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By-Product Formation from Select Pharmaceuticals during Drinking Water Ozonation Treatment

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by

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Declaration of Co-Authorship

I hereby declare that this thesis incorporates material that is result of joint research, as follows:

This thesis also incorporates the outcome of a joint research undertaken in collaboration with the Applied Chromatography Section, Laboratory Services Branch, Ontario Ministry of the Environment (MOE) under the supervision of Dr. Rajesh Seth and Dr. Shahram Tabe. The collaboration which includes preparation of pharmaceutical spiking stocks and analysis of pharmaceuticals and their by-products formed during ozonation process is covered in Chapter 3 of the thesis.

I am aware of the University of Windsor Senate Policy on Authorship and I certify that I have properly acknowledged the contribution of other researchers to my thesis.

I certify that, with the above qualification, this thesis, and the research to which it refers, is the product of my own work. I acknowledge supportive guidance of my supervisors Dr. Rajesh Seth and Dr. Shahram Tabe throughout the research and thesis work.

Abstract

Formation of ozonation products of pharmaceutical compounds bezafibrate and carbamazepine was studied under four varying ozone and hydroxyl radical exposures under typical drinking water ozonation treatment. The results indicate that the concentration of carbamazepine was reduced to below method detection limit within 2 minutes of ozonation. Oxidation efficiency of bezafibrate exceeded 95% after 5 min of ozonation in all settings. Direct attack by ozone was responsible for oxidation of carbamazepine under all experimental conditions. Major ozonation products of carbamazepine were observed to be susceptible to oxidation by hydroxyl radicals. Oxidation of bezafibrate and product formation was affected by both ozone and hydroxyl radical exposure, depending on their relative magnitude. While one of ozonation products of bezafibrate by direct attack of ozone was observed to be relatively resistant to further oxidation, the others were either completely or partially transformed mostly through reaction by hydroxyl radicals.

Dedication

To my lovely Shahin

and

fantastic Ardalan

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List of Abbreviations

Carbamazepine	CBZ
Dissolved Organic Carbon	DOC
Endocrine Disrupting Compound	EDC
Granular Activated Carbon	GAC
High-Pressure Liquid Chromatography	HPLC
Second-order rate constant of reaction between O_3 and a compound	k_{O3}
Second-order rate constant of reaction between $^{\cdot}OH$ and a compound	k_{OH}
Method Detection Limit	MDL
Ministry of the Environment, Ontario, Canada	MOE
Natural Organic Matter	NOM
Non-Steroidal Anti-Inflammatories	NSAIDS
Powder Activated Carbon	PAC
ρ-Chlorobenzoic Acid	p-CBA
Pharmaceutical and Personal Care Product	PPCP
Reverse Osmosis	RO
Sewage Treatment Plant	STP
Total Organic Carbon	TOC
Ultraviolet	UV
Water Treatment Plant	WTP

Chapter 1: Introduction

The presence of pharmaceutically active compounds (PhACs) as a sub set of emerging contaminants in the aquatic environment has attracted public attention and raised some concerns regarding potential impacts on the environment as well as human health (Schwarzenbach et al., 2006; Ternes et al., 2001; Ternes et al., 2002). Several studies have reported the occurrence of a great variety of pharmaceuticals in surface waters (Kolpin et al., 2002; Ternes, 1998). As some of these contaminated water sources are used as drinking water resources, the presence of pharmaceuticals and their natural metabolites in these aquatic environments has resulted in increased concern due to suspected toxic or other harmful effects, specifically because some of these pharmaceuticals are designed to affect human organs and their performance (Jones et al., 2002). Although only a few pharmaceuticals at low ng/L level have been detected in drinking water (Jones et al., 2005) and so far no obvious health effects due to contamination of water with trace levels of pharmaceuticals have been proved, chronic effects of long term digestion of very low levels of pharmaceuticals are not well understood. As a precautionary rule human drinking water must be free of pharmaceutical (Huber et al., 2003), thus efforts towards the elimination or reduction of presence of these compounds in drinking water are of the best interest to humans.

In considering different water treatment steps it has been shown that only advanced oxidation processes, separation by membranes (reverse osmosis (RO) and nano filtration (NF)) and adsorption to activated carbon (granular or powdered) can effectively remove pharmaceuticals from water (Adams et al., 2002; Ternes et al., 2002; Vieno et al., 2007; Zwiener & Frimmel, 2000). Each of these treatment methods has its own advantages and disadvantages. For instance studies have shown that hydrophilic pharmaceuticals pass through granular activated carbon (GAC) (Snyder et al., 2007; Vieno et al., 2007). Generally regular regeneration of GAC is essential in assuring good removal efficiency and this adds to operation costs. The disposal costs of used activated

carbon must be taken into account as well. Similarly, the use of membranes is generally considered an effective method for the removal of organic micropollutants. However, as reported by several studies, removal of less polar or neutral compounds with this method is not efficient (Snyder et al., 2003). Fouling of membranes and disposal of brine stream are the disadvantages of the application of membranes. Moreover, when membranes are used in series they have high operating costs and therefore, are not commonly used in municipal water treatment plants (Adams et al., 2002; Snyder et al., 2003). Advanced oxidation processes including the use of ozone, hydrogen peroxide, ultraviolet light or any combination of these methods have been proved to be effective for the oxidation of a large variety of pharmaceutical compounds (Acero & Von Gunten, 2001; Ternes et al., 2002; Ternes et al., 2003; Westerhoff et al., 2005; Zwiener & Frimmel, 2000).

As ozonation is commonly used for controlling microbiological concerns in drinking water treatment plants , optimizing this method for oxidation of pharmaceuticals and other organic micropollutants in water would only be an added benefit with potentially no or minor additional cost. However, the application of ozonation has its own challenges as well. As utilized ozone dose must be sufficient for disinfection purposes, it must also be optimized to avoid formation of disinfection by-products which could be of potential health concerns. Oxidation of pharmaceuticals and other emerging contaminants usually does not result in complete mineralization of these compounds and formation of ozonation by-products is very likely. Identification of these newly formed compounds and assessment of their potential harmful effects on the environment and human health is yet to be further studied.

In a previous study the detection of 51 pharmaceuticals, personal care products and endocrine disrupting chemicals in Detroit River watershed and removal of these compounds in three different full scale water treatment plants in Windsor and Detroit was examined (Tabe et al., 2009). Also, in a follow up study oxidation of 16 PPCP/EDCs with ozone both in bench scale experiments and in a pilot scale in the A.H. Weeks water

treatment plant (Windsor, ON) was investigated (Tabe et al., 2009). Results of that study showed that a group of compounds which had a high reaction rate constant with ozone (i.e. fast-reacting compounds) were successfully transformed with even low ozone exposure levels, while transformation of those compounds that had lower reaction rates with ozone (i.e. slow-reacting compounds) could benefit from increased ozone exposure. The results generally showed that close to complete transformation of target substances with the exception of ibuprofen and clofibric acid could be obtained. Under varying conditions, average conversion rate of these two compounds remained at 50%. Bezafibrate had varying oxidation efficiencies between 50 and 90%. Moreover the degree of mineralization, the detailed chemistry of the oxidation reactions and the characteristics and environmental effects of potential ozonation by-products formed remains unknown. In response to the need for more detailed information on ozonation of pharmaceutical compounds and their potential products, the current study conducts a more in depth investigation of ozonation of two selected compounds bezafibrate and carbamazepine. The criteria for selecting these two compounds was their wide occurrence in surface waters all around the world and in Ontario specifically, as well as the availability of prior literature on identification of ozonation by-products of these two compounds, and the toxicity assessment of them.

1.1. Objectives of the Study

The objective of this study was to investigate the formation of ozonation by-products of carbamazepine and bezafibrate in water under varying ozone and hydroxyl radical exposures that are expected during typical drinking water ozonation treatment processes. The following list of specific objectives was established:

- Select experimental settings to establish different ozone and hydroxyl radical exposures.
- Conduct experiments under selected experimental settings for each of the two pharmaceuticals and monitor residual pharmaceutical and ozone concentrations, and by-products formed.

- Conduct experiments under the selected experimental settings to quantify the expected hydroxyl radical exposure using p-chlorobenzoic acid (pCBA) as a probe compound.
- Quantify ozone and hydroxyl radical exposures under various experimental settings.
- Examine the results to interpret the effect of varying ozone and hydroxyl radical exposure on the formation of by-products during ozonation treatment of drinking water containing carbamazepine and bezafibrate.
- Identification of ozonation by-products of selected pharmaceutical compounds
- Examination of the effects of process conditions on the formation of by-products

Chapter 2: Literature Review

"Emerging contaminants" are substances that are not normally tested for in water quality tests. They can broadly be defined as "any synthetic or naturally occurring chemical or microorganism that is not commonly monitored in the environment but has the potential to enter the environment and cause known or suspected adverse ecological and human health effects" (USGS, 2009). Pharmaceuticals constitute a major subgroup of these contaminants. Other than pharmaceuticals, the term includes a number of industrial chemicals used in plastics, cleaning agents, personal care products and pesticides that are suspected Endocrine Disrupting Substances (USGS, 2009).

Testing for emerging contaminants in water did not begin until late 1990s. Much of this kind of testing has been done in Europe and the United states. One of the most extensive surveillance programs was started in 1999 in the United States by the U.S. Geological Survey. They tested for more than 150 different compounds in surface water, groundwater, and streambed sediments all over the United States. The results from this program showed that emerging contaminants were found everywhere (USGS, 2009).

Among all new emerging water contaminants, pharmaceuticals are attracting increasing attention. The reason is that they are widely used in significant quantities as human or veterinary medicines and thus are almost permanently present in aquatic environments. Pharmaceutically active compounds have been detected in rivers and streams of the United States, Canada and Europe (Boyd et al., 2003; Heberer et al., 1998; Heberer, 2002; Hua et al., 2006; Ikehata et al., 2006; Kolpin et al., 2002; Metcalfe et al., 2003; Tabe et al., 2009; Ternes, 1998). Pharmaceuticals are designed to impose some biological effects; therefore their presence in water sources and their pharmacological characteristics are a matter of concern (Boxall et al., 2003).

2.1. Occurrence of Pharmaceuticals

In a survey by the U.S. Geological Survey in 1999-2000 (Kolpin et al., 2002), the concentration of 95 organic wastewater contaminants was measured in 139 water

streams downstream of intense urbanization and livestock production across 30 states. Amongst these organic wastewater contaminants, there were 22 veterinary and human antibiotics, 14 other prescription and 5 nonprescription drugs. Erythromycin-H_2O (erythromycin metabolite), lincomycin, sulfamethoxazole, trimethoprim, acetaminophen (antipyretic), caffeine (stimulant), cotinine (nicotine metabolite) and 1,7-dimethylxanthine (caffeine metabolite) were the most frequently detected pharmaceutical compounds in this study. Furthermore nonprescription drugs were detected in more than 80%, antibiotics detected in close to 50% and other prescription drugs were detected in more than 30% of the streams (Kolpin et al., 2002). In Berlin, Germany, several studies conducted between 1996 and 2000 demonstrated the presence of instances of pharmaceutically active compounds including clofibric acid, diclofenac, ibuprofen, propyphenazone, primidone and carbamazepine in surface water samples downstream of sewage treatment plants (STPs) (Heberer, 2002). Sewage treatment plants have been shown to be important sources of contamination of water streams in several studies where a step increase in concentration of pharmaceuticals is observed downstream from major waste water treatment plants alongside the flow of rivers and streams that were comparably lower in headwaters (Barber et al., 2006; Heberer, 2002; Metcalfe et al., 2003).

In Canada studies have detected different pharmaceuticals in samples collected from surface waters and influent to water treatment plants across Ontario (Boyd et al., 2003; Hua et al., 2006; Metcalfe et al., 2003). Boyd et al. (2003) have reported presence of clofibric acid and naproxen in the Detroit River. In a study by Hua et al. (2006) carbamazepine and cotinine were detected in all the samples collected at different time intervals between September 2002 – June 2003 at concentrations between 0.3 to 3.8 ng/L and 0.1 to 1.6 ng/L respectively, whereas caffeine was detected in half of the samples in the range of 2.3 to 24.0 ng/L at raw water intake to A.H. Weeks water treatment plant (Windsor, ON, Canada). The most frequently and highly detected compounds in the study by Metcalfe et al. (2003) include acetaminophen, caffeine, carbamazepine, cotinine (metabolite of nicotine), diclofenac, fenoprofen, fluoxetine,

gemfibrozil, ibuprofen, indomethacin, naproxen, and trimethoprim. Pharmaceutical compounds were detected in higher concentrations at the effluents of sewage treatment plants (such as the Little River STP in Windsor, Ontario) (Metcalfe et al., 2003). Carbamazepine had the highest concentration amongst the compounds detected in both Little River and Hamilton Harbour in the survey conducted in 2000 , with values of 0.65 and 0.31 µg/L respectively (Metcalfe et al., 2003). Gemfibrozil and bezafibrate the most commonly prescribed fibrates in Canada were detected at high concentrations in surface waters (Metcalfe et al., 2003). This study shows that pharmaceutical compounds are generally detected at levels lower than 1 µg/L in surface water samples collected close to discharge points from sewage treatment plants. At sampling sites remote from STPs, in the Great Lakes region the levels of pharmaceuticals is very low (<0.01 µg/L). These detection levels are similar to those identified in studies in the northern Europe and the USA (Metcalfe et al., 2003).

In yet another study by the Ontario Ministry of the Environment (MOE) the presence of 51 PPCPs and EDCs was investigated in Detroit River which provides drinking water to the two cities of Windsor and Detroit (Tabe et al., 2009). The pharmaceuticals investigated included examples of analgesic/anti-inflammatories, antibiotics, lipid regulators, antiepileptics, antidepressants and antipyretics.

The list of pharmaceuticals detected in different sampling sites across Ontario is presented in Table 2.1. As some of the sampling sites were located at or close to the intakes of water treatment facilities, the detection of these pharmaceuticals brings about some concerns from the perspective of potential contamination of drinking water. In fact an investigation conducted by Edmonton's Envrio-Test Laboratories in 2003 found detectable concentrations of antiepileptic drug carbamazepine and lipid regulator gemfibrozil in drinking water samples from four out of ten Canadian cities, including carbamazepine in Brooks, Alberta; Montreal, Quebec; and Hamilton, Ontario; and gemfibrozil in Portage La Prairie, Manitoba (Tauber, 2003 as cited in Jones et al.,

2005). The results were confirmed by a second lab in Trent University, Ontario (CTV News, 2003) but not published in the peer-reviewed literature.

Table 2.1 Occurrence of pharmaceuticals in surface waters of Canada

Pharmaceutical	Group	Concentration (ng/L) Max.	Median	Mean	Location/Date(year)	Reference
Trimethoprim	Antibiotic			43	Hamilton Harbour/2002	Metcalfe et al., 2003
				134	Little River	Metcalfe et al., 2003
		0.3			intake of A.H. Weeks WTP/2006	Tabe et al., 2009
Lincomycin	Antibiotic	1.8			intake of A.H. Weeks WTP/2006	Tabe et al., 2009
Erythromycin	Antibiotic	33.9			intake of A.H. Weeks WTP	Tabe et al., 2009
Roxithromycin	Antibiotic	1.7			intake of A.H. Weeks WTP/2006	Tabe et al., 2009
Chlorotetracycline	Antibiotic	18.1			intake of A.H. Weeks WTP	Tabe et al., 2009
Oxytetracline	Antibiotic	2.9			intake of A.H. Weeks WTP/2006	Tabe et al., 2009
Sulfamethazine	Antibiotic	1.1			intake of A.H. Weeks WTP	Tabe et al., 2009
Sulfachloropyridazine	Antibiotic	1.3			intake of A.H. Weeks WTP/2006	Tabe et al., 2009
Sulfamethoxazole	Antibiotic	2.4			intake of A.H. Weeks WTP/2006	Tabe et al., 2009
Doxycycline	Antibiotic	3.7			intake of A.H. Weeks WTP/2006	Tabe et al., 2009
Tylosin	Antibiotic	39.5			intake of A.H. Weeks WTP/2006	Tabe et al., 2009
Sulfadiazine	Antibiotic	12			intake of A.H. Weeks WTP/2006	Tabe et al., 2009
Carbamazepine	Antiepileptic, psychiatric drug	650	185		Detroit River/2000	Metcalfe et al., 2003
				4	Detroit River/2002	Metcalfe et al., 2003
				80	Little River/2002	Metcalfe et al., 2003
		310	120		Hamilton Harbour/2000	Metcalfe et al., 2003
				23	Hamilton Harbour/2002	Metcalfe et al., 2003
		20	20		Western Lake Ontario/2000	Metcalfe et al., 2003
				2	Otonabee River/2002	Metcalfe et al., 2003
		3.8			A.H. Weeks pilot plant raw water/2002	Hua et al., 2006
		1.9			intake of A.H. Weeks WTP/2006	Tabe et al., 2009
Fluoxetine	Antidepressant			13	Hamilton Harbour/2002	Metcalfe et al., 2003
				46	Little River/2002	Metcalfe et al., 2003
Pentoxifylline	Vasodilator			8	Hamilton Harbour/2002	Metcalfe et al., 2003
				9	Little River/2002	Metcalfe et al., 2003

Pharmaceutical	Group	Concentration (ng/L)			Location/Date(year)	Reference
		Max.	Median	Mean		
Diclofenac	Analgesic/anti-inflammatory	42	26		Detroit River/2000	Metcalfe et al., 2003
				50	Little River/2002	Metcalfe et al., 2003
		194	194		Hamilton Harbour/2000	Metcalfe et al., 2003
				18	Hamilton Harbour/2002	Metcalfe et al., 2003
		21.6			intake of A.H. Weeks WTP/2006	Tabe et al., 2009
Naproxen	Analgesic/anti-inflammatory	551	207		Detroit River/2000	Metcalfe et al., 2003
				73	Little River/2002	Metcalfe et al., 2003
		139	94		Hamilton Harbour/2000	Metcalfe et al., 2003
				39	Hamilton Harbour/2002	Metcalfe et al., 2003
		63			Detroit River (A.H. Weeks WTP)/2002	Boyd et al., 2003
		3.6			intake of A.H. Weeks WTP/2006	Tabe et al., 2009
Ibuprofen	Analgesic/anti-inflammatory	790	141		Detroit River/2000	Metcalfe et al., 2003
				8	Little River/2002	Metcalfe et al., 2003
		93	64		Hamilton Harbour/2000	Metcalfe et al., 2003
				27	Hamilton Harbour/2002	Metcalfe et al., 2003
		23.5			intake of A.H. Weeks WTP/2006	Tabe et al., 2009
Fenoprofen	Analgesic/anti-inflammatory	_	_		Detroit River/2000	Metcalfe et al., 2003
				132	Little River/2002	Metcalfe et al., 2003
		64	45		Hamilton Harbour/2000	Metcalfe et al., 2003
				142	Hamilton Harbour/2002	Metcalfe et al., 2003
		59	59		Niagara River(Fort Erie)/2000	Metcalfe et al., 2003
Ketoprofen	Analgesic/ anti-inflammatory	17	12		Detroit River/2000	Metcalfe et al., 2003
		47	31		Hamilton Harbour/2000	Metcalfe et al., 2003
		50	50		Wolfe Island (Eastern Lake Ontario)/2000	Metcalfe et al., 2003
		0.8			intake of A.H. Weeks WTP/2006	Tabe et al., 2009
Indomethacin	Analgesic/anti-inflammatory			5	Hamilton Harbour/2002	Metcalfe et al., 2003
				18	Little River/2002	Metcalfe et al., 2003
Bezafibrate	Lipid regulator	200	52		Detroit River/2000	Metcalfe et al., 2003
				137	Little River/2002	Metcalfe et al., 2003
		_	_		Hamilton Harbour/2000	Metcalfe et al., 2003
				10	Hamilton Harbour/2002	Metcalfe et al., 2003

Pharmaceutical	Group	Concentration (ng/L) Max.	Median	Mean	Location/Date(year)	Reference
		4.2			intake of A.H. Weeks WTP/2006	Tabe et al., 2009
Gemfibrozil	Lipid regulator	112	66		Detroit River/2000	Metcalfe et al., 2003
				2	Detroit River/2002	Metcalfe et al., 2003
				34	Little River/2002	Metcalfe et al., 2003
				38	Hamilton Harbour/2002	Metcalfe et al., 2003
		67	12		Hamilton Harbour/2000	Metcalfe et al., 2003
		3.5			intake of A.H. Weeks WTP/2006	Tabe et al., 2009
Clofibric acid	Lipid regulator (active metabolite)	175	59		Detroit River/2000	Metcalfe et al., 2003
				3	Little River/2002	Metcalfe et al., 2003
		101	77		Hamilton Harbour/2000	Metcalfe et al., 2003
				1	Hamilton Harbour/2002	Metcalfe et al., 2003
		9	9		Niagara River (Fort Erie)/2000	Metcalfe et al., 2003
		15	15		Wolfe Island (Eastern Lake Ontario)/2000	Metcalfe et al., 2003
		103			Detroit River (A.H. Weeks WTP)/2002	Boyd et al., 2003
		12.1			intake of A.H. Weeks WTP/2006	Tabe et al., 2009
Atorvastatin	Lipid regulator			10	Hamilton Harbour/2002	Metcalfe et al., 2003
				15	Little River/2002	Metcalfe et al., 2003
Cyclophosphamide	Antineoplastic			5	Little River/2002	Metcalfe et al., 2003
Cotinine	Marker compound	1.6			A.H. Weeks pilot plant raw water/2003	Hua et al., 2006
				4	Otonabee River/2002	Metcalfe et al., 2003
				14	Little River/2002	Metcalfe et al., 2003
				13	Detroit River/2002	Metcalfe et al., 2003
Caffeine	Marker compound	24			A.H. Weeks pilot plant, raw water/2003	Hua et al., 2006
				14	Otonabee River/2002	Metcalfe et al., 2003
				33	Hamilton Harbour/2002	Metcalfe et al., 2003
				17	Little River/2002	Metcalfe et al., 2003
				46	Detroit river/2002	Metcalfe et al., 2003

Results of different studies show that analgesic/anti-inflammatories, lipid regulators, and antiepileptic drug carbamazepine are detected in highest concentrations in Ontario

surface waters. In the following section the different pathways through which the contaminants reach water sources are described.

2.2. Sources of Contamination of Water with Pharmaceuticals

2.2.1. Human Use of Pharmaceuticals

According to a study conducted by the USA National Center for Health Statistics, (NCHS, 2006), prescription drug use is increasing among all age ranges in the United States. This annual report shows that almost half of Americans take at least one prescription drug; one in six is taking three or more. Between the time periods 1988 – 1994 and 1999 – 2000, the number of people taking at least one drug has increased by 13% in the U.S. At the same time the number of people taking three or more drugs has increased by 40 % (Holtz, 2006). The situation is similar in Canada. In 1985, therapeutic drugs accounted for 9.5% of Canadian health care dollars (Canadian Institute for Health Information, 2004). Expenditures on pharmaceuticals in Canada in 2002 equaled 18.4 billion Canadian dollars. According to the same study, drugs were the second largest category of health care spending after hospital services since 1997 (Canadian Institute for Health Information, 2004). Between July 2001 and August 2002, there were 326.2 million human medical prescriptions filled in Canada (Holtz, 2006).

2.2.2. Veterinary Pharmaceuticals

In Health Canada's Drug Products Database, disinfectants and veterinary medication are listed besides Human medicine (Health Canada, 2008). The database listing of products has increased from approximately 17,000 in 1987 to more than 24,000 in 2004. Out of this latter number, 2500 are veterinary products (Holtz, 2006).

There are relatively a small number of drugs developed specifically for veterinary use. That is because; many diseases and conditions of animals are similar to those in humans. Therefore, veterinarians prescribe human drugs as well (Holtz, 2006). This practice is called extra-label or off-label use. Pharmaceutical drugs used for animals are almost as diverse and in the same categories as human used pharmaceuticals. In

addition, there are some special products for some species, like oral medications for dogs and cats used against fleas and ticks. The use of veterinary drugs has increased in the past years for the 5 million dogs, 7 million cats and under a million horses that are present in Canada. The corresponding numbers in the United States are 77 million cats, 65 million dogs and 9 million horses (Holtz, 2006).

When it comes to farm animals however, therapeutic drugs are less important than preventive ones, such as in crowded cattle feedlots. Furthermore, since it was discovered in 1949 that sub-therapeutic doses of antibiotics result in faster growth of chickens, adding antibiotics to animal food has become a routine. Considered food additives some drugs improve the growth of animals and allow them to get to market faster and with lower costs (Boxall et al., 2003; Jones et al., 2004). In a study by the Union of Concerned Scientists in 2003, it was stated that 70% of all antibiotics produced in the United States was used for farm animals, specifically for pigs, cows and chickens (Mellon et al., 2001). The non-therapeutic use of antibiotics and related pharmaceuticals for animals in the Unites States is estimated to be around 25 million pounds which is more than 8 times of what is used for treatment of human diseases (Mellon et al., 2001). The non-therapeutic use of antibiotics increased 1.5 times between 1985 and 2001. An estimated 25 to 75% of antibiotics taken by animals and humans pass to the environment through urine and manure (Sustainable table, Antibiotic use in animal; Mellon et al., 2001.)

In the aquaculture industry, disinfection agents, antibiotics for disease prevention and therapy, hormones for control of spawning and for growth promotion by sex selection (in certain species either males or females grow larger) are used. Seven chemicals including one anesthetic, two fungicides/disinfectants, and four antibiotics are approved to be sold in Canada when labeled for food fish use (Holtz, 2006). In 2002, estimates of antibacterial use in the U.S. aquaculture ranged from 92,500 to 196,400 kg annually (Benbrook, 2002).

Considering all different applications of veterinary chemicals, direct discharge of aquaculture products, excretions via urine and feces, and washoff of topical treatment products are the most important routes of entry of these chemicals into the environment.

2.2.3. Pathways of Contamination of Water Sources

This wide variety of use of chemicals for therapeutic and non-therapeutic purposes happens virtually everywhere from households to animal farms. Water is inseparably accessible everywhere humans are present, so there is no wonder there are various pathways that link use of chemicals to their occurrence in water streams. Following is a summary of the major pathways that result in presence of pharmaceuticals in water. These pathways are depicted in Figure 2.1 as well:

- Human use: a great fraction of the active ingredients in pharmaceuticals that are consumed by humans are excreted in their original chemical form and their metabolites in urine and feces and enter wastewater stream directly. (Batt, 2004; Boyd et al., 2003; Ternes, 1998)
- Production facilities: excess and unused compounds in the process of producing pharmaceuticals are discharged to wastewater (Ternes et al., 2001; Ternes, 1998)
- Household disposal: unused or expired drugs that are discharged into wastewater system (Ternes, 1998; Ternes et al., 2002)
- Runoffs: Run-off from private septic systems, treatment facilities for livestock waste and aquaculture operations carry excreted substances that may contain pharmaceuticals. Animal waste on farm fields together with sewage sludge used as agricultural fertilizers on farms are eventually washed away with run-offs as well (Boxall et al., 2003; Holtz, 2006).

As a result, the concentration of contaminants are expected to be higher downstream from manufacturing firms, sewage treatment facilities and livestock operations and

these are considered the major sources of contamination of surface waters with pharmaceuticals (Heberer, 2002; Holtz, 2006; Kolpin et al., 2002).

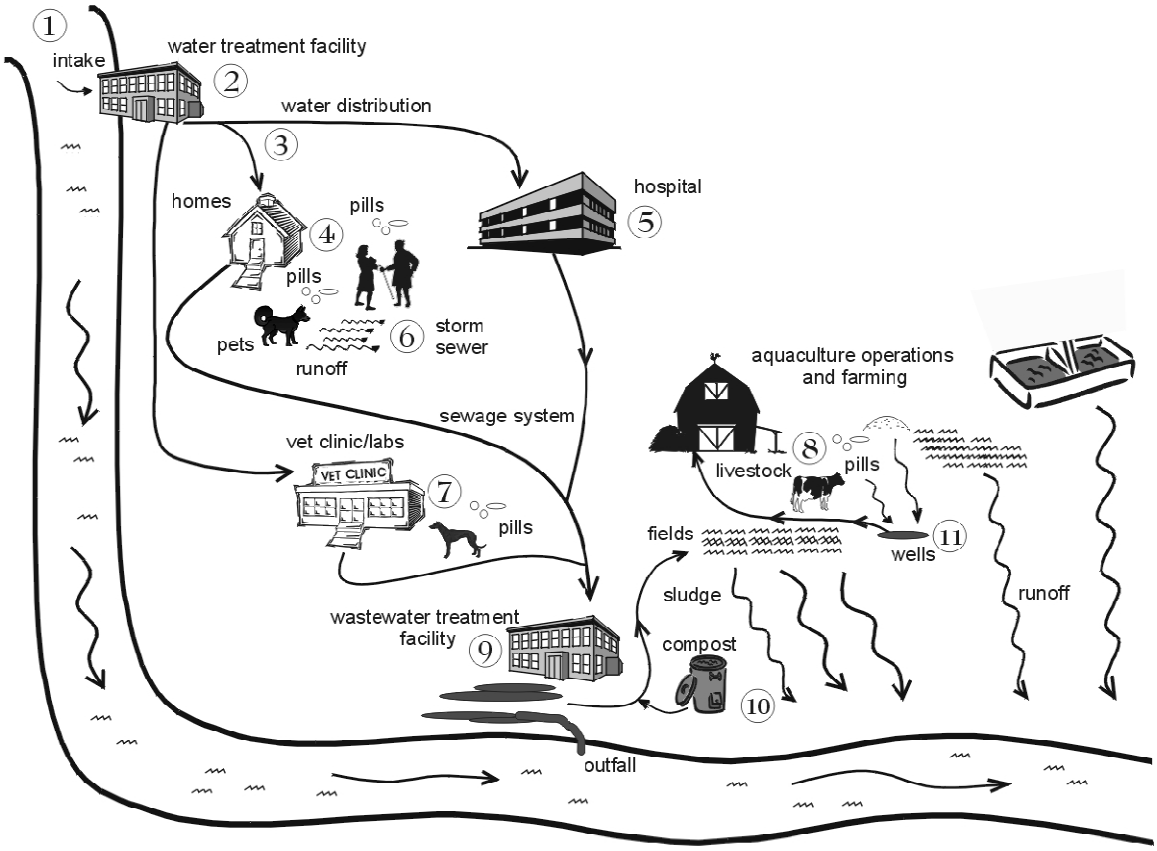

Figure 2.1 Pathways through which Emerging contaminants enter Water (Holtz, 2006).

1. Water Source,

2. Water Treatment Facility,

3. Water Distribution System,

4. Household Wastewater System,

5. Hospital Waste,

6. Pets' Manure on Lands Which Is Washed Away,

7. Veterinary Clinics, Hospitals and Labs,

8. Pharmaceuticals Drugs Used on Farms and Aquaculture Fields,

9. Wastewater Treatment Plant,

10. Municipal Composts,

11. Municipal Runoffs

2.3. Role of Wastewater Treatment Plants in Removal of Pharmaceuticals

Wastewater treatment plants are considered a key factor in the elimination of all kinds of contaminants from waters. Sewage treatment processes are designed to remove suspended solids, particulate contaminants, nutrients and dissolved biodegradable organic matter. The quality of a wastewater treatment effluent is measured against the amount of conventional chemicals they carry. For example, bulk organic matter is measured as COD (Chemical Oxygen Demand) and BOD (Biological Oxygen Demand) or TOC (Total Organic Carbon) (Nakada et al., 2007). During the treatment processes in a STP, pharmaceuticals may get mineralized, partially degraded, or remain unchanged. For instance those water soluble pharmaceuticals that are poorly biodegradable, such as carbamazepine, crotamiton, naproxen, ketoprofen and triclosan, show little or no removal by conventional sewage treatment processes (Nakada et al., 2007).

Different water quality parameters such as turbidity, pH, temperature, and flow rate affect natural degradation of various compounds in surface waters (Qiang, A. and Adams, C., 2004, as cited in Holtz, (2006)). A study on Santa Cruz River in Arizona showed that while concentration of some contaminants decreased downstream from the effluent, others that were not detected in the effluent appeared in the samples collected downstream. This phenomenon was contributed to desorption of some contaminants further downstream from the effluent (Cordy, G. et al., 2004 as cited in Holtz, (2006)).

