Exploring ozonation as treatment alternative for methiocarb and formed transformation products abatement

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ABSTRACT

Despite the high toxicity and resistance to conventional water treatments exhibited by methiocarb (MC), there are no reports regarding the degradation of this priority pesticide by means of alternative purification technologies. In this work, the removal of MC by means of ozonation was studied for the first time, employing a multi-reactor methodology and neutral pH conditions. The second-order rate constants of MC reaction with molecular ozone (O_3) and formed hydroxyl radicals (OH·) were determined to be $1.7 \cdot 10^6$ and $8.2 \cdot 10^9$ M^{-1} s^{-1}, respectively. During degradation experiments, direct ozone reaction was observed to effectively remove MC, but not its formed intermediates, whereas OH· could oxidize all species. The major identified TPs were methiocarb sulfoxide (MCX), methiocarb sulfoxide phenol (MCXP) and methiocarb sulfone phenol (MCNP), all of them formed through MC oxidation by O_3 or OH· in combination with hydrolysis. A toxicity assessment evidenced a strong dependence on MCX concentration, even at very low values. Despite the OH· capability

to degrade MC and its main metabolites, the relative resistance of TPs towards ozone attack enlarged the oxidant dosage (2.5 mg O_3/mg DOC) necessary to achieve a relatively low toxicity of the medium. Even though ozonation could be a suitable technique for MC removal from water compartments, strategies aimed to further promote the indirect contribution of hydroxyl radicals during this process should be explored.

KEYWORDS

Methiocarb, ozonation, hydroxyl radicals, second-order rate constants, reaction pathways, toxic intermediates

1. Introduction

Methiocarb (mesurol, 3,5-dimethyl-4-(methylthio)phenyl methylcarbamate) (MC) is one of the most common carbamate pesticides worldwide, employed in agriculture as insecticide, acaricide, molluscicide and bird repellent (Altinok et al., 2006; Blažková et al., 2009; Gitahi et al., 2002; Keum et al., 2000; Sinclair et al., 2006). This chemical has been detected in natural waters of several countries (APVMA, 2005; Barceló et al., 1996; Fytianos et al., 2006; García de Llasera and Bernal-González, 2001; Squillace et al., 2002) at concentration levels ranging from ng L^{-1} to µg L^{-1}. It also has been detected in wastewater effluents (Campo et al., 2013; Masiá et al., 2013), this last suggesting the resistance of MC to conventional wastewater treatments. Although the detected concentrations of this micropollutant in water compartments are generally low, it represents a serious threat to the aquatic and human life considering its high toxicity and

that of some of its water metabolites (UNFAO (Food and Agriculture Organization of the United Nations) and WHO (World Health Organization), 1999). For example, methiocarb sulfoxide (MCX), which is one of the typical MC natural transformation products (TPs), has been reported to be even more toxic than the parent compound (Marss, 1998), and is currently included on the Priority List of Transformation Products in Great British Drinking Water Supplies (Sinclair et al., 2006). Because of all these reasons, the World Health Organization has classified MC as a highly hazardous pesticide (World Health Organization, 2010). Furthermore, MC has been included in the recently launched 1st watch list of Decision 2015/495/EU for European monitoring (The European Comission, 2015), among other micropollutants considered as priority substances.

Several studies regarding the fate of MC during conventional wastewater and simulated drinking water treatment have been reported during the last few years. For wastewater treatment, no concluding results have been obtained about MC fate. For example, higher concentrations were found in the effluents than in the influents in a Spanish sewage treatment plant, probably due to limitations in sampling procedure (Barbosa et al., 2016; Campo et al., 2013). Studies regarding the fate of MC in simulated drinking water treatment have demonstrated that reactions between this pesticide and most commonly used disinfectants (i.e.: free chlorine, ClO_2 and NH_2Cl), which also possess a certain oxidizing power, yield transformation products (TPs) more toxic and persistent than the parent compound, even though this one becomes degraded (Qiang et al., 2014; Tian et al., 2013, 2010). However, despite the safety concern regarding the presence of this pesticide and their TPs in the aqueous systems, no studies related to the removal of MC by advanced treatment options have been found in literature, as also stated in a

recent review by Barbosa et al. (Barbosa et al., 2016). A possible explanation for this lack of data could be related to the moderate-high hydrophobic character of MC (log Kow = 3.2 and water solubility 27 mg L^{-1}, at 20ºC (UNFAO (Food and Agriculture Organization of the United Nations) and WHO (World Health Organization), 1999)), which could complicate the handling of MC during the experimental work due to its probable tendency of becoming adsorbed to other hydrophobic materials.

Ozonation for the abatement of micropollutants has been demonstrated to be an effective process (Dantas et al., 2008, 2007; Huber et al., 2003; Jin et al., 2012; Vel Leitner and Roshani, 2010), thus indicating the great potential of this advanced technology for that purpose. Ozone (O_3) is a strong oxidant that also undergoes self-decomposition in water to release hydroxyl radicals (OH·), under neutral and alkaline conditions, with stronger oxidizing capability than O_3 (Gligorovski et al., 2015). Since this technology is increasingly employed in wastewater and drinking water treatment, detailed information about kinetics, intermediates generation and associated toxicity changes during the process is essential, even more with the detection of new micropollutants.

