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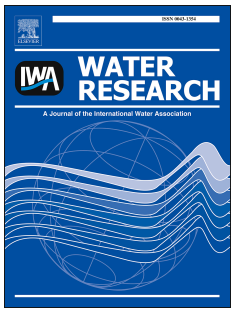

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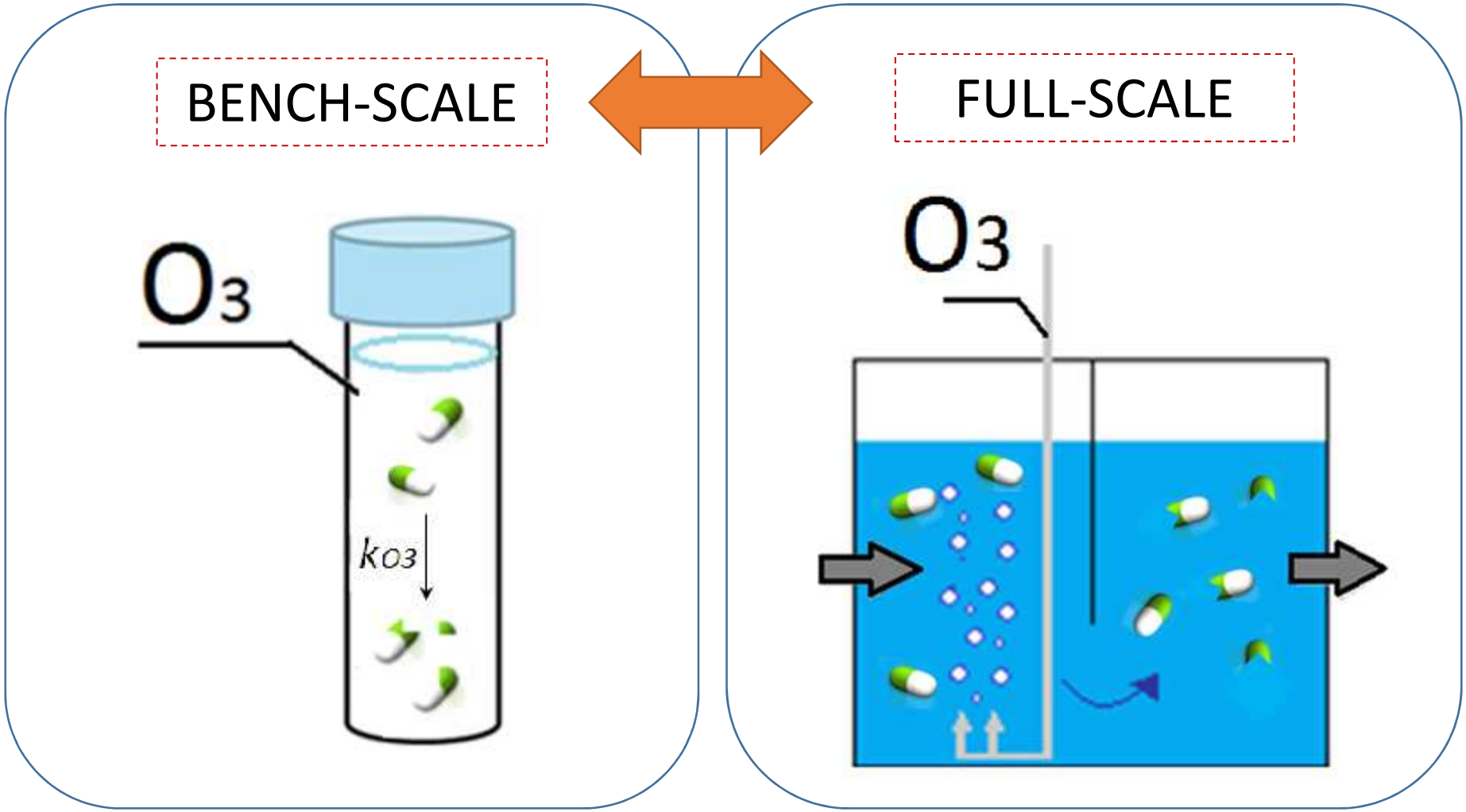
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Oxidation of cetirizine, fexofenadine and hydrochlorothiazide during ozonation: Kinetics and transformation products

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Abstract

The efficiency of wastewater ozonation for the abatement of three nitrogen-containing pharmaceuticals, two antihistamine drugs, cetirizine (CTR) and fexofenadine (FXF), and the diuretic drug, hydrochlorothiazide (HCTZ), was investigated. Species-specific second-order rate constants for the reactions of the molecular, protonated (CTR, FXF) or deprotonated (HCTZ) forms of these compounds with ozone were determined. All three compounds are very reactive with ozone (apparent second order rate constants at pH 7: $k_{O3,pH7}$ = $1.7 \cdot 10^5$ $M^{-1}s^{-1}$, $8.5 \cdot 10^4$ $M^{-1}s^{-1}$ and $9.0 \cdot 10^3$ $M^{-1}s^{-1}$ and, for CTR, HCTZ and FXF, respectively). Transformation product (TP) structures were elucidated using liquid chromatography coupled with high-resolution tandem mass spectrometry, including isotope-labeled standards. For cetirizine and hydrochlorothiazide 8 TPs each and for fexofenadine 7 TPs were identified. The main TPs of cetirizine and fexofenadine are their respective *N*-oxides, whereas chlorothiazide forms to almost 100% from hydrochlorothiazide. In the bacteria bioluminescence assay the toxicity was slightly increased only during the ozonation of cetirizine at very high cetirizine concentrations. The main TPs detected in bench-scale experiments were also detected in full-scale ozonation of a municipal wastewater, for >90% elimination of the parent compounds.

Key words: ozonation, second-order rate constant, kinetics, transformation products, high-resolution mass spectrometry

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1. Introduction

Ozone-based processes for the abatement of micropollutants have been studied intensively in bench-scale (Acero et al. 2000, Dantas et al. 2007, Garoma et al. 2010, Mawhinney et al. 2012, McDowell et al. 2005), pilot-scale (Gerrity et al. 2011, Huber et al. 2005, Lajeunesse et al. 2013, Margot et al. 2013) and a few full-scale systems (Hollender et al. 2009, Nakada et al. 2007) indicating the huge potential of these methods. Unfortunately, ozonation does not cause full mineralization and therefore transformation products (TPs) are formed (von Sonntag and von Gunten 2012). Literature concerning biodegradability of ozonation products is limited, but it is assumed that introduction of oxygen atoms in the molecules potentially leads to an increased biodegradability (Hübner et al. 2015). Therefore, it is often recommended to add biological post-treatment such as sand filtration after ozonation of wastewater effluents (Hollender et al. 2009, Zimmermann et al. 2011). For instance, Hübner and co-workers showed that the primary ozone transformation product of carbamazepine was more effectively biodegraded in sand columns than the parent compound (Hübner et al. 2014). However, as recently concluded in a review, especially oxidation products of nitrogen containing compounds such as *N*-oxides and hydroxylamines are not better biodegradable than the parent compounds (Hübner et al. 2015). Therefore, there is a need for the elucidation of structures of ozonation TPs and their potential ecotoxicological impact on the aquatic environment (Bourgin et al. 2013, Carbajo et al. 2015).

Because ozonation is already used in drinking water treatment and also increasingly in wastewater treatment for micropollutant abatement, detailed knowledge on the kinetics of micropollutant abatement and the formation of TPs is essential. To this end, there is already significant information in literature, which allows to estimate the abatement of selected compounds during wastewater ozonation (von Sonntag and von Gunten 2012). However, with the discovery of new classes of compounds, there is a need for more information on their behavior during ozonation.

This study concentrates on cetirizine and fexofenadine, two antihistamine drugs used for the treatment of allergic reactions, and hydrochlorothiazide - a diuretic drug commonly used in the treatment of hypertension. These compounds are frequently detected in municipal wastewater effluents and natural aquatic systems (Al-Qaim et al. 2014, Bahlmann et al. 2012, Kosonen and Kronberg 2009, Oosterhuis

et al. 2013). Cetirizine was found in wastewater effluents in Germany in concentrations of up to 510 ng L^{-1} (Bahlmann et al. 2012), in river water in Finland in concentrations of up to 9 ng L^{-1} (Kosonen and Kronberg, 2009) and in the San Francisco Bay and the Baltic Sea (German coast line) in concentrations of up to 6 and 13 ng L^{-1} (Nödler et al. 2014). In an extreme case, cetirizine was detected in concentrations of up to 1.2 mg L^{-1} in lake water, in an area with pharmaceutical industry in India (Fick et al. 2009). Fexofenadine was found in municipal wastewater effluent in Finland at a maximum concentration of 100 ng L^{-1}, and its elimination during a biological process was calculated to be 18% (Kosonen and Kronberg 2009). In river water up to 11 ng L^{-1} fexofenadine were measured (Kosonen and Kronberg 2009). Hydrochlorothiazide was detected in very high frequency (>85%) in high concentrations (hundreds of ng L^{-1} to 17.2 µg L^{-1}) in municipal wastewater samples in the Netherlands (Oosterhuis et al. 2013), Spain (Bueno et al. 2012) and Canada (Kim et al. 2014) and the elimination of this compound was demonstrated to be incomplete (0-77%) during conventional biological wastewater treatment (Castiglioni et al. 2006). Hydrochlorothiazide was detected at low ng L^{-1} levels (up to 54 ng L^{-1}) in Malaysian river waters (Al-Qaim et al. 2014).

Information on effects of these substances is scarce. For cetirizine hydrochloride, an EC_{50} of 330 mg L^{-1} was reported for the water flea *Daphnia magna* (Webb 2001) and a 96-h LC_{50} for the flatworm *Dugesia japonica* of 209.5 mg L^{-1} (Li 2013). The lowest concentration causing toxicity of fexofenadine hydrochloride was estimated to be 0.387 mg L^{-1} for *Daphnia magna* and 114 mg L^{-1} for fish (based on ECOSAR Data) (Sanderson et al. 2004). Hydrochlorothiazide elicited effects on algae growth with a 72-h EC_{50} of 34.35 mg L^{-1} (Fernández et al. 2010), whilst the 5-d LC_{25} for zebrafish embryos and larvae was above 1000 µM (300 mg L^{-1}) (Gustafson et al. 2012). However, there is no information concerning effects of their transformation products.

