

1 **Priority pesticide dichlorvos removal from water by ozonation process: reactivity,**  
2 **transformation products and associated toxicity**

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11

12 **ABSTRACT**

13

14 The treatability of waters contaminated with priority pesticide dichlorvos (DDVP) by  
15 means of ozonation has been assessed for the first time. In order to do so, reaction kinetics,  
16 transformation mechanisms and associated toxicity have been inspected in detail. Second-  
17 order rate constants of DDVP reactions with  $O_3$  and  $OH\cdot$  were determined to be 590 and  
18  $2.2 \cdot 10^9 \text{ M}^{-1}\text{s}^{-1}$ , respectively. These values partly explained the degradation profiles  
19 obtained during experiments with and without the presence of an  $OH\cdot$  scavenger, in which  
20 the significant contribution of the indirect degradative route in the removal of DDVP was  
21 revealed. LC-MS analyses for ozonated samples allowed the elucidation of desmethyl  
22 dichlorvos (d-DDVP), dichloroacetic acid (DCA) and dimethyl phosphate (DMP) as main  
23 transformation products (TPs). DMP was found to be present in both  $O_3$  and  $OH\cdot$ -  
24 mediated oxidation pathways. Three possible degradation routes were proposed for  $OH\cdot$ -  
25 degradation, whereas the direct oxidation by  $O_3$  was only well-explained by the addition  
26 of this oxidant to the double bond of DDVP dichlorovinyl moiety. Microtox<sup>®</sup> bioassays  
27 revealed the inability of molecular ozone to reduce the toxicity of the medium and pointed  
28 out the importance of  $OH\cdot$  contribution in the degradation process. In general, ozonation  
29 could be a suitable treatment alternative for DDVP, formed TPs and associated toxicity  
30 abatement.

31

32 **KEYWORDS**

33

34 Dichlorvos, ozone oxidation, hydroxyl radicals, second-order rate constants, toxic  
35 intermediates

36

## 37 **1. Introduction**

38

39 Although discharge limits for micropollutants have not been legally established yet, some  
40 regulations regarding the identification, monitoring and control of some of these  
41 substances in the aquatic environment have been released over the last few years [1].  
42 Directive 2013/39/EU, for instance, recommended attention to the monitoring of 45  
43 compounds/groups of compounds considered as priority substances, giving special  
44 importance to the development and implementation of innovative remediation  
45 technologies aimed to remove these chemicals from water and wastewater [2].

46

47 About half (22/45) of the priority substances considered in the list are pesticides [2], fact  
48 that points out the special concern regarding the presence of this kind of pollutants in  
49 water compartments. Increases in cancer incidence, endocrine disruption, birth defects  
50 and genetic mutations are some of the chronic consequences to human health that have  
51 been directly linked to pesticide exposure [3]. Also, it is well-known that pesticides can  
52 pose a risk to other living species [4]. However, since the agricultural use of these  
53 chemicals is still necessary to ensure harvest quality and food protection, pesticides  
54 continuously enter aquatic resources mainly via runoff, soil leaching or spray drifting [5].  
55 Moreover, and far of contributing to improve this problematic situation, most of these  
56 compounds are resistant to conventional water and wastewater treatments [6] thus  
57 increasing their environmental persistence and associated potential risks.

58

59 Dichlorvos (2,2-Dichlorovinyl dimethyl phosphate, DDVP) is a chlorinated  
60 organophosphorus pesticide. It has been traditionally employed as insecticide in  
61 agriculture, food storage areas, workplaces and homes [7], as well as to treat parasite  
62 infections in livestock and domestic animals [8]. As most organophosphorus insecticides,  
63 DDVP is highly toxic to humans: the acute exposure to this chemical can cause breathing  
64 problems, coma or even death [9]. Chronic exposure to DDVP has been related to  
65 increased risk of diabetes [10], whereas some studies suggest this pesticide could  
66 negatively affect liver [11] and renal [12] function. Moreover, and according to the  
67 International Agency for Research on Cancer (IARC), DDVP probably presents

68 carcinogenic activity in humans [13]. DDVP can also affect other living species. For  
69 instance, it has been found that the chronic exposure to this chemical induces oxidative  
70 damage, developmental changes, mutagenesis and carcinogenicity in fish [14]. Because  
71 of all these reasons, DDVP has been classified by the World Health Organization (WHO)  
72 as a highly hazardous pesticide [15] and has been excluded from the list of insecticides  
73 approved for use in some world regions like the European Union [16]. In other countries  
74 like USA [17] or Australia [18], the use of DDVP has been restricted but still continues.  
75 Moreover, DDVP is still largely used in many developing countries where poor  
76 environmental controls are applied [19,20]. The widespread employment of this  
77 insecticide has therefore caused its detection in surface waters worldwide, in  
78 concentrations ranging from 1.4 to 5630 ng L<sup>-1</sup> [21–25].

79

80 Despite the serious risks to human and environmental health posed by the presence of  
81 DDVP in water resources, the knowledge regarding the removal of this compound by  
82 means of unconventional water treatments is still incomplete. Concerning the use of  
83 Advanced Oxidation Processes (AOPs) on that purpose, some works in which  
84 heterogeneous photocatalysis [8,26–28], Fenton [29] and different combinations of  
85 oxidative processes [30] were applied can be found in literature. Even so, the use of other  
86 advanced treatments like ozonation has been barely explored.