To the best of our knowledge, this is the first report on MC removal by means of ozonation. The study aimed to determine the kinetics of the process considering both, direct reaction with molecular ozone and indirect reaction through hydroxyl radicals. The possible reaction pathways of MC ozonation were also explored by means of its main formed intermediates elucidation and finally, the potential ecotoxicological effects of MC and its TPs during the process were assessed by means of bacteria luminescence inhibition assays.

2. Materials and methods

2.1. Chemicals and reagents

Methiocarb, sulfamethoxazole and phenol analytical standards were acquired from Sigma-Aldrich (Germany). NaH_2PO_4, Na_2HPO_4, H_3PO_4, *tert*-butanol and acetonitrile were purchased from Panreac (Spain), and were all analytical grade. Milli-Q water was produced by a filtration system (Millipore, USA). Finally, all the reagents employed during toxicity bioassays were purchased from Modern Water (UK).

As early commented, MC was suspected to be adsorbed to some non-polar materials, due to its hydrophobicity. In order to be sure about that, some preliminary experiments were performed. Results revealed important losses of MC when aqueous solutions of this chemical were put in contact with plastic elements (i.e. filters, tubing), whereas this was not observed when working with glassware. Therefore, glass was selected as material for handling MC solutions during experimentation.

2.2. Ozonation experiments

All ozonation experiments were carried out at 20 ± 2 ºC and pH 7, in Milli-Q water. Preliminary hydrolysis tests at pH 7 were performed in order to determine the influence of this mechanism on the overall MC removal. Reaction solution did not show hydrolysis after a period of 2 h, which is exactly the time interval employed for ozonation experiments, including analysis. Due to the tendency of MC to become adsorbed onto many materials, as well as to the fast reaction kinetics also exhibited

during the preceding assays, ozonation runs were carried out employing a multi-reactor methodology, successfully used in several works (Borowska et al., 2016; Ning et al., 2007). Detailed information of ozone stock solutions preparation can be found in the supplementary information (Text and Fig. S1). All ozonation experiments were done in triplicate.

Since preliminary experiments showed fast reaction rates ("*k*" values > 1000 M^{-1} s^{-1}), the extensively-employed competition kinetics method (Borowska et al., 2016; Buxton et al., 1988; Hoigné and Bader, 1983; Huber et al., 2003; Jin et al., 2012) must be used to determine the kinetic constants of the reaction between MC and both, molecular ozone and hydroxyl radicals.

For $k_{MC,O3}$ measurement, experiments were carried out in a series of 25 mL vials containing 20 µM of MC and 20 µM of sulfamethoxazole (SMX), the reference compound. The competitor was selected considering the high-reactivity of MC with molecular ozone. To avoid reactions involving hydroxyl radicals (OH·), *tert*-butanol was employed as OH· scavenger (100 mM). Adequate quantities of a $H_2PO_4^-/HPO_4^{2-}$ buffer were also added in order to maintain the medium pH at a constant value of 7. Different doses (from 5 to 50 µM) of the ozone stock solution were injected to each vial as reactant. The mixtures were vigorously shaken for a few seconds, to completely mix the ozone in. Samples were withdrawn when the total consumption of ozone was achieved, and quickly analyzed. The residual concentrations of MC and SMX were determined by HPLC-DAD.

For $k_{MC,OH\cdot}$ determination, a similar procedure was followed. The multi-reactor system was used again, with initial concentrations of 20 µM for all compounds and without the presence of a radical scavenger. Two references were employed since two reactions (i.e. MC with both, O_3 and OH·) took place at the same time and needed to be considered due to their expected important contribution to MC depletion. SMX and phenol (PH) were chosen as competitors, since both were expected to present similar overall reactivity than MC.

Two extra sets of experiments were performed in order to demonstrate the relative contribution of hydroxyl radicals on MC removal. For direct reaction with ozone, each one of the 25 mL reaction vials contained 20 µM of MC, 25 mM of *tert*-butanol and adequate quantities of the pH 7 phosphate buffer. For reaction involving molecular ozone and hydroxyl radicals, the same procedure was followed but no scavenger was added. For both experiments, a wider range of ozone doses were applied (from 5 to 140 µM) in order to achieve the complete depletion of the pesticide. Once analyzed, the samples withdrawn in these experiments were frozen and lately employed for TPs and toxicity determinations.

2.3. Analytical procedures

The concentrations of MC, SMX and PH were quantified by means of an HPLC equipped with a diode array detector (DAD), all supplied by Agilent (1260 Infinity). For MC, PH and SMX analysis, the column employed was a Teknokroma Mediterranea Sea18 (250 mm x 4.6 mm and 5µm size packing). The chromatographic conditions for

each compound separation and detection are summarized in Table S1 (supplementary information).

In order to elucidate the possible reaction pathways during MC ozonation, samples in which different ozone doses were applied were analyzed by 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 conditions were the same ones employed for DAD quantification.

To assess the acute toxicity as a function of the applied ozone dose, Microtox® bioassays were performed. This method measures the inhibition of light emission of bioluminescent bacteria *Vibrio fischeri* caused by the presence of toxic compounds in the aqueous media. 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 a contact time of 15 minutes. All the tests were carried out in duplicate in a Microtox® M500 (Modern Water, UK) toxicity analyzer.