2.4. Adverse Effects of Chemicals in Water

Present levels of pharmaceuticals in the environment and specifically in water bodies are at least an order of magnitude smaller than those identified levels than can cause any acute toxic effects on standard test organisms. Still some studies have suggested

that low concentration (less than a few ng/L) presence of pharmaceutically active compounds can be associated with some health concerns (Kidd et al., 2007; Länge et al., 2001) and in addition, impacts on wildlife has been reported (Richardson, 2003). Many of these effects may remain unknown or subtle for long periods of time (Andreozzi et al., 2003). These concerns fall into different categories. The first category is the development of antimicrobial resistance which is the development of resistance to antibiotic drugs in pathogens. Antimicrobial resistance can result in a rise in number of diseases as well as appearance of cases where infections do not respond to antibiotics. These resistant bacterial strains develop in locations where there is a significant quantity of antibiotics in use like hospitals, livestock growing fields, fish farms and wastewater treatment plants (Holtz, 2006; Jones et al., 2004).

Another category is development of disruptive effects on endocrine system (vital systems) in aquatic organisms (Kidd et al., 2007; Länge et al., 2001). However impact on the other species and concerns associated with long term subtle effects of these chemicals have not been clearly understood. The category of chemicals associated with these effects include birth control pills, hormones, some other pharmaceuticals and industrial chemicals like PCBs, metals and plasticizers, surfactants, fragrances, preservatives in cleaning and personal care products and pesticides. Studies on fish, birds, and other wildlife have shown effects like reproductive impairment or failure, deformities and feminization (Holtz, 2006; Kidd et al., 2007; Länge et al., 2001).

Just as many drugs have side effects in humans, there could be numerous unexpected effects from pharmaceuticals on non-target organisms. Effects on growth, reproduction and spawning of different kinds of fish have been observed due to presence of pharmaceuticals such as beta blocker propranolol and certain antidepressants such as fluoxetine in micrograms to low milligrams per liter range (Jones et al., 2004). Inhibition of sperm activity in aquatic animals exposed to calcium-channel blockers and effects on other aquatic organisms due to presence of pharmaceuticals such as diazepam, digoxin and amlodipine with concentrations as low as 10 µg/L have also been reported in

various studies (Jones et al., 2004). The numerous detected effects of pharmaceutical compounds on different organisms at a wide range of concentrations could be an indication of potential adverse effects of these compounds at even lower concentrations. Chronic toxicity effects as well as genotoxicity or mutagenicity are considered to be potential effect of long time exposure to pharmaceutical compounds. In summary, little is known on effects from long term or multiple exposures to pharmaceuticals (Jones et al., 2004; Ongerth & Khan, 2004).

Another aspect of the problem is exposure to a mixture of chemicals. Interactions between chemicals such as addition, antagonism and synergism could alter the extent of environmental impacts of pharmaceuticals (Boxall et al., 2003; Stackelberg et al., 2004).

In addition to the direct impacts of pharmaceuticals, hydrolytic or enzymatic processes that affect pharmaceuticals form multiple products with different behaviors that:

- May keep parent compound's characteristics and thus have a similar level of toxicity to the environment (Boxall et al., 2003; Halling-Sørensen et al., 2002), or
- May impose a greater adverse environmental effect compared to parent compound (Boxall et al., 2003; Halling-Sørensen et al., 2002), or
- have synergistic or additive effect when combined with other chemicals, or
- Have less or no toxicological impact compared to parent compound (Boxall et al., 2003; Halling-Sørensen et al., 2002) which is the most likely scenario. Still activity and toxicity of these less potent degradation products could be of great importance.

2.5. Drinking Water Treatment

As mentioned before, some pharmaceuticals and other contaminants pass through wastewater treatment process and are eventually discharged to surface waters. A portion of these contaminants may then evaporate and leave water, adsorbed by sediments in the river and water stream banks and bottoms, photo-degraded (i.e. degraded by exposure to light), biodegraded (i.e. degraded through biological

processes) or go through other chemical interactions in waters. Thus the question of whether the pharmaceuticals survive processes in a water treatment plant or not still remains critical (Holtz, 2006). Consumption of drinking water is the route of exposure of humans to pharmaceuticals present in surface waters and therefore the potential persistence of these chemical compounds in treated drinking water raises health concerns.

Some persistent pharmaceuticals and/or their metabolites can survive drinking water treatments, mainly those using conventional treatment processes and in some exceptions advanced treatment processes (Heberer, 2002; Stackelberg et al., 2004; Vieno et al., 2005; Zuccato et al., 2000). A survey of more than 100 compounds in an American drinking water treatment plant showed that 18 of them survived treatment (Stackelberg et al., 2004). Four of these non-degraded compounds were prescription and non-prescription drugs. Out of these four caffeine, carbamazepine and cotinine were consistently found in raw water samples. Dehydronifedipine was detected in half of samples. A separate study in Minnesota has identified carbamazepine as persistent and mobile and thus of concern (Lee et al., 2004)

The concentrations of these compounds in water are extremely low compared to therapeutic doses. With most of medicines prescribed in several hundred milligrams doses, a person would need to drink thousands of liters of water to ingest an amount equivalent to only one medical dose (Holtz, 2006; Stackelberg et al., 2004). Most of the studies conducted on therapeutic effects of pharmaceutical compounds have only considered the effect of short term consumption of high doses of these drugs. There are relatively limited studies on effects of long term ingestion of very low doses of pharmaceuticals through drinking water (Stackelberg et al., 2004). Moreover although research work has been done on effects of low-level exposure to pharmaceutically active compounds (PhACs) on organisms, the effects of these chemicals on human health cannot be defined with certainty as no such experiments can be performed on humans. The human health effects will rather be determined by accumulation of clinical

observations and connections made to studies on other organisms, which is a lengthy process (Adams et al., 2002; Holtz, 2006).

2.5.1. Application of Advanced Treatment Process in Drinking Water Treatment

The main intention of treating surface water for drinking purposes is to remove natural organic matter (NOM), turbidity, and microorganisms from it. Techniques such as coagulation and sand filtration are effective in removing turbidity and NOM. Studies have shown that these techniques do not have much of success when it comes to the removal of PPCPs and EDCs (Adams et al., 2002; Boyd et al., 2003; Hua et al., 2006; Stackelberg et al., 2004; Ternes et al., 2002; Vieno et al., 2005; Westerhoff et al., 2005). Therefore the efficiency of advanced treatment processes such as oxidation, adsorption, and membrane removal of pharmaceuticals and other chemical compounds has been studied in the past years.

2.5.1.1. Adsorptive Processes

Activated Carbon

Activated carbon adsorbs a number of organic pollutants. Two types of activated carbon, packed-bed granular activated carbon (GAC) and powder activated carbon (PAC) are used for this purpose, both of which are effective for removal of numerous organic pollutants. PAC is utilized to control organic pollutants which cause taste and odor problems (Adams et al., 2002; Westerhoff et al., 2005). It has also been effective in removing some pesticides and herbicides (Westerhoff et al., 2005). However activated carbon capacity for removal of trace organic contaminants is greatly affected by presence of NOM that could block activated carbon pores (Snyder et al., 2007). Yet addition of PAC (and/or ozone) to conventional treatments (e.g., coagulation plus chlorination) improves the removal rate of many PPCP/EDCs (Westerhoff et al., 2005). During GAC filtration, adsorption mainly occurs by hydrophobic interactions. Ion exchange processes may contribute as well (Snyder et al., 2003). For instance, neutral pharmaceutical carbamazepine has a higher adsorption to GAC compared to ionic pharmaceuticals such as naproxen (Snyder et al., 2007). More hydrophobic compounds

like carbamazepine have been reported to be efficiently removed by GAC filtration and the more hydrophilic pharmaceuticals such as atenolol, sotalol and ciprofloxacin are reported to pass through GAC treatment (Snyder et al., 2007; Vieno et al., 2007). Regular regeneration of the carbon plays an important role in assuring successful removal of pharmaceuticals in full-scale GAC filtration (Snyder et al., 2007). Due to seasonal use of PAC at some treatment facilities the operating costs of this process would increase proportionally if applied year round (Adams et al., 2002). The disposal cost of used PAC and regeneration/disposal cost of GAC should also be considered when estimating the environmental impacts of this process (Snyder et al., 2007).

2.5.1.2. Membrane Filtration

Reverse osmosis (RO) membrane filtration has been shown to be an effective technology for removal of PPCPs and EDCs from water. As several studies have reported, polar and charged compounds are more effectively removed by membranes compared to less polar or neutral compounds (Snyder et al., 2003). With increased pH, electrostatic repulsion between RO membrane and dissociated organic compounds increases thus a better removal can be obtained (Ozaki and Li, 2002 as cited in Snyder et al., (2003)). Adams et al. (2002) investigated removal of antibiotics from water using RO membranes. They reported 99 and 99.9% rejection when two or three RO units are used in series, respectively. Similarly, Huang and Sedlak (2001) have reported greater than 90% efficiency in removal of steroid hormones (as cited in Snyder et al., (2003)). Overall, membrane separation is a successful method for removal of most PPCPs and EDCs (Snyder et al., 2003); however, RO technology is not commonly used in municipal water treatment plants due to its high operating costs. The cost of disposal of the concentrated reject stream (brine) is also a major economic obstacle (Adams et al., 2002). Furthermore, putting two or three RO membranes in series would increase the operating costs tremendously, rendering the process uneconomical. From an environmental point of view, the relatively high operating pressure of RO and nano-filtration (NF) membranes can easily be translated into high energy consumption which is a negative impact (Snyder et al., 2007).

2.5.1.3. Oxidative Treatments

Ultraviolet Irradiation

For purpose of water disinfection, ultraviolet irradiation is typically used at the order of 30 $mJ.cm^{-2}$ ($mW.s.cm^{-2}$) (Adams et al., 2002). A study that investigated the effect of conventional water treatment processes in removing select common antibiotics showed that ultraviolet irradiation at disinfection dosages does not result in successful removal of these compounds (Adams et al., 2002). Even though many pharmaceutical compounds absorb UV light, the UV intensity used for disinfection is too low to cause any transformation of the pharmaceuticals (Snyder et al., 2003). In the study conducted by Adams (2002), UV at 100 times typical dosage could only remove the target compounds at 50 to 80% efficiency. In another study, elimination of low concentration of ciprofloxacin, naproxen and sotalol with UV treatment was investigated. This treatment was effective in reducing low concentration of naproxen and sotalol to below detection limits in treated water. However, ciprofloxacin was not affected by UV treatment. Vieno et al. (2007) concluded that pharmaceuticals pass through UV treatment more or less unaffected.

Chlorination

Chlorination is primarily used as a disinfectant in drinking water treatment. Concentration-time (CT) values for free chlorination to result in 99.9% successful removal of *Giardia lamblia* varies from 56 mg.min/L (at 20 °C and pH 7) to 312 mg.min/L (at 5°C and pH 9) (Letterman, 1999). Often a 0.5 log removal is required for chlorine disinfection stage in surface water treatment plants (Adams et al., 2002). There are conflicting reports on removal of estrogenic compounds by chlorine (Snyder et al., 2003) In a study effect of chlorination along with some other treatment processes has been investigated on removal of seven antibiotics in both distilled/deionized and Missouri River water (Adams et al., 2002). In this study, in a time interval required for 0.5 log removal with chlorine under experimental conditions a reduction rate of minimum 50% to more than 90% can be obtained. Chlorination of organic compounds resulted in

formation of chlorinated by-products. Possible formation and potential toxicity of such by-products should be considered (Adams et al., 2002).

Chlorine Dioxide

Chlorine dioxide is reported to be effective in the removal of herbicides, pesticides and polycyclic aromatic hydrocarbons (Snyder et al., 2003). Chlorine dioxide is a stronger oxidant compared to chlorine and is more effective in oxidation of amine-containing pesticides (several studies in Snyder et al., (2003)). It is expected to react with compounds with phenolic amino and thiol functuional groups (Hoigne and Bader, 1994, as cited in Snyder et al., (2003)) but no further information is available on oxidation of pharmaceuticals with this oxidant.

Ozonation

The first application of ozone as a water treatment disinfectant occurred in 1893 at Oudshoorn, The Netherlands. The oldest ozonation installed in continuous operation dates back to 1906 in Nice, France. In the same year, ozone was first used in the United States for odor and taste control in New York City. By 1987, five treatment facilities in the United States were utilizing ozone treatment. The Milwaukee Cryptosporidium outbreak in 1993 attracted more attention in ozone as a disinfectant (Letterman, 1999).

It is because of ozone's substantial reactivity with microbial constituents that it has been used mainly as a disinfectant in water treatment for almost a century (Elovitz & Von Gunten, 1999; Von Gunten, 2003b). It is even able to inactivate more resistant pathogenic microorganisms such as Protozoa, e.g., *Cryptosporidium parvum* oocysts, that chlorine and chlorine dioxide are not capable of inactivating (Elovitz & Von Gunten, 1999; Von Gunten, 2003b).

Several studies have considered the effect of ozonation on removal of micro-contaminants such as PPCPs and EDCs. The categories of substances studied included:

- Antiphlogistics, analgesics or anti-inflammatories (e.g. naproxen, mefenamic acid, ketoprofen, fenoprofen, ibuprofen)

- Lipid regulator metabolites (e.g. clofibric acid, fenofibric acid)
- Beta blockers (e.g. atenolol, sotalol, celiprolol, propranolol, metoprolol)
- Anticonvulsants or antiepileptics (e.g. carbamazepine, diazepam)
- Antimicrobial
    - Phenolic antiseptics (e.g. triclosan, thymol)
    - Sulfonamide antibiotics (sulfapyridine, sulfamethoxazole)
    - Macrolide antibiotics (azithromycin, erythromycin anhydride, clarithromycin, roxithromycin, tylosin)
    - Fluoroquinolones (e.g. ciprofloxacin, enrofloxacin)
    - Dihydrofolate reductase inhibitor (trimethoprim)

Ozone is effective in oxidation of most of antibiotics. This reactivity toward ozone (k_{O3} $>10^5$) is attributed to having at least one active functional group in compounds' structure such as amine nitrogen, sulfur, or carbon-carbon double bound. Despite effective conversion of antibiotics by ozonation, their mineralization remains uncertain. As a result of incomplete mineralization, some by-products with unknown identity and characteristics could be formed. Unlike antibiotics which were mostly reactive toward ozone, anticonvulsants have different reactivity toward ozone. As can be observed in Table 2.2 carbamazepine is easily oxidized by ozone while diazepam and primidone are relatively resistant to ozonation. The situation is similar for non-steroidal anti-inflammatories (NSAIDs). Ibuprofen a member of this class of pharmaceuticals has a low reactivity toward ozone while diclofenac another NSAID is completely oxidized with ozone in the reviewed studies. Other NSAIDs have shown between 50 to more than 90% conversion with ozonation (Ikehata et al., 2006). There is limited literature on oxidation of β-blockers by ozone and this information is mostly on ozonation of these compounds in wastewater. However based on the existing information all the compounds in this group can effectively be oxidized by ozone. This reactivity has been referred to presence of a secondary amine group and a weakly/ moderately activated aromatic ring (Ikehata et al., 2006). Ozonation is not completely successful in conversion of lipid regulators and ozonation rates of these pharmaceuticals are generally in the low levels. However,

addition of hydroxyl radicals has been shown to be helpful in increasing the rate of oxidation of bezafibrate and clofibric acid (Huber et al., 2005; Zwiener & Frimmel, 2000). Based on the reviewed studies the extent of ozonation of different pharmaceutical classes varies from fairly effective for antibiotics and β-blockers to poorly effective for lipid regulators. Table 2.2 provides details of oxidation of some pharmaceuticals by ozone.

Ozonation has been identified as one of the most effective processes in removing compounds such as naproxen, ketoprofen, triclosan, crotamiton, sulfapyridine, macrolide antibiotics (erythromycin, clarithromycin), and carbamazepine (Nakada et al., 2007; Ternes et al., 2003). However as it can be observed from the data in Table 2.2 ozonation at doses used for water treatment does not result in complete mineralization for some pharmaceuticals. A drug is considered inactivated if its functional group is oxidized and transformed by ozone even if the rest of the molecule remained intact (Dodd et al., 2006). The steroid hormone 17α-ethinylestradiol is an instance where ozone selectively reacts with the phenolic moiety of the molecule responsible for drug's estrogenic activity (Huber et al., 2004). Dodd and coworkers (2006) discuss that while similar results are likely for many antibacterial molecules, they might still react differently with ozone. In some molecules the active moiety might be ozone refractory. In others, moieties of molecule with less biochemical activity might have higher reactivity with ozone. In such cases the effect of ozonation on deactivation of pharmaceuticals is reduced. Ciprofloxacin and enrofloxacin from fluoroquinolones, N(4)-acetylsulfamethoxazole from sulfonamides, penicillin G and cephalexin from β-lactams are examples of pharmaceuticals that have recalcitrant active moieties which react with ozone at lower rates (Dodd et al., 2006).

Table 2.2 Oxidation of pharmaceutical compounds by ozone

Pharmaceutical	Group/subgroup	Initial conc. (µg/L)	Ozone range	Oxidation range	Type of water	Reference
Erythromycin	Antibiotics/macrolide	2 - 620	3.5 - 5mg/L	<LOQ	municipal WW	Huber et al., 2005; Ternes et al., 2003
		0.2-1.5	1-3 mg/L	77%	simulated water	Tabe et al., 2009
Roxithromycin	Antibiotics/macrolide	_	_	< LOQ	_	Huber et al., 2003; 2005; Ternes et al., 2003
Lincomycin	Antibiotic/lincosamide	0.2-1.5	1-3 mg/L	<LOQ	simulated water	Tabe et al., 2009
		0.2-1.5	0.3-1.5 mg/L (2 to 8.6 min)	>90%	Detroit river water	Tabe et al., 2009
Ofloxacin	Antibiotics/quinolone	560	13.9 mg/L absorbed O_3 in 2 min	complete conversion	aqueous pharmaceutical mixture	Andreozzi et al. 2004 as cited in Ikehata et al., 2006
Sulfachlorpyridazine	Antibiotics/sulfonamide	50	0.3 mg/L (1.3 min)	95%	pre-filtered river water	Adams et al., 2002
		0.2-1.5	1-3 mg/L	<LOQ	simulated water	Tabe et al., 2009
		0.2-1.5	0.3-1.5 mg/L (2 to 8.6 min)	>90%	Detroit river water	Tabe et al., 2009
Sulfadimethoxine	Antibiotics/sulfonamide	_	_	rapid conversion	river water	Adams et al., 2002; Huber et al., 2005
Sulfamerazine	Antibiotics/sulfonamide	_	_	rapid conversion	river water	Adams et al., 2002; Huber et al., 2005
Sulfathiazole	Antibiotics/sulfonamide	_	_	rapid conversion	river water	Adams et al., 2002; Huber et al., 2005
Sulfadimethoxine, sulfamerazine, sulfathiazole	Antibiotics/sulfonamide	_	_	rapid conversion	river water	Adams et al., 2002; Huber et al., 2005
Sulfamethazine	Antibiotics/sulfonamide	50	0.3 mg/L (1.5 min)	>95%	pre-filtered river water	Adams et al., 2002
		0.2-1.5	1-3 mg/L	<LOQ	simulated water	Tabe et al., 2009
		0.2-1.5	0.3-1.5 mg/L (2 to 8.6 min)	>90%	Detroit river water	Tabe et al., 2009
Sulfamethoxazole	Antibiotics/sulfonamide	0.62	0.5 - 5 mg/L	< LOQ	natural river water	Huber et al., 2003; Ternes et al., 2003
		0.2-1.5	1-3 mg/L	<LOQ	simulated water	Tabe et al., 2009
		0.2-1.5	0.3- 1.5 mg/L	>90%	Detroit river water	Tabe et al., 2009
Tetracycline	Antibiotic/bacteriostatic	0.2-1.5	1-3 mg/L	93%	simulated water	Tabe et al., 2009

Pharmaceutical	Group/subgroup	Initial conc. (µg/L)	Ozone range	Oxidation range	Type of water	Reference
Carbadox	Antibiotic/veterinary	50	0.3 mg/L (1.5 min)	>95%	pre-filtered river water	Adams et al., 2002
Tylosin	Antibiotic/respiratory infection in animals	0.2-1.5	1-3 mg/L	96%	simulated water	Tabe et al., 2009
		0.2-1.5	0.3-1.5 mg/L (2 to 8.6 min)	>90%	Detroit river water	Tabe et al., 2009
Trimethoprim	Antibiotic/chemotherapeutic	50	0.3 mg/L (1.5 min)	>95%	pre-filtered river water;	Adams et al., 2002; Ternes et al., 2003
Monensin	Antibiotic	0.2-1.5	1-3 mg/L	90% (avg.)	simulated water	Tabe et al., 2009
Carbamazepine	Anticonvulsant (antiepileptics) and anti-anxiety	1	0.5 mg/L	>97%	surface water	Ternes et al., 2002
			5 mg/L	< LOQ	_	Ternes et al., 2003
		1.18×10^{5}	1.0 mg/L (1 h)	< LOQ	_	Andreozzi et al., 2002
		_	>0.5 mg/L	>97%	_	Huber et al., 2003
		0.2-1.5	1-3 mg/L	<LOQ	simulated water	Tabe et al., 2009
		0.2-1.5	0.3-1.5 mg/L (2 to 8.6 min)	>90%	Detroit river water	Tabe et al., 2009
Diazepam	Anti-anxiety	142	2 mg/L (10 min)	24-65% conversion	natural water	Huber et al., 2003
Primidone	Anticonvulsant/ pyrimidinedione	1	3 mg/L	87% converted	flocculated surface water	Ternes et al., 2002
Diclofenac	Non-steroidal anti-inflammatory drugs (NSAIDs) or antiphlogistic	1-2	0.5,1, 5 and 15 mg/L	< LOQ	flocculated surface water; pure water	Ternes et al., 2002; 2003; Zwiener & Frimmel, 2000; Vogna et al., 2004
Ibuprofen	NSAID	_	2 mg/L	40-77 %	lake water, well water	Huber et al., 2003
		_	2.5-4 mg/L	80% (avg.)	3 drinking water and 1 model water supplies	Westerhoff et al., 2005
		2	1 mg/L (10 min)	12% conversion	distilled water	Zwiener & Frimmel, 2000
		_	> 2mg/L	>62%	_	Ternes et al., 2003
		0.2-1.5	1-3 mg/L	31->97%	simulated Water	Tabe et al., 2009
		0.2-1.5	0.3-1.5 mg/L (2 to 8.6 min)	10-46%	Detroit river water	Tabe et al., 2009
Indomethacin	NSAID	0.1	5 mg/L	effective	_	Huber et al., 2005; Ternes et al., 2003

Pharmaceutical	Group/subgroup	Initial conc. (µg/L)	Ozone range	Oxidation range	Type of water	Reference
		0.2-1.5	1-3 mg/L	<LOQ	simulated water	Tabe et al., 2009
		0.2-1.5	0.3-1.5 mg/L (2 to 8.6 min)	>90 %	Detroit river water	Tabe et al., 2009
Naproxen	NSAID	0.1	5 mg/L	effective (>50%)	_	Huber et al., 2005; Ternes et al., 2003
		0.2-1.5	1-3 mg/L	<LOQ	simulated water	Tabe et al., 2009
		0.2-1.5	0.3-1.5 mg/L (2 to 8.6 min)	>87%	Detroit river water	Tabe et al., 2009
Salicylic acid*	NSAID	0.65	17.5 mg/L (15 min)	60% degradation	advanced water recycling demonstration plant, Australia	Khan et al, 2004 as cited in Ikehata et al., 2006
Paracetamol (acetaminophen)	NSAID – antipyretic	8×10^5	flow 72 g/h (30 min)	effectively degraded	_	Andreozzi et al., 2003 a as cited in Ikehata et al., 2006
Propranolol	β-blockers	0.18	5 mg/L	effective degradation	effluent of municipal STP	Ternes et al., 2003
		325.5	4.75 mg/L absorbed O_3 (2 min)	high reactivity	mixture of pharmaceuticals	Andreozzi et al., 2004 as cited in Ikehata et al., 2006
Bezafibrate	Lipid regulator	_	0.1-2.0 mg/L	<5 - >97%	lake water	Huber et al., 2003
		_	1.5 & 3 mg/L	50 & 80%	flocculated natural water	Ternes et al., 2002
		0.2-1.5	1-3 mg/L	37->99%	simulated water	Tabe et al., 2009
		0.2-1.5	0.3-1.5 mg/L (2 to 6 min)	46-98%	Detroit river water	Tabe et al., 2009
Clofibric acid	Lipid regulator	2	1 mg/L (10 min)	8% relatively resistant	pure water	Zwiener & Frimmel, 2000
		_	1.2 mg/L	57±17 %	simulated water	Ternes et al., 2002
		_	2.5-3.0 mg/L	<40 %	flocculated natural water	Ternes et al., 2002
		_	>2 mg/L	>59%	_	Ternes et al., 2003
		3.22×10^5	0.48 mg/L (continuous in bulk liquid for 1h)	< LOQ	_	Andreozzi et al., 2003b as cited in Ikehata et al., 2006
		0.2-1.5	1-3 mg/L	35->97%	simulated water	Tabe et al., 2009
		0.2-1.5	0.3-1.5 mg/L (2 to 6 min)	11-46%	Detroit river water	Tabe et al., 2009

Pharmaceutical	Group/subgroup	Initial conc. (µg/L)	Ozone range	Oxidation range	Type of water	Reference
Gemfibrozil	Lipid regulators	0.2-1.5 0.2-1.5	1-3 mg/L 0.3-1.5 mg/L (2 to 8.6 min)	<LOQ >90 %	simulated water Detroit river water	Tabe et al., 2009 Tabe et al., 2009

WW= wastewater; DL= detection limit; LOQ = limit of quantification

* Decomposition product of acetylsalicylic acid (aspirin)

The degree of mineralization of pharmaceuticals with high removal rates by ozonation is not thoroughly known yet. In cases where oxidation by-products do not show similar bioactive characteristics as the parent compounds, complete mineralization may not be necessary for purpose of inhibiting biological effects. However, these newly formed by-products need to be fully studied for their potential undesirable effects on both aquatic organisms and humans. Nevertheless, ozonation of pharmaceutical compounds and their oxidation mechanisms are poorly understood and limited information is available on the pathways leading to formation of these by-products. Few studies have investigated products of ozonation of pharmaceuticals in water and assessed their undesirable effects and characteristics. (Andreozzi et al., 2002; Dantas et al., 2007; McDowell et al., 2005; Shang et al., 2006; Vogna et al., 2004). Further studies are required to clarify the environmental risk of ozonation of residual pharmaceuticals (Boyd et al., 2003).

2.5.2. Mechanism of Oxidization by Ozone

The compounds in water either directly react with ozone molecules or indirectly with hydroxyl radicals that are formed when ozone decomposes. The idea was first proposed by Hoigne and Bader (1976). They stated that ozone either directly reacts with substrates or, at a certain pH level, it decomposes to secondary oxidants such as hydroxyl radicals ($^{\cdot}OH$) that demonstrate higher oxidation rates with substrates.

O_3+ Compounds → Products

Or:

O_3→hydroxyl radicals

Hydroxyl radicals+ Compounds→ Products

During ozonation process organic and inorganic micro pollutants in water are oxidized by ozone or by hydroxyl radicals or by a combination of the two. The pathway of the reaction depends on the relative concentration of ozone to hydroxyl radicals and the kinetics of corresponding reactions. Most of inorganic compounds in drinking water

have fast reactions with ozone; however the reaction of ozone with organic micro pollutants is highly selective. When the reaction of ozone with a specific compound is slow, hydroxyl radicals play a more important role in transformation of that compound. Ozone is an electrophile, so it has an enhanced reaction with any electron donating group (e.g. $–CH_3$, O^-, $–OCH_3$), and reduced reactivity with any electron withdrawing group (e.g. –Cl, $–NO_2$) (Von Gunten, 2003a). Generally, ozone reacts with phenols, double bonds in aliphatic compounds, activated aromatic systems and non-protonated amines (Snyder et al., 2003; Snyder et al., 2006; Von Gunten, 2003a). On the other hand, hydroxyl radical reaction rate with both inorganic and organic compounds is nearly diffusion-controlled (Von Gunten, 2003a).

2.5.2.1. Factors Affecting Mechanism of Attack of Ozone

The conversion of ozone to hydroxyl radicals has a complicated sequence of reactions (Acero & Von Gunten, 2001). Stability of ozone largely depends on the water matrix, especially on its pH. Decomposition of ozone initiates by either hydroxide (HO^-), de-protonated hydrogen peroxide (HO_2^-) or some organic compounds of Natural Organic Material (NOM) that exist in water (as cited from Staehelln & Hoigne, 1982 in (Acero & Von Gunten, 2001)).

Initiation reaction with OH^-: (Elovitz & Von Gunten, 1999; Von Gunten, 2003a)

$$O_3 + OH^- \rightarrow HO_2^- + O_2 \, , k = 70 M^{-1}s^{-1} \quad (2.1)$$

Transformation of O_3 to $^{\cdot}OH$ by reaction with the chain carriers HO_2^- and $O_2^{-\cdot}$ follows: (Elovitz & Von Gunten, 1999)

$$O_3 + HO_2^- \rightarrow O_2^{-\cdot} + O_2 + {}^{\cdot}OH \ , \ k = 2.8 \times 10^6 \ M^{-1}s^{-1} \quad (2.2)$$

$$O_3 + O_2^{-\cdot} \rightarrow O_3^{-\cdot} + O_2 \, , k = 1.6 \times 10^9 \ M^{-1}s^{-1} \quad (2.3)$$

If

$$pH<\approx 8: \quad O_3^{-\cdot} + H^+ \rightleftharpoons HO_3^{\cdot} \tag{2.4}$$

then

$$HO_3^{\cdot} \rightarrow {}^{\cdot}OH + O_2 \tag{2.5}$$

But if

$$pH>\approx 8 \quad O_3^{-\cdot} \rightleftharpoons O^{-\cdot} + O_2 \tag{2.6}$$

then

$$O^{-\cdot} + H_2O \rightarrow {}^{\cdot}OH + OH^- \tag{2.7}$$

In a water matrix different hydroxyl radical scavengers may promote or inhibit chain process of ozone decomposition. The term "˙OH scavenger" refers to any compound that consumes ˙OH whether it is a promoter or an inhibitor of ozone decomposition chain reaction (Elovitz & Von Gunten, 1999).

Promoters are compounds that react with hydroxyl radicals to promote ozone decomposition cycle by producing superoxides such as $HO_2^{\cdot}$ or $O_2^{\cdot -}$.

$$^{\cdot}OH + \text{ Promoter } \rightarrow HO_2^{\cdot} \text{ or } O_2^{\cdot -} \tag{2.8}$$

These superoxide radicals then react with ozone to form hydroxyl radical again (Acero & Von Gunten, 2001). Humic acids and primary alcohols are examples of promoters.

$$HO_2^{\cdot} \text{ or } O_2^{\cdot -} + O_3 \rightarrow {}^{\cdot}OH \tag{2.9}$$

When hydroxyl radicals react with an inhibitor no superoxide radical is produced. So the cycle of reactions is discontinued (Acero & Von Gunten, 2001).Therefore inhibitors are hydroxyl radical scavengers that do not enhance the chain reaction of ozone decomposition since when scavenging ˙OH they do not produce any superoxide radicals. Inhibitors include acetate, tert-butanol (t-BuOH) and bicarbonate (HCO_3^-/ CO_3^{2-}) (Elovitz

& Von Gunten, 1999). A fraction of NOM and carbonate/bicarbonate are the primary inhibitors in natural water (Von Gunten, 2003a).

$$^{\cdot}\mathrm{OH} + \text{inhibitor} \rightarrow \text{products} \qquad (2.10)$$

2.5.2.2. Prediction of Oxidation by Ozone and Hydroxyl Radical

As mentioned before in process of ozonation of a micropollutant the dominant pathway of reaction is decided by both the ratio of concentration of ozone to hydroxyl radicals and properties and kinetics of reactions of that micropollutant with each of the oxidants. As the quality of water matrix affects stability of ozone and can alter the mechanism of attack of ozone on the same micropollutant, the by-products formed are expected to be different depending on the dominant pathway (mechanism of attack). The overall oxidation in a water matrix is a combination of effects of molecular ozone and hydroxyl radicals. This concept is formulated as (Von Gunten, 2003a):

$$-\frac{\mathrm{d[C]}}{\mathrm{d}t} = k_{\mathrm{O_3}}[\mathrm{C}][\mathrm{O_3}] + k_{\mathrm{OH}}[\mathrm{C}][\ ^{\cdot}\mathrm{OH}] \qquad (2.11)$$

Where [C], [O_3], and [$^{\cdot}$OH] are concentrations of compound, ozone, and hydroxyl radical, respectively; k_{O3} and k_{OH} are rate constants for reaction of the compound with ozone and hydroxyl radical, respectively, and *t* is time.

