Transformation products of pharmaceutically active compounds during drinking water ozonation
L. Tootchi, R. Seth, S. Tabe and P. Yang

ABSTRACT
Ozonation and ozone-based advanced oxidation processes have been shown to be effective in the oxidation of several pharmaceutically active compounds (PhACs) routinely detected in surface waters. Under typical operating conditions of these processes, most of the parent compound oxidized is expected to lead to the formation of transformation products (TPs). For a target ozone exposure, the resulting hydroxyl radical exposure depends on the water matrix or process chosen (e.g. peroxone) which in turn may influence the degradation pathway and the TPs formed. This study was undertaken to examine the expected impact that varying ozone and hydroxyl radical exposures may have on TP formation from the oxidation of PhACs during typical drinking water ozonation. Two representative PhACs were selected for the study. Carbamazepine was chosen to represent PhACs with a fast reaction rate with ozone ($k_{O3} > 10^4$ M$^{-1}$ s$^{-1}$) and bezafibrate was chosen to represent PhACs with a slow to moderate reaction rate with ozone ($k_{O3} < 10^4$ M$^{-1}$ s$^{-1}$). The results show that under varying ozone and hydroxyl exposure scenarios examined, the major oxidation pathway for the parent compound was dominated by reaction with ozone for carbamazepine while for bezafibrate it varied.

Key words | bezafibrate, carbamazepine, ozonation, transformation products

INTRODUCTION
Pharmaceutically active compounds (PhACs) are routinely being detected in rivers and streams in the United States, Canada and Europe (Ternes 1998; Heberer 2002; Kolpin et al. 2002; Metcalfe et al. 2003; Tabe et al. 2009) as well as several other countries. These compounds are designed to impose certain biological effects under prescribed conditions and at low concentrations. Although the detected concentrations in most instances are extremely low (parts per trillion), the possibility of unintended and undesirable effects on living organisms due to their highly active nature is of concern. In addition, synergistic effects of these substances are not fully understood (Boxall et al. 2003; Jones et al. 2004; Stackelberg et al. 2004). Some effects such as development of antimicrobial resistance in microorganisms and disruption of the endocrine system in aquatic organisms have already been documented (Länge et al. 2001; Jones et al. 2004; Kidd et al. 2007).

Many PhACs survive the wastewater treatment processes and are discharged into surface waters that serve as drinking water sources. Conventional water treatment plants (WTPs) are also not designed to remove these contaminants of emerging concern, and some persistent PhACs and/or their metabolites can survive conventional drinking water treatment processes (Adams et al. 2002; Ternes et al. 2002; Stackelberg et al. 2004; Westerhoff et al. 2005). Ozonation and ozone-based advanced oxidation processes (AOP) have been shown to be effective in the oxidation of a large number of PhACs routinely detected in surface waters. In the process, most of the parent compound oxidized is expected to lead to the formation of by-products with little mineralization expected under typical ozonation treatment conditions. Although numerous studies have examined the oxidation of the parent compound for a wide range of contaminants and in various different water matrices, only a limited few have attempted to address the formation of by-products. Lack of detailed understanding of ozone chemistry as affected by water matrix and treatment conditions, analytical methods, and unavailability of
commercial standards for the by-products expected to be formed are major reasons for the lack of effort and progress in this regard. The intermediates formed can be more persistent and/or toxic than the parent compound. There is therefore an ongoing need for research on the identification and risk assessment of by-products formed during ozonation treatment of contaminants of emerging concern.

There are two major pathways of degradation for PhACs during ozonation treatment: (1) direct reaction with molecular ozone or (2) reaction with hydroxyl radicals formed during ozone decomposition. The expected transformation of a micropollutant due to both *OH and ozone exposures over a given time ‘t’ can be mathematically represented by the following equation (Elovitz & von Gunten 1999):

$$\ln \frac{[C]}{[C]_0} = -(k_{O3} + k_{OH}R_{CT}) \int [O_3] \, dt$$  \hspace{1cm} (1)

where [C] and [O3] are concentrations of the micropollutant and ozone, respectively; kO3 and kOH are rate constants for reaction of the micropollutant with ozone and hydroxyl radicals, and RCT is the ratio of exposures to hydroxyl radicals and ozone. The first and the second terms on the right represent micropollutant oxidation attributable to ozone and hydroxyl radical exposures, respectively. The fraction of micropollutant oxidation attributable to oxidation by hydroxyl radicals can be predicted by (von Gunten 2005):

$$f(*OH) = \frac{k_{OH}R_{CT}}{(k_{O3} + k_{OH}R_{CT})}$$  \hspace{1cm} (2)

