384	360.1381	0.7573	5.0	$C_{15}H_{25}N_4O_2SCl$
	360.1394	**−2.9709**	**4.5**	**$C_{17}H_{27}NO_3SCl$**
	360.1360	6.3919	9.5	$C_{20}H_{23}NO_3Cl$
	360.1410	−7.2527	4.5	$C_{15}H_{26}N_3O_3S_2$
224.1264	224.1267	−1.6837	5.0	$C_{10}H_{16}N_4O_2$
	224.1281	**−7.6745**	**4.5**	**$C_{12}H_{18}NO_3$**
	224.1308	−19.6320	9.0	$C_{15}H_{16}N_2$
286.1452	286.1457	−2.0947	4.0	$C_{12}H_{22}N_4O_2S$
	286.1437	4.9969	8.5	$C_{15}H_{26}N_3O_3S_2$
	286.1471	**−6.7871**	**3.5**	**$C_{14}H_{24}NO_3S$**
	286.1424	9.6892	9.0	$C_{15}H_{18}N_4O_2$
376.1325	376.1330	−1.4407	5.0	$C_{15}H_{25}N_4O_3SCl$
	376.1310	3.9543	9.5	$C_{20}H_{23}NO_4Cl$
	376.1343	**−5.0104**	**4.5**	**$C_{17}H_{27}NO_4SCl$**
	376.1359	−9.1101	4.5	$C_{15}H_{26}N_3O_4S_2$
270.1520	**270.1522**	**−0.8419**	**3.5**	**$C_{14}H_{24}NO_2S$**
	270.1508	4.1281	4.0	$C_{12}H_{22}N_4OS$
	270.1533	−5.1310	0.0	$C_8H_{22}N_4O_6$
360.1382	360.1381	0.2019	5.0	$C_{15}H_{25}N_4O_2SCl$
	360.1394	**−3.5262**	**4.5**	**$C_{17}H_{27}NO_3SCl$**
	360.1360	5.8366	9.5	$C_{20}H_{23}NO_3Cl$
	360.1410	−7.8081	4.5	$C_{15}H_{26}N_3O_3S_2$

[a] The chosen best-fit assignments are shown in bold.

clethodim fragmentation (**Table 3**). The $[M + H]^+$ ion at *m*/*z* 360.1384 was fragmented to yield a product ion at *m*/*z* 268.1378 corresponding to the loss of the oxime moiety. The ion at *m*/*z* 240.1049 is consistent with oxime moiety and ethene molecule loss, while the ion at *m*/*z* 206.1152, corresponding to the elemental composition of $C_{12}H_{16}NO_2^+$, is associated with the loss of the oxime group moiety and the ethylthiol moiety. Once again, the loss of the oxime moiety and the complete lateral sulfur chain at position 5 of the ring gave a product ion at *m*/*z* 164.0711.

Byproduct C9 was a protonated molecule whose nominal mass is the same as that of the $[M + H]^+$ ion of clethodim, and the mass spectra presented an identical fragmentation pattern (**Figure 5**). Chlorine and sulfur isotopic signals confirm the presence of these moieties in the molecule, offering added information for the correct identification of the compound.

Accurate mass measurement of C9 gave four possibilities (**Table 2**), and the only one consistent with the number of nitrogens, the number of sulfur atoms, and the DBE corresponded to the same elemental composition of *E*-clethodim, $C_{17}H_{27}NO_3SCl^+$. Therefore, C9 was identified as the *Z*-isomer of clethodim at the oxime ether double bond. The *Z*-isomer is much more polar than *E*-clethodim [$t_{R(Z-)} = 26.09$ min vs $t_{R(E-)} = 41.30$ min] as it can form an internal hydrogen bond between the oxime oxygen and the hydroxyl group of the cyclohexane ring. Several authors have already stated that some *E*-isomer of cyclohexanedione herbicides may equilibrate with the *Z*-isomer in polar solvents (*15*, *23*) or in chlorinated water (*10*). Moreover, it has been reported that isomerization can be induced by light and temperature (*7*, *24*).

