

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 O₃ and OH· were determined to be 590 and
18 2.2·10⁹ M⁻¹s⁻¹, respectively. These values partly explained the degradation profiles
19 obtained during experiments with and without the presence of an OH· 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 O₃ and OH·-
24 mediated oxidation pathways. Three possible degradation routes were proposed for OH·
25 degradation, whereas the direct oxidation by O₃ was only well-explained by the addition
26 of this oxidant to the double bond of DDVP dichlorovinyl moiety. Microtox[®] bioassays
27 revealed the inability of molecular ozone to reduce the toxicity of the medium and pointed
28 out the importance of OH· 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⁻¹ [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 (O₃) 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). NaH₂PO₄, Na₂HPO₄, H₃PO₄, *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⁻¹. 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 H₃PO₄/Na₂HPO₄ 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 μ M of DDVP and
141 20 μ 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 μ 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 μ M of DDVP, 25 mM of *tert*-butanol
160 and adequate quantities of the pH 7 phosphate buffer. Different doses (from 5 to 175 μ 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 H₃PO₄. The flow rate was set to 1 mL min⁻¹ 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[®] 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*_{50,15min}, 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[®] 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 O₃ and both DDVP and MPL.

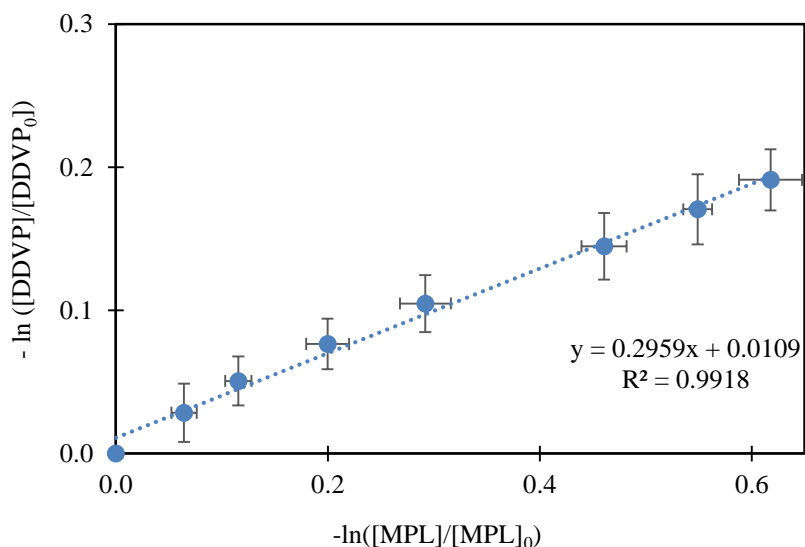
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$$-ln\left(\frac{[DDVP]}{[DDVP]_0}\right) = \frac{k_{DDVP,O_3}}{k_{MPL,O_3}} \left(-ln\left(\frac{[MPL]}{[MPL]_0}\right)\right) \quad (1)$$

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 O₃ 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 O₃ 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

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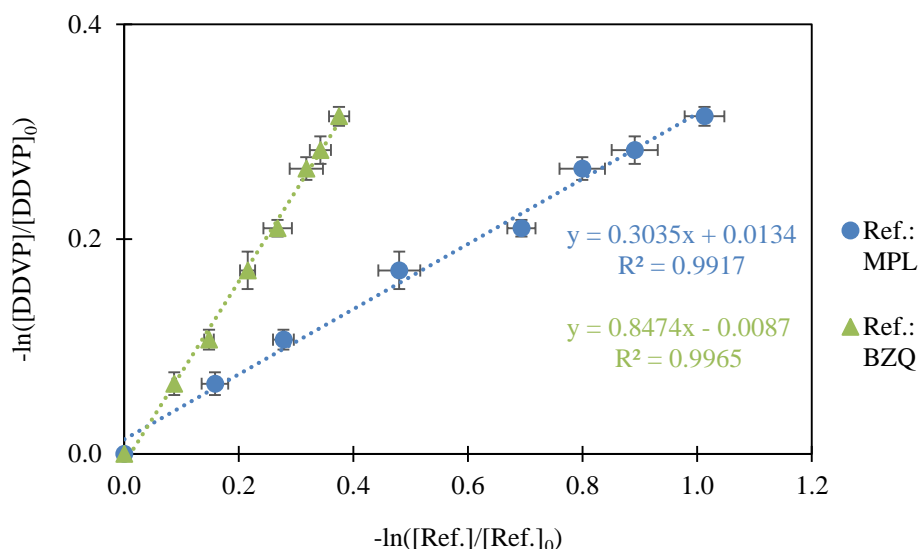
Table 1. Reactions considered during competition experiments for $k_{DDVP,OH}$ determination.