Equations (1) and (2) help predict the portion of a micropollutant oxidized by the two major pathways (direct reaction with ozone and reaction with hydroxyl radicals). Under typical conditions of ozonation treatment of drinking water, the resulting ozone and hydroxyl radical exposures are expected to be different based on characteristics of the water matrix and the target ozone exposure. The present study was undertaken to examine the extent of impact of these varying ozone and hydroxyl radical exposures on the by-products formed from PhACs. Since the reaction rate of PhACs with ozone can vary over several orders of magnitude, two representative PhACs were selected for the study. Carbamazepine was chosen to represent PhACs with a fast reaction rate with ozone ($k_{O3} < 10^4 \text{ M}^{-1} \text{s}^{-1}$) and bezafibrate was chosen to represent PhACs with a slower reaction rate with ozone ($k_{O3} > 10^4 \text{ M}^{-1} \text{s}^{-1}$). Both of these compounds are widely prescribed and commonly found in source waters around the world. Carbamazepine is a carboxamide type antiepileptic (anticonvulsant) drug which is widely used to control generalized tonic-clonic seizures (Ikehata et al. 2006). Fibrates are a group of drugs used to control the level of triglyceride in blood. Bezafibrate is one of the most highly prescribed fibrates in the world (Cermola et al. 2005).

**METHODOLOGY**

Experiments were conducted with both carbamazepine and bezafibrate under different conditions to represent a range of ozone and hydroxyl radical exposures expected under typical drinking water ozonation treatment. This range is from dominantly ozone only exposure (such as in waters with low dissolved organic carbon (DOC) or with hydroxyl radical scavengers) to both ozone and hydroxyl radical exposures (such as in waters with high DOC or in ozone-based AOP). Experiments were conducted in simulated water prepared using ultrapure Milli-Q water which was left overnight to saturate with carbon dioxide from air. The pH was then adjusted with 1 M sodium bicarbonate solution to 7.3 ± 0.2. Carbamazepine and bezafibrate were provided as stock solutions in pure methanol at concentrations of 3.62 and 2.36 mg/mL by the Laboratory Services Branch of the Ontario Ministry of the Environment (Toronto, Canada). Methanol addition was minimized by evaporation of a requisite aliquot of stock solution using nitrogen blow-down prior to spiking to simulated water to obtain a concentration of approximately 278 and 426 μg/L for carbamazepine and bezafibrate, respectively. These concentrations are much higher than the typical low to high ng/L concentrations observed in drinking water sources to allow for monitoring of degradation kinetics and transformation product (TP) formation. The spiked simulated water was modified as follows before the addition of ozone in three experimental settings to obtain a range of ozone and hydroxyl exposure conditions:

- **Experimental Setting A**: Scenario where only ozone is present in the water matrix. This was achieved by adding tert-butyl alcohol to the simulated water to act as a hydroxyl radical scavenger.
• Experimental Setting B: Scenario with both ozone and hydroxyl radical exposures. Hydrogen peroxide was added at a molar ratio of 0.25 to ozone to promote formation of hydroxyl radicals.

• Experimental Setting C: Scenario with both ozone and hydroxyl radical exposures. Hydrogen peroxide was added at a molar ratio of 0.5 to ozone to promote formation of hydroxyl radicals.

Duplicate and separate experiments were conducted at each of the three settings (A, B, and C) at ozone contact times of 2, 5 and 10 min, for both carbamazepine and bezafibrate.