Byproducts C4, C5, C7, and C8 exhibited $[M + H]^+$ ions at *m*/*z* 376 (**Figure 5**), and the observed fragmentation patterns were essentially the same for the four compounds. The mass of the precursor ion was 16 Da higher than that of clethodim, suggesting the presence of one additional oxygen atom. Mass spectra of byproducts presented a chlorine and sulfur isotope signature, as also observed for clethodim and C9, showing the presence of the oxime and the sulfur chain. Calculation of possible elemental compositions resulted in four formulas (**Table 2**). On the basis of the presence of heteroatoms and plausible DBE values, the empirical formula for these compounds was determined to be $C_{17}H_{27}NO_4SCl^+$. Fragmentation of the *m*/*z* 376 ion led to the formation of four ions at *m*/*z* 298, 284, 206, and 164 (**Table 3**). The ion at *m*/*z* 298 is probably derived from a cleavage of the C−S bond after migration of the hydrogen to the oxygen on the sulfur atom and the loss of the cation radical CH_3CH_2SOH as previously described as a common fragmentation of sulfoxides (*25*). The importance of DBE values is noteworthy. In this case, these values remained constant with respect to clethodim (4.5), confirming that oxidation occurred on the sulfur atom, where the double bond (S═O) does not compute for the DBE value. The loss of the oxime moiety (*m*/*z* 92) from the precursor ion led to the formation of the ion at *m*/*z* 284, as it was also observed in clethodim and C9. The consecutive losses of the oxime moiety and the cation radical CH_3CH_2SOH gave the formation of the ion at *m*/*z* 206. The ion at *m*/*z* 164 corresponds to the loss of the oxime moiety and the alkyl sulfoxide chain at position 5 of the ring.

The oxidation of the sulfur atom of *E*-clethodim originates a new chiral center. As the neighboring carbon atom is also chiral, two pairs of enantiomers formed (*RR*+*SS* and *RS*+*SR*), and diastereomers are chromatographically separated in two peaks, containing a pair of enantiomers each. Since the reaction took place without stereogenic control in the water achiral medium, similar amounts of each isomer are expected. In the same way, the *Z*-isomer of clethodim (C9) gave rise to the corresponding pairs of enantiomers of *Z*-clethodim sulfoxide. Therefore, on the basis of the retention time and the amount formed of the two pairs of enantiomers (**Figure 3**), C4 and C5 have been assigned to the sulfoxides of *Z*-clethodim and C7 and C8 to the sulfoxides of *E*-clethodim. However, assignation of each pair of enantiomers of *E*- and *Z*-isomers of clethodim (*RR*+*SS* and *RS*+*SR*) was no possible on the basis of the MS spectra alone.

It is known that one of the most important degradative pathways for sulfide pesticides in the environment is oxidation to the respective sulfoxide and sulfone due to the high reactivity of the sulfur moieties. In soils, sulfoxidation is so rapid and complete that sulfoxides are often the dominant species found shortly after application of the parent sulfide. In most cases, the sulfoxides also have pesticidal activity. Sulfoxide byproducts have much higher water solubility, lower sorption to soil, and a slower degradation rate compared to those of the parent sulfides, and therefore a higher potential for groundwater contamination (*8*).

Byproduct C6 ($t_R = 20.17$ min) exhibited an $[M + H]^+$ ion at *m*/*z* 270.1520 (**Table 3**). The isotope signature suggested the absence of chlorine and the presence of the sulfur chain.

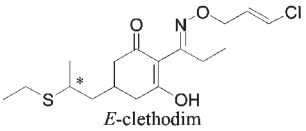
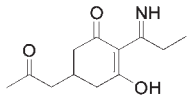
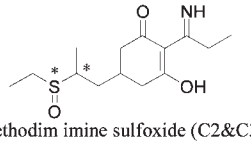
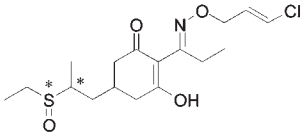
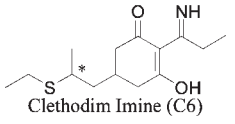
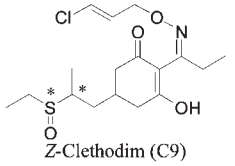
The calculation of the possible elemental composition results in only one possible formula, $C_{14}H_{24}NO_2S^+$ (**Table 2**), consistent with DBE values and the number of heteroatoms.