Reaction	2 nd -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].

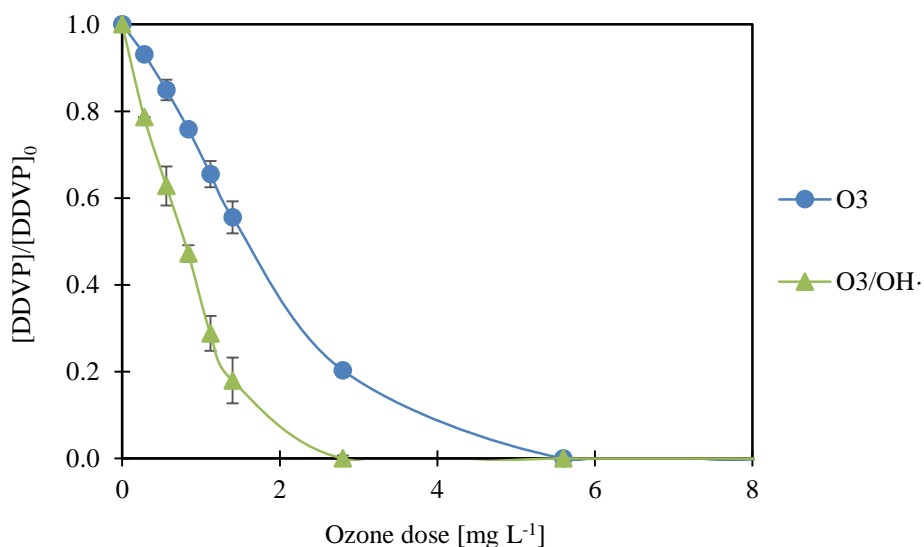
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253 3.2. DDVP degradation by ozone and hydroxyl radicals

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 (O₃) and without
 262 (O₃/OH·) the presence of *tert*-butanol (25 mM). Conditions: [DDVP]₀ = 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 O₃: an ozone dose of approximately 2.8 mg L⁻¹ was required for
 267 the complete removal of DDVP, while the double of this dose (5.5 mg L⁻¹) 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

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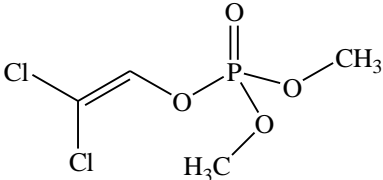
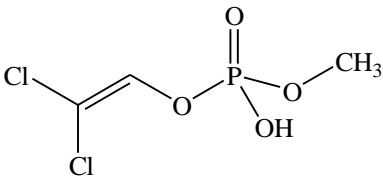
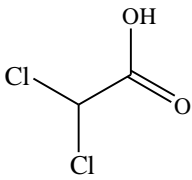
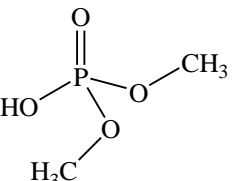
285 Relevant TPs generated during DDVP ozonation with and without radical scavenger
 286 addition were identified by means of LC-MS, on the basis of the detected masses. The
 287 proposed molecular structures are shown in Table 2.

