Oxidative transformation of oxcarbazepine by Cl_2, ClO_2 and O_3: characteristics and pathways

H. F. Miao, H. H. Han, X. P. Ji, M. F. Lu, Z. X. Huang and W. Q. Ruan

ABSTRACT

In this research, the degradation efficiency, mechanism and intermediates' toxicities of oxcarbazepine (OXC) upon chlorination, chlorine dioxide oxidation and ozonation were investigated. Results showed that three degradation approaches followed second-order kinetics, and ozonation had the highest removal efficiency both of OXC and dissolved organic carbon (DOC). Reaction intermediates were evaluated by ultra performance liquid chromatography in combination with time-of-flight mass spectrometry (UPLC-Q-TOF-MS). Totals of 11, six and 10 intermediates were detected during the oxidation processes of chlorination, chlorine dioxide oxidation and ozonation, respectively. Although three oxidation approaches had similar pathways in *N*-heterocyclic ring cleavage and reorganization, ozonation was much more focused on attacking by hydroxyl radicals ($OH^{\bullet}$), while chlorination had significant Cl-substitution by-products. Chlorine dioxide oxidation brought about fewer degradation by-products than the other two approaches. The above-mentioned oxidation intermediates according to EPA TEST were predicted to be more toxic than OXC, especially those from chlorination. Further test results of the eco-toxicities of oxidized mixtures to the bioluminescent marine bacterium *Vibrio fischeri* demonstrated the chlorinated samples could lead to the accumulation of toxic transformation products, while chlorine dioxide oxidation and ozonation had detoxication impacts during these processes.

Key words | by-products, degradation pathway, oxcarbazepine, oxidation, toxicity

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INTRODUCTION

Micropollutants in the environment are of great concern due to their threat to the preservation and sustainability of the environment. Increasing levels of micropollutants in soil and in ground and surface water could lead to a potentially serious risk to ecology and to human health by direct or indirect exposure to these chemicals (Qiao *et al.* 2011; Sun *et al.* 2014). Among emerging micropollutants, pharmaceuticals and personal-care products (PPCPs) have been frequently detected and widely evaluated in the aquatic environment with concentrations in the ng L^{-1} to μg L^{-1} range, in the last decade (Yang *et al.* 2014; Sun *et al.* 2015). Oxcarbazepine (10,11-dihidro-10-oxo-5H-dibenzo-*[b,f]*azepine-5-carboxamide, OXC), as a 10-keto analogue of carbamazepine (CBZ), is an anti-epileptic drug with chemical and therapeutic similarities to CBZ but a more favorable pharmacokinetic profile than CBZ (Zou *et al.* 2015). Hence, the production and prescription of OXC are on the rise. OXC consumption increased from 2.9 t $year^{-1}$ to 6.7 t $year^{-1}$ during 2002–2005 in France (Cui *et al.* 2014). Furthermore, OXC was introduced into Germany in 2000, and prescribed in an amount of 12.8 t in 2012 (Bahlmann *et al.* 2014). Although OXC was reported to be degraded to some extent during conventional wastewater treatment (~37.2%), it was present in the surface water at a maximum concentration of 0.234 μg L^{-1} (Kaiser *et al.* 2014). It may cause a potential risk in drinking water to human health, due to its biologically active nature, accumulation and persistent physico-chemical properties. Based on the calculated LC_{50} values according to advanced quantitative structure–activity

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relationship (QSAR) computational approaches, OXC is considered to be possibly toxic for bacteria, algae and other tested aquatic species (fathead minnow LC_{50} 9.53 mg L^{-1} and *Daphnia magna* LC_{50} 1.50 mg L^{-1}). Even though no adverse effects on human health are expected from the low concentration of OXC, the occurrence of this kind of compound in drinking water is undesirable.

As was mentioned above, OXC removal by conventional wastewater treatment was not effective due to its persistent physico-chemical properties. Recent studies have utilized lots of approaches regarding chemical disinfection for such kinds of compounds. Traditionally, chlorination is the most dominant method of disinfection: it has low oxidation potential and extreme dependence on the speciation of chlorine (Cl_2) as a function of pH, and allows for the removal of a limited number of PPCPs during water treatment (Sharma 2008; Qin *et al.* 2014). Chlorine dioxide (ClO_2) oxidation is also widely applied in drinking water treatment because of its stability in aqueous matrices, powerful oxidation potential and especially lower chlorine disinfection by-product production. The reactivity of ClO_2 is supposed to be more effective with compounds containing phenolic and tertiary amino groups, and is significantly influenced by the pH (Sharma 2008). Ozonation has recently appeared as an important technology for the removal of most organic pollutants in water treatment and is extensively utilized in drinking water facilities (Gerrity & Snyder 2011). Although ozone (O_3) is unstable both in gaseous and aqueous phase, it may react indiscriminately with PPCPs through molecular O_3 and produce free radicals (mainly the hydroxyl radical, $OH^{\bullet}$).

Another concern for the chemical treatment of PPCPs is the formation of transformation products, some of which have been identified to be potentially more toxic than their precursors. For instance, chlorination and/or ClO_2 disinfection of PPCPs containing amine groups such as ranitidine, chlorpheniramine and chlortetracycline hydrochloride, etc. could result in the production of *N*-nitrosodimethylamine (Zhang *et al.* 2014). Cl_2 reacts with CBZ leading to the generation of the carcinogenic compounds acridine, acridine-9-carbaldehyde and 9-hydroxy-acridine (Postigo & Richardson 2014). Moreover, ozonation of CBZ results in the formation of 1-(2-benzaldehyde)-4-hydro-(1H,3H)-quinazoline-2-one, 1-(2-benzaldehyde)-(1H,3H)-quinazoline-2,4-dione, 1-(2-benzoic acid)-4-hydro-(1H,3H)-quinazoline-2-one and 1-(2-benzoic acid)-(1H,3H)-quinazoline-2,4-dione (Hübner *et al.* 2014). To the best of our knowledge, there has been little study of OXC removal in drinking water treatment, and fewer publications have reported the degradation intermediates and mechanism of OXC during disinfection (Li *et al.* 2011). Furthermore, there has not been any investigation of the degradation efficiency, degradation mechanism and intermediates' toxicity of OXC with chlorination, ClO_2 disinfection and ozonation.