87

88 Ozonation process has extensively demonstrated to have a great potential for  
89 micropollutant abatement [31–34]. Ozone (O<sub>3</sub>) is a strong oxidizing agent that also  
90 decomposes in water to yield hydroxyl radicals (OH·), a transient species with  
91 extraordinary oxidation capacity [35]. However, although ozonation can be a suitable  
92 alternative to remove these contaminants from water it is important, prior to its full-scale  
93 application, to deeply investigate the fundamentals of the degradative process by studying  
94 the kinetics and mechanisms of the principal reactions involved. This information is  
95 crucial to properly assess the process efficiency since harmful transformation products  
96 (TPs) can be formed during the treatment, even though the parent compound becomes  
97 completely removed [35].

98

99 To the best of our knowledge this is the first time in which the degradation of priority  
100 pesticide DDVP by means of ozonation process is explored. The study aimed to determine  
101 the reaction kinetics of this compound with both, molecular ozone and hydroxyl radicals,

102 as well as to elucidate the possible reaction mechanisms involved in the process. In order  
103 to evaluate the potential risks that formed TPs could pose, the residual toxicity of the  
104 treated water was also determined.

105

## 106 **2. Materials and methods**

107

### 108 *2.1. Chemicals and reagents*

109

110 Dichlorvos, metoprolol, and *p*-benzoquinone analytical standards were acquired from  
111 Sigma-Aldrich (Germany). NaH<sub>2</sub>PO<sub>4</sub>, Na<sub>2</sub>HPO<sub>4</sub>, H<sub>3</sub>PO<sub>4</sub>, *tert*-butanol and acetonitrile  
112 were purchased from Panreac (Spain), and were all analytical grade. Milli-Q water was  
113 produced by a filtration system (Millipore, USA). Pure oxygen (>99.999%) was supplied  
114 by Abelló Linde (Spain). Finally, all the reagents employed during toxicity bioassays  
115 were purchased from Modern Water (UK).

116

### 117 *2.2. Ozonation experiments*

118

119 Several control experiments were performed in order to determine the possible effects of  
120 hydrolysis, adsorption or UV-Vis photolysis on DDVP disappearance during ozonation  
121 experiments. All the assays were carried out in 250 mL closed glass beakers, with initial  
122 DDVP concentrations of 1 mg L<sup>-1</sup>. For hydrolysis and adsorption experiments, the  
123 beakers were covered with aluminum foil in order to prevent the possible influence of  
124 radiation. The pH in hydrolysis tests was adjusted to a value of 7 by adding adequate  
125 quantities of a H<sub>3</sub>PO<sub>4</sub>/Na<sub>2</sub>HPO<sub>4</sub> buffer solution. For adsorption experiments, different  
126 types of silicones, PVDF and PTFE were put in contact with the pesticide solutions.  
127 Samples were taken at 0, 1, 5 and 24 h, and analyzed by HPLC-DAD. Results showed  
128 that DDVP remained stable for hydrolysis, photolysis and most of the adsorption  
129 experiments. Only in the case of silicones a significant disappearance of DDVP (5-10%)  
130 was observed, after 24 h. The use of this kind of materials was therefore discarded during  
131 experimentation.

132

133 All ozonation experiments were carried out in triplicate, at a controlled temperature of  
134 20±2 °C and neutral pH conditions, in Milli-Q water. A multi-reactor methodology,  
135 successfully employed in several previous works [31,36,37], was employed for kinetic

136 and degradation experiments. Detailed information regarding ozone stock solutions  
137 preparation can be found elsewhere [36].

138

139 For  $k_{DDVP,O_3}$  measurement, competition kinetics method [31,38] was employed.  
140 Experiments were carried out in a series of 25 mL vials containing 20  $\mu$ M of DDVP and  
141 20  $\mu$ M of metoprolol (MPL), the reference compound. This competitor was selected  
142 considering the moderate reactivity of DDVP with molecular ozone, revealed during  
143 preliminary experiments. To avoid the presence of hydroxyl radicals ( $OH\cdot$ ), *tert*-butanol  
144 (100 mM) was employed as scavenger. Adequate quantities of the phosphate buffer were  
145 also added in order to maintain the medium pH at a constant value of 7. Different doses  
146 (from 5 to 40  $\mu$ M) of the ozone stock solution ( $[O_3] = 14 \text{ mg L}^{-1}$ ) were injected to each  
147 vial to initiate the reaction. The mixtures were shaken for a few seconds to obtain  
148 homogeneous conditions. Samples were withdrawn when the total consumption of ozone  
149 was achieved (all within 2 h). The residual concentrations of DDVP and MPL were  
150 determined by HPLC-DAD. For  $k_{MC,OH\cdot}$  determination, a similar procedure was followed.  
151 This time, however, two references were employed instead of one since reactions of  
152 DDVP with  $O_3$  and  $OH\cdot$  took place at the same time and needed to be considered due to  
153 their significant contribution to DDVP degradation. MPL and *p*-benzoquinone (pBZQ)  
154 were selected as competitors since both were expected to present similar overall reactivity  
155 than DDVP. pBZQ residual concentration was also determined by HPLC-DAD.

