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1 **Impact of EfOM in the Elimination of PPCPs by UV/chlorine:**

2 **Radical chemistry and toxicity bioassays**

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16 **Abstract:** The UV/chlorine process as a potential tertiary municipal wastewater  
17 treatment alternative for removing refractory PPCPs has been widely investigated.  
18 However, the role of effluent organic matter (EfOM) on the radical chemistry and  
19 toxicity alteration is unclear. The elimination of two model PPCPs, primidone (PRM)  
20 and caffeine (CAF), by the co-exposure of UV and free chlorine was investigated to  
21 elucidate the impact of EfOM. Experimental results indicated that both  $\cdot\text{OH}$  and  
22 reactive chlorine species (RCS) were importantly involved in the decay of PRM at  
23 acidic condition, while  $\text{ClO}\cdot$  played dominant role at alkaline pH. The decay of CAF  
24 was dominated by  $\text{ClO}\cdot$  under all conditions. Chlorine dose, initial contaminant  
25 concentration, solution pH, and water matrix affect the process efficiency at varying  
26 degree resulting from their specific effect on the radical speciation in the system.

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27 Presence of EfOM isolate remarkably inhibited the decay of PRM and CAF by  
28 preferentially scavenging RCS and particularly ClO<sup>•</sup>. Good correlations (linear for  
29 PRM and exponential for CAF) between UV absorbance at 254 nm and the observed  
30 pseudo first-order rate constants ( $k'_{\text{obs}}$ ) for all EfOM solutions were obtained,  
31 demonstrating the importance of aromatic moieties in inhibiting the degradation of  
32 targeted contaminants by UV/chlorine process. Degradation of PRM/CAF in  
33 reconstituted effluent spiked with the major effluent constituents (i.e., EfOM isolates,  
34 Cl<sup>-</sup>, HCO<sub>3</sub><sup>-</sup>, and NO<sub>3</sub><sup>-</sup>) was comparable to the results obtained with the real WWTP  
35 effluent and fit well to the correlation between  $k'_{\text{obs}}$  and UV absorbance at 254 nm,  
36 suggesting that EfOM isolates can be used to determine the efficiency of UV/chlorine  
37 process in real effluent. EfOM serves as the main precursors of adsorbable organic  
38 chlorine in the UV/chlorine treatment. Bioassays indicated that chlorine-containing  
39 compounds could induce oxidative stress, mitochondrial dysfunction, and increase the  
40 cell DNA damage. Among evaluated treatment conditions, the nature of EfOM,  
41 hydrophobic versus transphilic fraction, is likely the predominant factor affecting the  
42 cytotoxicity. Meanwhile the UV/chlorine treatment can significantly reduce the  
43 cytotoxicity of the EfOM isolates. However, adding high level of selected  
44 contaminants (e.g., PRM and CAF) can inhibit this phenomenon due to competition  
45 with reactive radicals.

46 **Key words:** Effluent organic matter, Primidone, Caffeine, Reactive chlorine species,  
47 Adsorbable organic chlorine

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## 51 **1. Introduction**

52 The increasing occurrence of pharmaceutical and personal care products (PPCPs)  
53 in environmental matrix has raised great public concerns. These complex  
54 compounds are frequently detected at  $\text{ng L}^{-1}$  to  $\mu\text{g L}^{-1}$  concentration levels in varied  
55 aquatic environments (Yang et al., 2017). The presence of PPCPs induces potential  
56 threat to aquatic organisms and human health (Hamid et al., 2021). Many PPCPs  
57 cannot be effectively eliminated by conventional wastewater treatment processes,  
58 leading to their continuous discharge into the environment (Yang et al., 2017). It is of  
59 great importance to develop and incorporate advanced techniques to efficiently  
60 remove PPCPs from WWTPs effluent before their discharge.

61 Recently, the UV/chlorine process has emerged as a promising alternative for  
62 PPCP removal. UV irradiation of free chlorine (FC, HOCl/OCl<sup>-</sup>) can generate  
63 non-selective hydroxyl radical (<sup>•</sup>OH) and selective reactive chlorine species (RCS,  
64 e.g., Cl<sup>•</sup>, Cl<sub>2</sub><sup>•-</sup>, and ClO<sup>•</sup>), leading to the elimination of PPCPs resistant to both UV  
65 photolysis and chlorination (Guo et al., 2017). Compared to H<sub>2</sub>O<sub>2</sub> and persulfate,  
66 chlorine species have relative higher molar absorbance coefficients (i.e., HClO: 59  
67 M<sup>-1</sup> cm<sup>-1</sup>; ClO<sup>-</sup>: 66 M<sup>-1</sup> cm<sup>-1</sup>) and higher quantum yields ( $>1.0 \text{ M E}^{-1} \text{ s}^{-1}$ ) at UV<sub>254</sub>  
68 wavelength (Feng et al., 2007; Watts and Linden, 2007), leading to a higher radical  
69 production. Particularly, the simultaneous generation of diverse and more or less  
70 selective radical species with high redox potentials ( $E_0 = 1.8\text{-}2.7, 2.4, \text{ and } 2.0 \text{ V}$  for  
71 <sup>•</sup>OH, Cl<sup>•</sup>, and Cl<sub>2</sub><sup>•-</sup>, respectively (Buxton et al., 1988; Beitz et al., 1998) provides the

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72 UV/chlorine process complementary reactivities toward a broad spectrum of  
73 molecular structures.  $\text{Cl}^\bullet$  preferentially reacts with organic compounds bearing  
74 electron-rich moieties at comparable or higher reaction rate constants compared to  
75  $^\bullet\text{OH}$ , while  $\text{Cl}_2^\bullet$  exhibits considerable reactivity toward olefinic compounds and  
76 molecules containing phenol, alkoxy benzene, and aniline groups (Lei et al., 2019).  
77 Additionally, recent studies have demonstrated the predominant role of  $\text{ClO}^\bullet$  (1.5-1.8  
78 V) in degrading specific PPCPs, such as trimethoprim, carbamazepine, and caffeine  
79 with high rate constants ranging from  $10^8$ - $10^{10} \text{ M}^{-1} \text{ s}^{-1}$  (Guo et al., 2018).

80 However, effluent organic matter (EfOM) contained in WWTP effluent is of  
81 significant concern for the application of UV/chlorine process in practical conditions.  
82 EfOM is a major component of effluent matrix and occurs at concentration ( $\text{mg L}^{-1}$ )  
83 several orders of magnitude higher than that of micropollutants ( $\text{ng L}^{-1}$ ) (Crittenden et  
84 al., 2005). EfOM is generally recognized as a heterogenous mixture of  
85 non-biodegradable dissolved organic matter (DOM) derived from drinking water,  
86 trace levels of refractory organic micropollutants (e.g., PPCPs), and soluble microbial  
87 products (O'Connor et al., 2019). The presence of EfOM remarkably impacts the  
88 radical chemistry of UV/chlorine process by scavenging radical species, consuming  
89 FC, and inducing UV light attenuation via the inner filter effect. Our recent study  
90 observed that EfOM isolate exerted a much stronger inhibition on the UV/chlorine  
91 process compared with surface water DOM fractions possibly due to its higher  
92 abundance in sulfur-containing functional groups (i.e., sink of  $^\bullet\text{OH}/\text{Cl}^\bullet$  radicals)  
93 (Wang et al., 2020). The scavenging effect of EfOM on  $\text{ClO}^\bullet$  is even more pronounced,

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94 thereby significantly reducing degradation kinetics of PPCPs dominated by  $\text{ClO}^\bullet$   
95 oxidation (Guo et al., 2017). Formation of chlorinated byproducts (e.g., DBPs) and  
96 the associated toxicity is another important issue of concern. EfOM enriched in  
97 nitrogenous moieties serves as a major source of precursors for a wide range of DBPs  
98 (Krasner et al., 2009; Le Roux et al., 2017). Compared to chlorination, the  
99 UV/chlorine process alters the organic matter through synergistic mechanisms of UV  
100 photolysis, chlorination, and reactive radical species.  $\text{Cl}^\bullet/\text{Cl}_2^\bullet$  can induce the  
101 formation of chlorinated byproducts via direct addition to DOM/EfOM, meanwhile  
102 RCS also enhances the yield of high molecular weight nitrogen-containing DBPs  
103 (Bulman and Remucal, 2020). Increased formation of adsorbable organic halogen  
104 (AOX) and cytotoxicity was observed in UV/chlorine treatment of DOM compared  
105 with that of chlorination alone (Wang et al., 2017). Therefore, it is of great importance  
106 to assess the impact of EfOM on the efficacy and toxicity of UV/chlorine process for  
107 PPCPs removal. Past studies mainly focused on the occurrence of regulated or known  
108 DBPs from UV/chlorine treatment of a specific contaminant or DOM fraction and  
109 individual toxicity assay. The vast majority of unidentified chlorinated byproducts as  
110 indicated by analysis of AOX (i.e., a collective parameter of halogenated DBPs) and  
111 their contribution to toxicity remain unclear.

112       Herein, this study aimed to reveal the impact of EfOM on the radical chemistry  
113 and toxicity alteration of UV/chlorine process for PPCPs removal. Primidone (PRM)  
114 and caffeine (CAF) were selected as the model PPCPs due to their wide occurrence in  
115 aquatic environment and well elucidated radical mechanisms in UV/chlorine process

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116 under varying water scenarios which allow the comparison with our study (Sun et al.,  
117 2016; Guo et al., 2018; Wang et al., 2020). Experiments in ultrapure water, real  
118 WWTP effluent, and reconstituted effluent spiked with contaminants were conducted  
119 to elucidate the key factors affecting the treatment efficiency. Adsorbable organic  
120 chlorine (AOCl) analysis in conjunction with multiple *in vitro* bioassays on HepG2  
121 cells were conducted to comprehensively evaluate the formation and cytotoxicity of  
122 chlorinated byproducts under complex EfOM matrix.

