

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

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10

11 **ABSTRACT**

12

13 Despite the high toxicity and resistance to conventional water treatments exhibited by
14 methiocarb (MC), there are no reports regarding the degradation of this priority
15 pesticide by means of alternative purification technologies. In this work, the removal of
16 MC by means of ozonation was studied for the first time, employing a multi-reactor
17 methodology and neutral pH conditions. The second-order rate constants of MC
18 reaction with molecular ozone (O_3) and formed hydroxyl radicals ($OH\cdot$) were
19 determined to be $1.7 \cdot 10^6$ and $8.2 \cdot 10^9 M^{-1} s^{-1}$, respectively. During degradation
20 experiments, direct ozone reaction was observed to effectively remove MC, but not its
21 formed intermediates, whereas $OH\cdot$ could oxidize all species. The major identified TPs
22 were methiocarb sulfoxide (MCX), methiocarb sulfoxide phenol (MCXP) and
23 methiocarb sulfone phenol (MCNP), all of them formed through MC oxidation by O_3 or
24 $OH\cdot$ in combination with hydrolysis. A toxicity assessment evidenced a strong
25 dependence on MCX concentration, even at very low values. Despite the $OH\cdot$ capability

26 to degrade MC and its main metabolites, the relative resistance of TPs towards ozone
27 attack enlarged the oxidant dosage (2.5 mg O₃/mg DOC) necessary to achieve a
28 relatively low toxicity of the medium. Even though ozonation could be a suitable
29 technique for MC removal from water compartments, strategies aimed to further
30 promote the indirect contribution of hydroxyl radicals during this process should be
31 explored.

32

33 **KEYWORDS**

34

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

37

38 **1. Introduction**

39

40 Methiocarb (mesurol, 3,5-dimethyl-4-(methylthio)phenyl methylcarbamate) (MC) is
41 one of the most common carbamate pesticides worldwide, employed in agriculture as
42 insecticide, acaricide, molluscicide and bird repellent (Altinok et al., 2006; Blažková et
43 al., 2009; Gitahi et al., 2002; Keum et al., 2000; Sinclair et al., 2006). This chemical has
44 been detected in natural waters of several countries (APVMA, 2005; Barceló et al.,
45 1996; Fytianos et al., 2006; García de Llasera and Bernal-González, 2001; Squillace et
46 al., 2002) at concentration levels ranging from ng L⁻¹ to µg L⁻¹. It also has been detected
47 in wastewater effluents (Campo et al., 2013; Masiá et al., 2013), this last suggesting the
48 resistance of MC to conventional wastewater treatments. Although the detected
49 concentrations of this micropollutant in water compartments are generally low, it
50 represents a serious threat to the aquatic and human life considering its high toxicity and

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

62

63 Several studies regarding the fate of MC during conventional wastewater and simulated
64 drinking water treatment have been reported during the last few years. For wastewater
65 treatment, no concluding results have been obtained about MC fate. For example,
66 higher concentrations were found in the effluents than in the influents in a Spanish
67 sewage treatment plant, probably due to limitations in sampling procedure (Barbosa et
68 al., 2016; Campo et al., 2013). Studies regarding the fate of MC in simulated drinking
69 water treatment have demonstrated that reactions between this pesticide and most
70 commonly used disinfectants (i.e.: free chlorine, ClO₂ and NH₂Cl), which also possess a
71 certain oxidizing power, yield transformation products (TPs) more toxic and persistent
72 than the parent compound, even though this one becomes degraded (Qiang et al., 2014;
73 Tian et al., 2013, 2010). However, despite the safety concern regarding the presence of
74 this pesticide and their TPs in the aqueous systems, no studies related to the removal of
75 MC by advanced treatment options have been found in literature, as also stated in a

76 recent review by Barbosa et al. (Barbosa et al., 2016). A possible explanation for this
77 lack of data could be related to the moderate-high hydrophobic character of MC (log
78 $K_{ow} = 3.2$ and water solubility 27 mg L^{-1} , at 20°C (UNFAO (Food and Agriculture
79 Organization of the United Nations) and WHO (World Health Organization), 1999)),
80 which could complicate the handling of MC during the experimental work due to its
81 probable tendency of becoming adsorbed to other hydrophobic materials.

