

Photocatalytic ozonation of clopyralid, picloram and triclopyr. Kinetics, toxicity and operational parameters influence

Rafael R. Solís ^a, F. Javier Rivas^{*,a}, Olga Gimeno ^a and José Luis Pérez-Bote ^b

Abstract

BACKGROUND: The systems photolysis, photocatalysis, single ozonation and photocatalytic ozonation in the presence of titania have been applied to the elimination of three pyridine carboxylic acids herbicides (clopyralid, picloram and triclopyr) in water which could be considered as priority organic pollutants.

RESULTS: Combination of radiation, titania photocatalyst and ozone led to the best results both in terms of herbicides elimination rate and mineralization. All the herbicides were relatively recalcitrant to direct ozonation showing second order rate constants in the proximity of $20 \text{ M}^{-1} \text{ min}^{-1}$ for clopyralid and triclopyr, and $105 \text{ M}^{-1} \text{ min}^{-1}$ in the case of picloram. Herbicides degradation is supposed to develop by the action of generated hydroxyl radicals, calculated second order rate constants with these species were 0.73, 3.80 and $1.73 \cdot 10^9 \text{ M}^{-1} \text{ s}^{-1}$ corresponding to clopyralid, picloram and triclopyr, respectively. Monitoring of some ionic species such as chloride and nitrates suggests the dechlorination of the herbicides as the primary stages in the process. Ring opening would follow leading to the accumulation of nitrates and the appearance of some low weight carboxylic acids. Toxicity drastically decreases at the end of the process.

**Correspondence to: FJ Rivas, Departamento de Ingeniería Química y Química Física, Universidad de Extremadura, Avda. Elvas s/n, 06071, Badajoz (Spain). Email: fjrivas@unex.es, Phone 00 34 289300, FAX 00 34 289385*

^a Departamento de Ingeniería Química y Química Física, Universidad de Extremadura, Avda. Elvas s/n, 06071, Badajoz (Spain)

^b Departamento de Anatomía, Biología Celular y Zoología. Universidad de Extremadura Av. Elvas s/n, 06071, Badajoz (Spain)

CONCLUSION: Picloram, clopyralid and triclopyr are relatively recalcitrant to ozonation. Photocatalytic ozonation shows different degrees of synergism. O₃/UVA/TiO₂ leads to 80% mineralization in less than one hour. Small amounts of TiO₂ as low as 0.005 g L⁻¹ could keep the efficiency of the photocatalytic ozonation without a significant loss in the herbicide abatement rate. Quantitative free chloride and nitrates rapidly appear in the O₃/UVA/TiO₂ system. First intermediates generated in the photocatalytic ozonation of clopyralid show a higher toxicity than the parent compound.

Keywords: Clopyralid, picloram, triclopyr, photocatalysis, photocatalytic ozonation, titania

Introduction:

Picloram (4-Amino-3,5,6-trichloro-2-pyridinecarboxylic acid), clopyralid (3,6-dichloro-2-pyridinecarboxylic acid) and triclopyr (3,5,6-Trichloro-2-pyridinyloxyacetic acid) are three chlorinated pyridine selective herbicides used for control of broadleaf weeds.

The principal environmental risks of these herbicides relate to contamination of surface and ground water, and damage to non-target terrestrial plants including crops adjacent to areas of application via runoff or drift. Such damage to plants also may emanate from more distant areas where ground water is used for irrigation or is discharged into surface water. Non-target plants adjacent to areas of application may be exposed to concentrations many times the levels that have been associated with toxic effects. In addition, EPA has concerns related to endangered terrestrial mammals and endangered aquatic animals. These herbicides are highly soluble in water, resistant to biotic and abiotic degradation processes, and mobile under both laboratory and field conditions.

Clopyralid is an herbicide that has recently been reported to occur in drinking water at concentrations above the Permitted Concentration Value (PCV) for an individual pesticide (EU directive 98/83/EC).¹ Triclopyr acid is somewhat persistent, and is mobile. The predominant degradation pathway for triclopyr in water is photodegradation (EPA (<http://www.epa.gov/oppsrrd1/REDs/factsheets/2710fact.pdf>)). EPA is concerned about the potential chronic toxicity and persistence of the triclopyr degradate, 3,5,6-trichloro-2-pyridinol, in the aquatic environment and is requiring additional confirmatory data to better characterize the fate of this metabolite. Picloram is highly soluble in water, resistant to biotic and abiotic degradation processes, and mobile under both laboratory and field conditions. It is stable to hydrolysis and anaerobic degradation, and degrades very slowly with half-lives ranging from 167 to 513 days (EPA (<http://www.epa.gov/oppsrrd1/REDs/factsheets/0096fact.pdf>)). EPA is concerned about

degradation of water quality in picloram use areas. Eventual contamination of ground water is virtually certain in areas where picloram residues persist in the overlying soil. Once in ground water, picloram is unlikely to degrade, even over a period of several years. Picloram also has a high potential to contaminate surface water by runoff from use areas. In this work, several advanced oxidation systems have been applied to remove these pesticides from water. Amongst them, special emphasis has been paid to the photocatalytic ozonation in the presence of TiO_2 . This powerful process is capable of combining the positive effect of three simpler processes (ozonation, photocatalysis and catalytic ozonation) leading to a global synergistic effect, especially significant when analyzing the mineralization extent.

Experimental:

Photoreactor and procedure

A 1L capacity perfectly mixed borosilicate glass photoreactor was used in all the experiments. The reactor was placed in the middle of a 31 cm external diameter pipe (54 cm height). The internal wall of the pipe was covered by aluminium foil to increase the photons reflection towards the reaction media. Four black light lamps (41 cm length) were evenly distributed and attached to the pipe. The lamps (LAMP15TBL HQPOWER™ manufactured by Velleman®) had a nominal power of 15 W mainly emitting within the range 350-400 nm, the maximum being located at 365 nm. Actinometry experiments in the presence of ferryxalate led to a value of $6.86 \cdot 10^{-5}$ Einstein $\text{min}^{-1} \text{L}^{-1}$ when 4 lamps were used.

Oxygen, nitrogen or a mixture oxygen-ozone was continuously bubbled into the water bulk by means of a diffuser placed at the reactor bottom. The gas flow rate was kept constant at 30 L h^{-1} in all the experiments. Photocatalysts were maintained in suspension

by magnetic stirring. Previously to the photodegradation experiments, the mixture water-photocatalyst, was stirred for 30 min in the dark to achieve the herbicide adsorption equilibrium on the photocatalyst surface. Prior to the analysis, the solid was removed from samples by filtration through Millex-HA filters (Millipore, 0.45 μm).

Ozone was produced from pure oxygen by using a Sander Laboratory Ozone Generator. Ozone in the gas phase was monitored by means of an Anseros Ozomat ozone analyser, which analysis is based on the absorbance at 254 nm.

Materials

Herbicides were purchased from Sigma-Aldrich and were used as received. Cliophar 425 from AGRIPHAR® (42.5% in the amine salt of clopyralid) was considered for ecotoxicity bioassays. Organic solvents were HPLC grade obtained from VWR Chemicals. Commercial photocatalyst TiO₂ Aeroxide® P25 from Evonik Industries (70% anatase and 30% rutile) with an average particle size of 30 nm and BET surface area of 50 m² g⁻¹ was used. Water purified by a Milli-Q water system (Millipore) was used in the preparation of solutions and suspensions.

Analysis

Herbicides were analysed by high-performance liquid chromatography (Agilent 1100). The column used was a Kromasil 100 5C18. The mobile phase was pumped at a flow rate of 1 mL min⁻¹, and the acetonitrile: acidified water (0.1% phosphoric acid) volume percentages applied were 40:60, 20:80 and 25:75 for tryclopir, clopyralid and picloram, respectively. Detection was conducted at 230, 200 and 220 nm, respectively.

