Priority pesticides abatement by advanced water technologies: the case of acetamiprid removal by ozonation

A. Cruz-Alcalde*, C. Sans, S. Esplugas

Department of Chemical Engineering and Analytical Chemistry, Faculty of Chemistry, Universitat de Barcelona, C/Martí i Franqués 1, 08028 Barcelona, Spain. Tel: +34934029789; fax: +34934021291

*Corresponding Author: alberto.cruz@ub.edu

ABSTRACT

With the aim of exploring treatment alternatives for priority insecticide acetamiprid (ACMP) abatement, the removal of this compound from water by ozonation was studied for the first time, paying special attention to the kinetic, mechanistic and toxicological aspects of the process. The second order rate constants of reactions between ACMP and both molecular ozone (O_3) and hydroxyl radicals (OH·) were determined to be 0.25 $M^{-1}s^{-1}$ and $2.1 \cdot 10^9$ $M^{-1}s^{-1}$, respectively. On the basis of kinetic results, the degradation of ACMP during ozonation could be well-explained by the reactivity of this pesticide with OH·. HPLC/MS analysis of the ozonated ACMP showed ACMP-*N*-desmethyl, 6-chloronicotinic acid, *N'*cyano-*N*-methyl acetamidine and *N'*-cyano acetamidine as the major transformation products (TPs), all of them formed through amine α carbon oxidation in combination with hydrolysis. Microtox bioassays revealed an increase in the toxicity of the medium during ACMP ozonation process, followed by a decrease to relatively low values. These changes could be attributed to the synergistic effects between TPs as well as to the presence of toxic intermediate aldehydes. Even though adopting strategies to further promote ozone decomposition to hydroxyl radicals appears to be essential, ozonation can be an effective treatment process for ACMP removal and associated toxicity abatement.

KEYWORDS

Acetamiprid, priority pesticides, ozonation, hydroxyl radical oxidation, reaction mechanisms, toxicity assessment

1. Introduction

Since 2013 some regulations regarding the identification, monitoring and control of priority substances/groups of substances in aquatic compartments have been published [1,2]. For example, the 1st watch list of substances for Union-wide monitoring, Decision 2015/495/EU [3], promotes the study of alternative water and wastewater treatment options aimed to remove these substances from aqueous resources. Several pesticides belonging to different families are included as priority pollutants. One of these groups, neonicotinoids, are nowadays one of the most employed class of pesticides [4]. They offer insect selectivity, excellent physicochemical properties, wide spectrum of efficacy and a relative safe use in comparison with other pesticide classes like organophosphorus, carbamates or pyrethroids [5,6]. The widespread use of these chemicals have resulted in their occurrence in all environment compartments, including water [5]. According to previous studies, the presence of neonicotinoids in nature could be harmful to a broad range of invertebrate [7] and also vertebrate [8] non-target organisms. Regarding the risks for human health, several recent studies have associated the chronic exposure to neonicotinoids with certain types of developmental disorders like congenital heart defects (CHD) [9], neural tube defects (NTD) [10] and autism spectrum disorder (ASD) [11].

(*E*)-N-(6-chloro-3-pyridylmethyl)-*N*'-cyano-N-methylacetamidine, better known as acetamiprid (ACMP) is a pesticide belonging to neonicotinoid insecticides class. It is one of the most applied insecticides nowadays, being the fourth most employed neonicotinoid in USA [4] and representing more than a 10% of the total sales of this group of insecticides in the last years [12]. China, one of the largest ACMP producers, had in 2013 a production of 8000 tons of this insecticide, 5000 of which were exported [13]. Because of its extensive usage, this micropollutant has been detected in surface (20-380 ng L^{-1} [14]; 2.7-59.3 ng L^{-1} [15]; 2-410 ng L^{-1} [16]; up to 41 µg L^{-1} [17]) and also wastewater (50 ng L^{-1} [18]) samples worldwide. The presence of ACMP in the environment can pose risks to human health. Based on a previous work by Kimura-Kuroda [19], the European Food Safety Authority (EFSA) recently delivered a

Scientific Opinion concluding that chronic exposure to ACMP could affect neural development and function in humans [20]. A more recent study associated the chronic exposure to this insecticide with some adverse effects on human health, including memory loss and finger tremors [21]. Moreover, ACMP has been demonstrated to negatively affect other species like aquatic [22] and soil [23] microorganisms, as well as beneficial insects [24,25]. However, despite its presence in water compartments pose a serious threat to environmental and human safety, scientific literature regarding the removal of ACMP by means of non-conventional treatment technologies is still incomplete [1]. Regarding the use of Advanced Oxidation Processes (AOPs) for this purpose, some studies concerning the application of Fenton-based treatments [26,27], heterogeneous photocatalysis [28,29] and other related technologies like the innovative low temperature plasma [30] have been published in the last few years, all of them demonstrating their potential for efficiently remove ACMP from different water matrices. However, no reports concerning the employment of ozone for ACMP abatement have been found.

Ozone-based processes have demonstrated to have great potential for micropollutants removal from water [31–36]. This technology is based on the strong oxidizing capacity of ozone (O_3), which also yields hydroxyl radicals (OH·) during ozone decay [37]. Although ozone and hydroxyl radicals can be effective in removing pollutants, transformation products (TPs) which may also be toxic can be formed during ozonation. It is important, therefore, to possess full knowledge of this process by studying reaction kinetics, transformation products, and residual toxicity of the treated water.

The present work aimed, for the first time, to go in-depth with the fundamentals (i.e., reaction kinetics, transformation products and associated toxicity evolution) of ACMP ozonation process. The objective was to determine the reaction kinetics of this pesticide when reacting with both, molecular ozone and formed hydroxyl radicals, as well as to elucidate the possible reaction pathways and potential negative effects of the resulting transformation products from ACMP degradation.