3. Results and discussion

3.1. Rate constant for the reaction between MC and O_3

The second-order rate constant for the reaction of MC with molecular ozone was calculated from Eq. 1, being this one obtained by dividing the kinetic equations

corresponding to the direct reactions between MC and SMX with O_3, as described elsewhere (Dantas et al., 2008, 2007).

$$-ln\left(\frac{[MC]}{[MC]_0}\right) = \frac{k_{MC,O_3}}{k_{SMX,O_3}}\left(-ln\left(\frac{[SMX]}{[SMX]_0}\right)\right) \quad (1)$$

As shown in this expression, a linear dependence between the natural logarithm of the relative MC concentration and the natural logarithm of the relative SMX concentration is expected, with the ratio between the second-order kinetic constants of the target and the reference compound being the slope. For the three replicates that were performed, linear regression coefficients greater than 0.99 were obtained, together with a good agreement between the corresponding slope values (0.87 ± 0.01, see Fig. S2 of the supplementary information). Considering a value of $2.0 \cdot 10^6$ M^{-1} s^{-1} for $k_{SMX,O3}$ at pH 7 (Huber et al., 2003; Jin et al., 2012), the second-order rate constant for reaction between MC and molecular ozone, $k_{MC,O3}$, was determined to be $(1.7 \pm 0.1) \cdot 10^6$ M^{-1} s^{-1}. As suspected during preliminary experiments, the rate of the reaction between MC and O_3 is considerable fast. MC molecule contains a thioether moiety, which has been considered to be the main responsible for the fast kinetics (between $2.0 \cdot 10^5$ and $6.7 \cdot 10^5$ M^{-1} s^{-1}) presented by other compounds containing this functional group in their corresponding reactions with molecular ozone (Dodd et al., 2006; Jeon et al., 2016). Since MC does not show basic or acidic properties in aqueous systems (UNFAO (Food and Agriculture Organization of the United Nations) and WHO (World Health Organization), 1999), the reactivity of this compound with ozone is not expected to be dependent on the medium pH, as reported for many other dissociating chemicals (Borowska et al., 2016; Dantas et al., 2008, 2007; Hoigné and Bader, 1983).

3.2. Rate constant for the reaction between MC and OH·

For $k_{MC,OH\cdot}$ determination, a different protocol was employed: since no radical scavenger was added to the reaction medium, the contribution of molecular ozone and hydroxyl radicals to the overall MC depletion must be considered. Because of that, two reference compounds (SMX and PH) were needed in order to later solve the corresponding mathematical equations. This method was successfully employed for similar purposes in a previous study (Vel Leitner and Roshani, 2010). In the present case, six reactions were considered to simultaneously take place in the described system. They are gathered in Table 1, along with the corresponding kinetic constant values found in literature.

Table 1. Reactions considered during competition experiments for $k_{MC,OH\cdot}$ determination.

Reaction	k **[M^{-1} s^{-1}]**	**Reference**
$MC + O_3 \rightarrow k_{MC,O3}$	$1.7 \cdot 10^6$	This study
$MC + OH\cdot \rightarrow k_{MC,OH\cdot}$	Unknown	-
$SMX + O_3 \rightarrow k_{SMX,O3}$	$2.0 \cdot 10^6$	(Jin et al., 2012)
$SMX + OH\cdot \rightarrow k_{SMX,OH\cdot}$	$5.5 \cdot 10^6$	(Huber et al., 2003)
$PH + O_3 \rightarrow k_{PH,O3}$	$1.8 \cdot 10^6$	(Hoigné and Bader, 1983)
$PH + OH\cdot \rightarrow k_{PH,OH\cdot}$	$6.6 \cdot 10^9$	(Buxton et al., 1988)

The second-order rate constant for the reaction of MC with hydroxyl radicals was calculated by solving the system formed by Eq. 2 and 3. Detailed information about the obtaining of these expressions can be found in Text S2 (supplementary information).

$$ln\frac{[MC]}{[MC]_0} = \frac{(k_{MC,O_3} + k_{MC,OH\cdot}Rct)}{(k_{SMX,O_3} + k_{SMX,OH\cdot}Rct)} ln\frac{[SMX]}{[SMX]_0} \quad (2)$$

$$ln\frac{[MC]}{[MC]_0} = \frac{(k_{MC,O_3} + k_{MC,OH\cdot}Rct)}{(k_{PH,O_3} + k_{PH,OH\cdot}Rct)} ln\frac{[PH]}{[PH]_0} \quad (3)$$

240

241 From the above equations, it can be deduced that by plotting the natural logarithm of the
242 relative concentration of MC versus the natural logarithm of the relative concentration
243 of each one of the competitors, per separate, two linear relations are obtained. Together
244 with the experimentally obtained slopes, if all the required kinetic constant values are
245 known the system can be solved in order to determine $k_{MC,OH\cdot}$, as well as *Rct*. The latter
246 corresponds to a time-independent relation which represents the ratio $[OH\cdot]/[O_3]$ in a
247 reaction medium subject to ozonation (Elovitz and Von Gunten, 1999).