Ionic short chain organic and inorganic compounds were monitored by ionic chromatography (Metrohm 881 Compact Pro), whose performance is based on high selectivity conductivity measure.

In order to assess the degree of mineralization, total organic carbon (TOC) was determined by a Shimadzu TOC 5000A analyser by directly injecting the aqueous solution.

Dissolved ozone in solution was determined by the indigo method, the analysis is based on the decolouration of the 5,5,7-indigo trisulfonate based on Bader and Hoigné method.² The pH of the reaction media was measured by means of a GLP 21+ CRISON pH-meter.

Eco-toxicity bioassays with *Daphnia parvula* and *Culex pipiens* larvae

The acute toxicity tests using *Daphnia parvula* were conducted using US EPA standard operating procedures.³ This procedure was extrapolated to carry out similar trials with mosquito larvae *Culex pipiens*. A culture of *D. parvula* or *C. pipiens* larvae was received from installations of Extremadura's University, where organisms were naturally cultured in artificial ponds.

20 young *D. parvula* or 15 second-instar *C. pipiens* larvae were placed in 100 mL of test solution using a disposable plastic transfer pipette, and were subjected to 16:8 light: dark photoperiods at room temperature. Survival numbers were recorded and monitored at 6, 24 and 48 hours for *D. parvula*; 24, 46, 72 and 96 hours for *C. pipiens*. Organisms were not fed during the experiments. In eco-toxicity tests, commercial clopyralid solutions were prepared in mineral water (10.7 mg L⁻¹ HCO₃⁻, 5.3 mg L⁻¹ SO₄²⁻, 19.0 mg L⁻¹, Cl⁻, 2.7 mg L⁻¹, Ca²⁺ 2.7 mg L⁻¹ Mg²⁺, 14.7 mg L⁻¹, Na⁺, and 14.3 mg L⁻¹ SiO₂). pH was adjusted to 7 ± 0.1. Three blank tests, without commercial herbicide addition, were considered per each run.

Initially trials were carried out at various parent compound initial concentrations in order to determine the concentration which causes the 50 % mortality (LC₅₀). After this preliminary study, samples from photocatalytic ozonation experiments at different stages

of oxidation extent were taken. An additional sample was also considered when TOC removal reached the steady state level.

Survival percentage was corrected to account for natural mortality in control runs by means of the expressions ⁴:

$$\% \text{ corrected mortality} = \frac{\% \text{ MWC} - \% \text{ CM}}{100 - \% \text{ CM}} \times 100 \quad (1)$$

$$\% \text{ Corrected survival} = 100 - \% \text{ corrected mortality} \quad (2)$$

In Eq. 1, MWC and CM stand for mortality without correction and control mortality (mortality in blank runs), respectively.

Results and discussion:

Technologies comparison

In a preliminary experimental series, different systems were applied to individual herbicides to assess their potential to remove these substances from water. Accordingly, UVA (365 nm) photolysis, UVA (365 nm) photocatalysis in the presence of titania, single ozonation and UVA (365 nm) photocatalytic ozonation in the presence of titania were investigated. Fig. 1 shows the normalized remaining concentration of clopyralid, picloram and triclopyr with time and the corresponding mineralization degree in terms of total organic carbon conversion. From this figure a similar behaviour is observed in all cases. Hence, none of the herbicides presents photolytic activity at the wavelength used (365 nm). Ozonation of clopyralid requires almost 180 minutes to reduce its concentration above 99% while in the case of picloram the reaction is slightly faster, showing conversions >99% in roughly 140 min. Triclopyr is the most recalcitrant compound towards single ozonation. The presence of halogens in the molecules of the herbicides tested suggests their recalcitrant nature towards ozonation. The electrophilic nature of

halogens produces the reactive molecular sites electron-deficient, inhibiting the ozonation process.⁵ Also, due to large size of halogen atoms such as chlorine, bromine, etc.; they create a steric hindrance for attack by ozone on the herbicides. Single ozonation led to poor results in terms of mineralization reduction (20-30% TOC conversion after 180 min).

The low efficiency of the ozonation process could be corroborated by calculating the direct rate constant between the different herbicides and molecular ozone. Accordingly, some experiments were conducted in the presence of tert-butanol (t-BuOH), a well-known free hydroxyl radical scavenger. Under these conditions, the parent compound removal rate can be formulated as:

$$-\frac{dC_i}{dt} = k_{O_3,i} C_i C_{O_3} = k_{O_3,i} C_i \left[a \times t + b \times t^2 + c \times \text{atan}(t) + d \times \tanh(t) \right] \quad (3)$$

In Eq. 3, the experimental ozone concentration has been fitted to a mathematical expression with parameters a, b, c and d. Additionally $k_{O_3,i}$ stands for the direct ozonation rate constant of compound i and C_i , C_{O_3} the concentration of parent compound and ozone in the water bulk, respectively. Eq. 3 applies when slow kinetic regimes develop in the system, that is, when Hatta number values are below 0.02. This expression was numerically solved by using the 4th order Runge Kutta method. Unknown $k_{O_3,i}$ was optimized to minimize the squared differences between experimental and calculated herbicide concentrations. The SOLVER add-in included in the EXCEL spreadsheet was used in the optimization process. Fig. 2 shows the experimental and calculated results obtained in ozonation experiments in the presence of t-BuOH. The optimization process led to $k_{O_3,i}$ values of $20 \text{ M}^{-1} \text{ min}^{-1}$ ($R^2=0.97$), $105 \text{ M}^{-1} \text{ min}^{-1}$ ($R^2=0.99$) and $20 \text{ M}^{-1} \text{ min}^{-1}$ ($R^2=0.96$) corresponding to the direct ozonation rate of clopyralid, picloram and triclopyr,

respectively. The value obtained for picloram is below the interval 300-9000 M⁻¹ min⁻¹ proposed by Yao and Haag ⁶; and, there are no more available data for the rest.

The values of k_{O₃,i} confirm the low reactivity of these herbicides towards ozone. Hatta number is below 0.02 in all cases, validating the use of Eq. 1. In an attempt to validate the calculated k_{O₃,i} values, a series of competitive experiments were carried out. Hence, when two herbicides (i and j) were simultaneously ozonated at pH 7 in the presence of tert-butanol, the following equation apply (slow regime):

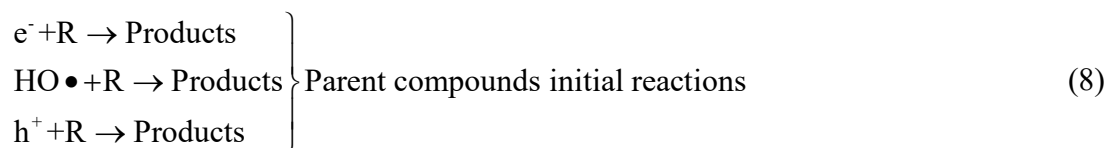
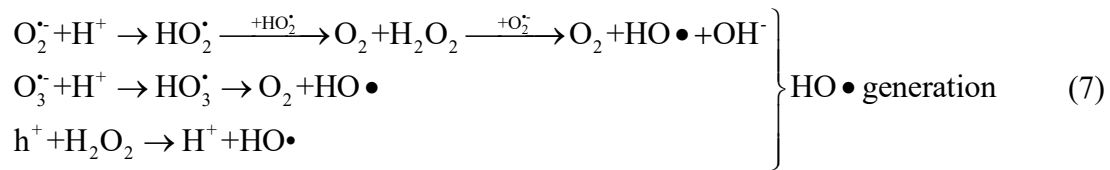
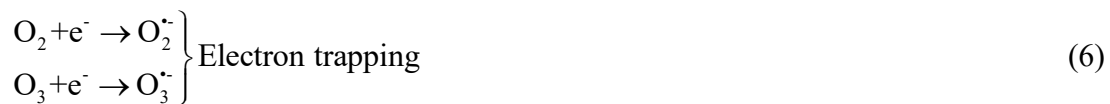
$$\ln\left(\frac{C_i}{C_{i0}}\right) = \frac{k_{O_3,i}}{k_{O_3,j}} \ln\left(\frac{C_j}{C_{j0}}\right) \quad (4)$$