Since the concentration of hydroxyl radicals cannot be measured directly, the term R_{CT} is introduced as the ratio of exposure of hydroxyl radicals to exposure of ozone. Exposure being the concentration integrated over contact time. Then, substituting the hydroxyl radical concentration in equation 2.11 with R_{CT} [O_3] and integrating:

$$\ln\frac{[\mathrm{C}]}{[\mathrm{C}]_0} = -(k_{\mathrm{O_3}} + k_{\mathrm{OH}}R_{\mathrm{CT}})\int[\mathrm{O_3}]\,\mathrm{d}t \qquad (2.12)$$

The latter equation determines the extent of ozonation of a micropollutant. The details of calculation of R_{CT} are discussed in the next section.

Oxidation by OH radicals can be predicted from equation 2.13 and therefore comparison of the predicted oxidized micropollutant with hydroxyl radicals to the overall oxidized compound can be done using equations 2.12 and 2.13:

$$f(\cdot OH) = k_{OH} R_{CT} / (k_{O_3} + k_{OH} R_{CT}) \quad (2.13)$$

2.5.2.3. Hydroxyl Radical Quantification

Ozone concentration can be measured using electrochemical or colorimetric methods (APHA, 1998; Acero & Von Gunten, 2001) but there is no fast and easy method for measuring hydroxyl radical concentration during an ozonation process (Acero & Von Gunten, 2001). In 1999 Elovitz and Von Gunten developed an experimental approach for measuring transient concentration of hydroxyl radical during an ozonation process. This approach is based on the concept of R_{CT} as the ratio of exposure of hydroxyl radicals to exposure of ozone. Exposure being the concentration integrated over contact time. In this method the concentration data from removal of an ozone resistant probe compound combined with measured exposure of ozone during contact time and kinetic rate constants of reactions of ozone and hydroxyl radical with the removed compounds provide information on R_{CT} (Acero & Von Gunten, 2001).

2.5.3. Basic Ozonation Chemistry

Ozone is the triatomic form of oxygen. The molecular structure is a neutral but polar one. Figure 2.2 presents the two most stable Lewis structures of ozone:

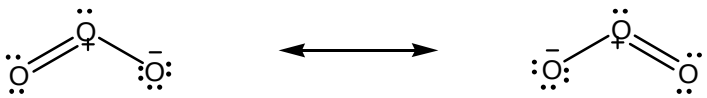

Figure 2.2 Two Lewis structures of ozone (Carey, 2006).

Ozone is a powerful electrophile. In chemistry, an electrophile is a molecule which has an affinity to electrons and is ready to participate in a chemical reaction with a nucleophile (a species with excess electron density). Most electrophiles are positively charged, have an atom which carries a partial positive charge, or do not have an octet of

electrons (in the case of first row element-based electrophiles). As an electrophile, ozone goes through some reactions with alkenes where both (sigma (σ) and pi (π)) bonds of carbon-carbon double bond are cleaved to produce an ozonide (Figure 2.3).

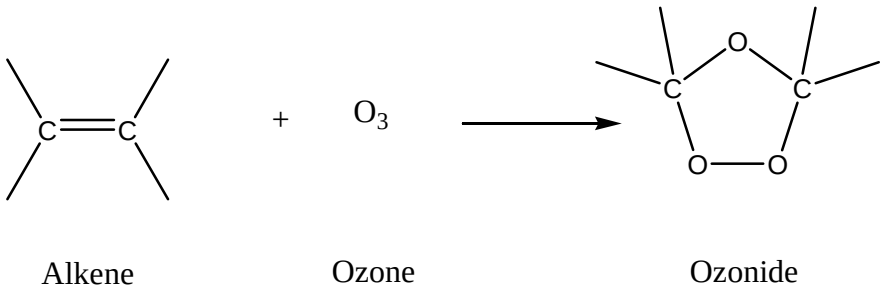

Figure 2.3 Reaction of ozone with alkenes that results in formation of ozonides (Carey, 2006).

In aqueous medium ozonides undergo hydrolysis and give two carboxyl compounds (Figure 2.4) (Carey, 2006)

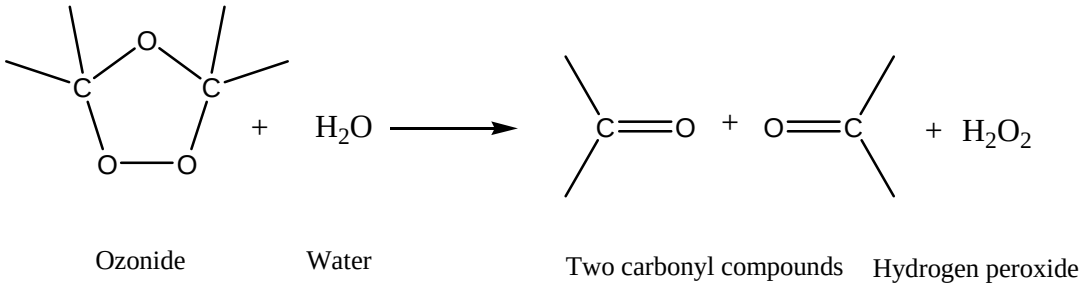

Figure 2.4 Hydrolysis of ozonide and formation of carboxyl compounds (Carey, 2006).

Alkenes that can go through ozonolysis include cyclic variants. If the double bond in the alkene is connected to an electron-donating group the ozonolysis reaction can be many times faster compared to the case where an electron-withdrawing group is connected to that double bond. Ozonolysis usually involves compounds that have more than one double bond; where generally all double bonds are cleaved. In presence of bulky groups, conversion of substrate compound to an epoxide is an important side reaction; sometimes the main reaction. Rearrangement is likely in ozonolysis of some compounds. Compared to double bonds, ozonolysis of triple bonds is less likely. Ozone being an electrophile prefers double to triple bonds where reactions proceed more

easily. Aromatic compounds compared to alkenes are attacked less readily, but reaction often proceeds and the compound is cleaved. In ozonolysis, aromatic compounds behave as if the double bonds in the Kekulé structure were real. With polycyclic aromatic compounds the site of attack of ozone depends on the structure of the molecule (Smith & March, 2007).

Despite large amount of work that has been done on mechanism of ozonization, not all the details involved in this process are known. The basic mechanism of ozonization was formulated by Criegee (Smith & March, 2007) based on which, a 1,3-dipolar addition of ozone to substrate forms the primary or initial ozonide (1,2,3-trioxolane in Figure 2.5). This compound is highly unstable, it cleaves to an aldehyde or ketone and an intermediate compound showed as a zwitterion or a diradical, referred to as a carbonyl oxide. The carbonyl oxide part can then undergo various reactions (Smith & March, 2007). A zwitterion is defined as a compound that carries a net charge of zero and thus is electrically neutral, but carries formal positive and negative charges on different atoms. Sometimes the term is restricted to be applied for those compounds with non-adjacent positive and negative charges. Zwitterions are polar and usually very water soluble. In a broad definition a diradical is defined as an even electron molecule that has one bond less than the number permitted by the standard rules of valance. Diradicals are known by their higher activity and shorter lifetime.

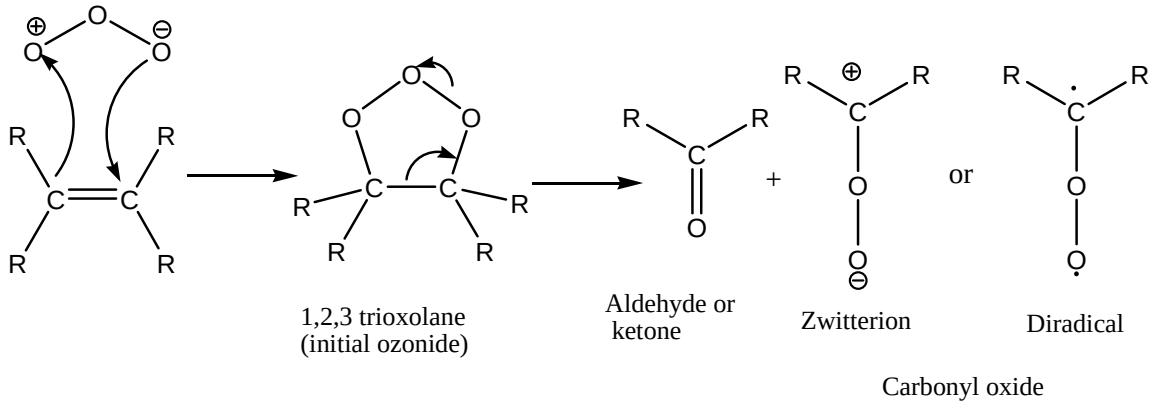

Figure 2.5 Basic mechanism of ozonation by Criegee (Smith & March, 2007).

Based on ozone chemistry the potential site of attack of ozone on different pharmaceutical compounds is proposed in several studies (Andreozzi et al., 2002; Dantas et al., 2007; Ikehata et al., 2006; McDowell et al., 2005). Examples of ozone attacking aromatic rings are in indomethacin, naproxen, gemfibrozil, bezafibrate, clofibric acid and ibuprofen (Yue, 2008).

2.6. Selected Pharmaceuticals

2.6.1. Carbamazepine

Carbamazepine presented in Figure 2.6 is a carboxamide type antiepileptic (anticonvulsant) drug which is widely used to control generalized tonic-chronic seizures (Ikehata et al., 2006).

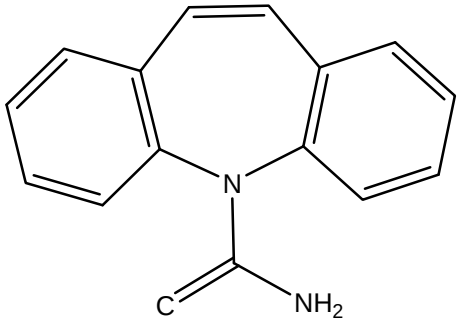

Figure 2.6 Carbamazepine (5H-Dibenz[b,f]azepine-5-carboxamide) (McDowell et al., 2005).

It was amongst the compounds vastly present in German rivers and streams in 1998 (Ternes, 1998). The maximum detected level of carbamazepine in those surface waters was 1.1 µg/L with a median concentration of 0.25 µg/L (Ternes, 1998). Between 1996 and 2000 carbamazepine was detected in surface water samples collected downstream from sewage treatment plants, in several monitoring studies in Berlin (Heberer, 2002). In the United States presence of carbamazepine was reported at a mean level of 0.011 µg/L in all of 12 samples collected from stream waters or raw water supplies for a drinking water treatment plant (Stackelberg et al., 2004). It was also detected at ng/L level in surface water samples collected from sites 100 – 400 m away from sewage treatment plants in city of Windsor (ON, Canada) (Metcalfe et al., 2003). In Hamilton

Harbour (ON, Canada), carbamazepine was the most prevalent drug in the samples collected during summer and fall 2000 (Metcalfe et al., 2003). In the same study carbamazepine was also detected at sites in Lake Ontario and one site in Niagara River (Fort Erie, ON, Canada) which were remote from sewage treatment plants discharges. In another study conducted by Great Lakes Institute for Environmental Research in University of Windsor, carbamazepine was amongst few compounds that were consistently detected in raw water intake of the A.H. Weeks drinking water treatment plant in city of Windsor (ON, Canada) in sub-ng/L levels (Hua et al., 2006). Carbamazepine was one of the two compounds noted as particular concern by a study in Minnesota because of its persistence and being readily transported in water (Lee et al., 2004). Because of its continual input into the environment and resistance to biological treatment, carbamazepine and its metabolites are of considerable environmental concern (Kosjek et al., 2007). Carbamazepine has even been detected in concentrations up to 24 ng/L in finished drinking waters in Canada (Tauber, 2003 as cited in Jones et al., (2005) and 258 ng/L in the United States (Stackelberg et al., 2004). A summary of detection of carbamazepine in streams and surface waters is presented in Table 2.3.

Table 2.3 Occurrence of carbamazepine in surface and drinking waters

Type of water	Location	levels of detection (ng/L)				Reference
		Maximum	Median	Mean	Detected	
surface water	Germany	140-7100	65-1200	70-133.3	45-80	Heberer, 2002; Liebig et al., 2006; Reddersen & Heberer, 2003; Sacher et al., 2001; Ternes, 1998; Ternes et al., 2002; Wiegel et al., 2004
	the U.S.	1500			11	Stackelberg et al., 2004
	Ontario	20-650	20-185	2-23		Hua et al., 2006; Metcalfe et al., 2003; Tabe et al., 2009
finished drinking	Canada	24				Tauber from Jones et al., 2005
	the U.S.	258				Stackelberg et al., 2004

2.6.1.1. Oxidation of Carbamazepine by Ozone

Complete removal of carbamazepine (with a starting concentration of ~800 µg/L) by ozonation has been reported in the literature (Andreozzi et al., 2002). The results of a study by Hua et al. (2006) at the A.H. Weeks water treatment plant showed that ozonation removed an already low concentration of carbamazepine from raw water to below the method detection limit. Snyder et al. (2006) state that based on occurrence and removal data, carbamazepine is one of the compounds that can be used as an indicator for O_3/H_2O_2 performance as it is expected to have a complete removal even with the lowest dose of oxidant. However, a low degree of mineralization (i.e., complete oxidation to carbon dioxide and water) was observed after 60 min of ozonation and the carbon balance remained lacking for even long ozonation times (Andreozzi et al., 2002). A summary of oxidation rates of carbamazepine in simulated and surface water samples is presented in Table 2.4.

Table 2.4 Oxidation rates for ozonation of carbamazepine in simulated and surface waters

Type of water	Ozone dose (mg/L)	Initial concentration	Extent of oxidation	Reference
bi-distilled water	1	780 µg/L	< MDL	Andreozzi et al., 2002
intake to Windsor WTP	1.5-2	0.3-3.8 ng/L	< MDL	Hua et al., 2006
surface water	1-1.3	30-40 ng/L	< MDL	Vieno et al., 2007
surface water	0.7	80 ng/L	< MDL	Ternes et al., 2002
flocculated surface water	0.5	1 µg/L	< MDL	Ternes et al., 2002
surface water	0.2-0.5	118 µg/L	< MDL	Huber et al., 2003
pre-filtered surface water	1.25-2.5	100-373 ng/L	< MDL	Snyder et al., 2006
surface water	1-3	3.4-16 ng/L	<MDL	Snyder et al., 2006
simulated water	1-3	0.2-1.5 µg/L	< MDL	Tabe et al., 2009
surface water	0.3-1.5	0.2-1.5 µg/L	< MDL	Tabe et al., 2009
intake of Windsor WTP	1-3	0.2-1.9 ng/L	< MDL	Tabe et al., 2009

MDL= method detection limit

Despite the widely studied effect of ozonation on reduction of concentration of carbamazepine in pure, simulated and surface water samples, only two studies have attempted to propose a pathway for oxidation of this compound and identify ozonation products (Andreozzi et al., 2002; McDowell et al., 2005). According to Andreozzi et al.

(2002) ozonolysis of carbamazepine can proceed via two pathways as shown in Figure 2.7.

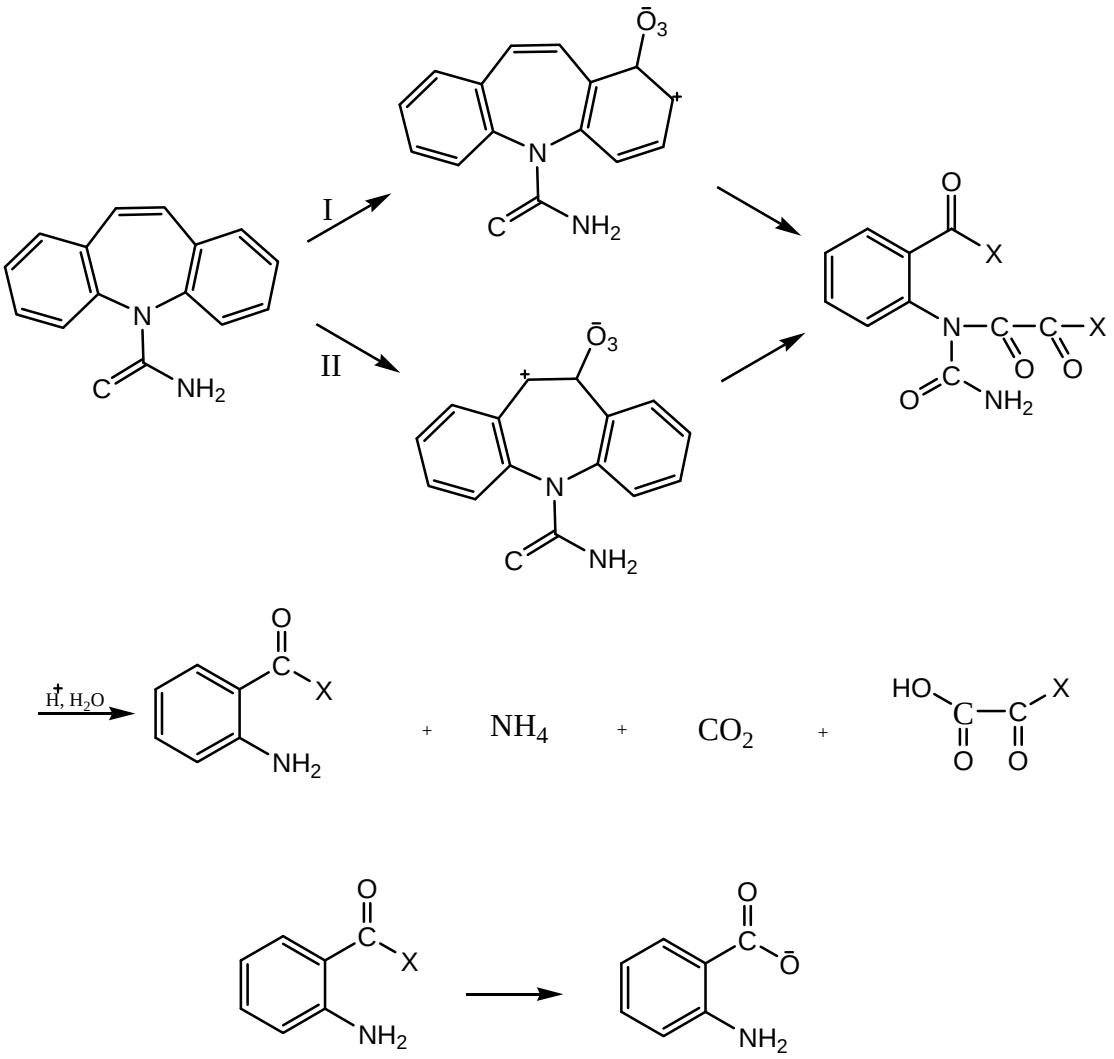

Figure 2.7 Two pathways of ozone attack to carbamazepine according to Andreozzi et al. (2002).

Andreozzi et al., discuses that the oxidation process mainly proceeds through path I. However both pathways result in formation of anthranilic acid. Glyoxal, glyoxilic and oxalic acid have been identified as products of ozonolysis of carbamazepine as well.

These identified compounds only account for 30% of initial carbon content of carbamazepine, meaning that some of the by-products remain non-identified.

McDowell et al. (2005) identified three oxidation products of carbamazepine using mass spectrometric and NMR techniques as shown in Figure 2.8. Unlike Andreozzi et al. (2002) who suggested attack of ozone on benzene ring as the dominant pathway for ozonation of carbamazepine, all the three identified by-products in McDowell et al.'s study are the result of attack of ozone on the non-aromatic carbon-carbon double bond of carbamazepine molecule, proceeding to cleavage of ring according to the Criegee mechanism as discussed in Section 2.5.3. These identified ozonation products are:

-1-(2-benzaldehyde)-4_hydro-(1H,3H)-quinazoline-2-one (BQM)

-1-(2-benzaldehyde)-(1H,3H)-quinazoline-2,4-dione (BQD)

-1-(2-benzoic acid)-(1H,3H)-quinazoline-2,4-dione (BaQD)

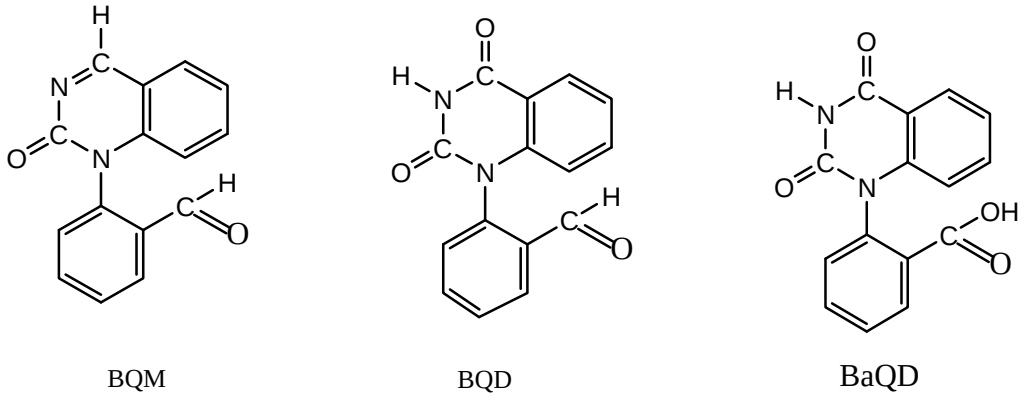

Figure 2.8 The structure of three new oxidation products of carbamazepine elucidated by a combination of mass spectrometric and NMR techniques (McDowell et al., 2005).

The mechanism of formation of BQM, one of these three compounds, is illustrated in Figure 2.9.

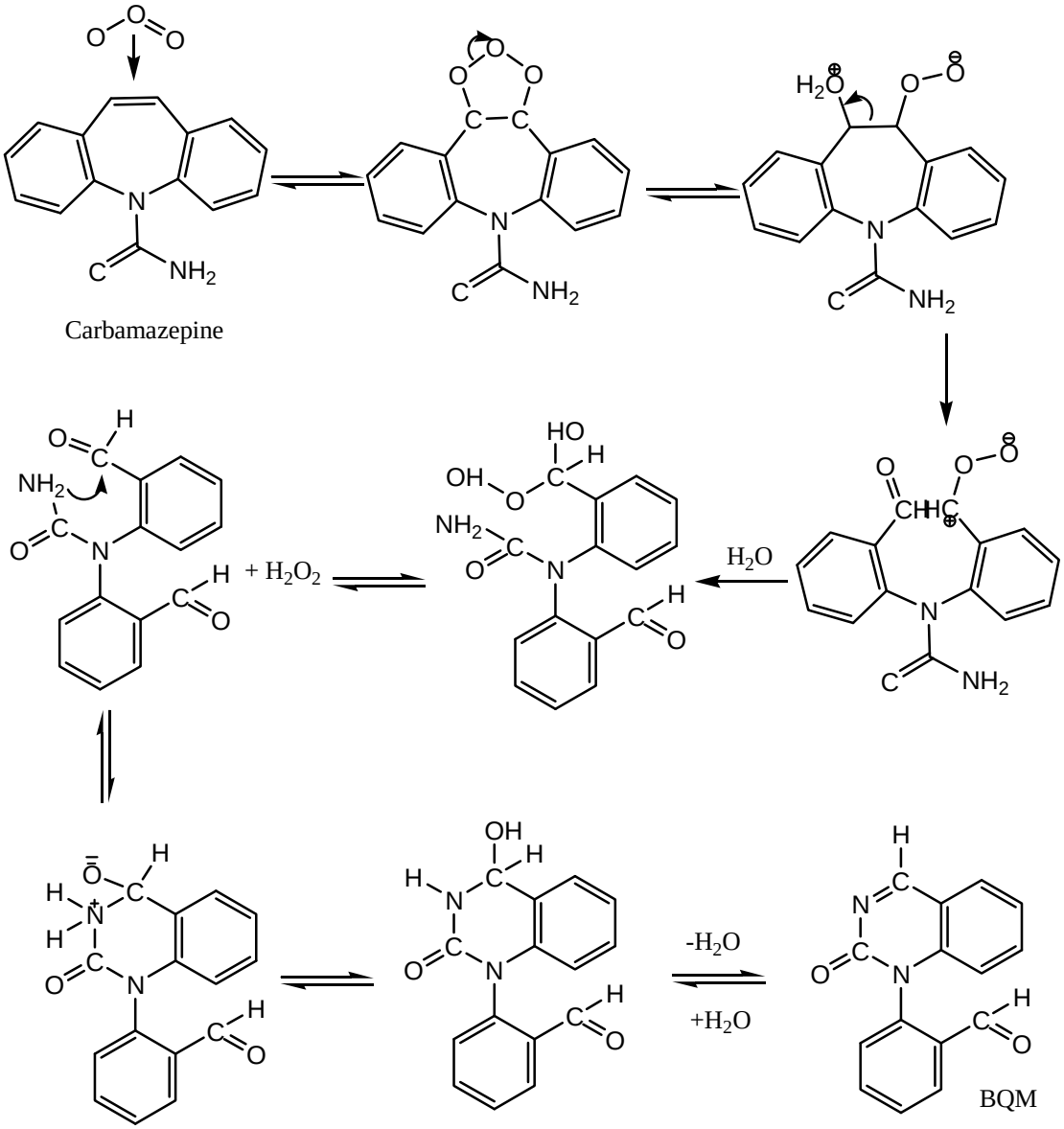

Figure 2.9 Proposed mechanism for direct attack of ozone to carbamazepine and formation of BQM (McDowell et al., 2005).

Kinetic studies by McDowell et al., 2005 on identified ozonation products have shown that BQM and BQD have very low second order rate constants with ozone; k_{O3}=~7 $M^{-1}s^{-1}$ and ~1 $M^{-1}s^{-1}$ at pH=6 respectively. With much higher values for reaction rate constants with hydroxyl radicals; k_{OH}=~7×10^9 $M^{-1}s^{-1}$ and ~ 5×10^9 $M^{-1}s^{-1}$ for BQM and BQD respectively, it was concluded that further oxidation of these two compounds was

feasible by hydroxyl radicals rather than ozone (McDowell et al., 2005). Production of the two major ozonation products (BQM and BQD) identified in lab scale experiments were verified in ozonation of carbamazepine spiked natural waters in full scale water treatment plant as well. Lake Zurich water samples spiked with 236 µg/L (1 µM) of carbamazepine were determined to have 0.48 and 0.15 µM of BQM and BQD respectively after ozonation with 1.9 mg/L of ozone. The pathway in Figure 2.10 demonstrates a proposed oxidation pattern of carbamazepine with ozone and hydroxyl radicals leading to formation and subsequent degradation of intermediate compounds (McDowell et al., 2005).

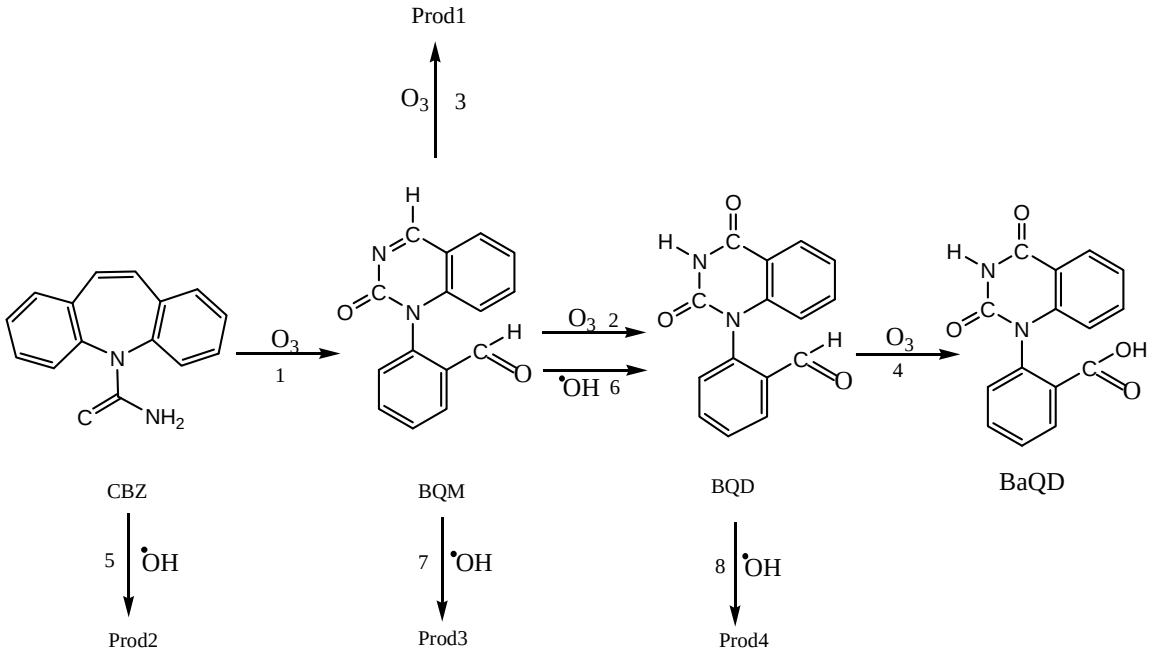

Figure 2.10 Oxidation pathway of carbamazepine with ozone and hydroxyl radicals from McDowell et al. (2005).

1 $CBZ + O_3 \longrightarrow BQM$
2 $BQM + O_3 \longrightarrow BQD$
3 $BQM + O_3 \longrightarrow Prod1$
4 $BQD + O_3 \longrightarrow BaQD$
5 $CBZ + OH \longrightarrow Prod2$
6 $BQM + OH \longrightarrow BQD$
7 $BQM + OH \longrightarrow Prod3$
8 $BQD + OH \longrightarrow Prod4$

As mentioned before the extent of available studies on identification of ozonation products of carbamazepine is limited and moreover the relative influence of ratio of concentration of ozone to hydroxyl radicals (ratio of ozone exposure to hydroxyl radical exposure) in the oxidation medium on formation of products was not clarified in these studies.

2.6.1.2. Toxicity Effects of Carbamazepine and Its By-products

Andreozzi et al. (2002) have reported that carbamazepine is not toxic towards *Selenastrum capricornutum* and *Ankistrodesmus braunii* and no accumulation of the compound into algae has been observed. Toxicity tests on carbamazepine solution after ozonation with different reaction times of 5, 10, 20, 30, and 40 minutes have been conducted. No inhibition to the experimental organisms was observed during assessment tests that took from 96 hour to 30 days.

2.6.2. Bezafibrate

Fibric acid derivatives (fibrates) are a group of drugs marketed since 1963 to control the level of triglyceride in blood. This group is most often given to patients with mixed and combined hyperlipidaemia, when raised cholesterol levels are associated with raised levels of triglycerides (Cermola et al., 2005). Fibrates act by reducing the liver's production of VLDL (triglyceride-carrying particle that circulates in the blood) and by speeding up the removal of triglycerides from blood (Isidori et al., 2007). Examples of fibrates that are prescribed in the United States and Europe are bezafibrate, gemfibrozil, fenofibrate and clofibric acid. Bezafibrate is one of the most highly used pharmaceuticals in the world (Cermola et al., 2005). In Canada, bezafibrate together with gemfibrozil are the most highly prescribed fibrates (Metcalfe et al., 2003). In Germany alone, the estimated prescription amount of bezafibrate was 30 tons in 1995 (Ternes, 1998). Estimated sales data for this drug in Italy was 7.60 tons in 2001 (Calamari et al., 2003). The chemical structure of bezafibrate is given in Figure 2.11.

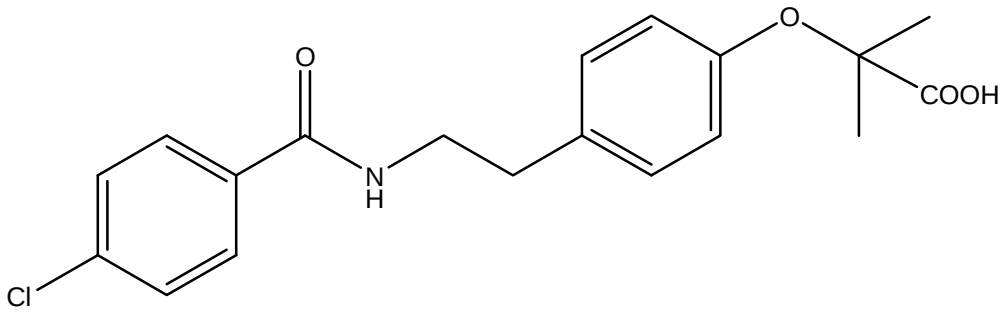

Figure 2.11 Bezafibrate (2-[4-2-[4-chlorobenzamido] ethylphenoxy]-2-methylpropanoic acid) (Dantas et al., 2007).