All three pharmaceuticals, cetirizine, fexofenadine and hydrochlorothiazide (Fig. 1), are nitrogen-containing compounds. Cetirizine and fexofenadine contain tertiary amine moieties whereas hydrochlorothiazide has an aniline-like moiety within a saturated ring structure and two sulfonamide groups. According to the pK_a-values recorded in literature, differently charged nitrogen species occur under environmental conditions. This is especially relevant as only the non-protonated nitrogen atoms exhibit enough electron density to react with ozone (von Sonntag and von Gunten 2012). Due to their

94 occurrence in wastewater effluents, the expected high reactivity with ozone and the possible formation
95 of recalcitrant TPs such as *N*-oxides, the three compounds were selected to assess their fate during
96 ozonation. The kinetics of the reaction of ozone with the target compounds and some identified TPs
97 were investigated over a wide pH range to determine species-specific second-order rate constants.
98 Additionally, TPs formed during ozonation were identified by liquid chromatography coupled with
99 high-resolution tandem mass spectrometry to propose reaction mechanisms. Potential ecotoxicological
100 effects of the compounds and their TPs on bacteria were assessed with a bacteria luminescence
101 inhibition assay. The abatement of the selected compounds and the formation of TPs were also
102 investigated in wastewater during full-scale ozonation.

103 **2. Material and methods**

104 **2.1. Chemicals and reagents**

105 All chemicals used in the study were of the highest purity available (Supplementary Information (SI)
106 Text S1). Ozone stock solutions were prepared as described in Text S2.

107 **2.2. Measurement of reaction kinetics**

108 *2.2.1 Ozonation experiments*

109 All kinetic experiments were carried out at 20±2 ºC. Apparent second-order rate constants k_{O3} for the
110 reaction of cetirizine, fexofenadine and hydrochlorothiazide (initial concentration 70 µM) with ozone
111 were determined using competition kinetics (Muñoz and von Sonntag 2000a, von Sonntag and von
112 Gunten 2012) with *trans*-cinnamic acid, *p*-cresol, 1-penten-3-one, 1,4-benzoquinone or orotic acid as
113 competitors (initial concentration 100 µM) (Table S1, Text S3). Kinetic experiments for cetirizine
114 were performed in the pH range 2-11, for fexofenadine in the pH range 7-12, and for
115 hydrochlorothiazide in the pH range 2-12. The pH of the solutions was kept constant by a 40-50 mM
116 phosphate buffer adjusted by 1 M HCl or 1 M NaOH. To verify the buffer capacity, the pH was also
117 measured in ozonated samples and a deviation of <0.1 pH unit was observed. Reactions of hydroxyl
118 radicals ($^{\bullet}$OH) were suppressed by addition of 20-100 mM *tert*-butanol (*t*-BuOH) used as $^{\bullet}$OH
119 scavenger. Similarly, k_{O3} values for highly reactive TPs (cetirizine *N*-oxide, norchlorcyclizine and
120 azacyclonol) were determined at pH 7, using competition kinetics as described above. Second-order
121 rate constants for TPs with low ozone reactivity - 4-chlorobenzophenone, fexofenadine *N*-oxide and

chlorothiazide - were determined under pseudo-first order conditions (Yao and Haag 1991), using ≥ 20-fold excess of ozone relative to the target compound. Further information about this method is given in Text S4.

2.2.2. Analysis by HPLC-DAD

The residual concentrations of the target compounds during kinetic experiments were measured by high performance liquid chromatography with a diode-array detector HPLC-DAD (UltiMate3000, Dionex) using an Atlantis® T3 3µm 3.0 x 150 mm column (Waters). A gradient program was applied with 0.1% formic acid in nanopure water (NPW) and acetonitrile for cetirizine and hydrochlorothiazide and their TPs. Fexofenadine was analyzed using a modified method described elsewhere (Vaghela et al. 2012), with 0.05% triethylamine in NPW and acetonitrile as mobile phases. For information about detection wavelengths and gradient programs see Table S3.

2.3. Identification of TPs

2.3.1. Sample preparation

The experimental solution contained 40 µM of a target compound. $^{\bullet}OH$ were scavenged using 100 mM of *t*-BuOH. The solutions were buffered with 50 mM phosphate at pH 7. The experimental mixtures were ozonated by addition of an aliquot of the ozone stock solution to achieve varying ozone:target compound molar ratios, ranging from an excess of compound (ratio=0.1) to an excess of ozone (ratio=10). The analyses were performed after complete depletion of ozone. Additionally, as a control, non-ozonated samples were prepared, for which the ozone stock solution was replaced by NPW.

2.3.2. Identification of TPs by LC- HRMS/MS

Separation of TPs was achieved with an Atlantis® T3 3µm 3.0 x 150 mm column (Waters). Analytes were eluted with a gradient program using MeOH and water, both acidified with 0.1% formic acid. MS data were acquired by a ThermoScientific™ Q-Exactive™ Hybrid Quadrupole-Orbitrap Mass Spectrometer. MS data were collected in parallel full scan mode (60-700 m/z) at 70'000 resolution, using both positive and negative electrospray ionization. Data were analyzed with Xcalibur™ (Thermo Scientific™, Switzerland) in the Qual Browser. Before injection, samples were spiked with the internal standard of the parent compounds - CTR-d_8, FXF-d_6 or HCTZ-^{13}C,d_2 (Fig S4) - for the

calculation of normalized area, *i.e.* the ratio between the peak area of the analyte of interest and the peak area of the internal standard of the corresponding parent compound. To obtain MS^2 spectra of the potential TPs, ozonated samples were re-analyzed with the same analytical method, and using different collision energies to reveal all relevant signals of the structure fragments. Values of HCD (Higher-energy collisional dissociation) were in the range 10-70 %.

Detailed information about analytical conditions are presented in Table S4.

2.3.3. Non-target screening

The search for unknown TPs was performed by differential analysis. The collected full-scan MS spectra of ozonated samples were analyzed by the SIEVE™ Thermo Scientific software. Non-spiked, ozonated water and non-ozonated solutions of the compounds served as the control samples. By comparison of treated samples (spiked and ozonated) with control samples, the peaks potentially corresponding to TPs were selected. MS^2 spectra of detected peaks were subsequently acquired to propose the structure of TPs.

2.3.4. Structural confirmation by comparison with ozonated labeled compounds

The labeled compounds cetirizine-d_8 and hydrochlorothiazide-$^{13}C,d_2$ (Fig. S4) were ozonated using the same protocol as for non-labeled compounds. The comparison of the MS and MS^2 spectra for TPs formed from labeled and non-labeled compounds, especially the comparison of signal shifts caused by the labeled atoms, can provide additional evidence for the possible structure.

2.4. Quantification and ecotoxicological evaluation of formed TPs in bench-scale experiments

For the quantification of commercially available TPs, solutions containing the parent compounds (CTR, FXF and HCTZ) at a concentration of 35 µM, buffered at pH 7 (50 mM phosphate buffer) were ozonated. $^{\bullet}OH$ were scavenged using 100 mM *t*-BuOH. Aliquots of ozone stock solution were added to reach ozone:target compound ratios of 0.1, 0.2, 0.5, 1, 2, 5, 10 and 20. Compounds (parent compounds and their TPs) were quantified using LC-HRMS (Table S5). Additionally, ecotoxicological effects of samples without ozone and with ozone:target compound ratios of 1, 5, and 20 on bacteria were assessed in a bacteria luminescence inhibition assay as described in SI Text S7.

2.5. Determination of the yields of $^{\bullet}OH$ and chlorothiazide during hydrochlorothiazide ozonation.

To pH 7-buffered solutions of hydrochlorothiazide (1.6 µmol HCTZ in 8 mL NPW with an excess of *t*-BuOH, *i.e.* 400 mM), an amount of 0.07-1.07 µmol ozone was dosed. Under these conditions, $^{\bullet}OH$ reacts preferentially (>99.5%) with *t*-BuOH ($k_{.OH} = 6 \cdot 10^8$ M^{-1} s^{-1}) compared to hydrochlorothiazide ($k_{.OH} = 5.7 \cdot 10^9$ M^{-1} s^{-1}) (Real et al. 2010). The $^{\bullet}OH$ yield was directly determined by quantifying the formation of formaldehyde. Two moles of $^{\bullet}OH$ are produced per mole of formaldehyde (Nöthe et al. 2009). HCTZ and chlorothiazide were quantified using the HPLC-DAD method described in Table S6.

2.6. Analysis of wastewater samples from a full-scale ozonation reactor

The efficiency of ozonation for CTR, FXF and HCTZ abatement was investigated in a full-scale ozonation facility (WWTP Neugut, Dübendorf, Switzerland, population equivalent 105'000) using 4 ozone doses (specific ozone doses), namely, 2 g O_3 m^{-3} (0.35 g O_3 g^{-1} DOC), 3 g O_3 m^{-3} (0.54 g O_3 g^{-1} DOC), 4 g O_3 m^{-3} (0.67 g O_3 g^{-1} DOC) and 5 g O_3 m^{-3} (0.97 g O_3 g^{-1} DOC). Three flow proportional 24-h composite samples on three consecutive dry days were taken from two sampling points: (i) after the secondary clarifier (O_3-INF) and (ii) after the ozone reactor (O_3-EFF). Samples were filtered through two layers of Whatman® glass microfiber filters (bottom layer: GF/F, pore size 0.7 µm, top layer: GF/D, pore size 2.7 µm). To improve the extraction efficiency and reduce matrix interferences, the wastewater samples were diluted with NPW (4-fold for the O_3-INF and 2-fold for the O_3-EFF). All samples were spiked with internal standards (cetirizine-d_8, fexofenadine-d_6, hydrochlorothiazide-$^{13}C,d_2$) and analyzed using online solid-phase extraction coupled to the same LC-HRMS described above (online-SPE LC-HRMS). The analytical method used for the measurement is described elsewhere (Jeon et al. 2013).