Eq. 4 is plotted in Fig. 3. The slopes k_{O₃,Picloram} / k_{O₃,Triclopyr} (4.1) and k_{O₃,Picloram} / k_{O₃,Clopyralid} (4.7) were acceptably close to the expected value of 5.2, however the slope k_{O₃, Triclopyr} / k_{O₃,Clopyralid} (2.5, R² = 0.99 in duplicated experiments) substantially differed from the expected value of roughly 1.0. By considering the evolution of the dissolved ozone concentration in the latter experiments the value of k_{O₃,Clopyralid} was verified in the proximity of 20 M⁻¹ min⁻¹; in other words, the presence of clopyralid increases the apparent value of k_{O₃,Triclopyr} by a factor of 2.5. Given the experimental conditions used in the competitive experiments, any role played by hydroxyl radicals could be a priori neglected. The activity of organic peroxyradicals and other organic radicals formed upon ozonation of clopyralid might explain the observed results.

From Fig. 1 it can be inferred that photocatalytic ozonation was the most efficient technology in terms of herbicide removal rate and mineralization degree achieved after three hours of treatment. Photocatalysis also led to acceptable results if compared to the inefficient photolysis or even single ozonation of herbicides.

In order to assess the potential synergistic effect of the simultaneous application of ozone, UVA radiation and TiO₂, the apparent pseudofirst order rate constant was calculated for the system O₃/UVA/TiO₂ and compared to the sum of the corresponding k-values for O₃ and O₂/UVA/TiO₂. Obviously, the pseudofirst order rate constants calculated have no rigorous kinetic basis and they are just considered as a mere comparison tool. Table 1 shows the three constants obtained under the experimental conditions used and the synergistic effect observed.

As displayed in Table 1, the efficiency observed in the photocatalytic ozonation cannot be explained by the simple summation of the single ozonation and photolysis processes. Thus, it is expected a higher generation of hydroxyl radicals in the photocatalytic system when ozone is present in the media. Ozone is capable of trapping photocatalytic generated electrons more efficiently than oxygen, avoiding, therefore the recombination of the electron-hole pair according to the following mechanism.⁷



In the above mechanism one molecule of ozone necessitates only one electron to produce one hydroxyl radical, compared to three electrons required by oxygen to get the same HO• yield.

Mineralization of the herbicides is highly improved when TiO₂ is in the reaction media. The system O₃/UVA/TiO₂ leads to a final TOC conversion in the range 70-80% regardless of the oxidized herbicide. Additionally, it should be highlighted that TOC elimination preferentially occurs in the first 40-50 min of photocatalytic ozonation, that is, TOC concentration comes to a halt after the first hour of treatment. The photocatalytic oxidation by HO• radicals is hypothesized to be the main path to TOC abatement. Accordingly, the rate constants between this species and the used herbicides were calculated. The photolysis of each herbicide in the presence of a high concentration of H₂O₂ was completed by using UVC radiation. In this system, the following differential equations apply ⁸:

$$-\frac{dC_i(t)}{dt} = \varphi_i \frac{\varepsilon_i C_i(t)}{A_{254nm}(t)} I_0 [1 - \exp(-2.303 L A_{254nm}(t))] + k_{HO\bullet,i} C_i(t) C_{HO\bullet}(t) \quad (9)$$

$$-\frac{dC_{H_2O_2}(t)}{dt} = \varphi_{H_2O_2} \frac{\varepsilon_{H_2O_2} C_{H_2O_2}(t)}{A_{254nm}(t)} I_0 [1 - \exp(-2.303 L A_{254nm}(t))] + k_{HO\bullet,H_2O_2} C_{H_2O_2}(t) C_{HO\bullet}(t) \quad (10)$$

$$\frac{dC_{HO\bullet}(t)}{dt} = 2\varphi_{H_2O_2} \frac{\varepsilon_{H_2O_2} C_{H_2O_2}(t)}{A_{254nm}(t)} I_0 [1 - \exp(-2.303 L A_{254nm}(t))] - k_{HO\bullet,i} C_i(t) C_{HO\bullet}(t) - k_{HO\bullet,H_2O_2} C_{H_2O_2}(t) C_{HO\bullet}(t) - k_{HO\bullet,Intermediates} C_{Intermediates}(t) C_{HO\bullet}(t) \quad (11)$$

In the above mechanism, φ_i and ε_i are the quantum yield and molar absorptivity at 254 nm of compound i, respectively. $A_{254nm}(t)$ accounts for the absorption of UV light of all the species present in solution at t time, I_0 is the incident radiation intensity per volume ($1.73 \cdot 10^{-6}$ Einstein L⁻¹ s⁻¹) and L is the radiation pathlength in the reactor (3 cm).

Since H₂O₂ is in excess, complete adsorption of UVC radiation by H₂O₂ can be assumed. Generated hydroxyl radicals are mainly trapped by H₂O₂ and the parent compounds

studied. Also, given the high hydrogen peroxide concentration used, its concentration does not appreciably vary with time. As a consequence, applying the steady state assumption to hydroxyl radical concentration, the herbicide removal rate simplifies to:

$$-\frac{dC_i(t)}{dt} = k_{HO\bullet,i} C_i(t) \frac{2\phi_{H_2O_2} I_0 \left[1 - \exp\left(-2.303 L \varepsilon_{H_2O_2} C_{H_2O_2_0}\right) \right]}{k_{HO\bullet,i} C_i(t) + k_{HO\bullet,H_2O_2} C_{H_2O_2_0}} \quad (12)$$

After integration:

$$\ln \frac{C_{i_0}}{C_i} = k_{HO\bullet,i} \frac{2\phi_{H_2O_2} I_0 \left[1 - \exp\left(-2.303 L \varepsilon_{H_2O_2} C_{H_2O_2_0}\right) \right]}{k_{HO\bullet,i} C_i(t) + k_{HO\bullet,H_2O_2} C_{H_2O_2_0}} \times t \quad (13)$$

From Eq. 13 (see Fig. 3), the following $k_{HO\bullet,i}$ values were obtained: 0.73, 3.80 and $1.73 \cdot 10^9 \text{ M}^{-1} \text{ s}^{-1}$ corresponding to clopyralid, picloram and triclopyr, respectively.

Photocatalytic ozonation. Variables influence

Catalyst concentration

Catalyst concentration effect was investigated in the range $0.5\text{-}0.005 \text{ g L}^{-1}$, in individual experiments. Fig. 4 shows the results obtained.