156

157 In order to demonstrate the relative contribution of each oxidant (i.e.,  $O_3$  and  $OH\cdot$ ) to  
158 DDVP degradation, two extra sets of experiments were carried out. For direct degradation  
159 by molecular ozone, each reaction vial contained 20  $\mu$ M of DDVP, 25 mM of *tert*-butanol  
160 and adequate quantities of the pH 7 phosphate buffer. Different doses (from 5 to 175  $\mu$ M)  
161 of the ozone stock solution were applied. Samples were withdrawn when the total  
162 consumption of ozone was achieved (all within 2 h). Once the DDVP concentrations were  
163 analyzed by HPLC-DAD, samples were frozen and lately employed for TPs and toxicity  
164 determinations.

165

### 166 2.3. Analytical procedures

167

168 The concentrations of DDVP, MPL and pBZQ were quantified by means of a high  
169 performance liquid chromatograph (HPLC) equipped with a diode array detector (DAD),

170 all supplied by Agilent (1260 Infinity). The column employed was a Teknokroma  
171 Mediterranea Sea18 (250 mm x 4.6 mm and 5µm size packing). The mobile phase  
172 consisted on a 35:65 volumetric mixture of acetonitrile and Milli-Q water acidified at pH  
173 3 by the addition of H<sub>3</sub>PO<sub>4</sub>. The flow rate was set to 1 mL min<sup>-1</sup> and the UV detection  
174 was performed at 205, 220 and 254 nm for DDVP, MPL and pBZQ, respectively.

175

176 In order to elucidate the possible reaction pathways during DDVP ozonation, samples in  
177 which different ozone doses were applied were analyzed by LC-MS. An Agilent 1100  
178 HPLC coupled with a G1969A LC/MSD-TOF mass spectrometer was employed. MS data  
179 were collected in full scan mode (25-1100 m/z), employing negative electrospray  
180 ionization.

181

182 With the aim of assessing the toxicity changes during DDVP ozonation process,  
183 Microtox<sup>®</sup> assays were performed for samples withdrawn from degradation experiments.  
184 This method measures the inhibition of light emission of bioluminescent bacteria *Vibrio*  
185 *fischeri* caused by the presence of toxic compounds in the aqueous media. The results of  
186 this assay are usually expressed as *EC*<sub>50,15min</sub>, which represents the percentage of sample  
187 dilution (v/v) that causes a 50% reduction in bacteria luminescence after a contact time  
188 of 15 minutes. All the tests were carried out in duplicate in a Microtox<sup>®</sup> M500 (Modern  
189 Water, UK) toxicity analyzer.

190

### 191 **3. Results and discussion**

192

#### 193 *3.1. Kinetics of DDVP reactions with ozone and hydroxyl radicals*

194

195 The second-order rate constant of DDVP reaction with molecular ozone was calculated  
196 from Eq. 1, obtained by dividing the kinetic equations corresponding to reactions between  
197 O<sub>3</sub> and both DDVP and MPL.

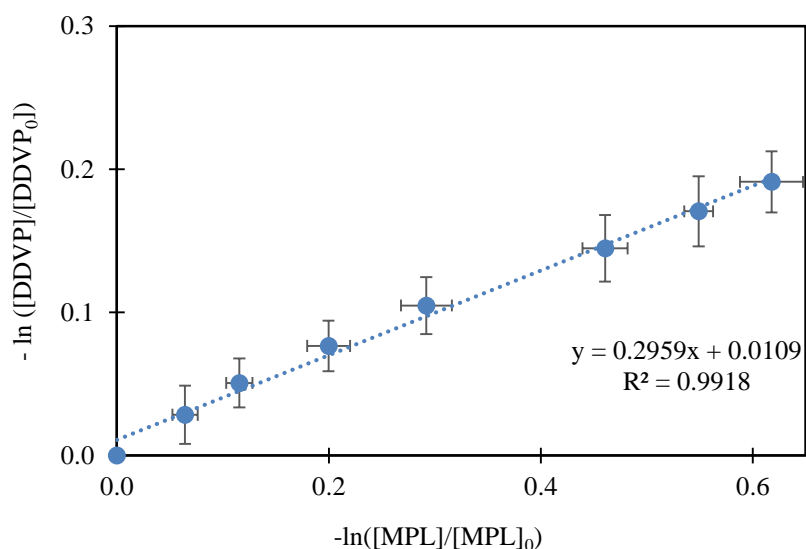
198

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

199

200 According to this expression, a linear dependence between the natural logarithm of the  
201 relative DDVP concentration and the natural logarithm of the relative MPL concentration

202 was expected. The ratio between the second-order rate constants of O<sub>3</sub> with target  
203 (DDVP) and reference (MPL) compound would represent the slope of this line.  
204



205  
206 Figure 1. Determination of second-order rate constant ( $k_{DDVP-O_3}$ ) for the reaction of DDVP and ozone by  
207 competition kinetics. Conditions:  $[DDVP]_0 = [MPL]_0 = 20 \mu\text{M}$ , pH 7, temperature =  $20 \pm 2 \text{ }^\circ\text{C}$ .  
208