248

249 A good agreement was observed between the three replicates that were performed: slope
250 values of 0.90 ± 0.02 and 0.98 ± 0.01 resulted for MC-SMX and MC-PH corresponding
251 relationships, respectively. All the linear coefficients were above 0.99 (see Fig. S3 of
252 the supplementary information). The kinetic constant was determined to be (8.2 ±
253 0.2)·10^9 M^{-1} s^{-1}, which indicates the high reactivity of MC with hydroxyl radicals. In
254 that case, the fast kinetics was not attributable to a single specific reaction like the one
255 exhibited between molecular ozone and the thioether group: although this sulfur moiety
256 is also highly reactive to hydroxyl radicals, with reported rate constants in the order of
257 10^9 to 10^{10} M^{-1} s^{-1} (Rózsa et al., 2017; Szabó et al., 2015), the characteristic non-
258 selectivity of OH· could promote other reaction mechanisms usually exhibited by this
259 transient species (e.g. electron or hydrogen abstraction) (Gligorovski et al., 2015).

260

3.3. *MC degradation by ozone and hydroxyl radicals*

During the ozonation process, methiocarb can react with molecular ozone but also with hydroxyl radicals generated by ozone decomposition (Gligorovski et al., 2015). Degradation experiments applying different ozone dosages were performed in order to observe the removal profile by means of the direct route (attack of molecular ozone), as well as to demonstrate the contribution of hydroxyl radicals to the overall depletion of MC at neutral pH. Results are shown in Fig. 1. Transformation by means of O_3/OH· combination was more effective than the removal only due to O_3 attack: the ozone dose required to deplete over a 99% of the initial MC concentration was about 2.5 times lower in the first case than in the second (1.1 mg L^{-1} vs 2.8 mg L^{-1}). At pH 7, therefore, the indirect degradation pathway could play an important role in the global depletion of MC. This means that efficiency of ozonation for MC depletion will strongly depend on water pH, among other characteristics, since decomposition of ozone in hydroxyl radicals is accelerated under alkaline conditions (Gligorovski et al., 2015).

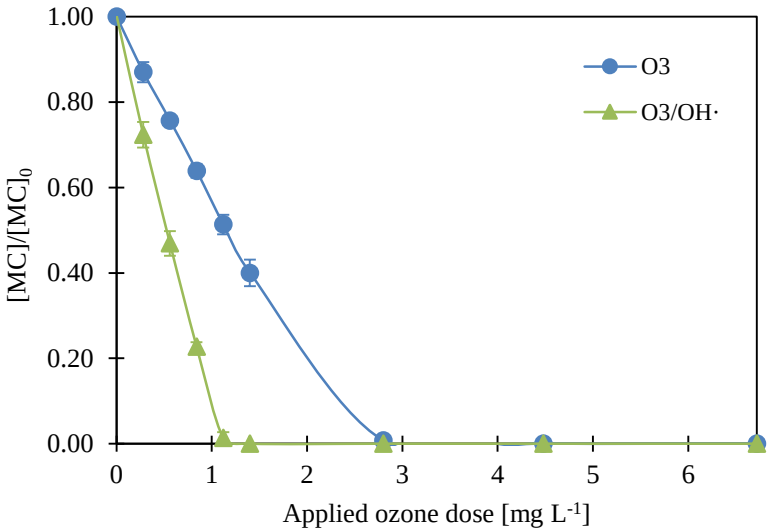


Figure 1. Profile of MC degradation ($[MC]_0$ = 20 µM) as a function of the applied ozone dose, for experiments with (O_3) and without (O_3/OH·) radical scavenger.

According to bibliography (Gerrity et al., 2012; Lee et al., 2013), doses until 1 mg O_3/mg DOC are considered reasonable for disinfection and trace pollutant oxidation in drinking and wastewater treatment plants. In the case of study, the normalized ozone dose of 0.4 mg O_3/mg DOC was calculated to be enough to completely remove MC by means of the O_3 and OH· joint action. As stated before, besides the chemical reactivity of the pollutant with ozone, the efficiency of the process strongly depends on water characteristics like pH or organic matter content. For instance, and given the high value obtained for the second-order rate constant of MC reaction with O_3 ($1.7 \cdot 10^6$ $M^{-1}s^{-1}$), in waters containing natural organic matter (NOM) or soluble microbial compounds (SMP) at concentration levels of mg L^{-1}, MC (from ng L^{-1}-µg L^{-1}) removal is expected to happen mainly via ozone oxidation. It is clear, therefore, that the required ozone doses to deplete MC in real waters are difficult to determine only on the basis of the above results. Carrying out experiments with realistic matrices and MC concentrations could constitute a good idea on that purpose. However, inherent difficulties related to

MC hydrophobic properties, which led to the adsorption of this chemical onto many materials, made complicated the performance of this experimental work. A good alternative could be the use of kinetic models, like the ones based on the *Rct* concept (Elovitz and Von Gunten, 1999; Ning et al., 2007): the obtaining of this parameter for particular waters, together with the second-order rate constants provided in this study, should allow the prediction of MC removal and thus the estimation of the ozone dosage required for the complete depletion of this contaminant in real aqueous matrices (Elovitz and Von Gunten, 1999; Lee et al., 2013).