## 123 **2. Materials and methods**

### 124 **2.1 Chemicals and materials**

125 All chemicals were of analytical-reagent grade or higher and used as received.  
126 The details of chemical sources and stock solutions are provided in Text S1 in the  
127 Supplementary Information (SI).

128 A secondary wastewater effluent sample was collected from a municipal WWTP  
129 in Xi'an, China and used for the EfOM isolation and real wastewater effluent test. A  
130 XAD-8<sup>®</sup>/XAD-4<sup>®</sup> resin adsorption chromatography protocol applied by Drewes and  
131 Croué (2002) was employed in this study to adsorb and isolate the organic matter  
132 present in the secondary wastewater effluent after reducing pH to 2 and prefiltering  
133 with 0.45 µm. Accordingly, two EfOM fractions were obtained, i.e., the hydrophobic  
134 (HPO) EfOM fraction retained by XAD-8<sup>®</sup> resin and transphilic (TPI) EfOM fraction  
135 adsorbed by the XAD-4<sup>®</sup> resin. Adsorbed organics were eluted from the XAD resins  
136 by the acetonitrile/water desorption method and then lyophilized to get the solid  
137 EfOM fraction (i.e., EfOM isolate).

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## 138 2.2 Experimental procedures

139 Experiments were conducted under magnetic stirring and temperature control (23  
140  $\pm 2$  °C) in a UV apparatus equipped with six 8 W low-pressure UV lamps emitting at  
141 254 nm above the petri dish (inner diameter = 95 mm, volume = 250 mL) containing  
142 reaction solution (Figure S1). The average UV fluence rate entering the solution was  
143 determined as  $1.04 \text{ mW}\cdot\text{cm}^{-2}$  using the iodide/iodate chemical actinometry (Bolton et  
144 al., 2011).

145 For kinetic experiments, a 100 mL phosphate buffer solution (PBS, 5 mM)  
146 containing 5  $\mu\text{M}$  PRM or CAF was exposed to UV irradiation in the presence of 70  
147  $\mu\text{M}$  (i.e.,  $5 \text{ mg L}^{-1}$ ) FC for 10 min. The UV lamp was warmed up at least 30 min prior  
148 to the experiments. Samples (1 mL) were withdrawn at given time intervals and  
149 quenched by slight stoichiometry excess of  $\text{Na}_2\text{S}_2\text{O}_3$  before HPLC analysis.  
150 Experiments with higher concentrations of contaminant (50  $\mu\text{M}$ ) and FC (200  $\mu\text{M}$ )  
151 were conducted to identify the byproducts during PRM and CAF degradation by the  
152 UV/chlorine process and sample preparation is detailed in Text S2.

153 AOC<sub>l</sub> formation and toxicity experiments were conducted with a 200 mL  
154 solution of 5  $\mu\text{M}$  micropollutant and 70  $\mu\text{M}$  FC buffered at pH 7 with 5 mM PBS in  
155 the presence or absence of EfOM isolate ( $5 \text{ mg-C L}^{-1}$ ). After 10 min UV exposure,  
156 residual chlorine was quenched with slight stoichiometry excess of  $\text{Na}_2\text{S}_2\text{O}_3$  which  
157 was selected as quenching agent rather than sodium sulfite to minimize the  
158 interference for subsequent analysis (Croué and Reckhow, 1989; Ina et al., 2014).

159 Dark chlorination experiments in amber bottles with headspace-free were conducted



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160 following the same procedure. For toxicity assay, 4 identical experiments were  
161 conducted in a row, thereby 800 mL sample was obtained and subjected to solid phase  
162 extraction (SPE, see [Text S2](#) for the extraction procedure) using a Bond Elut-PPL  
163 cartridge (1 g, 6 mL, Agilent). The cartridge was then eluted with 10 mL methanol of  
164 LC-MS grade and the eluate was blow-dried with a gentle stream of nitrogen. DMSO of  
165 200  $\mu$ L was added to the eluate as a keeper and served as the final reconstitution solvent  
166 ([Dabrowski, 2016](#)).

### 167 **2.3 Analytical methods**

168 PRM, CAF, and nitrobenzene (NB) were quantified by a Dionex UltiMate 3000  
169 HPLC system. Tentative identification of PRM and CAF transformation byproducts  
170 following UV/chlorine treatment was conducted by high resolution LC-MS (Orbitrap  
171 Q-Exactive, Thermo Scientific, USA) and GC-MS (GC-QTOF, Agilent, USA). The  
172 presence of selected contaminants in the EfOM isolates was quantified by LC-MS/MS  
173 (Shimadzu 8060 LC-MS equipped with an electrospray ionization source). AOCl was  
174 determined by an adsorption/pyrolysis/microcoulometric analytical method using an  
175 Analytik Jena multiX 2500 AOX/TOX analyser equipped with an APU 28 adsorption  
176 module. The detailed HPLC, high resolution LC-MS and GC-MS, LC-MS/MS, and  
177 AOCl analysis methods are provided in SI [Text S3](#). Chlorine concentration was  
178 determined by the DPD method ([Moore et al., 1984](#)). The specific UV absorbance  
179 (SUVA) for EfOM isolate was calculated as the ratio of the UV absorption coefficient  
180 ( $\text{cm}^{-1}$ ) at  $\lambda = 254$  nm to the concentration of total organic carbon in solution (Vario  
181 TOC analyzer, Elementar Analysensysteme GmbH, Germany).

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## 182 2.4 Kinetic modeling simulation

183 A previously developed and validated kinetic model using Kintecus, version  
184 V6.70 (Ianni, 2018) was updated and modified based on current experimental  
185 conditions to evaluate the steady-state concentrations of reactive radicals (Wang et al.,  
186 2020). The rate constants of reactions involved in the UV/chlorine process for the  
187 modelling were provided in Table S1. The validation of the current model was further  
188 verified through the comparison of experimental data vs. model prediction (Table S2)

## 189 2.5 Toxicity bioassays

190 Cell-based assays were selected to evaluate cytotoxicity, induction of cellular  
191 reactive oxygen species, DNA damage, and mitochondrial dysfunction. Selection  
192 criteria is further discussed under section 3.6.

193 **Cell Culture:** HepG2 cells were purchased from the American Type Culture  
194 Collection (ATCC HB-8065); cells were cultured in Dulbecco's Modified Eagle  
195 Medium (DMEM) with 10% fetal bovine serum (FBS) at 37°C and 5% carbon  
196 dioxide atmosphere.

197 **Cytotoxicity, reactive oxygen species (ROS), and DNA damage:** A colorimetric  
198 method (MTS assay; Cell Aqueous, Promega) was used for determining cell viability  
199 (i.e., cytotoxicity). A fluorogenic probe (CellROX Green Reagent, Invitrogen) was  
200 used for oxidative stress detection. For DNA damage detection, a phosphorylation of  
201 histone H2AX (pH2AX) detection kit was used according to manufacturer  
202 instructions (HCS DAN Damage Kit, Invitrogen). In a 96 well plate, 40,000 cells  
203 were seeded into each plate well with 100 µL of phenol red free DMEM/2%FBS and

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204 incubated for 16 hours. Cell culture media was replaced with fresh media and wells  
205 were treated for 24 hours with either SPE extracts or assay controls (n=5) to a final  
206 DMSO concentration of 1% DMSO for cytotoxicity (equivalent to an enrichment  
207 factor of 40X) and 0.5% DMSO for ROS and DNA damage (equivalent to an  
208 enrichment factor of 20X). Details of the three test methods are given in [Text S4](#).  
209 Results are presented as signal fold change (ROS and DNA damage) over negative  
210 control (0.5% DMSO) and % increase (cytotoxicity).

211 **Mitochondrial function:** Oxygen consumption rate (OCR) and extracellular  
212 acidification rate of live cells were measured using Seahorse XFe96 Analyzer  
213 (Agilent Technologies), mitochondrial function measurements were obtained using  
214 the Agilent Seahorse XF Cell Mito Stress Test Kit using different modulators of the  
215 electron transport chain. Basal respiration, ATP-linked respiration, maximal and  
216 reserve capacities, and non-mitochondrial respiration were monitored. Assay  
217 conditions used followed manufacturer instruction, 40,000 cells were used in each  
218 experimental well, modulators concentration selected were oligomycin 1.0  $\mu$ M, FCCP  
219 1.0  $\mu$ M, and rotenone + antimycin A 0.5  $\mu$ M. A seeding time of 24 hours was used.  
220 For acute experiments 8 measurements cycles were used (120 min) and for long term  
221 experiments a 24 hours exposure time was used. An enrichment factor of 20X was  
222 used (maximum of 0.5% DMSO). All experiments were done in five replicates.