209 Fig. 1 shows the experimental results, in which a good linear adjustment was obtained  
210 ( $R^2 > 0.99$ ). By knowing the second-order rate constant value for the reaction between  
211 ozone and the reference compound, the second-order rate constant for the reaction  
212 between DDVP and molecular ozone could be easily calculated. Under pH 7 conditions,  
213 and according to the results obtained in previous works [39], MPL reaction with ozone  
214 presented a second-order rate constant of  $2.0 \cdot 10^3 \text{ M}^{-1}\text{s}^{-1}$ . A value of  $590 \pm 20 \text{ M}^{-1}\text{s}^{-1}$  was  
215 finally determined for  $k_{DDVP-O_3}$  at pH 7 and  $20 \text{ }^\circ\text{C}$ , which indicates a moderate reaction  
216 rate of DDVP with molecular ozone, according to the classifications established by Von  
217 Gunten and coworkers regarding the reactivity of micropollutants with this oxidant  
218 [40,41]. Since DDVP does not show basic or acidic properties in water, the reactivity of  
219 this compound with O<sub>3</sub> was not expected to change with the medium pH, as happens with  
220 many other chemicals [38].

221  
222 The second-order rate constant of DDVP reaction with hydroxyl radicals was calculated  
223 by solving the system formed by Eq. 2 and Eq. 3. Detailed information about the obtaining  
224 of these expressions can be found in previous works, in which this methodology was  
225 successfully employed [36,42].

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

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

227 It is important to note that since this protocol employed two reference compounds (MPL  
 228 and pBZQ) and each one of them could react with both ozone and hydroxyl radicals, six  
 229 reactions needed to be considered to simultaneously take place in the studied system.  
 230 These are gathered in Table 1 along with their corresponding second-order rate constant  
 231 values.

232

233

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

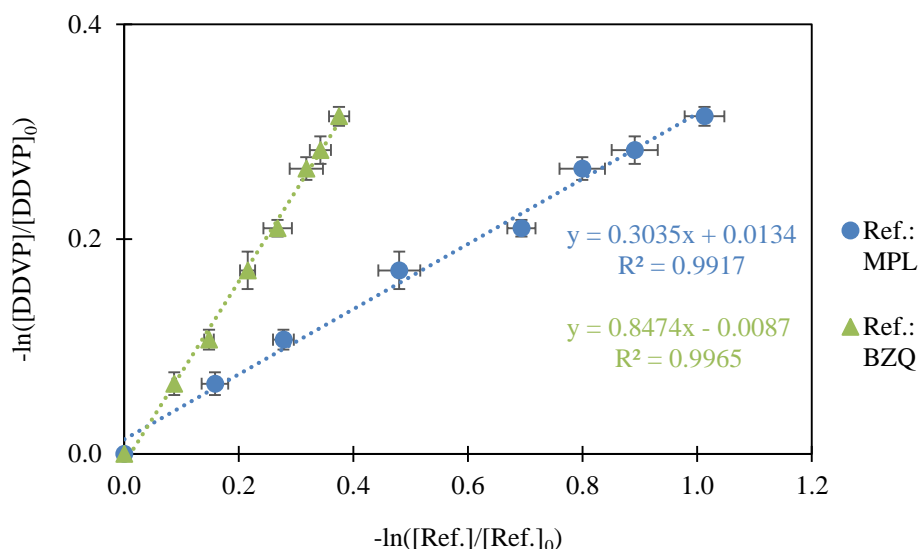
Reaction	2 <sup>nd</sup> -order $k$ value [ $M^{-1}s^{-1}$ ]	Reference
$DDVP + O_3 \rightarrow k_{DDVP,O_3}$	590	This study
$DDVP + OH \cdot \rightarrow k_{DDVP,OH}$	Unknown	-
$MPL + O_3 \rightarrow k_{MPL,O_3}$	$2.0 \cdot 10^3$	[39]
$MPL + OH \cdot \rightarrow k_{MPL,OH}$	$7.3 \cdot 10^9$	[39]
$pBZQ + O_3 \rightarrow k_{pBZQ,O_3}$	$2.5 \cdot 10^3$	[43]
$pBZQ + OH \cdot \rightarrow k_{pBZQ,OH}$	$1.2 \cdot 10^9$	[44]

234

235 From Eq. 2 and Eq. 3 it was deduced that by plotting the natural logarithm of DDVP  
 236 relative concentration versus the natural logarithm of both MPL and pBZQ relative  
 237 concentrations, two linear relationships could be obtained. The slope values of these lines,  
 238 together with the second-order rate constant values shown in Table 1 were required to  
 239 solve the equation system.