With this in mind, the objectives of this study are (1) to investigate degradation efficiencies and kinetics of OXC upon the three different disinfection approaches of chlorination, ClO_2 oxidation and ozonation, (2) to identify the transformation products and propose the tentative degradation pathways during the different oxidation approaches and (3) to evaluate the toxicities of the by-products, comparatively.

MATERIALS AND METHODS

Oxidation experiments

Reactions of OXC (≥98%, HPLC grade) with Cl_2, ClO_2 and O_3 were conducted in a 1,000 mL cylindrical jacketed borosilicate glass reactor, in batch mode. Chlorination was carried out with NaClO (4.00%–4.99%) and the procedure was as follows. Firstly, the stock solution of Cl_2 was prepared with 5 mM phosphate buffer solution (pH 7.0) and kept at 4 °C. Aqueous concentration of available Cl_2 in the stock solution was tested right before the chlorination experiments. Secondly, the chlorination was started by injecting OXC solution into the Cl_2 stock solution at a dosage of $[Cl_2]_0$:$[OXC]_0 = 20{:}1$ (mole ratio), and shaking the reaction vessel vigorously for 10 s. The reaction was operated in the dark and the temperature was kept at 25 ± 1 °C. Finally, samples for analysis were obtained at defined time intervals and quenched immediately by adding 0.1 mL fresh sodium sulfite solution (Na_2SO_3, 20 mM) to remove the residual Cl_2. The same as with chlorination, ClO_2 disinfection was also performed under conditions of pH 7.0, temperature 25 ± 1 °C and dosage $[ClO_2]_0$:$[OXC]_0 = 20{:}1$ (mole ratio). ClO_2 stock solution was prepared by adding sulfuric acid to $NaClO_2$ (80% RT) solution, and the produced ClO_2 solution was stored in an amber glass bottle at 4 °C. Ozonation was also according to the above-mentioned protocol, with the

experimental conditions of pH 7.0, temperature 25 ± 1 °C and dosage $[O_3]_0$:$[OXC]_0$ = 20:1 (mole ratio). O_3 stock solution was freshly prepared by bubbling O_3 gas through the 5 mM phosphate buffer solution. O_3 was produced from purified O_2 (99.8%) by a COM-AD-01 O_3 generator (Anseros, Germany). Due to the high degradation rate of OXC ozonation, the reaction time of ozonation was set at 30 min, while those of chlorination and ClO_2 oxidation were prolonged to 6 h and 5 h, respectively. Residual oxidants such as ClO_2 and O_3 were also removed by the addition of 20 mM Na_2SO_3. All the experiments were repeated at least three times and the data were presented as mean value ± deviations.

Analytical methods

The concentrations of available Cl_2 and ClO_2 in the solution were determined by the *N,N*-diethyl-*p*-phenylenediamine (DPD) colorimetric methods (Hach, PCII 58700-00 and PCII 58700-51, USA). The aqueous concentration of O_3 was measured with the indigo colorimetric method (Bader & Hoigné 1981). Dissolved organic carbon (DOC) analysis was carried out by a TOC-VCPH analyzer equipped with an ASI-V auto-sampler (Shimadzu, Japan). Nitrate (NO_3^-) formation during disinfection was determined using a Metrohm ion chromatograph (883 Compact IC Pro, Switzerland) equipped with a Metrosep A Supp 5 (250 × 4.0 mm^2) analytical column (Ismail *et al.* 2013). OXC analysis was performed by high-performance liquid chromatography (HPLC) (Agilent 1200, USA) equipped with an Acclaim 120 C18 (250 × 4.6 mm^2, I.D. 5 μm) column. The mobile phase was a mixture of acetonitrile (ACN) and water (60%:40%) at an isocratic flow rate of 1.0 mL min^{-1} and temperature of 30 °C. Injections were performed with a 20 μL loop and the wavelength of the UV absorbance detector was 220 nm (Li *et al.* 2011). OXC transformation products were identified by ultra performance liquid chromatography in combination with time-of-flight mass spectrometry (UPLC-Q-TOF-MS). Chromatography was performed on a Waters Acquity UPLC BEH C18 column (2.1 × 100 mm^2, 1.7 μm particle) (Miao *et al.* 2015).

Toxicity evaluations

Toxicities of the degradation by-products from OXC oxidation were performed by using the US-EPA TEST (version 4.1). According to the methods introduced by Rodríguez-Álvarez, the 96-hour fathead minnow LC_{50}, 48-hour *Daphnia magna* LC_{50} and oral rat LD_{50} were calculated (Rodríguez-Álvarez *et al.* 2013). Moreover, the acute toxicities of OXC as well as its degradation by-products from oxidation experiments were further evaluated by the bioluminescence inhibition test with *Vibrio fischeri* according to the ISO 11348 standard protocol (ISO 2007).

RESULTS AND DISCUSSION

OXC degradation kinetics

Reactions between the disinfectants and the target inorganic and/or organic compounds can be presented by the well-established second-order kinetics, and by first-order with respect to both reactants (Sharma 2008). Therefore, the reaction rates of OXC with the selected oxidants may be expressed as follows:

$$-\frac{d[\mathrm{OXC}]}{dt} = k_{app}[\mathrm{OXC}][\mathrm{Oxidant}] = k_{obs}[\mathrm{OXC}] \quad (1)$$

$$k_{app} = \frac{k_{obs}}{[\mathrm{Oxidant}]} \quad (2)$$

where [OXC] was the total molar concentration of OXC; [Oxidant] was the total molar concentrations of the disinfectants (Cl_2, ClO_2 and O_3) as well as their active species (HOCl, ClO^-, ClO_2^-, ClO_3^- and $OH^{\bullet}$, etc.); k_{app} and k_{obs} are the apparent second-order and rate constant and the corresponding pseudo-first-order rate constant during OXC disinfection. Integration of each side of Equation (1) can result in Equation (3):

$$\ln\frac{[\mathrm{OXC}]}{[\mathrm{OXC}]_0} = -k_{app}\int[\mathrm{Oxidant}]dt = -k_{obs}t \quad (3)$$