In a study by Ternes (1998), concentrations of 20 drugs and 4 related metabolites were measured in rivers and stream waters of Germany. Bezafibrate with detected concentration of 3.1 µg/L had the highest concentration amongst all ubiquitously found pharmaceuticals. Lipid regulators, antiphlogistics, antiepileptic and beta blockers were detected at ng/L range (Ternes, 1998). Furthermore, the median concentration for bezafibrate found in this study, i.e., 0.35 µg/L, in 22 rivers and streams in Germany was only one order of magnitude lower than the median concentration in STP effluents. Also, bezafibrate was detected in drinking water in Germany at levels as high as 27 ng/L (Stumpf et al., 1996 as cited in (Jones et al., 2005)). In Italy, bezafibrate was detected in the Po and Lambro rivers at concentrations up to 57.15 ng/L (Calamari et al., 2003). It is widely detected in high concentrations in Canada as well (Metcalfe et al., 2003). A summary of available data is presented in Table 2.5

Table 2.5 Occurrence of bezafibrate in surface and drinking waters

Type of water	Location	levels of detection (ng/L)			Reference
		Maximum	Median	Mean	
surface water	Europe	57.15-3100	350		Calamari et al., 2003; Ternes, 1998
	Ontario	4.2-200	52	10-137	Metcalfe et al., 2003; Tabe et al., 2009
drinking water	Germany	27			Stumpf et al., 1996 as cited in Jones et al., 2005

2.6.2.1. Oxidation of Bezafibrate by Ozone

Relative oxidation of bezafibrate along with other pharmaceuticals has been investigated in several studies. The results reported by Vieno et al. (2007) indicated that bezafibrate was detected in ozonated water samples (0.2 – 0.4 mg O_3/mg of TOC) at concentrations lower than limits of quantification. Bezafibrate had an overall reduction of >77% in this study at ozone doses of 1.0 – 1.3 mg/L. Other studies have reported that ozone doses of 1-3 mg/L are required for 70-80 % reduction of bezafibrate (Huber et al., 2003; Ternes et al., 2002). This level of ozone matches 0.3 – 2.3 mg of O_3/mg of TOC. These studies show that compared to other pharmaceuticals such as carbamazepine and diclofenac, bezafibrate is more resistant to oxidation by ozone. Lab scale experiments in a study by Ternes et al. (2002) showed that 1.5 mg/L of ozone reduced bezafibrate by only 50% from its initial concentration of 1 µg/L. Increasing ozone dose to 3.0 mg/L increased the reduction to 80%. Table 2.6 summarizes the oxidation data of bezafibrate by ozone found in the literature.

Table 2.6 Oxidation of bezafibrate by ozone in different waters

Type of water	Ozone dose mg/L	Initial concentration	Extent of oxidation	Reference
surface water	1.0-1.3	< 10ng/L	>77%	Vieno et al., 2007
flocculated surface water	1-3	1 µg/L	50-80 %	Ternes et al., 2002
surface water	1-2	181 µg/L	70 to >97%	Huber et al., 2003
–	35	181 mg/L	< MDL	Dantas et al., 2007
intake of Windsor WTP	1-3	0.5-4.2 ng/L	< MDL	Tabe et al., 2009

MDL= method detection limit

Among the studies that have investigated ozonation of bezafibrate, only one has focused on identification of by-products formed (Dantas et al., 2007). Mechanism of attack of ozone and the proposed by-products formed in literature are presented here.

The two aromatic rings in the structure of bezafibrate are two possible site of reaction with ozone as shown in Figure 2.12 (Bailey, 1982 as cited in Dantas et al. (2007)).

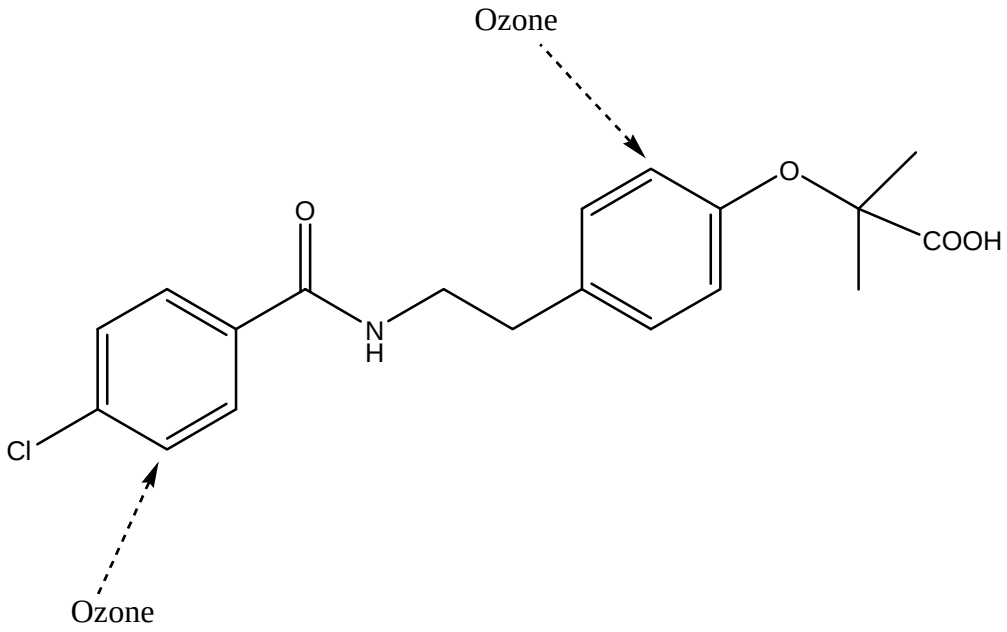

Figure 2.12 Possible sites of attack of ozone on bezafibrate from Dantas et al. (2007).

As discussed in Section 2.5.2, ozone, as an electrophile, has a tendency to attack aromatic rings. One of the aromatic rings in bezafibrate is chlorinated. Since chlorine is an electron withdrawing group, the chlorinated aromatic ring is expected to be a less desirable site of attack for ozone. In a study by Dantas et al. (2007) 0.5 mM (181 mg/L) of bezafibrate was completely reduced with 10 minutes of ozonation. However the mineralization degree was only 20% which increased to 30% with continued ozonation up to 103 minutes. In this study, HPLC-MS analysis of ozonation of bezafibrate at different contact times was performed and some proposed structures of products were assigned to detected peaks.

The formation of two of these proposed structures, one with a molecular weight of 367 and the other with a molecular weight of 393 is justified by a direct ozone attack to un-chlorinated aromatic ring in bezafibrate molecule resulting in aldehydic and ketonic products (Dantas et al., 2007). The proposed structures for these two compounds are shown in Figures 2.13 and 2.14.

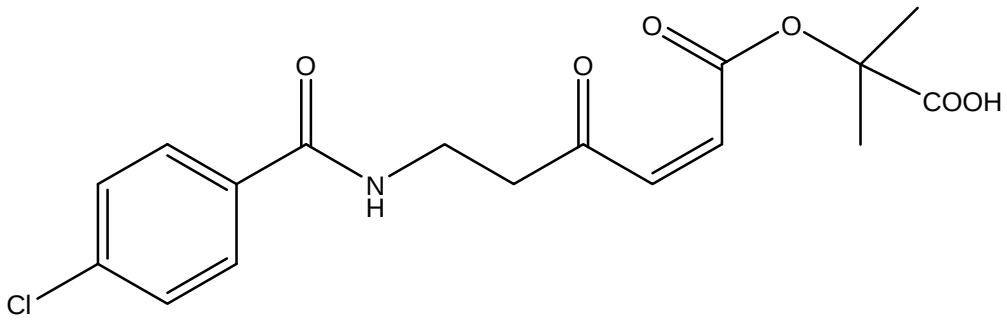

Figure 2.13 Proposed structure for one of ozonation products of bezafibrate with a molecular weight equal to 367 (Dantas et al., 2007).

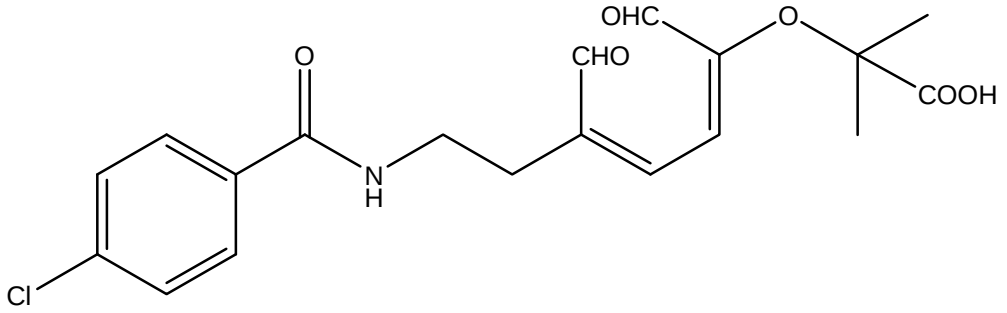

Figure 2.14 Proposed structure for an ozonation product of bezafibrate with molecular weight equal to 393 (Dantas et al., 2007).

It has been stated that an anomalous ozonolysis mechanism on the species which is identified here with molecular weight 393 forms another compound with molecular weight of 227 (Dantas et al., 2007). The structure of this compound is shown in Figure 2.15.

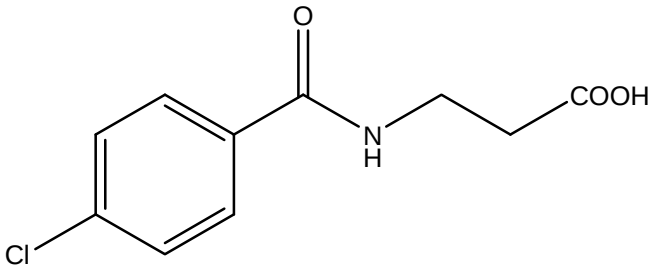

Figure 2.15 Proposed structure of an ozonation product of bezafibrate with molecular weight of 227 (Dantas et al., 2007).

In addition, some ozone attacks on aromatic rings are suggested to be accompanied by hydroxylation reactions. Two structures, both with corresponding molecular weights of 409 are proposed for this reaction (Dantas et al., 2007) as shown Figure 2.16.

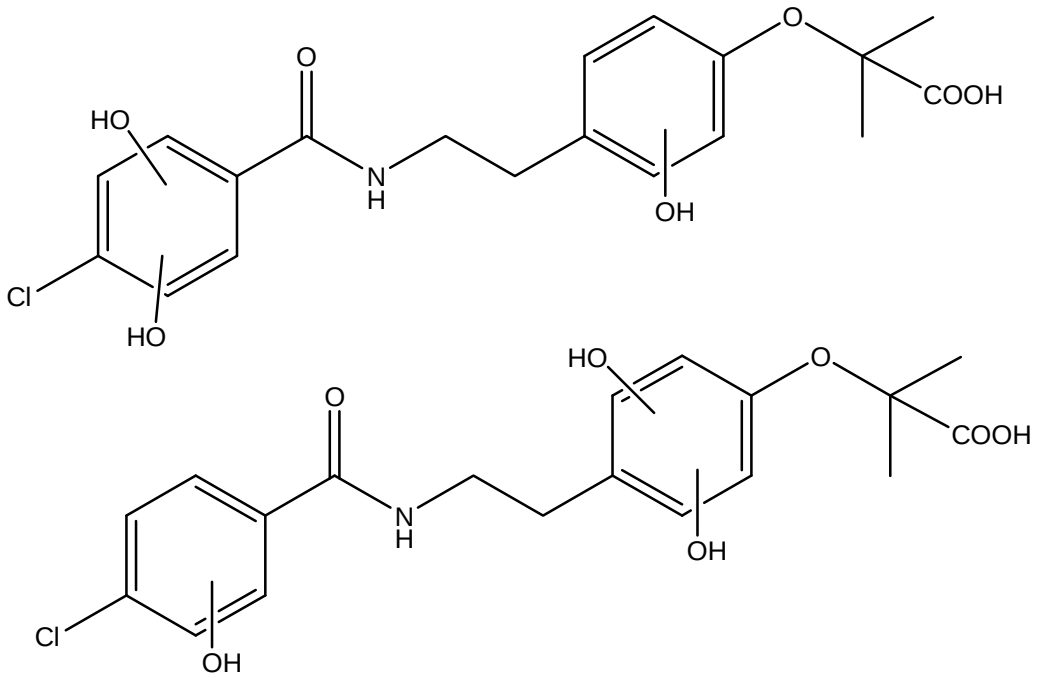

Figure 2.16 Two proposed structures for ozonation products of bezafibrate with their molecular weight equal to 408 (Dantas et al., 2007).

2.6.2.2. Toxicity Effects of Bezafibrate

A limited number of studies report the formation of by-products during ozonation of bezafibrate. Among them, only one investigated the toxicity of intermediates formed during ozonation of bezafibrate (Dantas et al., 2007). In that study, the tests performed to assess the acute toxicity of ozonated water samples showed that the toxicity of waters containing bezafibrate was reduced after ozonation. Microtox® test was used for measuring acute toxicity of ozonation of bezafibrate under different experimental conditions. In Microtox® test the inhibition of the light emission of *Vibrio fischeri* bacteria is an indication of toxicity of tested samples. Starting with bezafibrate solution the measured acute toxicity had been observed to be low. The results of that study

showed an initial increase in toxicity in early stages of ozonation. That is, for less than ~10 minutes. With increased ozonation the measured levels of toxicity declined and after 105 minutes resulted in values lower than that of starting solution (Dantas et al., 2007).

Although the results from this study are indicative of complete removal of bezafibrate by ozonation, the contact time required to achieve such high level of efficiency is beyond the common practice. For this reason, formation of oxidation by-products during this process and potential increased toxicity is of concern. However, ozonation has remained the most effective way of reducing concentration of bezafibrate as other oxidation processes can only remove this pollutant by 12% (Isidori et al., 2007).

Chapter 3: Materials and Methods

3.1. Experimental Design

Parameters like temperature, pH, DOC (Dissolved Organic Carbon) level, ozone concentration and ozone contact time can influence the extent of micro pollutant transformation during ozone treatment. In a systematic study, Tabe et al. (2009) examined the effect of pH (6.8 and 8.1), temperature (5°C and 23°C), DOC concentration (0.8 and 4.5 mg/L), ozone dose (1 and 3 mg/L) and ozone contact time (2 and 6 minutes) on the transformation of 16 PPCPs and EDCs during ozonation treatment of drinking water. The results showed that DOC concentration as well as ozone dose and ozone contact time had the greatest influence on the ozonation process in a way that high DOC loading and low ozone dose and contact time were accounted for low oxidation efficiencies. The effect of ozone concentration and DOC can be explained in terms of direct and indirect mechanism of attack by ozone, as discussed in Section 2.5.2. The contact time influences the corresponding exposure to ozone and hydroxyl radicals. The extent of transformation of a micro pollutant during the ozonation process is therefore largely influenced by the relative exposure to ozone and hydroxyl radicals. The range and concentrations of by-products formed during the transformation of a micro pollutant in the ozonation process are also therefore expected to be influenced by the relative exposure to ozone and hydroxyl radicals.

In the present study, by-products formed from the transformation of carbamazepine and bezafibrate during ozonation treatment of drinking water under varying ozone and hydroxyl exposures were examined in simulated water. Temperature, pH and ozone dose were maintained in the range shown in Table 3.1 for all experiments.

Table 3.1 Values of experimental conditions.

	PH	Temperature (°C)	O_3 Conc. (mg/L)
value	7.3±0.2	23±2	2

3.1.1. Simulated Water

In this study experiments were conducted using simulated water. The simulated water consisted of ultra pure Milli-Q water (Environmental Engineering Lab, University of Windsor) with its pH adjusted to 7.3±0.2 using sodium bicarbonate (sodium hydrogen carbonate, $NaHCO_3$, ACS reagent grade, CAS Number 144-55-8) as buffer solution. This target pH range is the typical of water during drinking water treatment. Conducting experiments with simulated water rather than any given natural water would provide capability of extending and extrapolating the obtained results to various real water treatment conditions.

3.1.2. Experimental Variables

Four experimental settings (designated Settings A, B, C and D) were selected to influence the formation of hydroxyl radicals as discussed below.

Setting A: Tert-butanol was added to act as hydroxyl radical scavenger. Tert-butanol (anhydrous grade, assay ≥99.5%, CAS Number 75-65-0) was purchased from Sigma-Aldrich. A concentration of 30 mM was used. Micro pollutant transformation due to ozone exposure alone is expected under these conditions. Experiments were conducted at ozone contact times of 2, 5 and 10 min to further vary the extent of ozone exposure.

Setting B: Simulated water under conditions specified in Table 3.1 was used. No additional chemicals were added. Micro pollutant transformation due to both ozone and hydroxyl radical exposures, is expected under these conditions. Experiments were conducted at ozone contact times of 2, 5 and 10 min to further vary the extent of ozone and hydroxyl radical exposures.

Settings C and D: Hydrogen peroxide was added to promote the formation of hydroxyl radicals. ACS reagent grade hydrogen peroxide (30% solution), calculated to have a concentration of 9.703 M, was purchased from ACP Chemicals Inc., Montreal, Quebec.

In setting C, 0.123 ml/L of hydrogen peroxide solution (100 mM) was added to the matrix resulting in a hydrogen peroxide to ozone molar ratio of 0.25 in the reactor. A

hydrogen peroxide to ozone molar ratio of 0.5 was obtained in experimental setting D using 0.245 ml/L of hydrogen peroxide solution (100 mM). Experiments were conducted at ozone contact times of 2, 5 and 10 min under each experimental setting to further vary the extent of ozone and hydroxyl radical exposures.

The stoichiometric molar ratio between ozone consumption and hydroxyl radical formation as per reactions 2.2 to 2.7 is one to one. Micro pollutant transformation due to both ozone and hydroxyl radical exposures, is expected under these conditions. Since ozone is consumed in this reaction, ozone exposure is expected to be lower and hydroxyl radical exposure is expected to be higher than under settings A and B.

3.2. Experimental Procedures

The current study examined ozonation transformation by-products of two selected pharmaceuticals: bezafibrate and carbamazepine (both obtained from Sigma-Aldrich, catalogue numbers: bezafibrate B7273, carbamazepine C4034). A set of 12 experiments (three ozone contact times for each of four experimental settings as discussed in Section 3.1.2) were conducted for each one of these two pharmaceuticals. The experimental design is shown in Table 3.2. Although it is desirable to have larger number of experimental runs to statistically compare results obtained, the number of runs in current study was limited to two, due to high analytical expenses and limited funding.

Table 3.2 Design of experiments for each of the two studied chemicals

Experimental Setting	Experiment	Hydroxyl radical controlling compound	Contact time (min)
Setting A	T1 T2 T3	tert-butanol	2 min 5 min 10 min
Setting B	T1 T2 T3	N.A.	2 min 5 min 10 min
Setting C	T1 T2 T3	Hydrogen peroxide molar ratio to ozone 0.25	2 min 5 min 10 min
Setting D	T1 T2 T3	Hydrogen peroxide molar ratio to ozone 0.5	2 min 5 min 10 min

3.2.1. Preparation of Water Matrix

For preparing water matrix, ultra pure water was collected from MQ water dispenser system (Environmental Engineering Lab, University of Windsor). The water was collected the evening before the experiment and was allowed to be saturated with CO_2 from air overnight. A 9.5 L glass container was used for collection of Matrix water. pH was adjusted with 1 M sodium bicarbonate solution to a value between 7.1 to 7.5. VWR micropipettes of 20-200 µL or 100-1000 µL capacities were used for measurements of buffering solution and all other low volume contents.

Depending on experimental settings tert-butanol (tert-butyl alcohol), hydrogen peroxide or neither of the two was added to water matrix. Tert-butyl alcohol was added in 10-50 mM to simulated water media as the OH radical scavenger (Huber et al., 2003). In all setting A experiments 25 ml of tert-butanol was added to 9 L of simulated water to yield a concentration of approximately 30 mM. A 100 mM solution of hydrogen peroxide prepared by dilution of the original stock was used for setting C and setting D experiments. The molar concentration of hydrogen peroxide in water matrix in settings C and D was 0.0122 and 0.0245 mM respectively. These quantities correspondingly

result in molar ratios of 0.25 and 0.5 of hydrogen peroxide to ozone in settings C and D experiments.

3.2.2. Spiking the Compound

Pharmaceutical compounds were provided as a stock solution in pure methanol by MOE Laboratory Services. The concentrations of bezafibrate and carbamazepine stocks were 3.62 and 2.36 mg/ml, respectively. Target concentrations for these pharmaceuticals in simulated water were approximately 426 and 278 µg/L for bezafibrate and carbamazepine, respectively. In order to prevent methanol from entering simulated water matrix, the required volume of stock solution, combined with the same volume of ultra pure Milli-Q Water was blown down with a constant flow of Nitrogen gas for 15 minutes prior to mixing. In this way methanol from spiking solution was evaporated before adding the pharmaceutical compounds to simulated water media.

3.2.3. Ozone Treatment

After the spiked pharmaceutical was mixed with simulated water, required volume of water media was dispensed to 2.5 L silanized reactor bottles to go through ozone treatment. Ozone solution was produced by passing ozone gas, generated from oxygen by a bench scale ozone generator (Ozonia North America, NJ), through approximately 1500 ml of cold ultra pure water, the container was immersed in an ice bath for keeping the solution temperature low and preventing ozone from degradation. After 30 minutes of running ozone generator the ozone concentration of stock solution was measured according to 4500-O_3 Indigo Colorimetric Method (APHA, 1998). A calculated volume of ozone stock solution was then added to the reactor bottles to bring about an initial ozone concentration of 2 mg/L. Contact time was recorded with a stopwatch and at the end a 100 ml sample was taken from the reactor for measuring residual ozone concentration using the same colorimetric method. The rest of reactor content was quenched with 25 mg/L sodium thiosulfate ($Na_2S_2O_3$) solution for bezafibrate experiments and 30.286 mg/L (ten fold alcohol to ozone) 3-Buten-2-ol (methyl vinyl carbinol, CAS number 598-32-3, assay 97% purchased from Sigma-Aldrich) for

carbamazepine experiments (Bader & Hoigné, 1981; McDowell et al., 2005). Reactor contents were then dispensed to 1 L amber glass sample bottles provided by MOE laboratory and were shipped in ice packed coolers to the Laboratory Services Branch, Ontario Ministry of the Environment by courier within 48 hours for parent compound and by-product analysis. The experimental procedure is shown in Figure 3.1.

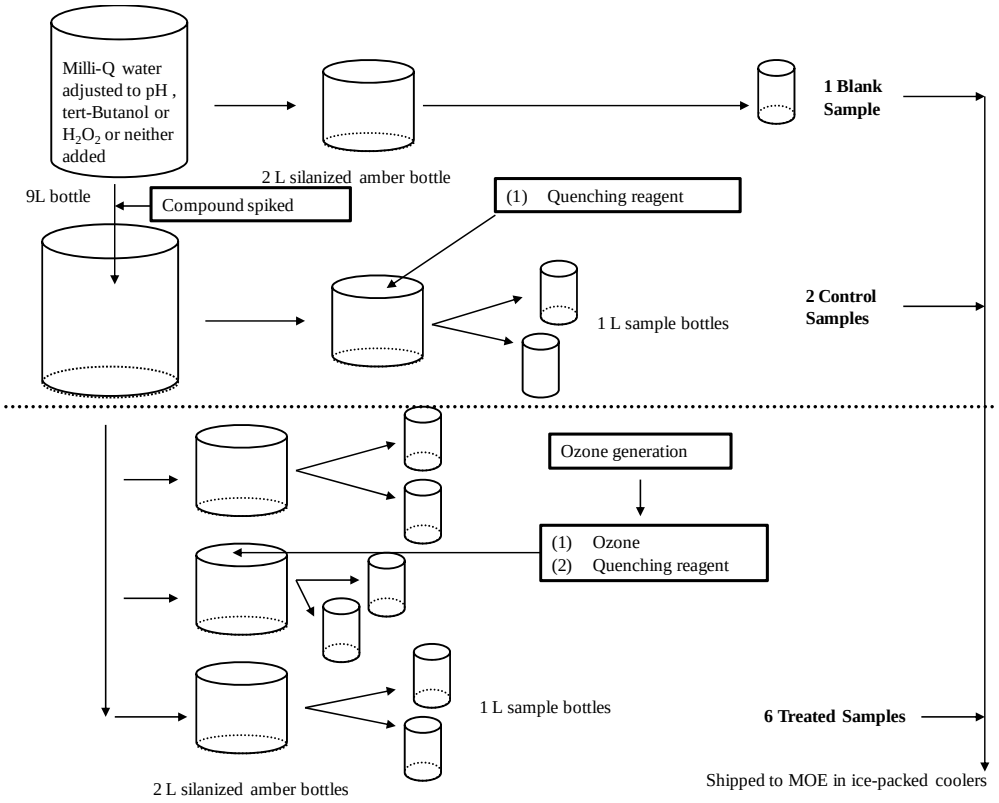

Figure 3.1 Procedure for conducting experiments (Yue, 2008).

3.3. Hydroxyl Radical Quantification

Since hydroxyl radicals are present in low transient concentrations in water media there is no quick and simple way of measuring them. Based on literature an indirect method of quantifying hydroxyl radical level present in experimental simulated water was used in the current study (Elovitz & Von Gunten, 1999). This method is based on the removal of an ozone resistant probe compound with hydroxyl radicals present in simulated

water matrix. p-chlorobenzoic acid (4- chlorobenzoic acid 99%, MW 156.57, CAS Number 74-11-3 purchased from Acros organics) was chosen for this purpose.

Hydroxyl radical exposure was quantified under each experimental setting A, B, C and D. The matrix used was similar to the one used in main experiments. For details on preparation of matrix refer to Section 3.2.1. Following a similar procedure to that explained in Section 3.2.2 the water matrix was then spiked with 1 µM of p-chlorobenzoic acid (pCBA) (500 ppm stock solution in pure methanol) after evaporation of methanol with Nitrogen stream flow. This water matrix containing pCBA was then treated with ozone in a similar fashion to ozone treatment of water matrix spiked with pharmaceuticals (Section 3.2.3). Sampling was done at one minute time intervals in first 5 minutes of contact with ozone (i.e. 1, 2, 3, 4, 5 minutes after addition of ozone solution) and then every 90 seconds for up to 14 minutes of contact time (i.e. 6:30, 8, 9:30, 11, 12:30 and 14 minutes). At every sampling instance two sample aliquots were taken simultaneously. Those were quenched with two different quenching reagents. One sample was a 10 ml aliquot dispensed to a 20 ml vial containing sodium thiosulfate 0.5% (W/W) (10 times diluted sodium thiosulfate 5% (W/W)) and was used for HPLC analysis. The other sample (approximately 10-20 ml) was quenched with indigo solution and used for measuring the ozone residual.

3.4. Analytical Work

3.4.1. Measurement of Experimental Variables

3.4.1.1. Residual Ozone Measurement

Ozone concentration of prepared stock solution, as well as ozone residuals in each reactor sample at the end of contact time and ozone concentration in samples taken in hydroxyl radical quantification experiments were measured by a Spectronic 20D+ spectrophotometer (at wavelength 600) according to standard method (4500-O_3)/Indigo Colorimetric Method (APHA, 1998).

3.4.1.2. pH Measurement

pH value of simulated water was measured using a VWR pH meter model 8100 (VWR scientific products) and a Ag/AgCl Symphony VWR pH electrode. The pH meter was calibrated using buffer standard pH 7 and pH 10 (ACP CHEMICALS INC. Montreal, Quebec) at the beginning of each experiment according to pH probe instruction manual. Used buffers were traceable to N.I.S.T. (National Institute of Standards and Technology).

3.4.1.3. Measurement of p-Chlorobenzoic Acid

As mentioned before p-chlorobenzoic acid was used as a probe compound for indirect measurement of hydroxyl radical concentration. The analysis for pCBA was conducted with a Dionex UltiMate 3000 high-pressure liquid chromatograph (HPLC) (Dionex, Sunnyville, CA). The analytical column used was a Zorbax $\mathrm{C18}\ 3.5\ \mu\mathrm{m},\ 3.0\ \mathrm{mm\ ID} \times 100\ \mathrm{mm}$ (Chromatographic Specialties Inc.). Quantification of pCBA was done by reverse-phase HPLC, using a two pump system of mobile phase 70:30 Methanol: 10% Acetic acid in water at a flow of 0.4 ml/min. Analysis was performed at 240±10 nm wavelength and injection volume was 50 µL. pCBA had a retention time of 2 minutes with a total analysis time of 3 minutes. Oven temperature was set at 50 °C. Detection limit for pCBA was 10 µg/L. Standards were run at the beginning of each batch of samples. All chemicals used in solutions and eluents were reagent grade and were used without further purification.

3.4.2. Analysis of Parent Compounds and By-Products in the MOE Laboratory

All the analytical work determining the present concentration of parent compounds as well as ozonation products in all of experimental samples were performed in Applied Chromatography Section, Laboratory Services Branch, Ontario Ministry of the Environment. Complete description of Methods and protocols can be found in a journal publication by Hao et al., 2008 (Hao et al., 2008). A brief explanation is presented here.

After samples were received by the Laboratory Services at MOE, they were stored in the dark between 2 to 6 °C until analysis. 99.9% N_2 gas (Parker Balston, MA) was used in

both sample preparation and MS-MS analysis. Solid phase extraction was performed using Waters (Millford, MA) HLB SPE cartridges (6 ml, 200 mg).

Analysis were performed using an Agilent 1100 LC (Mississauga, Ontario, Canada) coupled with an Applied Biosystems API 4000 Q-trap mass spectrometer (Foster City, CA) using an ESI interface. LC column (Thermo Electron, Bellefonte, PA, Hypersil Gold, C-18, 100*2.1 mm, 3 µm) was used. In these analyses Instrument detection limits (i.e., the amount of analyte at which the multiple reaction monitoring (MRM) chromatogram has a signal to-noise ratio of 5) for carbamazepine and bezafibrate were 0.004 and 0.01 ng/L respectively. Method detection limits were 4.3 and 3.0 ng/L for carbamazepine and bezafibrate respectively. Magnitudes of detected by-products were reported as peak area counts. Concentrations of by-products could not be determined due to unavailability of reference compounds.

Chapter 4: Results and Discussion

4.1. Bezafibrate

4.1.1. Bezafibrate Oxidation

The results for residual fraction of bezafibrate as a function of contact time for the four experimental settings, as discussed in Section 3.1.2, are shown in Figure 4.1. The obtained results during the two runs under each experimental setting were very similar and agreed within a factor of 1.74 of the geometric mean of duplicate values for all contact times. Most of the bezafibrate was oxidized during the first 2 min under all the experimental settings. After 2 min contact time, average oxidation was 89% during setting A, which increased to more than 95% in settings B, C and D. Oxidation efficiency exceeded 95% after 5 min contact time for all settings.

Variable oxidation efficiencies have been reported for bezafibrate in the literature depending on the characteristics of the water and the applied ozone dose. In simulated water under laboratory conditions, Tabe et al. (2009) reported >95% oxidation of bezafibrate at lower DOC concentration of 0.8 mg/L at both ozone doses of 1 and 3 mg/L. Oxidation efficiencies were lower at higher DOC concentration of 4.5 mg/L and ranged between 21-41% at ozone dose of 1 mg/L and 87-94% at ozone dose of 3 mg/L. In pilot-scale experiments using Detroit River water (Windsor, Ontario) in the same study, bezafibrate oxidation varied between 53-99% at ozone dose of 1 mg/L and exceeded 97% at ozone dose of 1.5 mg/L. Bezafibrate oxidation to less than detection limit was reported in River Seine water (Paris, France) within 5 min of ozonation with an ozone dose of 2 mg/L (Huber et al., 2003). Using an ozone dose of 1.0 to 1.5 mg/L, Ternes et al. (2002) have observed a reduction of only 50% in spiked bezafibrate concentration in a natural water sample in lab scale experiments. Increasing ozone dose to 3.0 mg/L improved transformation of bezafibrate to 80%.