2. Materials and methods

2.1. Chemicals and reagents

Acetamiprid and *p*-chlorobenzoic acid analytical standards, as well as potassium indigotrisulfonate, were acquired from Sigma-Aldrich (Germany). Na_2HPO_4, NaH_2PO_4, H_3PO_4 and acetonitrile were purchased from Panreac (Spain), and were all analytical grade. Milli-Q water was produced by a filtration system (Millipore, USA). Pure oxygen ($\geq$ 99.999%) was supplied by Abelló Linde (Spain).

In order to control the effects of side mechanisms like hydrolysis, adsorption or UV-Vis photolysis on ACMP disappearance during ozonation experiments, several control assays were performed. All runs were carried out in 250 mL closed glass beakers, with initial ACMP concentrations of 1 mg L^{-1}. For hydrolysis and adsorption experiments, the beaker was covered with aluminum foil in order to avoid the possible influence of ambient radiation. The pH in hydrolysis tests was adjusted to a value of 2 or 7 by adding adequate quantities of H_3PO_4 and Na_2HPO_4. For adsorption experiments, several plastic materials (different types of silicones, PVDF and PTFE) usually employed in laboratory were put in contact with the pesticide solution. In all experiments, the medium was under stirring conditions. Samples were withdrawn at 0, 1, 5 and 24 h, and analyzed by HPLC-DAD. Results showed that ACMP remained stable prior to oxidant addition.

2.2. Ozonation experiments

All experiments in this work were performed by mixing aqueous ozone stock solutions with aqueous stock solutions of ACMP. Ozone stock solutions (10-12 mg L^{-1}) were prepared in a 1000 mL jacketed reactor by continuously bubbling a gaseous ozone/oxygen mixture (~ 48 mg L^{-1}) into Milli-Q water at a rate of 40 L/h, using a 301.19 Sander Labor Ozonator (Germany). The medium was maintained at a temperature of 10±1 ºC. The O_3 concentration in aqueous phase was continuously monitored by means of a Q45H/64 ozone probe (Analytical technology, US) connected to a liquid recirculation stream. All kinetic and degradation experiments were performed in triplicate, at a controlled temperature of 20±2 ºC.

The second-order rate constant for the reaction between ACMP and molecular ozone was directly determined under pseudo-first order conditions [38], with a 50-fold molar excess of ozone with respect to the target compound. In order to avoid the presence of hydroxyl radicals in the system, the reaction medium was adjusted to pH 2 by adding adequate quantities of H_3PO_4 and Na_2HPO_4 [39]. Experiments were performed in a closed 250 mL bottle, in which the headspace was almost removed in order to avoid aqueous ozone losses. ACMP and ozone stock solutions were mixed to reach initial reactant concentrations of 4 and 200 µM, respectively, and the medium was stirred for 10 seconds to obtain homogeneous conditions. Aliquots of 0.5 mL were withdrawn at prefixed reaction times, and immediately quenched with 2.5 mL of an indigo solution. These samples were finally employed to determine the dissolved ozone concentration [40], as well as to quantify the remaining concentration of ACMP by HPLC-DAD.

Due to the fast reaction rates expected for the reaction between ACMP and indirectly formed hydroxyl radicals, competition kinetics method [31,35] must be employed in order to determine the corresponding second-order rate constant. The selected reference was *p*-chlorobenzoic acid (pCBA), since the reactivity of this chemical with molecular ozone is very low (≤ 0.15 $M^{-1}s^{-1}$, [38]), whereas its reaction with hydroxyl radicals is fast ($5 \cdot 10^9$ $M^{-1}s^{-1}$, [41]). In order to guarantee the proper generation of OH· while maintaining a relative stability of aqueous ozone, experiments were performed at pH 7 by adding adequate quantities of a $H_2PO_4^-/HPO_4^{2-}$ buffer (1 mM). Reactions were conducted employing a multi-reactor system, successfully used in several previous works [32,42]: in a series of 25 mL vials containing 4 µM of ACMP and 4 µM of pCBA, different doses (from 5 to 35 µM) of the ozone stock solution were injected and mixed. Samples were taken when the total consumption of ozone was achieved (all within 2 h). The residual concentrations of ACMP and pCBA were determined by HPLC-DAD.

With the aim of demonstrating the relative contribution of each oxidant involved in ozonation (i.e., molecular ozone and hydroxyl radicals) to ACMP degradation, two additional sets of experiments were performed. Again, the multi-reactor methodology was used. For direct reaction with ozone, each reaction vial (total volume of 25 mL) contained 10 µM of ACMP and 25 mM of *tert*-butanol as radical scavenger. The pH of the solution was adjusted to 7 by adding adequate quantities of a phosphate buffer (1

mM). For reaction involving both, the attack of ozone and hydroxyl radicals, a similar procedure was followed but no scavenger was employed. In all experiments, ozone doses between 5 and 175 µM were injected to each vial. Samples were withdrawn when the total consumption of ozone was achieved (all within 2 h). Once the residual concentration of ACMP was chromatographically determined, the corresponding samples were frozen and later employed for TPs and toxicity determinations.