As observed from this figure, TiO_2 amounts as low as $5 \cdot 10^{-3} \text{ g L}^{-1}$ are able to degrade the target pesticides without significantly decreasing the oxidation rate if compared to the experiments completed with higher titania concentrations. In the specific case of picloram, no significant differences could be appreciated between the experiment conducted with $5 \cdot 10^{-3} \text{ g L}^{-1} \text{ TiO}_2$ and the experiment carried out with a concentration 100 times higher. In the case of triclopyr and clopyralid, a slight decrease in the efficiency can be envisaged, which is in agreement with the existence of an optimum concentration (m_{\max}) in TiO_2 . This optimum concentration seems to be $m_{\max} > 0.5 \text{ g L}^{-1}$ for clopyralid,

$0.05 > m_{\max} > 0.1 \text{ g L}^{-1}$ for triclopyr and $m_{\max} < 0.005 \text{ g L}^{-1}$ for picloram. Similar results (not shown) were obtained when TOC evolution was analysed.

Catalyst reuse

One of the most important withdrawals in heterogeneous catalysis is the loss of activity of the solid after several cycles. In the particular case of titania this situation is aggravated by the difficulty in recovering the 100% of the catalyst amount used in each run. However, the latter drawback is minimized when experiments are carried out at pilot plant scale. In this work an attempt was made to reuse TiO_2 in consecutive photocatalytic ozonation experiments by utilizing each time, the amount recovered after centrifugation. With this methodology, the potential deactivation of TiO_2 is not hindered by the addition of fresh catalyst required to get the same initial solid concentration in all experiments.

Fig. 5 depicts the results obtained. As inferred from the figure caption, the amount of catalyst used in each cycle decreases to 65%, 45% and 25% of the initial TiO_2 added corresponding to the first, second and third reuses, respectively. From Fig. 5 it is observed that catalyst deactivates in consecutive runs; in other words, by comparing the results in Figs. 4 and 5, the loss in TiO_2 concentration due to the recovery process does not explain the loss in herbicides removal efficiency. Amazingly, the mineralization degree did not follow the same pattern. Thus, besides some differences in the TOC abatement rate, at the end of the process the TOC conversion is similar regardless of the reuse cycle of the catalyst.

Initial herbicide concentration

Fig. 6 shows two experiments carried out with initial herbicide concentrations of 5 and 20 mg L^{-1} , respectively. As observed, increasing the initial herbicide load leads to the displacement of the curve to the right, so the time needed for complete herbicide removal

is practically doubled. This curve shift discards the development of first order kinetics, a complex reaction mechanism proceeds in this type of systems. Previous adsorption of the pesticides onto the catalyst likely occurs, so Langmuir type kinetics develops.

Additionally, TOC evolution should be highlighted. Hence, TOC conversion increases as the initial concentration of clopyralid or triclopyr is increased, while in the case of picloram no clear trend was experienced. These results suggest the development of autocatalytic mechanisms in TOC abatement, likely due to the generation of reactive organic radicals. The TOC removal when 20 mg L^{-1} of initial herbicide concentration was used was correlated to the evolution of some ionic species such as nitrate, chloride and some low weight organic acids (end-products). Fig. 7 shows the evolution of the aforementioned species. As seen in Fig. 7 some low weight organic acids such as acetic, formic, and oxalic acids were tentatively identified during the process. Particular importance is given to the evolution of chloride. This anion immediately reaches the maximum amount that can be released from the herbicide in just 10-15 min (represented by the dashed line Cl^-_{MAX}), in agreement with the total abatement of the parent compound. These results suggest the primary attack to the Cl-C bond in the herbicides. Some authors have reported the generation of intermediates keeping the Cl moiety in their structures. Thus, Sojic et al.⁹ claim the formation of 3,6-dichloropyridin-2-ol, 3,6-dichloro-4-hydroxypyridine-2-carboxylic acid and 3,6-dichloro-5-hydroxypyridine-2-carboxylic acid in the first stages of the photocatalysis of clopyralid. Also, 2,3,5-Trichloropyridine-4-ylamine, 3,5,6-Trichloropyridine-2-carboxylic acid, 4-Amino-5,6-dichloro-3-hydroxypyridine-2-carboxylic acid and 5,6-Dichloro-3-hydroxypyridine-2-carboxylic acid were detected in the degradation of picloram by the electro-Fenton process.¹⁰

From chloride evolution in this work, dechlorination of herbicides seems to be the initial mechanism of parent compound abatement. These results are also substantiated in other works.¹¹

Additionally, nitrate accumulation curves follow the same pattern than TOC conversion indicating that ring opening and pyridinic-nitrogen loss are the key stages in mineralization. Fig. 7 shows the maximum nitrate concentration expected in solution from the pyridinic nitrogen. Nitrate final concentration reached 70-80% of the maximum amount in the case of clopyralid and triclopyr and 100% in the case of picloram. In the latter case formation of oxamic acid from the amine group is likely to occur.¹²

Amongst the identified low weight carboxylic acids, formic acid predominates over the rest, suggesting the breakage of the COOH moiety in the herbicides.

Photocatalytic ozonation of commercial clopyralid. Toxicity evolution.

Given the similarity between the structures of the used herbicides, it was decided to evaluate the toxicity of only one of them in its commercial form. Hence, branded clopyralid was oxidized and the toxicity of generated intermediates evaluated at different parent compound conversions. Previously, some preliminary tests were carried out to assess the potential toxicity of the non-treated herbicide. Several herbicide concentrations were used and the toxicity to *D. parvula* and *C. pipiens* larvae monitored at 6, 24, 48, 72 and 96 exposure hours.

Fig. 8 shows a higher toxicity of clopyralid to *D. parvula*. In any case, toxicity levels are relatively low for both the crustacean and the larvae.

From Fig. 8 the following LC₅₀ values towards *D. parvula* can be calculated, 184 mg L⁻¹ (24 h) and 70 mg L⁻¹ (48 h). An EC₅₀ (48h) value of 225 mg L⁻¹ can be found in the EPA data base (EPA (<http://cfpub.epa.gov/ecotox/>)) when using *D. magna*. In the case of *C.*

pipiens larvae, clopyralid tested concentrations are not sufficiently high to achieve the zero survival limit; however, assuming that 100% mortality can be achieved at high concentrations, calculated LC₅₀ values are 3550 mg L⁻¹ (72 h) and 3100 mg L⁻¹ (96 h).

As stated previously, photocatalytic ozonation is a suitable process to eliminate the herbicides used in this study. Nevertheless, the toxicity of intermediates and accumulated end products in the reaction media requires attention. Subsequently, toxicity bioassays were completed by considering different stages in the oxidation process, i.e. considering several values of clopyralid conversion. Additionally, different clopyralid initial concentrations were also used in these runs. Fig. 9 shows a general trend in toxicity profiles. Apparently, the first intermediates generated in clopyralid oxidation are more toxic than the parent herbicide. For instance, when 100 ppm of clopyralid was treated, *D. parvula* mortality at 24 and 48 h of exposure reached the top limit of 100% when the herbicide was present in solution. Even so, the survival percentage drastically increased once clopyralid was removed from solution. As expected, the experiment carried out with the lowest clopyralid concentration (5 ppm) barely exhibited some toxicity regardless of the time exposure.

As stated previously, *C. pipiens* larvae are less sensitive to the herbicide than Daphnis. This can also be translated to intermediates, although again the “U” type profiles in survival percentage (mainly after 72-96 h exposure) can be envisaged when high clopyralid concentration is used. Again, the lowest value of clopyralid initial concentration led to solutions of negligible toxicity towards the larvae.

Conclusions

The following conclusions can be listed:

-Clopyralid, picloram and triclopyr are relatively recalcitrant towards ozone. These species are better degraded in those systems capable of generating radical species.

-The combination of ozone and photocatalysis lead to the best results in terms of herbicide abatement rate and mineralization extent.

-In the photocatalytic ozonation process, TOC conversion is not negatively affected by an increase in the initial herbicide concentration, suggesting the development of autocatalytic reactions.