3. Results and discussion

3.1. Kinetic experiments

3.1.1. Reactivity of cetirizine with ozone

Cetirizine contains two tertiary amines (Fig. 1a), which are expected to react with ozone. The pK_a values have previously been determined (Tam and Quere 2001) as 2.1 (pK_{a1}), 2.9 (pK_{a2}) and 8.0

204 (pK_{a3}), corresponding to the tertiary amine close to the biphenyl moiety (*N1*), the carboxylic group and
205 a tertiary amine next to the aliphatic chain (*N2*), respectively.
206 At low pH (2-5) no significant change of reactivity was observed (Fig. 1a). In this pH range mainly the
207 *N1*-non-protonated, *N2*-protonated form of CTR is present (Fig. 1a CTR_{PROT}). However, in contrast to
208 a prediction based on the reported pK_{a1} value, which suggests the presence of 50% of *N1*,*N2*-
209 diprotonated CTR at pH 2 (Fig.1a CTR_{DIPROT}), we did not observe a decrease of the apparent second-
210 order rate constant at pH 2. This might be an indication that the actual pK_{a1} value is lower than the
211 reported one. Based on the data at pH < 5 a second order rate constant for the reaction of the CTR_{PROT}
212 can be estimated as $(6.0\pm0.1)\cdot 10^3$ M^{-1} s^{-1} (Table 1). At pH>5, the reactivity of cetirizine with ozone
213 increases due to the presence of the molecular form of *N2*-amine (Fig. 1a CTR_{MOL}), which is assumed
214 to be the most reactive site of CTR. The apparent second-order rate constant for the reaction of CTR
215 with ozone increased to a value of $(1.7\pm0.1)\cdot 10^5$ M^{-1} at pH 7 (Fig. 1a, Table 1). The species-specific
216 rate constant of CTR_{MOL} was determined as $2.8\cdot 10^5$ M^{-1} s^{-1} (Text S5), which is comparable with the
217 experimental values at pH 9 ($(2.8\pm0.1)\cdot 10^5$ M^{-1} s^{-1}) and pH 11 ($(3.4\pm0.1)\cdot 10^5$ M^{-1} s^{-1}).
218 The obtained k_{O3} values for the reaction of CTR with ozone are comparable to other non-protonated
219 tertiary amines such as tramadol ($1.0\cdot 10^6$ $M^{-1}s^{-1}$) and tylosin ($2.7\cdot 10^6$ $M^{-1}s^{-1}$) (Dodd et al. 2006,
220 Zimmermann et al. 2012).
221 The data in Fig. 1a were fitted (line) with a pK_{a3} = 7.0 for the *N2* tertiary amine, which is lower than
222 the previously reported pK_{a3} of 8.0 (Tam and Quere 2001). Our kinetic data from a large pH range
223 point clearly towards this lower pK_a value of the *N2* tertiary amine group (Text S5, Fig. S1a).
224 *3.1.2. Reactivity of fexofenadine with ozone*
225 Fexofenadine contains a tertiary amine group, which is the most probable site of ozone attack. The pK_a
226 values reported for fexofenadine are 4.2 and 9.5, for the carboxylic and the amine group, respectively
227 (Ming et al. 2011, Yasui-Furukori et al. 2005). The carboxylic group is not relevant for ozone attack,
228 wherefore the FXF reactivity with ozone was investigated only in the pH range 7-12 (Fig. 1b). The k_{O3}
229 value determined at pH 12 for fexofenadine was $(5.7\pm0.1)\cdot 10^5$ M^{-1} s^{-1} (Table 1), which is in agreement
230 with the value of $5.6\cdot 10^5$ M^{-1} s^{-1} obtained for the species-specific rate constant of the molecular form of
231 FXF (Fig. 1b FXF_{MOL}, Text S5). The data suggests that the pK_a value of the amine group of

fexofenadine is lower than previously reported. The determined apparent second-order rate constants shown in Fig. 1b were better modeled by a pK_a of 9.0 (Fig. S1b), which is below the previously reported value of 9.5 (Ming et al. 2011, Yasui-Furukori et al. 2005). It has to be emphasized that in none of these publications, any details on the determination of the pK_a values were provided.

At pH 7 k_{O3} of FXF was determined as $(9\pm0.1)\cdot 10^3$ M^{-1} s^{-1} (Table 1). At this pH, 1% of FXF is still in molecular form (assuming a pK_a of 9.0), contributing strongly to the k_{O3} value. k_{O3} for the protonated FXF (Fig. 1b FXF_{PROT}) should be considerably lower when comparing to other protonated tertiary amines (e.g k_{O3} = 77 $M^{-1}s^{-1}$ for tramadol (Zimmermann et al. 2011), 5 $M^{-1}s^{-1}$ for triethylamine (Pryor et al. 1984).

3.1.3. Reactivity of hydrochlorothiazide with ozone

Hydrochlorothiazide contains one aniline-like moiety and two sulfonamide (cyclic and free) groups (Fig. 1c). The free sulfonamide group (S^2) does not react with ozone (von Sonntag and von Gunten 2012). The reported pK_a values of hydrochlorothiazide show considerable variability (Hennig et al. 1981, Mollica et al. 1971, Vujić et al. 2012). The pK_a of aniline (Ph-NH_2/PhNH^-) was reported to be in the range 9.0-11.0 while the pK_a of the cyclic sulfonamide (R-SO_2NH_2/R-SO_2NH^-) was estimated to be in the range of 6.5-8.5.

Apparent second-order rate constants for the reaction of hydrochlorothiazide with ozone were determined in the pH range 2-12. At pH>11, apparent second-order rate constants remained constant at $(5.1\pm0.1)\cdot 10^5$ M^{-1} s^{-1} (Fig. 1c $HCTZ_{DIDEPROT}$), reflecting the rate constants of the completely deprotonated molecule (both the sulfonamide and the aniline-like moiety are deprotonated; Table 1). From pH 11 to pH 8, the apparent second-order rate constants decreased by a factor of 2 to a value of $\sim 3\cdot 10^5$ M^{-1} s^{-1} (Fig. 1c), indicating that the protonation of the negatively-charged aniline moiety affects the reactivity of hydrochlorothiazide with ozone only slightly. At pH<8, the k_{O3} value decreased significantly to 590 (± 70) M^{-1} s^{-1} at pH<3.5, showing that a protonation of the N-atom in the cyclic sulfonamide group (S^1, Fig.1c $HCTZ_{MOL}$) contributes distinctly to the overall reactivity of hydrochlorothiazide with ozone. In a previous publication (Real et al. 2010) apparent second-order rate constants for the reaction of hydrochlorothiazide with ozone were determined at four pH values (3, 5, 7 and 9). However, the values they obtained were about one order of magnitude lower than the

values presented in this paper. The second-order rate constants reported in the aforementioned paper were determined using the competition method in a semi-batch reactor with metoprolol as a competitor. The complication with ozone mass transfer and the use of a different competitor might explain the discrepancies between the second order rate constants presented in both studies.

Based on the previous kinetic data, species specific second-order rate constants were calculated to be 590 (± 70) M^{-1} s^{-1} for the $HCTZ_{MOL}$, ~$3 \cdot 10^5$ M^{-1} s^{-1} for $HCTZ_{DEPROT}$ and 5.1 (±0.1)$\cdot 10^5$ M^{-1} s^{-1} for the $HCTZ_{DIDEPROT}$ (Fig. 1c, Table 1).

The pH dependence of the apparent second-order rate constants for the reaction of hydrochlorothiazide with ozone was best modeled by pK_a values of the cyclic sulfonamide (S^1) and the aniline moieties aof 7.0 and 10.5, respectively (Fig. 1c), which is in the range of the previously reported pK_a values.

Detailed results on the kinetics of HCTZ oxidation by ozone are compiled in the Table S2.

3.2. Identification of TPs

3.2.1. TPs of the reaction of cetirizine with ozone

Eight TPs of cetirizine ozonation were detected in the screening (Fig. 2a). As expected, the tertiary amine groups were the main sites of ozone attack. *N*-oxide formation has been previously reported for the reaction of tertiary amines with ozone (Muñoz and von Sonntag 2000b, Zimmermann et al. 2012). Due to the presence of two tertiary amines in the structure of cetirizine, *N*-oxidation may occur at two sites. Based on the information provided by MS^2 spectrum of CTR-TP1 (Fig. 2a), it was impossible to distinguish which *N*-oxide was formed. However, as discussed above, kinetic data showed that the *N2*-amine is more reactive than the *N1*-amine. Therefore cetirizine *N2*-oxide is more likely formed than *N1*-oxide. Additionally the available standard of cetirizine *N2*-oxide had a similar HPLC RT and MS^2 spectrum as CTR-TP1 (Fig. S11) which also confirmed the kinetics-based assumption about the structure of CTR-TP1.