2.3. Analytical procedures

The concentrations of ACMP and pCBA were quantified by means of a high performance liquid chromatograph (HPLC) equipped with a diode array detector (DAD), all supplied by Agilent (1260 Infinity). The column employed was a Teknokroma Mediterranea Sea18 (250 mm x 4.6 mm and 5µm size packing). For ACMP analysis, the mobile phase consisted on a 30:70 volumetric mixture of acetonitrile and Milli-Q water acidified at pH 3 by the addition of H_3PO_4. The flow rate was maintained at 1.4 mL min^{-1}, and the detection wavelength was set to 250 nm. For pCBA quantification, the mobile phase consisted on a 50:50 volumetric mixture of acetonitrile and pH 3 Milli-Q water. The flow rate was set to 1 mL min^{-1} and the UV detection was performed at 236 nm. The limits of detection (LODs) for ACMP and pCBA were 0.018 and 0.029 µM, respectively.

With the aim of elucidating the ACMP degradation pathways given in ozonation process, samples in which different ozone doses were applied were analyzed by Liquid Chromatography-Mass Spectrometry (LC-MS). An Agilent 1100 HPLC coupled with a G1969A LC/MSD-TOF mass spectrometer was employed. MS data were collected in full scan mode (25-1100 m/z), employing positive electrospray ionization. The separation of chemical species was achieved by operating with the following elution program: a 5:95 volumetric mixture of ACN and Milli-Q (pH 3) was maintained as initial mobile phase for 5 min; then, a linear gradient changed the eluent composition from 5:95 to 30:70 in 5 min; the 30:70 mixture was maintained during the next 10 min and, finally, a linear gradient returned back the eluent initial composition (5:95) in 5 min.

In order to assess the toxicity changes along the ACMP ozonation process, Microtox® bioassays were performed. This method measures the inhibition of light emission of

bioluminescent bacteria *Vibrio fischeri* caused by the presence, in aqueous media, of toxic compounds. The results of this assay are usually expressed as $EC_{50,15min}$, which represents the percentage of sample dilution (% v/v) that causes a 50% reduction in bacteria luminescence after 15 min of exposure. All the tests were carried out in duplicate in a Microtox® M500 (Modern Water, UK) toxicity analyzer.

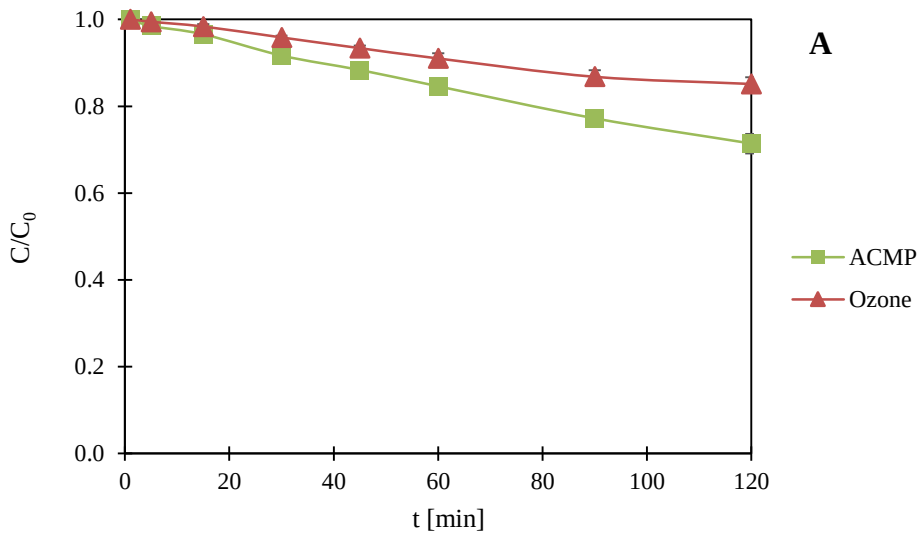
3. Results and discussion

3.1. Kinetics of ACMP reactions with ozone and hydroxyl radicals

Under the experimental conditions employed in these assays (i.e. pH 2), the half-life of molecular ozone in pure aqueous solutions was observed to be more than 6 h, thus evidencing that no radical chain reaction occurred. It was assumed, therefore, that molecular ozone was the only oxidant in the reaction medium. Thus, the second-order rate constant for the reaction between ACMP and O_3 could be calculated from Eq. 1, being obtained by integrating the corresponding kinetic equation.

$$-ln\left(\frac{[ACMP]}{[ACMP]_0}\right) = k_{MC,O_3}\int_0^t [O_3]dt \quad (1)$$

By plotting the natural logarithm of the relative residual concentration of ACMP against the ozone exposure, $\int[O_3]dt$, a linear relation was obtained. The slope of the function corresponds to the second-order kinetic constant for the reaction between ACMP and molecular ozone. Figure 1 shows the relative concentration of ACMP and ozone during the experiment (A), as well as the logarithmic relative concentration of ACMP as a function of $\int[O_3]dt$ (B). The ozone exposure was determined by calculating the area under the ozone decay curve, employing the trapezoidal method of numerical integration. $k_{ACMP\text{-}O3}$ was determined to be 0.25 ± 0.02 $M^{-1}s^{-1}$.