The plots of $\ln([OXC]/[OXC]_0)$ vs $\int[Cl_2]dt$, $\int[ClO_2]dt$ and $\int[O_3]dt$ shown in Figure S1 (available with the online version of this paper) exhibit straight lines with slopes of k_{app}. All the reactions strictly followed the second-order kinetic model with the coefficient of determination (R^2) > 0.99. In addition,

the plots of $\ln([OXC]/[OXC]_0)$ vs reaction time (t) show satisfactory linear form ($R^2 > 0.99$), which mean pseudo-first-order kinetics with respect to OXC concentration during all processes (Figure S2, available with the online version of this paper). The calculated k_{app}, k_{obs} and $t_{1/2}$ (half-life of OXC oxidation) are compiled in Table 1. Ozonation was most effective with regard to OXC degradation with a k_{app} value of 3.02×10^3 M^{-1} s^{-1}, while ClO_2 oxidation and chlorination were of much lower efficiency with k_{app} values of 3.42×10^2 M^{-1} s^{-1} and 1.46×10^2 M^{-1} s^{-1}, respectively. The values of $t_{1/2}$ calculated from Figure S2 also demonstrated that ozonation was fastest in OXC degradation with the value of 239 s.

Table 1 | Rate constants and $t_{1/2}$ for OXC oxidation by Cl_2, ClO_2 and O_3

OXC[a] degradation	k_{app} (M^{-1} s^{-1})	R^2	k_{obs} (s^{-1})	R^2	$t_{1/2}$ (s)
By Cl_2	1.46×10^2	0.990	1.19×10^{-4}	0.994	6,766
By ClO_2	3.42×10^2	0.994	1.29×10^{-4}	0.997	5,218
By O_3	3.02×10^3	0.995	2.72×10^{-3}	0.993	239

[a]$[OXC]_0$: 0.40 μmol L^{-1}.

OXC mineralization efficiencies

Further investigation of OXC mineralization was carried out with DOC and NO_3^- variations compared to OXC removal. Results presented in Figure 1 show that all the three oxidation approaches had OXC mineralization capability to some extent. During ClO_2 oxidation and ozonation processes, DOC was observed to decrease by 27.93% and 21.93% after 30 min ozonation and 5 h ClO_2 oxidation, respectively. This meant that OXC mineralization by O_3 was much higher than by ClO_2. The same results were obtained from NO_3^- production. The short time of ozonation (30 min) resulted in the formation of NO_3^- with a concentration of 0.278 μmol L^{-1}, while the prolonged time of ClO_2 oxidation (5 h) brought about 0.143 μmol L^{-1} NO_3^-. In addition, chlorination

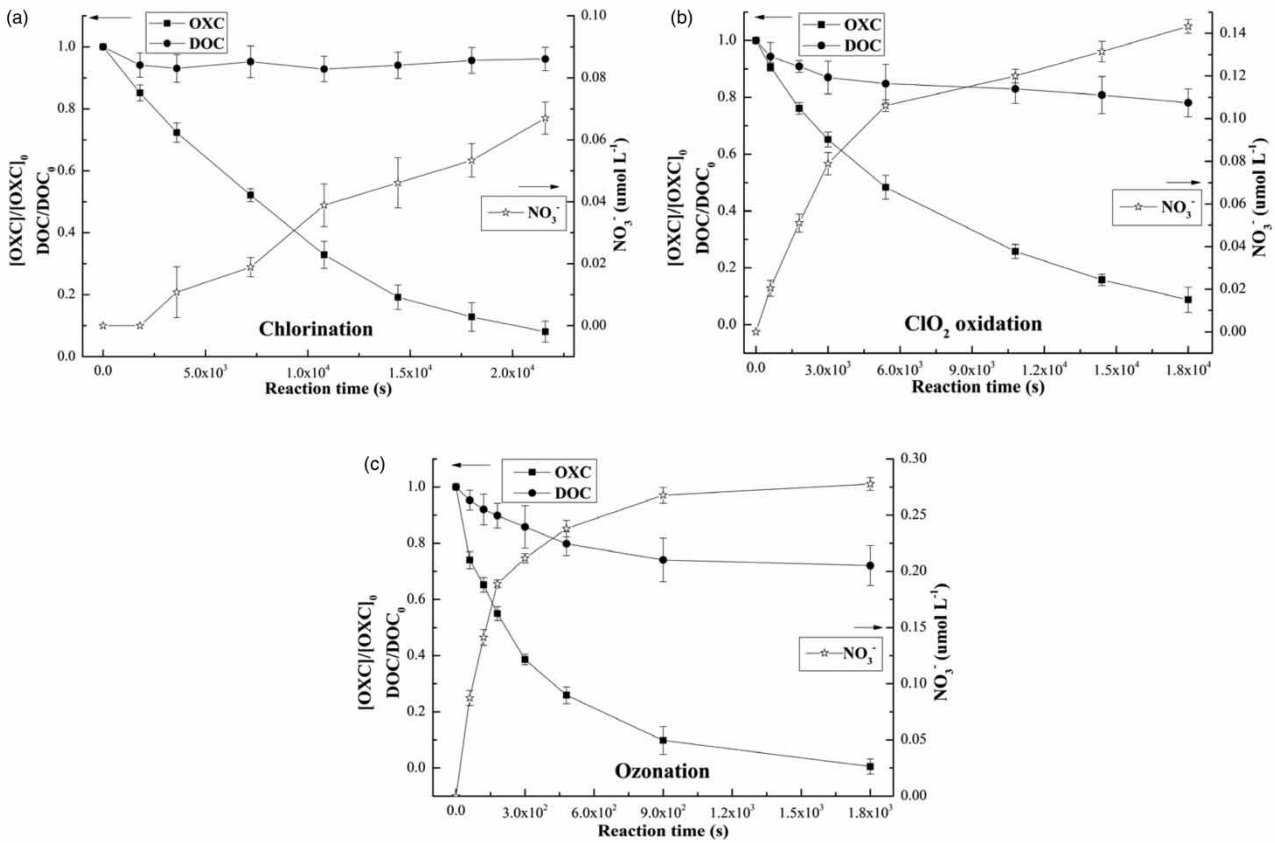

Figure 1 | Changes of normalized OXC and DOC concentrations as well as NO_3^- formations during OXC degradation by (a) Cl_2, (b) ClO_2 and (c) O_3.

showed much lower mineralization capability than ClO_2 oxidation and ozonation, with little DOC removal and 0.067 μmol L^{-1} NO_3^- formation after 6 h reaction.