The oxidation of CTR with higher ozone:CTR ratios resulted in the formation of a molecule with a *m/z* signal shifted by 15.995 Da from CTR-TP1, corresponding to an additional oxygen in the structure (CTR-TP5). A *N*-oxidation at the N1 position can be proposed, leading to the formation of CTR-*N,N'*-dioxide. This hypothesis was also supported by ozonation experiments with CTR-*N*-oxide as starting compound. While the signal of CTR-*N*-oxide decreased, the formation of CTR-TP5 (CTR-*N,N'*-

dioxide) was observed (Fig. S6). Alternatively to an *N*-oxidation, tertiary amines can undergo a dealkylation, though this reaction has generally a much lower efficiency (Zimmermann et al. 2012). Nevertheless, signals corresponding to dealkylated TPs were detected. A dealkylation of the tertiary amine groups resulted in the formation of a secondary amine and a carbonyl-containing molecule. In the case of cetirizine, a disruption of the C-N bond (position *N1*) resulted in the formation of CTR-TP2 (4-chlorobenzophenone, 4-CBP) and CTR-TP3 (Fig. 2a). The presence of CTR-TP2 could be confirmed by an available standard (Fig. S12). Furthermore, during the ozonation of CTR-d_8, a compound with a similar HPLC RT and the same *m/z* value as in the ozonated non-labeled CTR samples was observed, which confirmed that CTR-TP2 most likely contained the aromatic moiety of cetirizine. CTR-TP3, which contains one secondary amine and one tertiary amine group, can be further oxidized at the *N*-atoms to form the corresponding *N*-oxide (CTR-TP6) and/or a hydroxylamine (CTR-TP7) (*m/z* signal shift of 15.995 Da from CTR-TP3). Another TP (CTR-TP8) with an additional increase of the *m/z* value of 15.995 Da compared to CTR-TP6/7 was detected, suggesting formation of the corresponding *N*-oxide,*N*'-hydroxylamine (CTR-TP8, Fig. 2a). All these oxidative transformations were supported by observed signal shifts of 8.0509 Da in the CTR-d_8 TPs. Dealkylation at the *N2*-atom of cetirizine resulted in the formation of CTR-TP4 (norchlorcyclizine, NCC), which was also confirmed by comparison with a commercial standard (Fig. S13). This compound could be further oxidized at the tertiary amine group. However, the signal of CTR-TP4 was low, which could be explained by a transient character of this compound. A more detailed discussion about the significance of the different ozone reactions is provided in section 3.4. Detailed information on CTR-TPs is provided in Table S7. MS^2 spectra of CTR TPs without commercial standards are presented in Figs. S17-S20. A discussion about using labeled compounds for structure confirmation is presented in Text S8.

All TPs were proposed based on knowledge of ozonation mechanisms and classified by confidence levels according to Schymanski et al. (2014). CTR-TP1, CTR-TP2 and CTR-TP4 are assigned to confidence level 1 (confirmation with commercial standard), CTR-TP3, CTR-TP5 and CTR-TP8 have a confidence level 2b (probable structure based on MS^2 spectra interpretation, information from ozonation of labeled compound and experimental context), CTR-TP6 and CTR-TP7 have a confidence

level 3 (tentative candidates based on MS^2 spectra interpretation, information from ozonation of labeled compound and experimental context).

3.2.2. TPs of the reaction of fexofenadine with ozone

Seven fexofenadine TPs were identified (Fig. 2b). One of the most prominent ozone TPs (FXF-TP1) was found to be fexofenadine *N*-oxide (FXF-*N*-oxide). The structure was confirmed by comparison with the commercial standard FXF-*N*-oxide (Fig. S14). A difference of 2.0158 Da in FXF-TP4 compared to FXF-TP1 could be explained by the oxidation of a hydroxyl group to a ketone (loss of two hydrogen atoms). Further evidence for the formation of FXF-TP4 from FXF-TP1 is provided with the ozonation of a standard of FXF-*N*-oxide by various ozone:target compound ratios (Fig. S8). However, this reaction is very slow (von Sonntag and von Gunten 2012) and is rather unlikely to happen under realistic ozonation conditions with ozone.

Apart from an *N*-oxidation, dealkylation of FXF was also observed. The disruption of the N-C bond in the heterocycle resulted in the formation of FXF-TP2, for which the structure was confirmed by comparison with a commercial standard (azacyclonol ACC, Fig. S15). FXF-TP2, as a secondary amine, could be further oxidized yielding TPs with the *m/z* signal shifted by 15.995 Da (an additional oxygen atom). For this signal, two TPs were proposed: FXF-TP5, a hydroxylamine, and FXF-TP6, a molecule where the N-C bond is cleaved, with an aldehyde formation after ring opening. The second transformation is less likely than hydroxylation. Data provided by the MS^2 spectrum of the signal 284.1644 *m/z* were not sufficient to confirm the structure of the corresponding TPs.

FXF-TP3, for which a primary amine was proposed, resulted from di-dealkylation of FXF. For FXF-TP7, which contained an oxygen more than FXF-TP3, a hydroxylamine structure is proposed. However, the formation of a primary amine is rather unlikely and has a minor relevance in comparison to *N*-oxidation. This is discussed in more detail in section 3.4.

Detailed information about FXF TPs is presented in Table S7 and their structures are proposed based on the knowledge of ozone chemistry. MS^2 spectra of FXF TPs without commercial standards are presented in SI Fig. S21-S24. FXF-TP1 and FXF-TP2 belong to confidence level 1, whereas the rest of the proposed TPs (FXF-TP3-TP7) correspond to level 3.

3.2.3. TPs of the reaction of hydrochlorothiazide with ozone

Screening provided information about eight TPs of hydrochlorothiazide for which structures were proposed (Fig. 2c).

For HCTZ-TP1, chlorothiazide (CTZ) was proposed based on MS^2 spectra. The standard of chlorothiazide showed the same HPLC RT and HRMS/MS spectra as HCTZ-TP1 (Fig. S16). Chlorothiazide was already assumed to be formed from hydrochlorothiazide during photodegradation (Brigante et al. 2005) and biotransformation in river sediments (Li et al. 2014).

During ozonation of hydrochlorothiazide, the $^{\bullet}OH$ yield was determined to be 37 ± 1% (Fig. 3). Chlorothiazide formation was proposed to consist of two successive electron-transfer reactions (Fig. 4a). In this mechanism, considering the high selectivity of the reaction (> 99%, as described later in the section 3.4.3.), 2 moles of ozone are consumed per mole of HCTZ removed, thereby producing 1 mole of chlorothiazide and 2 moles of $^{\bullet}OH$ from the decomposition of the ozonide radical anion ($O_3^{\bullet-}$) and the reaction of ozone with superoxide ($O_2^{\bullet-}$) (reactions (1-4)).

$$O_3^{\bullet-} \rightleftarrows O_2 + O^{\bullet-} \quad (1)$$

$$O^{\bullet-} + H_2O \rightleftarrows {}^{\bullet}OH + OH^- \quad (2)$$

$$HO_2^{\bullet} \rightleftarrows O_2^{\bullet-} + H^+ \quad (3)$$

$$O_2^{\bullet-} + O_3 \rightleftarrows O_2 + O_3^{\bullet-} \quad (4)$$

Considering the $^{\bullet}OH$ yield, the electron-transfer mechanism accounts only for about 18.5% of the formed of CTZ. Another mechanism (81.5%) involving only one mole of ozone without $^{\bullet}OH$ production, is proposed by the formation of a hydroxylamine and the subsequent loss of a hydroxyl ion to form the corresponding imine (oxygen transfer pathway, Fig.4b). Considering the respective yield and the number of ozone molecules involved in each mechanism, 1.2 moles ozone should be consumed per mole of HCTZ. However, Fig. 3 shows that 1.5 moles of ozone were consumed per mole of HCTZ removed. The difference may be explained by a catalytic destruction of ozone by nitrogen- and carbon-centered radicals as presented in Fig. 4a (von Sonntag and von Gunten, 2012).

The seven other hydrochlorothiazide TPs were also formed when chlorothiazide was ozonated., confirming that chlorothiazide is a key intermediate during hydrochlorothiazide ozonation (Fig. 2c).

The proposed chemical formula of HCTZ-TP2 differed from HCTZ-TP1 only by the loss of one nitrogen and one hydrogen atom and by the addition of one oxygen atom (mass shift of *m/z* 0.9838). HCTZ-TP2 was therefore suspected to be the sulfonate analogue of HCTZ-TP1. This assumption could be confirmed by the MS^2 fragmentation of both compounds: while the HCD fragmentation of HCTZ-TP1 induced the formation of a SNO_2^- fragment ion (*m/z* 77.96502), the formation of a SO_3^- fragment ion (*m/z* 79.95686) was observed during the fragmentation of HCTZ-TP2.

HCTZ-TP3 differed from hydrochlorothiazide by the addition of one oxygen atom. Hydroxylation or heteroatomic ring opening/amide formation were suspected to occur, to explain the formation of HCTZ-TP3. Ozonation experiments were carried out with hydrochlorothiazide labeled on the methylene bridge with one ^{13}C and two deuterium (HCTZ-$^{13}C,d_2$, Fig. S4c) atoms between the aniline moiety and sulfonamide group (S^1). The resulting TP3 showed a mass shift of one ^{13}C and one deuterium compared to non-labeled hydrochlorothiazide, indicating that the site of ozone attack was located at the alkyl group. Therefore, a hydroxylation of the aromatic ring was ruled out in this case. The MS^2 experiments showed the loss of CO (*m/z* 27.995) from the parent ion of HCTZ-TP3, further pointing to the formation of an amide group.

Transformation product HCTZ-TP5 was formed by addition of 2 oxygen atoms and loss of 2 hydrogen atoms compared to HCTZ-TP3. HCTZ-TP5 might be formed from the oxidation of the aromatic ring in HCTZ-TP3 resulting in a 1,4-benzoquinone derivative (see Text S6, Fig. S2).

HCTZ-TP7 was proposed to be a compound with only 6 carbon atoms, instead of 7 carbons for HCTZ-TP5. The ozonation of the non-labeled and the labeled HCTZ induced the formation of the same compound as HCTZ-TP7, without any mass shift from the isotopes. Therefore, this result confirmed that the carbon atom lost during the oxidation is the carbon at the heteroatomic ring.

In analogy to HCTZ-TP1 and HCTZ-TP2, the TPs HCTZ-TP4, HCTZ-TP6 and HCTZ-TP8a/b were assumed to be the sulfonate analogues of HCTZ-TP3, HCTZ-TP5 and HCTZ-TP7, respectively.