The product ion mass spectrum (**Figure 5**) displays a main fragment at *m*/*z* 208.1316 corresponding to the ethanethiol loss, and the subsequent loss of ethene gives rise to the ion at *m*/*z* 180.1373. A minor fragment is the ion at *m*/*z* 166.0851 that corresponds to the loss of the alkyl sulfide chain.

The information obtained from the product ion spectrum and the elemental composition led to the conclusion that byproduct C6 corresponds to the imine of clethodim. Besides, the lack of the loss of the ion at *m*/*z* 92, observed in clethodim and also in other cyclohexanedione mass spectra (*10*, *11*), suggested the absence of the oxime moiety and supports imine formation.

Imine degradate has been observed in degradation processes of other cyclohexanedione herbicides like alloxydim in different matrices such as soil, water, and plants (*26*−*28*). In this sense, photodegradation studies conducted by our group gave rise to

Table 3. Accurate Mass Measurements and Elemental Compositions of Clethodim, Its Photodegradation Products, and Their Major Ions Obtained by LC-ESI-QTOF

Compound	Experimental mass ($[M+H]^+$)	Major ions	Calculated mass	Elemental composition	DBE	Error (ppm)
 *E*-clethodim	360.1384	382.1215	382.1214	$C_{17}H_{26}NO_3SClNa^+$	4.5	0.22
		360.1384	360.1394	$C_{17}H_{27}NO_3SCl^+$	4.5	-2.97
		268.1378	268.1365	$C_{14}H_{22}NO_2S^+$	4.5	4.56
		240.1049	240.1052	$C_{12}H_{18}NO_2S^+$	4.5	-1.57
		206.1152	206.1175	$C_{12}H_{16}NO_2^+$	5.5	-11.43
		164.0711	164.0706	$C_9H_{10}NO_2^+$	5.5	3.02
 Ketone of clethodim imine (C1)	224.1264	246.1121	246.1100	$C_{12}H_{17}NO_3Na^+$	4.5	8.27
		224.1264	224.1281	$C_{12}H_{18}NO_3^+$	4.5	-7.67
		196.1329	196.1332	$C_{11}H_{18}NO_2^+$	3.5	-1.56
		166.0854	166.0862	$C_9H_{12}NO_2^+$	4.5	-5.15
		111.0434	111.0440	$C_6H_7O_2^+$	3.5	-5.91
 Clethodim imine sulfoxide (C2&C3)	286.1452	308.1267	308.1290	$C_{14}H_{23}NO_3SNa^+$	3.5	-7.75
		286.1452	286.1471	$C_{14}H_{24}NO_3S^+$	3.5	-6.79
		208.1319	208.1332	$C_{12}H_{18}NO_2^+$	4.5	-1.30
		166.0853	166.0862	$C_9H_{12}NO_2^+$	4.5	-6.27
 Clethodim sulfoxide (C4&C5, C7&C8)	376.1327	398.1161	398.1163	$C_{17}H_{26}NO_4SClNa^+$	4.5	0.57
		376.1327	376.1343	$C_{17}H_{27}NO_4SCl^+$	4.5	-4.48
		298.1202	298.1204	$C_{15}H_{21}NO_3Cl^+$	5.5	-0.83
		284.1301	284.1314	$C_{14}H_{22}NO_3S^+$	4.5	-4.90
		206.1168	206.1175	$C_{12}H_1NO_2^+$	5.5	-3.66
		164.0702	164.0706	$C_9H_{10}NO_2^+$	5.5	-2.47
 Clethodim Imine (C6)	270.1520	292.1331	292.1341	$C_{14}H_{23}NO_2SNa^+$	3.5	-1.27
		270.1520	270.1522	$C_{14}H_{24}NO_2S^+$	3.5	-0.84
		208.1316	208.1332	$C_{12}H_{18}NO_2^+$	4.5	-7.71
		180.1373	180.1382	$C_{11}H_{18}NO^+$	3.5	-5.50
		166.0851	166.0862	$C_9H_{12}NO_2^+$	4.5	-6.95
 Z-Clethodim (C9)	360.1382	382.1215	382.1214	$C_{17}H_{26}NO_3SClNa^+$	4.5	0.22
		360.1382	360.1394	$C_{17}H_{27}NO_3SCl^+$	4.5	-3.53
		268.1369	268.1365	$C_{14}H_{22}NO_2S^+$	4.5	1.20
		240.1050	240.1052	$C_1H_{18}NO_2S^+$	4.5	-1.15
		206.1166	206.1175	$C_{12}H_{16}NO_2^+$	5.5	-4.63
		164.0700	164.0706	$C_9H_{10}NO_2^+$	5.5	-3.69