Results indicated that, although chlorination, ClO_2 oxidation and ozonation could degrade OXC with different efficiencies, oxidation by-products produced that are more resistant to further mineralization still existed in the water samples, especially for chlorination. Moreover, the formation of NO_3^- might be due to deamination from the *N*-heterocyclic ring in OXC (Chang *et al.* 2012). However, only a tiny fraction of organic nitrogen was observed to be transformed into NO_3^-, which meant large amounts of organic nitrogen existed in the solution with other forms or structures.

OXC degradation by-products

During the degradation of OXC by chlorination, ClO_2 oxidation and ozonation processes, series intermediates were identified with UPLC-Q-TOF-MS operated in the full scan and production ion scan modes. A total of 27 OXC degradation by-products are compiled in Table S1 (available with the online version of this paper), in which 11 came from chlorination, six from ClO_2 oxidation and 10 from ozonation, respectively. Table S1 presents the retention times (RTs), proposed formulas, main mass fragments, double-bond equivalences (DBEs) of OXC and their degradation by-products during different processes. Figure S3 (available with the online version of this paper) shows the chromatographic profiles of 27 oxidation by-products as well as OXC during different oxidation processes. As should be pointed out, among all the OXC degradation by-products, there were several by-products identified during the two or three oxidation processes. For example, CL-3, CD-3 and O-2 shared similar RT and mass fragments, and CL-4, CD-4 and O-3 also showed similar chromatographic and mass profiles. They were supposed as having the same structures, respectively. Proposed degradation pathways of OXC by chlorination, ClO_2 oxidation and ozonation are provided in Figure 2 and summarized as follows.

Cl_2 degradation pathway

The OXC degradation pathway during chlorination is illustrated in Figure 2 with the dotted line. Firstly, OXC could be converted into CL-1 with hydroxylation on the ^{5}C position in *N*-heterocyclic ring. With the reaction progressing, the hydroxylated OXC (CL-1) was transformed to CL-2 with the *N*-heterocyclic ring opening and hexahydropyrimidine moiety re-arrangement. Then, CL-2 was degraded into CL-3 and CL-4 with further oxidation on the phenyl-methanol moiety. The degradation mechanism from CL-1 to CL-4 was mainly due to the oxidative effects during chlorination. Secondly, Cl-addition and/or Cl-substitution also exerted its impacts on OXC degradation. CL-5 was caused by the deamidation process on the ^{1}N position in the *N*-heterocyclic ring, from CL-1. And CL-5 could be quickly converted to CL-6 by further dehydroxylation and deoxygenation in the *N*-heterocyclic ring. CL-7 and CL-8 were supposed as being chlorinated CL-6. Although accurate positions were not determined by the current methods, the different RTs and MS fragment ions of CL-7 and CL-8 could help to illustrate that Cl radicals attacked different moieties such as the benzene ring and *N*-heterocyclic ring (Table S1). Furthermore, CL-9 and CL-10 were supposed as being CL-1 transformation products with one and two Cl-substitutions on the ^{5}C position in the *N*-heterocyclic ring, respectively (Li *et al.* 2011). Finally, another Cl-substitution intermediate (CL-11) was observed, which was supposed as being produced with Cl attacking the acetamide group, directly from OXC.

As shown in Figure 3, CL-5, CL-6, CL-7, CL-8 and CL-10 were identified as the most accumulated degradation intermediates during OXC chlorination, especially CL-6, CL-7 and CL-8, which were observed to rapidly accumulate to 5,950 counts, 2,030 counts and 2,810 counts, respectively, during the chlorination time of 6 h. Among these compounds, CL-5 was found to be rapidly formed, then decreasing slowly with the reaction time. However, CL-6, CL-7, CL-8 and CL-10 were observed to have an accumulating tendency with the reaction time.

ClO_2 degradation pathway

The OXC degradation pathway during ClO_2 oxidation is illustrated in Figure 2 with the semi-solid line. OXC was firstly degraded to CD-1 with hydroxylation on the ^{5}C position in the *N*-heterocyclic ring, which was the same as with that from OXC to CL-1 during chlorination. CD-1 was then further