240





241

242 Figure 2. Determination of  $k_{DDVP,OH}$  by competition kinetics:  $-\ln([DDVP]/[DDVP]_0)$  versus  $-\ln([Ref.]/[Ref.]_0)$  for the simultaneous reaction of ozone and hydroxyl radicals with DDVP and the  
 243 references (Ref.) MPL and pBZQ. Conditions:  $[DDVP]_0 = [MPL]_0 = [pBZQ]_0 = 20 \mu\text{M}$ , pH 7, temperature  
 244 =  $20 \pm 2 \text{ }^\circ\text{C}$ .  
 245

246

247 Fig. 2 shows the experimental results, in which good linear adjustments ( $R^2 > 0.99$ ) were  
 248 obtained for both plots. The second-order rate constant of DDVP reaction with hydroxyl  
 249 radicals was determined to be  $(2.2 \pm 0.1) \cdot 10^9 \text{ M}^{-1}\text{s}^{-1}$ , thus pointing out the high reactivity  
 250 of DDVP with  $\text{OH}\cdot$ . This was explained by the non-selective character of the oxidant,  
 251 which can readily undergo reactions with different points of organic molecules [38].

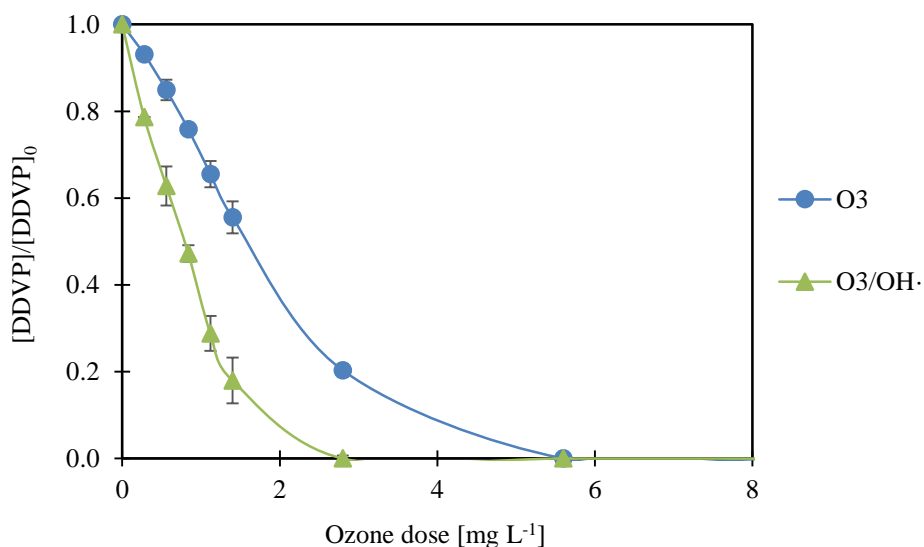
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### 253 3.2. DDVP degradation by ozone and hydroxyl radicals

254

255 During ozonation, DDVP could react with molecular ozone and also with hydroxyl  
 256 radicals formed as a consequence of ozone decay. In order to determine the relative  
 257 contribution of  $\text{OH}\cdot$  to overall DDVP removal, degradation experiments were conducted  
 258 at pH 7 with and without the presence of *tert*-butanol. Results are presented in Fig. 3.

259



260

261 Figure 3. DDVP degradation as a function of the ozone dose, for experiments with (O<sub>3</sub>) and without  
 262 (O<sub>3</sub>/OH·) the presence of *tert*-butanol (25 mM). Conditions: [DDVP]<sub>0</sub> = 20 μM, pH 7, temperature = 20 ±  
 263 2 °C.

264

265 Degradation through the combination of ozone and hydroxyl radicals was more effective  
 266 than the lonely attack by O<sub>3</sub>: an ozone dose of approximately 2.8 mg L<sup>-1</sup> was required for  
 267 the complete removal of DDVP, while the double of this dose (5.5 mg L<sup>-1</sup>) was necessary  
 268 in the presence of *tert*-butanol. Therefore, the contribution of hydroxyl radicals to the  
 269 pesticide removal was significant at neutral pH. Higher pH values, of course, would  
 270 probably enhance this contribution since ozone decomposition is accelerated under basic  
 271 conditions [38]. Besides the pH, other water characteristics like the organic and inorganic  
 272 matter content can also affect the process efficiency. Thus, performing experiments with  
 273 real water matrices and more realistic pesticide concentrations would be convenient in  
 274 order to properly determine the ozone dose necessary to remove DDVP. Also, models  
 275 based on the kinetic data here determined and water specific information [41,46] could  
 276 be useful on that purpose.

277

278 Besides the degradation of DDVP, the study of the formed TPs was thought to be essential  
 279 in order to assess the process efficiency: intermediates presenting higher toxicity than the  
 280 parent compound could be formed, thus enlarging the oxidant dosage necessary to obtain  
 281 a water relatively free of harmful substances.

282

283 3.3. Reaction intermediates and possible mechanisms

284

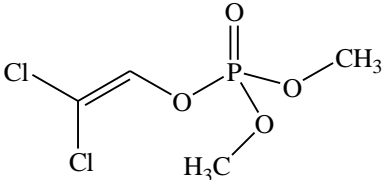
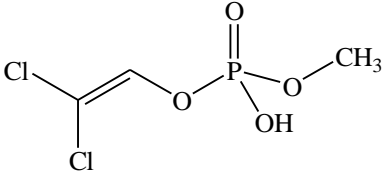
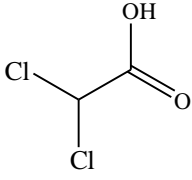
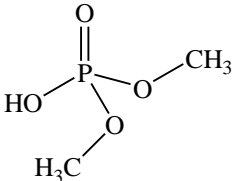
285 Relevant TPs generated during DDVP ozonation with and without radical scavenger  
 286 addition were identified by means of LC-MS, on the basis of the detected masses. The  
 287 proposed molecular structures are shown in Table 2.