imine as the main transformation product of alloxydim/sodium aqueous media (*7*).

However, to the best of our knowledge, an imine byproduct has not previously been observed in clethodim degradation studies.

Byproducts C2 and C3 (t_R = 7.72 and 8.21 min, respectively) exhibited $[M + H]^+$ ions at m/z 286 (**Table 3**). This mass corresponded to a molecular mass 16 Da higher than that of imine, suggesting that this molecule has one additional oxygen atom. Both compounds contained an odd number of nitrogen atoms, and the detected m/z 286 isotopic signature reveals the absence of chlorine and the presence of a sulfur atom. The accurate mass measurements gave four hits (**Table 2**), and the only one consistent with previously remarks corresponded to the $C_{14}H_{24}NO_3S^+$ empirical formula, which confirms oxidation.

The mass spectra exhibited two important fragments for these transformation products at m/z 208.1319 and 166.0853 (**Figure 5**). The former fragment corresponds to the neutral loss of ethyl sulfoxide, while the latter derives from the loss of the complete chain at position 5 of the ring.

Comparing the fragments of these byproducts with the parent compound, we can note that the typical loss of the ether moiety (m/z 92) is now absent.

These features led to the assignment of the structure of the two detected isomers to clethodim imine sulfoxides. As in the case of clethodim, the oxidation of the sulfur atom generates a new chiral center, and therefore, two pairs of enantiomers were also formed corresponding for each chromatographic peak to one pair of enantiomers (*RR*+*SS* and *RS*+*SR*). In the same way that was demonstrated for clethodim sulfoxides, no assignation of each peak to the corresponding pair of enantiomers is feasible with the mass spectrometric technique.

Degradation product C1 exhibited an $[M + H]^+$ ion at m/z 224.1264 (**Figure 5**). Among the three possible empirical molecular formulas (**Table 2**), only $C_{12}H_{18}NO_3^+$ is consistent according to the number of nitrogen atoms and DBE values.

This byproduct contains an odd number of nitrogen atoms, so the structure maintains the nitrogen atom of the oxime moiety. The lack of isotopic signatures revealed the absence of both chlorine and sulfur atoms. The absence of the loss of m/z 92, typical of cyclohexanedione oximes, allows us to rule out the presence of the oxime moiety and support the imine formation.