82

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

93

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

101 **2. Materials and methods**

102

103 *2.1. Chemicals and reagents*

104

105 Methiocarb, sulfamethoxazole and phenol analytical standards were acquired from
106 Sigma-Aldrich (Germany). NaH₂PO₄, Na₂HPO₄, H₃PO₄, *tert*-butanol and acetonitrile
107 were purchased from Panreac (Spain), and were all analytical grade. Milli-Q water was
108 produced by a filtration system (Millipore, USA). Finally, all the reagents employed
109 during toxicity bioassays were purchased from Modern Water (UK).

110

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

117

118 *2.2. Ozonation experiments*

119

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

126 during the preceding assays, ozonation runs were carried out employing a multi-reactor
127 methodology, successfully used in several works (Borowska et al., 2016; Ning et al.,
128 2007). Detailed information of ozone stock solutions preparation can be found in the
129 supplementary information (Text and Fig. S1). All ozonation experiments were done in
130 triplicate.

131

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

137

138 For k_{MC,O_3} measurement, experiments were carried out in a series of 25 mL vials
139 containing 20 μM of MC and 20 μM of sulfamethoxazole (SMX), the reference
140 compound. The competitor was selected considering the high-reactivity of MC with
141 molecular ozone. To avoid reactions involving hydroxyl radicals (OH·), *tert*-butanol
142 was employed as OH· scavenger (100 mM). Adequate quantities of a H₂PO₄⁻/HPO₄²⁻
143 buffer were also added in order to maintain the medium pH at a constant value of 7.
144 Different doses (from 5 to 50 μM) of the ozone stock solution were injected to each vial
145 as reactant. The mixtures were vigorously shaken for a few seconds, to completely mix
146 the ozone in. Samples were withdrawn when the total consumption of ozone was
147 achieved, and quickly analyzed. The residual concentrations of MC and SMX were
148 determined by HPLC-DAD.

149

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

157

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

167

168 2.3. Analytical procedures

169

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

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

176

177 In order to elucidate the possible reaction pathways during MC ozonation, samples in
178 which different ozone doses were applied were analyzed by LC-MS. An Agilent 1100
179 HPLC coupled with a G1969A LC/MSD-TOF mass spectrometer was employed. MS
180 data were collected in full scan mode (25-1100 m/z), employing positive electrospray
181 ionization. The separation conditions were the same ones employed for DAD
182 quantification.

183

184 To assess the acute toxicity as a function of the applied ozone dose, Microtox[®]
185 bioassays were performed. This method measures the inhibition of light emission of
186 bioluminescent bacteria *Vibrio fischeri* caused by the presence of toxic compounds in
187 the aqueous media. The results of this assay are usually expressed as $EC_{50,15min}$, which
188 represents the percentage of sample dilution (v:v) that causes a 50% reduction in
189 bacteria luminescence after a contact time of 15 minutes. All the tests were carried out
190 in duplicate in a Microtox[®] M500 (Modern Water, UK) toxicity analyzer.

191

192 **3. Results and discussion**

193

194 *3.1. Rate constant for the reaction between MC and O₃*

195

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

198 corresponding to the direct reactions between MC and SMX with O₃, as described
199 elsewhere (Dantas et al., 2008, 2007).

200

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

201

202 As shown in this expression, a linear dependence between the natural logarithm of the
203 relative MC concentration and the natural logarithm of the relative SMX concentration
204 is expected, with the ratio between the second-order kinetic constants of the target and
205 the reference compound being the slope. For the three replicates that were performed,
206 linear regression coefficients greater than 0.99 were obtained, together with a good
207 agreement between the corresponding slope values (0.87 ± 0.01 , see Fig. S2 of the
208 supplementary information). Considering a value of $2.0 \cdot 10^6 \text{ M}^{-1} \text{ s}^{-1}$ for k_{SMX,O_3} at pH 7
209 (Huber et al., 2003; Jin et al., 2012), the second-order rate constant for reaction between
210 MC and molecular ozone, k_{MC,O_3} , was determined to be $(1.7 \pm 0.1) \cdot 10^6 \text{ M}^{-1} \text{ s}^{-1}$. As
211 suspected during preliminary experiments, the rate of the reaction between MC and O₃
212 is considerable fast. MC molecule contains a thioether moiety, which has been
213 considered to be the main responsible for the fast kinetics (between $2.0 \cdot 10^5$ and
214 $6.7 \cdot 10^5 \text{ M}^{-1} \text{ s}^{-1}$) presented by other compounds containing this functional group in their
215 corresponding reactions with molecular ozone (Dodd et al., 2006; Jeon et al., 2016).
216 Since MC does not show basic or acidic properties in aqueous systems (UNFAO (Food
217 and Agriculture Organization of the United Nations) and WHO (World Health
218 Organization), 1999), the reactivity of this compound with ozone is not expected to be
219 dependent on the medium pH, as reported for many other dissociating chemicals
220 (Borowska et al., 2016; Dantas et al., 2008, 2007; Hoigné and Bader, 1983).