-The photocatalyst partially deteriorates after several reuses when the herbicide evolution was monitored; however, the solid kept its mineralization activity after the third reuse.

-Free chloride immediately accumulates in the reaction media, dechlorination is supposed to occur in the first stages of the photocatalytic ozonation process.

-In order to decrease the toxicity of treated effluents, special care must be considered to completely remove herbicides from water. In any case, intermediates generated in the photocatalytic ozonation of diluted herbicides do not present toxicity problems.

Acknowledgements

The authors thank the economic support received from Gobierno de Extremadura and CICYT of Spain through Projects GRU10012 and CTQ2012-39789-C02-01, respectively. Mr. Rafael Rodríguez Solís also thanks Gobierno de Extremadura, Consejería de Empleo, Empresa e Innovación, and FEDER Funds for his PhD grant (PD12058).

References

- 1 Tizaoui C, Mezughi, K, Bickley, R, Heterogeneous photocatalytic removal of the herbicide clopyralid and its comparison with UV/H₂O₂ and ozone oxidation techniques, *Desalination* 273(1): 197–204 (2011).
- 2 Bader H, Hoigné J, Determination of ozone in water by the indigo method, *Water Res.* 15(4): 449-456 (1981).
- 3 EPA, *Methods for measuring the acute toxicity of effluents and receiving water to freshwater and marine organisms*. U.S. Environmental Protection Agency Office of Water, Washington (2002).
- 4 Abbott WS, A method of computing the effectiveness of an insecticide, *J. Econ. Entomol.* 18(2): 265-267 (1925).
- 5 Lei H, Snyder SA, 3D QSPR models for the removal of trace organic contaminants by ozone and free chlorine, *Water Res.* 41(18), 4051-4060 (2007).
- 6 Yao CCD, Haag WR, Rate constants for direct reactions of ozone with several drinking water contaminants, *Water Res.* 25(7): 761-773 (1991).
- 7 Agustina TE, Ang HM, Vareek VK, A review of synergistic effect of photocatalysis and ozonation on wastewater treatment, *J. Photoch. Photobio. C* 6(4): 264–273 (2005).
- 8 Rivas J, Gimeno O, Borrallho T, Carbajo M, UV-C photolysis of endocrine disruptors. The influence of inorganic peroxides, *J. Hazard. Mater.* 174(1-3): 393-397 (2010).
- 9 Sojic DV, Anderluh VB, Orcic DZ, Abramovic BF, Photodegradation of clopyralid in TiO₂ suspensions: Identification of intermediates and reaction pathways, *J. Hazard. Mater.* 168(1): 94–101 (2009).
- 10 Özcan A, Şahin Y, Koparal AS, Oturan MA, Degradation of picloram by the electro-Fenton process, *J. Hazard. Mater.* 153(1-2): 718-727 (2008).

- 11 Westphal K, Saliger R, Jäger D, Teevs L, Prüße U, Degradation of Clopyralid by the Fenton Reaction, *Ind. Eng. Chem. Res.* **52(39)**: 13924-13929 (2013).
- 12 Özcan A, Oturan N, Şahin Y, Oturan MA, Electro-Fenton treatment of aqueous clopyralid solutions, *Int. J. Environ. An. Ch.* **90(3-6)**: 478-486 (2010).

Table 1. Observed pseudofirst order rate constant in different systems. Synergistic effect.

	Observed pseudo-k rate constant ($\cdot 10^2 \text{ min}^{-1}$)			$k_3/(k_1+k_2)$
	O_3 (k_1)	$\text{O}_2/\text{UVA}/\text{TiO}_2$ (k_2)	$\text{O}_3/\text{UVA}/\text{TiO}_2$ (k_3)	
Clopyralid	2.3	3.9	22.6	3.7
Picloram	4.1	8.5	103.8	8.2
Triclopyr	0.42	3.1	67.4	18.9

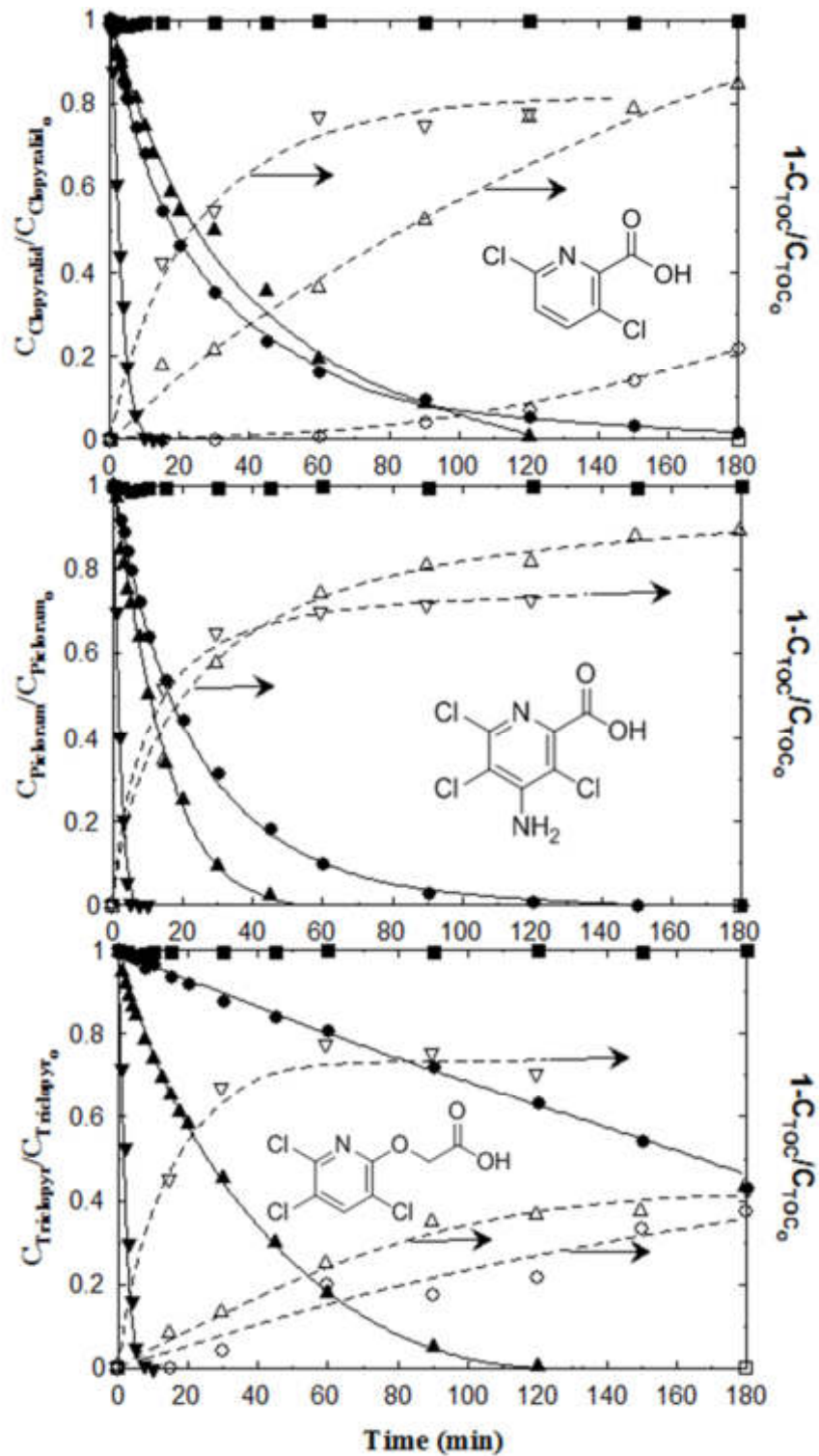


Figure 1. Removal of herbicides by different technologies. Experimental conditions: $V = 1.0 \text{ L}$, $C_{\text{TiO}_2} = 0.5 \text{ g L}^{-1}$; $Q_{\text{Gas}} = 30 \text{ L h}^{-1}$; $C_{\text{O}_3\text{in}} = 10 \text{ mg L}^{-1}$, $\text{pH} = 5.0$; $C_{\text{Herbicide}} = 5.0 \text{ mg L}^{-1}$. Symbols: ●, O_3 ; ■, UVA; ▲, $\text{O}_2/\text{UVA}/\text{TiO}_2$; ▼, $\text{O}_3/\text{UVA}/\text{TiO}_2$.