Fragmentation of the ion at m/z 224.1264 led to the formation of product ions at m/z 196.1329, 166.0854, and 111.0434

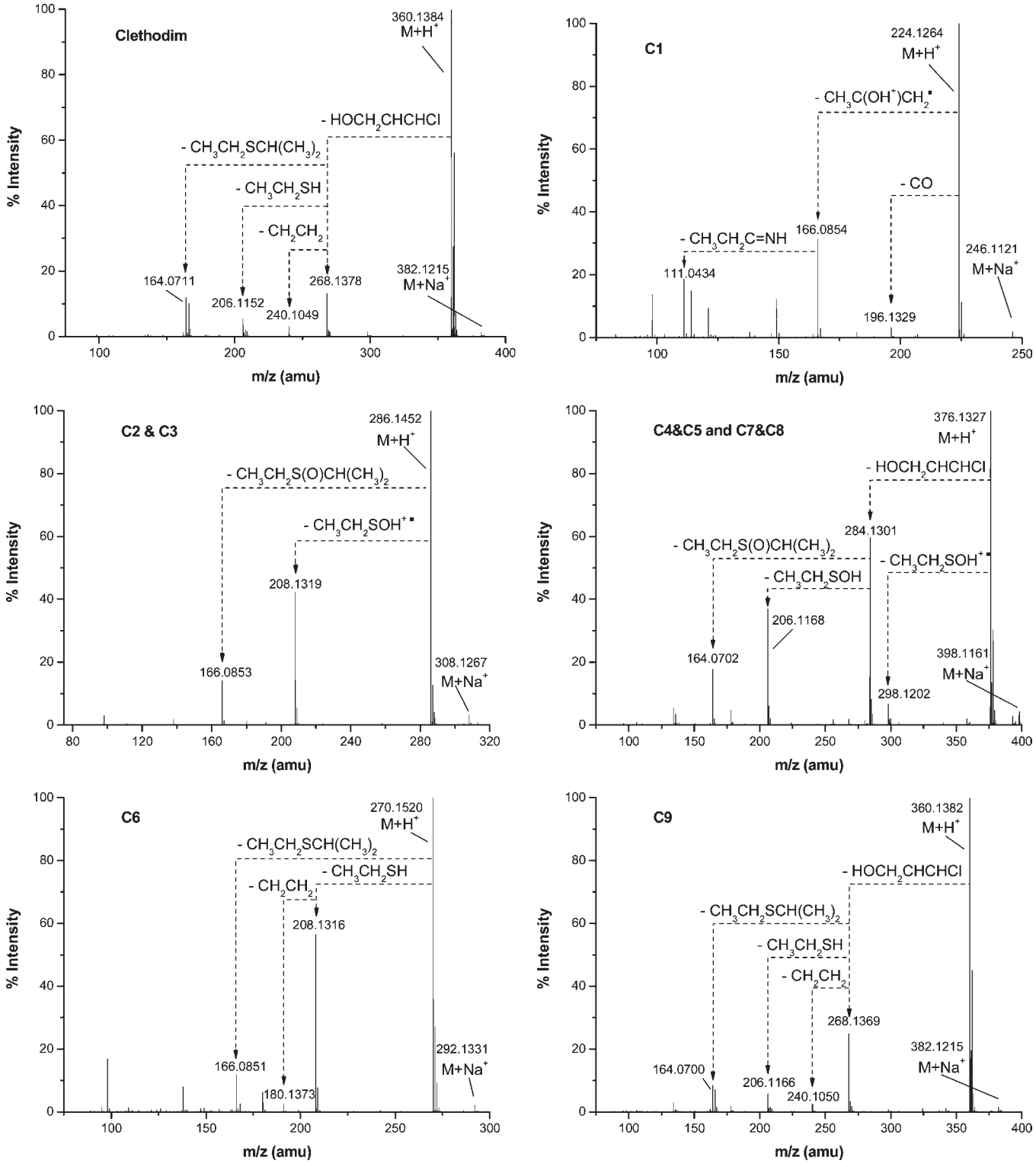


Figure 5. LC-QTOF mass spectra of clethodim and its photodegradation products.

(**Figure 5**). The ion at *m*/*z* 196.1329 is formed as a result of a loss of 28 Da attributed to CO. Accurate mass measurement of the product ions made it possible to remove ambiguities between CO and C_2H_4 loss, both of which have distinguished the same nominal mass (error for C_2H_4 = −2.1398 ppm, and error for CO = −0.5966 ppm).