After viewing the results obtained up to this point of the study, and without forgetting their limitations, what is clear is that ozonation could constitute a suitable treatment option regarding MC removal from water. Its high reactivity with O_3 and $OH\cdot$, together with the significant contribution of hydroxyl radicals to its degradation at neutral pH, allows to suggest that this contaminant could be totally removed during ozonation stages. However, and because of mineralization capability of ozonation is low, it was necessary to explore other basic aspects of the process like the generation of reaction intermediates and the associated toxicity changes.

3.4. Reaction intermediates and possible mechanisms

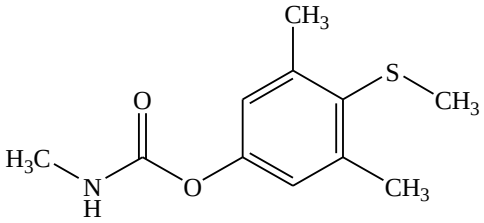
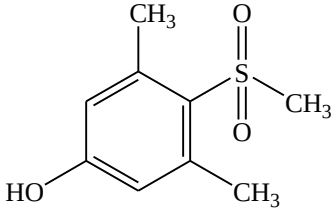
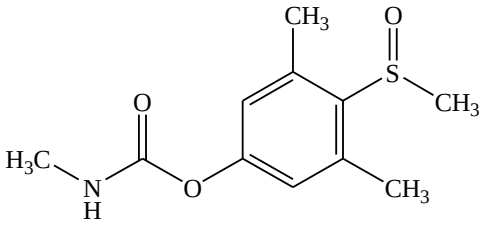
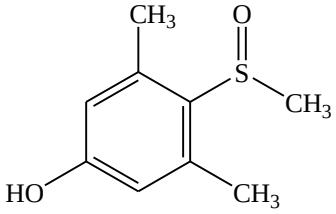
The identification of relevant TPs generated during MC ozonation, with or without the presence of a radical scavenger was performed by means of HPLC-MS. The same signals were observed in both processes, leading this fact to the idea that all the identified TPs could have been formed simultaneously through the two possible

ozonation degradative routes. An example of chromatogram illustrating the appearance of TPs signals can be found in Fig. S2 (supplementary information).

The proposed molecular structures of peaks for which the identification procedure was successful are gathered in Table 2. The observed differences between them were two: the relative degree of oxidation presented by the original thioether moiety, on one hand, and the loss of the carbamate group caused by hydrolysis of TP's, on the other. Both reactions are possible, well-known, and indeed have been considered in several papers regarding the environmental fate and degradation of MC by other oxidants (Qiang et al., 2014; Tian et al., 2013, 2010; UNFAO (Food and Agriculture Organization of the United Nations) and WHO (World Health Organization), 1999). In the case of O_3 and OH· attack to thioether groups, the mechanisms are reported in studies concerning the degradation of organic compounds containing this moiety (Jeon et al., 2016; Jin et al., 2012; Szabó et al., 2015). By this way, methiocarb sulfoxide (MCX), methiocarb sulfoxide phenol (MCXP), and methiocarb sulfone phenol (MCNP) were identified as the major TPs formed during MC ozonation process. The generation of these species, as well as the formation of other byproducts like methiocarb sulfone (MCN) and methiocarb phenol (MCP) (not observed in this work), are reported in previous studies regarding MC oxidation by chlorine dioxide (Tian et al., 2010), free chlorine (Tian et al., 2013) and monochloramine (Qiang et al., 2014).

Table 2. Detected TPs and corresponding structures.

m/z	Name	Proposed structure

226 (m+1)	Methiocarb (MC)	
201 (m+1)	Methiocarb sulfone phenol (MCNP)	
242 (m+1)	Methiocarb sulfoxide (MCX)	
185 (m+1)	Methiocarb sulfoxide phenol (MCXP)	

341

342 Fig. 2 shows the semi-quantitative evolution of MC and its major formed TPs during
343 ozonation process, as a function of the applied ozone dose. Since non-reference
344 standards were available for all the species, the graphs provide the relative variation of
345 the TPs presence in the reaction medium, but not the evolution of their absolute
346 concentrations. As early commented, the major formed byproducts were the same ones
347 in the process only involving the direct attack of ozone (Fig. 2 A) than in the process
348 that involved the attack of both, molecular ozone and hydroxyl radicals (Fig. 2 B).
349 Besides, the evolution profiles as a function of the applied ozone dose presented shape
350 similarities, being the main difference related to the relative efficiency of each
351 degradative process in terms of required oxidant dosage: since the removal of MC for
352 the process only involving the attack of molecular ozone needed larger oxidant doses
353 than for the process in which both ozone and hydroxyl radicals participated, the

generation and subsequent destruction of the TPs also required larger ozone doses in the first case than in the second. Another key difference was the relative residual signal of these products at each experimental point, always significantly lower when the radical route played its role. Without the presence of *tert*-butanol, formed hydroxyl radicals were supposed to oxidize part of the remaining MC and TPs, thereby contributing to their global depletion. Since these organic species are generally more reactive to hydroxyl radicals than molecular ozone, that contributed to an enhanced efficiency of the degradative process.