Detailed information concerning identified HCTZ TPs is presented in Table S7 and their structures are proposed based on the knowledge of ozone chemistry. HCTZ-TP1 was assigned to confidence level 1, HCTZ-TP2 and HCTZ-TP3 have confidence level 2b, whereas the rest of the proposed TPs (HCTZ-

TP4- HCTZ-TP8a/b) correspond to level 3. MS^2 spectra of HCTZ TPs without commercial standards are presented in SI Fig. S25-S32.

3.3. Reactivity of TPs with ozone

3.3.1. Cetirizine TPs

Apparent second-order rate constants at pH 7 for the reaction of ozone with the TPs with available commercial standards were also determined (Table 1). For CTR-*N*-oxide (CTR-TP1, Fig. 2a) the apparent second-order rate constant of $(8.3\pm0.1)\cdot 10^3$ $M^{-1}s^{-1}$ was determined, which is significantly lower than the corresponding rate constant for CTR ($(1.7\pm0.1)\cdot 10^5$ $M^{-1}s^{-1}$). 4-chlorobenzophenone (CTR-TP2, Fig. 2a) has no ozone-reactive moieties (ketone and inactivated benzene rings), explaining its very low reactivity (k_{O3} = (0.40±0.05) $M^{-1}s^{-1}$). The apparent second-order rate constant of NCC (CTR-TP4, Fig. 2a) at pH 7 was determined to be $(2.1\pm0.1)\cdot 10^4$ $M^{-1}s^{-1}$, reflecting the reaction of ozone with a tertiary amine. At pH 7, the secondary amine is protonated, since the pK_a values corresponding to the tertiary and the secondary amine of NCC are predicted with the ChemAxon software (http://www.chemicalize.org) as 3.9 and 9.2, respectively.

3.3.2. Fexofenadine TPs

The apparent second-order rate constant for the reaction of ozone with FXF-*N*-oxide (FXF-TP1, Fig. 2b) at pH 7 was ~6.0±2.0 $M^{-1}s^{-1}$ (Table 1). This value is substantially lower than for the parent compound ($k_{O3,FXF}$ = $(9.0\pm0.1)\cdot 10^3$ $M^{-1}s^{-1}$). The apparent second-order rate constant at pH 7 for the reaction of ozone with ACC (FXF-TP2, Fig. 2b) containing a secondary amine was 350±10 $M^{-1}s^{-1}$ (Table 1). This is in a similar range as apparent second-order rate constants for the reaction of protonated secondary amines with ozone at pH 7 (*e.g.*, metoprolol $2\cdot 10^3$ $M^{-1}s^{-1}$, atenolol $1.7\cdot 10^3$ $M^{-1}s^{-1}$, (Benner et al. 2008, Benner and Ternes 2009). The value of $k_{O3,ACC}$ is lower than $k_{O3,FXF}$, based on the fact that secondary amines are less reactive towards ozone than tertiary amines (von Sonntag and von Gunten, 2012).

3.3.3. Hydrochlorothiazide TPs

The k_{O3} of CTZ (HCTZ-TP1, Fig. 2c) at pH 7 was determined to be 1.5±0.1 $M^{-1}s^{-1}$, whereas under the same conditions the k_{O3} of HCTZ is $8.5\cdot 10^4$ $M^{-1}s^{-1}$ (Table 1). This difference of 5 orders of magnitude

indicates that the imine group in the chlorothiazide structure strongly deactivates this compound compared to hydrochlorothiazide.

3.4. Evolution of TPs at different ozone to target compound ratios

3.4.1. Evolution of cetirizine TPs

Complete depletion of CTR was observed for ozone:CTR molar ratios > 2 (Fig. 5a). CTR-*N*-oxide was the most prominent TP of cetirizine for ozone:CTR ratio ≤2. According to mass balance, CTR was completely converted into CTR-*N*-oxide and, to a lower extend, to 4-CBP, up to ozone:CTR ratio of 1. For a molar excess of ozone relative to cetirizine, the *N*-oxide decreased with the formation of other TPs as outlined above.

4-CBP (CTR-TP2) increased with increasing ozone:CTR ratios, however, less pronounced than CTR-*N*-oxide (CTR-TP1). At an ozone:CTR molar ratio of 10, 35% of the initial concentration of CTR was transformed into 4-CBP. The accumulation of 4-CBP is expected from its low apparent second-order rate constant at pH 7 (see above). In all ozonated samples, NCC (CTR-TP4) was below LOQ, which indicates that this branch of the ozonation pathway (dealkylation at the N-atom of the heterocycle) is not relevant.

Due to the lack of commercial standards the rest of CTR-TPs could only be determined semi-quantitatively, based on the normalized peak area of the TPs divided by the peak area of CTR-d_8. CTR-*N*-oxide oxidation led to the formation of CTR-*N,N'*-dioxide (CTR-TP5, Fig. S5, Fig. S6 see section 3.2.1.). While the normalized area of CTR-*N*-oxide decreased, the normalized area of CTR *N,N'*-dioxide (CTR-TP5) increased. This confirms that this TP belongs to a second generation of cetirizine TPs. In Fig. S5 the change of the normalized area of CTR-TP6/7 as a function of the O_3 dose is illustrated. In all samples its normalized area was low, which demonstrates that de-alkylation of CTR-*N*-oxide is a minor pathway compared to *N*-oxidation of the two tertiary amine moieties.

CTR-TP3 was formed 1:1 with the formation of 4-CBP (CTR-TP2) (Fig. 2a). In contrast to 4-CBP, CTR-TP3 was quite reactive with ozone leading quickly to the next generation of TPs, namely TP6/7 (Fig. S5), subsequently oxidized to CTR-TP8.

3.4.2 Evolution of fexofenadine TPs

Complete depletion of FXF was observed for ozone:FXF molar ratios ≥2 (Fig. 5b). Based on the mass balance, the formation yield of FXF-*N*-oxide from FXF was determined to be almost 100% up to an ozone:FXF ratio of 0.5. Additionally, based on the mass balance, other FXF TPs are formed at ozone:FXF ratios ≥0.5. Unfortunately, due to the lack of commercial standards, they could not be quantified.

For higher ozone:FXF ratios, FXF-*N*-oxide was further oxidized to the FXF-TP4 (see section 3.2.2), which could be determined semi-quantitatively (Fig. S7, Fig. S8). ACC (FXF-TP2) was not formed in high concentrations (< 1μM for all ozone:FXF ratios), which suggests that *N*-dealkylation in the heterocycle is a minor reaction pathway during ozonation. The normalized areas of the peaks corresponding to FXF-TP5 and FXF-TP6, which were proposed as ACC-TPs, were small (data not shown). Therefore, their formation is highly unlikely under realistic conditions. Similarly, semi-quantitative determinations of FXF-TP3 and FXF-TP7 showed that these compounds are formed to a negligible extent.

3.4.3. Evolution of hydrochlorothiazide TPs

Fig. 5c shows that a complete depletion of the hydrochlorothiazide was observed for ozone:HCTZ molar ratios ≥ 2 with an almost quantitative formation (>99%) of CTZ (HCTZ-TP1, Fig 3, inset). For ozone:HCTZ molar ratios >2 an abatement of CTZ was observed, suggesting that CTZ was further oxidized with the formation of a second generation of TPs. However, as shown above, this reaction was very slow (k_{O3}= 1.5±0.1 M^{-1} s^{-1} at pH 7) and is hence not relevant under realistic conditions. Additional ozonation experiments with CTZ as parent compound led to the formation of the 7 other identified TPs (Fig. 2, Fig. S10). As no standards are available for these TPs, only a semi-quantitative analysis was possible (see section 3.4.1) (Fig. S9). HCTZ-TP2, the second predominant HCTZ-TP, started to increase significantly for ozone:HCTZ ratios≥2, simultaneously with the decrease of chlorothiazide (HCTZ-TP1). This could indicate that HCTZ-TP2 was formed directly from CTZ (HCTZ-TP1, Fig. 2c). HCTZ-TP3 increased at the lowest ozone:HCTZ molar ratio (up to stoichiometric conditions), and subsequently decreased with increasing ozone doses, showing the relatively significant reactivity of this compound with ozone and the formation of the next generation

TPs. HCTZ-TPs 4-8 increased as a function of the ozone:HCTZ molar ratio, but their normalized areas were very low, indicating that these reactions have minor relevance.

3.5. Ecotoxicological effects of cetirizine, fexofenadine and hydrochlorothiazide and their TPs in bacteria bioluminescence tests

No effects of the parent compounds on bacteria luminescence were detected at 31-38 µM. Cetirizine samples treated with ozone elicited biological effects on bacteria bioluminescence with a 50% effect concentration of 10.2 µM CTR (initial concentration, 4.0 mg L^{-1}) and a 10% effect concentration of 1.1 µM (0.4 mg L^{-1}) at an ozone:CTR ratio of 5 (Fig. S3). Taking the results discussed in section 3.4.1 (Fig. 5a) for the high excess of ozone into account, no CTR or CTR-*N*-oxide was present anymore in these samples. This could indicate that the observed toxicity was caused by 4-CBP or by other CTR TPs, which could not be confirmed with commercial standards.

Ozone-treated fexofenadine and hydrochlorothiazide samples did not exceed a 10% inhibition at the highest concentrations tested. This clearly indicates that parent compounds and the corresponding transformation products are not harmful towards the tested bacteria. For all three compounds, the effect concentrations are well above environmentally relevant concentrations in the ng L^{-1} or low µg L^{-1} range (see introduction).

Additional bioluminescence tests were also performed on wastewater effluent, showing a decrease of the toxicity of up to 71% after ozonation. These results are discussed in detail elsewhere (McArdell et al., 2015).