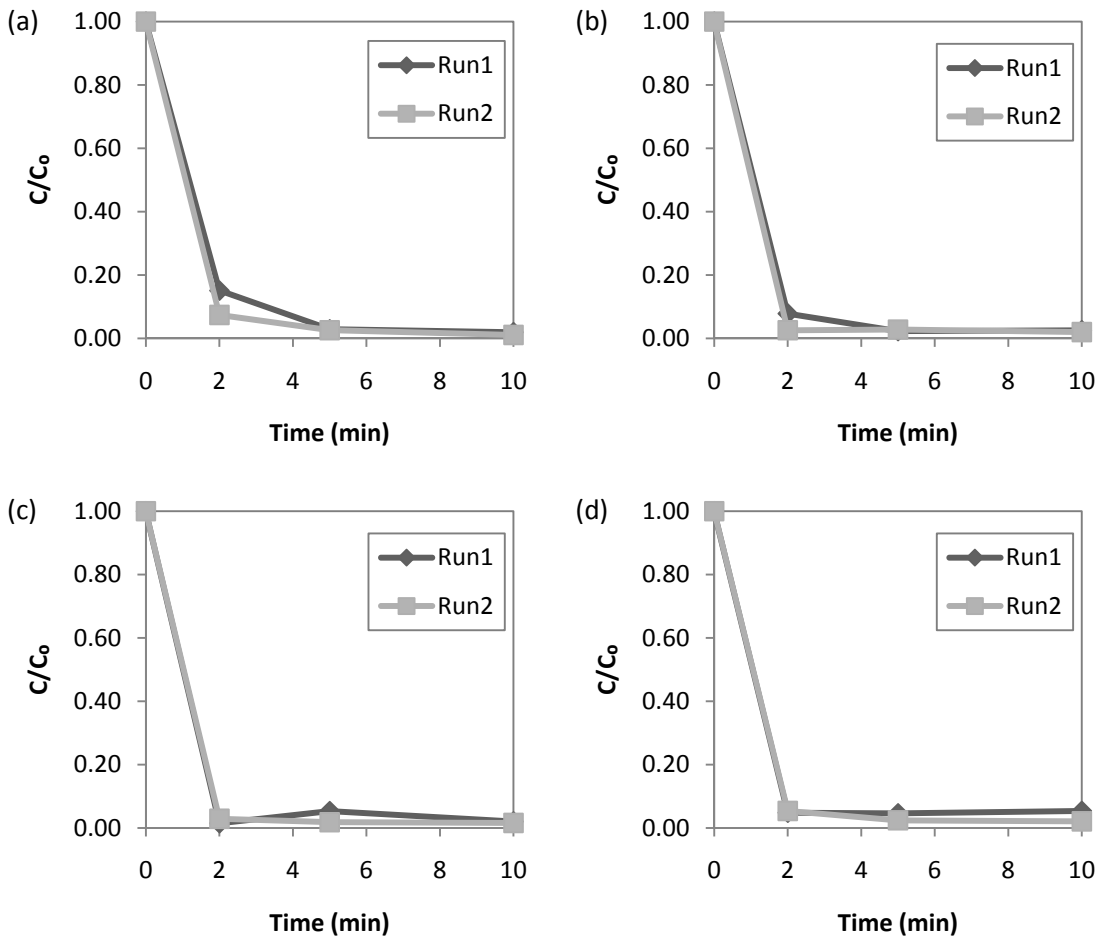

Figure 4.1 Relative bezafibrate concentration [C/C_o] as a function of ozone contact time in a) Experimental settings A, b) Experimental setting B, c) Experimental setting C and d) Experimental setting D. Runs 1 and 2 are duplicate experiments within each setting.

The trends for ozone residual concentrations during the four experimental settings are shown in Figure 4.2 . Unlike the oxidation of bezafibrate, the trends for ozone residual concentrations were quite different during the four experimental settings. The results obtained during the two duplicate runs under each experimental setting were very similar. For residual ozone concentrations above 0.1 mg/L the values were within a factor of 1.73 of geometric mean for all contact times. During setting A, the average ozone concentration declined to 1.1 mg/L in 5 minutes after which the decline was much more gradual to 1.0 mg/L after 10 min. The trend was similar during setting B

with a slightly larger decline during the first 5 min to an average of 0.6 mg/L followed by a more gradual decline to 0.5 mg/L after 10 min. Settings C and D followed similar trends with a rapid consumption of almost all the applied ozone concentration of 2 mg/L during the first 2 min. The average residual ozone concentration after 2 min was 0.4 mg/L for setting C and 0.2 mg/L for setting D.

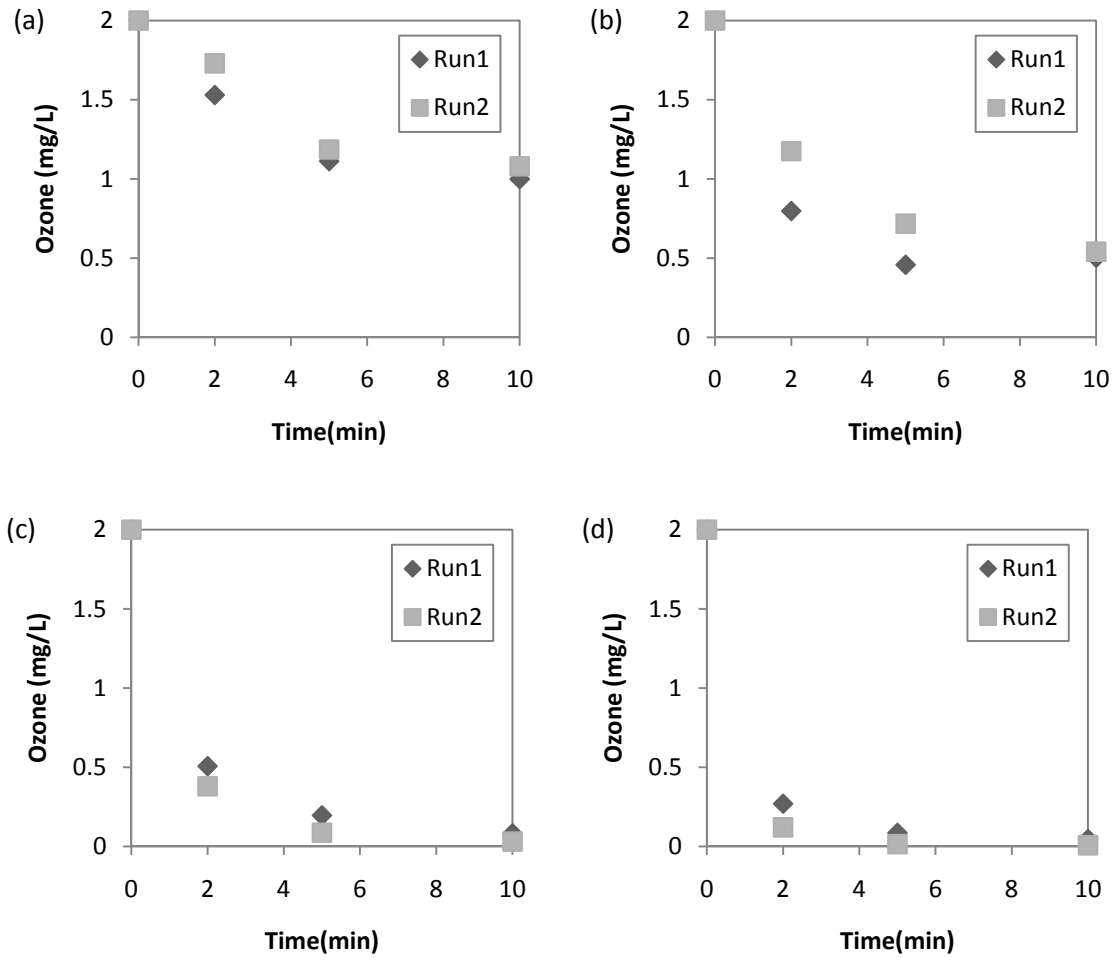

Figure 4.2 Residual ozone concentrations as a function of contact time during bezafibrate oxidation under the four experimental settings. a) Experimental setting A. b) Experimental setting B. c) Experimental setting C. d) Experimental setting D.

During ozonation treatment, ozone is expected to be consumed by its direct reaction with the components of the water matrix and in reactions associated with the hydroxyl radical chemistry. The production of hydroxyl radicals can be initiated by the following reactions (Elovitz & Von Gunten, 1999; Von Gunten, 2003a):

$$O_3 + OH^- \rightarrow HO_2^- + O_2\,, \qquad k = 70\ M^{-1}s^{-1} \tag{4.1}$$

$$O_3 + HO_2^- \rightarrow O_2^{-\cdot} + O_2 + {}^{\cdot}OH\,, \qquad k = 2.8 \times 10^6\ M^{-1}s^{-1} \tag{4.2}$$

Given the near neutral pH of the experiments (7.3±0.2) and the small rate constant for equation 4.1, the production of hydroxyl radicals from this reaction is expected to be slow. Any hydroxyl radicals formed during setting A are consumed by the added tert-butanol which acts as a hydroxyl radical scavenger that terminates its further reaction. In settings C and D where hydrogen peroxide is added to the matrix, presence of HO_2^-, based on reaction (4.2) contributes to formation of hydroxyl radicals and that can explain rapider consumption of ozone in first two minutes of these experimental settings.

Using the measured residual ozone concentration in various reactor samples in different experimental settings, ozone exposures in these reactor samples were determined. Calculated ozone exposures in different experimental settings are shown in Table 4.1. This table shows a decrease in ozone exposure from setting A to setting D at the three experimental contact times. The details are included in Appendix A.

During ozonation the residual fraction of any micro pollutant C in a media can be estimated using the following equation (Von Gunten, 2003a)

$$\ln([C]/[C]_0) = -(k_{O_3} \int [O_3]\,dt + k_{OH} R_{CT} \int [O_3]\,dt) \tag{4.3}$$

Table 4.1 Calculated ozone exposure during oxidation of bezafibrate at three different ozone contact times under four experimental settings

Experimental setting	Ozone exposure (mol.L^{-1}.sec) in contact time of:		
	2 min	5 min	10 min
A	0.0075	0.0127	0.0195
B	0.0036	0.0065	0.0100
C	0.0026	0.0037	0.0043
D	0.0019	0.0024	0.0026

Where k_{O3} is the second order rate constant for reaction of ozone and respective micro pollutant and k_{OH} is the second order rate constant for reaction of the same micro pollutant with hydroxyl radicals and R_{CT} is the ratio of hydroxyl radical exposure to ozone exposure. The fraction of compound oxidized by hydroxyl radicals can be determined based on the following equation (Von Gunten, 2003a):

$$f(\cdot OH) = k_{OH} R_{CT} / (k_{O_3} + k_{OH} R_{CT}) \quad (4.4)$$

The first and second terms in equation 4.3 account for oxidations due to ozone and hydroxyl radical exposures respectively. By ignoring the second term, the expected residual fraction due to ozone exposure alone can be calculated:

$$\ln([C]/[C]_0) = -k_{O_3} \int [O_3]\, dt \quad (4.5)$$

Using k_{O3} value from the literature (Huber et al., 2003) and ozone exposure values presented in Table 4.1, the predicted oxidation efficiencies for bezafibrate due to ozone exposure alone (using equation 4.5) are compared against those measured under various experimental settings for a contact time of 2 min in Table 4.2.

In Table 4.2 the predicted oxidation efficiency due to exposure to ozone alone was seen to decline from 99 to 68% whereas the measured efficiency was maintained at 89% or better. This difference in trends indicates an additional oxidation due to exposure to hydroxyl radicals, particularly during settings C and D. The results for predicted oxidation efficiencies due to exposure to both ozone and hydroxyl radicals (estimated from equation 4.3) are presented in Table 4.3. The fraction of bezafibrate oxidation attributed to hydroxyl radical exposure (estimated from equation 4.4) is also presented in the same table. k_{OH} value for reaction with bezafibrate was obtained from Huber et al. (2003).

Table 4.2 Comparison of predicted oxidation efficiency for bezafibrate due to ozone exposure alone against actual measured oxidation rates for ozone contact time of 2 minutes under experimental conditions

Experimental setting	$[C]/[C]_0$[1]	P Ox[2]	M Ox[3]
A	0.01	99%	89%
B	0.12	88%	95%
C	0.22	78%	98%
D	0.32	68%	95%

1. Residual fraction calculated using equation 4.5 and ozone exposure during 2 minutes of ozone contact time
2. Predicted oxidation is estimated ignoring the effect of hydroxyl radicals and at the end of the first 2 minutes of ozonation
3. Measured oxidation of bezafibrate by both ozone and hydroxyl radicals at the end of two minutes of ozonation

The value of R_{CT} (presented in Table 4.3), which is the ratio of hydroxyl radical exposure to ozone exposure, for the four experimental settings was determined using the procedure proposed by Elovitz and Von Gunten (1999), using pCBA as the probe compound (details of calculation in the Appendix). In experimental settings B, C and D, at the end of 2 minutes of contact time, no pCBA could be detected; therefore method detection limit (MDL) value was used to determine a minimum value for R_{CT} in these

settings. R_{CT} and hydroxyl radical exposures are shown as being greater than this calculated value in Table 4.3. With the estimates of R_{CT} available for different experimental settings, using equation (4.3) the expected overall oxidation of bezafibrate can be calculated.

Table 4.3 Determined values for R_{CT}, anticipated oxidation efficiencies, estimated OH radical exposure and fractional removal of bezafibrate due to oxidation by hydroxyl radicals during two minutes of ozone contact

Experimental setting	R_{CT}	$^{\cdot}$OH exposure[1]	$[C]/[C]_0$[2]	P Ox[3]	$f(^{\cdot}OH)$[4]
A	0	0	0.01	99%	0
B	>1.43E-07	>5.13E-10	0.00	100%	>0.64
C	>2.05E-07	> 5.30E-10	0.00	100%	>0.72
D	>3.65E-07	>7.00E-10	0.00	100%	>0.82

1. Hydroxyl radical exposure (mol.L^{-1}.sec) quantified for ozone contact time of 2 minutes as resultant of R_{CT} and ozone exposure at the same time interval
2. Residual fraction of bezafibrate calculated using equation 4.3 and ozone exposure values from Table 4.1
3. Predicted oxidation calculated for ozone contact time of 2 min based on residual fraction values
4. Fraction of bezafibrate oxidized with OH radicals estimated using equation 4.4

Results in Table 4.3 validate the basis for the design of the experiments (Section 3.1.2). Although the measured bezafibrate oxidation (89-98%) at 2 min is similar for the four experimental settings (Table 4.2), the dominant pathways of reaction are expected to be different. R_{CT} value is zero during setting A, which means that bezafibrate oxidation is due to exposure to ozone only. Due to the addition of hydrogen peroxide in settings C and D, ozone exposure declines (Table 4.1) and hydroxyl radical exposure is enhanced (Table 4.3) resulting in an estimated >72% bezafibrate oxidation due to exposure to hydroxyl radicals. Bezafibrate oxidation is thus dominated by ozone exposure during setting A and by hydroxyl radical exposure during settings C and D, and somewhere in between during setting B.

4.1.2. By-Product Formation

In a previous study on ozonation of bezafibrate, five different by-products were identified which were labelled as MW367, MW393, MW227 and MW409 (2 isomers) (Dantas et al., 2007). The same by-products were monitored in the current study and the distribution of the by-products identified in each one of experimental settings is shown in Table 4.4.

Table 4.4 Distribution of bezafibrate ozonation by-products in different experimental settings

Experimental setting	MW367	MW393	MW227
A	✓	✓	✓
B	✓	✓	✓
C			✓
D			✓

In current study only three of ozonation by-products as identified by Dantas et al. (2007) were detected. The structure of these three by-products is shown in Figure 4.3 .The level of bezafibrate used in the present study was 500 times lower than the mentioned reference, which could explain detection of fewer numbers of ozonation by-products in the current study. This confirms that these three are the major by-products of oxidation of bezafibrate as shown in (Figure 4.3). If the other two by-products were formed their concentrations were below MDL. The by-products identified by Dantas et al (2007) were limited to the experimental setting A in the current study only. The differences in the relative abundance of by-products observed in Table 4.4 will be discussed individually in the subsequent sections.

Since the two aromatic rings in bezafibrate are the most electron rich sites of molecule, they are the most likely sites of attack for ozone as an electrophile (Section 2.6.2.1), as discussed by Dantas et al. (2007) and shown in Figure 4.4. Due to withdrawing effect of chlorine, chlorinated aromatic ring is expected to have a lower reactivity compared to the non-chlorinated aromatic ring which is therefore expected to be a more susceptible site of attack for ozone (Dantas et al., 2007).

The results of the current study support this hypothesis. Formation of all the by-products detected in the present study can be explained on the basis of attack of ozone on the non-chlorinated aromatic ring while the other aromatic ring remains intact. The possible pathways of formation of these by-products are presented in Figures 4.5 to 4.8 (Thadani, 2009). By products MW367 and MW393 are formed by direct attack of ozone on bezafibrate (Figures 4.5 and 4.6). The formation of by-product MW227 can be explained through an anomalous ozonolysis mechanism (Dantas et al., 2007; Yamamoto et al., 1979). It is a product of further oxidation of the other two ozonation products of bezafibrate MW367 and MW393 (Figures 4.7 and 4.8).

A general scheme for ozonation products of bezafibrate is presented in Figure 4.9. Oxidation of bezafibrate or any one of the above mentioned by-products with hydroxyl radicals can form other potential by-products that are represented by BZF-Prod1 through 4 in Figure 4.9. Bezafibrate could be directly oxidized by hydroxyl radicals to form one or a group of by-products abbreviated as BZF-Prod1 in Figure 4.9. Reaction of MW367, MW393 and MW227 with hydroxyl radicals can potentially form BZF-Prod2, BZF-Prod3 and BZF-Prod4, respectively. The structure of bezafibrate by-products BZF-Prod1, 2, 3 and 4 is not identified.

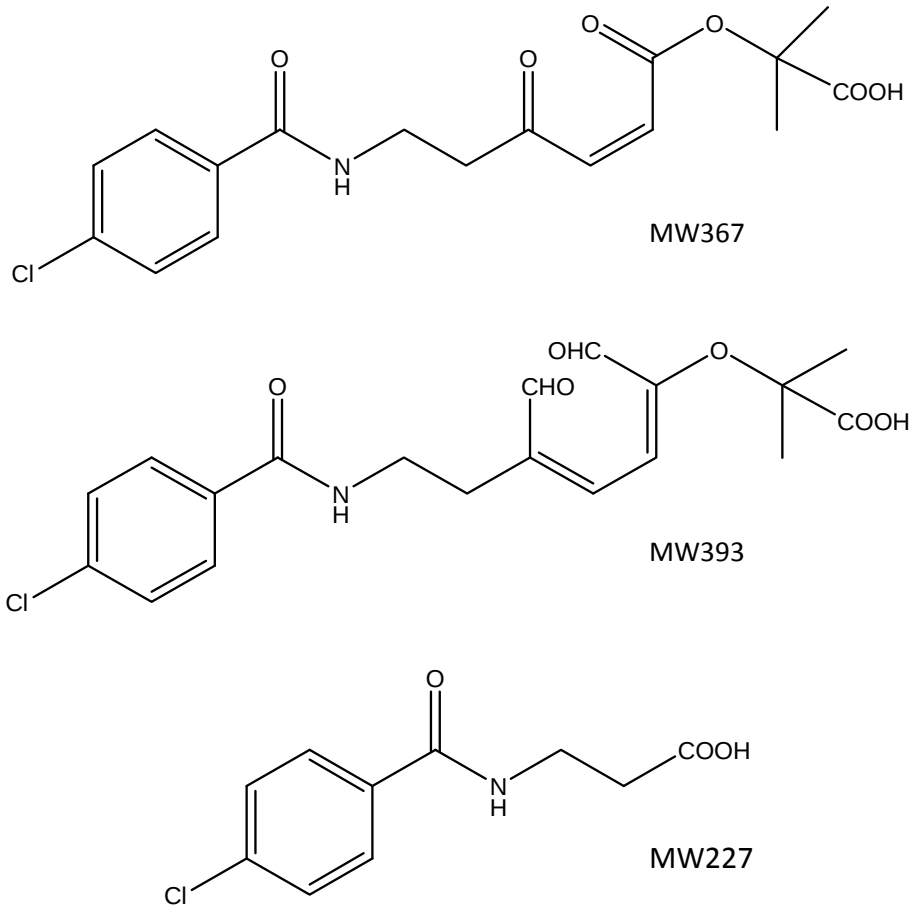

Figure 4.3 Structures of ozonation by-products of bezafibrate observed in the current study

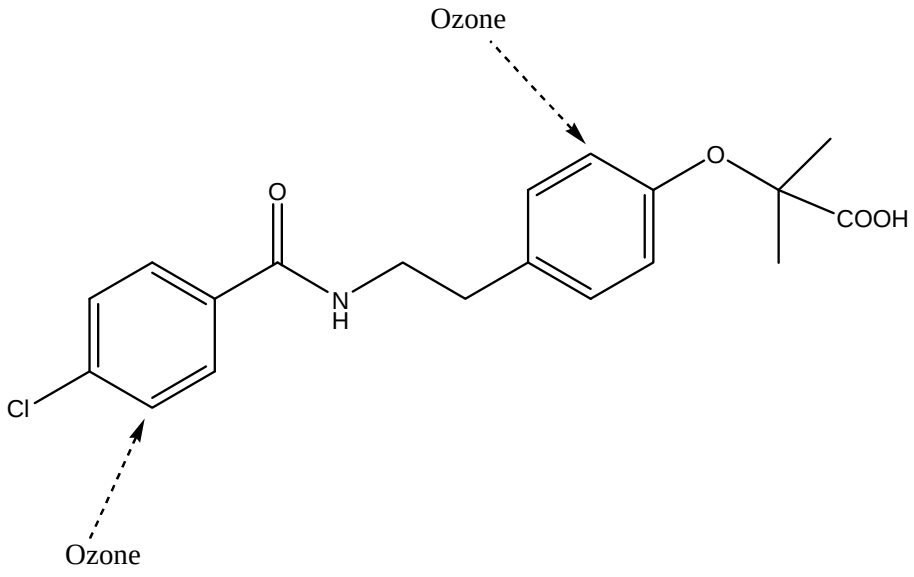

Figure 4.4 Sites of attack of ozone on bezafibrate molecule.

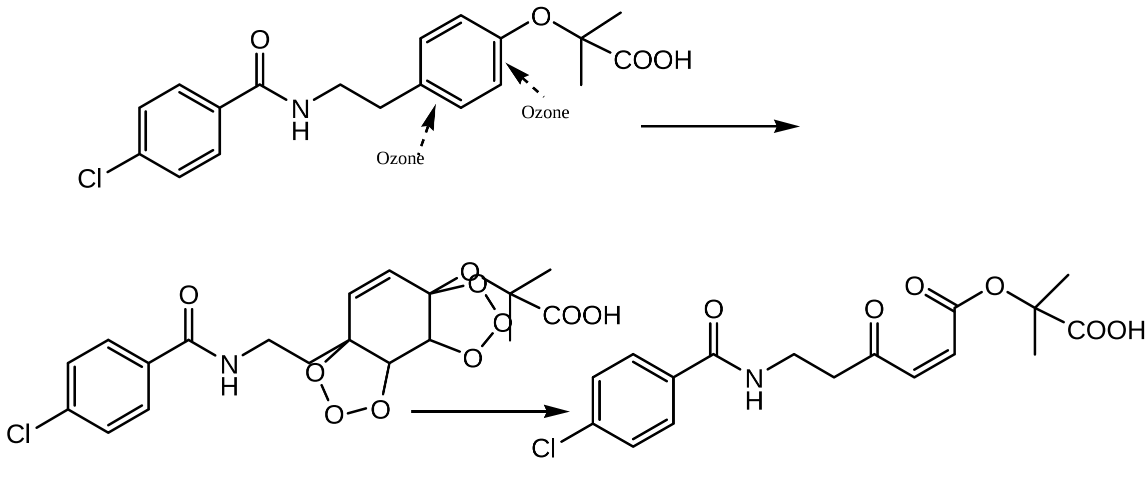

Figure 4.5 Formation of MW367 by attack of ozone on non-chlorinated aromatic ring in bezafibrate.

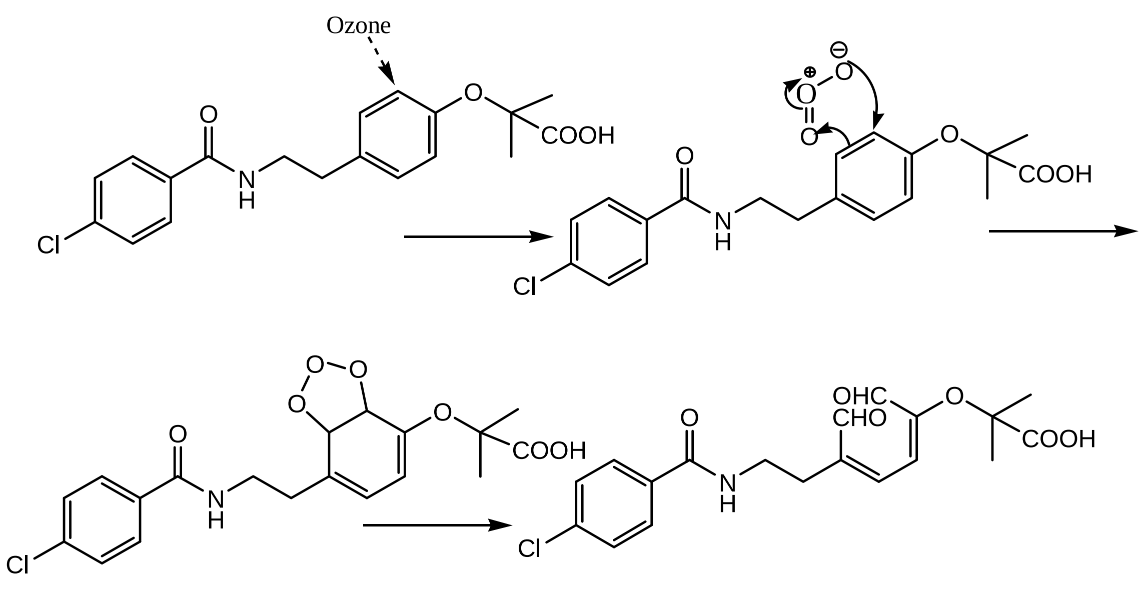

Figure 4.6 Formation of MW393 by attack of ozone on non-chlorinated aromatic ring in bezafibrate.

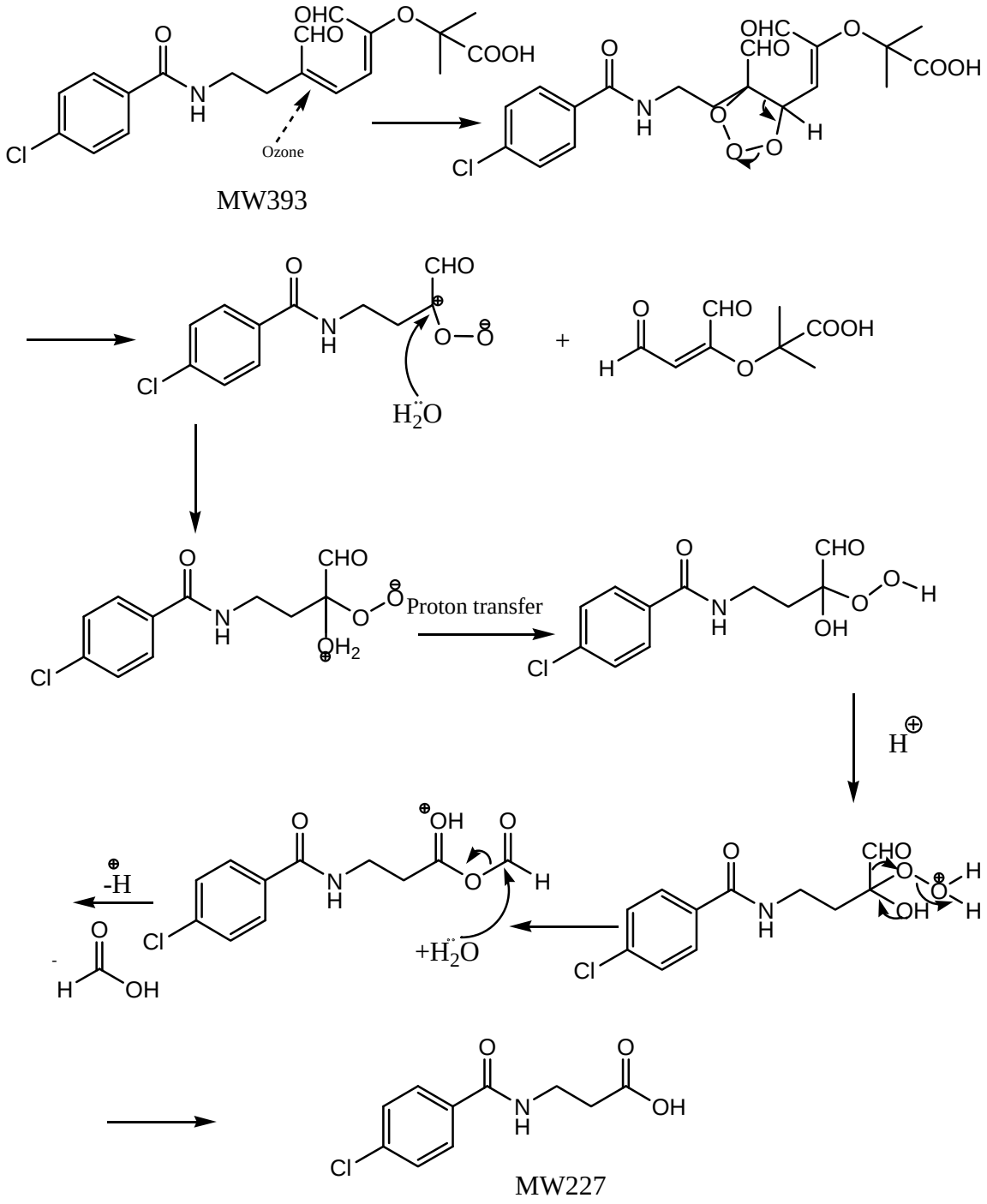

Figure 4.7 Formation of by-product MW227 through further ozonation of MW393.

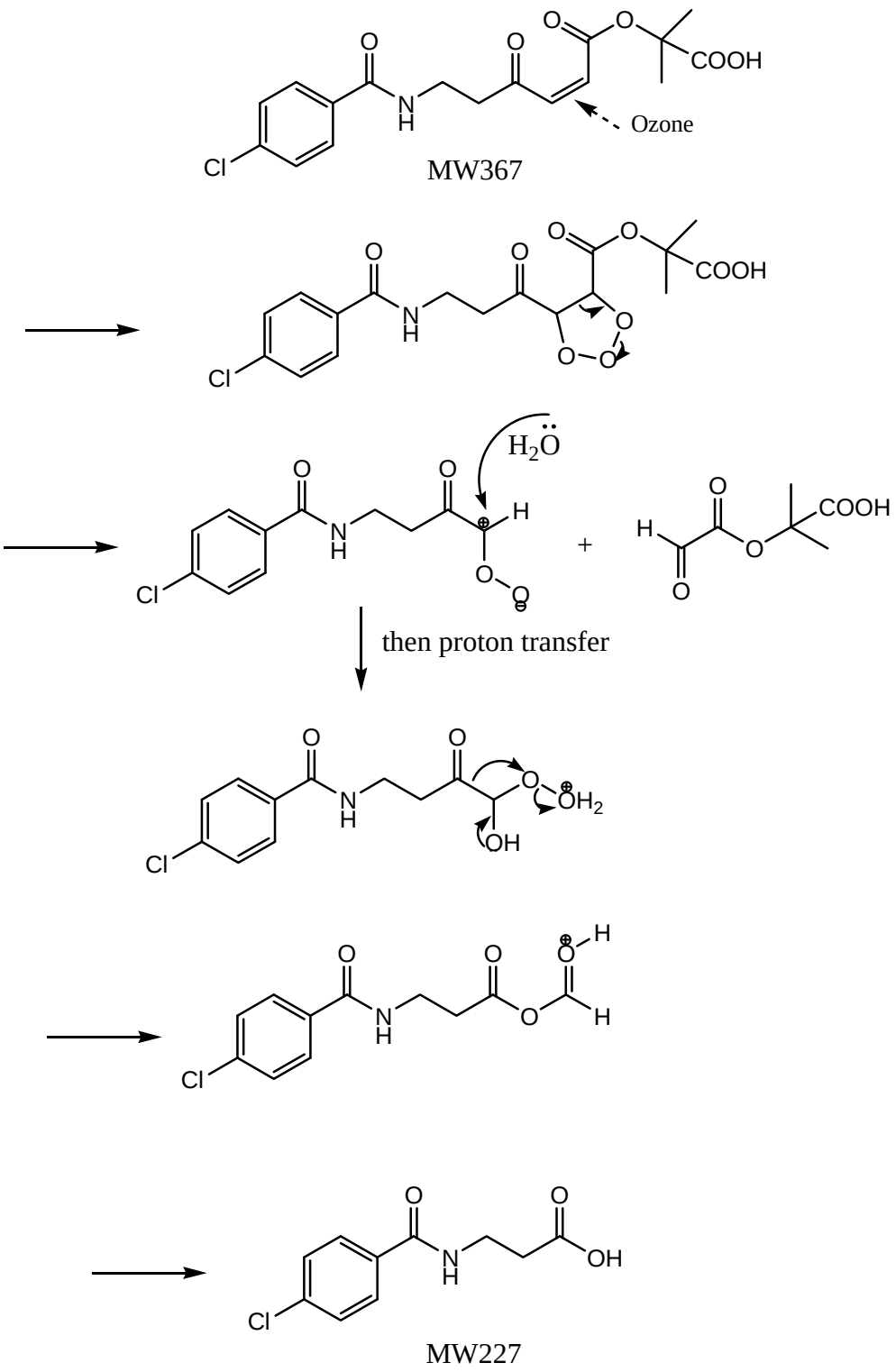

Figure 4.8 Formation of by-product MW227 by further ozonation of MW367.