221

222 3.2. Rate constant for the reaction between MC and OH·

223

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

233

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

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

235

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

239

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

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

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 $k_{MC,OH}$, 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 $(8.2 \pm$
 253 $0.2) \cdot 10^9 \text{ M}^{-1} \text{ s}^{-1}$, 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 $10^{10} \text{ M}^{-1} \text{ s}^{-1}$ (Rózsa et al., 2017; Szabó et al., 2015), the characteristic non-
 258 selectivity of $OH\cdot$ could promote other reaction mechanisms usually exhibited by this
 259 transient species (e.g. electron or hydrogen abstraction) (Gligorovski et al., 2015).

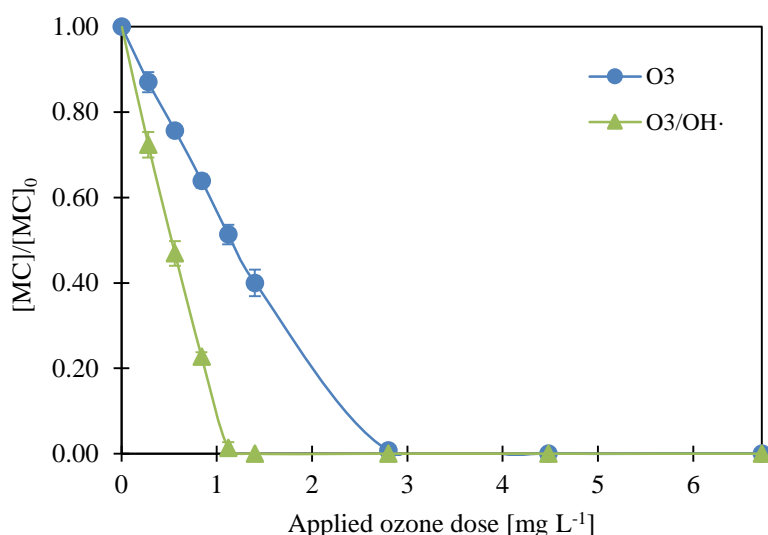
260

261 3.3. *MC degradation by ozone and hydroxyl radicals*

262

263 During the ozonation process, methiocarb can react with molecular ozone but also with
264 hydroxyl radicals generated by ozone decomposition (Gligorovski et al., 2015).
265 Degradation experiments applying different ozone dosages were performed in order to
266 observe the removal profile by means of the direct route (attack of molecular ozone), as
267 well as to demonstrate the contribution of hydroxyl radicals to the overall depletion of
268 MC at neutral pH. Results are shown in Fig. 1. Transformation by means of O₃/OH·
269 combination was more effective than the removal only due to O₃ attack: the ozone dose
270 required to deplete over a 99% of the initial MC concentration was about 2.5 times
271 lower in the first case than in the second (1.1 mg L⁻¹ vs 2.8 mg L⁻¹). At pH 7, therefore,
272 the indirect degradation pathway could play an important role in the global depletion of
273 MC. This means that efficiency of ozonation for MC depletion will strongly depend on
274 water pH, among other characteristics, since decomposition of ozone in hydroxyl
275 radicals is accelerated under alkaline conditions (Gligorovski et al., 2015).

276



277

278 Figure 1. Profile of MC degradation ($[MC]_0 = 20 \mu\text{M}$) as a function of the applied ozone dose, for
 279 experiments with (O_3) and without ($\text{O}_3/\text{OH}\cdot$) radical scavenger.