### 223 **3. Results and discussion**

#### 224 **3.1 Kinetics of PRM and CAF degradation in buffered ultrapure water**

225 Both PRM and CAF are characterized as resistant to UV irradiation and direct

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226 oxidation by FC or H<sub>2</sub>O<sub>2</sub> (Guo et al., 2018; Miklos et al., 2019). Control experiments  
227 confirmed their negligible role in PRM and CAF degradation (< 7%, Figure 1). In  
228 contrast, significant enhancement in contaminant elimination (84% for PRM and 99%  
229 for CAF in 10 min) was obtained in the UV/chlorine process due to the generation of  
230 reactive radical species, meanwhile UV irradiation led to approximately 43% of FC  
231 consumption in the system. The loss of PRM by UV/chlorine followed pseudo  
232 first-order kinetics (Figure S2). However, the loss of CAF deviated from first-order  
233 kinetics and accelerated with reaction progress as observed by previous studies (Sun  
234 et al., 2016; Cheng et al., 2018). These differences might be associated with the highly  
235 selective characteristic of RCS whose reactivity toward aromatic PPCPs strongly  
236 depends on the nature of substituents on aromatic ring and the core structures (Guo et  
237 al., 2017). The electron-donating 1-methylimidazole moiety in CAF induced its high  
238 reactivity with ClO<sup>•</sup> related reactive species, which was claimed as the reason for its  
239 unique kinetics (Sun et al., 2016). It was reported that ClO<sup>•</sup> played a major role rather  
240 than <sup>•</sup>OH and Cl<sup>•</sup> in degrading CAF, while contribution of <sup>•</sup>OH to the decay of PRM  
241 bearing an electron-withdrawing amide group was more substantial compared with  
242 that of CAF (Guo et al., 2017).

243 The observed pseudo first-order rate constants ( $k'_{\text{obs}}$ ) of PRM and CAF  
244 degradation in the UV/chlorine process (determined from the first 5 min of reaction)  
245 were 0.187 and 0.339 min<sup>-1</sup>, respectively. Our results are several times higher or  
246 comparable compared to those from previous studies (Guo et al., 2017; Wang et al.,  
247 2020), while the difference among the  $k'_{\text{obs}}$  values could be attributed to the use of

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248 different experimental setup and conditions (Table S3). Comparison using an equal  
249 molar dose of FC or H<sub>2</sub>O<sub>2</sub> under UV exposure showed that UV/chlorine exhibited a  
250 much higher removal efficacy for both contaminants. The  $k'_{\text{obs}}$  values for PRM and  
251 CAF were 3.96 and 7.72 times of those obtained from the UV/H<sub>2</sub>O<sub>2</sub> process,  
252 respectively. Higher performance of UV/chlorine in CAF degradation in buffered  
253 ultrapure water, natural water, and wastewater effluent has been reported, owing to the  
254 formation of high ClO<sup>•</sup> content (~3 orders of magnitude higher than that of <sup>•</sup>OH) in  
255 the UV/chlorine process and its preferential attack toward CAF (Yang et al., 2016;  
256 Guo et al., 2018; Wang et al., 2019). Our previous study also observed a better  
257 removal of PRM in the UV/chlorine process under similar experimental conditions at  
258 pH 7, where RCS species dominated ~55% of PRM decay kinetics (Wang et al., 2020).  
259 Conflicting result was obtained for PRM decay by Guo et al. (2018) using a mixture  
260 of 28 PPCPs at 1 μg L<sup>-1</sup> with 2 mM PBS where UV/H<sub>2</sub>O<sub>2</sub> demonstrated a better  
261 performance. This contradiction may be attributed to the different scavenging capacity  
262 of PBS exerted on the kinetics of contaminant degradation (i.e., single contaminant of  
263 mg L<sup>-1</sup> level vs. mixture of μg L<sup>-1</sup> level with a similar PBS).

264 In the UV/chlorine process, FC serves as the precursor and also major scavenger  
265 (including Cl<sup>-</sup> from the FC stock) of <sup>•</sup>OH and Cl<sup>•</sup>, meanwhile secondary ClO<sup>•</sup> and Cl<sub>2</sub><sup>•</sup>  
266 radicals were produced through the scavenging effect (see reactions 38, 40, 41, 65,  
267 and 68 in Table S1). As FC increased from 10 to 35 μM, the modelled steady-state  
268 contents of <sup>•</sup>OH and Cl<sup>•</sup> increased from  $3.3 \times 10^{-14}$  M to  $8.8 \times 10^{-14}$  M and from  $1.6 \times$   
269  $10^{-14}$  M to  $2.4 \times 10^{-14}$  M, and then maintained at  $\sim 1.3 \times 10^{-13}$  M and  $2.6 \times 10^{-14}$  M in

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270 the FC range of 70 - 210  $\mu\text{M}$  (Figure S3), respectively. In contrast, continuous  
271 increase in  $\text{ClO}^\bullet$  ( $10^{-10}$  -  $10^{-9}$  M) and  $\text{Cl}_2^{\bullet-}$  ( $10^{-15}$  -  $10^{-14}$  M) concentration was observed  
272 at FC dose of 70 - 210  $\mu\text{M}$  due to the conversion from  $^\bullet\text{OH}$  and  $\text{Cl}^\bullet$ . Obviously,  
273 variation in FC dose remarkably changed the speciation of radical species, which in  
274 turn induced divergent degradation kinetics of PPCPs based on their reactivity toward  
275 specific radical species. As shown in Figure 2a,  $k'_{\text{obs}}$  of PRM exhibited a first increase  
276 and then reached a plateau similar to those of  $^\bullet\text{OH}$  and  $\text{Cl}^\bullet$  with increasing FC dose,  
277 while  $k'_{\text{obs}}$  of CAF linearly increased by 8.9-fold with the increase of FC from 10 to  
278 210  $\mu\text{M}$ , following a similar evolution profile of  $\text{ClO}^\bullet$  and  $\text{Cl}_2^{\bullet-}$ .

279 Increase in initial contaminant concentration resulted in a decrease in  $k'_{\text{obs}}$  as  
280 shown in Figure 2b, likely attributed to the enhanced demand for reactive radicals at  
281 higher contaminant concentration. However,  $k'_{\text{obs}}$  of PRM just slightly decreased from  
282 0.187 to 0.109  $\text{min}^{-1}$  with the increase of initial concentration from 2 to 50  $\mu\text{M}$ , while  
283  $k'_{\text{obs}}$  of CAF exhibited an exponential decline from 1.022 to 0.041  $\text{min}^{-1}$ . Kinetic  
284 modelling showed a decline in steady-state concentrations of both  $^\bullet\text{OH}$  and RCS  
285 (Figure S4). However,  $\text{ClO}^\bullet$  content was more significantly affected than other radical  
286 species and was reduced by ~3 orders of magnitude with increasing contaminant  
287 concentration from 2 to 50  $\mu\text{M}$ . Consequently, the degradation of CAF dominated by  
288  $\text{ClO}^\bullet$  oxidation was remarkably inhibited at higher initial contaminant concentration.  
289 Besides, high concentration of CAF solution induced a much higher UV absorbance  
290 at 254 nm (0.205 at 1 cm for 50  $\mu\text{M}$  CAF), while it was negligible for PRM (<0.01).  
291 The enhanced inner filter effect at high CAF concentration also contributed to its

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292 sharp decline in decay rate constants.

### 293 **3.2 Elucidation of reactive species during UV/chlorine treatment**

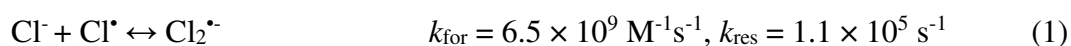
294 [Figure 3](#) depicts the specific  $k'_{\text{obs}}$  of UV light, chlorination,  $\cdot\text{OH}$ , and RCS for  
295 PRM and CAF degradation in the pH range of 6.2-9 based on the loss of NB i.e., in  
296 *situ*  $\cdot\text{OH}$  probe ([Figure S5](#), calculation detailed in SI [Text S5](#)). Contributions of direct  
297 photolysis and chlorination to the decay of PRM/CAF were negligible. The  
298 UV/chlorine treatment demonstrated a considerably higher decay kinetics in CAF  
299 removal across pH 6.2-9 than that of PRM, which was rational considering the  
300 comparable rate constants of CAF with  $\cdot\text{OH}$  but much higher reactivity toward the  
301 main RCS species compared to PRM (rate constants of contaminants with  $\cdot\text{OH}$  and  
302 RCS are provided in [Table S4](#)). Both PRM and CAF exhibited a decreasing trend in  
303  $k'_{\text{obs}}$  with increasing solution pH, consistent with previous literature ([Sun et al., 2016](#);  
304 [Guo et al., 2017](#); [Wang et al., 2020](#)). Given that PRM ( $\text{p}K_{\text{a}}$  of 12.3) and CAF ( $\text{p}K_{\text{a}}$  of  
305 6.1 and 10.4) occurred mainly as one speciation form under the investigated pH range,  
306 the shift in chlorine speciation from HOCl toward  $\text{ClO}^-$  species with lower quantum  
307 yield and higher scavenging effect on  $\cdot\text{OH}$  and  $\text{Cl}\cdot$  was the main reason accounting for  
308 the decreased efficiency at elevated pH ([Watts and Linden, 2007](#); [Fang et al., 2014](#)).  
309 Meanwhile, pH variation exerted a negligible impact on the steady-state concentration  
310 of  $\text{ClO}\cdot$  ([Wu et al., 2017](#)). For PRM, both  $\cdot\text{OH}$  and RCS were importantly involved in  
311 the decay process at pH 6.2, and contribution of  $\cdot\text{OH}$  (i.e., 53%) was even slightly  
312 higher than that of RCS ([Figure S6](#)). However, as the pH increased from 6.2 to 9,  $k'_{\cdot\text{OH}}$   
313 for PRM significantly decreased by 80% whereas  $k'_{\text{RCS}}$  slightly increased from pH 6.2

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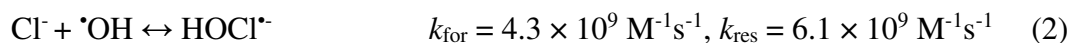
314 to 7 and then kept constant at higher pH, indicating the predominant role of ClO<sup>•</sup> and  
315 negligible role of Cl<sup>•</sup> in alkaline conditions. For CAF, both  $k'_{\text{OH}}$  and  $k'_{\text{RCS}}$  decreased  
316 with increasing pH. RCS dominated the degradation of CAF across pH 6.2-9,  
317 contributing to 72.9% of  $k'_{\text{obs}}$  at pH 6.2 and 86% - 87.8% at neutral to alkaline  
318 conditions (Figure S6). These results confirmed that the degradation of CAF was  
319 primarily attributable to RCS and particularly ClO<sup>•</sup> oxidation.