The ion at *m*/*z* 166.0854 corresponds to an elemental composition of $C_9H_{12}NO_2^+$ obtained after a loss of *m*/*z* 58.0410 which is due to the loss of the ketone chain via a McLafferty rearrangement that involves an intramolecular proton abstraction in the γ position (carbon in position 6 of the ring) by the ketone oxygen and elimination of the CH_3-C═OH^+-$CH_2^•$ chain.

The consecutive loss of the ketone chain and the imine group leads to the formation of the ion at *m*/*z* 111.0434. On the basis of the previous discussion (**Table 2**), a structure of photoproduct C1 has been proposed (**Figure 6**), suggesting the oxidative cleavage of the C−S bond of clethodim imine. This photooxidation reaction has been also observed by others (*29*, *30*) in sulfide compounds.

A summary of the proposed degradation pathway resulting from the photolysis of clethodim in water is shown in **Figure 6**.

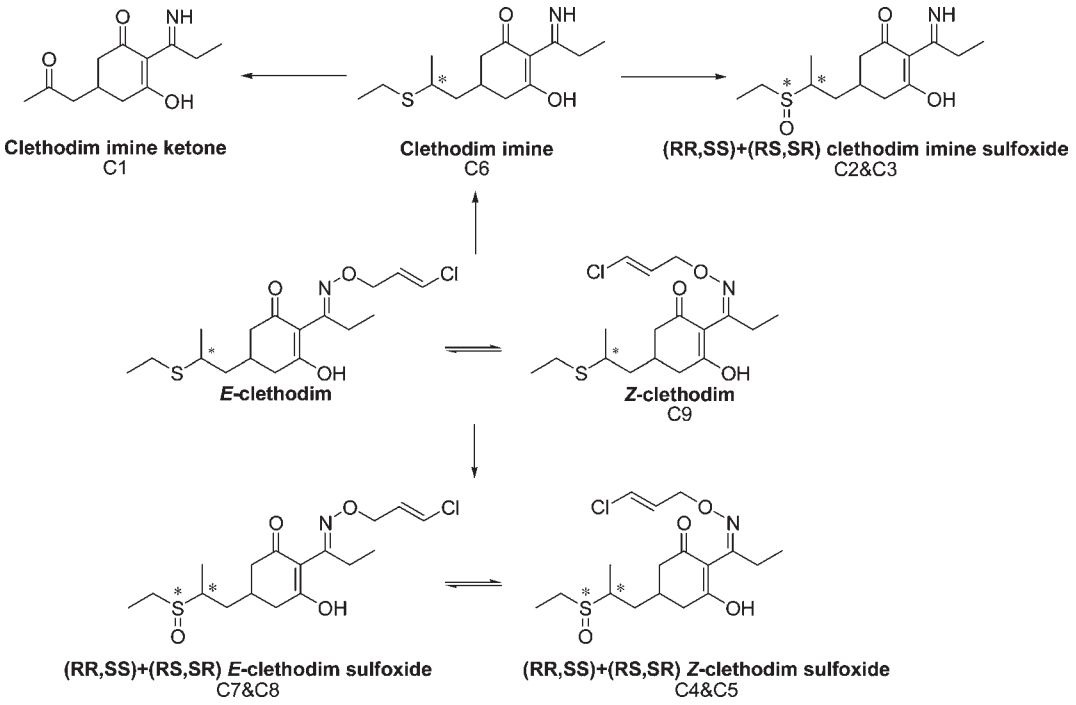


Figure 6. Proposed photodegradation pathway of clethodim in aqueous solution.

In summary, the effects of different natural substances on the photolysis behavior of clethodim have been investigated. HA exerted a retardant effect on the photodegradation rates of the herbicide, but the presence of nitrate ions has no influence. On the other hand, half-lives of clethodim were significantly diminished in the presence of Fe(III) ions. Furthermore, in our work, the QTOF mass spectrometer has been used as a valuable tool for the identification of unknown degradation products resulting from photolysis reactions in water. On the basis of the exact mass measurements and fragmentation patterns, it has been possible to elucidate the structures of nine clethodim byproducts resulting from isomerization, S-oxidation, reduction of the oxime moiety chain, and oxidative C−S bond cleavage. A future work on the study of the toxicity of these byproducts is planned to improve our understanding of the environmental impact of clethodim herbicide in water.