280

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

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

303

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

312

313 *3.4. Reaction intermediates and possible mechanisms*

314

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

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

321

322 The proposed molecular structures of peaks for which the identification procedure was
323 successful are gathered in Table 2. The observed differences between them were two:
324 the relative degree of oxidation presented by the original thioether moiety, on one hand,
325 and the loss of the carbamate group caused by hydrolysis of TP's, on the other. Both
326 reactions are possible, well-known, and indeed have been considered in several papers
327 regarding the environmental fate and degradation of MC by other oxidants (Qiang et al.,
328 2014; Tian et al., 2013, 2010; UNFAO (Food and Agriculture Organization of the
329 United Nations) and WHO (World Health Organization), 1999). In the case of O₃ and
330 OH· attack to thioether groups, the mechanisms are reported in studies concerning the
331 degradation of organic compounds containing this moiety (Jeon et al., 2016; Jin et al.,
332 2012; Szabó et al., 2015). By this way, methiocarb sulfoxide (MCX), methiocarb
333 sulfoxide phenol (MCXP), and methiocarb sulfone phenol (MCNP) were identified as
334 the major TPs formed during MC ozonation process. The generation of these species, as
335 well as the formation of other byproducts like methiocarb sulfone (MCN) and
336 methiocarb phenol (MCP) (not observed in this work), are reported in previous studies
337 regarding MC oxidation by chlorine dioxide (Tian et al., 2010), free chlorine (Tian et
338 al., 2013) and monochloramine (Qiang et al., 2014).

339

340

Table 2. Detected TPs and corresponding structures.

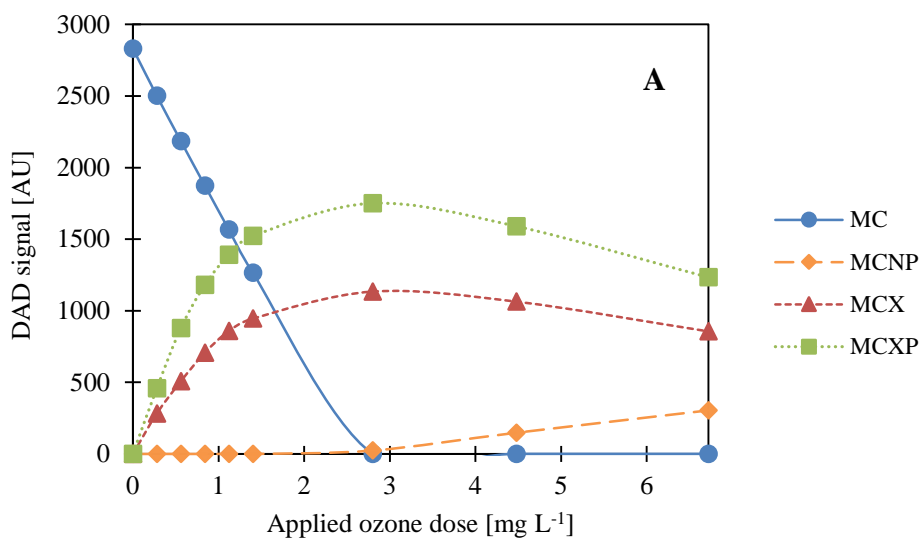
m/z	Name	Proposed structure
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226	Methiocarb	
(m+1)	(MC)	
201	Methiocarb	
(m+1)	sulfone phenol (MCNP)	
242	Methiocarb	
(m+1)	sulfoxide (MCX)	
185	Methiocarb	
(m+1)	sulfoxide phenol (MCXP)	