288

289

290

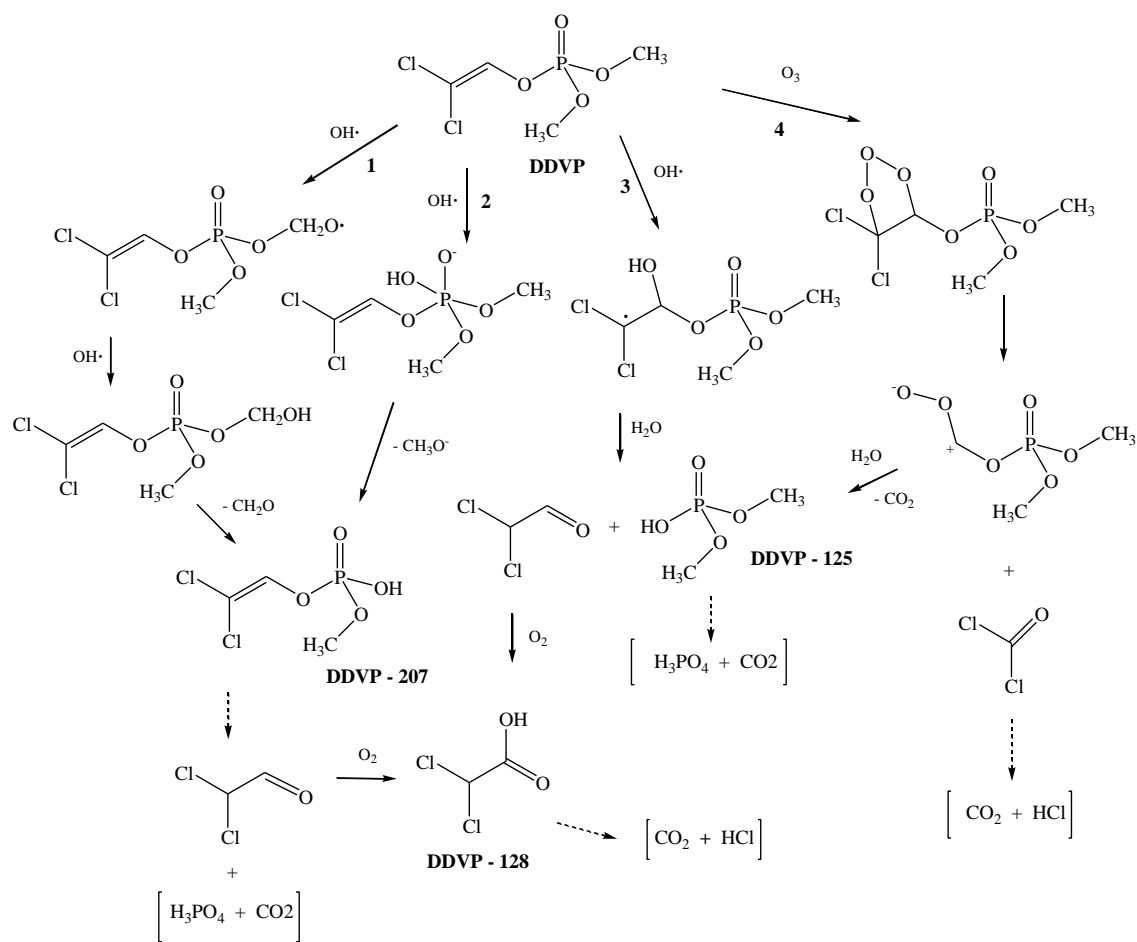
Table 2. DDVP, detected TPs and their corresponding molecular structures.

m/z	Name	Proposed structure	Observations
220 (m-1)	Dichlorvos (DDVP)		Parent compound in both experiments
207 (m-1)	DDVP-207 Desmethyl Dichlorvos (d-DDVP)		Only detected in the presence of OH·
128 (m-1)	DDVP-128 Dichloroacetic Acid (DCA)		Only detected in the presence of OH·
125 (m-1)	DDVP-125 Dimethyl phosphate (DMP)		Detected in both experiments

291

292 Only one of the observed intermediates, DDVP-125 or dimethyl phosphate (DMP), was  
 293 detected in both experiments (i.e., experiments with and without the presence of hydroxyl  
 294 radicals). For their part, DDVP-207 and DDVP-128 (desmethyl dichlorvos (d-DDVP)  
 295 and dichloroacetic acid (DCA), respectively) were only detected in experiments in which  
 296 OH· were present in the reaction medium. These findings appeared to indicate that DMP  
 297 could be generated through both molecular ozone and hydroxyl radicals mediated  
 298 oxidation, whereas d-DDVP and DCA were only formed via OH·. The first degradation  
 299 steps during DDVP ozonation process, therefore, could be explained by the reaction  
 300 pathways illustrated in Fig. 4.