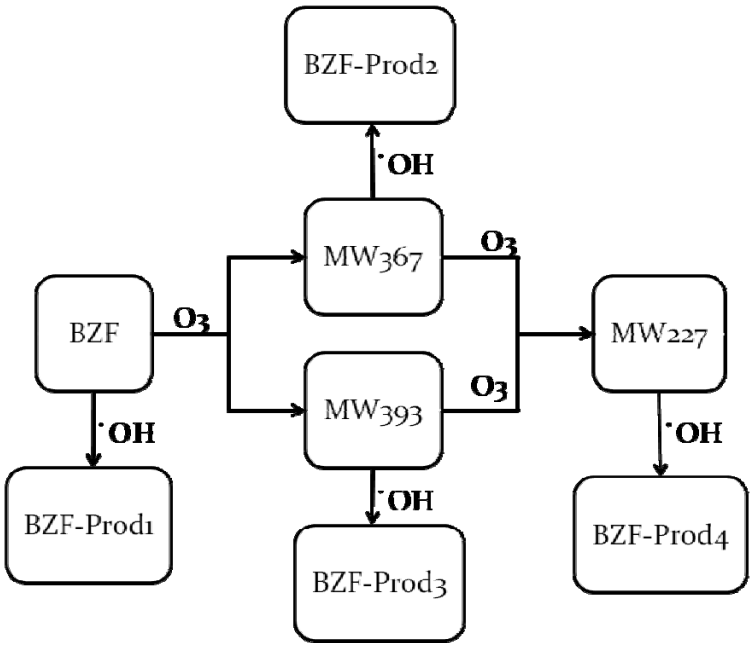

Figure 4.9 Proposed reaction pathways for the oxidation of bezafibrate with ozone and OH radicals (Thadani, 2009)

4.1.2.1. MW367

Figure 4.10 shows quantified peak area counts for MW367 in duplicate runs of experimental settings A and B for three different contact times. These duplicate area counts at any given contact time of the two experimental settings were similar and agreed within a factor of 1.63 of the geometric mean of duplicate values. In both runs of setting A from T1 to T2 levels of peak area counts are similar and within 5% of the average, followed by a decrease of about 33 to 52% from T2 to T3 (as shown in Tables B.1 to B.3 in Appendix B). Overall results indicate the maintenance of peak area counts under this setting suggesting relative resistance of MW367 to degradation by ozone. In the experimental setting B, after 2 minutes of ozonation the average level of MW367 detected was a factor of 0.02 of that in experimental setting A. In this setting from T1 to T2 peak area counts unlike setting A declined by 47 and 75% in runs 1 and 2, followed by a 93% decline from T2 to T3 in run 2. A peak for MW367 was not detected in reactor T3 of run1, the reason for which is not known (as shown in Tables B.4 to B.6 in Appendix B).

The rate of decline of this by-product in setting B seems to be larger than setting A. As the ozone exposure in setting A was larger than setting B (Table 4.1), this higher rate of decline can suggest that this by-product is more susceptible to attack by hydroxyl radicals than ozone itself.

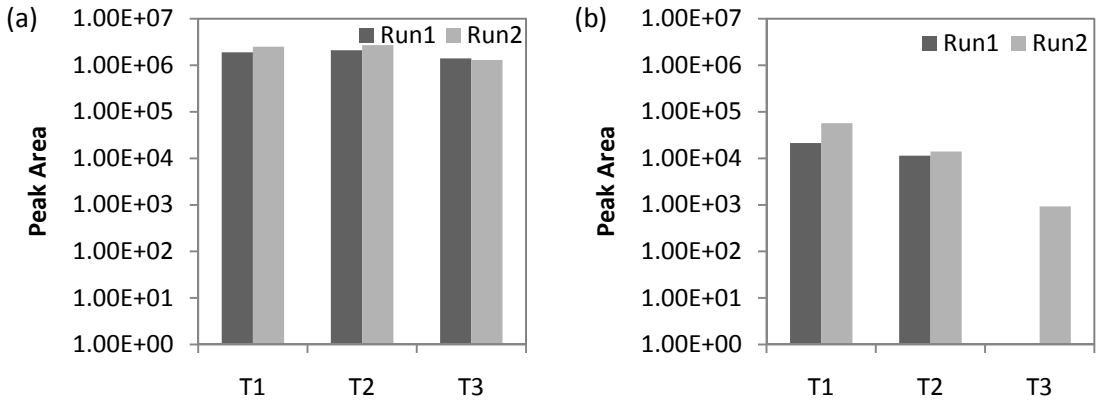

Figure 4.10 Peak area counts for MW367, an ozonation product of bezafibrate, which was only detected in experimental settings A and B. T1=2 min, T2=5 min and T3=10 min. a) Experimental setting A. b) Experimental setting B.

4.1.2.2. MW393

MW393 is a by-product of bezafibrate which, similar to MW367, was only detected in experimental settings A and B. Peak area counts in duplicate runs under each experimental setting which are shown in Figure 4.11 were similar and agreed within a factor of 1.63 of the geometric mean of peak area counts in duplicate runs. In experimental setting A, from T1 to T2 peak area counts for this by-product in runs 1 and 2 decreased by 29 and 21% respectively. This initial decrease was followed by a 63% decrease from T2 to T3 in run2 (as shown in Tables B.1 to B.3 in Appendix B). A peak for this by-product was not detected in T3 of run1, reason for which is not clear. The decreasing trend of MW393 in this setting is an indication of susceptibility of this compound to attack by ozone. Although MW393 was detected in both experimental settings A and B, the average detected level of this by-product in setting B after 2 minutes of contact with ozone was about 120 times lower. In setting B from T1 to T2

peak area counts for this by-product decreased by 52 and 81% in runs 1 and 2 respectively. A further decrease by 74% happened from T2 to T3 in run 2. In run 1 the respective peak detected for MW393 at T3 was a trace and no area count was determined for it (as shown in Tables B.4 to B.6 in Appendix B). The rate of decline of MW393 in setting B was larger than setting A. This higher rate of decline is suggestive of attack of hydroxyl radicals on this by-product.

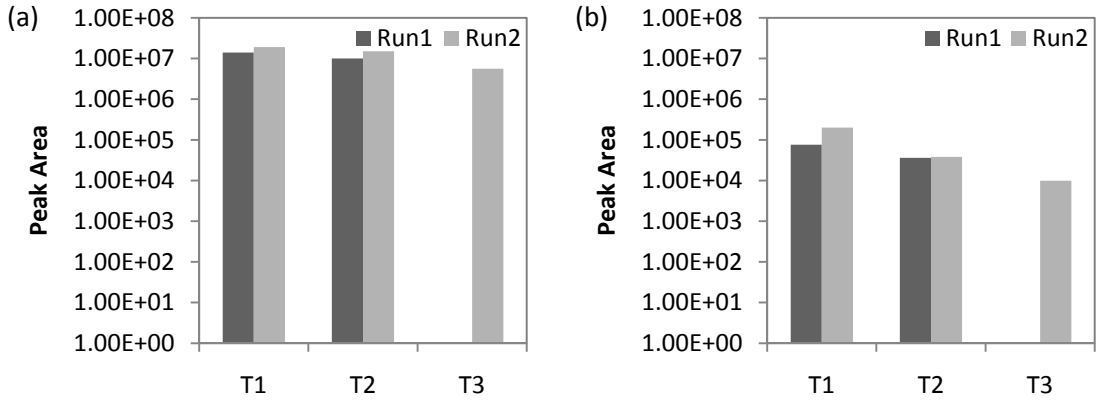

Figure 4.11 Peak area counts for by-product MW393, an ozonation product of bezafibrate, only detected in two experimental settings A and B. T1=2 min, T2=5 min and T3=10 min. a) Experimental setting A. b) Experimental setting B.

4.1.2.3. MW227

As observed in Figure 4.12 by-product MW227 was detected in all four experimental settings. Detected peak area counts in duplicate runs were very close and agreed within a factor of 2.06 of geometric mean of duplicate area count values at each contact time. The results observed in experimental settings A and B showed that at the end of 2 minutes of contact time peak area count levels in the two settings are similar and comparable. Average of detected peak area counts for MW227 at the end of 2 minutes of contact with ozone in experimental settings C and D were respectively 191 and 338 times lower compared to the similar level in experimental setting A. The details are included in Appendix B. Based on the proposed pathway (Figures 4.7 and 4.8) MW227 is expected to be formed as a further ozonation product from MW367 and MW393, and

since the levels of these two by-products in settings C and D were lower, the lower amounts of MW227 seen in settings C and D are consistent with this proposed mechanism. Based on the same reason lower levels of MW227 were expected at the end of 2 minutes of ozonation in setting B, but were not observed. In both runs of setting A detected peak area count levels from T1 to T2 were similar and within 8% of the average. Following that from T2 to T3 a 39 and 32% increase in peak area count for MW227 was observed in runs 1 and 2 respectively (Details are included in Tables B.1 to B.3 in Appendix B). Whereas in setting B from T1 to T2, 44 and 40% decrease was observed in peak area count of this by-product in runs 1 and 2 respectively, followed by a further decline of 70 and 71% from T2 to T3 of runs 1 and 2 respectively (The details are included in Tables B.4 to B.6 in Appendix B). The relative decline observed in setting B compared to small increase observed in setting A suggests that this by-product is more susceptible to attack by hydroxyl radicals. An overall observation of the peak area counts relatively maintained in these two experimental settings would suggest that MW227 is resistant to both ozone and hydroxyl radicals.

In experimental setting C the trends observed for this by-product were different. In run 1 from T1 to T2 detected peak area count showed a 345% increase; from T2 to T3 detected peak areas were within 5% of the average value and indicated a maintained level. However in run 2 from T1 to T2 peak area showed a 39% decrease, followed by a 69% increase from T2 to T3. The averages of area counts showed an overall increasing trend (The details are included in Tables B.7 to B.9 in Appendix B). In experimental setting D from T1 to T2 area count increased by 214% in run 1 whereas a 20% decrease was observed in run 2. In this setting from T2 to T3, increases by 122% and 111% were observed in runs 1 and 2 respectively. However averages of area counts indicate an always increasing trend (The details are included in Tables B.10 to B.12 in Appendix B). At all contact times the values of area counts in settings C and D were at least a factor of 100 times lower than similar peaks in setting A. Therefore differences in trends in the duplicate runs could be attributed to the levels of by-product being so close to method detection limit. As indicated previously the two by-products, MW367 and MW393,

which result in formation of MW227 were not detected in these two settings. Yet the low level of MW227 detected is an indication of some MW227 formed.

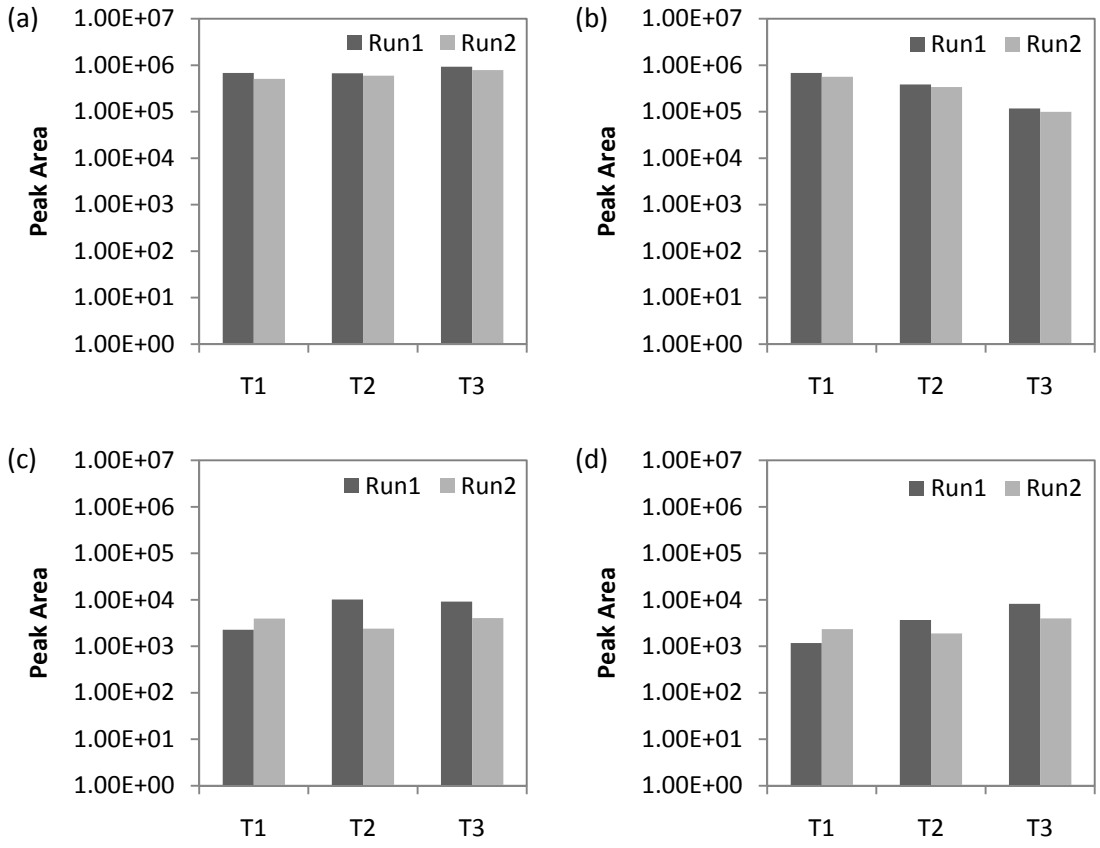

Figure 4.12 Peak area counts for ozonation product MW227 in four experimental settings A, B, C and D are shown in duplicate runs 1 and 2 at different contact times of T1=2 min, T2=5 min and T3= 10 min. a) Experimental setting A. b) Experimental setting B. c) Experimental setting C. d) Experimental setting D.

4.1.3. Discussion

The comparison of time trend of by-products in all experimental settings show that all three monitored by-products were detected in experimental settings A and B which are expected to have relatively the lowest presence of hydroxyl radicals (Section 4.1.1). In experimental settings C and D where hydrogen peroxide was added, only by-product

MW227 was detected, still quantified levels of MW227 were the lowest in settings C and D compared to other two settings. The absence of MW367 and MW393 in experimental settings C and D could either imply that these two compounds were not formed in relative absence of molecular ozone or they were formed but immediately reduced by hydroxyl radicals to below MDL. Nevertheless this difference in presence pattern of by-products can be explained by domination of hydroxyl radicals in experimental settings C and D.

The results of these experiments are in agreement with a study conducted by Dantas et al., 2007. In that study it was proposed that formation of species MW367 and MW393 may be explained by direct ozone attack to un-chlorinated aromatic ring in bezafibrate (Dantas et al., 2007) (Section 2.6.2.1). In current study these two by-products (MW367 and MW393) were only detected in settings A and B where highest ozone exposure levels among four experimental settings were observed. Especially the highest detected levels of these two by-products in experimental setting A can be explained by presence of hydroxyl radical scavenger tert-butanol in matrix in that setting (Section 3.1.2) which results in the highest ozone exposure level compared to other experimental settings.

The increasing trend of by-product MW227 in experimental setting A in parallel to decreasing trend of by-product MW393 confirms the proposed formation of MW227 from MW393 through an anomalous ozonolysis mechanism (Section 4.1.2) (Dantas et al., 2007; Yamamoto et al., 1979). Based on the same anomalous mechanism the by-product MW367 can contribute to formation of MW227 as well. However the case was slightly different in setting B, where the decreasing trend of MW393 was accompanied by a severely decreasing trend of MW227 (83% decrease in average of detected peak area counts between 2 and 10 minutes of contact time) which could perhaps be related to interference of hydroxyl radicals present in experimental setting B. If the reaction of hydroxyl radicals with MW227 is assumed to be fast enough, even though MW227 is formed through reaction of molecular ozone with MW393, the rate of decline of MW227 will be greater than its rate of formation. At the same time a portion of MW393

itself could be reduced through reaction with hydroxyl radicals and thus not result in formation of MW227. The low level presence (peak area count 1.76E+03 to 6.60E+03) of MW227 in settings C and D could be explained to be formed from undetectable levels of MW393 in these same settings. In these settings, as a result of relative abundance of hydroxyl radicals and lower ozone exposure, formation of MW393 had a lower level compared to settings A and B at the first place. In addition to that, formation of MW227 from MW393 through an anomalous ozonation will then be affected by hydroxyl radicals that would be competing with molecular ozone for reaction with MW393. This altogether will explain lower level of MW227 in settings C and D.

4.1.4. Summary of Bezafibrate Oxidation

Based on the results discussed in Section 4.1.1, more than 89% of bezafibrate was oxidized within 2 minutes of ozonation under all experimental conditions. Although the level of oxidation achieved in all four experimental settings was similar, the magnitude and distribution of ozonation by-products formed were different as shown in Figures 4.10 to 4.12 and discussed in Section 4.1.2. Similar ozonation by-products were detected in experimental settings A and B as shown in Figures 4.10 to 4.12 and discussed in Sections 4.1.2.1 to 4.1.2.3. Only one of major ozonation by-products was detected in experimental settings C and D as shown in Figure 4.12 and discussed in Section 4.1.2.3.

As discussed in Section 4.1.2 and shown in Figures 4.5 and 4.6, by-products MW367 and MW393 are formed by direct attack of ozone on bezafibrate. Formation of MW227 could be explained through further ozonation of MW393 and MW367 as shown in Figures 4.7 and 4.8, respectively.

As discussed in Section 4.1.1, in experimental setting A oxidation of bezafibrate was dominated by direct reaction with ozone. Based on the observed results under conditions of ozone exposure alone formation of MW367, MW393 and MW227 is expected. As discussed in Sections 4.1.2.1 to 4.1.2.3 and shown in Figures 4.10 to 4.12, highest levels of all three by-products were detected under this same experimental

setting. Under these conditions by-products MW367 and MW227 are relatively resistant to degradation by ozone as shown in Figures 4.10a and 4.12a respectively.

By-products observed under experimental setting B were similar to those in experimental setting A. Levels of detected by-products MW367, MW393 and MW227 are lower compared to experimental setting A as shown in Figures 4.10 to 4.12.

In experimental settings C and D, where exposure to hydroxyl radicals is the dominant reaction pathway for oxidation of bezafibrate, only by-product MW227 was detected as discussed in Section 4.1.2.3 and shown in Figure 4.12. Level of detection of this by-product in these two settings was the lowest compared to the other two experimental settings A and B (Figure 4.12). As discussed in Section 4.1.2.3 results suggest that this by-product is relatively resistant to degradation by hydroxyl radicals.

4.2. Carbamazepine

4.2.1. Carbamazepine Oxidation

Figure 4.13 demonstrates the results of oxidation of carbamazepine in terms of fractional residual peak area counts as a function of contact time for four experimental settings (Section 3.1.2). Quantified concentrations for carbamazepine in duplicates of control samples in four experimental settings were very close and agreed within a factor of 1.05 of the geometric mean of the two values. Within 2 minutes of ozonation the concentration of carbamazepine in all four experimental settings dropped to below MDL. Only in T3 of one of the duplicate runs for setting C the detected concentration of carbamazepine was 133 ng/L, which is equivalent to 0.06% of initial concentration of carbamazepine in the reactor.

These observations for oxidation of carbamazepine with ozone are in agreement with previous studies by Huber et al. (2003), Andreozzi et al. (2002) and Tabe et al. (2009). Bench scale experiments on simulated water samples have shown >99% oxidation of carbamazepine at lower DOC level of 0.8 mg/L, at ozone doses of 1 and 3 mg/L (Tabe et al., 2009; Yue, 2008). Only at cases where higher DOC level of 4.5 mg/L was combined

with low ozone dose of 1 mg/L oxidation efficiencies dropped to 58 – 84%. In the same study, pilot-scale experiments using Detroit River water (Windsor, Ontario) indicated oxidation efficiencies exceeding 99% using ozone doses of 0.3 – 1.5 mg/L. Huber et al. (2003) have observed more than 97% transformation of carbamazepine in surface water samples at ozone doses of 0.2 – 0.5 mg/L. Similarly using 1 mg/L ozone Andreozzi et al. (2002) observed a complete abatement of carbamazepine in bi-distilled water samples.

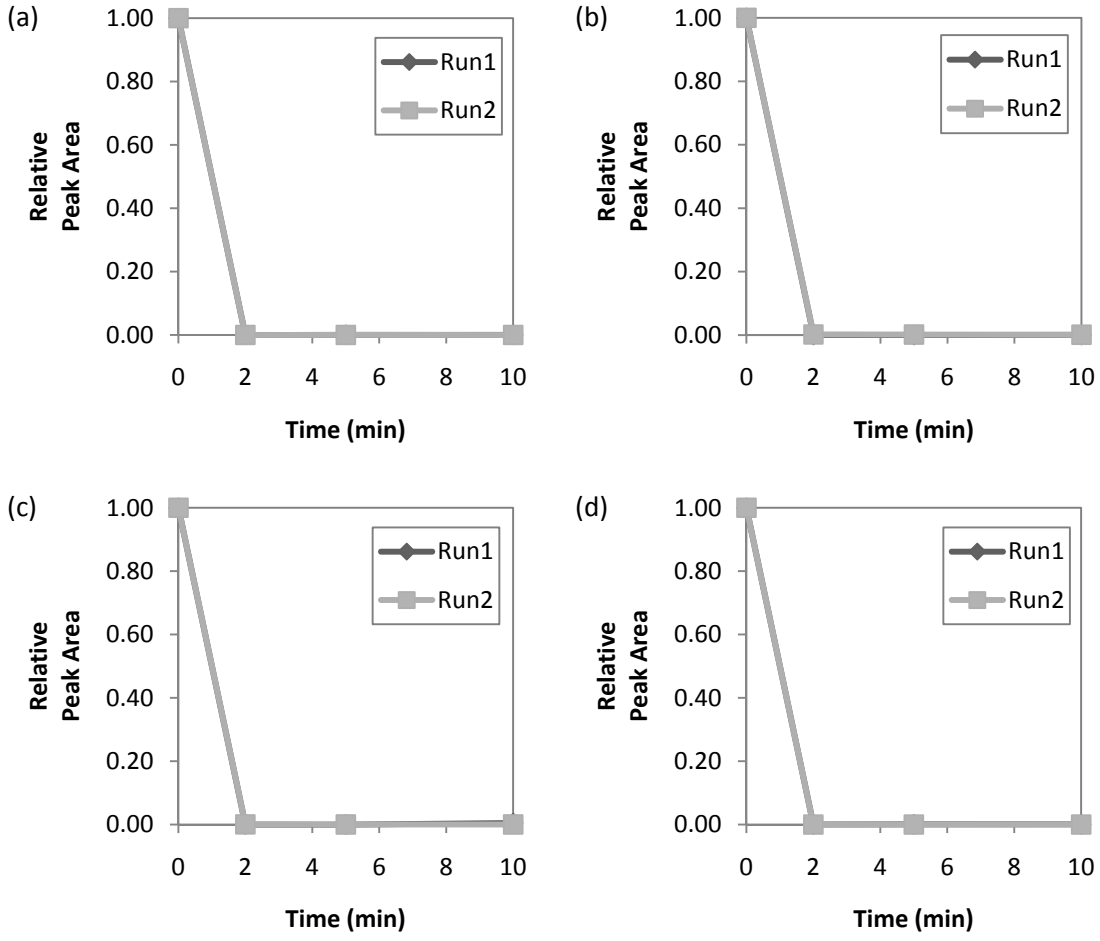

Figure 4.13 Fractional residual peak area count of carbamazepine in four experimental settings. a) Experimental setting A. b) Experimental setting B. c) Experimental setting C. d) Experimental setting D. Run1 and run2 are duplicate experiments within each experimental setting.

Figure 4.14 shows the trend of residual ozone decline in four experimental settings of carbamazepine. The measured residual ozone concentrations for all contact times in duplicate runs of four experimental settings were close and for values above 0.1 mg/L agreed within a factor of 1.41 of the geometric mean. Unlike the trend observed in decline of carbamazepine in four experimental settings (Figure 4.13), the trend of residual ozone concentrations differs greatly from one experimental setting to another. In experimental setting A average ozone concentration declined to 1.2 mg/L in 5 minutes of ozonation, after that the decline in ozone concentration is more gradual and results in average residual ozone concentration of 1.0 mg/L in 10 minutes. In experimental setting B the rate of decline of ozone residual in the first 5 minutes is faster compared to experimental setting A and ozone concentration drops to an average of 0.3 mg/L. Between 5 and 10 minutes of ozonation the rate of decline in ozone concentration is much more gradual and average ozone concentration only declines to 0.2 mg/L at the end of 10 minutes of ozonation. Rates of ozone decline in experimental settings C and D were similar and most of applied ozone concentration of 2 mg/L was consumed in the first 2 minutes of ozonation in both of these experimental settings. In both of these settings, the average residual ozone concentration declined to 0.1 mg/L at the end of two minutes of contact time.

As discussed in Section 4.1.1., reactions associated with hydroxyl radical chemistry are expected to affect ozone consumption in different experimental settings. Due to varying conditions across four experimental settings and the effects they have on ozone decomposition chemistry the levels of ozone exposure will not be the same in comparable contact times of different settings. Using measured residual ozone concentrations, average ozone exposure values for different contact times in each of four settings are calculated and shown in Table 4.5. Corresponding to what is expected theoretically based on variations in water matrix settings, calculated values for ozone exposure show a decreasing pattern form setting A to setting D.

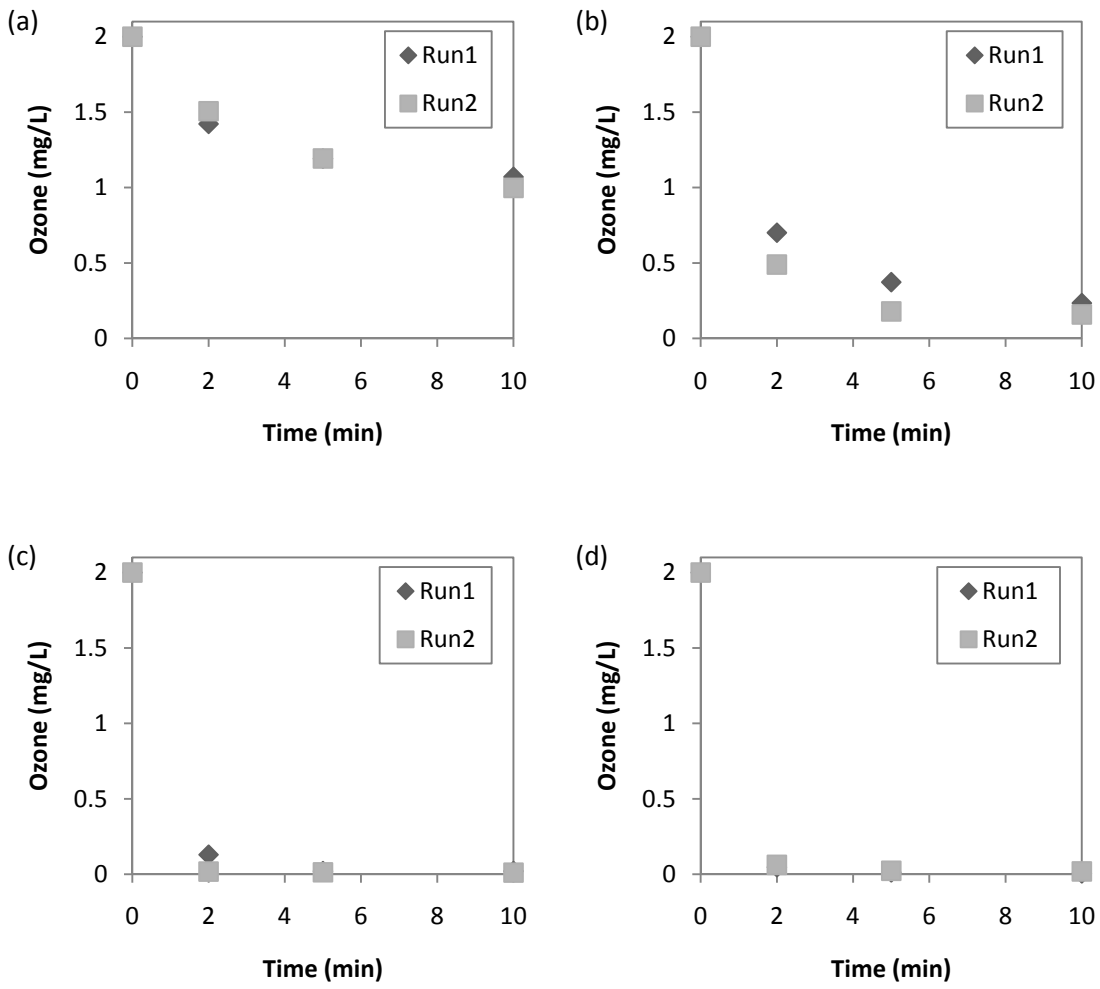

Figure 4.14 Residual ozone concentration as a function of contact time during carbamazepine oxidation under four experimental settings. a) Experimental setting A. b) Experimental setting B. c) Experimental setting C. d) Experimental setting D.

Table 4.5 Calculated values for average ozone exposure during oxidation of carbamazepine in various contact times under four experimental settings.

Experimental setting	Ozone exposure ($mol.L^{-1}.sec$) in contact time of:		
	2 min	5 min	10 min
A	0.0043	0.0093	0.0162
B	0.0029	0.0045	0.0060
C	0.0014	0.0016	0.0017
D	0.0013	0.0015	0.0016

Using ozone exposure values from Table 4.5 and substituting for k_{O3} value from the literature (Huber et al., 2003) in equation 4.5, an estimation of residual fraction of carbamazepine concentration at the end of 2 minutes of ozonation in different experimental settings can be determined. The effect of hydroxyl radicals has been ignored in these calculations. In Table 4.6 the predicted oxidation efficiencies for carbamazepine due to ozone exposure alone is compared against the experimentally measured oxidation efficiencies obtained during two minutes of ozonation under four experimental settings.

Table 4.6 Comparison of predicted oxidation efficiency for carbamazepine due to ozone exposure alone against actual measured oxidation rates in experiments for ozone contact time of 2 minutes.

Experimental setting	$[C]/[C]_0$[1]	P Ox[2]	M Ox[3]
A	0.00	100%	100%
B	0.00	100%	100%
C	0.00	100%	100%
D	0.00	100%	100%

1. Residual fraction of carbamazepine concentration using equation 4.5 and ozone exposure data during two minutes of contact time from Table 4.5
2. Predicted oxidation for carbamazepine in 2 min of contact time due to ozone exposure alone ignoring the effect of hydroxyl radicals on overall oxidation efficiency
3. Measured oxidation of carbamazepine obtained by ozonation of the compound for two minutes

Comparing value of predicted oxidation of carbamazepine after 2 minutes of ozonation in setting A from Table 4.6 with real oxidation rates determined on the basis of analytical measurements indicates that unlike bezafibrate, the ozone exposure value existing in this setting is adequate for transformation of carbamazepine. In order to identify the effect of hydroxyl radicals in oxidation of carbamazepine, as described in

Section 4.1.1., the R_{CT} values were obtained from a parallel set of experiments on pCBA using the procedure proposed by Elovitz and Von Gunten (1999). Substituting for k_{O3} and k_{OH} values obtained from Huber et al. (2003), and R_{CT} values , determined for the first two minutes of ozonation under different experimental settings, in equation 4.4, the fraction of carbamazepine oxidized by hydroxyl radicals were calculated and are shown in Table 4.7. Estimated oxidation efficiencies due to exposure to both ozone and hydroxyl radicals from equation 4.3 are also presented in Table 4.7.