### 320 3.3 Effects of anions

321 Figure 4 illustrates the impact of Cl<sup>-</sup>, HCO<sub>3</sub><sup>-</sup>, and NO<sub>3</sub><sup>-</sup> on PRM and CAF  
322 degradation kinetics by the UV/chlorine treatment at pH 7. The presence of Cl<sup>-</sup>  
323 induced a negligible inhibition on the  $k'_{\text{obs}}$  of both PPCPs, in agreement with Sun et al.  
324 (2016) and Wang et al. (2020). According to eqs. 1 and 2 (Grebel et al., 2010; Wu et  
325 al., 2016), formation of Cl<sub>2</sub><sup>•-</sup> would be enhanced through the scavenging of Cl<sup>•</sup> by Cl<sup>-</sup>,  
326 while <sup>•</sup>OH was not significantly affected with the addition of Cl<sup>-</sup> due to the rapid  
327 reversible dissociation of HOCl<sup>•-</sup> back into <sup>•</sup>OH. Kinetic modelling demonstrates the  
328 role of Cl<sup>-</sup>, where 3 orders of magnitude increase in Cl<sub>2</sub><sup>•-</sup> concentration, 36%-53%  
329 decrease in Cl<sup>•</sup>, and insignificant variation in <sup>•</sup>OH and ClO<sup>•</sup> were obtained in the  
330 presence of 10 mM Cl<sup>-</sup> compared with the control. Given the above variations of main  
331 radical species in the system and the rate constants of two PPCPs with <sup>•</sup>OH and RCS  
332 (see Table S4), the negligible inhibition on PRM/CAF degradation in the presence of  
333 Cl<sup>-</sup> was probably due to that the increased contributions of Cl<sub>2</sub><sup>•-</sup> compensated for the  
334 decrease of Cl<sup>•</sup>.







335 The ubiquitous  $\text{HCO}_3^-$  presence in aquatic systems serves as important sink of  
 336  $\cdot\text{OH}$ ,  $\text{Cl}^\cdot$ , and  $\text{Cl}_2^\cdot$  through eqs. 3-5 (Fang et al., 2014), resulting in a decrease of these  
 337 radical species as well as significant buildup of selective carbonate radical ( $\text{CO}_3^\cdot$ ).  
 338 Modelling results showed that 10 mM  $\text{HCO}_3^-$  scavenged approximately 57%  $\cdot\text{OH}$ , 89%  
 339  $\text{Cl}^\cdot$ , and 99%  $\text{Cl}_2^\cdot$ , while  $\text{ClO}^\cdot$  remained almost unchanged due to its low reactivity  
 340 with  $\text{HCO}_3^-$  (Table S5). The steady-state concentration of  $\text{CO}_3^\cdot$  was simulated at level  
 341 of  $10^{-9}$  M, several orders of magnitude higher than those of  $\cdot\text{OH}$  and  $\text{Cl}^\cdot$ . The role of  
 342  $\text{CO}_3^\cdot$  in the degradation of micropollutants is compound-specific. Both PRM and  
 343 CAF react with  $\text{CO}_3^\cdot$  at a low second-order rate constants ( $<10^{-6} \text{ M}^{-1}\text{s}^{-1}$ , Text S6 and  
 344 Figure S7) (Wu et al., 2017). Consequently,  $\text{HCO}_3^-$  induced an inhibitory effect on  
 345 PRM and CAF degradation in the UV/chlorine process. As  $\text{HCO}_3^-$  increased from 0 to  
 346 10 mM  $\text{HCO}_3^-$ ,  $k'_{\text{obs}}$  of PRM and CAF decreased from 0.186 to 0.113  $\text{min}^{-1}$ , from  
 347 0.339 to 0.230  $\text{min}^{-1}$ , respectively.



348 In contrast to  $\text{Cl}^-$  and  $\text{HCO}_3^-$ , the presence of  $\text{NO}_3^-$  promoted the loss of PRM and  
 349 CAF. This could be explained by the extra generation of  $\cdot\text{OH}$  and reactive nitrogen  
 350 species (RNS, e.g.,  $\text{NO}_2^\cdot$ ) induced by photolysis of  $\text{NO}_3^-$  (Mack and Bolton, 1999).  
 351 Experiments using NB as a  $\cdot\text{OH}$  probe confirmed the increasing contribution of  $\cdot\text{OH}$   
 352 to the decay kinetics of both PPCPs with the increase of  $\text{NO}_3^-$  (Figure S8) in the

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353 UV/chlorine process. The  $k'_{\text{RCS+RNS}}$  of CAF significantly increased with increasing  
354  $\text{NO}_3^-$ , while that of PRM kept almost constant. Degradation of PRM and CAF in the  
355 UV/ $\text{NO}_3^-$  system (see [Figure S8](#)) showed that  $\cdot\text{OH}$  radical dominated the decay of  
356 contaminants, followed by RNS. Meanwhile  $k'_{\text{RNS}}$  of CAF increased from 0.07 to 0.11  
357  $\text{min}^{-1}$  with the increase of  $\text{NO}_3^-$  from 50 to 100 mM, while  $k'_{\text{RNS}}$  of PRM kept constant.  
358 Nevertheless, experimental results suggest a negligible enhancement from  $\text{NO}_3^-$  under  
359 common WWTP effluent condition where concentration of  $\text{NO}_3^-$  is generally below 1  
360 mM.

### 361 **3.4 Radical chemistry in simulated EfOM-containing water**

362 The degradation of PRM and CAF by UV/chlorine decreased with an increase in  
363 EfOM concentration ([Figure 5a-d](#)). Both EfOM isolates inhibited the decay of CAF to  
364 a substantial higher extent compared with that of PRM. Meanwhile, based on the  
365 same DOC concentration, HPO fraction has a stronger impact on the degradation  
366 kinetics of both contaminants than TPI fraction. The  $k'_{\text{obs}}$  of PRM reduced by 65.0%  
367 and 31.7% in the presence of 10  $\text{mg-C L}^{-1}$  HPO and TPI ([Figure S9](#)), respectively,  
368 whereas that for CAF was 95.7% and 90.1%, respectively. These experimental results  
369 indicate that the radical chemistry in the UV/chlorine system varied in the presence of  
370 different EfOM isolates. The two EfOM isolates bearing different physicochemical  
371 properties likely account for their different influence. The SUVA values of HPO and  
372 TPI were determined as 3.10 and 1.96  $\text{L mg}^{-1} \text{m}^{-1}$ , respectively. HPO with higher  
373 SUVA is more enriched in aromatic moieties than TPI considered as more hydrophilic  
374 in nature with a higher abundance of aliphatic carbon ([Weishaar et al., 2003](#)). The

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375 good correlations (linear for PRM and exponential for CAF) obtained between UV  
376 absorbance at 254 nm and  $k'_{\text{obs}}$  for all HPO and TPI solutions (Figure 6) demonstrate  
377 that the larger the presence of aromatic moieties into the solution the stronger the  
378 inhibition of the degradation of targeted contaminants by the UV/chlorine process.  
379 Figure 6 confirms that the presence of UV absorbing moieties has a stronger effect on  
380 CAF degradation compared to PRM.

381 Figure 5e compares the impact of the two EfOM isolates at 5 mg-C L<sup>-1</sup> on the  
382 contribution of  $k'_{\text{OH}}$  and  $k'_{\text{RCS}}$  for PRM and CAF decay at pH 7. It can be seen that  
383 decay of contaminants due to RCS oxidation was significantly inhibited in  
384 comparison with the moderate decrease in  $k'_{\text{OH}}$ , suggesting that RCS is more  
385 vulnerable to EfOM. Typically,  $k'_{\text{RCS}}$  for CAF dropped by ~94.5%, contributing to  
386 approximately 82.5% of the total loss in  $k'_{\text{obs}}$ . Since the decay of CAF is primarily due  
387 to  $\text{ClO}^\bullet$ , EfOM isolates preferentially scavenging  $\text{ClO}^\bullet$  could be the main reason  
388 causing the sharp decline of  $k'_{\text{RCS}}$  for CAF. The scavenging capacity of DOM isolates  
389 on  $\text{ClO}^\bullet$  shows a strong dependency upon SUVA (Wang et al., 2020). As a result, at  
390 pH 7 where  $\text{ClO}^\bullet$  dominated the decay of PPCPs, HPO fraction induced a higher  
391 degree of inhibition on the UV/chlorine process. The reactivity of DOM (EfOM) with  
392  $\text{Cl}_2^\bullet$  was also found to be positively correlated with aromatic carbon content (i.e.,  
393 SUVA) (Lei et al., 2021). The role of physicochemical properties of EfOM in the  
394 reactivity toward  $^\bullet\text{OH}$  and  $\text{Cl}^\bullet$  is complicated, as some nonaromatic moieties (e.g.,  
395 thiols and sulfonate group) of EfOM in addition to aromatic components exhibit high  
396 vulnerability to  $^\bullet\text{OH}$  or  $\text{Cl}^\bullet$  attack (Westerhoff et al., 2007; Varanasi et al., 2018). No

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397 correlation was found between SUVA and rate constants of DOM with  $\text{Cl}^\bullet$  (Lei et al.,  
398 2021). Studies on the relationship of SUVA with  $\bullet\text{OH}$  rate constants of EfOM (i.e.,  $k_{\bullet\text{OH}}$ ,  
399  $k_{\bullet\text{OH}, \text{EfOM}}$ ) remain inconsistent. Keen et al. (2014) observed some correlation ( $R^2 = 0.75$ )  
400 between SUVA and  $k_{\bullet\text{OH}, \text{EfOM}}$ , while Westerhoff et al. (2007) found no strong  
401 statistical relevant trend.