301 The identification of d-DDVP suggests an initial H-abstraction, carried out by OH·, at  
302 one of the methyl groups of DDVP's phosphate moiety (pathway 1). A subsequent OH·  
303 addition after this first step, followed by the yielding of a formaldehyde molecule from  
304 the resulting structure would finally end in the d-DDVP (DDVP-207) formation.  
305 Alternatively, this TP could be formed through OH· addition to the phosphate moiety of  
306 DDVP, followed by the elimination of a methanolate (CH<sub>3</sub>O<sup>-</sup>) group from the resulting  
307 structure (pathway 2). The attack by hydroxyl radicals to DDVP molecule could also take  
308 place at its dichlorovinyl moiety. This pathway would consist on the addition of OH· to  
309 the corresponding double bond, followed by the hydrolysis of the resulting radical to  
310 yield DMP (DDVP-125) and dichloroacetaldehyde (pathway 3). Under the oxidizing  
311 conditions of the reaction medium, the latter would easily undergo oxidation to yield  
312 DCA, the last detected TP (DDVP-128). As before commented, in addition to the OH·-  
313 mediated oxidation and according to the LC-MS results obtained for samples in which  
314 *tert*-butanol was added as OH· scavenger, the intermediate DDVP-125 (DMP) could be  
315 formed via double bond cleavage by O<sub>3</sub> (pathway 4). The primary ozonide would be  
316 rapidly decomposed into a phosgene molecule and a zwitterion, being the latter  
317 subsequently transformed into DMP through CO<sub>2</sub> release.



318

319

Figure 4. Proposed reaction pathways for DDVP oxidation by molecular ozone and hydroxyl radicals during ozonation process.

320

321

322

As an organophosphate, the insecticide properties of DDVP are based on the irreversible inhibition of the vital enzyme acetyl cholinesterase. This inhibition is called cholinergic effect and is caused by the phosphorylation of the active site of the enzyme, which is no longer able to hydrolyze the acetylcholine neurotransmitter. This causes the overstimulation of the insect nervous system [9]. Since the presence of the phosphate structure is necessary to maintain the cholinergic effect, the intermediate DDVP-128 was not expected to present that pesticide mode of action. In addition, DDVP dichlorovinyl moiety enhances the binding between the enzyme's active site and the pesticide due to the electronegativity of its terminal chlorine. Because of that, it is probable that DDVP-127 had lost its cholinergic properties. It is important to note, however, that losing the cholinergic capacity does not mean to be non-toxic since other toxicity mechanisms affecting the life of target and non-target organisms could be manifested. For its part, intermediate DDVP-207 still maintains the DDVP dichlorovinyl phosphate structure and,

334

335 as a consequence, this compound was expected to present similar insecticide activity than  
336 the parent compound.

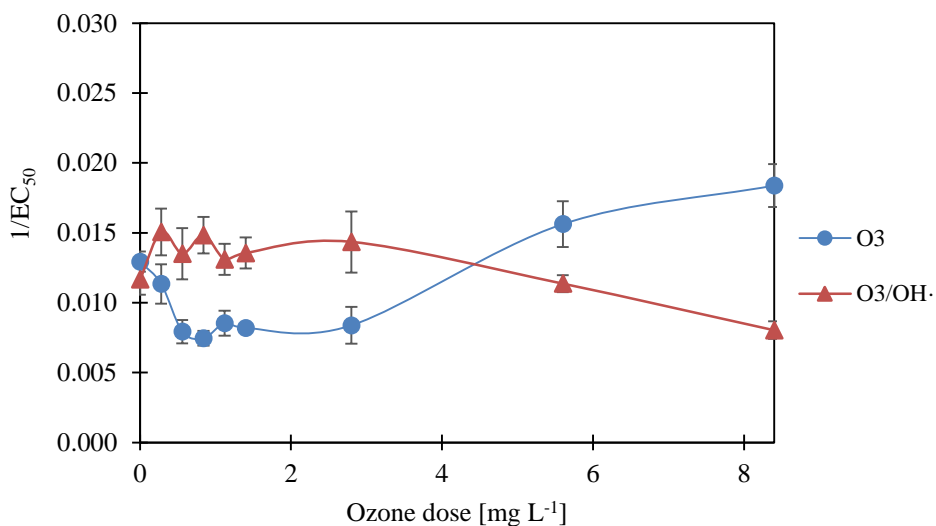
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### 338 3.4. Toxicity evolution during DDVP ozonation

339

340 Changes in the chemical species involved in DDVP ozonation process could imply  
341 alterations in the properties of the reaction medium. Among these properties, toxicity is  
342 of special interest since DDVP and formed TPs could affect target (insects) but also non-  
343 target organisms. The evolution of  $1/EC_{50}$  as a function of the ozone dose is presented in  
344 Fig. 5, for samples corresponding to degradation experiments with and without the  
345 presence of a radical scavenger. It has to be remembered that higher  $1/EC_{50}$  values mean  
346 higher inhibition of bacteria luminescence (i.e., higher toxicity), and vice versa. Initial  
347  $EC_{50}$  value, that is, the sample concentration that causes mortality in the 50% of the  
348 bacteria population after 15 min contact was determined to be  $3.8 \text{ mg L}^{-1}$  ( $EC_{50}$  about  
349 80% in terms of sample dilution). For the rest of samples, only  $EC_{50}$  expressed as sample  
350 dilution could be obtained since the medium compositions were unknown.