3.6. Determination of CTR, FXF, HCTZ and their TPs during ozonation of real wastewater samples

Analyses of wastewater samples from the WWTP Neugut (Switzerland) showed that the investigated compounds of this study are not completely removed during activated sludge treatment. They are present in the effluent of the secondary clarifier, at the influent to the ozone reactor (O_3-INF), at average concentrations of approximately 50 ng L^{-1}, 150-500 ng L^{-1} and 1000 ng L^{-1} for CTR, FXF and HCTZ, respectively (Table 2). As expected from their reactivity, ozonation resulted in significant abatement of these compounds. A comparison of the concentration in a sample before (O_3-INF) and after ozonation (O_3-EFF) showed that an application of a low ozone dose of 2.03±0.15 g O_3 m^{-3}

506 (0.35±0.02 g O_3 g^{-1} DOC) caused an elimination of 92%, 86% and 83% for CTR, HCTZ and FXF,
507 respectively (Table 2). This is in agreement with the observed apparent second-order rate constants at
508 circumneutral pH ($k_{O3,CTR} > k_{O3,HCTZ} > k_{O3,FXF}$). An increase of the ozone dose to 3.00±0.09 g O_3 m^{-3}
509 (0.54±0.05 g O_3 g^{-1} DOC) resulted in a higher elimination of 90-99% for all investigated compounds.
510 Concomitant to the abatement of the parent compounds, the formation of TPs was investigated. From
511 the three CTR-TPs confirmed with commercial standards in the laboratory studies, cetirizine *N*-oxide
512 (CTR-*N*-oxide), 4-chlorobenzophenone (4-CBP) and norchlorcyclizine (NCC), only CTR-*N*-oxide was
513 found at a low concentration (4±2 ng L^{-1}), only for the lowest ozone dose (Table 2). The formation
514 yield of CTR-*N*-oxide was lower than 10%, which is significantly lower than in the laboratory studies.
515 Higher ozone doses might have caused further oxidation of CTR-*N*-oxide to the dioxide and therefore
516 its concentration was below LOQ. A possible explanation for the absence of the TPs at higher ozone
517 doses is their reaction with $^{\bullet}$OH, which are formed from ozone decomposition (von Sonntag and von
518 Gunten 2012). This may lead to different products, which were not identified in the current study
519 where $^{\bullet}$OH were scavenged. The extent of direct reaction with ozone or oxidation by $^{\bullet}$OH is controlled
520 by the water matrix and has been discussed in the literature (Lee et al., 2013, Lee et al., 2014).
521 None of the other cetirizine TPs was detected in ozonated wastewater samples.
522 FXF-*N*-oxide was identified in ozonated wastewater at a concentration of 141 ng L^{-1} at an ozone dose
523 of 2.72±0.13 g O_3 m^{-3} (0.54±0.04 g O_3 g^{-1} DOC), corresponding to a yield of FXF-*N*-oxide formation
524 from FXF of ~35%. The other proposed TPs from above were not detected.
525 CTZ was already detected in the influent of the WWTP as it is also used as a pharmaceutical
526 compound, and at the O_3-INF an average concentration of 55 ng L^{-1} was still observed (Table 2). A
527 significant formation of CTZ in wastewater was observed during ozonation with a concentration of up
528 to 436 ± 13 ng L^{-1} in the O_3-EFF for the lowest ozone dose (2.03±0.15 g O_3 m^{-3} = 0.35±0.02 g O_3 g^{-1}
529 DOC) with a yield of about 40% from HCTZ. With increasing ozone doses, the concentration of CTZ
530 decreased (Table 2), suggesting a further oxidation of CTZ by $^{\bullet}$OH and a formation of second
531 generation TPs. This was confirmed by the detection of HCTZ-TP3, -TP4, -TP5 and -TP7 in O_3-EFF
532 in addition to CTZ (data not shown).

4. Conclusions

- Apparent second-order rate constants for the reactions of cetirizine (CTR), fexofenadine (FXF) and hydrochlorothiazide (HCTZ) with ozone at pH 7 are high ($k_{O3,pH7}$ of $1.7 \cdot 10^5$ $M^{-1}s^{-1}$, $9.0 \cdot 10^3$ $M^{-1}s^{-1}$ and $8.5\ 10^4$ $M^{-1}s^{-1}$, for CTR, FXF and HCTZ, respectively) warranting an efficient abatement of these compounds during ozonation of wastewater effluents.
- Ozone TPs were determined and the mechanisms for the formation of 8 TPs of cetirizine and hydrochlorothiazide, respectively, and 7 TPs of fexofenadine, were elucidated.
- *N*-oxides were quantified with high yields as the primary TPs for the tertiary amines of CTR and FXF.
- Chlorothiazide was shown to be the main ozone transformation product of HCTZ, being formed partly by an electron transfer mechanism (18.5%) and mainly an oxygen transfer mechanism (81.5%).
- Ecotoxicological evaluation with bacteria bioluminescence showed only for the ozonation products of cetirizine slightly increased effects compared to the parent compound. However, the effect concentrations are well above environmentally relevant concentrations.
- Investigations of ozonation performed on a full-scale municipal wastewater treatment plant confirmed the high efficiency of the abatement of the three target compounds. Application of the lowest studied ozone dose (2 g O_3 m^{-3}, 0.35 g O_3 g^{-1} DOC) resulted in an elimination of 92%, 86% and 83% for CTR, HCTZ and FXF, respectively, which is in agreement with the observed apparent second-order rate constants at circumneutral pH ($k_{O3,CTR} > k_{O3,HCTZ} > k_{O3,FXF}$). Concomitantly, the formation of the main TPs, CTR-*N*-oxide, FXF-*N*-oxide and CTZ, was observed.

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SUPPORTING INFORMATION

Additional information presented in Text S1-S8, Tables S1-S7 and Fig. S1-S32 are shown in the Supplementary Information.

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739 Figures captions

740 Fig. 1. pH dependence of the apparent second-order rate constants for the reaction of ozone with (a)
741 cetirizine (CTR), (b) fexofenadine (FXF), (c) hydrochlorothiazide (HCTZ), at T=20 ^{o}C, $[CTR]_0$ =
742 $[FXF]_0$ = $[HCTZ]_0$ = 40 µM. Values of k_{O3} were determined by competition kinetics using the
743 following competitors: (♦) *p*-cresol; (●) *trans*-cinnamic acid; (▲) 1,4-benzoquinone; (■) orotic acid.
744 The lines correspond to the calculated pH-dependence of the apparent second-order rate constants (see
745 Table 1) using for (i) cetirizine, pK_{a1}=2.1, pK_{a3}=7.0, (ii) fexofenadine, pK_{a2}=9.0, (iii)
746 hydrochlorothiazide, pK_{a1} = 7.0, pK_{a2} = 10.5.

747 Fig. 2. Ozonation pathways for (a) cetirizine (CTR), (b) fexofenadine (FXF) and (c)
748 hydrochlorothiazide (HCTZ). TPs marked with dashed frames are available as commercial standards,
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750 compound molar ratio 0.1-10).

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757 Fig. 5. Abatement of (a) cetirizine (CTR), (b) fexofenadine (FXF) and (c) hydrochlorothiazide
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759 compound molar ratios of 0.1-10 (pH 7, T=20 ^{0}C, [*t*-BuOH]=100 mM). For all TPs shown commercial
760 standards were available. For evolution of TPs without standards see Figs. S5-S10.

761

762 Tables captions

763 Table 1

764 Species-specific and apparent second-order rate constants for the reaction of ozone with the parent
765 compounds and with selected TPs (T=20 ^{0}C, [*t*-BuOH] =20-100 mM).

766 Table 2

767 Average concentrations (± standard deviation) (ng L^{-1}), % elimination of the target compounds
768 cetirizine, fexofenadine and hydrochlorothiazide and the formation of TPs during full-scale ozonation
769 at WWTP Neugut, Dübendorf, Switzerland (pH 7.4-7.9, T=18.2-22.3 ^{0}C, 0.01 - 0.04 mg NO_2^-N L^{-1},
770 DOC= 4.7-5.7 mg L^{-1}, alkalinity 4-5 mM).

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Table 1 Species-specific and apparent second-order rate constants for the reaction of ozone with the parent compounds and with selected TPs (T=20 ^{0}C, [*t*-BuOH] =20-100 mM).

Compound		Type	Method	pH	k_{O3} $M^{-1}s^{-1}$
Cetirizine	CTR	Parent	Competition kinetics with *trans*-cinnamic acid	7	$(1.7\pm0.1)\cdot 10^5$
	protonated [a]		Competition kinetics with *p*-cresol	2	$(6.0\pm0.1)\cdot 10^3$
	molecular [a]		Competition kinetics with *p*-cresol and *trans*-cinnamic acid	2-11	$2.8\cdot 10^5$ [b]
Cetirizine *N*-oxide	CTR-*N*-oxide	CTR-TP1	Competition kinetics with penten-3-one	7	$(8.1\pm0.1)\cdot 10^3$
4-chlorobenzophenone	4-CBP	CTR-TP2	Monitoring of target compound	7	0.40±0.05
Norchlorcyclizine	NCC	CTR-TP4	Competition kinetics with penten-3-one	7	$(2.1\pm0.1)\cdot 10^4$
Fexofenadine	FXF	Parent	Competition kinetics with 1,4-benzoquinone	7	$(9.0\pm0.1)\cdot 10^3$
	molecular [a]		Competition kinetics with 1,4-benzoquinone and *trans*-cinnamic acid	7-12	$5.6\cdot 10^5$ [b]
Fexofenadine *N*-oxide	FXF-*N*-oxide	FXF-TP1	Monitoring of target compound	7	~ 6.0±2.0
Azacyclonol	ACC	FXF-TP2	Competition kinetics with 1,4-benzoquinone	7	350±10
Hydrochlorothiazide	HCTZ	Parent	Competition kinetics with *trans*-cinnamic acid	7	$(8.5\pm0.2)\cdot 10^4$
	molecular [a]		Competition kinetics with orotic acid	2	590±70
	deprotonated [a]		Estimated from apparent second-order rate constants	8-11	$\sim 3\cdot 10^5$
	dideprotonated [a]		Competition kinetics with *trans*-cinnamic acid	12	$(5.1\pm0.1)\cdot 10^5$
Chlorothiazide	CTZ	HCTZ-TP1	Monitoring of target compound	7	1.5±0.1