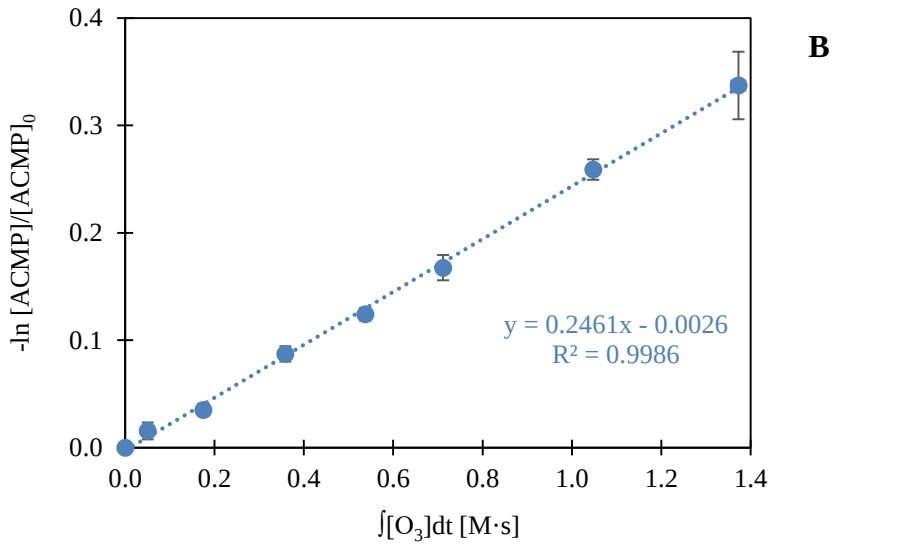


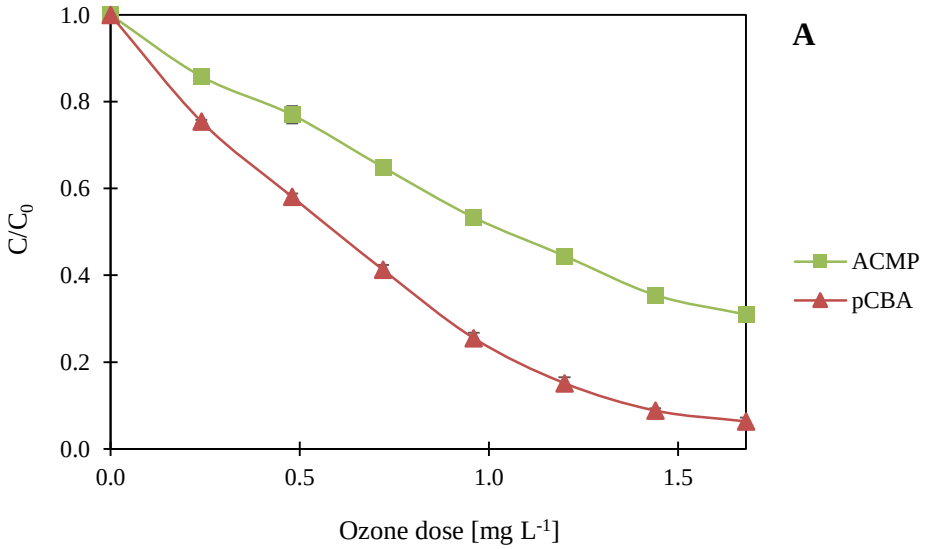
Figure 1. Determination of second-order rate constant ($k_{ACMP\text{-}O3}$) for the reaction of ACMP and ozone. A) Relative concentration of ACMP and ozone vs reaction time. B) Natural logarithm of the relative concentration of ACMP vs ozone exposure. Conditions: $[ACMP]_0$ = 4 µM, $[O_3]_0$ = 200 µM, pH 2, temperature = 20 ± 2 ºC.

In the view of the above results it is clear that reactivity of ACMP towards direct ozone attack is very low, as expected from preliminary experiments. It is important to note that the determined value corresponds to ACMP deprotonated form, since the pKa value of this pesticide is 0.7 [43]. Therefore, the rate constant value should remain unaltered for higher pH values, including the ones exhibited by most water and wastewater real matrices.

Since the reactivity of ACMP and pCBA with molecular ozone is very low, and reactions between these chemicals and OH· were expected to be fast, it was assumed that depletion of both compounds under the employed experimental conditions (pH=7) was only due to OH· attack. Therefore, the second-order rate constant for the reaction between ACMP and OH· could be calculated from Eq. 2, being obtained by dividing the kinetic equations corresponding to reactions between OH· and both ACMP and pCBA.

$$-ln\left(\frac{[ACMP]}{[ACMP]_0}\right) = \frac{k_{ACMP,OH\cdot}}{k_{pCBA,OH\cdot}}\left(-ln\left(\frac{[pCBA]}{[pCBA]_0}\right)\right) \quad (2)$$

According to the above expression, a linear dependence between the natural logarithm of the relative ACMP concentration and the natural logarithm of the relative pCBA concentration was expected. The slope of this relationship represents the ratio between the second-order rate constants of OH· with target (ACMP) and reference (pCBA) compound, respectively. Figure 2 shows the relative concentration of both ACMP and pCBA as a function of the ozone dose (A), as well as the natural logarithm of ACMP relative concentration as a function of the natural logarithm of the relative pCBA concentration (B). A value of (2.1 ± 0.1)·10^9 $M^{-1}s^{-1}$ was finally determined for $k_{ACMP,OH\cdot}$. The high reactivity of ACMP with hydroxyl radicals is explained by the non-selective character of the oxidant [37] which readily undergo reactions with different points of organic molecules.