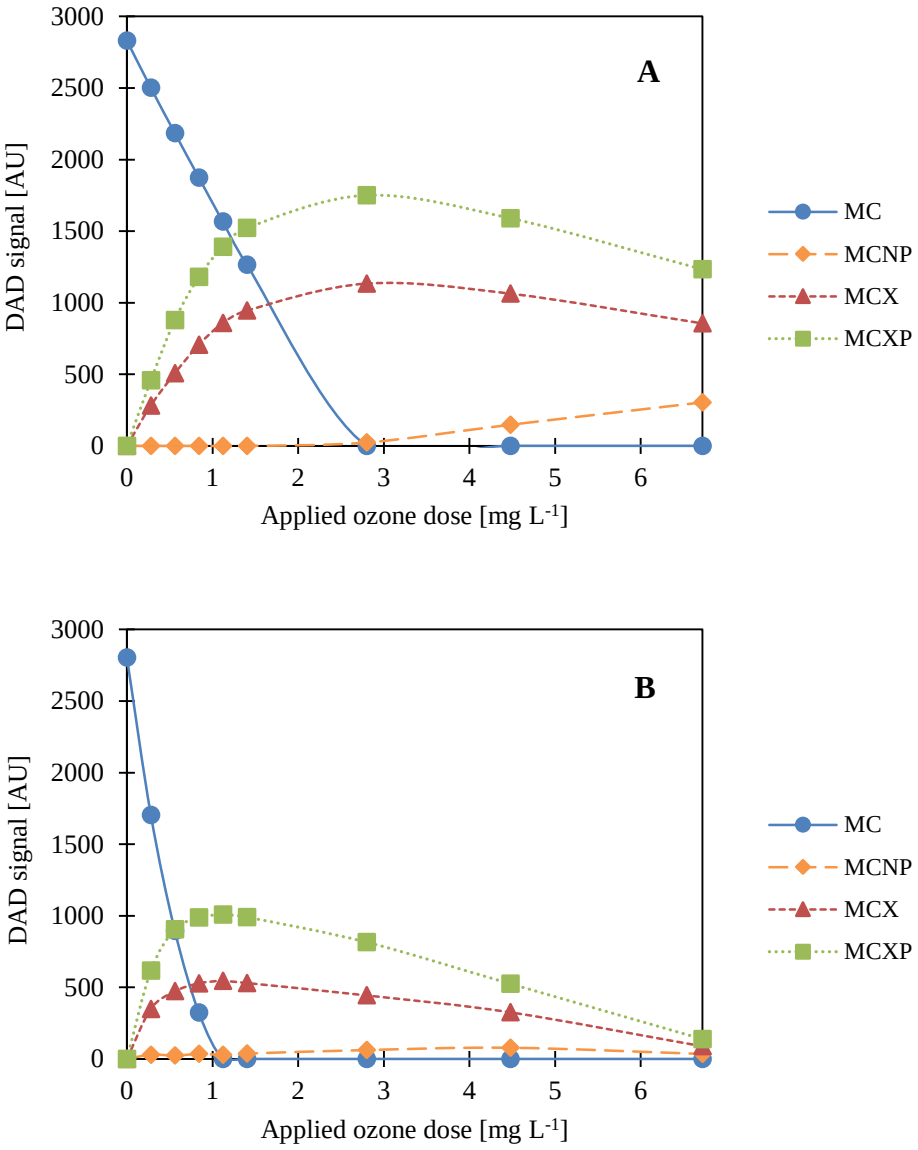


Figure 2. Semi-quantitative monitoring of MC TPs generated during reaction of the pesticide (C_0 = 20 µM) with O_3 (A) and O_3/OH· (B), as a function of the applied ozone dose.

Taking into account the observed TPs and their semi-quantitative evolution with the ozone dosage, the sequential pathway shown in Fig. 3 was proposed for MC ozonation. It has to be cleared that this mechanism attempted to explain what seems to constitute the first steps of the degradation pathway followed by the pesticide during the process, considering that further oxidation products were not possible to be identified. MC oxidation by ozone or hydroxyl radicals firstly occurred at the thioether moiety.

Additionally, and due to its demonstrated potential to undergo hydrolysis in water at pH values up to 6.5 (Qiang et al., 2014; Tian et al., 2013), the carbamate group was prone to disintegration generating MCXP as side-product. Then, the concentrations of MCX and MCXP increased by means of these mechanisms until MC was totally depleted. In the view of the profiles presented in Fig. 2, it seems like MCX and MCXP signals decreased at different rates from this moment, being the disappearance of MCXP slightly faster than the one for MCX. It is possible that MCXP was easier to oxidize than MCX, as happened in the study employing monochloramine as oxidant (Qiang et al., 2014). Finally, higher ozone doses allowed further oxidation of MCXP, leading to MCNP generation.