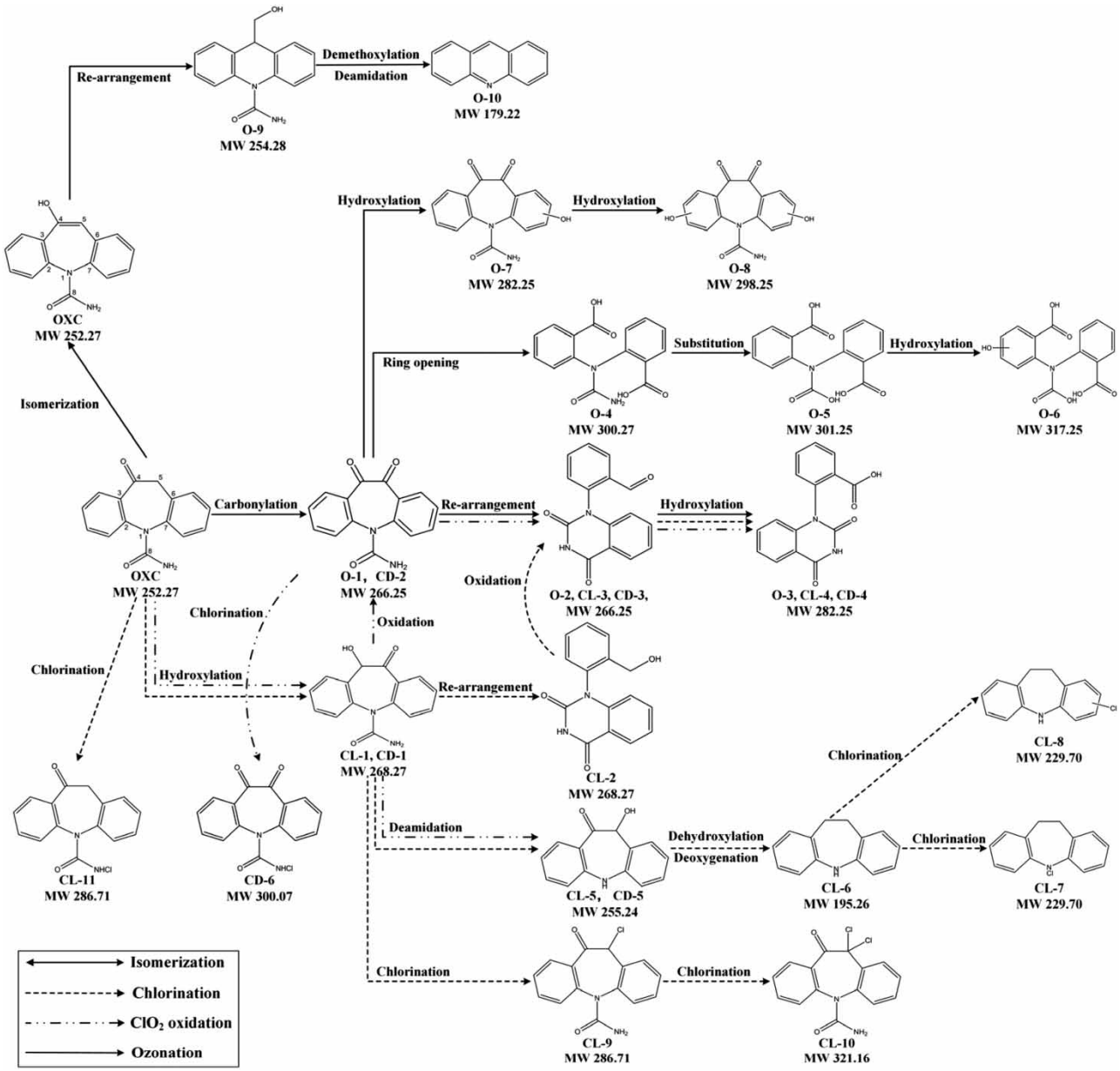

Figure 2 | Proposed degradation pathways for OXC chlorination, ClO_2 oxidation and ozonation.

oxidized to CD-2, possibly due to the much higher oxidation potential of ClO_2 than Cl_2 (Kim & Hensley 1997; Sylvia *et al.* 2000). *N*-heterocyclic ring opening and hexahydropyrimidine moiety re-arrangement could result in CD-3 from CD-2. Finally, CD-3 was observed to transform into CD-4 with hydroxylation on the benzaldehyde group. This reaction pathway was similar to those from OXC to CL-4 during chlorination, except for the formation of CD-2. Moreover, CD-5 (the same structure as CL-5) was also identified during ClO_2 oxidation, which was because of the deamidation process on the ^{1}N position in the *N*-heterocyclic ring from CD-1. During ClO_2 oxidation, only one chlorinated intermediate (CD-6) was observed. It was assumed that CD-6 was formed from CD-2 with Cl-substitution on the acetamide group.

Figure 3 also displays the degradation of OXC and the evolution of its intermediates during ClO_2 oxidation. Results

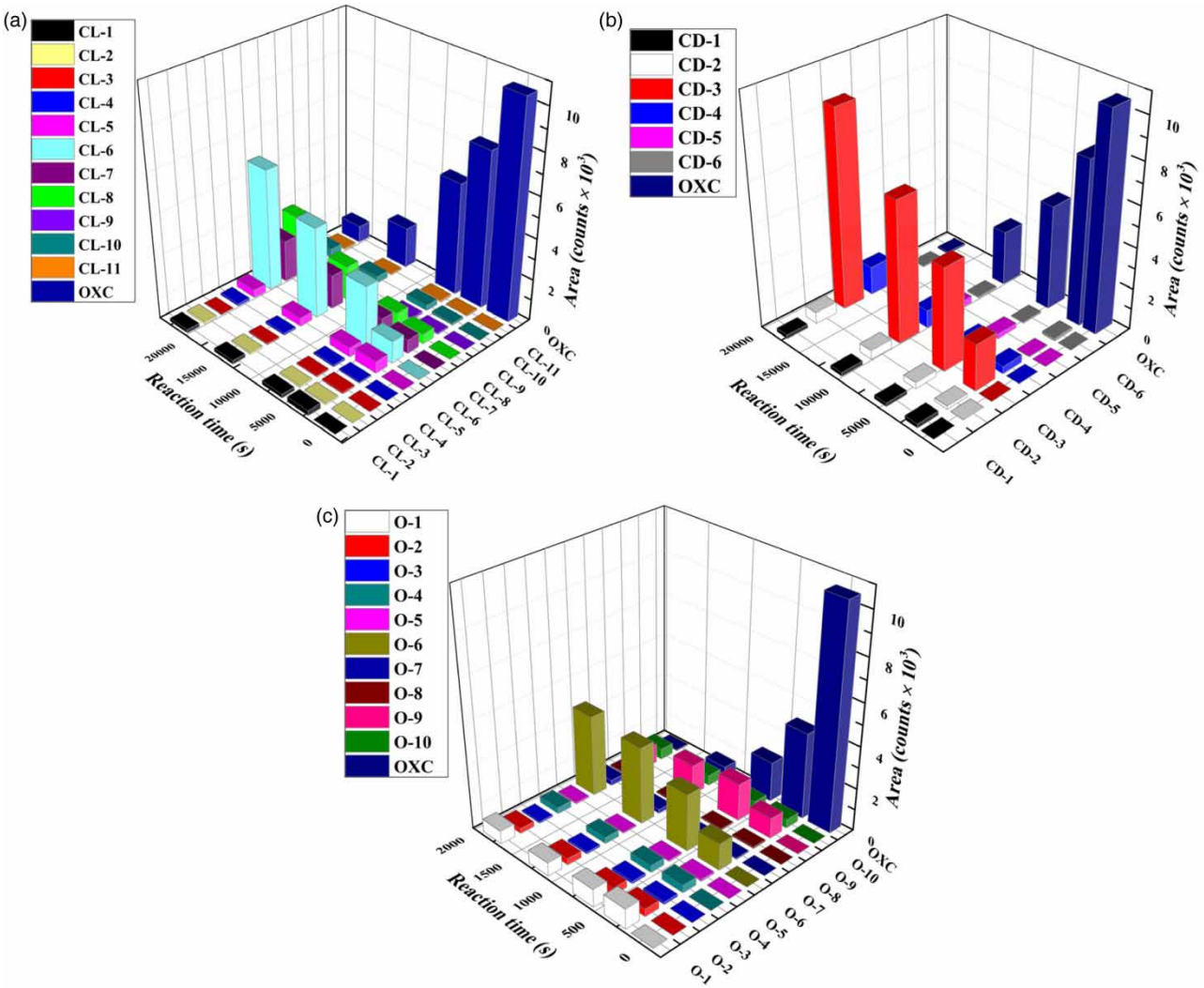

Figure 3 | Responses of OXC degradation by-products under different oxidation times during (a) chlorination, (b) ClO_2 oxidation and (c) ozonation.