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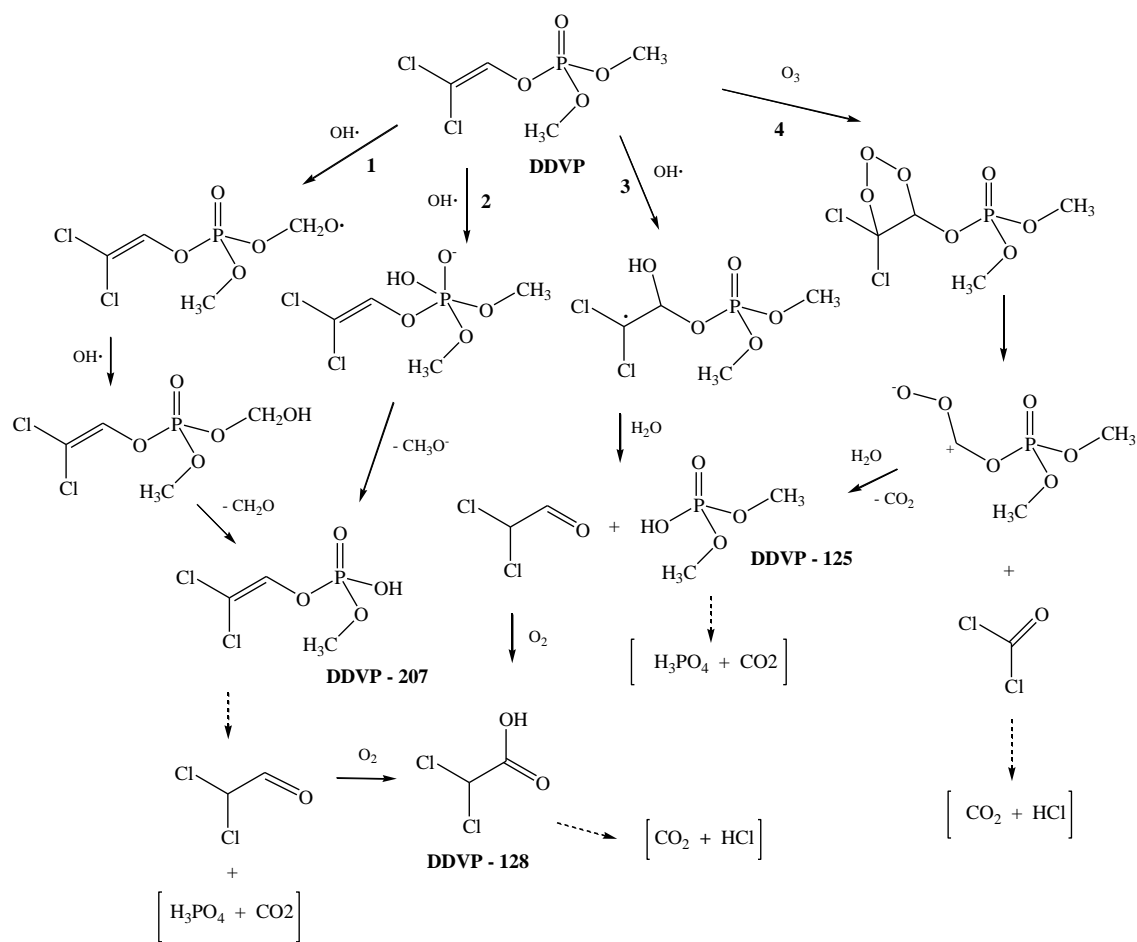
Table 2. DDVP, detected TPs and their corresponding molecular structures.

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 (CH₃O-) 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 O₃ (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 CO₂ 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.

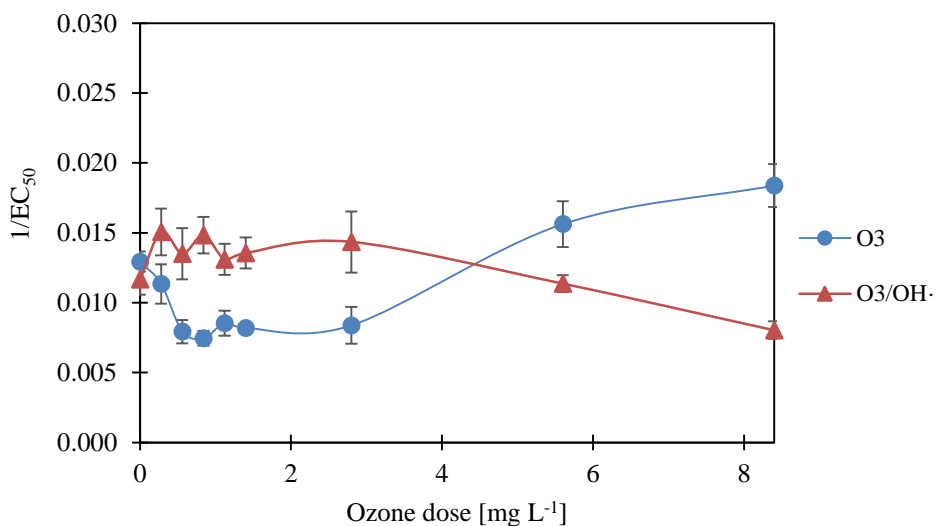
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338 3.4. Toxicity evolution during DDVP ozonation