Table 4.7 Calculated values for R_{CT}, predicted oxidation efficiencies for carbamazepine and fraction of carbamazepine oxidized by hydroxyl radicals during two minutes of ozone contact are presented

Experimental setting	R_{CT}	$^{\cdot}OH$ Exposure[1]	$[C]/[C]_0$[2]	P Ox[3]	$f(^{\cdot}OH)$[4]
A	0	0	0.00	100%	0
B	>1.43E-07	>4.15E-10	0.00	100%	0
C	>2.05E-07	> 2.84E-10	0.00	100%	>0.0060
D	>3.65E-07	>4.92E-10	0.00	100%	>0.0106

1. Hydroxyl radical exposure (mol.L^{-1}.sec) quantified using the R_{CT} concept, calculated as resultant of R_{CT} and ozone exposure
2. Residual fraction of carbamazepine concentration calculated using equation 4.3 and ozone exposure values form Table 4.5 for 2 minutes of contact time
3. Predicted oxidation for carbamazepine accounting for both ozone and hydroxyl radical exposures calculated based on residual fraction values ($[C]/[C]_o$)
4. Fraction of carbamazepine oxidized by hydroxyl radicals during 2 min of contact time estimated using equation 4.4

In experimental setting A, the fraction of carbamazepine removed by hydroxyl radicals is zero. The level of hydroxyl radical exposure in setting B is lower than setting C but higher compared to setting A. Although the fraction of carbamazepine oxidized by hydroxyl radicals is expected to be more than 0.0060 in setting C, overall it seems that hydroxyl radical exposure is not playing a significant role in oxidation of carbamazepine in the first two minutes of ozonation with applied dose of 2 mg/L. It could then be

stated that with varying levels of presence of hydroxyl radicals, direct attack by ozone remains the dominant pathway of oxidation of carbamazepine and ozonation products are not expected to change significantly under different experimental settings.

4.2.2. By-Product Formation

In a study by McDowell et al., three major ozonation products of carbamazepine in MQ water were identified. These three by-products were abbreviated as BQM, BQD and BaQD (Section 2.6.1.1). In the current study these by-products as well as four natural metabolites of carbamazepine (Miao and Metcalfe 2003) which could also be produced as a result of ozonation were monitored. A summary of distribution of by-products found in different experimental settings is presented in Table 4.8.

Table 4.8 Ozonation by-products of carbamazepine in different experimental settings

Experimental setting	**BQM**	**BQD**	**CBZ-EP**	**CBZ-DiOH**	**CBZ-OH**
A	✓	✓	✓	✓	✓
B	✓	✓			
C	✓				
D	✓	✓			

In current study in experimental conditions similar to the simulated water matrix in McDowell's study two of the major by-products BQM and BQD were detected. However the third by-product (BaQD) was not detected in the samples. Three natural metabolites of carbamazepine were detected in the same setting (Figure 4.15). Detection and magnitude of BQM and BQD as major ozonation by-products of carbamazepine varied across different experimental settings in the present study. The scheme in Figure 4.16 gives an overall picture of carbamazepine oxidation in ozonation process. More details of individual by-products are further discussed in the following sections.

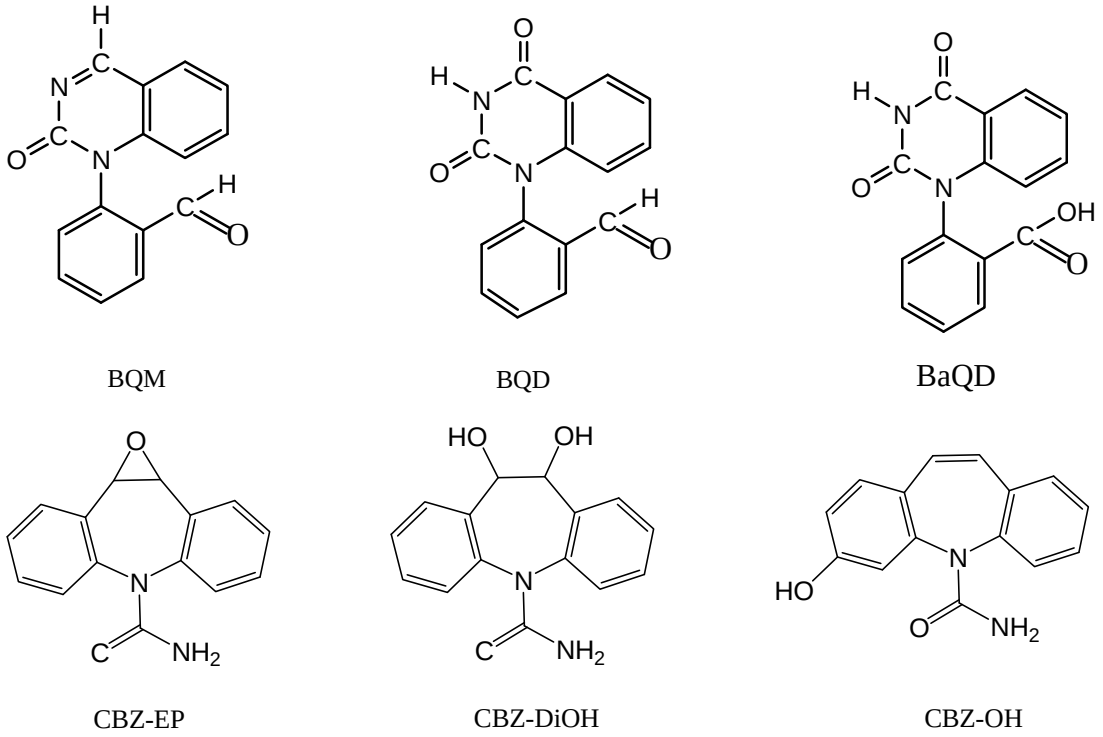

Figure 4.15 Molecular structures of six ozonation products of carbamazepine from McDowell et al. (2005) and Miao and Metcalfe (2003).

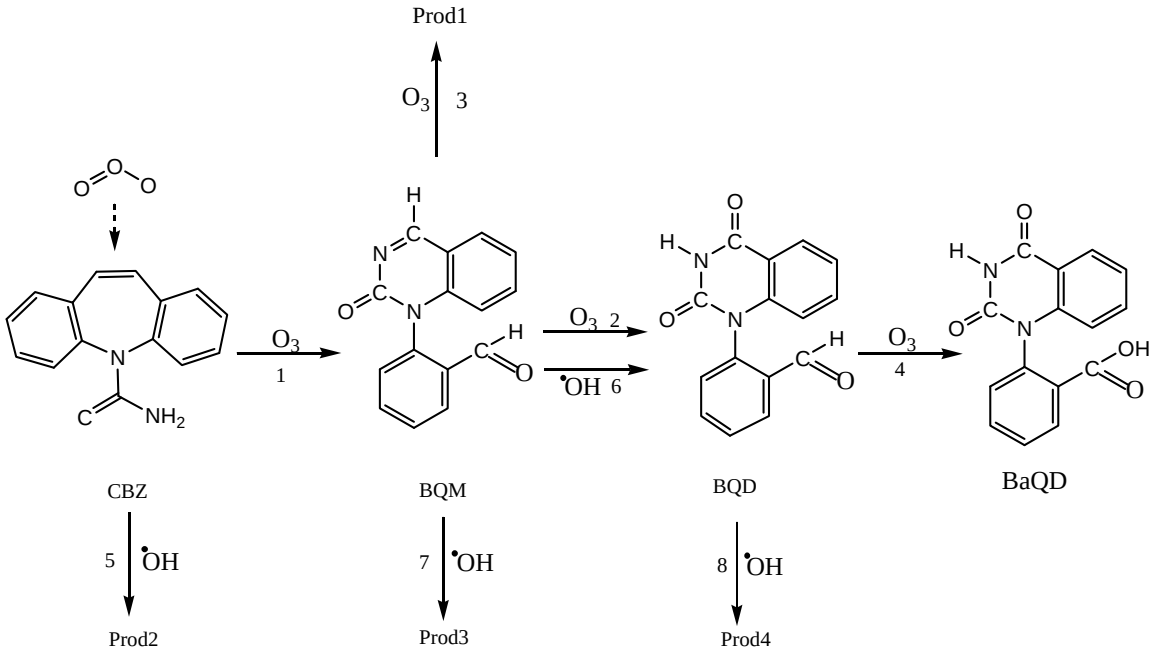

Figure 4.16 Proposed reaction pathway for the oxidation of carbamazepine with ozone and OH radicals (adapted from McDowell et al., 2005).

Rate constants for the reactions labeled in the scheme of Figure 4.16 were measured by McDowell et al. (2005):

Label number	Reaction	k_{O3} ($M^{-1}s^{-1}$) or k_{OH} ($M^{-1}s^{-1}$)
1	$CBZ + O_3 \longrightarrow BQM$	3×10^5
2	$BQM + O_3 \longrightarrow BQD$	~ 3
3	$BQM + O_3 \longrightarrow Prod1$	~ 4
4	$BQD + O_3 \longrightarrow BaQD$	1
5	$CBZ + {}^{\cdot}OH \longrightarrow Prod2$	$(8.8 \pm 1.2) \times 10^9$
6	$BQM + {}^{\cdot}OH \longrightarrow BQD$	$\sim 2 \times 10^9$
7	$BQM + {}^{\cdot}OH \longrightarrow Prod3$	$\sim 5 \times 10^9$
8	$BQD + OH^{\cdot} \longrightarrow Prod4$	5×10^9

The mechanisms for formation of minor ozonation by-products are presented in Figures 4.17 to 4.19 (Thadani, 2009).

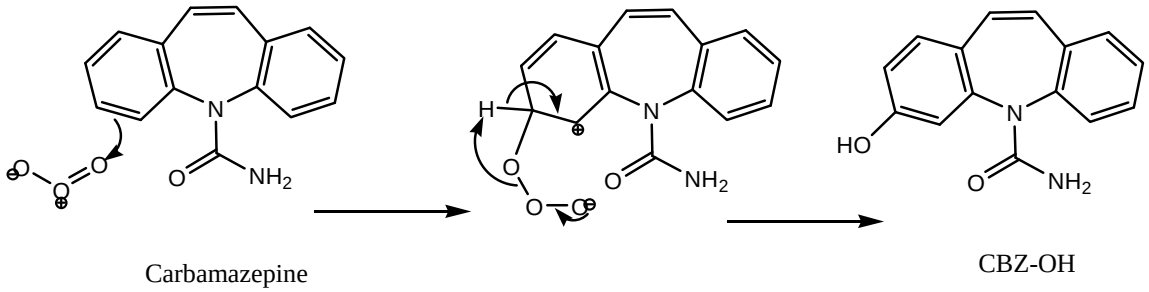

Figure 4.17 Formation of CBZ-OH by attack of ozone on benzene ring in carbamazepine.

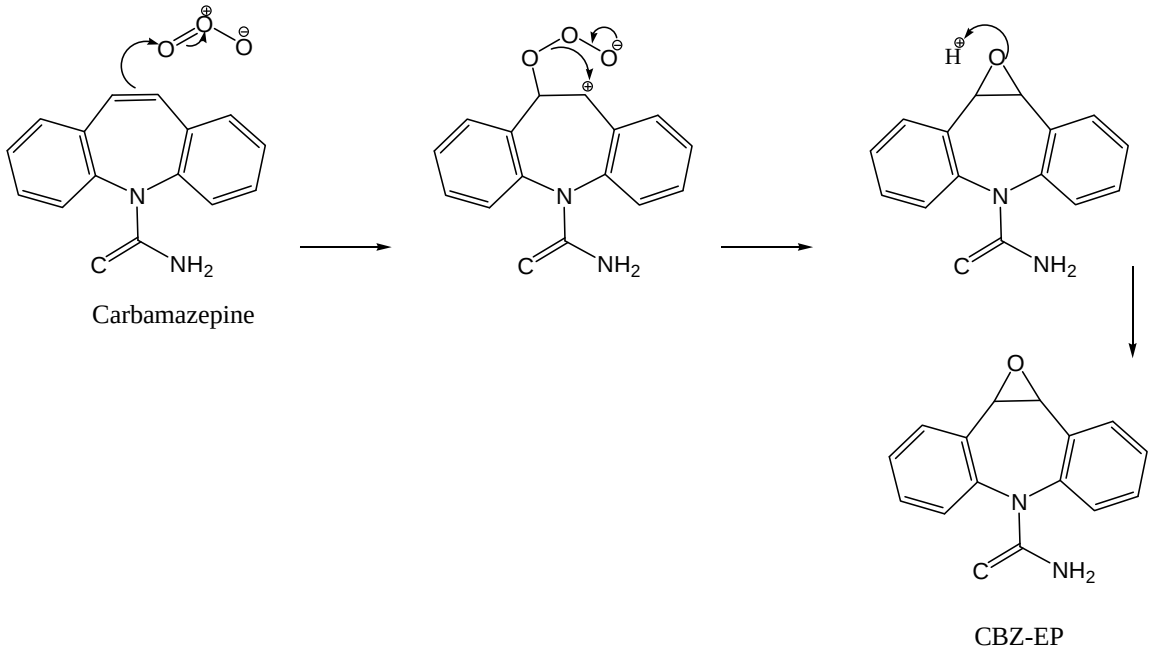

Figure 4.18 Formation of CBZ-EP by attack of ozone on aliphatic double bond in carbamazepine.

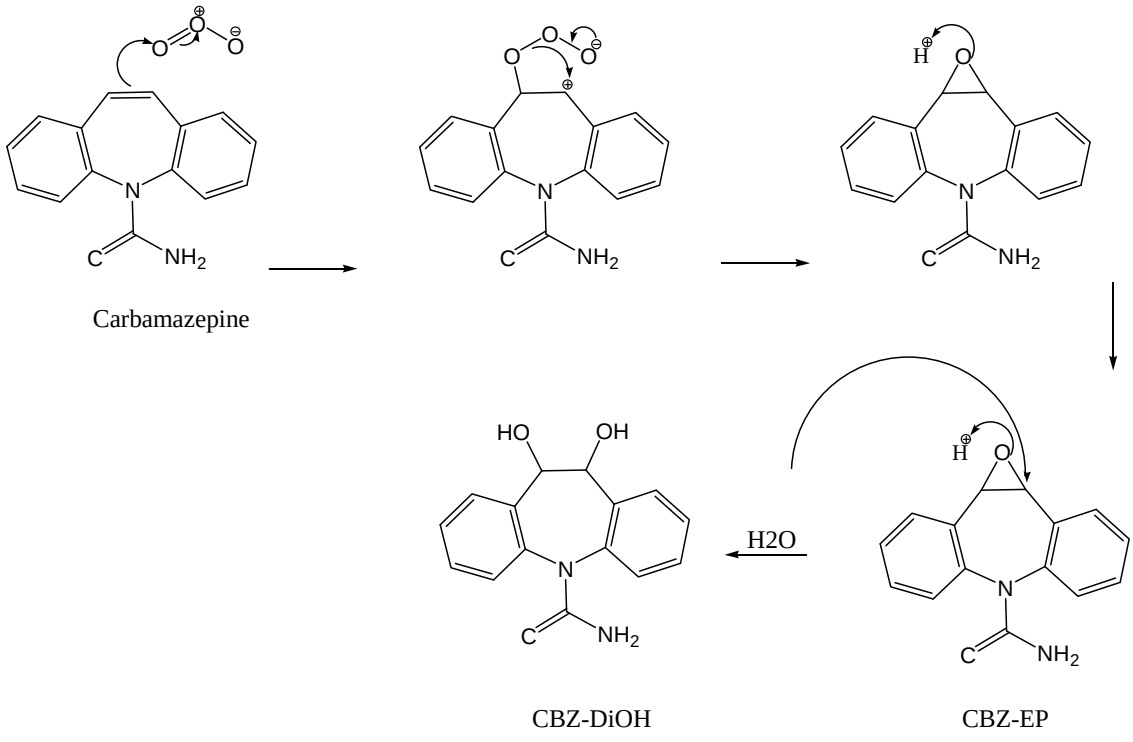

Figure 4.19 Formation of by-product CBZ-DiOH by attack of ozone on aliphatic double bond in carbamazepine.

4.2.2.1. BQM

Figure 4.20 shows detected peak area counts for an ozonation by-product of carbamazepine, BQM, in duplicate runs of four different experimental settings at all contact times. Peak area counts detected for duplicates at different contact times were similar in all settings except the last one. These area counts were within a factor of 3.26 of the geometric mean of duplicate values in experimental settings A, B and C. In experimental setting D, the duplicates were different from the geometric mean value by a factor of 10.72 times at T1. The results in Figure 4.20 show that in experimental setting A from T1 to T2 levels of detected peak area counts in both experimental runs were similar and within 1% of the average. This maintenance in levels of peak area counts continued from T2 to T3, where area counts in both runs were within 2% of the average (The details are included in Tables C.1 to C.3 in Appendix C). The maintenance observed in detected peak area counts for BQM in this setting compared to declining trend of residual ozone concentration with time in Figure 4.14 for setting A suggests relative resistance of this compound to ozonation. The small value of k_{O3} for reaction 2 (~3 $M^{-1}s^{-1}$) from the scheme in Figure 4.16, confirms the relative resistance of BQM to ozonation.

In experimental setting B, unlike setting A, from T1 to T2 detected peak area counts for BQM declined by 90% in both runs, followed by 87% decline from T2 to T3 in run 2. Because of unknown reasons the analogous peak for T3 in run 1 was not detected (The details are included in Tables C.4 to C.6 in Appendix C). The high rate of decline of BQM in setting B compared against the maintenance of this compound in setting A indicates susceptibility of this compound to oxidation by hydroxyl radicals.

The trends observed in experimental setting C are different. In first run, detected peak area counts for BQM decreased by 48% from T1 to T2, followed by a further 34% decline from T2 to T3. In second run detected peak area count first increased by 19% from T1 to T2, but then from T2 to T3 area counts were similar and within 9% of the average (The details are included in Tables C.7 to C.9 in Appendix C). Since all of carbamazepine was removed in 2 minutes of ozonation and most of ozone concentration was consumed in

the same time interval as well, no change in detected peak area counts is expected after 2 minutes of ozonation. As changes in ozone exposure after 2 minutes of ozonation in setting D similar to setting C were negligible, the decreasing trend of BQM observed in the results cannot be validated (The details are included in Tables C.10 to C.12 in Appendix C).

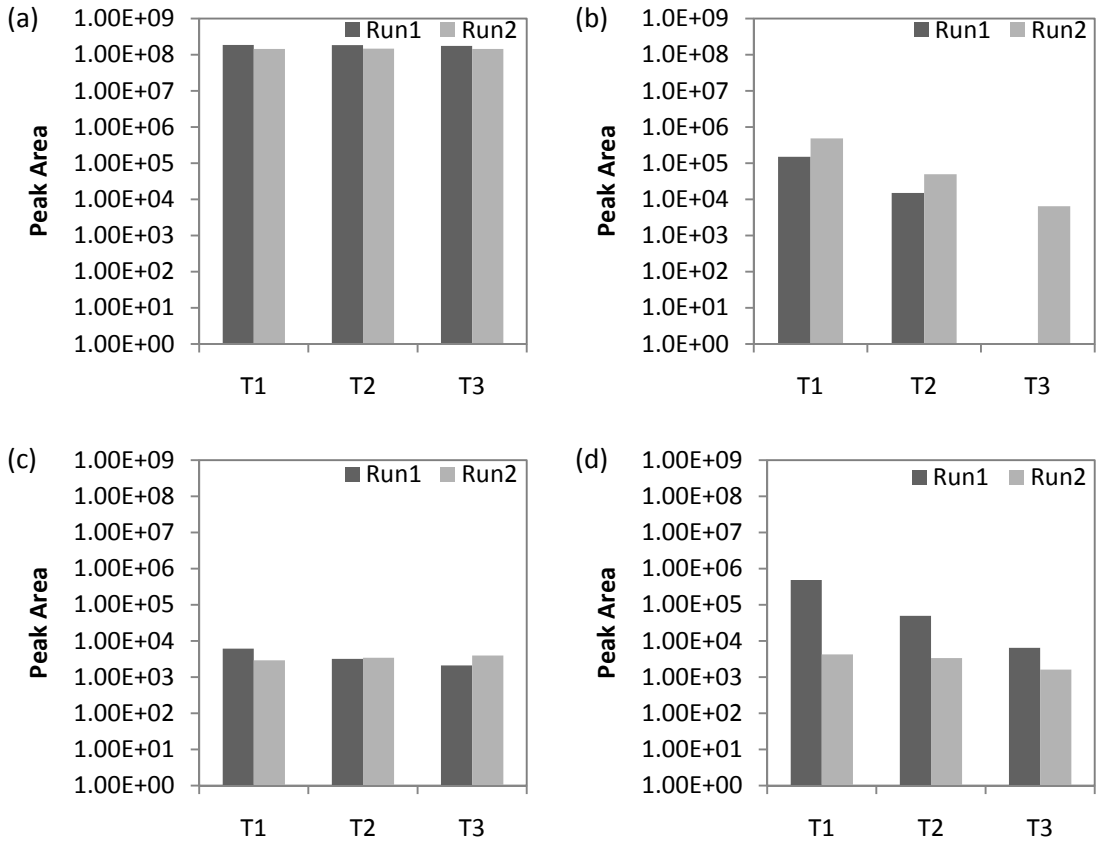

Figure 4.20 Peak area counts of ozonation product of carbamazepine, BQM, in a) Experimental setting A, b) Experimental setting B, c) Experimental setting C and d) Experimental setting D. T1=2 min, T2=5 min and T3=10 min.

In the experimental setting B after 2 minutes of ozonation the average of detected peak area count for BQM was lower by 520 times compared to setting A. similarly at the end of 2 minutes of ozonation average detected peak area counts for BQM in settings C and

D were lower approximately by 35000 and 675 times from the analogous average area count in setting A, respectively. At the same time since all carbamazepine was oxidized in first two minutes of ozonation in all settings, it could be suggested that this difference in present levels of BQM in 2 minutes of ozonation confirms the fact that direct attack of ozone is the dominant pathway for formation of BQM, and the BQM formed in three latter settings were immediately oxidized by present hydroxyl radicals.

4.2.2.2. BQD

As the results in Figure 4.21 show detected peak area counts for BQD in all contact times of experimental settings A, B and D were similar for the duplicate runs and were within a factor of 2.54 of the geometric mean of the duplicate values. This by-product was not detected in any of reactor samples in experimental setting C.

As observed in the results, average detected peak area counts for BQD at the end of two minutes of ozonation in experimental settings B and D, were lower compared to the corresponding average area count in experimental setting A by a factor of 180 and 20500 respectively. This could be explained to be the effect of combination of two factors. Based on the proposed reaction pathway of carbamazepine in Figure 4.16, BQD can be formed from oxidation of (reactions 2 and 6). Since detected levels of BQM in experimental setting A were higher compared to other settings, higher levels of BQD in experimental setting A were expected as well. Other than that in the conditions where attack by ozone was the dominant pathway of formation of BQD (absence of hydroxyl radicals, this is the case in setting A) comparing rate constants for reactions 2 and 4 shows that the rate of formation of BQD can be three times higher than the rate of consumption of this compound. On the other hand if hydroxyl radicals can be found in the matrix although BQD is produced at a higher rate by hydroxyl radicals than molecular ozone (k_{OH} = ~2×10^{9} $M^{-1}s^{-1}$ and k_{O3}= ~3 $M^{-1}s^{-1}$), comparison of rate constants for reactions 6 and 8 in Figure 4.16 shows that the rate of degradation of BQD to other by-products by hydroxyl radicals is 2.5 times greater than its formation rate. This confirms lower detected level of BQD in experimental settings B and D.

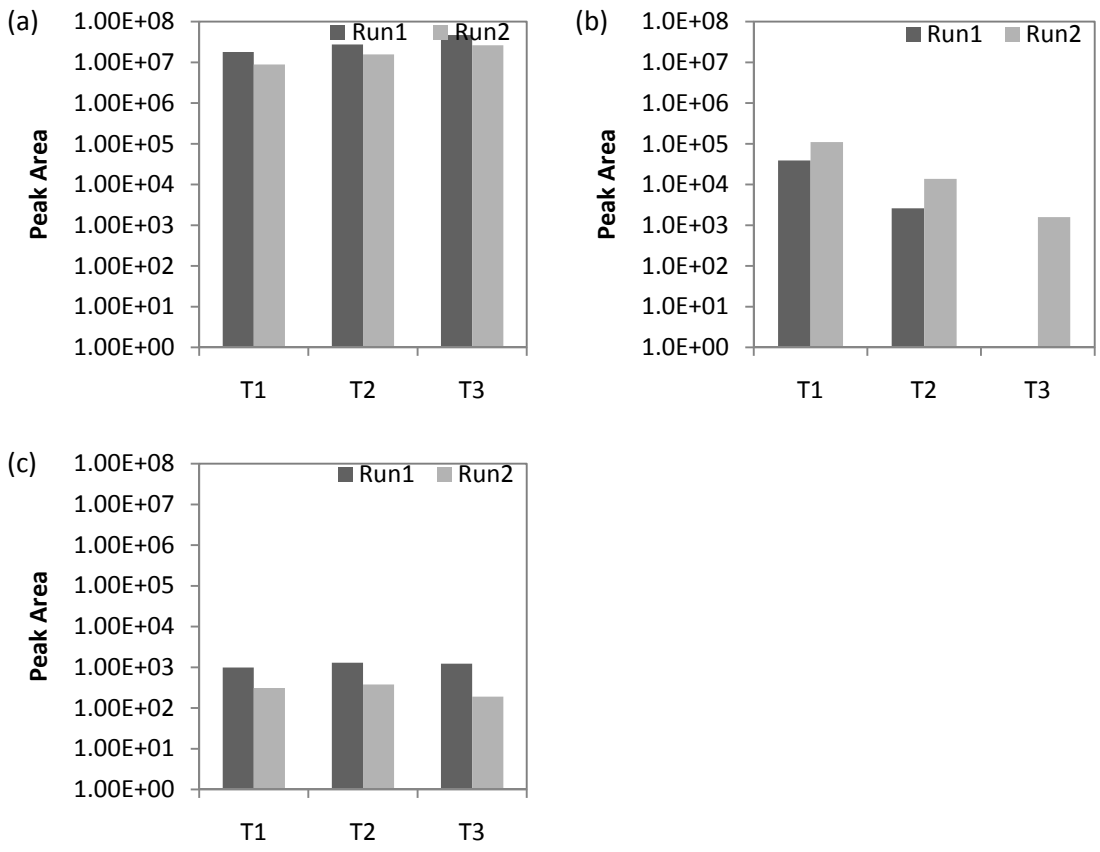

Figure 4.21 Peak area counts for carbamazepine ozonation product, BQD, in a) Experimental setting A, b) Experimental setting B and c) Experimental setting D. This by-product was not detected in experimental setting C. T1=2min, T2=5 min and T3=10 min.

In experimental setting A detected peak area count for by-product BQD increased for 54 and 70% from T1 to T2 and from T2 to T3 in run 1 respectively. In the second run, area count increased for 77 and 67% from T1 to T2 and then from T2 to T3 respectively (The details are included in Tables C.1 to C.3 in Appendix C). This increasing trend with increasing ozone exposure which was expected based on the ozonation rate constants for reactions 2 and 4 in Figure 4.16 indicates that this compound is relatively resistant to oxidation by ozone (k_{O3}= 1 $M^{-1}s^{-1}$).

In setting B from T1 to T2, 93 and 88% decline was observed in peak area counts for BQD in runs 1 and 2 respectively. Following that, from T2 to T3, detected peak area

count for BQD decreased by another 88% in second run. The corresponding peak for T3 in run 1 was not detected due to unknown reasons (The details are included in Tables C.4 to C.6 in Appendix C). The observed decrease in setting B compared to the increase in setting A is suggestive of susceptibility of this compound to attack by hydroxyl radicals. This is consistent with the rate constant for reaction of BQD with hydroxyl radicals (k_{OH}= 5×10^9, reaction 8 in Figure 4.16).

As can be seen in Figure 4.21, levels of detected BQD remain relatively constant in setting D (The details are included in Tables C.10 to C.12 in Appendix C). Since the entire applied ozone dose of 2 mg/L was consumed within the first two minutes of ozonation in this setting, maintained levels of BQD are acceptable. Moreover detected levels were approximately 20000 times lower than setting A and could be close to detection limits for this by-product.

4.2.2.3. CBZ-EP

This by-product was only detected in experimental setting A (Figure 4.22). Detected peak area counts for CBZ-EP at T1 and T2 were within 1% of the average in both experimental runs. Similarly peak area counts for CBZ-EP at T2 and T3 were within 5% of the average in both runs 1 and 2. The details of peak area counts are included in Tables C.1 to C.3 in Appendix C. Since there was a considerable amount of ozone available in this setting at all contact times approximate maintenance of levels of area count for CBZ-EP could either be an indication of resistance of this compound to ozonation or be related to low levels of detection which were potentially close to MDL.

4.2.2.4. CBZ-DiOH

This compound (Figure 4.23) which is only detected in experimental setting A is a further oxidation product of CBZ-EP. Similar to the case of CBZ-EP, no trend was observed for this product. Detected peak area counts for this product at varying contact times in duplicate runs were very close and within a factor of 1.08 of the geometric mean of duplicate values. Detected peak area counts from the two runs at T1 and T2 are very similar and vary within 2% of the average. Similarly detected peak area counts for T2

and T3 are within a factor of 1% of the average value in both runs. The details of peak area counts are included in Tables C.1 to C.3 in Appendix C.

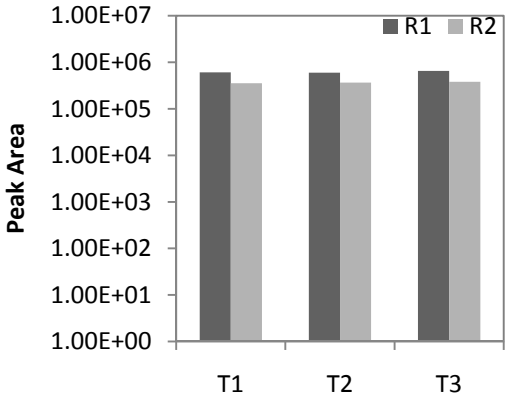

Figure 4.22 Peak area counts for ozonation product of carbamazepine, CBZ-EP, which is only detected in experimental setting A. T1=2 min, T2=5 min and T3=10 min.

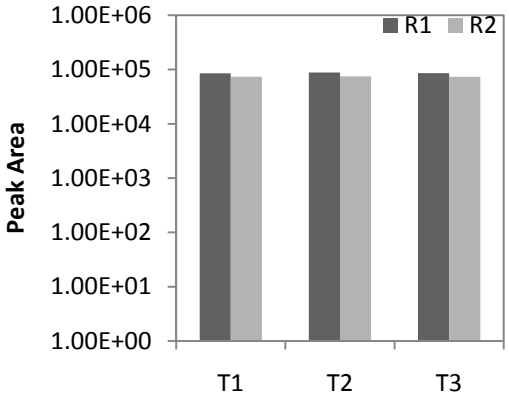

Figure 4.23 Detected peak area counts for ozonation product of carbamazepine, CBZ-DiOH, in different contact times of T1=2 min, T2= 5 min and T3=10 min in experimental setting A.

4.2.2.5. CBZ-OH

Levels of detected peak area counts for this by-product which was only detected in setting A (Figure 4.24) were close in duplicate runs and within a factor of 1.10 of geometric mean value. The level of this ozonation product was maintained with increasing contact time. Since peak area counts were small and could be close to MDL, the maintenance of respective peak area counts of CBZ-OH in this experimental setting cannot be validated. The details of peak area counts are included in Tables C.1 to C.3 in Appendix C.

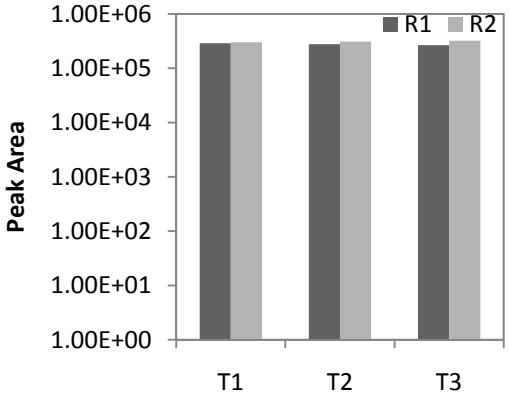

Figure 4.24 Detected peak area counts for ozonation product of carbamazepine, CBZ-OH, at three different contact times T1=2 min, T2=5 min and T3=10 min. This product is only detected in experimental setting A.