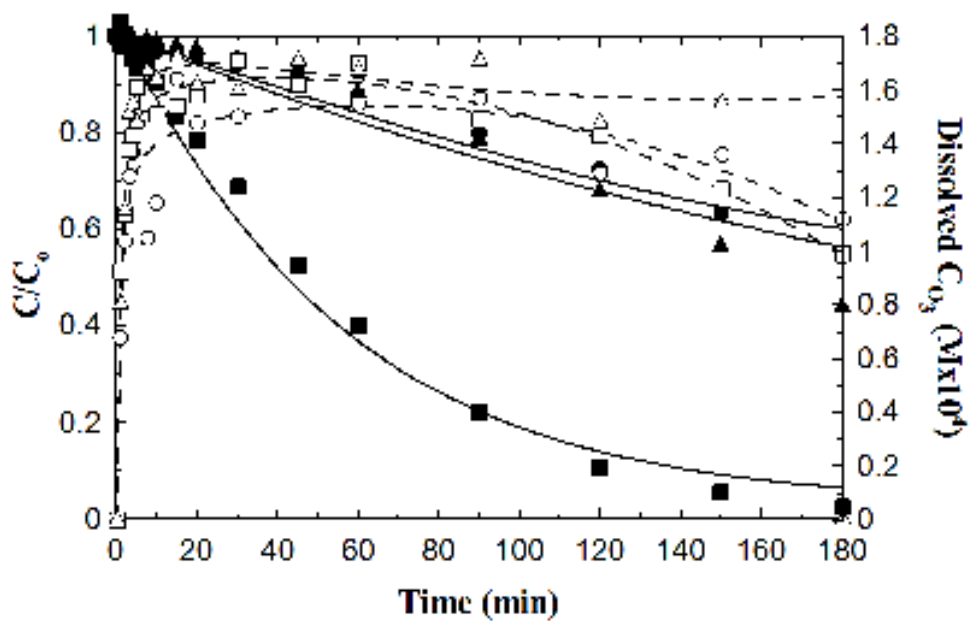


Figure 2. Removal of herbicides by ozonation in the presence of 0.01 M of t-BuOH. Experimental conditions: $V = 1.0 \text{ L}$, $Q_{\text{Gas}} = 30 \text{ L h}^{-1}$; $C_{\text{O}_3\text{in}} = 50 \text{ mg L}^{-1}$; $\text{pH} = 7.0$; $C_{\text{Herbicide}} = 5.0 \text{ mg L}^{-1}$. Symbols: ●, clopyralid; ■, picloram; ▲, triclopyr. (Open symbols correspond to dissolved ozone). Solid line = model calculations, dashed lines = mathematical fitting.

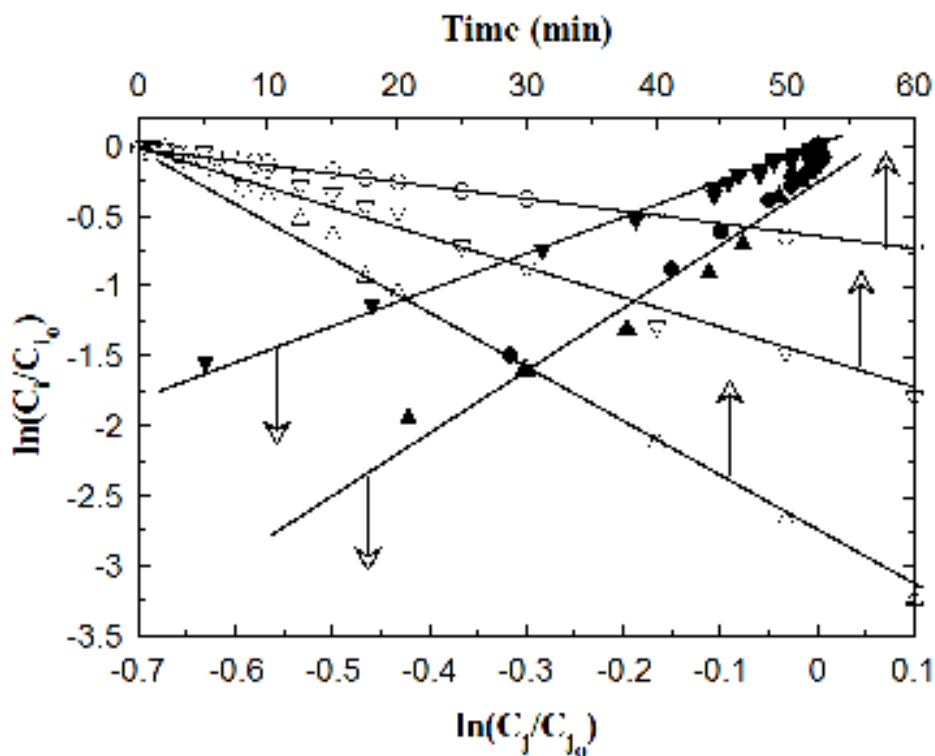


Figure 3. Competitive ozonation of herbicides in the presence of 0.01 M of t-BuOH. Experimental conditions: $V = 1.0$ L; $\text{pH} = 7.0$; $C_{\text{Herbicide}} = 5.0 \text{ mg L}^{-1}$. Symbols: \bullet , i =Triclopyr j =Picloram; \blacktriangledown , i =Triclopyr j =Clopyralid; \blacktriangle , i =Picloram j =Clopyralid. UVC Photolysis in the presence of 0.5 M of H_2O_2 . $V = 1.0$ L; $\text{pH} = 7.0$; $C_{\text{Herbicide}} = 5.0 \text{ mg L}^{-1}$. Symbols: \circ , Clopyralid; Δ , Picloram; ∇ , Triclopyr.