402 The presence of  $5 \text{ mg-C L}^{-1}$  EfOM resulted in significantly faster consumption of  
403 FC in the UV/chlorine process. As shown in Figure 5f, the chlorine residual was  
404 barely detected in 10 min for HPO and TPI in the UV/chlorine process. In comparison,  
405 photolysis of FC without EfOM only consumed 21% of FC, while dark chlorination  
406 of the two EfOM isolates led to less than 46% decay of FC. HPO with higher SUVA  
407 exhibits a higher chlorine demand, in agreement with previous studies (Westerhoff et  
408 al., 2004; Wang et al., 2020). Besides, SUVA of EfOM causes light absorption during  
409 the UV/chlorine process. The higher the SUVA, the stronger the light attenuation. The  
410 applied UV fluence was reduced by 21.3% and 14.2% in the presence of  $5.0 \text{ mg-C L}^{-1}$   
411 HPO and TPI, respectively. The above effects from EfOM play additional roles in  
412 lowering the steady-state concentration of radicals, therefore reducing the process  
413 efficiency and driving the different impact of EfOM with diverse properties.

### 414 3.5 Degradation in real and reconstituted WWTP effluent

415 The degradation of PRM and CAF in real WWTP effluent and in reconstituted  
416 effluent by adding the major matrix components (i.e., HPO and TPI EfOM isolates  
417 added in representative relative abundance,  $\text{Cl}^-$ ,  $\text{HCO}_3^-$ , and  $\text{NO}_3^-$ ) based on the real  
418 wastewater effluent quality (see Table S6) was investigated. Preliminary experiments

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419 demonstrated an insignificant role of UV-induced photolysis of PRM and CAF in  
420 WWTP effluent (Figure S10). As illustrated in Figure 7a and 7b, the degradation of  
421 PRM and CAF was retarded when UV/chlorine was applied to WWTP effluent,  
422 showing a strong scavenging effect from the wastewater matrix. Compared to the  
423 control,  $k'_{\text{obs}}$  of PRM was reduced by 43.3%. Specifically, the decay kinetics of CAF  
424 was almost completely suppressed (>90%) in WWTP effluent. Additionally, the  
425 relatively high EfOM content (6.07 mg-C L<sup>-1</sup>) in WWTP effluent led to a rapid  
426 depletion (>95%) of 70 μM FC in 4 min (Figure S11) through the synergistic effects  
427 of directly reacting with FC and accelerating its photolysis. As a result, a rapid decay  
428 of PRM in the first 4 min followed by a retardation decay stage was observed.  
429 Increasing FC dose can progressively offset the inhibitory effect by providing more  
430 chlorine residual (Figure S11 and S12). The removals obtained at chlorine dose of 210  
431 μM for PRM and CAF were 79.4% and 75.0%, respectively, while they were 40.4%  
432 and 14.2% at 70 μM FC. Contributions of  $k'_{\text{OH}}$  and  $k'_{\text{RCS}}$  to PRM/CAF decay both  
433 increased with the increase of FC dose (Figure 7c).

434 Results obtained with reconstituted effluent are comparable to the ones obtained  
435 with real WWTP effluent, suggesting that the major influencing factors inhibiting the  
436 PPCPs removal were taken into account. It is important to note that the reconstituted  
437 effluent was prepared by adding similar quantities of HPO and TPI (same amount of  
438 HPO and TPI were isolated from WWTP effluent) to obtain the same UV<sub>254</sub>  
439 absorbance as the real WWTP effluent (i.e., 0.133; 1 cm). The strong inhibitory effect  
440 of the water matrix could be mainly attributed to the relatively high EfOM content

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441 (DOC # 6 mg L<sup>-1</sup>) and alkalinity (3.6 mM), as organics and HCO<sub>3</sub><sup>-</sup> both adversely  
442 lower the process efficiency. The results obtained with the two waters fit well to the  
443 correlation between  $k'_{\text{obs}}$  and UV absorbance at 254 nm established in [Figure 6](#). This  
444 observation provides evidence that the presence of organic matter and more  
445 specifically UV absorbing organic moieties, is likely the predominant factor affecting  
446 the UV/chlorine process in this WWTP effluent. These findings demonstrate that  
447 EfOM isolates can be used to determine the efficiency of the UV/chlorine process in  
448 real effluent. Nevertheless, we speculate that using HPO and TPI EfOM isolates  
449 (which represent around 50% of the DOC and more than 80% of UV absorbance) to  
450 simulate the whole DOC in real effluent may slightly magnify the reactive EfOM  
451 fraction, as the XAD<sup>®</sup> resin protocol cannot effectively extract the hydrophilic or very  
452 polar nonaromatic organics which generally have less reactivity toward free chlorine  
453 and radicals.

454 Compared with WWTP effluent, decay of PRM and CAF was obviously  
455 enhanced in tap water, leading to 66.0% and 75.6% of removal, respectively. It can be  
456 concluded that in waters with low levels of impurities after removing DOC and  
457 alkalinity or even in complex wastewater effluent with sufficient chlorine residual  
458 supply, UV/chlorine is a promising alternative for PPCPs elimination.

### 459 **3.6 Toxicity assessment**

460 Previous studies have drawn inconsistent findings on the associations between  
461 AOX concentration and toxicity. Chlorinated byproducts were reported to correlate  
462 with the variation in the toxicity during UV/chlorine and chlorination treatment of

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463 trimethoprim (Wu et al., 2016). The evolution of chlorine-containing intermediates  
464 showed no relationship with toxicity changes in the degradation of gemfibrozil by  
465 UV/chlorine (Kong et al., 2018). Wang et al. (2017) found that cytotoxicity positively  
466 correlated with AOX formation based on the comparison between UV/chlorine and  
467 chlorination treatment of natural organic matter, but weakly related to AOX  
468 concentrations by comparing among different reaction times. In our study, attempt  
469 was also made to evaluate the AOCl formation in the context of potential toxicity.  
470 Figure 8 presents the formation of AOCl after 10 min of UV/chlorine treatment.  
471 Significant formation of AOCl was observed in the presence of 5 mg-C L<sup>-1</sup> EfOM  
472 isolate, i.e., 7.51 and 5.94 μM from HPO and TPI, respectively. The addition of 5 μM  
473 contaminant induced negligible impact on the yield of AOCl. High resolution LC/MS  
474 and GC/MS analyses of the SPE extracts did not provide any evidence of the presence  
475 of chlorinated by-products for both PRM and CAF under the applied experimental  
476 conditions. Lists of identified transformation intermediates are provided in Table S7  
477 and S8. It should be noted that the applied analytical methods were not appropriate to  
478 analyze low molecular weight chlorinated by-products that could account for the  
479 produced AOCl.

480       Considering the production of intermediates and chlorinated by-products in the  
481 presence of EfOM isolate, evaluation of the risk caused by the UV/chlorine process is  
482 of great significance. Therefore, different toxic effects of treated samples on HepG2  
483 cells were investigated.

484       As shown in Figure 9a, compared to experimental controls (PBS), a significant

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485 increase in the cellular oxidative stress was observed for most experiments containing  
486 HPO. The untreated HPO showed a (4.1±0.4)-fold increase in ROS signal over the  
487 negative control (P<0.0001), which was much higher than the TPI fraction. Higher  
488 toxicity of HPO EfOM than TPI was also reported (Le Roux et al., 2017). Both  
489 UV/Cl<sub>2</sub>+HPO+PRM (1.9±0.2, P<0.005) and UV/Cl<sub>2</sub>+HPO+CAF (2.97 ± 0.6,  
490 P<0.0001) extracts showed a significantly increased ROS production; fold change for  
491 UV/Cl<sub>2</sub>+HPO was not statistically significant. The induction of oxidative stress  
492 response pathway is an indicator of the presence of chemical and non-chemical  
493 stresses in cells, and, for water samples, it is believed that most oxidative stress  
494 response comes from unknown chemicals (Escher et al., 2013). Many DPBs have  
495 been implicated with the increase in adaptive stress responses following water  
496 disinfection, toxicity is also highly dependent on treatment technique and disinfection  
497 mechanism (Lundqvist et al., 2019). In our study, UV/chlorine treatment of the  
498 contaminants did not significantly alter the oxidative stress. On the other hand, the  
499 presence of PRM and CAF seems to hinder the reduction of HPO cellular ROS  
500 production. We believe that these compounds are consuming a significant part of the  
501 radicals produced which will not react with the PPCPs or other unknown chemicals  
502 initially present in the HPO or TPI isolate. The targeted 11 PPCPs (not necessarily  
503 considered as responsible for the toxicity of EfOM isolates) were present in the  
504 untreated EfOM isolates and the UV/chlorine treatment of EfOM isolate generally  
505 degrade these PPCPs to a certain extent (Table S9).