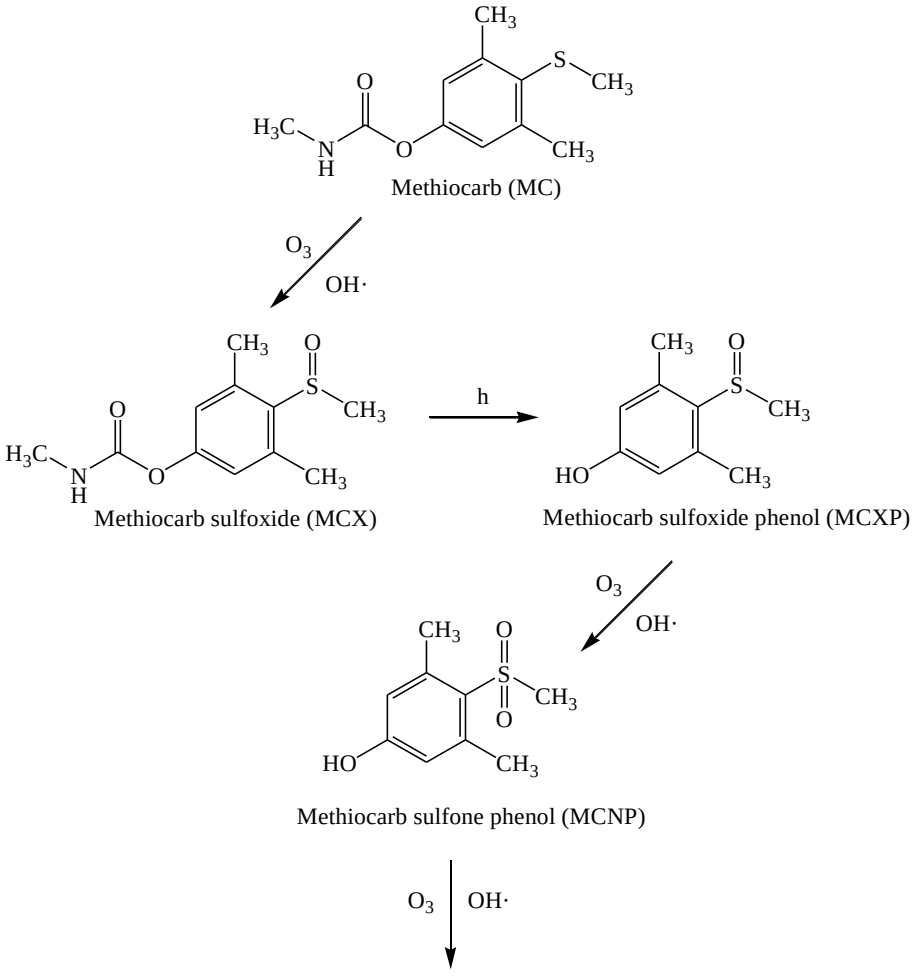


Figure 3. Proposed reaction mechanism for MC attack by molecular ozone and formed hydroxyl radicals.

It is known that as a carbamate pesticide, the specific action of MC against pests is based on the inhibition of the vital enzyme cholinesterase by its carbamate group (Padilla et al., 2007). Because of that, MCNX and MCNP probably did not maintain their activity as pesticides after losing their carbamate moiety, which did not mean that these species were non-toxic. Following the same reasoning, MCX probably kept its activity as pesticide, since its carbamate moiety remained unaltered. Because this TP is more toxic than the parent compound (Marss, 1998), the observed increase in toxicity could be attributable to the only difference between both molecular structures: the sulfoxide moiety of MCX, in contrast with the sulfur group presented by MC.

3.5. Toxicity during MC ozonation process as a function of the applied ozone dose

The chemical alterations produced during the ozonation-hydrolysis process implied changes in the properties of the resulting species, including toxicity. The variation of $1/EC_{50}$ versus the applied ozone dose is presented in Fig. 4. This parameter provides a direct idea about the ecotoxicity of the solution, since higher values imply a higher inhibition in bacteria bioluminescence. Initial toxicity for both experiments was relatively high (EC_{50} about 4.5%), which is not surprising considering that MC had already demonstrated to be highly toxic (Marss, 1998; World Health Organization, 2010). For the untreated solution, the corresponding EC_{50} value expressed in concentration units could be also calculated, since the composition of this sample was known. With an EC_{50} of 0.2 mg L^{-1}, and according to the toxicity classification stablished in Directive 93/67/EEC (very toxic to aquatic organisms (0.1-1 mg L^{-1}), toxic (1-10 mg L^{-1}), harmful (10-100 mg L^{-1}), non-toxic (>100 mg L^{-1})) (European

410 Commission Joint Research Centre, 2003), MC would be considered as very toxic to
411 aquatic organisms, which would confirm again the previous knowledge about MC
412 ecotoxicity. For the rest of experimental points, only the EC_{50} in terms of sample
413 dilution (% v/v) could be provided since the corresponding samples compositions were
414 unknown.
415
416 For experiments in the presence of *tert*-butanol, solution toxicity increased to a
417 maximum (EC_{50} about 1.5%) for applied O_3 concentrations about 3.5-4 mg L^{-1}, and
418 decreased for higher dosages. However, this decrement was not significant and for
419 larger ozone doses the acute toxicity was even higher (EC_{50} about 2%) than that for the
420 untreated solution. For experiments without the presence of radical scavenger, a small
421 increase in toxicity (EC_{50} about 4%) was observed at O_3 doses about 0.5-1 mg L^{-1},
422 followed by a significant drop of this parameter: for ozone doses about 7 mg L^{-1}, EC_{50}
423 value was about 95%, which represented a relatively low toxicity.
424
425 Changes in toxicity observed in both experiments can be attributed to the generation of
426 TPs more toxic than the parent compound, as concluded in many other related studies
427 (Borowska et al., 2016; Dantas et al., 2008, 2007). In the current case, reaction
428 intermediate MCX is the main suspect of increasing toxicity, since is known to be about
429 2-3 times more toxic than MC based on oral LD_{50} values in rats (Marss, 1998). Also,
430 other TPs like MCXP and MCNP, as well as hydrogen peroxide formed through ozone
431 decomposition (Hoigné, 1982) could contribute to this increase in bacteria
432 bioluminescence inhibition. Of course, it was not possible to exactly quantify the
433 contribution of this species to the total toxicity of the solution, but by comparing the
434 MCX and toxicity profiles, a correlation between both parameters appears to be