indicated that CD-2, CD-3, CD-4 and CD-5 were the most accumulated intermediates. And CD-3 and CD-4 were observed with much stronger responses than others, accumulated responses of 9,630 counts and 1,380 counts, respectively, after the reaction time of 5 h. Differently to chlorination, oxidation exerted the dominant role during this process. Intermediates from CD-1 to CD-4 contributed the most by-products both in quantities and amounts. Chlorinated intermediate CD-6 only contributed low responses in the range of 36–133 counts to the total by-products. This implied that ClO_2 oxidation and chlorination showed totally different mechanisms for OXC degradation.

O_3 degradation pathway

The OXC degradation pathway during ozonation is illustrated in Figure 2 with the solid line. Firstly, OXC was degraded to O-1, O-2 and O-3 by series carboxylation, rearrangement and hydroxylation, respectively. This degradation mechanism was similar to that during ClO_2 oxidation, but without CD-1 formation. Secondly, O-1 underwent an *N*-heterocyclic ring opening process probably upon $OH^{\cdot}$ attacking to produce O-4. Further substitution of the -OH group for an -NH_2 group on the ^{8}C position of O-4 could result in the formation of O-5. O-6 was supposed as being the further hydroxylation by-product on the benzene

ring from O-5. Thirdly, O-7 and O-8 were principally from hydroxylation and di-hydroxylation on the benzene ring of O-1. Finally, O-9 was produced from the OXC enol tautomer with a ring contraction reaction, possibly due to attacking by $OH^{\cdot}$ (Figure 2). Subsequently, O-9 could be further degraded to O-10 after demethoxylation and deamidation on the dihydropyridine ring (Li *et al.* 2011).

Among the 10 OXC degradation by-products during ozonation, O-1, O-6, O-9 and O-10 were the most accumulated intermediates, as is displayed in Figure 3. O-1 was observed to be rapidly formed with a response of 936 counts at a reaction time of 5 min, then decreasing slowly to 612 counts until the final time of 30 min probably due to its further degradation. O-9 also showed the characteristic of an intermediate by-product, with an accumulative tendency at the initial 11 min then decreasing in the latter reaction time. O-6 and O-10, as the final by-products in this pathway, showed an accumulative tendency with the ozonation time.

Toxicity evaluation

In accordance with the proposed structures of OXC and its degradation by-products, their eco-toxicities were preliminarily estimated with the US-EPA TEST. TEST can predict eco-toxicity by relating the biological activity of chemicals to theoretically calculated or experimental descriptors of their chemical structure, according to the global QSAR models (Rodríguez-Álvarez *et al.* 2013). Table 2 presents those estimated values for fathead minnow LC_{50} (96 h), *Daphnia magna* LC_{50} (48 h) and oral rat LD_{50} of OXC as well as its degradation

Table 2 | US-EPA TEST of predicted toxicological LC_{50} and LD_{50} values of OXC and its degradation by-products

	Fathead minnow LC_{50} (96 h)		*Daphnia magna* LC_{50} (48 h)		Oral rat LD_{50}	
Compounds	**$-\log$ (mol L^{-1})**	**mg L^{-1}**	**$-\log$ (mol L^{-1})**	**mg L^{-1}**	**$-\log$ (mol kg^{-1})**	**mg kg^{-1}**
OXC	4.42	9.53	5.23	1.50	2.10	2,000.03
CL-1 (CD-1)	4.62	6.39	5.41	1.04	2.10	2,124.94
CL-2	4.49	8.63	5.22	1.60	2.30	1,350.79
CL-3 (CD-3, O-2)	4.76	4.60	5.18	1.77	2.03	2,494.82
CL-4 (CD-4, O-3)	4.27	15.01	5.66	0.62	2.10	2,244.96
CL-5 (CD-5)	4.89	2.89	5.26	1.24	2.63	522.87
CL-6	5.40	0.77	5.09	1.60	2.52	590.69
CL-7	5.10	1.81	NA[a]	NA	2.33	1,064.61
CL-8	5.66	0.50	5.73	0.43	2.53	670.59
CL-9	5.15	2.02	6.33	0.13	1.97	3,044.25
CL-10	5.42	1.23	5.55	0.91	1.78	5,283.93
CL-11	5.01	2.82	NA	NA	NA	NA
CD-2 (O-1)	5.02	2.55	4.44	9.63	2.66	585.78
CD-6	5.36	1.31	NA	NA	NA	NA
O-4	NA	NA	5.15	2.13	1.74	5,449.15
O-5	4.32	14.47	4.98	3.18	1.68	6,312.40
O-6	4.92	3.82	5.19	2.05	1.79	5,124.54
O-7	5.01	2.74	5.57	0.77	2.49	911.60
O-8	5.07	2.54	5.16	2.05	2.52	901.23
O-9	3.98	26.73	5.84	0.37	2.12	1,933.45
O-10	5.00	1.79	4.35	8.10	2.05	1,611.18

[a]NA: No reliable prediction was obtained.