[a] *Forms of the investigated compounds are presented in Fig. 1.*
[b] *Values for the molecular form of CTR and FXF were obtained from the relationship between the apparent second-order rate constant of their reaction with ozone in the investigated pH range and the degree of dissociation (Text S5).*

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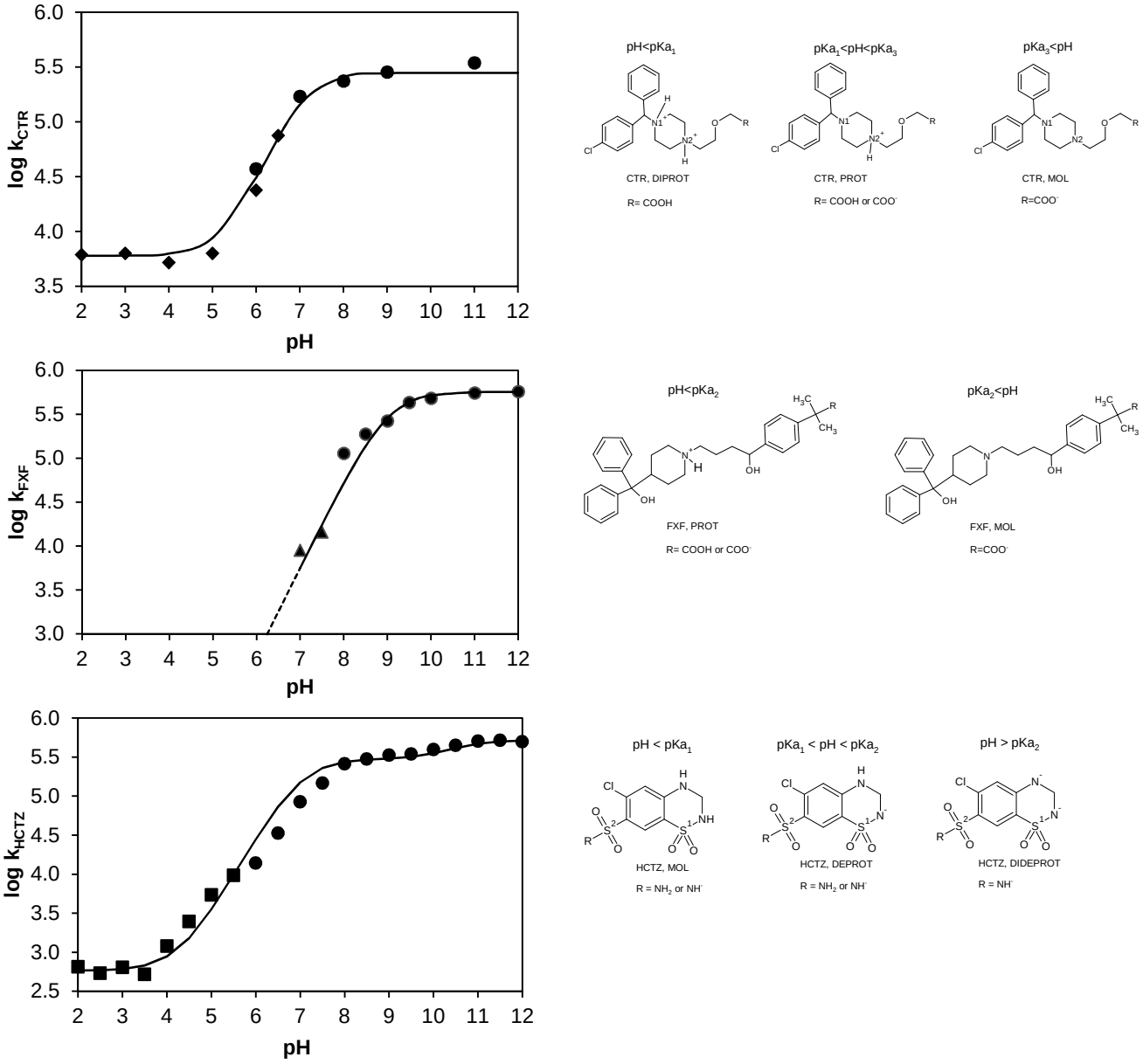
Table 2 Average concentrations (± standard deviation) (ng L^{-1}), % elimination of the target compounds cetirizine, fexofenadine and hydrochlorothiazide and the formation of TPs during full-scale ozonation at WWTP Neugut, Dübendorf, Switzerland (pH 7.4-7.9, T=18.2-22.3 ^{0}C, 0.01 - 0.04 mg NO_2-N L^{-1}, DOC= 4.7-5.7 mg L^{-1}, alkalinity 4-5 mM).

	Ozone dose Specific ozone dose											
	2.03±0.15 g O_3 m^{-3} 0.35±0.02 g O_3 g^{-1} DOC			**3.00±0.09 g O_3 m^{-3}** 0.54±0.05 g O_3 g^{-1} DOC			**3.95±0.13 g O_3 m^{-3}** 0.67±0.03 g O_3 g^{-1} DOC			**4.90±0.2 g O_3 m^{-3}** 0.97±0.09 g O_3 g^{-1} DOC		
	O_3-INF	O_3-EFF	Elimination (%)	O_3-INF	O_3-EFF	Elimination (%)	O_3-INF	O_3-EFF	Elimination (%)	O_3-INF	O_3-EFF	Elimination (%)
Cetirizine	52±6	4±1	92±1	51±3	<LOQ	93±0	53±2	<LOQ	95±0	58±7	<LOQ	> 95±1
Cetirizine *N*-oxide	<LOQ	4±2	n.a	<LOQ	<LOQ	n.a.	<LOQ	<LOQ	n.a.	6±1	<LOQ	n.a.
Fexofenadine	149±6	25±4	83±3	493±26*	72±6*	85*	177±5	9±4	96±1	164±16	<LOQ	> 91±1
Fexofenadine *N*-oxide	n.d.	n.d.	n.d.	<LOQ*	141±30*	n.d	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Hydrochlorothiazide	1126±116	158±39	86±2	976±2	33±10	98±3	969±36	40±21	97±3	1093±156	8±1	99±0
Chlorothiazide	57±7	436±13	n.a	49±4	373±11	n.a	56±1	326±33	n.a	58±12	247±75	n.a

O_3-INF*: concentration in the effluent of the secondary clarifier, which is the influent of the ozone reactor;* O_3-EFF*: concentration in the effluent of the ozone reactor;*
<LOQ: below limit of quantification; LOQ in the range of 2-10 ng L^{-1}.
n.a.: not applicable
n.d..: not determined (due to the lack of the commercial standard at the time of these measurements)
**Concentrations measured for ozone dose of 2.72±0.13 g O_3 m^{-3} (0.54±0.04 g O_3 g^{-1} DOC)*



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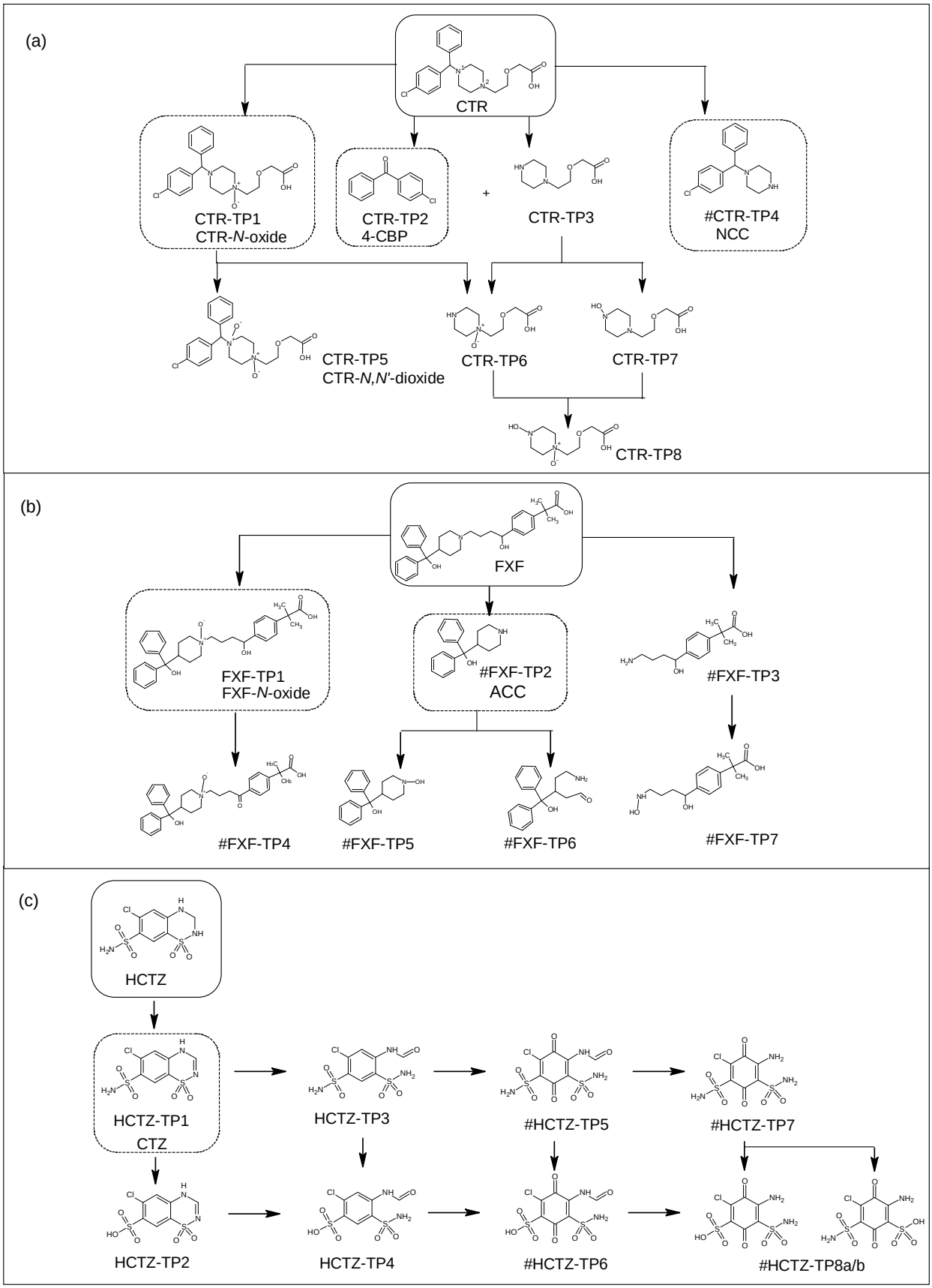