suitable: in all experiments, the maximum *1/EC$_{50}$* value is reached at approximately the O_3 dosage in which the maximum relative amount of MCX (DAD signal) is also observed. In addition, when the concentration of this intermediate starts to decrease, the toxicity of the solution also starts to diminish. Since toxicity changes appears to be mainly caused by variations of the MCX concentration, the rest of TPs generated during the process and contained in the analyzed samples should necessarily present similar or lower toxicity levels than MC. This fact would be in total agreement with the previous literature regarding this issue (Marss, 1998; Tian et al., 2013, 2010).

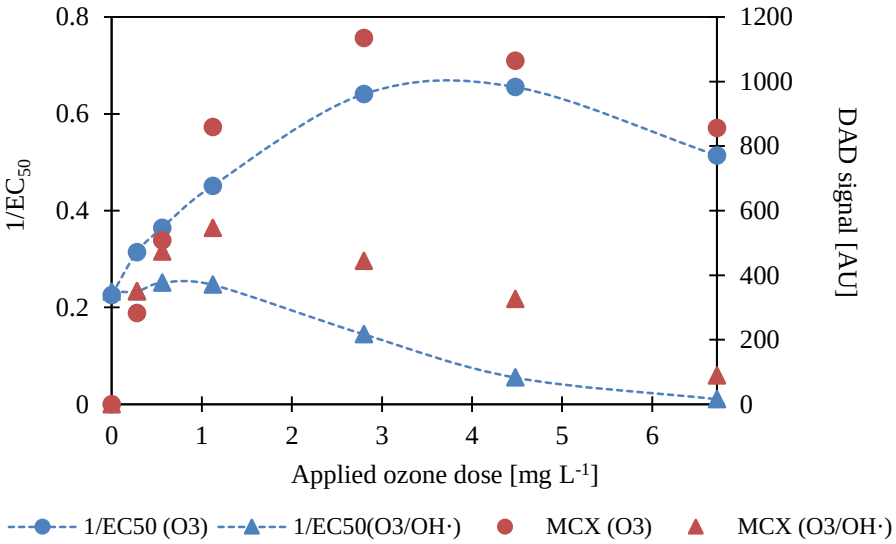


Figure 4. Acute toxicity and remaining DAD signal of MCX, as a function of the applied ozone dose.

In the view of the last results, ozonation of MC contributed to increase the toxicity of the reaction medium when low oxidant doses were applied. The relative resistance of the formed TPs towards direct ozone attack caused a drop in the overall efficiency of the process, thus enlarging the oxidant dosage required to reach relatively low toxicities. Thus, a normalized ozone dose of approximately 2.5 mg O_3/mg DOC, which probably would be considered as economically non-reasonable (Gerrity et al., 2012; Lee et al.,

2013), would be needed under the studied conditions. Since the attack of hydroxyl radicals during MC ozonation revealed to be essential if the removal of its toxic TPs (especially MCX) is wanted to be achieved, an enhancement of the indirect degradation route should be promoted. It is important to mention, however, that in waters with pH values up to 7 the indirect degradative route would be naturally favored, thus enlarging the process efficiency.

4. Conclusions

For the first time, the kinetics, pathways and toxicity changes associated to MC degradation during ozonation process at neutral pH were investigated. The second-order rate constants for reactions of MC with ozone and hydroxyl radicals were determined to be $1.7 \cdot 10^6$ and $8.2 \cdot 10^9$ M^{-1} s^{-1}, respectively. Both ozone and hydroxyl radicals showed to play an important role in the overall depletion of MC at neutral pH, thus indicating the potential of the ozonation process to remove MC from water. Specifically, the OH· attack highly contributed to increase the efficiency of the process by reducing to more than half the oxidant dose necessary to completely degrade MC. MCX, MCXP and MCNP were the major intermediates identified in the MC ozonation process. These byproducts were generated through a sequential combination of both O_3 and OH· oxidation and hydrolysis. The toxicity changes observed in MC ozonation were principally attributed to variations in the MCX concentration. Despite its demonstrated capacity to oxidize MC, direct ozone attack was unable to completely degrade MCX. Although the oxidation by OH· showed its ability to degrade MC and all its TPs, the resistance towards ozone attack exhibited by these compounds increased the oxidant dosage necessary to achieve a relative low toxicity in the medium. In order to overcome

these problems and enhance the overall efficiency of the process, the indirect degradation route through hydroxyl radicals should be favored.

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