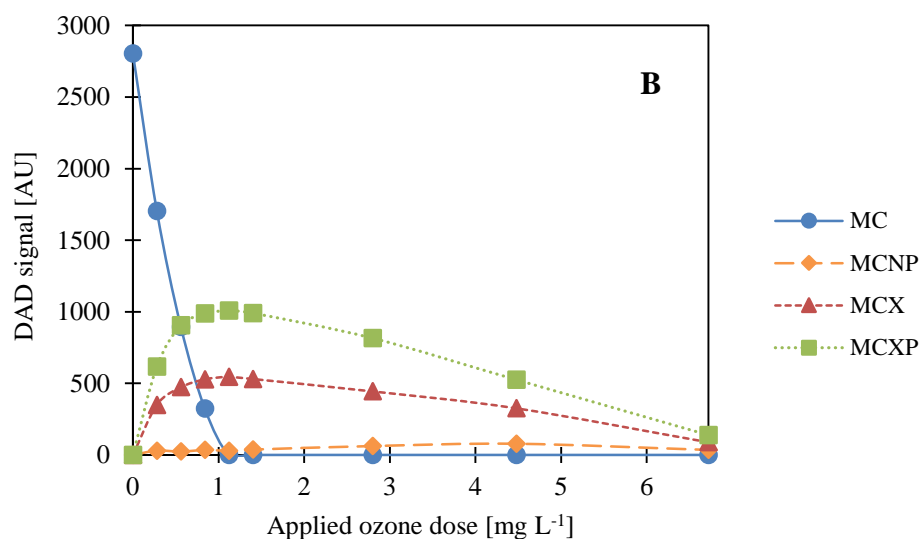
341

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

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



362



363

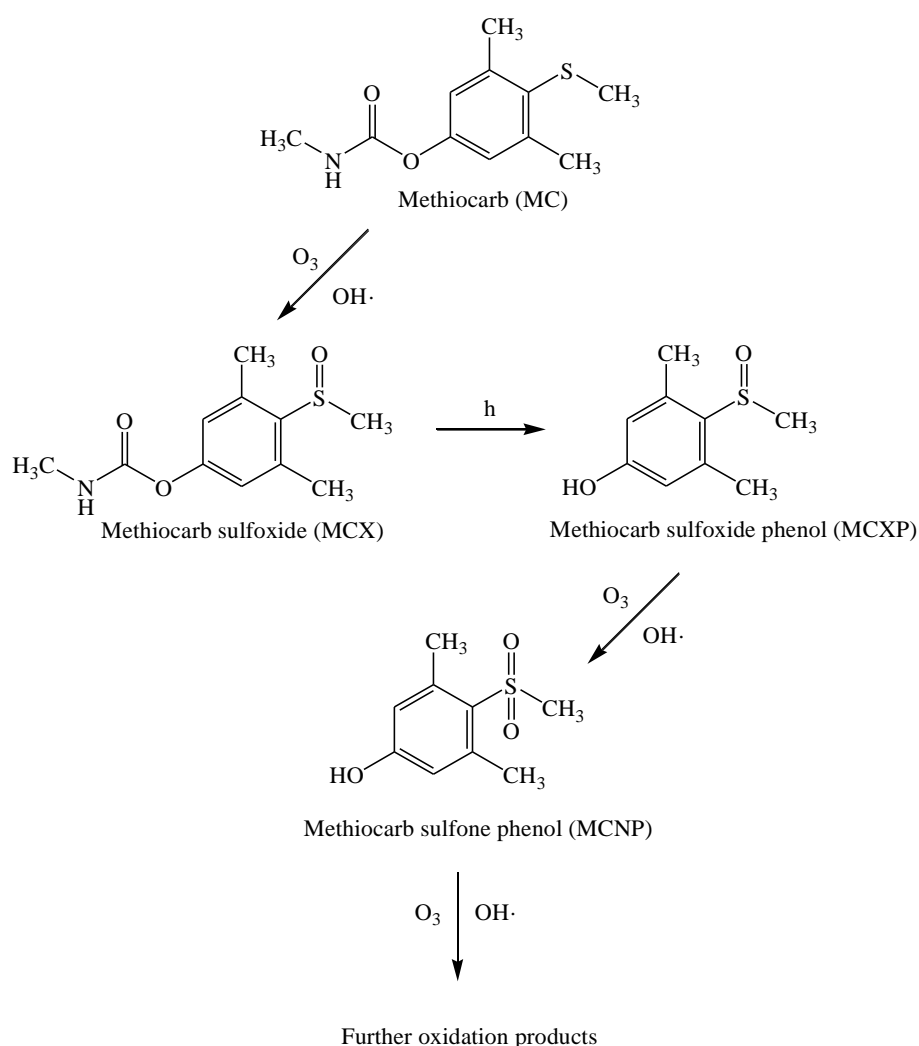
364 Figure 2. Semi-quantitative monitoring of MC TPs generated during reaction of the pesticide ($C_0 = 20$
 365 μM) with O_3 (A) and $\text{O}_3/\text{OH}\cdot$ (B), as a function of the applied ozone dose.

366

367 Taking into account the observed TPs and their semi-quantitative evolution with the
 368 ozone dosage, the sequential pathway shown in Fig. 3 was proposed for MC ozonation.