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LITERATURE CITED

(1) Konstantinou, I. K.; Hela, D. G.; Albanis, T. A. The status of pesticide pollution in surface waters (rivers and lakes) of Greece. Part I. Review on occurrence and levels. *Environ. Pollut.* **2006**, *141* (3), 555–570.

(2) Rebich, R. A.; Coupe, R. H.; Thurman, E. M. Herbicide concentrations in the Mississippi River Basin: The importance of chloroacetanilide herbicide degradates. *Sci. Total Environ.* **2004**, *321*, 189–199.

(3) Marín, J. M.; Sancho, J. V.; Pozo, O. J.; López, F. J.; Hernández, F. Quantification and confirmation of anionic, cationic and neutral pesticides and transformation products in water by on-line solid phase extraction−liquid chromatography−tandem mass spectrometry. *J. Chromatogr., A* **2006**, *1133*, 204–214.

(4) Iwataki, I. *Cyclohexanedione herbicides: Their activities and properties*; CRC Press: Boca Raton, FL, 1992; Vol. XVI, pp 397−426.

(5) Mazellier, P.; Jirkovsky, J.; Bolte, M. Degradation of diuron photoinduced by iron(III) in aqueous solution. *Pestic. Sci.* **1997**, *49* (3), 259–267.

(6) Quivet, E.; Faure, R.; Georges, J.; Païssé, J. O.; Herbreteau, B.; Lantéri, P. Photochemical degradation of imazamox in aqueous solution: Influence of metal ions and anionic species on the ultraviolet photolysis. *J. Agric. Food Chem.* **2006**, *54* (10), 3641–3645.

(7) Sevilla-Morán, B.; Sandín-España, P.; Vicente-Arana, M. J.; Alonso-Prados, J. L.; García-Baudín, J. M. Study of alloxydim photodegradation in the presence of natural substances: Elucidation of transformation products. *J. Photochem. Photobiol., A* **2008**, *198* (2−3), 162–168.

(8) Somasundaram, L.; Coats, J. R. *Pesticide transformation products. Fate and significance in the environment*; ACS Symposium Series; American Chemical Society: Washington, DC, 1991; Vol. 459, p 305.

(9) European Commission Report. Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption, **1998**, pp 32−54.

(10) Sandín-España, P.; Magrans, J. O.; García-Baudín, J. M. Study of clethodim degradation and by-product formation in chlorinated water by HPLC. *Chromatographia* **2005**, *62* (3−4), 133–137.

(11) Marek, L. J.; Koskinen, W. C.; Bresnahan, G. A. LC/MS analysis of cyclohexanedione oxime herbicides in water. *J. Agric. Food Chem.* **2000**, *48* (7), 2797–2801.

(12) Ibáñez, M.; Sancho, J. V.; Pozo, O. J.; Hernández, F. Use of quadrupole time-of-flight mass spectrometry in environmental analysis: Elucidation of transformation products of triazine herbicides in water after UV exposure. *Anal. Chem.* **2004**, *76* (5), 1328–1335.

(13) Aguera, A.; Estrada, L. A. P.; Ferrer, I.; Thurman, E. M.; Malato, S.; Fernandez-Alba, A. R. Application of time-of-flight mass spectrometry to the analysis of phototransformation products of diclofenac in water under natural sunlight. *J. Mass Spectrom.* **2005**, *40* (7), 908–915.

(14) Falb, L. N.; Bridges, D. C.; Smith, A. E. Separation of clethodim herbicide from acid and photodegradation products by liquid chromatography. *J.—Assoc. Off. Anal. Chem.* **1991**, *74* (6), 999–1002.