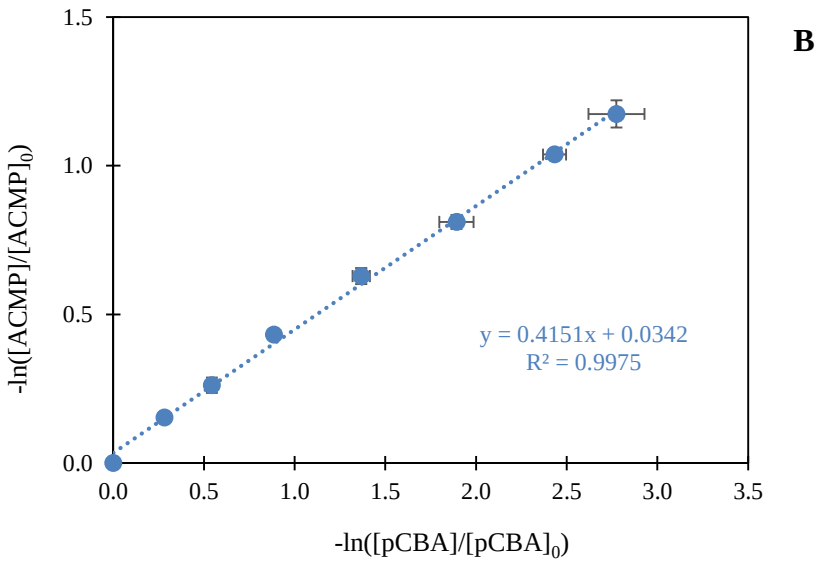


Figure 2. Determination of second-order rate constant ($k_{ACMP,OH\cdot}$) for the reaction of ACMP and OH· by competition kinetics. A) Relative concentrations of ACMP and pCBA as a function of the ozone dose. B) Natural logarithm of ACMP relative concentration vs natural logarithm of pCBA relative concentration. Conditions: $[ACMP]_0 = [pCBA]_0$ = 4 µM, pH 7, temperature = 20 ± 2 ºC.

3.2. ACMP degradation by ozone and hydroxyl radicals

It is well known that during ozonation, a compound can directly react with molecular ozone but also with hydroxyl radicals formed through O_3 decomposition [37]. With the aim of observing and comparing the removal of ACMP by both possible transformation routes, degradation experiments were conducted at pH 7 with and without the presence of a radical scavenger. Results are shown in Figure 3. Degradation by means of the direct reaction barely occurred, which was not surprising considering the extremely low rates exhibited by the reaction between ACMP and O_3 during the preceding kinetic runs. Besides, under the employed neutral pH conditions, ozone self-decomposition becomes relevant and therefore the stability of this oxidant in the medium is reduced with respect to more acidic conditions. For its part, indirect transformation trough hydroxyl radicals demonstrated the effectiveness usually exhibited by this transient species in organics oxidation, and showed to be in total agreement with the findings of the previously mentioned studies dealing with the removal of ACMP by means of other AOPs [26,28]. With an ozone dosage of approximately 5.50 mg L^{-1}, the complete removal of ACMP was achieved.

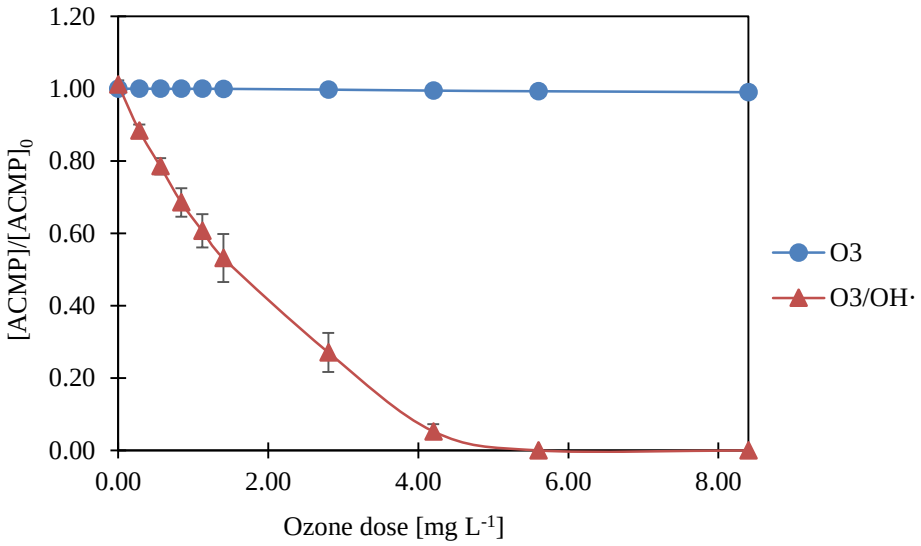


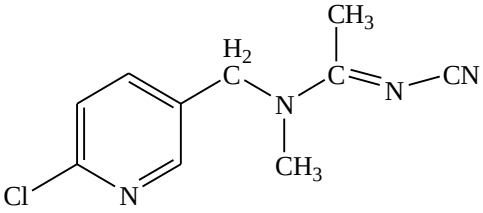
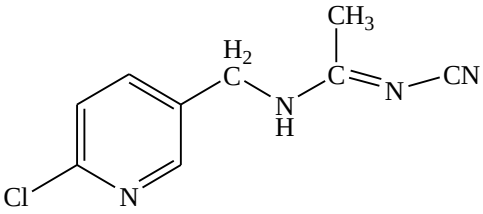
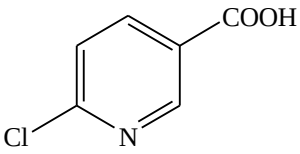
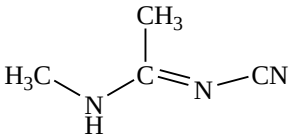
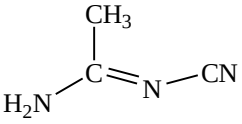
Figure 3. Profile of ACMP degradation as a function of the ozone dose, for experiments with (O_3) and without (O_3/OH·) the presence of *tert*-butanol (25 mM). Conditions: $[ACMP]_0$ = 10 µM, pH 7, temperature = 20 ± 2 ºC.