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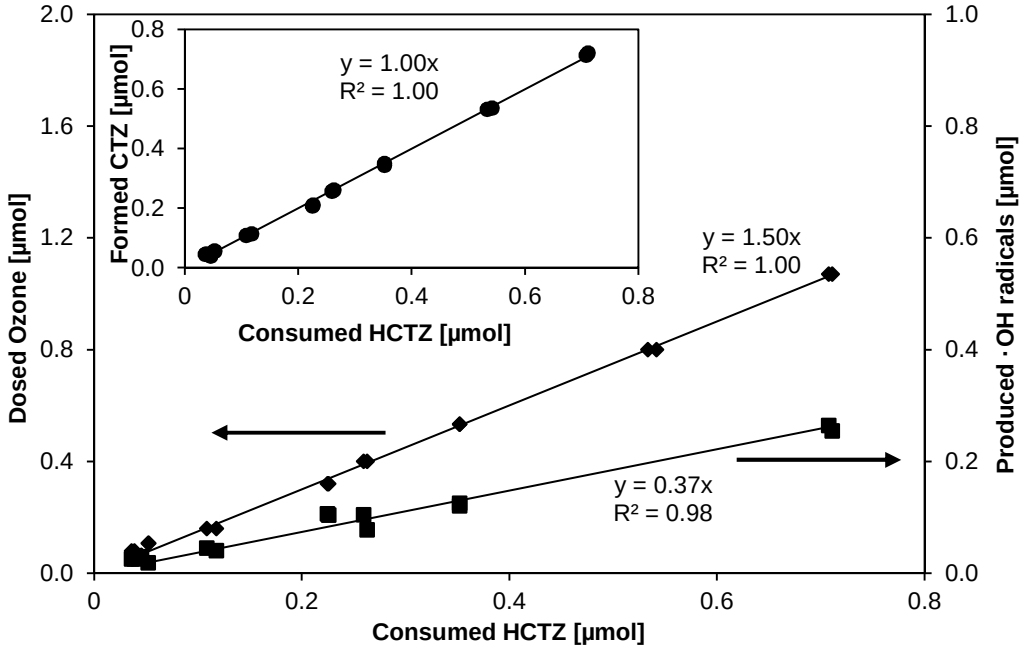

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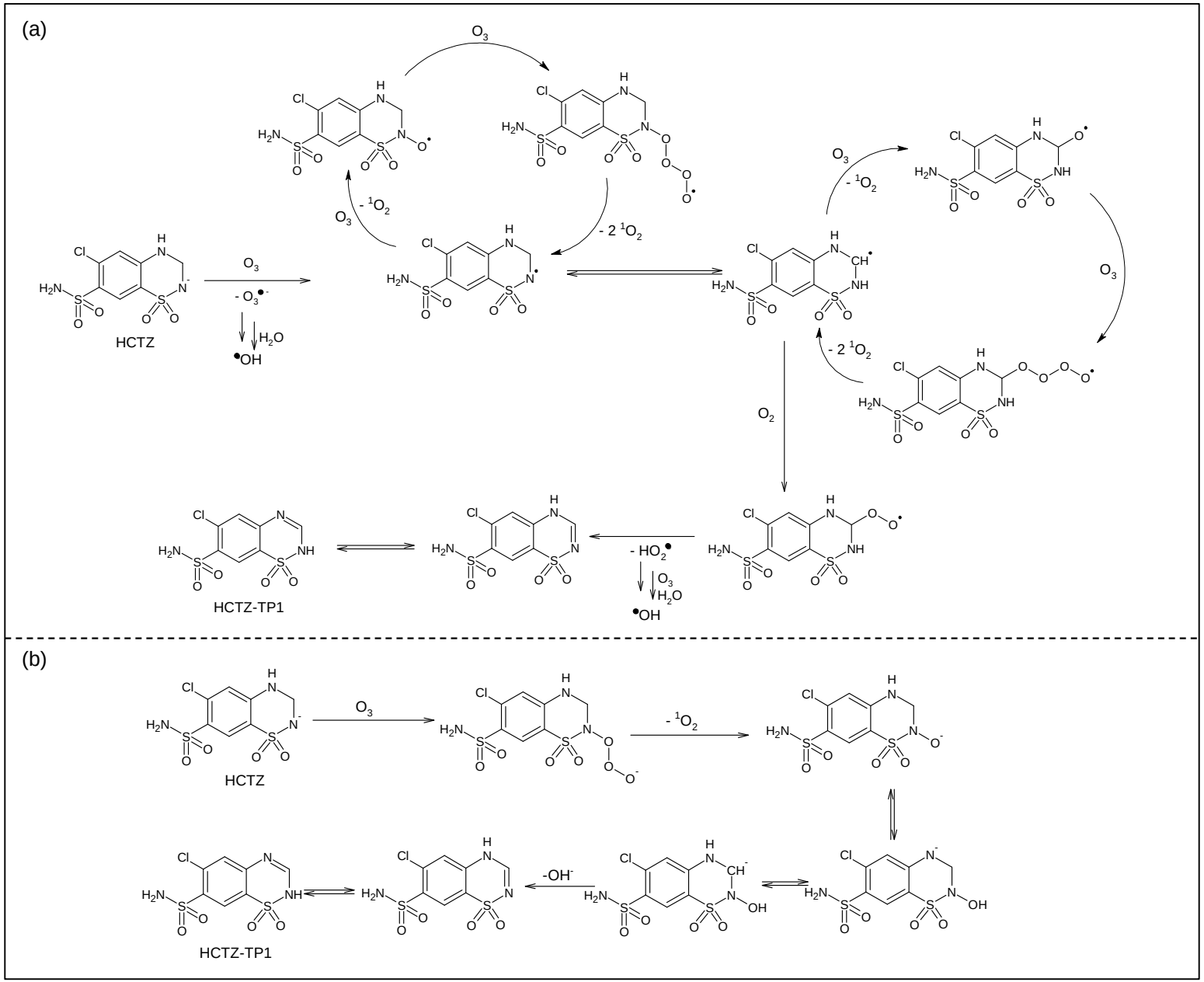

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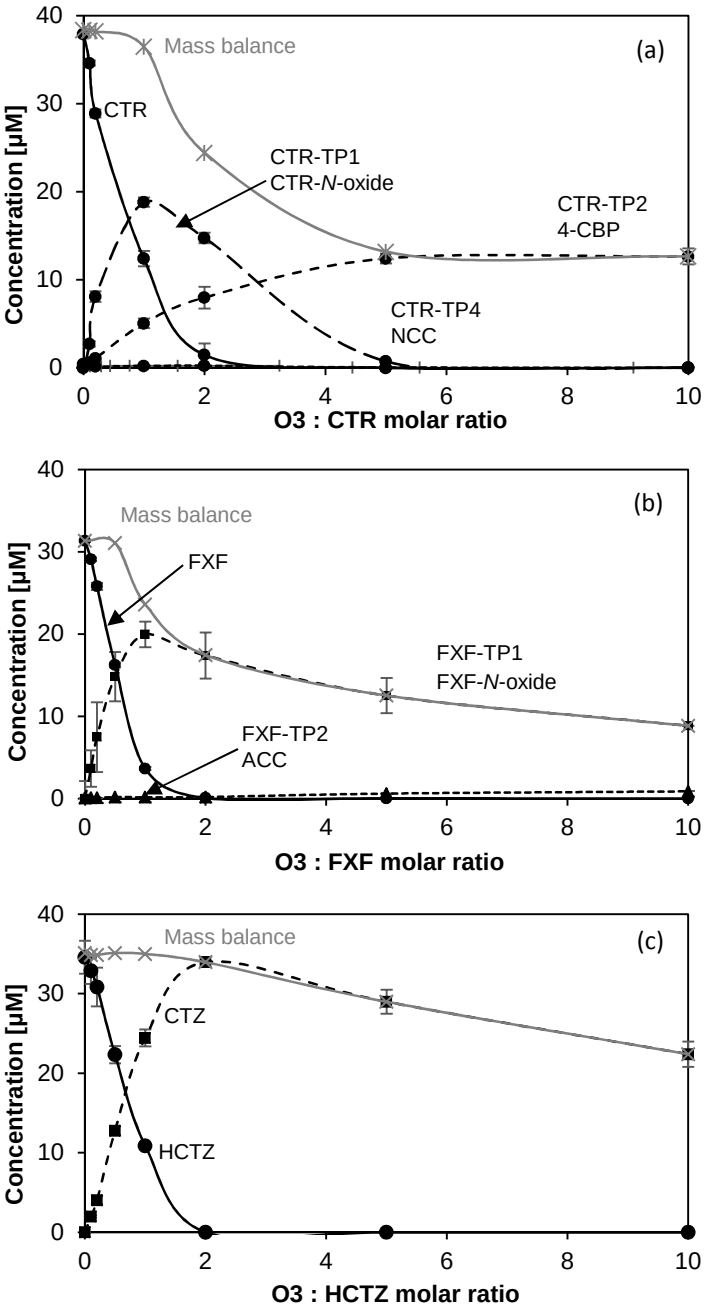

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Research highlights

Reactivity of three pharmaceuticals with ozone was studied over a wide pH-range

Identification of transformation products (TPs) was performed

N-oxides were the predominant TPs of cetirizine and fexofenadine

Chlorothiazide was formed from hydrochlorothiazide

Identified TPs were found during full-scale wastewater ozonation

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