Ozone gas was generated from oxygen using a bench-scale ozone generator (Ozonia North America, NJ). This ozone gas was then passed through approximately 1,500 mL of cold ultrapure water to produce the ozone stock solution. A calculated volume of ozone stock solution was added to the reactor bottles with simulated water containing carbamazepine or bezafibrate under a given experimental setting (A, B, or C) to obtain an initial ozone concentration of 2 mg/L. After the desired contact time, a sample was drawn to measure the residual ozone concentration using Standard Methods (APHA 1998). The residual ozone in the remaining simulated water was then quenched with 30.3 mg/L 3-buten-2-ol for carbamazepine experiments and 25 mg/L sodium thiosulphate solution for bezafibrate experiments. Samples packed in ice were shipped to the Mass Spectroscopy Laboratory of the Ontario Ministry of the Environment (Toronto, Canada) for the analyses of the carbamazepine, bezafibrate and their TPs using liquid chromatography/tandem mass spectrometry (LC/MS-MS). Quantification of actual concentrations for the TPs was not possible due to non-availability of standards and therefore the results are presented and interpreted based on area counts. For compounds with high ionization efficiency, a peak intensity of 10^5 in MS/MS roughly corresponds to a concentration of about 5 ng/L (Kern et al. 2009).

For an ozone resistant compound, Equation (1) reduces to the following:

\[ \ln \left( \frac{[C]}{[C]_0} \right) = -k_{OH} R_{CT} \left[ O_3 \right] dt \] (3)

Experiments were conducted with the same water matrices and ozone treatment as in experimental settings A, B, and C but with an ozone resistant probe compound, p-chlorobenzoic acid (pCBA) instead of the pharmaceuticals. Residual pCBA and ozone concentrations were monitored with time and the corresponding \( R_{CT} \) values were calculated using Equation (3) as per the procedure described by Elovitz & von Gunten (1999). These \( R_{CT} \) values together with the ozone exposure estimated from experiments conducted with carbamazepine and bezafibrate under experimental settings A, B, and C were then used to predict their oxidation attributable to ozone and hydroxyl radical exposure using Equations (1) and (2).

RESULTS AND DISCUSSION

Carbamazepine oxidation

The concentration of carbamazepine in all three experimental settings dropped below the method detection limit (MDL) within the first 2 min of exposure to ozone. The rate of consumption of ozone was slower during experimental setting A and much faster in settings B and C, where the residual ozone dropped to close to zero during the first 2 min. Using \( k_{O_3} = 3 \times 10^5 \text{ M}^{-1} \text{s}^{-1} \) and \( k_{OH} = 8.8 \times 10^9 \text{ M}^{-1} \text{s}^{-1} \) (Huber et al. 2003), Table 1 presents the measured and predicted carbamazepine oxidation efficiencies under the various experimental conditions for a contact time of 2 min. Almost 100% of carbamazepine is predicted to be oxidized within the first 2 min under all experimental conditions, which is in agreement with the measured values. The oxidation is dominated by exposure to ozone under all experimental settings, with the fraction oxidized by exposure to hydroxyl radicals predicted to be in the range of 1%.

In the present study, the three major by-products of ozonation of carbamazepine as identified by McDowell et al. (2005) were monitored. Only two of these (BQM and BQD) were detected in any of the experimental settings. The possible pathways of carbamazepine oxidation, as discussed by McDowell et al. (2005), are presented in Figure 1. The results presented in Table 1 suggest that carbamazepine oxidation was dominated by Path 1 and the contribution of Path 5 was insignificant under all experimental conditions.
Relatively high levels (area count $>10^7$) were observed for BQM and BQD during setting A which persisted during the 10 min of exposure examined (Figure 2). These TPs were either at very low levels (area count $<10^5$) or not detected during settings B and C (data not reported). Given the domination of Path 1 (Figure 1), BQM is expected to be formed under all conditions. The high levels and persistence of BQM and BQD during setting A suggest that both of these TPs were relatively resistant to oxidation by ozone alone, which is consistent with their low $k_{O3}$ values ($<7$ M$^{-1}$s$^{-1}$; McDowell et al., 2005). Low levels of the two TPs under settings B and C indicate their susceptibility to oxidation by hydroxyl radicals (Paths 6, 7, and 8). High rate constants ($r_{OH} > 5 \times 10^9$ M$^{-1}$s$^{-1}$) for these reactions reported by McDowell et al. (2005) support this contention.