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 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 ± 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⁻¹. The final *EC*₅₀ 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 H₂O₂ 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⁻¹), remained without major variations for doses up
376 to 3 mg L⁻¹ and then started to decrease, reaching 1/*EC*₅₀ 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⁻¹, DDVP was no longer present in the reaction medium (see Fig. 3) and synergistic
381 effects apparently disappeared. From this point, therefore, further OH[·]-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[®] results, it can be stated that O₃ 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 O₃ 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 O₃ 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

415 **Acknowledgements**

416

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

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422 **References**

423

424 [1]A.R. Ribeiro, O.C. Nunes, M.F.R. Pereira, A.M.T. Silva, An overview on the advanced
425 oxidation processes applied for the treatment of water pollutants defined in the

426 recently launched Directive 2013/39/EU, *Environ. Int.* 75 (2015) 33–51.
427 doi:10.1016/j.envint.2014.10.027.

428 [2]The European Parliament and the Council of the European Union, Directive of 12
429 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards
430 priority substances in the field of water policy, Official Journal of the European
431 Union, 2013. [http://eur-lex.europa.eu/legal-](http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex:32013L0039)
432 [content/EN/TXT/?uri=celex:32013L0039](http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex:32013L0039).

433 [3]K.-H. Kim, E. Kabir, S.A. Jahan, Exposure to pesticides and the associated human
434 health effects, *Sci. Total Environ.* 575 (2017) 525–535.
435 doi:10.1016/j.scitotenv.2016.09.009.

436 [4]H. Kaur, H. Garg, Pesticides: Environmental Impacts and Management Strategies, in:
437 *Environ. Heal. Risk - Hazard. Factors to Living Species*, InTech, 2014: pp. 187–
438 203. doi:10.5772/57399.

439 [5]L.M.L. Nollet, H.S. Rathore, Fate of Pesticides and Transfer Processes, in: *Handb.*
440 *Pestic. Methods Pestic. Residues Anal.*, CRC Press/Taylor & Francis, 2010: p. 54.

441 [6]J. Campo, A. Masiá, C. Blasco, Y. Picó, Occurrence and removal efficiency of
442 pesticides in sewage treatment plants of four Mediterranean River Basins, *J.*
443 *Hazard. Mater.* 263 (2013) 146–157. doi:10.1016/j.jhazmat.2013.09.061.

444 [7]P.N. Patil, P.R. Gogate, Degradation of dichlorvos using hybrid advanced oxidation
445 processes based on ultrasound, *J. Water Process Eng.* 8 (2015) e58–e65.
446 doi:10.1016/j.jwpe.2014.10.012.

447 [8]T. Oncescu, M.I. Stefan, P. Oancea, Photocatalytic degradation of dichlorvos in
448 aqueous TiO₂ suspensions, *Environ. Sci. Pollut. Res.* 17 (2010) 1158–1166.
449 doi:10.1007/s11356-009-0292-4.

450 [9]K. V. Ragnarsdottir, Environmental fate and toxicology of organophosphate
451 pesticides, *J. Geol. Soc. London.* 157 (2000) 859–876. doi:10.1144/jgs.157.4.859.

452 [10] M.P. Montgomery, F. Kamel, T.M. Saldana, M.C.R. Alavanja, D.P. Sandler,
453 Incident diabetes and pesticide exposure among licensed pesticide applicators:
454 Agricultural Health Study, 1993–2003, *Am. J. Epidemiol.* 167 (2008) 1235–1246.
455 doi:10.1093/aje/kwn028.