506 As an early and sensitive indicator, ROS plays critical roles in cell survival,



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507 apoptosis, and death (Franklin, 2011). Previous studies showed that the gene  
508 expression pathways of monohaloacetic acids-treated cells would be modulated  
509 through the generation of ROS, causing DNA damage, ATP depletion and  
510 mitochondrial stress (Dad et al., 2013). Oxidative stress is one of the main causes of  
511 genotoxic insults together with exposure to UV irradiation and chemicals. Induction  
512 of pH2AX, a sensitive molecular marker of DNA damage and repair (Mei et al., 2015),  
513 is associated with double-strand breaks (DSBs) and is among the most serious forms  
514 of DNA damage that can lead to cellular death, chromosomal aberrations, mutations,  
515 and cancer (Mah et al., 2010). While exposure to HPO and TPI had no significant  
516 effect on cell DNA damage, as measured by pH2AX induction, treatments with  
517 UV/Cl<sub>2</sub>+HPO (fold change 2.2±0.5, P=0.0003), UV/Cl<sub>2</sub>+HPO+PRM (fold change  
518 3.0±0.7, P<0.0001), UV/Cl<sub>2</sub>+HPO+CAF (fold change 4.1±0.8, P<0.0001), and  
519 UV/Cl<sub>2</sub>+TPI+CAF (fold change 1.9 ± 0.4, P=0.0081) all showed a significant fold  
520 change for pH2AX induction (Figure 9b). Similar effects were also observed for  
521 chlorination only samples, with an increased role of HPO over TPI; a pH2AX  
522 induction of Cl<sub>2</sub>+HPO sample fold change of 2.2±0.3 versus Cl<sub>2</sub>+TPI sample fold  
523 change of 1.7±0.5, and a ROS induction of 2.1±0.3 versus 0.9±0.2, respectively (data  
524 not shown in graph).

525 While oxidative stress results for UV treated samples showed similar patterns, as  
526 those for pH2AX induction assay, the lack of DNA damage for HPO-only sample  
527 suggests that cellular ROS and oxidative stress are not the only factors responsible for  
528 the increase in pH2AX induction. The induction of genotoxic effects by different

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529 mechanisms was demonstrated by different studies on drinking water treatment  
530 processes ([Lundqvist et al., 2019](#)), and strands breaks have been shown to be caused  
531 by halogenated DBPs such as trihalomethanes ([Geter et al., 2004](#)).

532 To confirm the induction of oxidative stress caused by the tested samples,  
533 mitochondrial function assays were employed. ROS levels and mitochondrial function  
534 are closely related. Exogenous stimuli can induce mitochondrial ROS production, and  
535 oxidative stress causes the collapse of mitochondrial membrane potential, resulting in  
536 an increase in ROS in a positive feedback loop (ROS-induced ROS release) ([Zorov et  
537 al., 2000](#)). HPO-only, UV/Cl<sub>2</sub>+HPO+PRM and UV/Cl<sub>2</sub>+HPO+CAF extracts have a  
538 generalized effect of mitochondrial dysfunction, affecting different stages of the  
539 electron transport chain and reducing basal respiration, maximal respiration, and ATP  
540 production levels ([Figure 9c](#) and [Figures S13](#)). This effect is consistent with  
541 previously observed effects from induced oxidative stress ([Zorov et al., 2006](#)), and  
542 different studies showed similarly related mitochondrial-dependent toxicity through  
543 oxidative stress for different DBPs ([Zuo et al., 2017](#)).

544 As a sum parameter of overall toxicity, cytotoxicity serves as the overarching effect  
545 overlying each of the cellular toxicity pathways ([Escher et al., 2014](#)). No significant  
546 cytotoxicity was observed at the concentration (20x) used for oxidative stress,  
547 mitochondrial function, and DNA damage assays. General cell cytotoxicity was  
548 observed at higher exposure concentration (40x) for UV/Cl<sub>2</sub>+HPO+PRM and  
549 UV/Cl<sub>2</sub>+HPO+CAF samples possibly due to the increased DNA damage,  
550 mitochondrial and oxidative stress.

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551 Taken together, the observations demonstrate that chlorinated byproducts could  
552 induce oxidative stress, mitochondrial dysfunction, and increase DNA damage. The  
553 nature of EfOM present seems to be the predominant factor affecting toxicity and the  
554 presence of different model compounds such as PRM and CAF shows that the  
555 UV/chlorine treatment can have preferential targets that also impact in the overall  
556 toxic effects. Although AOCl results are consistent for all isolate samples, the  
557 composition of AOCl fraction can have a great impact on cellular toxicity (Du et al.,  
558 2020). The mechanism of action observed in our study, involving cytotoxicity,  
559 oxidative stress, mitochondrial dysfunction, and DNA damage are also comparable to  
560 previous studies (Wang et al., 2018).

#### 561 **4. Conclusion**

562 The UV/chlorine process was efficient for degrading UV- and chlorine-resistant  
563 PPCPs due to the generation of radical species. The decay of PRM and CAF was  
564 maximized at pH 6.2 and decreased with increasing solution pH. Both  $\cdot\text{OH}$  and RCS  
565 dominated the decay of PRM at acidic pH, while contribution of RCS became more  
566 important at alkaline condition. The loss of CAF was mainly attributed to  $\text{ClO}\cdot$ . The  
567 variation of chlorine dose and initial concentration of contaminant significantly alters  
568 the content of  $\text{ClO}\cdot$ , thereby leading to a more pronounced influence on the decay of  
569 CAF. The  $k'_{\text{obs}}$  for PRM and CAF degradation was not apparently impacted by  $\text{Cl}^-$   
570 ions but suppressed with the increase of  $\text{HCO}_3^-$  concentration, while  $\text{NO}_3^-$  enhanced  
571 the process efficiency through the extra generation of  $\cdot\text{OH}$  and RNS.

572 Degradation of PRM and CAF by UV/chlorine decreased with an increase in

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573 EfOM concentration. The HPO fraction with higher SUVA has a stronger impact than  
574 the TPI fraction. The presence of EfOM has a stronger inhibition on CAF degradation  
575 compared to that of PRM by preferentially scavenging  $\text{ClO}^\bullet$ . Good correlations (linear  
576 for PRM and exponential for CAF) were obtained between  $k'_{\text{obs}}$  of contaminants and  
577 UV absorbance at 254 nm of all EfOM solutions including the real effluent and  
578 reconstituted effluent, suggesting that the UV absorbing organic moieties in EfOM is  
579 likely the predominant factor affecting the UV/chlorine process in WWTP effluent.

580 The presence of EfOM isolate serves as important precursors of AOCl during  
581 UV/chlorine treatment, which induced significant effect on cellular DNA damage. A  
582 remarkable increase in intracellular oxidative stress was observed for most  
583 experiments containing HPO, while UV/chlorine treatment did not induce a  
584 significant increase in oxidative stress in our study, suggesting the nature of EfOM  
585 has a significant impact on the cytotoxicity.

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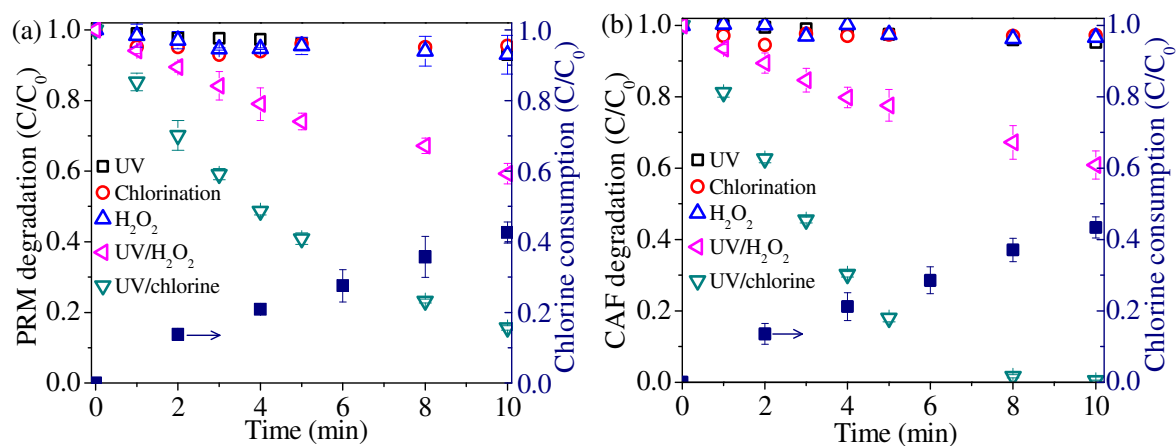


Figure 1. (a) PRM and (b) CAF degradation by UV irradiation, chlorination,  $H_2O_2$  oxidation, UV/ $H_2O_2$ , and UV/chlorine processes; and the corresponding chlorine consumption in the UV/chlorine process. Conditions:  $[PRM] = [CAF] = 5 \mu M$ ,  $[chlorine] = [H_2O_2] = 70 \mu M$ ,  $pH = 7$ .