351



352

353 Figure 5. Acute toxicity of the reaction medium during DDVP ozonation, in experiments with ( $O_3$ ) and  
354 without ( $O_3/OH\cdot$ ) the presence of *tert*-butanol. Conditions:  $[DDVP]_0 = 20 \mu\text{M}$ , pH 7, temperature =  $20 \pm 2$   
355 °C.

356

357 The toxicity during experiments with the addition of *tert*-butanol initially decreased to a  
358 minimum, being this caused by the reduction of DDVP concentration in the reaction

359 solution. For low ozone doses, the amount of formed TPs is low and thus the contribution  
360 of these intermediates and the synergies between them to the medium toxicity are not  
361 expected to be significant. After this first drop, toxicity started to increase for oxidant  
362 doses above 3 mg L<sup>-1</sup>. The final *EC*<sub>50</sub> value (51.3%), higher than the initial one (86.2%)  
363 indicates a global growth in the toxicity of the medium that could be caused by synergistic  
364 effects between residual DDVP and newly formed species like DDVP-125 (DMP) or  
365 phosgene, a well-known toxic agent. The latter, although not detected during LC-MS  
366 analyses, was expected to be generated according to the proposed degradation pathways  
367 (see Fig. 4). However, this compound rapidly hydrolyzes [47]. Perhaps other toxic, non-  
368 detected species formed during DDVP ozonation process (i.e. aldehydes or H<sub>2</sub>O<sub>2</sub> formed  
369 through ozone decomposition [35,48] could be present and contribute to enhance the  
370 medium toxicity. Also, dimethyl phosphate, the TP generated through direct molecular  
371 ozone attack, presents a saturated, aliphatic molecular structure. Therefore, the reactivity  
372 of this byproduct with molecular ozone is expected to be low [49,50], and this would  
373 cause its accumulation in the system thus increasing the residual toxicity of the medium.  
374 In the case of experiments without the presence of *tert*-butanol, toxicity slightly increased  
375 for low oxidant dosages (0-0.3 mg L<sup>-1</sup>), remained without major variations for doses up  
376 to 3 mg L<sup>-1</sup> and then started to decrease, reaching 1/*EC*<sub>50</sub> values below the initial one. The  
377 initial rise of this parameter, although not very significant, was probably due to synergistic  
378 effects between remaining DDVP and early-formed TPs like DDVP-207, which as before  
379 commented, could maintain a similar toxicity than the parent compound. For doses above  
380 3 mg L<sup>-1</sup>, DDVP was no longer present in the reaction medium (see Fig. 3) and synergistic  
381 effects apparently disappeared. From this point, therefore, further OH<sup>·</sup>-mediated  
382 oxidation of the TPs that were present in the reaction system allowed the final toxicity  
383 abatement of the solution.

384

385 In the view of the Microtox<sup>®</sup> results, it can be stated that O<sub>3</sub> oxidation alone contributed  
386 to increase the toxicity of the medium. The low reactivity of formed TP (i.e., DMP) with  
387 this oxidant probably caused the accumulation of this chemical species in the reaction  
388 medium, thus inducing the observed increase in toxicity. However, hydroxyl radicals  
389 formed through ozone decomposition allowed to solve this problem since this transient  
390 species were able to remove both DDVP and TPs. Therefore, it is concluded that  
391 ozonation process could be a suitable treatment alternative for DDVP removal and

392 associated toxicity abatement, provided that the indirect degradative route (i.e., OH·-  
393 mediated oxidation) is properly promoted.

394

#### 395 **4. Conclusions**

396

397 The obtained results allow drawing some conclusions regarding the degradation of  
398 dichlorvos by means of ozonation. The moderate and high values for second-order rate  
399 constants of DDVP reactions with O<sub>3</sub> and OH·, respectively, point out the treatability by  
400 ozone-based processes of water matrices containing traces of this priority pesticide. Both  
401 ozone and hydroxyl radicals can play an important role in DDVP abatement, although  
402 studies dealing with real waters and lower concentrations of the contaminant are required  
403 in order to properly assess the process performance. Proposed reaction mechanisms  
404 indicate the formation of dimethyl phosphate as a common TP for both O<sub>3</sub> and OH·  
405 degradation routes. This intermediate is expected to present low reactivity towards ozone,  
406 even though can be degraded by hydroxyl radical-mediated oxidation. The acute toxicity  
407 analyses in experiments with OH· scavenger demonstrates that a rise in the bacteria  
408 luminescence inhibition occur for increasing oxidant dosages. However, results of  
409 experiments without *tert*-butanol show that formed hydroxyl radicals are able to abate the  
410 toxicity of the reaction medium. It is clear, therefore, that the contribution of the OH·  
411 degradation route during ozonation process is required in order to remove DDVP, formed  
412 TPs and reduce the associated toxicity. Strategies aimed to enhance this indirect via need  
413 to be assessed to improve the process efficiency.

414

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416

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421

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