456 [11] P. Wang, H.P. Wang, M.Y. Xu, Y.J. Liang, Y.J. Sun, L. Yang, L. Li, W. Li, Y.J.
457 Wu, Combined subchronic toxicity of dichlorvos with malathion or pirimicarb in
458 mice liver and serum: A metabonomic study, *Food Chem. Toxicol.* 70 (2014) 222–
459 230. doi:10.1016/j.fct.2014.05.027.

- 460 [12] S.N. Desai, P. V. Desai, Changes in renal clearance and renal tubular function in
461 albino mice under the influence of Dichlorvos, *Pestic. Biochem. Physiol.* 91 (2008)
462 160–169. doi:10.1016/j.pestbp.2008.03.008.
- 463 [13] International Agency for Research on Cancer, IARC monographs on the evaluation
464 of carcinogenic risks to humans, Volume 53: occupational exposures in insecticide
465 application, and some pesticides, 1991.
- 466 [14] S. Das, A review of Dichlorvos Toxicity in Fish, *Curr. World Environ.* 8 (2013)
467 143–149. doi:http://dx.doi.org/10.12944/CWE.8.1.08.
- 468 [15] World Health Organization, The Who Recommended Classification of Pesticides
469 By Hazard and Guidelines To Classification: 2009, World Health Organization,
470 2010. doi:ISBN 978 92 4 154796 3.
- 471 [16] The European Commission, Decision 2012/254/EU concerning the non-inclusion of
472 dichlorvos for product type 18 in Annex I, IA or IB to Directive 98/8/EC of the
473 European Parliament and of the Council concerning the placing of biocidal
474 products on the market, *Official Journal of the European Union*, 2012. [http://eur-](http://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A22013D0066)
475 [lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A22013D0066](http://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A22013D0066).
- 476 [17] C.T. Miller, Controversial Insecticide Allowed to Stay on Market, *Los Angeles*
477 *Times.* (2017). <http://www.latimes.com/la-me-pesticide17may17-story.html>
478 (accessed May 31, 2017).
- 479 [18] Environmental Protection Authority, Application for the Reassessment of a Group
480 of Hazardous Substances under Section 63 of the Hazardous Substances and New
481 Organisms Act 1996. APP202097: Dichlorvos and its formulations, 2015.
482 www.epa.govt.nz/search-databases/HSNO Application Register
483 Documents/APP202097_Dichlorvos_Decision_FINAL.pdf.
- 484 [19] S. Gomez, C.L. Marchena, M.S. Renzini, L. Pizzio, L. Pierella, In situ generated
485 TiO₂ over zeolitic supports as reusable photocatalysts for the degradation of
486 dichlorvos, *Appl. Catal. B Environ.* 162 (2015) 167–173.
487 doi:10.1016/j.apcatb.2014.06.047.
- 488 [20] P. Nan, S. Yan, L. Li, J. Chen, Q. Du, Z. Chang, Toxicity effect of dichlorvos on
489 loach (*Misgurnus anguillicaudatus*) assessed by micronucleus test, hepatase
490 activity analysis and comet assay., *Toxicol. Ind. Health.* 31 (2013) 566–575.
491 doi:10.1177/0748233713475512.
- 492 [21] H. Heidar, N. Seyed Taghi Omid, Z. Abbasali, Monitoring Organophosphorous
493 Pesticides Residues in the Shahid Rajaei Dam Reservoir, Sari, Iran, *Bull. Environ.*