by-products. The predicted toxicity values of $-\log(LC_{50})$ or $-\log(LD_{50})$ according to the QSAR models, displayed as $-\log(\text{mol L}^{-1})$ or $-\log(\text{mol kg}^{-1})$, as well as their calculated LC_{50} or LD_{50} values (mg L^{-1} or mg kg^{-1}) were obtained. Results indicated that most OXC degradation by-products showed higher toxicity than OXC for at least one test. For OXC chlorination, CL-1, CL-2, CL-6, CL-7, CL-9 and CL-10 were more toxic than OXC for two tests. CL-5 and CL-8 were predicted more toxic than OXC for all the tests. This may be particularly relevant for CL-5, CL-6, CL-7, CL-8 and CL-10, as being the most accumulated by-products during chlorination, would pose a potential risk to drinking water quality. For OXC ClO_2 oxidation, CD-1, CD-2 and CD-5 showed higher toxicity than OXC for more than two tests, in which only CD-2 and CD-5 showed accumulative tendency during ClO_2 oxidation. Hence, ClO_2 oxidation was predicted to have less toxic by-products than chlorination. For OXC ozonation, O-1, O-7, O-8, O-9 and O-10 were predicted more toxic than OXC for more than two tests, especially O-9 and O-10, being the most accumulated by-products during ozonation, showing 3.05 times more toxicity than OXC for *Daphnia magna* LC_{50} (O-9) and 4.32 times more toxicity than OXC for fathead minnow LC_{50} (O-10), respectively.

Validation experiments on the acute toxicities of OXC degradation by-products were performed by the inhibition of bioluminescence of *Vibrio fischeri*. OXC degradation profiles and the total toxicities of OXC oxidized mixtures under different oxidation times are displayed in Figure 4. The background toxicity of the OXC solution before oxidation was determined as 28.62% inhibition, mainly due to the presence of 0.4 μmol L^{-1} OXC, buffer, oxidants (Cl_2, ClO_2 or O_3) and quenching agents (Na_2SO_3). OXC experienced degradation rates of 91.93%, 91.19% and 99.45% after 6 h chlorination, 5 h ClO_2 oxidation and 30 min ozonation, respectively. However, bioluminescence inhibitions from the oxidized mixtures showed absolutely different trends to OXC concentrations. For OXC chlorination, the toxicity of the oxidized mixture increased continuously with the chlorination time. Results indicated that OXC chlorinated by-products were more toxic than OXC, which was in accordance with those from EPA TEST (Table 2). Most chlorinated by-products such as CL-6, CL-7, CL-8 and CL-10, etc. not only showed obviously higher toxicities than OXC, but also had

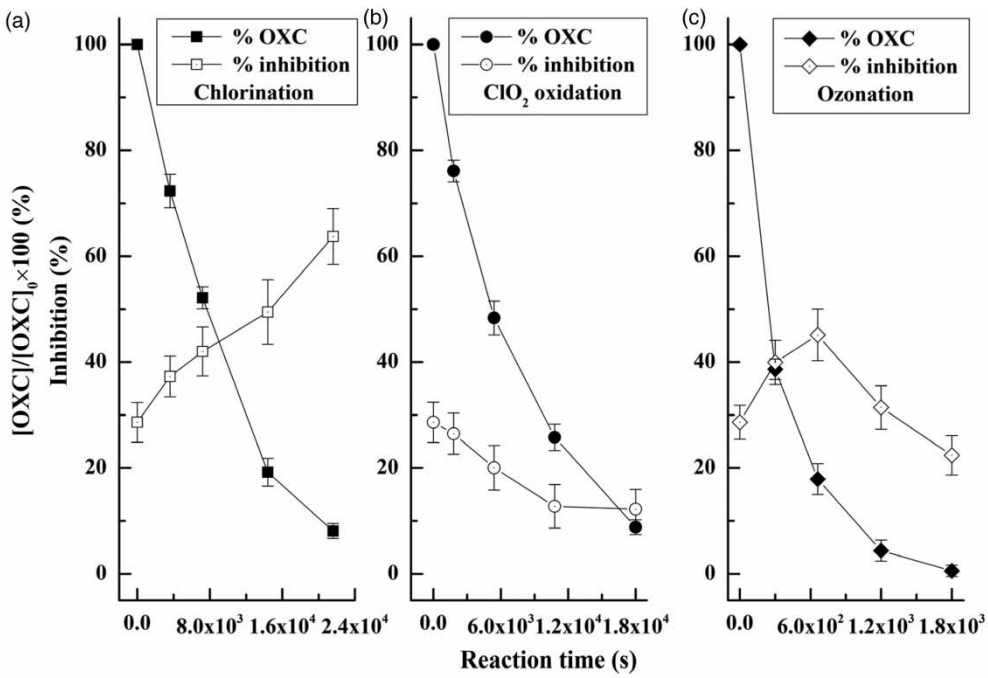

Figure 4 | Variations of OXC concentration and bioluminescence inhibition of *Vibrio fischeri* during (a) chlorination, (b) ClO_2 oxidation and (c) ozonation.

accumulative tendencies with reaction time. For ClO_2 oxidation, the toxicity of the oxidized mixture decreased slowly from 28.62% to 12.21% inhibition with reaction time, which meant that ClO_2 oxidation had a detoxication effect for OXC to some extent. This demonstrated that there were some by-products with toxic characteristics such as CD-1, CD-2 and CD-5. Interestingly, the toxicity of the oxidized mixture after ozonation was observed to increase sharply to 45.15% inhibition under a reaction time of 11 min, while further decreasing to 31.41% inhibition and 22.38% inhibition under reaction times of 20 min and 30 min, respectively. The enhancement of the toxicity can be correlated to formation of intermediates with higher toxicity (O-1, O-7, O-8, O-9 and O-10, etc.). The reduction of the toxicity may be due to their further degradation and even mineralization with prolonged ozonation time. In all, OXC chlorination would lead to toxicity increase after treatment. Although ClO_2 oxidation and ozonation had a detoxication effect during these processes, ClO_2 oxidation was more effective in toxicity reduction than ozonation.