(15) Falb, L. N.; Bridges, D. C.; Smith, A. E. Effects of pH and adjuvants on clethodim photodegradation. *J. Agric. Food Chem.* **1990**, *38* (3), 875–878.

(16) European Commission Report. Commission Directive 94/37/EC of 22 July 1994 amending Council Directive 91/414/EEC concerning the placing of plant protection products on the market, **1994,** pp 0065−0081.

(17) Dimou, A. D.; Sakkas, V. A.; Albanis, T. A. Trifluralin photolysis in natural waters and under the presence of isolated organic matter and nitrate ions: Kinetics and photoproduct analysis. *J. Photochem. Photobiol., A* **2004**, *163* (3), 473–480.
(18) Machado, F.; Boule, P. Photonitration and photonitrosation of phenolic derivatives induced in aqueous-solution by excitation of nitrite and nitrate ions. *J. Photochem. Photobiol., A* **1995**, *86* (1−3), 73–80.
(19) Andreozzi, R.; Raffaele, M.; Nicklas, P. Pharmaceuticals in STP effluents and their solar photodegradation in aquatic environment. *Chemosphere* **2003**, *50* (10), 1319–1330.
(20) Chaabane, H.; Vulliet, E.; Joux, F.; Lantoine, F.; Conan, P.; Cooper, J.-F.; Coste, C.-M. Photodegradation of sulcotrione in various aquatic environments and toxicity of its photoproducts for some marine micro-organisms. *Water Res.* **2007**, *41*, 1781–1789.
(21) Galichet, F.; Mailhot, G.; Bonnemoy, F.; Bohatier, J.; Bolte, M. Iron(III) photo-induced degradation of isoproturon: Correlation between degradation and toxicity. *Pest Manage. Sci.* **2002**, *58* (7), 707–712.
(22) Boule, P. *The handbook of environmental chemistry, Vol. II, Part L: Reactions and processes: Environmental photochemistry*; Springer-Verlag: Berlin, 1999; p 359.
(23) Sandín-España, P.; González-Blázquez, J. J.; Magrans, J. O.; García-Baudín, J. M. Determination of herbicide tepraloxydim and main metabolites in drinking water by solid-phase extraction and liquid chromatography with UV detection. *Chromatographia* **2002**, *55* (11/12), 681–686.
(24) Curtin, D. Y.; Grubbs, E. J.; McCarty, C. G. Uncatalysed syn-anti isomerization of imines, oxime ethers, and haloimines. *J. Am. Chem. Soc.* **1966**, *88* (12), 2775–2786.
(25) Khmel'nitskii, R. A.; Efremov, Yu. A. Rearrangements in sulphoxides and sulphones induced by electron impact. *Russ. Chem. Rev.* **1977**, *46* (1), 83–111.
(26) Ono, S.; Shiotani, H.; Ishihara, K.; Tokieda, M.; Soeda, Y. Degradation of the herbicide alloxydim-sodium in soil. *J. Pestic. Sci.* **1984**, *9* (3), 471–480.
(27) Hashimoto, Y.; Ishihara, K.; Soeda, Y. Fate of alloxydim-sodium on or in soybean plants. *J. Pestic. Sci.* **1979**, *4* (3), 299–304.
(28) Soeda, Y.; Ishihara, K.; Iwataki, I.; Kamimura, H. Fate of a herbicide ^{14}C-alloxydim-sodium in sugar beets. *J. Pestic. Sci.* **1979**, *4* (2), 121–128.
(29) Pasto, D. J.; Cottard, F.; Jumelle, L. Photooxidation of alkyl 4-nitrophenyl sulfides and sulfoxides. Observation of oxidative C-S bond cleavage and rearrangement reactions. *J. Am. Chem. Soc.* **1994**, *116* (20), 8978–8984.
(30) Sheu, C.; Foote, C. S.; Gu, C.-L. Photooxidation of 1,5-dithiacyclooctane. A novel carbon-sulfur bond cleavage. *J. Am. Chem. Soc.* **1992**, *114* (8), 3015–3021.

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