- 494 Contam. Toxicol. 98 (2017) 791–797. doi:10.1007/s00128-017-2080-z.
- 495 [22] J. Gao, L. Liu, X. Liu, H. Zhou, J. Lu, S. Huang, Z. Wang, The Occurrence and
496 Spatial Distribution of Organophosphorous Pesticides in Chinese Surface Water,
497 Bull. Environ. Contam. Toxicol. 82 (2009) 223–229. doi:10.1007/s00128-008-
498 9618-z.
- 499 [23] T. Nasrabadi, G. Nabi Bidhendi, A. Karbassi, P. Grathwohl, N. Mehrdadi, Impact
500 of major organophosphate pesticides used in agriculture to surface water and
501 sediment quality (Southern Caspian Sea basin, Haraz River), Environ. Earth Sci.
502 63 (2011) 873–883. doi:10.1007/s12665-010-0757-2.
- 503 [24] W. Sangchan, M. Bannwarth, J. Ingwersen, C. Hugenschmidt, K. Schwadorf, P.
504 Thavornnyutikarn, K. Pansombat, T. Streck, Monitoring and risk assessment of
505 pesticides in a tropical river of an agricultural watershed in northern Thailand,
506 (n.d.). doi:10.1007/s10661-013-3440-8.
- 507 [25] H. Chen, J. Zhu, Z. Li, A. Chen, Q. Zhang, The occurrence and risk assessment of
508 five organophosphorus pesticides in river water from Shangyu, China, Environ.
509 Monit. Assess. 188 (2016) 614. doi:10.1007/s10661-016-5612-9.
- 510 [26] M.A. Rahman, M. Muneer, Photocatalysed degradation of two selected pesticide
511 derivatives, dichlorvos and phosphamidon, in aqueous suspensions of titanium
512 dioxide, Desalination. 181 (2005) 161–172. doi:10.1016/j.desal.2005.02.019.
- 513 [27] E. Evgenidou, K. Fytianos, I. Poulios, Semiconductor-sensitized photodegradation
514 of dichlorvos in water using TiO₂ and ZnO as catalysts, Appl. Catal. B Environ.
515 59 (2005) 81–89. doi:10.1016/j.apcatb.2005.01.005.
- 516 [28] E. Evgenidou, I. Konstantinou, K. Fytianos, T. Albanis, Study of the removal of
517 dichlorvos and dimethoate in a titanium dioxide mediated photocatalytic process
518 through the examination of intermediates and the reaction mechanism, J. Hazard.
519 Mater. 137 (2006) 1056–1064. doi:10.1016/j.jhazmat.2006.03.042.
- 520 [29] M.-C. Lu, J.-N. Chen, C.-P. Chang, Oxidation of dichlorvos with hydrogen
521 peroxide using ferrous ion as catalyst, J. Hazard. Mater. 65 (1999) 277–288.
522 doi:10.1016/S0304-3894(98)00268-4.
- 523 [30] P.N. Patil, P.R. Gogate, Degradation of dichlorvos using hybrid advanced
524 oxidation processes based on ultrasound, J. Water Process Eng. 8 (2015) e58–e65.
525 doi:10.1016/j.jwpe.2014.10.012.
- 526 [31] M.M. Huber, S. Canonica, G.Y. Park, U. Von Gunten, Oxidation of
527 pharmaceuticals during ozonation and advanced oxidation processes, Environ. Sci.

Technol. 37 (2003) 1016–1024. doi:10.1021/es025896h.

529 [32] X. Jin, S. Peldszus, P.M. Huck, Reaction kinetics of selected micropollutants in
530 ozonation and advanced oxidation processes, *Water Res.* 46 (2012) 6519–6530.
531 doi:10.1016/j.watres.2012.09.026.

532 [33] Effect of operational and water quality parameters on conventional ozonation and
533 the advanced oxidation process O₃/H₂O₂: Kinetics of micropollutant abatement,
534 transformation product and bromate formation in a surface water, *Water Res.* 122
535 (2017) 234–245. doi:10.1016/J.WATRES.2017.05.018.

536 [34] Application of ozonation for pharmaceuticals and personal care products removal
537 from water, *Sci. Total Environ.* 586 (2017) 265–283.
538 doi:10.1016/J.SCITOTENV.2017.01.216.

539 [35] C. von Sonntag, U. von Gunten, *Chemistry of Ozone in Water and Wastewater*
540 *Treatment: From Basic Principles to Applications*, IWA Publishing, 2012.

541 [36] A. Cruz-Alcalde, C. Sans, S. Esplugas, Exploring ozonation as treatment
542 alternative for methiocarb and formed transformation products abatement,
543 *Chemosphere.* (2017). doi:10.1016/j.chemosphere.2017.08.040.