Considering the initial concentration of ACMP in degradation experiments, a normalized ozone dose of approximately 2.50 mg O_3/mg DOC (Dissolved Organic Carbon) was required to 100% eliminate ACMP under the studied conditions. This oxidant dosage, according to literature [45,46], would probably be considered expensive since doses up to 1 mg O_3/mg DOC are usually enough for disinfection and trace pollutant removal in drinking and wastewater treatment plants [45,46]. Because of that reason, and considering that ozone decomposition to hydroxyl radicals is the key of ACMP removal by ozonation, strategies aimed to further promote this indirect route should be pursued to make the process a competitive treatment option for waters contaminated by this compound. It is important to note, however, that since the process performance would always depend on water characteristics, like pH or inorganic and organic matter type and concentrations, the application in real matrices should be properly evaluated in future studies. With that purpose, experiments with real water matrices and pesticide concentrations should be performed. Another good option would be the employment of kinetic models based on the use of water specific information and the rate constants determined in this study [45,46].

3.3. Reaction intermediates and possible mechanisms

The identification of major TPs generated during ACMP ozonation was performed by means of LC-MS, being the corresponding chemical structures proposed on the basis of the detected masses. Since ozone was shown to be ineffective degrading ACMP molecules, it could be stated that all the detected species were reaction intermediates corresponding to products of OH· reactions, that is, the indirect reaction pathway. The molecular structures of the TPs that were identified are shown in Table 1.

Table 1. ACMP, detected TPs and corresponding molecular structures.

m/z	**Name**	**Proposed structure**
223 (m+1)	Acetamiprid (ACMP)	
209 (m+1)	ACMP-209 Acetamiprid-*N*-desmethyl	
158 (m+1)	ACMP-158 6-Chloronicotinic acid	
98 (m+1)	ACMP-98 *N'*-cyano-*N*-methyl acetamidine	
84 (m+1)	ACMP-84 *N'*-cyano acetamidine	

According to the detected structures and the experimental conditions employed in the study, the first stages of ACMP degradation during ozonation process could consist on a combination of OH· oxidation and fast hydrolysis of the metabolites generated during

the first step, as shown in Figure 4. The brackets in some of the proposed intermediates indicate that these species could not be detected during the analysis, probably due to their low concentration or fast tendency to undergo hydrolysis or become oxidized.

The identification of ACMP-158, ACMP-98 and ACMP-84 suggests that the initial attack by hydroxyl radicals takes place at the methylene group (C α) of the ACMP amine. After the fast H-abstraction carried out by OH·, further oxidation of the α-aminoalkyl radical by molecular oxygen in the presence of OH^- yields the corresponding α-hydroxymethylamine (reaction **1**). This mechanism is similar to the one reported by Das et al. for trimethylamine OH·-induced oxidation [47]. The hydrolysis of the hydroxymethylamine (reaction **2**) would lead to the generation of *N'*-cyano-*N*-methyl acetamidine (ACMP-98) and 6-chloronicotinoid acid (ACMP-158). The latter, however, would require a previous step which should involve the generation of the corresponding aldehyde hydrate and its subsequent transformation to a carboxylic acid (reaction **3**), being the latter step caused by the oxidizing conditions of the medium [48]. For its part, further attack to ACMP-98 by hydroxyl radicals in the presence of O_2 (reaction **4**) would result on the generation of its demethylated form, or *N'*-cyano acetamidine (ACMP-84), after the hydrolysis of the corresponding, previously formed hydroxymethylamine (reaction **5**). Instead of at the methylene group, the initial H-abstraction from ACMP structure can also take place at the methyl group of its amine moiety (also an alpha C) (reaction **7**). Hydrolysis of the resulting hydroxymethylamine (reaction **8**) would finally give ACMP-*N*-desmethyl (ACMP-209). It is interesting to mention that the hydrated form of formaldehyde would be yielded as a side product of ACMP-97 and ACMP-208 hydrolysis. Under the oxidizing conditions of the medium, this compound could eventually be transformed and yield carbonic acid as final product (reactions **6** and **9**).