**Bezafibrate oxidation**

The rate of consumption of ozone was slower during experimental setting A and much faster in settings B and C, where the residual ozone dropped to close to zero during the first 2 min. Using $r_{O3} = 590$ M$^{-1}$s$^{-1}$ and $r_{OH} = 7.4 \times 10^9$ M$^{-1}$s$^{-1}$ (Huber et al., 2003), Table 1 shows the measured and predicted bezafibrate oxidation efficiencies under the various experimental conditions for a contact time of 2 min. The difference between measured and predicted efficiency was

![Table 1](https://iwaponline.com/ws/article-pdf/13/6/1576/415186/1576.pdf)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Experimental setting</th>
<th>Ozone exposure (M s)</th>
<th>$R_{CT}$</th>
<th>$M^e$ (%)</th>
<th>$P^b$ (%)</th>
<th>$f\left(\cdot OH\right)^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamazepine</td>
<td>A</td>
<td>4.3 × 10$^{-3}$</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>1.4 × 10$^{-3}$</td>
<td>2.05 × 10$^{-7}$</td>
<td>100</td>
<td>100</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>1.3 × 10$^{-3}$</td>
<td>3.65 × 10$^{-7}$</td>
<td>100</td>
<td>100</td>
<td>0.01</td>
</tr>
<tr>
<td>Bezafibrate</td>
<td>A</td>
<td>7.5 × 10$^{-3}$</td>
<td>0</td>
<td>89</td>
<td>99</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>2.6 × 10$^{-3}$</td>
<td>2.05 × 10$^{-7}$</td>
<td>98</td>
<td>$&gt;99$</td>
<td>0.72</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>1.9 × 10$^{-3}$</td>
<td>3.65 × 10$^{-7}$</td>
<td>95</td>
<td>$&gt;99$</td>
<td>0.82</td>
</tr>
</tbody>
</table>

$^a$M = measured.
$^b$P = predicted (Equation (2)).
$^c$f (OH) = predicted fractional oxidation attributable to hydroxyl radical exposure.

![Figure 1](https://iwaponline.com/ws/article-pdf/13/6/1576/415186/1576.pdf)

**Figure 1** | Oxidation pathway of carbamazepine with ozone and hydroxyl radicals (adapted from McDowell et al. (2005)).
10% or less, which may be due to uncertainties associated with the rate constants and the measured ozone exposures. Most (89% and higher) of the bezafibrate was oxidized during the first 2 min in all experimental settings, which is in agreement with predictions using Equation (2) (99% or greater). However, unlike carbamazepine where the oxidation was dominated by exposure to ozone under all experimental conditions, the oxidation pathways for bezafibrate are predicted to vary. Under experimental setting A where a hydroxyl radical scavenger is added, the oxidation of bezafibrate is entirely due to exposure to ozone. In contrast, most of the bezafibrate (72 and 82%) is predicted to be oxidized by exposure to hydroxyl radicals under settings B and C.

Dantas et al. (2007) have examined and identified some of the major TPs that may be formed from the reaction of ozone with bezafibrate. Ozone is an electrophile and therefore has an affinity to attack the two aromatic rings in the structure of bezafibrate. Five major TPs with molecular weights (MWs) of 409, 393, 367, and 227 resulting from ozone oxidation were identified and interpreted by Dantas et al. (2007). These five TPs were monitored under the three experimental settings in the current study to examine the potential of their formation and expected persistence under varying ozone and hydroxyl exposure scenarios expected during typical drinking water ozonation. Only three of the TPs (MWs 393, 367, and 227) were detected in this study under any of the three experimental settings examined. The absence of the two by-products with MW 409 under any of the experimental settings examined would suggest that their concentrations might be too low to be detected in the current study. The formation of by-products with MW 393 and 367 can be explained based on preferential ozone attack on the non-chlorinated aromatic ring, which is consistent with the results. It can be also shown that MW 227 can be formed from further reaction of both MW 393 and 367 with ozone. Under experimental setting A, oxidation of bezafibrate is due to exposure to ozone alone, and all the three TPs were observed (Figure 2).