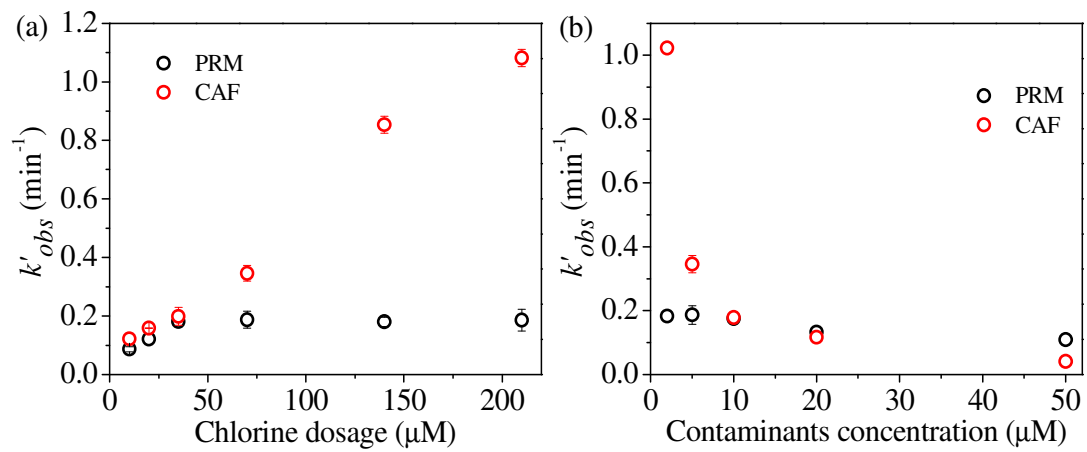


Figure 2. Observed rate constants ( $k'_{obs}$ ) for PRM and CAF decay in the UV/chlorine process under (a) various chlorine dose and (b) PPCPs concentration. Conditions: (a) [PRM] = [CAF] = 5 μM, pH = 7; (b) [chlorine] = 70 μM, pH = 7.

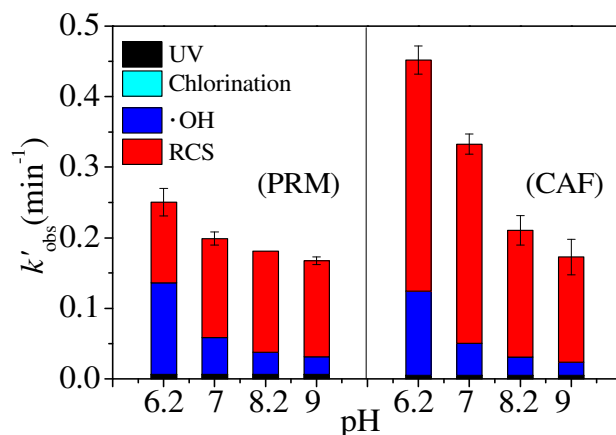


Figure 3. Observed rate constants ( $k'_{obs}$ ) and contributions of specific species for PRM and CAF decay in the UV/chlorine process at different pH. Conditions: [PRM] = [CAF] = 5  $\mu\text{M}$ , [chlorine] = 70  $\mu\text{M}$ , [NB] = 1  $\mu\text{M}$ .

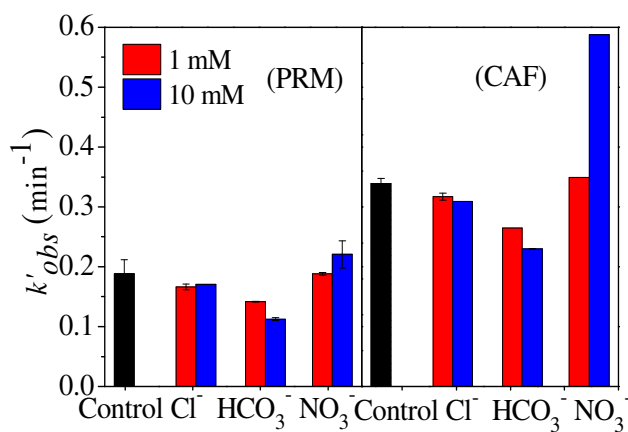


Figure 4. Effect of  $\text{Cl}^-$ ,  $\text{HCO}_3^-$ , and  $\text{NO}_3^-$  on the degradation of PRM and CAF in the UV/chlorine process. Conditions: [PRM] = [CAF] = 5  $\mu\text{M}$ , [chlorine] = 70  $\mu\text{M}$ , pH = 7.

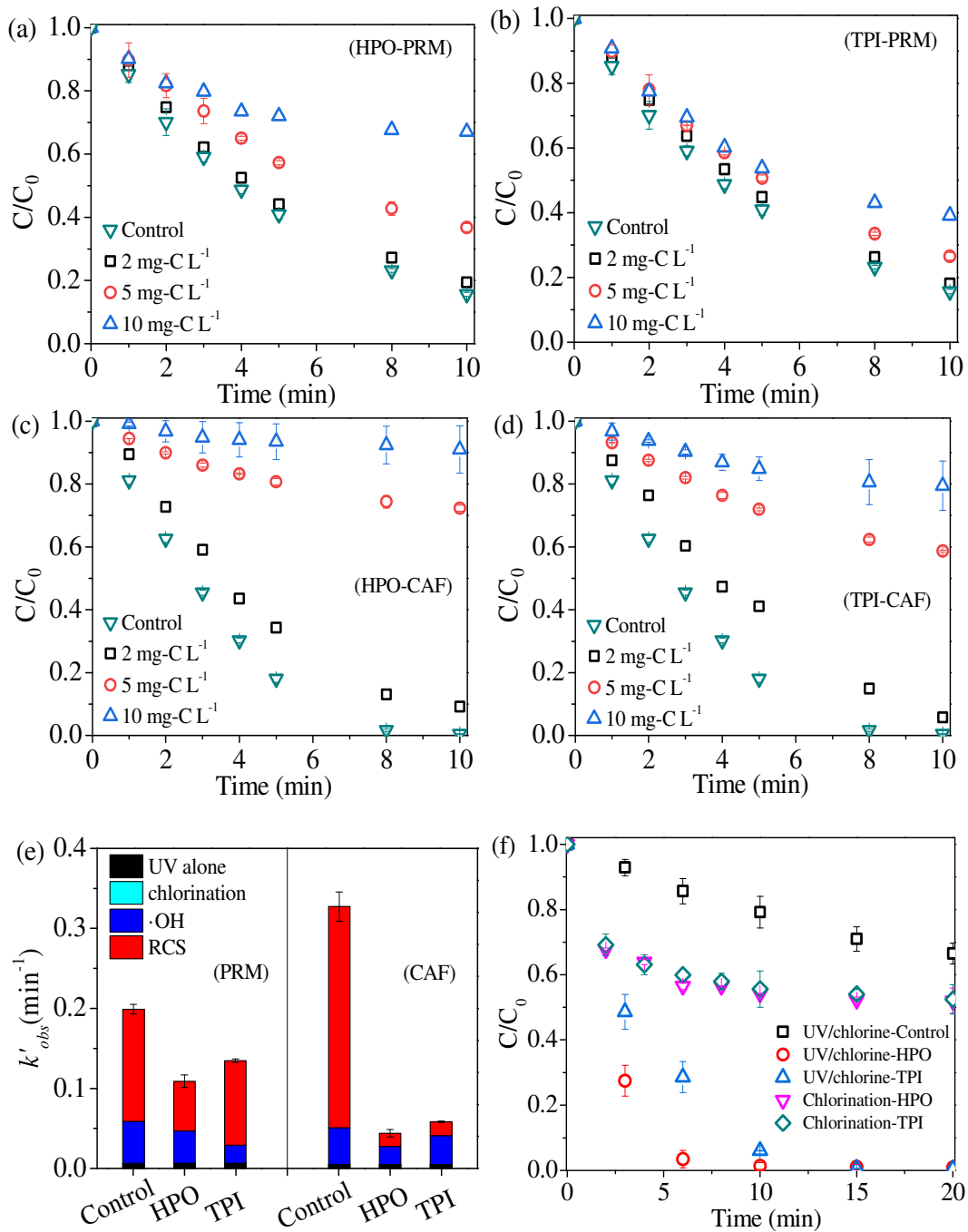


Figure 5. Effect of different concentrations of (a, c) HPO and (b, d) TPI on the degradation of PRM and CAF in the UV/chlorine systems; (e) effect of 5 mg-C L<sup>-1</sup> EfOM isolate on the contribution of  $k'_{\cdot\text{OH}}$  and  $k'_{\text{RCS}}$  for PRM and CAF decay at pH 7 with 1  $\mu\text{M}$  NB as radical probe, and (f)

corresponding chlorine consumption during the UV/chlorine process in comparison with dark chlorination of EfOM isolates. Conditions: [PRM] = [CAF] = 5  $\mu\text{M}$ , [chlorine] = 70  $\mu\text{M}$ , pH = 7.