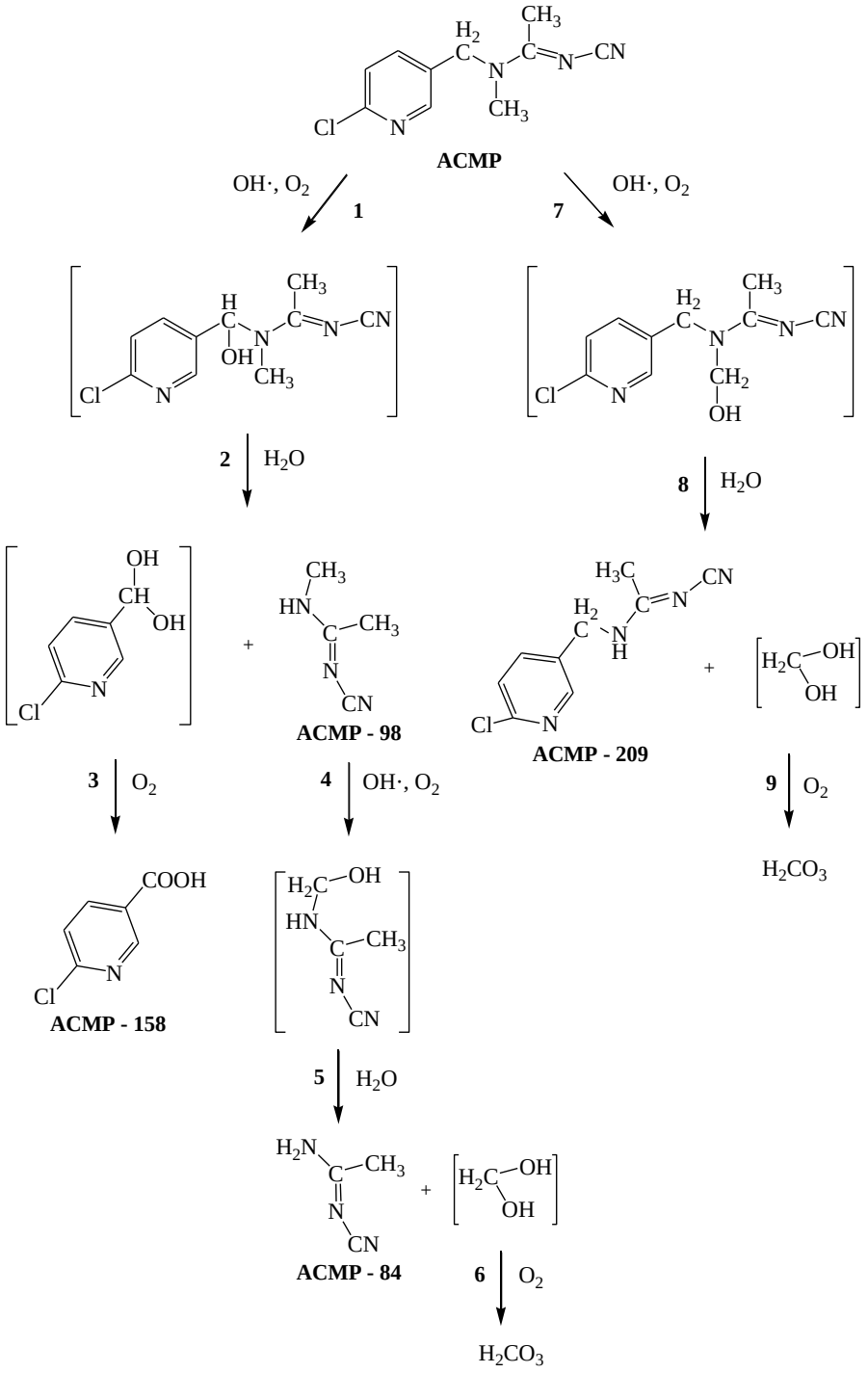


Figure 4. Proposed reaction pathways for ACMP degradation by OH· during ozonation process.

The pesticide properties of ACMP are based on its nicotinoid structure, which mimics the vital neurotransmitter acetylcholine (ACh) by binding to the corresponding nicotinic acetylcholine receptor (*n*AChR) [6]. Due to the fact that this specific neural pathway is more abundant in insects than in warm-blooded animals, ACMP and those of its family (i.e. neonicotinoids) are more toxic to insects than to mammals [49]. Since the presence of the nicotinoid structure appears to be fundamental to maintain this selectivity against

pests, TPs ACMP-98 and ACMP-84 could have lost its ability to bind the insect *n*AChRs and thus their insecticide features, which did not necessarily mean that these side products were non-toxic. By the same argument, the intermediates ACMP-209 and ACMP-158 (i.e. acetamiprid-*N*-desmethyl and 6-chloronicotinic acid) could still maintain certain specificity in their pesticide action. However, in order to ensure a proper interaction with the *n*AChRs and therefore a high selective action against insects, it is also important for neonicotinoid species to possess an electronegative moiety on their molecule to bind to the unique, positive charged amino acid residue present in the nicotinic cholinergic receptor [49,50]. In relation with that, it has been found that nitro or cyano substituents could be the most adequate electron-withdrawing moieties to enhance the affinity between the pesticide and the receptor subsite [49,50]. Therefore, in the present case it is expected for 6-chloronicotinic acid to present less affinity with *n*AChRs and thus, an also less selective pesticide action than the exhibited by acetamiprid-*N*-desmethyl, which would still keep the original cyano group of ACMP.

3.4. Toxicity evolution during ACMP ozonation process

Due to the changes on the reaction medium composition, caused by the generation of new products and the degradation of parent compound, ACMP ozonation process also involved changes in the solution toxicity. The evolution of this property is represented in Figure 6 by the $1/EC_{50}$ value for *Vibrio fischeri* assays, as a function of the ozone dosage. It is important to remember that higher $1/EC_{50}$ values mean higher toxicities, and vice versa. Initial EC_{50} value, that is, the effective concentration that inhibits a 50% of the bacteria light emission, was determined to be 86 mg L^{-1} (about 40 times the initial concentration, in terms of sample dilution), which clearly represents a low toxicity to non-target species. This value is lower than the one reported by Dell'Arciprete et al. (129 mg L^{-1}), which was obtained by exposing bioluminescent bacteria to solutions with different concentrations of ACMP. For the rest of samples, only EC_{50} in terms of percentage of dilution (% v/v) could be determined since their compositions were unknown. The toxicity of the medium notably increased for ozone doses above 3 mg L^{-1}, reaching a maximum (EC_{50} 51.6%) for an O_3 dosage of approximately 4.50 mg L^{-1}. The application of larger ozone doses resulted on significant toxicity abatement, as observed in the $1/EC_{50}$ profile. A similar value than the starting one was practically achieved for an ozone dose about 8.5 mg L^{-1}.

In previous works regarding ACMP degradation by oxidation processes in which hydroxyl radicals and other ROS (Reactive Oxygen Species) were involved, an increase in the medium toxicity was observed after the treatment with respect to the untreated solution [48,51]. The same happened in a previous research regarding the photocatalytic degradation of 6-chloronicotinic acid, one of the ACMP TPs detected in this work (ACMP-158) [52]. Although the results in this study agree with the previous related literature, a larger extent of the oxidation reaction in the present case with respect to the preceding researches also led to the degradation of the intermediate species that caused the observed increase in the medium toxicity.