Area counts of $\sim 10^7$ for MW 393, between $10^6$ and $10^7$ for MW 367, and between $10^5$ and $10^6$ for MW 227 under experimental setting A suggests formation of significant amounts of these products under ozone exposure alone. The persistence of all the three TPs during the 10 min exposure under experimental setting A indicates that they are resistant to degradation by ozone. The proposed reaction pathway for the oxidation of bezafibrate with ozone and hydroxyl radicals in presented in Figure 3. TPs with MWs 393, 367, and 227 detected in experimental setting A were either at very low levels (area count $< 10^5$) or not detected during settings B and C (data not reported). The results presented in Table 1 suggest that >70% of bezafibrate was oxidized by reaction with hydroxyl radicals (Path 4 in Figure 3), which may explain the lack of formation of TPs with MWs 393, 367, and 227 detected in experimental setting A. It is also possible that some of the TPs formed with MWs 393, 367, and 227 may have been degraded by Paths 5, 6, and 7 and this was responsible for the low levels or non-detects observed under experimental settings B and C.

CONCLUSIONS

This study shows that under typical drinking water ozonation, the following might be expected. (The concentrations
of TPs formed are however expected to be much lower since the spiked concentration of the pharmaceuticals was several orders of magnitude higher than those typically observed in drinking water sources.)

For carbamazepine (\(k_{O_3} > 10^4 \text{ M}^{-1} \text{s}^{-1}\)):

- \(\sim 100\%\) carbamazepine was oxidized under both ozone only and ozone + hydroxyl radical scenarios examined.
- Oxidation of carbamazepine was predicted to be dominated by reaction with ozone under all exposure scenarios examined. The oxidation of parent PhACs with \(k_{O_3} > 10^4 \text{ M}^{-1} \text{s}^{-1}\) is therefore expected to be dominated by reaction with ozone under all conditions of typical drinking water ozonation.
- Major ozonation TPs BQM and BQD were detected when exposed to only ozone. The two TPs were resistant to degradation by ozone and persisted during the 10 min exposure.
- Low levels or non-detects of BQM and BQD under scenarios of ozone + hydroxyl radical exposure (\(^{•}\)OH exposure \(> 5.3 \times 10^{-10} \text{ M s}\)) indicates their susceptibility to degradation by hydroxyl radicals. Hydroxyl radical exposure can therefore still be important in the transformation of TPs from parent compounds with a fast reaction rate with ozone (\(k_{O_3} > 10^4 \text{ M}^{-1} \text{s}^{-1}\)).

For bezafibrate (\(k_{O_3} < 10^4 \text{ M}^{-1} \text{s}^{-1}\)):

- \(>89\%\) of bezafibrate was oxidized under both ozone only and ozone + hydroxyl radical scenarios examined.
- Oxidation of bezafibrate was predicted to be dominated by reaction with ozone when only exposed to ozone. However, \(>70\%\) bezafibrate was predicted to be oxidized by hydroxyl radicals under scenarios of ozone + hydroxyl radical exposure examined (\(^{•}\)OH exposure \(> 5.3 \times 10^{-10} \text{ M s}\)). Hydroxyl radical exposure during conditions of typical drinking water ozonation is therefore important in the determination of oxidation pathways for parent PhACs with a slower reaction rate with ozone (\(k_{O_3} < 10^4 \text{ M}^{-1} \text{s}^{-1}\)).
- Major ozonation TPs with MWs 393, 367 and 227 were detected under the scenario of ozone exposure only. The three TPs were resistant to degradation by ozone and persisted during the 10 min exposure.
- Low levels or non-detects of TPs with MWs 393, 367, and 227 under scenarios of ozone + hydroxyl radical exposures indicate an alternate pathway of bezafibrate oxidation by hydroxyl radicals and susceptibility of TPs of MWs 393, 367 and 227 to further degradation by hydroxyl radicals.
ACKNOWLEDGEMENT

The authors acknowledge the assistance of Bill Middleton at the University of Windsor, and financial assistance from the Ontario Ministry of the Environment.

REFERENCES