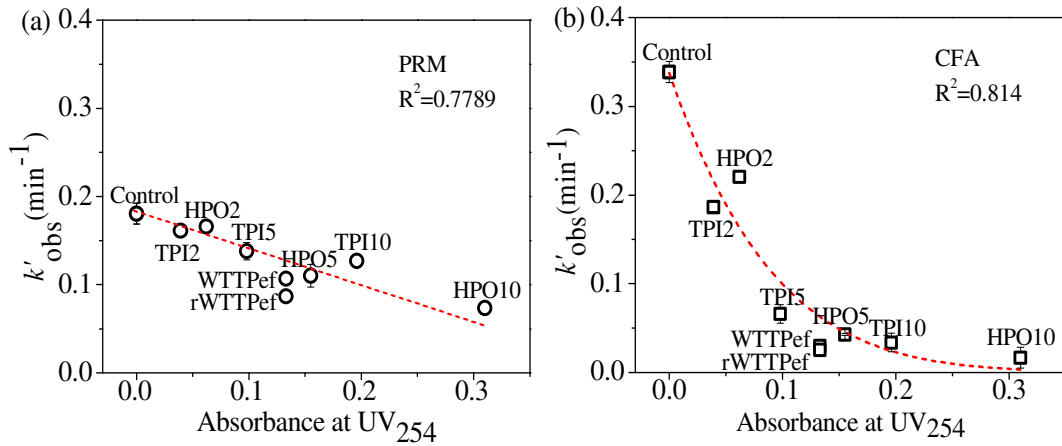


Figure 6. The linear correlation for (a) PRM and exponential correlation for (b) CAF obtained between the UV absorbance at 254 nm and  $k'_{\text{obs}}$  with all HPO and TPI solutions. HPO2-HPO10 and TPI2-TPI10 represent the solution with 2-10  $\text{mg-C L}^{-1}$  of EfOM isolate, WTTPEf and rWTTPEf represent the WWTP effluent and reconstituted WWTP effluent, respectively.

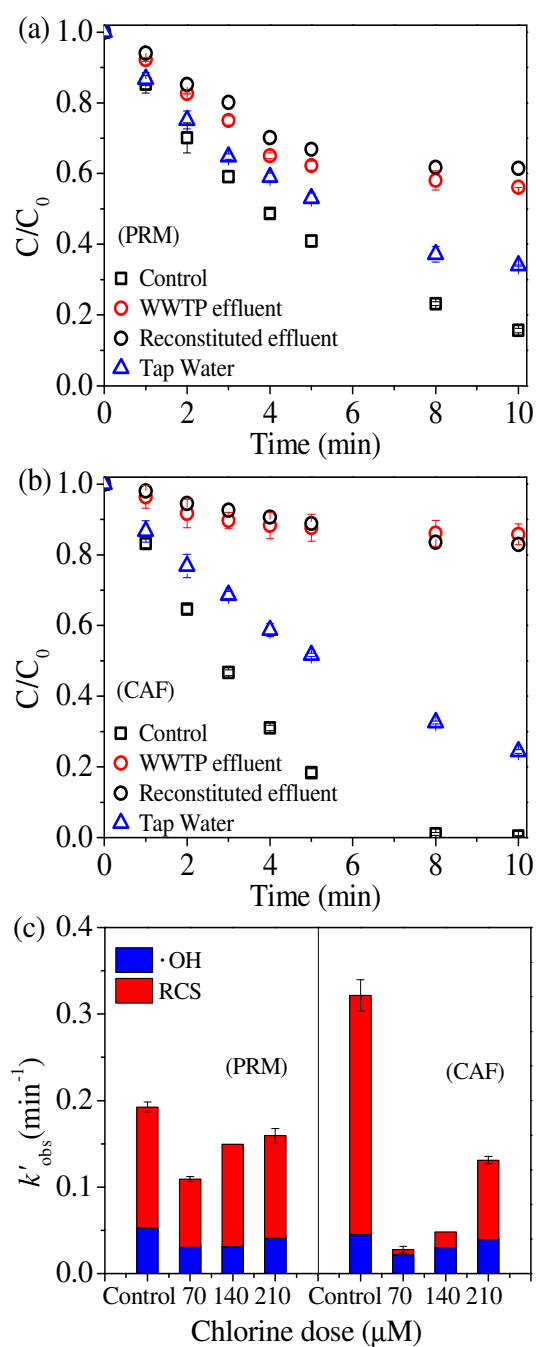


Figure 7. Decay of (a) PRM and (b) CAF in WWTP effluent, reconstituted effluent, and tap water in the UV/chlorine ( $70 \mu\text{M}$ ) process; (c)  $k'_{\text{obs}}$  of PRM and CAF at different chlorine dose in WWTP effluent using  $1 \mu\text{M}$  NB as radical probe. Conditions:  $[\text{PRM}] = [\text{CAF}] = 5 \mu\text{M}$ ,  $\text{pH}=7.76$  for WWTP and reconstituted effluent,  $\text{pH} = 7.21$  for tap water.

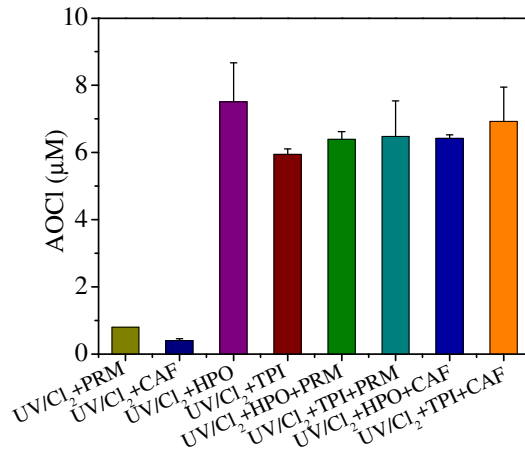


Figure 8. Formation of AOCI under different conditions. Conditions: [PRM] = [CAF] = 5 µM, [HPO] = [TPI] = 5 mg-C L<sup>-1</sup>, [chlorine] = 70 µM, pH = 7.

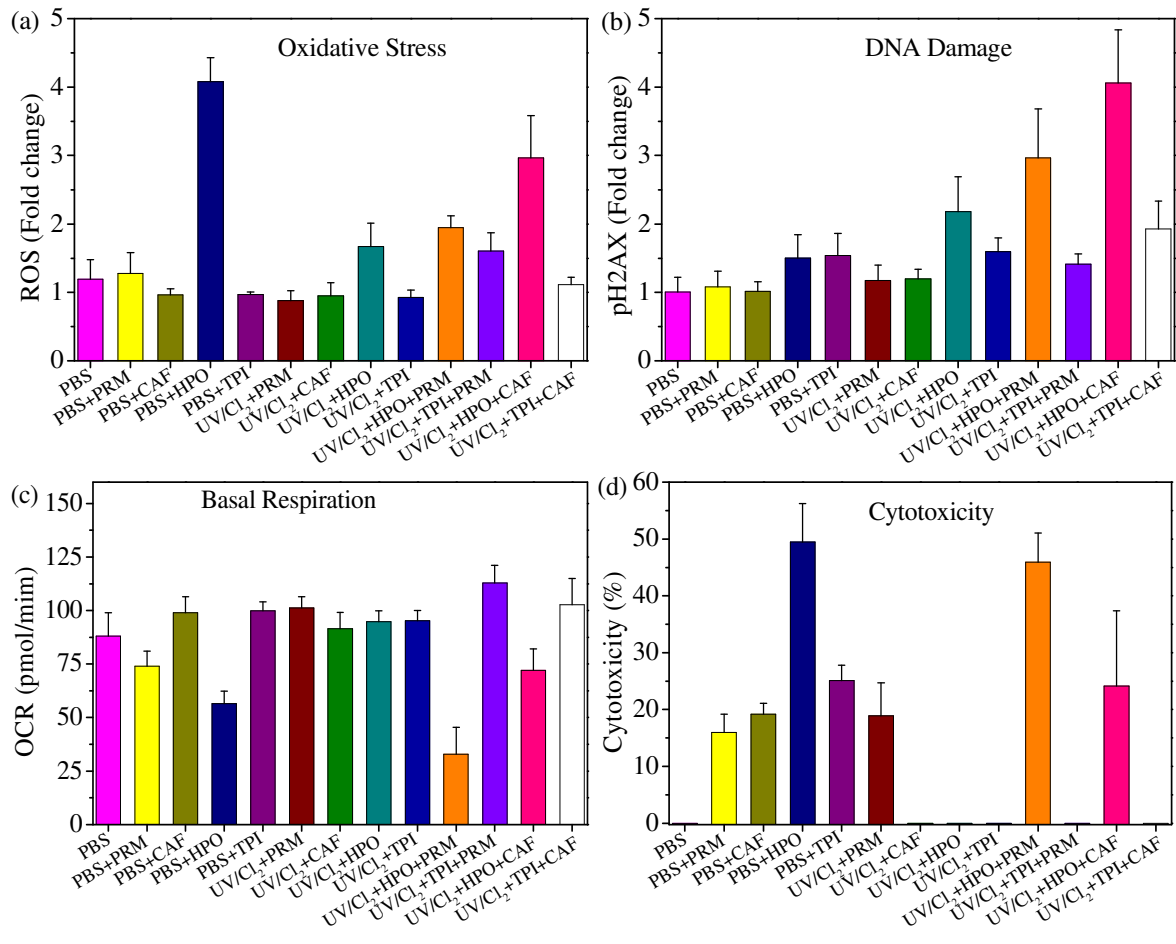


Figure 9. Impact of EfOM isolates on the oxidative stress, DNA damage, basal respiration, and cytotoxicity of UV/chlorine treatment of PPCPs. Conditions: [PRM] = [CAF] = 5  $\mu$ M, [HPO] = [TPI] = 5 mg-C L<sup>-1</sup>, [chlorine] = 70  $\mu$ M, pH = 7.



Graphical abstract:

Kinetics ← Role of EfOM in the UV/chlorine Process → Toxicity