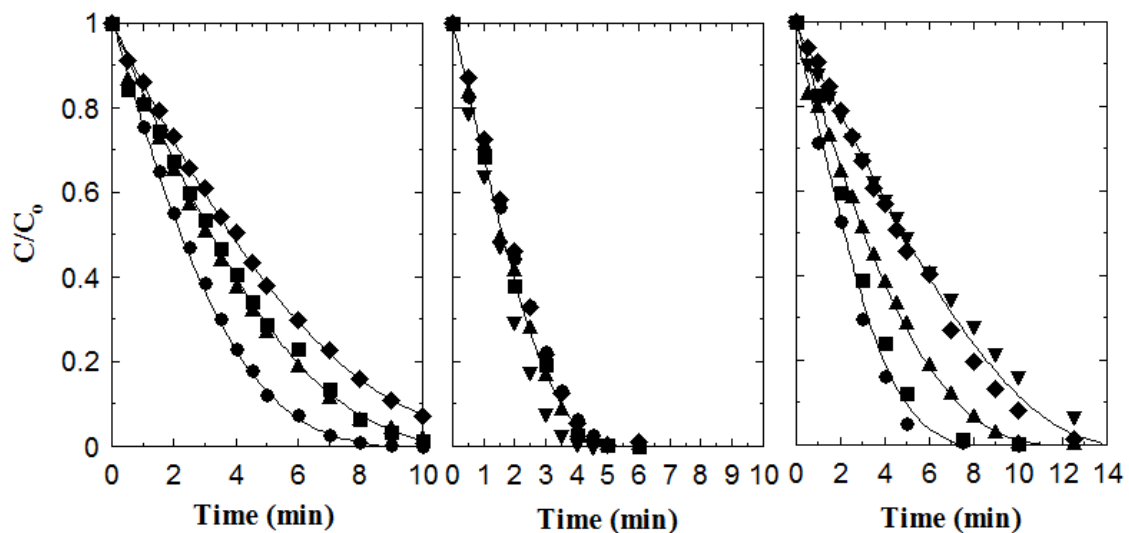


Figure 4. Photocatalytic ozonation of clopyralid (left), picloram (middle) and triclopyr (right). Influence of catalyst concentration. Experimental conditions: $V = 1.0 \text{ L}$; $\text{pH} = 5.0$; $C_{\text{Herbicide}} = 5.0 \text{ mg L}^{-1}$, $Q_{\text{Gas}} = 30 \text{ L h}^{-1}$; $C_{\text{O}_3\text{in}} = 10 \text{ mg L}^{-1}$. $\text{TiO}_2 \text{ (g L}^{-1}\text{)}$: ●, 0.5; ■, 0.1; ▲, 0.05; ▼, 0.01; ◆, 0.005.

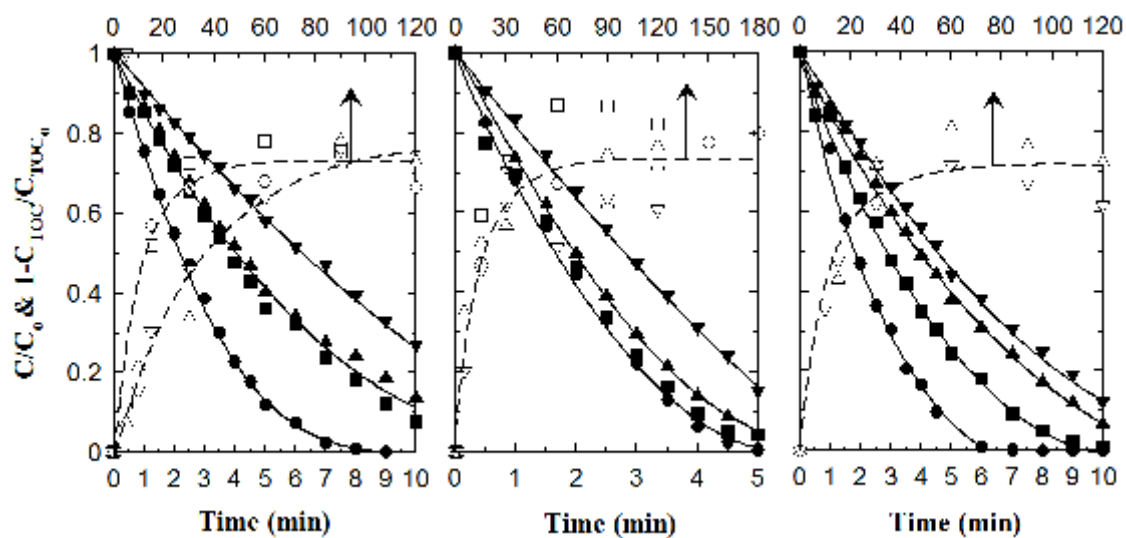


Figure 5. Photocatalytic ozonation of clopyralid (left), picloram (middle) and triclopyr (right). Influence of catalyst reuse. Experimental conditions: $V = 1.0 \text{ L}$; $\text{pH} = 5.0$; $C_{\text{Herbicide}} = 5.0 \text{ mg L}^{-1}$, $Q_{\text{Gas}} = 30 \text{ L h}^{-1}$; $C_{\text{O}_3\text{in}} = 10 \text{ mg L}^{-1}$; initial TiO_2 0.5 g L^{-1} . ●, fresh (0.5 g L^{-1}); ■, 1st reuse ($0.32\text{-}0.35 \text{ g L}^{-1}$); ▲, 2nd reuse ($0.22\text{-}0.25 \text{ g L}^{-1}$); ▼, 3rd reuse ($0.12\text{-}0.13 \text{ g L}^{-1}$).

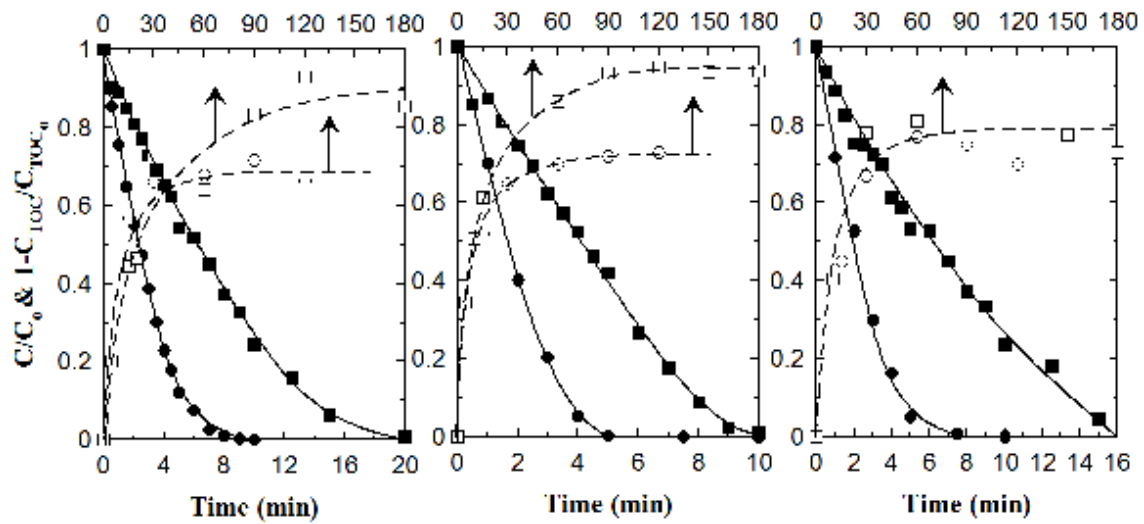


Figure 6. Photocatalytic ozonation of clopyralid (left), picloram (middle) and triclopyr (right). Influence of initial herbicide concentration. Experimental conditions: $V = 1.0$ L; $pH = 5.0$; $Q_{Gas} = 30$ L h^{-1} ; $C_{O_3in} = 10$ mg L^{-1} ; $C_{TiO_2} = 0.5$ g L^{-1} ; $C_{Herbicide}$ (mg L^{-1}), ●, 5; ■, 20.