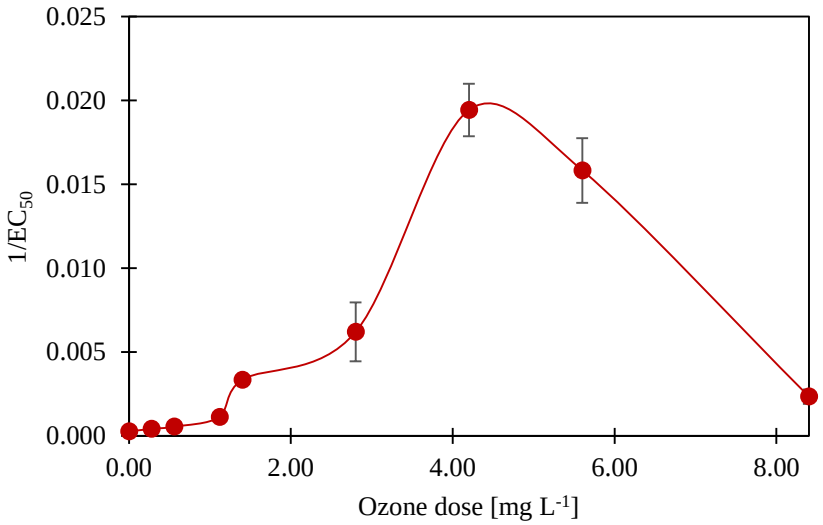


Figure 5. Acute toxicity of the reaction medium during ACMP ozonation, as a function of the ozone dose.

The changes in toxicity observed during ACMP ozonation can be attributed, as in many other works [33,34,52] to the generation of TPs more toxic than the parent compound, as well as to the possible synergistic effects between initial and newly formed species present in the reaction medium. Because of the relative loss in their pesticide selectivity, it would be reasonable to consider ACMP-158, ACMP-98 or ACMP-84 as the most suitable candidates leading to the bacteria bioluminescence inhibition, as *Vibrio fischeri* could be considered as a non-target species. ACMP-209, for its part, should still maintain a similar activity than the parent compound, and therefore a relative high specificity against insects. However, ACMP-158 (6-chloronicotinic acid) has been reported to be less toxic to *Vibrio fischeri* than ACMP itself [48], and no information regarding the response of this bioluminescent bacteria under ACMP-98 and ACMP-84

418 exposure has been found. Given the scarcity of data on that topic, it was not possible to
419 attribute the increase in toxicity to the single presence of one of the detected TPs. In
420 addition to the possible synergistic effects between all the present species, as earlier
421 mentioned, it is possible that the observed changes were related to the presence of other
422 toxic intermediates that could not be identified during the LC-MS analyses. In the
423 preceding section it has been stated that aldehyde hydrate compounds (formaldehyde
424 and 6-chloronicotinaldehyde hydrates) could be involved in the ACMP degradation
425 pathway. Since these compounds are typically in equilibrium with their parent
426 aldehydes, and the latter have already proven to be highly toxic to Microtox® bacteria
427 (EC_{50} of 1.35 mg L^{-1} for formaldehyde versus 89 mg L^{-1} for ACMP determined in this
428 study) [36,53], that could constitute an alternative good explanation to the observed
429 increase in toxicity.
430
431 Considering the Microtox results, it is clear that ozonation applied to waters
432 contaminated by ACMP could cause an increase in the toxicity of the medium, at least
433 within a certain range of ozone doses. Since the employment of this treatment will
434 always pursue the complete depletion of the pesticide while ensuring the lowest
435 possible toxicity in the treated water matrix, this could enlarge the necessary ozone
436 dosage to prohibitive values, economically speaking. Moreover, because of ACMP is
437 resistant to molecular ozone oxidation, the degradation through hydroxyl radicals will
438 be the main removal mechanism of this priority pesticide during ozonation. Therefore,
439 the water matrix characteristics will play a decisive role in the ACMP degradation
440 efficiency. In addition to the conditions that naturally favor the ozone decomposition
441 process to hydroxyl radicals, like neutral and alkaline pH conditions, strategies aimed to
442 further promote the indirect pathway should be equally investigated and employed.
443 This, of course, would be essential in order to enhance the degradation efficiency and
444 consequently reducing the oxidant dose to be applied.
445
446 **Conclusions**
447
448 The kinetics, reaction pathways and toxicity evolution during ACMP ozonation process
449 were explored for the first time. The second-order kinetic constant for the reactions of
450 ACMP with molecular ozone and hydroxyl radicals were determined to be 0.25 $M^{-1}s^{-1}$
451 and $2.1 \cdot 10^9$ $M^{-1}s^{-1}$, thus clearly indicating the resistance of the pesticide structure

towards O_3 attack. This ozone-recalcitrance was confirmed through degradation experiments at neutral pH, in which the direct reaction was barely observed. Formed hydroxyl radicals showed to completely remove ACMP (initial concentration 10 μM) with an ozone dosage of 5.5 mg L^{-1}, while their major intermediate products needed higher doses. The proposed ACMP degradation pathways consisted of combinations of oxidation and hydrolysis steps, which would yield different TPs depending on the initial site in which the hydrogen abstraction by hydroxyl radicals took place. Toxicity of the reaction medium increased to reach a maximum, and then decreased to relatively low values. Since these changes could not be related to the single presence of some of the detected TPs, they were attributed to synergistic effects among different species as well as to the presence, although not identified, of intermediate aldehydes which even at very low concentrations, exhibited acute toxicity to bacteria. In the view of the obtained results, further promoting ozone decomposition to hydroxyl radicals appears to be necessary to achieve a complete ACMP and associated toxicity abatement while maintaining a reasonable efficiency.

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