369 It has to be cleared that this mechanism attempted to explain what seems to constitute
 370 the first steps of the degradation pathway followed by the pesticide during the process,
 371 considering that further oxidation products were not possible to be identified. MC
 372 oxidation by ozone or hydroxyl radicals firstly occurred at the thioether moiety.

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



383

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

385

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

395

396 *3.5. Toxicity during MC ozonation process as a function of the applied ozone dose*

397

398 The chemical alterations produced during the ozonation-hydrolysis process implied
399 changes in the properties of the resulting species, including toxicity. The variation of
400 $1/EC_{50}$ versus the applied ozone dose is presented in Fig. 4. This parameter provides a
401 direct idea about the ecotoxicity of the solution, since higher values imply a higher
402 inhibition in bacteria bioluminescence. Initial toxicity for both experiments was
403 relatively high (EC_{50} about 4.5%), which is not surprising considering that MC had
404 already demonstrated to be highly toxic (Marss, 1998; World Health Organization,
405 2010). For the untreated solution, the corresponding EC_{50} value expressed in
406 concentration units could be also calculated, since the composition of this sample was
407 known. With an EC_{50} of 0.2 mg L^{-1} , and according to the toxicity classification
408 established in Directive 93/67/EEC (very toxic to aquatic organisms ($0.1\text{-}1 \text{ mg L}^{-1}$), toxic
409 ($1\text{-}10 \text{ mg L}^{-1}$), harmful ($10\text{-}100 \text{ mg L}^{-1}$), non-toxic ($>100 \text{ mg L}^{-1}$)) (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

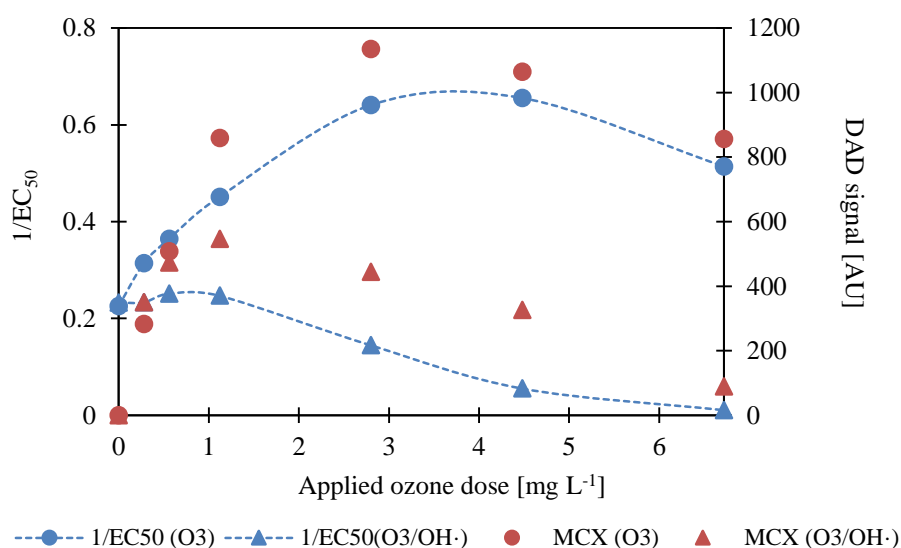
416 For experiments in the presence of *tert*-butanol, solution toxicity increased to a
417 maximum (EC_{50} about 1.5%) for applied O_3 concentrations about 3.5-4 mg L⁻¹, and
418 decreased for higher dosages. However, this decrement was not significant and for
419 larger ozone doses the acute toxicity 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

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

443



444

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

446

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

453 2013), would be needed under the studied conditions. Since the attack of hydroxyl
454 radicals during MC ozonation revealed to be essential if the removal of its toxic TPs
455 (especially MCX) is wanted to be achieved, an enhancement of the indirect degradation
456 route should be promoted. It is important to mention, however, that in waters with pH
457 values up to 7 the indirect degradative route would be naturally favored, thus enlarging
458 the process efficiency.

459

460 **4. Conclusions**

461

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

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

480

481 **Acknowledgements**

482

483 This work was financially supported by the Spanish Ministry of Science and Innovation
484 (project CTQ2014-52607-R), the Spanish Ministry of Economy and Competitiveness
485 (FPI research fellowship BES-2015-074109) and the Agency for Management of
486 University and Research Grants of the Government of Catalonia (project 2014SGR245).

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