CONCLUSIONS

Bench-scale experiments were performed to investigate the degradation efficiency, mechanism and intermediates' toxicities of OXC by chlorination, ClO_2 oxidation and ozonation. All the degradation profiles obeyed second-order kinetics, and ozonation was the most effective both for OXC reduction and mineralization. Totals of 11, six and 10 degradation intermediates were detected by UPLC-Q-TOF-MS during the oxidation processes of Cl_2, ClO_2 and O_3, respectively. All the oxidation approaches had similar pathways in the *N*-heterocyclic ring cleavage mechanism. However, chlorination and ozonation led to more Cl-substitution by-products and $OH^{\cdot}$ attacking intermediates, respectively, and ClO_2 oxidation brought about fewer degradation by-products. The above-mentioned oxidation by-products according to EPA TEST were predicted to be more toxic than OXC, especially those from chlorination. Further tests of the toxicities of oxidized mixtures to the bioluminescent *Vibrio fischeri* demonstrated the chlorinated samples could lead to the accumulation of toxic transformation products, and ClO_2 oxidation and ozonation had detoxication effects during these processes.

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REFERENCES

Bader, H. & Hoigné, J. 1981 Determination of ozone in water by the indigo method. *Water Research* **15** (4), 449–456.

Bahlmann, A., Brack, W., Schneider, R. J. & Krauss, M. 2014 Carbamazepine and its metabolites in wastewater: analytical pitfalls and occurrence in Germany and Portugal. *Water Research* **57** (15), 104–114.

Chang, E. E., Liu, T. Y., Huang, C. P., Liang, C. H. & Chiang, P. C. 2012 Degradation of mefenamic acid from aqueous solutions by the ozonation and O_3/UV processes. *Separation & Purification Technology* **98** (19), 123–129.

Cui, C. Z., Yang, Z. R., Zhou, Z. X., Cheng, W. H. & Lin, K. F. 2014 Oxidation of antiepileptic drug oxcarbazepine stimulated by thermally activated persulfate. *International Journal of Environmental Technology & Management* **17** (5), 418–429.

Gerrity, D. & Snyder, S. 2011 Review of ozone for water reuse applications: toxicity, regulations, and trace organic contaminant oxidation. *Ozone: Science & Engineering* **33** (4), 253–266.

Hübner, U., Seiwert, B., Reemtsma, T. & Jekel, M. 2014 Ozonation products of carbamazepine and their removal from secondary effluents by soil aquifer treatment – indications from column experiments. *Water Research* **49** (1), 34–43.

International Organization for Standardization 2007 *Water Quality – Determination of the Inhibitory Effect of Water Samples on the Light Emission of Vibrio fischeri (Luminescent Bacteria Test)*. ISO 11348-3 revised version, Austrian Standards Institute, Geneva, Switzerland.

Ismail, M., Khan, H. M., Sayed, M. & Cooper, W. J. 2013 Advanced oxidation for the treatment of chlorpyrifos in aqueous solution. *Chemosphere* **93** (4), 645–651.

Kaiser, E., Prasse, C., Wagner, M., Bröder, K. & Ternes, T. A. 2014 Transformation of oxcarbazepine and human metabolites of carbamazepine and oxcarbazepine in wastewater treatment

and sand filters. *Environmental Science & Technology* **48** (17), 10208–10216.

Kim, Y. H. & Hensley, R. 1997 Effective control of chlorination and dechlorination at wastewater treatment plants using redox potential. *Water Environment Research* **69** (5), 1008–1014.

Li, Z., Fenet, H., Gomez, E. & Chiron, S. 2011 Transformation of the antiepileptic drug oxcarbazepine upon different water disinfection processes. *Water Research* **45** (4), 1587–1596.

Miao, H. F., Cao, M., Xu, D. Y., Ren, H. Y., Zhao, M. X., Huang, Z. X. & Ruan, W. Q. 2015 Degradation of phenazone in aqueous solution with ozone: influencing factors and degradation pathways. *Chemosphere* **119**, 326–333.

Postigo, C. & Richardson, S. D. 2014 Transformation of pharmaceuticals during oxidation/disinfection processes in drinking water treatment. *Journal of Hazardous Materials* **279** (30), 461–475.

Qiao, T., Yu, Z., Zhang, X. & Au, D. W. T. 2011 Occurrence and fate of pharmaceuticals and personal care products in drinking water in southern China. *Journal of Environmental Monitoring* **13** (11), 3097–3103.

Qin, L., Lin, Y. L., Xu, B., Hu, C. Y., Tian, F. X., Zhang, T. Y., Zhu, W. Q., Huang, H. & Gao, N. Y. 2014 Kinetic models and pathways of ronidazole degradation by chlorination, UV irradiation and UV/chlorine processes. *Water Research* **65** (15), 271–281.

Rodríguez-Álvarez, T., Rodil, R., Quintana, J. B., Triñanes, S. & Cela, R. 2013 Oxidation of non-steroidal anti-inflammatory drugs with aqueous permanganate. *Water Research* **47** (9), 3220–3230.

Sharma, V. K. 2008 Oxidative transformations of environmental pharmaceuticals by Cl_2, ClO_2, O_3, and Fe(VI): kinetics assessment. *Chemosphere* **73** (9), 1379–1386.

Sun, Q., Lv, M., Hu, A., Yang, X. & Yu, C. P. 2014 Seasonal variation in the occurrence and removal of pharmaceuticals and personal care products in a wastewater treatment plant in Xiamen, China. *Journal of Hazardous Materials* **277** (30), 69–75.

Sun, J., Luo, Q., Wang, D. H. & Wang, Z. J. 2015 Occurrences of pharmaceuticals in drinking water sources of major river watersheds, China. *Ecotoxicology & Environmental Safety* **117**, 132–140.

Sylvia, E. B., Stuart, W. K. & Gary, L. A. 2000 *Natural Organic Matter and Disinfection By-Products*. American Chemical Society, Washington, DC.

Yang, G., Fan, M. H. & Zhang, G. M. 2014 Emerging contaminants in surface waters in China – a short review. *Environmental Research Letters* **9** (7), 1–13.

Zhang, A., Li, Y. M., Song, Y., Lv, J. & Yang, J. 2014 Characterization of pharmaceuticals and personal care products as *N*-nitrosodimethylamine precursors during disinfection processes using free chlorine and chlorine dioxide. *Journal of Hazardous Materials* **276** (15), 499–509.

Zou, X. M., Chen, J. N., An, D. M., Hao, N. Y., Hong, Z., Hao, X. T., Rao, P. & Zhou, D. 2015 Efficacy of low to moderate doses of oxcarbazepine in adult patients with newly diagnosed partial epilepsy. *Seizure* **29**, 81–85.

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