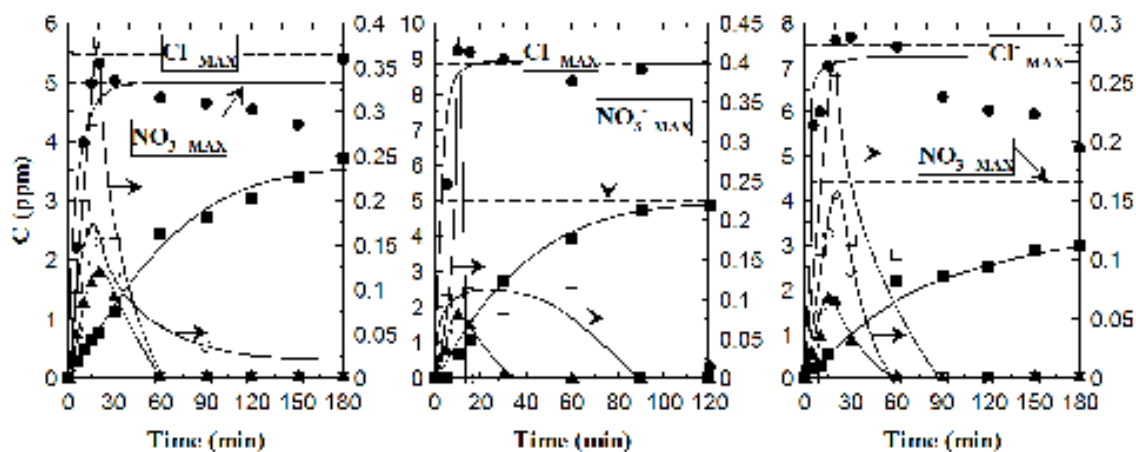


Figure 7. Photocatalytic ozonation of clopyralid (left), picloram (middle) and triclopyr (right). Ionic species evolution. Experimental conditions: $V = 1.0 \text{ L}$; $\text{pH} = 5.0$; $Q_{\text{Gas}} = 30 \text{ L}\cdot\text{h}^{-1}$; $C_{\text{O}_3\text{in}} = 10 \text{ mg L}^{-1}$; $C_{\text{TiO}_2} = 0.5 \text{ g L}^{-1}$; $C_{\text{Herbicide}} = 20 \text{ mg L}^{-1}$. ●, chloride (left y-axis); ■, nitrates (left y-axis); ▲, formic acid (left y-axis); ○, acetic acid (right y-axis); □, oxalic acid (right y-axis).

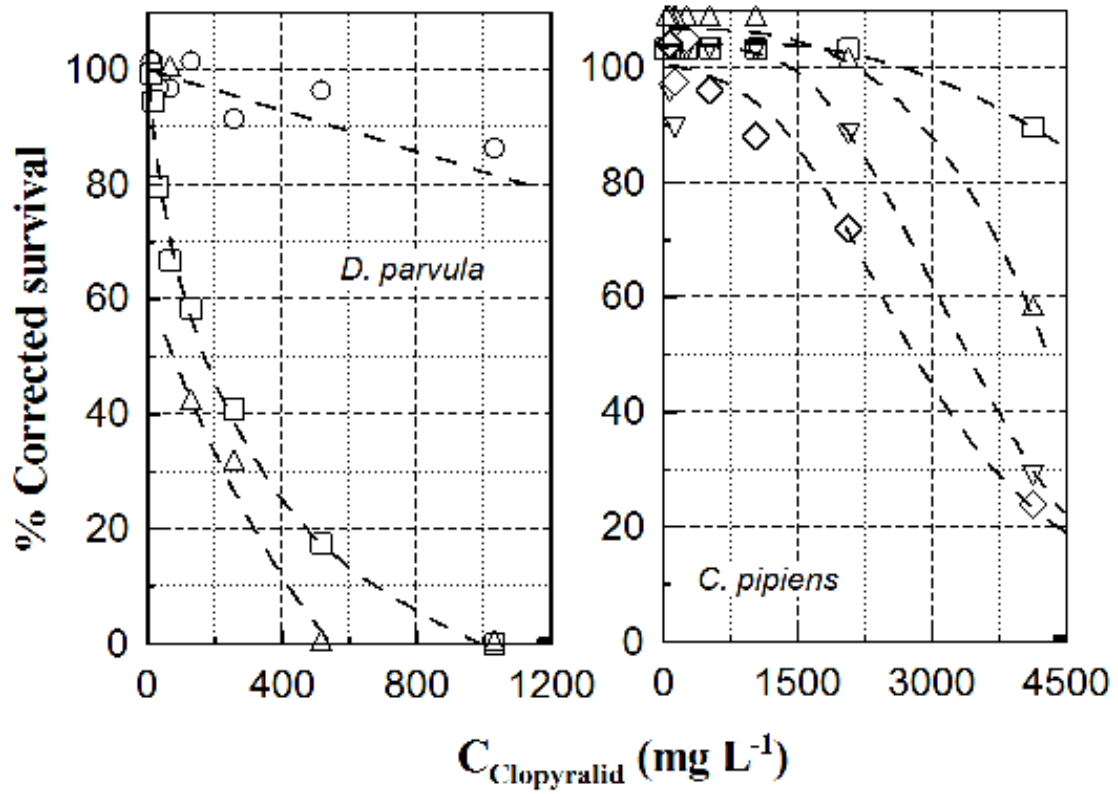


Figure 8. Percentage of survival of *D. parvula* and *C. pipiens* larvae at increasing values of clopyralid concentration. Exposure time (h): ○, 6.0; □, 24; △, 48; ▽, 72; ◇, 96.

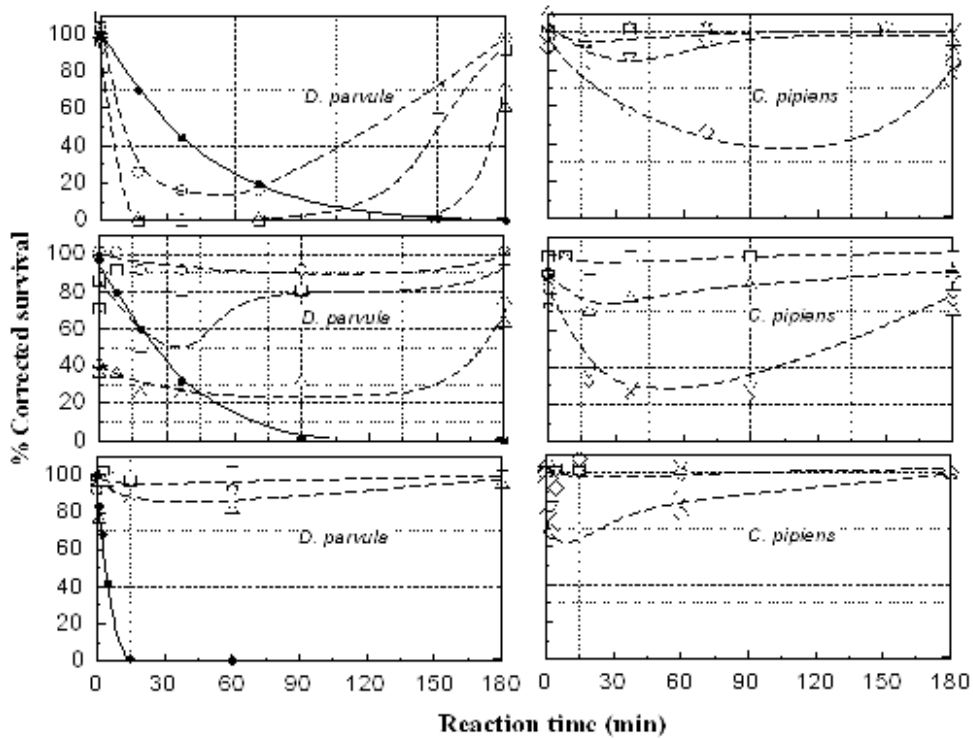


Figure 9. Percentage of survival of *D. parvula* and *C. pipiens* larvae in solutions of photocatalytic ozononated clopyralid at different reaction periods. Initial MCPA concentration: top figure = 100 mg L⁻¹; middle figure = 75 mg L⁻¹; bottom figure = 5 mg L⁻¹. Time of exposure (h): ○, 6.0; □, 24; Δ, 48; ∇, 72; ◇, 96 (solid circles = remaining clopyralid percentage).