544 [37] A. Cruz-Alcalde, C. Sans, S. Esplugas, Priority pesticides abatement by advanced
545 water technologies: The case of acetamiprid removal by ozonation, *Sci. Total*
546 *Environ.* 599–600 (2017) 1454–1461. doi:10.1016/j.scitotenv.2017.05.065.

547 [38] J. Hoigné, H. Bader, Rate constants of reactions of ozone with organic and
548 inorganic compounds in water—II, *Water Res.* 17 (1983) 185–194.
549 doi:10.1016/0043-1354(83)90099-4.

550 [39] J. Benner, E. Salhi, T. Ternes, U. von Gunten, Ozonation of reverse osmosis
551 concentrate: Kinetics and efficiency of beta blocker oxidation, *Water Res.* 42
552 (2008) 3003–3012. doi:10.1016/j.watres.2008.04.002.

553 [40] D. Gerrity, S. Gamage, D. Jones, G. V. Korshin, Y. Lee, A. Pisarenko, R.A.
554 Trenholm, U. von Gunten, E.C. Wert, S.A. Snyder, Development of surrogate
555 correlation models to predict trace organic contaminant oxidation and microbial
556 inactivation during ozonation, *Water Res.* 46 (2012) 6257–6272.
557 doi:10.1016/j.watres.2012.08.037.

558 [41] Y. Lee, D. Gerrity, M. Lee, A.E. Bogeat, E. Salhi, S. Gamage, R.A. Trenholm,
559 E.C. Wert, S.A. Snyder, U. Von Gunten, Prediction of micropollutant elimination
560 during ozonation of municipal wastewater effluents: Use of kinetic and water
561 specific information, *Environ. Sci. Technol.* 47 (2013) 5872–5881.

562 doi:10.1021/es400781r.

563 [42] N.K. Vel Leitner, B. Roshani, Kinetic of benzotriazole oxidation by ozone and
564 hydroxyl radical, *Water Res.* 44 (2010) 2058–2066.
565 doi:10.1016/j.watres.2009.12.018.

566 [43] E. Mvula, C. von Sonntag, Ozonolysis of phenols in aqueous solution., *Org.*
567 *Biomol. Chem.* 1 (2003) 1749–1756. doi:10.1039/b301824p.

568 [44] G. V Buxton, C.L. Greenstock, W.P. Helman, A.B. Ross, Critical Review of rate
569 constants for reactions of hydrated electrons, hydrogen atoms and hydroxyl
570 radicals in aqueous solution, *J. Phys. Chem. Ref. Data.* 17 (1988) 513.
571 doi:10.1063/1.555805.

572 [45] S. Gligorovski, R. Strekowski, S. Barbati, D. Vione, Environmental Implications
573 of Hydroxyl Radicals ($\cdot\text{OH}$), *Chem. Rev.* 115 (2015) 13051–13092.
574 doi:10.1021/cr500310b.

575 [46] M.S. Elovitz, U. Von Gunten, Hydroxyl Radical/Ozone Ratios During Ozonation
576 Processes. I. The Rct Concept, *Ozone Sci. Eng.* 21 (1999) 239–260.
577 doi:10.1080/01919519908547239.

578 [47] P. Dowideit, C. von Sonntag, Reaction of Ozone with Ethene and Its Methyl-and
579 Chlorine-Substituted Derivatives in Aqueous Solution, *Environ. Sci. Technol.* 32
580 (1998) 1112.

581 [48] J. Hoigné, *Handbook of Ozone Technology and Applications*, Ann Arbor Science
582 Publishers, 1982. doi:0250405776.

583 [49] F.J. Beltran, *Ozone Reaction Kinetics for Water and Wastewater Systems*, Lewis
584 Publishers, 2003.

585 [50] J.P. Pocostales, M.M. Sein, W. Knolle, C. Von Sonntag, T.C. Schmidt,
586 Degradation of ozone-refractory organic phosphates in wastewater by ozone and
587 ozone/hydrogen peroxide (peroxone): The role of ozone consumption by dissolved
588 organic matter, *Environ. Sci. Technol.* 44 (2010) 8248–8253.
589 doi:10.1021/es1018288.

590