4.2.3. Discussion

In setting A of experiments, where tert-butanol was used as a hydroxyl radical scavenger at 25 mM level, by-product BQD showed an increasing trend (Figure 4.21). In fact it increased by 54% from T1 to T2 in run 1 and then increased for 70% from T2 to T3 in the same run. Similarly in the second run, this by-product had an increasing rate of 77% from T1 to T2 and then 67% from T2 to T3. This increasing trend of BQD in presence of tert-butanol is in agreement with a study by McDowell et al., 2005 that reported an increase in BQD level with ozone exposure in a similar set up with tert-butyl alcohol level of 30 mM and initial ozone dose of 50 µM (2.4 mg/L). Unlike the latter study, the

increasing BQD level was not accompanied by a decrease in BQM level (Figure 4.20). However the range of ozone exposure over which the changes in levels of by-products were measured in that study (zero to 0.15 $mol.L^{-1}$.sec equivalent to 2.5 mM.min) was ten times larger compared to the present study which could be the main reason for difference in observed trends. Based on McDowell et al., 2005, starting with excess initial ozone, and in the presence of tert-butanol the reaction of carbamazepine with molecular ozone should be the dominant pathway of transformation of carbamazepine and result in formation of BQM. As can be observed in Figure 4.20, after 2 minutes of ozonation a noticeable amount of BQM was formed (an average of 1.65E+08 in peak area count) and the concentration of carbamazepine had decreased to below detection limit, since at this stage a considerable concentration of ozone (average of 1.5 mg/L) was left in the media, it was expected that the sequential reaction proposed by McDowell et al. (2005) proceeded and an increase in amount of BQD parallel to a decrease of BQM happened. However observations in this experimental setting show that an increase in the peak area count of BQD regardless of perseverance of BQM happened. According to the proposed mechanism by McDowell et al., 2005, in a similar setting there is rather no other pathway than transformation of BQM with ozonation for formation of BQD. At the same time, due to molecular ozone attack BQM can yield another by-product (named as Prod 1 in that study) as well.

In setting B, averages of peak area counts for both BQM and BQD decreased between 2 and 10 minutes of contact time (Figures 4.20 and 4.21, respectively). Considering the range of residual ozone concentration at this time interval (Figure 4.14), the decreasing trend of BQM can be related to ozonation of this compound (both direct and indirect). Both attacks of ozone and hydroxyl radicals can justify decreasing level of BQM. A decreasing trend for BQD was observed which could be justified by reduction of BQD to another by-product named Prod4 through oxidation by hydroxyl radical as proposed by McDowell et al., 2005 (Figure 4.16). Since the compound BaQD (formed by direct ozonation of BQD, reaction 4 in Figure 4.16) is not detected, no credit can be given to molecular ozone for oxidation of BQD at this time interval.

In McDowell's study rate constants of reaction of hydroxyl radicals (k_{OH}) with BQM and BQD were determined as ~6.8×10^9 and ~5.1×10^9 $M^{-1}s^{-1}$ respectively at pH=8. These high rate constants reveal quick oxidization of these two by-products with hydroxyl radicals during ozonation. As observed in Figures 4.20 and 4.21, in all other settings but setting A detected levels of BQM and BQD were low which could be related to oxidation with hydroxyl radicals. With negligible levels of residual ozone in settings C and D only after 2 minutes of contact time, it seems that BQM and BQD remain constant for up to 10 minutes of contact time due to lack of any oxidizing compounds in the medium. In experimental setting C, BQD was not detected at all. Because of high oxidation rates it could have been completely reduced and transformed to some other compounds that were not monitored in these experiments. It can be concluded that oxidation of these ozonation products mainly happen through reactions with hydroxyl radicals (McDowell et al., 2005).

4.2.4. Summary of Carbamazepine Oxidation

Based on the results discussed in Section 4.2.1 more than 99% of carbamazepine was oxidized in the first 2 minutes of ozonation under all experimental settings. Although the level of oxidation of carbamazepine is similar in all four experimental settings the distribution of ozonation by-products formed and their relative magnitudes varies in different experimental settings as shown in Figures 4.20 to 4.24. Five different ozonation by-products of carbamazepine were detected under conditions of experimental setting A. Number of by-products detected in experimental settings B and D are limited to two as shown in Figures 4.20 and 4.21. Only one is detected in experimental setting C as shown in Figure 4.20.

Although relative presence of ozone and hydroxyl radicals varies across different experimental settings, oxidation of carbamazepine mainly proceeds through reaction with ozone under all experimental settings as discussed in Section 4.2.1. However as mentioned in Section 4.2.2 relative magnitudes of carbamazepine by-products and their further oxidation can still be affected by presence of hydroxyl radicals in experimental

settings B, C and D. BQM is formed by attack of ozone on carbamazepine as shown in Figure 2.9. Further oxidation of BQM by both ozone and hydroxyl radicals results in formation of BQD as discussed in Section 4.2.2.2 and shown in Figure 4.16. Formation of CBZ-OH, CBZ-EP and CBZ-DiOH is explained by direct reaction by ozone as well, as shown in Figures 4.17 to 4.19.

As discussed in Section 4.2.1, in experimental setting A, the only present oxidant is ozone and there is no hydroxyl radical exposure in this experimental setting. As results in the current study show (Section 4.2.2) formation of BQM, BQD, CBZ-EP, CBZ-DiOH and CBZ-OH is expected under conditions similar to experimental setting A. Results in Sections 4.2.2.1 and 4.2.2.2 demonstrate that highest levels of carbamazepine by-products BQM and BQD are detected in experimental setting A with ozone exposure alone, as shown in Figures 4.20 and 4.21. Based on the results discussed in Sections 4.2.2.3 to 4.2.2.5 and shown in Figures 4.22 to 4.24, three other ozonation products of carbamazepine CBZ-EP, CBZ-DiOH and CBZ-OH are only expected in experimental setting A. Under conditions in this experimental setting, by-products BQM and BQD are resistant to further oxidation by ozone as shown in Figures 4.20a and 4.21a, respectively.

By-products BQM and BQD were detected in experimental settings B and D. In experimental setting C only BQM was detected. Although direct reaction by ozone is mainly responsible for oxidation of carbamazepine in all experimental settings, presence of hydroxyl radicals under conditions in experimental settings B, C and D affects level of BQM and BQD as discussed in Sections 4.2.2.1 and 4.2.2.2. As discussed in Section 4.2.2.1 and shown in Figure 4.20, due to presence of hydroxyl radicals, level of detected BQM is expected to be hundreds to thousands times lower in these experimental settings compared to experimental setting A. Similarly lower levels of BQD are expected in these latter experimental settings, as shown in Figure 4.21. These effects are attributed to further oxidation of these two by-products by hydroxyl radicals.

Chapter 5: Conclusions and Recommendations

5.1. Conclusions

A set of 12 experiments were conducted for each of the two target pharmaceuticals to investigate the effect of varying ozone and hydroxyl radical exposures on formation of ozonation by-products. The experiments were designed at typical dosages that are used in drinking water treatment. Based on analytical results the following conclusions are made.

5.1.1. Bezafibrate

- Ozonation was proved to be effective in oxidation of bezafibrate under all experimental settings. Using an ozone dose of 2 mg/L, more than 95% oxidation of bezafibrate was obtained in 5 minutes with an average of 89% of bezafibrate oxidized in the first 2 minutes of contact.
- Varying dominance of direct and indirect mechanism of attack of ozone was observed in ozonation treatment of bezafibrate depending on relative ozone and hydroxyl radical exposures.
- Three compounds, MW367, MW393 and MW227 were the major ozonation products of bezafibrate.
- MW367 was a by-product of ozonation of bezafibrate formed as the result of attack of molecular ozone on non-chlorinated aromatic ring of the compound.
    - This by-product was observed under ozone dominated conditions.
    - The results indicated a resistance of MW367 to oxidation in the presence of hydroxyl radical scavenger, tert-butanol.
    - MW367 was susceptible to oxidation by hydroxyl radicals.
    - Declining levels of this by-product were observed with increasing hydroxyl radical exposure.
- MW393 was a by-product of bezafibrate formed by direct attack of ozone on non-chlorinated aromatic ring of this compound.

  - Similar to MW367, this by-product was also only detected under ozone dominated experimental settings.
  - A decreasing trend with increasing contact time was observed for this by-product under ozone dominated conditions. This was indicative of susceptibility of this compound to attack by ozone.
  - MW393 was susceptible to degradation by hydroxyl radicals.
  - Declining levels were observed with increasing hydroxyl radical exposure.
- MW227 was a chain product of further ozonation of mainly MW393 and, to a lesser extent, MW367.
  - MW227 was detected under all experimental settings. However the amount of the by-product formed was the highest under ozone dominated conditions and varied with the experimental conditions.
  - MW227 was resistant to degradation by both ozone and hydroxyl radicals.
  - The formation of this by-product declined with reducing ozone exposure and increasing hydroxyl radical exposure.
- MW393 was still detectable after 10 minutes of contact time with ozone in the presence of tert-butanol. That was despite the fact that almost half of the ozone dose remained intact in the medium. Oxidation of MW367 and MW393 was not quite successful in experimental setting B (where no hydroxyl radical scavenger or hydrogen peroxide was added). In this setting, ozone and hydroxyl radical exposures were not sufficient for complete oxidation of these compounds. Ozonation at present dose was not successful in transformation of MW227 in any of experimental settings.
- The transformation of MW367 and MW393 were successful when hydrogen peroxide was present. In these cases oxidation happened instantaneously and both these two by-products were transformed to below detection limit in less than 2 minutes of ozonation.

5.1.2. Carbamazepine

- Ozonation was observed to be a successful method for oxidation of carbamazepine under all experimental settings. Starting with an ozone dose of 2 mg/L carbamazepine was oxidized to below detection limit even at the lowest contact time, i.e., 2 minutes.
- The results indicate that under typical water treatment conditions carbamazepine oxidation is dominated by direct attack of ozone.
- In this study formation of two major ozonation by-products of carbamazepine abbreviates as BQM and BQD (McDowell et al., 2005) and three other ozonation by-products (originally identified as natural metabolites of carbamazepine) was confirmed.
- BQM is an ozonation by-product of carbamazepine. This compound is suggested to be formed by direct attack of ozone on carbamazepine.
    - BQM was formed under all experimental conditions.
    - Observed levels of this by-product declined with increasing levels of hydroxyl radicals.
    - BQM was relatively resistant to oxidation by ozone, but susceptible to degradation by hydroxyl radicals.
- BQD is another ozonation by-product of carbamazepine that was formed by direct attack of ozone on BQM.
    - This compound was formed under all experimental conditions.
    - Highest level of this by-product was detected in the absence of hydroxyl radicals.
    - BQD is relatively resistant to degradation by ozone.
    - This compound is observed to be susceptible to degradation by hydroxyl radicals.
- Both major oxidation products of carbamazepine were shown to be resistant to ozonation but had a high reactivity with hydroxyl radicals. Therefore it was

concluded that oxidation of these ozonation by-products mainly occurs by hydroxyl radicals.

- Minor ozonation products of carbamazepine were only detected in relatively small levels in thepresence of tert-butanol, therefore their high reactivity towards hydroxyl radicals was suggested.

5.2. Recommendations

- As interpretations based on comparison of peak area counts has to be made with caution, preparation of standards for different ozonation products seem to be an important step in facilitating analytical interpretations. Availability of standards not only would allow better understanding of changes in levels of each ozonation product in different settings but it will also provide a means of comparison for relative presence of various by-products within each setting. As in the current study although the trend of changes in level of any by-product (e.g. BQM) in any given setting (e.g. setting B) can be investigated, no comment on relative presence of any two selected by-products (e.g. BQM and CBZ-EP) can be made.
- Having more data points, i.e. analysing for identified by-products in a wider range of ozone contact times (ozone exposure) could further clarify the results obtained in these experiments. In order to better realize the sequential reactions that result in formation of some ozonation by-products from others sampling could start at smaller ozone exposures which would require a more sophisticated experimental setting to assure proper mixing in reactor vessels under lower contact times and provisions for a rapid accurate sampling method must be maid. Moreover, in experiments on oxidation of carbamazepine in presence of hydrogen peroxide, approximately all the applied ozone dose is consumed in the first 2 minutes and therefore despite high reaction rates for oxidation of some of these by-products with hydroxyl radicals, after 10 minutes of ozonation they are

still detectable in most cases, effect of higher applied ozone doses and extended exposures could be studied on oxidation of these by-products.

- Since potential effects of long term exposure to low levels of any chemical compounds through consumption of drinking water cannot be compromised and based on precautionary rules drinking water must be free of any contaminants further studies on optimizing ozonation process for complete oxidation of any by-products is advised, however determination of any potential harmful or possibly toxic effects from these micropollutants could be helpful in prioritizing the areas of expected research work and perhaps in decision making measures when it comes to selecting the most suitable method for removal of pharmaceutical compound.

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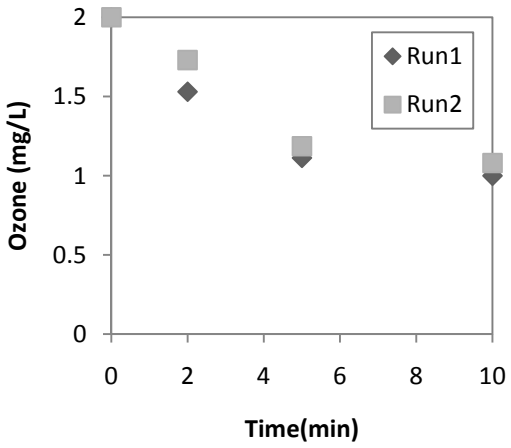
Appendix A

Ozone Exposure Calculations

As a sample, calculations for ozone exposure of setting A experiments of bezafibrate is explained below.

The table and chart below show residual ozone concentrations in duplicate runs in this experimental setting

Reactor	Contact time(min)	Residual ozone in Run1 (mg/L)	Residual ozone in Run2 (mg/L)
Control	0	2	2
T1	2	1.5	1.7
T2	5	1.1	1.2
T3	10	1.0	1.1



Ozone exposure which is the area beneath the data points for residual ozone concentrations is calculated by fitting an exponential curve for the first two minutes of ozonation and a linear trend line for the residual ozone data between 2 and 5 minutes as well as 5 to 10 minutes. Using this approach the following values were calculated for ozone exposure in this setting.

	Ozone exposure (M.sec)		
	2min	5min	10 min
run1	0.0091	0.0141	0.0207
run2	0.0058	0.0113	0.0184
avg. of 2 runs	0.0075	0.0127	0.0195

R_{CT} Calculations

Knowledge of ozone and hydroxyl radical concentrations in any ozonation process is necessary for predicting the extent of disinfection, oxidation and any potential disinfection by-product formation. However quantifying the steady state concentration of hydroxyl radicals is a challenging task. It has been figured out that investigating the transformation of an ozone resistant compound in any particular water can lead to determination of hydroxyl radical exposure in that water (Elovitz & Von Gunten, 1999).

The oxidation of any micropollutant C in an ozonation process can be formulated as follow (Von Gunten, 2003a)

$$-\frac{\mathrm{d[C]}}{\mathrm{d}t} = k_{\mathrm{O_3}}[\mathrm{C}][\mathrm{O_3}] + k_{\mathrm{OH}}[\mathrm{C}][\ ^{\cdot}\mathrm{OH}]$$

The probe compound used in this study is pCBA. Since this compound has a very low reactivity with ozone, its oxidation in a water media is dominantly controlled by reaction with hydroxyl radicals. So the equation above reduces to:

$$-\frac{\mathrm{d[pCBA]}}{\mathrm{d}t} = k_{\ ^{\cdot}\mathrm{OH,pCBA}}[\mathrm{pCBA}][\ ^{\cdot}\mathrm{OH}]$$

Rearranging and integrating

$$\ln\left(\frac{[\mathrm{pCBA}]}{[\mathrm{pCBA}]_0}\right) = -k_{\ ^{\cdot}\mathrm{OH,pCBA}} \int [\ ^{\cdot}\mathrm{OH}]\mathrm{d}t$$

This equation shows that relative reduction in concentration of this ozone resistant compound is an indirect measurement of the hydroxyl radical exposure (Elovitz & Von

Gunten, 1999). Defining the term R_{CT} as the ratio of hydroxyl radical exposure to ozone exposure and substituting for hydroxyl radical exposure in the equation above yields

$$\ln\left(\frac{[\mathrm{pCBA}]}{[\mathrm{pCBA}]_0}\right) = -k_{\cdot\mathrm{OH,pCBA}} R_{\mathrm{CT}} \int [\mathrm{O_3}]\,\mathrm{d}t$$

If reduction in concentration of pCBA is experimentally measured in a particular water, knowing the ozone exposure in that same setting using the equation above the R_{CT} parameter can be determined.

As an example the calculation of R_{CT} in experimental setting C is presented here.

Starting with pCBA initial concentration of 1 µM (156.57 µg/L) decrease in concentration of both pCBA and ozone is measured (for details on preparation of water Matrix and measurement of ozone and pCBA refer to Sections 3.3, 3.4.1.1. and 3.4.1.3 respectively).

Ozone exposure in the first two minutes of ozonation in this setting is calculated to be 0.0025 $mol.L^{-1}.sec$. Measurements of concentration of pCBA shows that the concentration of this compound drops to below method detection limit in 2 minutes of contact time. Method detection limit which is 10 ppb is then used for calculation of R_{CT}. This calculation then yields a lower limit value for R_{CT} in this setting. Using the equation above and substituting for the following values

$[pCBA]_{t=2}$=10 ppb,

$[pCBA]_0$=145.91 ppb,

$k_{OH,pCBA}$=5.2E+09,

The lower limit for R_{CT} is calculated to be 2.05E-07

Hydroxyl Radical Exposure

The ratio of hydroxyl radicals exposure to ozone exposure was introduced as a new parameter by Elovitz and von Gunten (Elovitz & Von Gunten, 1999). Since the low level, steady state transient concentration of hydroxyl radicals cannot be measured with an

easy method directly, calculation of this parameter and ozone exposure measurements can be used for determining this factor.

$$R_{\mathrm{CT}} = \frac{\cdot\mathrm{OH\ exposrue}}{\mathrm{O_3\ exposure}}$$

For example, having the value of R_{CT} and ozone exposure at 2 minutes of contact time for setting C experiments of bezafibrate from previous calculations, the hydroxyl radical exposure is determined to be greater than 5.30E-10 $mol.L^{-1}.sec$.

Appendix B

Analytical Data for Bezafibrate Experiments

The magnitudes of detected peak area counts for bezafibrate and its ozonation products MW367, MW393 and MW227 as well as determined concentration of bezafibrate in each of reactor samples under four experimental settings are presented in the following tables.

Experimental setting A

Table B.1 Detected peak area counts for bezafibrate and its ozonation by-products in duplicate samples of run1 of experimental setting A.

Sample	Concentration (ng/L)	Peak area			
	Bezafibrate	Bezafibrate	MW367	MW393	MW227
BZF T1	51900	2.06E+07	1.74E+06	1.31E+07	6.36E+05
BZF T1	57600	2.29E+07	2.15E+06	1.51E+07	7.20E+05
BZF T2	10800	4.29E+06	1.84E+06	9.72E+06	6.92E+05
BZF T2	10200	4.03E+06	2.26E+06	1.04E+07	6.52E+05
BZF T3	7140	2.83E+06	1.35E+06	No Peak	9.33E+05
BZF T3	Sample bottle broken				

Table B.2 Detected peak area counts for bezafibrate and its ozonation products in duplicate samples of run2 of experimental setting A.

Sample	Concentration (ng/L)	Peak area			
	Bezafibrate	Bezafibrate	MW367	MW393	MW227
BZF T1	26100	1.90E+07	2.20E+06	1.69E+07	5.60E+05
BZF T1	27600	2.02E+07	2.85E+06	2.01E+07	4.56E+05
BZF T2	9370	6.84E+06	2.62E+06	1.47E+07	5.44E+05
BZF T2	9390	6.85E+06	2.77E+06	1.56E+07	6.44E+05
BZF T3	3870	2.82E+06	1.37E+06	5.78E+06	7.29E+05
BZF T3	3960	2.89E+06	1.25E+06	5.40E+06	8.44E+05

Summary Table for Experimental Setting A

Table B.3 Average of determined peak area counts for bezafibrate and its ozonation products in duplicate samples of each run in experimental setting A.

Average of duplicates	Average concentration (ng/L)	Average peak area			
	Bezafibrate	Bezafibrate	MW367	MW393	MW227
BZF T1-Run1	54750	2.20E+07	1.90E+06	1.40E+07	6.80E+05
BZF T1-Run2	26850	1.96E+07	2.70E+06	1.90E+07	5.08E+05
BZF T2-Run1	10500	4.20E+06	2.10E+06	1.00E+07	6.70E+05
BZF T2-Run2	9380	6.85E+06	1.30E+06	1.50E+07	5.94E+05
BZF T3-Run1	7140	2.80E+06	1.40E+06	No peak	9.30E+05
BZF T3-Run2	3915	2.86E+06	2.70E+06	5.60E+06	7.87E+05

Experimental setting B

Table B.4 Detected peak area counts for bezafibrate and its ozonation by-products in duplicate samples of run1 of experimental setting B.

Sample	Concentration (ng/L)	Peak area			
	Bezafibrate	Bezafibrate	MW367	MW393	MW227
BZF T1	19900	7.90E+06	1.98E+04	7.22E+04	6.68E+05
BZF T1	36800	1.46E+07	2.30E+04	7.92E+04	6.97E+05
BZF T2	10300	4.08E+06	1.13E+04	3.33E+04	3.78E+05
BZF T2	6300	2.50E+06	1.15E+04	3.93E+04	3.90E+05
BZF T3	10100	4.00E+06	No Peak	trace	1.12E+05
BZF T3	8300	3.29E+06	trace	trace	1.21E+05

Table B.5 Detected peak area counts for bezafibrate and its ozonation by-products in duplicate samples of run2 of experimental setting B.

Sample	Concentration (ng/L)	Peak area			
	Bezafibrate	Bezafibrate	MW367	MW393	MW227
BZF T1	7980	5.82E+06	4.56E+04	1.57E+05	5.41E+05
BZF T1	10700	7.82E+06	6.85E+04	2.38E+05	5.88E+05
BZF T2	9600	7.00E+06	1.81E+04	5.54E+04	3.24E+05
BZF T2	10600	7.75E+06	9.91E+03	1.99E+04	3.56E+05
BZF T3	6680	4.87E+06	9.86E+02	9.87E+03	1.04E+05
BZF T3	7790	5.69E+06	8.64E+02	9.92E+03	9.44E+04

Summary Table for Experimental Setting B

Table B.6 Average of determined peak area counts for bezafibrate and its ozonation products in duplicate samples of each run in experimental setting B.

Average of duplicates	Average concentration (ng/L)	Average peak area			
	Bezafibrate	Bezafibrate	MW367	MW393	MW227
BZF T1-Run1	28350	1.10E+07	2.14E+04	7.57E+04	6.83E+05
BZF T1-Run2	9340	6.82E+06	5.70E+04	2.00E+05	5.65E+05
BZF T2-Run1	8300	3.30E+06	1.14E+04	3.63E+04	3.84E+05
BZF T2-Run2	10100	7.38E+06	1.40E+04	3.80E+04	3.40E+05
BZF T3-Run1	9200	3.60E+06	Trace	Trace	1.17E+05
BZF T3-Run2	7235	5.28E+06	9.30E+02	9.90E+03	9.92E+04

Experimental setting C

Table B.7 Detected peak area counts for bezafibrate and its ozonation by-products in duplicate samples of run1 of experimental setting C.

Sample	Concentration (ng/L)	Peak area			
	Bezafibrate	Bezafibrate	MW367	MW393	MW227
BZF T1	6040	4.58E+06	No Peak	No Peak	2.24E+03
BZF T1	4800	3.64E+06	No Peak	No Peak	2.30E+03
BZF T2	24700	1.88E+07	No Peak	No Peak	9.46E+03
BZF T2	14100	1.07E+07	No Peak	No Peak	1.08E+04
BZF T3	8410	6.38E+06	No Peak	No Peak	8.50E+03
BZF T3	6560	4.98E+06	No Peak	No Peak	9.82E+03

Table B.8 Detected peak area counts for bezafibrate and its ozonation by-products in duplicate samples of run2 of experimental setting C.

Sample	Concentration (ng/L)	Peak area			
	Bezafibrate	Bezafibrate	MW367	MW393	MW227
BZF T1	10800	6.21E+06	No Peak	No Peak	4.08E+03
BZF T1	10200	5.87E+06	No Peak	No Peak	3.82E+03
BZF T2	5870	3.38E+06	No Peak	No Peak	2.04E+03
BZF T2	7380	4.25E+06	No Peak	No Peak	2.73E+03
BZF T3	5900	3.40E+06	No Peak	No Peak	4.02E+03
BZF T3	5700	3.29E+06	No Peak	No Peak	4.77E+03

Summary Table for Experimental Setting C

Table B.9 Average of determined peak area counts for bezafibrate and its ozonation products in duplicate samples of each run in experimental setting C.

Average of duplicates	Average concentration (ng/L)	Average peak area			
	Bezafibrate	Bezafibrate	MW367	MW393	MW227
BZF T1-Run1	5420	4.11E+06	no peak	no peak	2.27E+03
BZF T1-Run2	10500	6.04E+06	no peak	no peak	3.95E+03
BZF T2-Run1	19400	1.48E+07	no peak	no peak	1.01E+04
BZF T2-Run2	6625	3.82E+06	no peak	no peak	2.39E+03
BZF T3-Run1	7485	5.68E+06	no peak	no peak	9.16E+03
BZF T3-Run2	5800	3.35E+06	no peak	no peak	4.04E+03

Experimental setting D

Table B.10 Detected peak area counts for bezafibrate and its ozonation by-products in duplicate samples of run1 of experimental setting D.

Sample	Concentration (ng/L)	Peak area			
	Bezafibrate	Bezafibrate	MW367	MW393	MW227
BZF T1	13600	1.03E+07	No Peak	No Peak	1.00E+03
BZF T1	21400	1.62E+07	No Peak	No Peak	1.34E+03
BZF T2	16900	1.29E+07	No Peak	No Peak	4.13E+03
BZF T2	16500	1.25E+07	No Peak	No Peak	3.20E+03
BZF T3	19800	1.50E+07	No Peak	No Peak	7.37E+03
BZF T3	19300	1.46E+07	No Peak	No Peak	8.92E+03

Table B.11 Detected peak area counts for bezafibrate and its ozonation by-products in duplicate samples of run2 of experimental setting D.

Sample	Concentration (ng/L)	Peak area			
	Bezafibrate	Bezafibrate	MW367	MW393	MW227
BZF T1	20700	1.19E+07	No Peak	No Peak	2.16E+03
BZF T1	18300	1.06E+07	No Peak	No Peak	2.51E+03
BZF T2	8140	4.69E+06	No Peak	No Peak	1.71E+03
BZF T2	9440	5.44E+06	No Peak	No Peak	2.05E+03
BZF T3	7040	4.06E+06	No Peak	No Peak	4.02E+03
BZF T3	8230	4.74E+06	No Peak	No Peak	3.91E+03

Summary Table for Experimental Setting D

Table B.12 Average of determined peak area counts for bezafibrate and its ozonation products in duplicate samples of each run in experimental setting D.

Average of duplicates	**Average concentration (ng/L)**	**Average peak area**			
	Bezafibrate	Bezafibrate	MW367	MW393	MW227
BZF T1-Run1	17500	1.33E+07	no peak	no peak	1.17E+03
BZF T1-Run2	19500	1.13E+07	no peak	no peak	2.34E+03
BZF T2-Run1	16700	1.27E+07	no peak	no peak	3.67E+03
BZF T2-Run2	8790	5.07E+06	no peak	no peak	1.88E+03
BZF T3-Run1	19550	1.48E+07	no peak	no peak	8.15E+03
BZF T3-Run2	7635	4.40E+06	no peak	no peak	3.97E+03

Appendix C

Analytical Data for Carbamazepine Experiments

The magnitudes of detected peak area counts for carbamazepine and its ozonation products BQM, BQD, CBZ-EP, CBZ-DiOH and CBZ-OH as well as determined concentration of carbamazepine in each of reactor samples under four experimental settings are presented in the following tables.

Experimental setting A

Table C.1 Detected peak area counts for carbamazepine and its ozonation by-products in duplicate samples of run1 of experimental setting A.

Sample	**Conc. (ng/L)**	**Peak area**					
	CBZ	CBZ	BQM	BQD	CBZ-EP	CBZ-DiOH	CBZ-OH
CBZ T1	< MDL	9.22E+03	1.84E+08	1.65E+07	5.90E+05	8.21E+04	2.82E+05
CBZ T1	< MDL	1.82E+04	1.87E+08	1.93E+07	6.24E+05	8.82E+04	2.96E+05
CBZ T2	< MDL	2.79E+04	1.80E+08	2.66E+07	5.80E+05	8.26E+04	2.74E+05
CBZ T2	< MDL	3.41E+04	1.87E+08	2.84E+07	6.11E+05	9.40E+04	2.79E+05
CBZ T3	< MDL	4.77E+04	1.76E+08	4.79E+07	6.57E+05	8.58E+04	2.68E+05
CBZ T3	< MDL	4.91E+04	1.76E+08	4.57E+07	6.48E+05	8.61E+04	2.63E+05

Table C.2 Detected peak area counts for carbamazepine and its ozonation by-products in duplicate samples of run2 of experimental setting A.

Sample	**Conc. (ng/L)**	**Peak area**					
	CBZ	CBZ	BQM	BQD	CBZ-EP	CBZ-DiOH	CBZ-OH
CBZ T1	< MDL	1.22E+04	1.49E+08	9.40E+06	3.70E+05	7.73E+04	3.14E+05
CBZ T1	< MDL	9.35E+03	1.38E+08	8.31E+06	3.39E+05	7.09E+04	2.87E+05
CBZ T2	< MDL	9.96E+03	1.51E+08	1.66E+07	3.73E+05	7.71E+04	3.11E+05
CBZ T2	< MDL	2.15E+04	1.42E+08	1.47E+07	3.56E+05	7.31E+04	3.07E+05
CBZ T3	< MDL	6.97E+03	1.42E+08	2.61E+07	3.52E+05	7.15E+04	3.23E+05
CBZ T3	< MDL	7.31E+03	1.48E+08	2.63E+07	4.07E+05	7.55E+04	3.20E+05

Summary Table for Experimental Setting A

Table C.3 Averages of determined peak area counts for CBZ and its ozonation products in duplicate samples of each run in experimental setting A.

Average of duplicates	**Avg. conc. (ng/L)**	**Average peak area**					
	CBZ	CBZ	BQM	BQD	CBZ-EP	CBZ-DiOH	CBZ-OH
CBZ T1-Run1	<MDL	1.40E+04	1.86E+08	1.79E+07	6.07E+05	8.52E+04	2.89E+05
CBZ T1-Run2	<MDL	1.08E+04	1.44E+08	8.86E+06	3.55E+05	7.41E+04	3.01E+05
CBZ T2-Run1	<MDL	3.10E+04	1.84E+08	2.75E+07	5.96E+05	8.83E+04	2.77E+05
CBZ T2-Run2	<MDL	1.57E+04	1.47E+08	1.57E+07	3.65E+05	7.51E+04	3.09E+05
CBZ T3-Run1	<MDL	4.80E+04	1.76E+08	4.68E+07	6.53E+05	8.60E+04	2.66E+05
CBZ T3-Run2	<MDL	7.14E+03	1.45E+08	2.62E+07	3.80E+05	7.35E+04	3.22E+05

Experimental setting B

Table C.4 Detected peak area counts for carbamazepine and its ozonation by-products in duplicate samples of run1 of experimental setting B.

Sample	**Conc. (ng/L)**	**Peak area**					
	CBZ	CBZ	BQM	BQD	CBZ-EP	CBZ-DiOH	CBZ-OH
CBZ T1	< MDL	3.90E+04	1.78E+05	3.84E+04	No Peak	No Peak	No Peak
CBZ T1	< MDL	3.28E+04	1.28E+05	4.03E+04	No Peak	No Peak	No Peak
CBZ T2	< MDL	3.32E+04	1.57E+04	2.06E+03	No Peak	No Peak	No Peak
CBZ T2	< MDL	1.46E+04	1.48E+04	3.06E+03	No Peak	No Peak	No Peak
CBZ T3	< MDL	5.25E+04	No Peak	No Peak	No Peak	No Peak	No Peak
CBZ T3	< MDL	7.12E+04	No Peak	No Peak	No Peak	No Peak	No Peak

Table C.5 Detected peak area counts for carbamazepine and its ozonation by-products in duplicate samples of run2 of experimental setting B.

Sample	**Conc. (ng/L)**	**Peak area**					
	CBZ	CBZ	BQM	BQD	CBZ-EP	CBZ-DiOH	CBZ-OH
CBZ T1	< MDL	2.95E+05	4.82E+05	1.06E+05	No Peak	No Peak	No Peak
CBZ T1	< MDL	1.40E+05	4.87E+05	1.13E+05	No Peak	No Peak	No Peak
CBZ T2	< MDL	2.08E+05	5.53E+04	1.75E+04	No Peak	No Peak	No Peak
CBZ T2	< MDL	1.84E+05	4.32E+04	9.87E+03	No Peak	No Peak	No Peak
CBZ T3	< MDL	1.18E+05	5.10E+03	1.62E+03	No Peak	No Peak	No Peak
CBZ T3	< MDL	1.52E+05	7.79E+03	1.53E+03	No Peak	No Peak	No Peak

Summary Table for Experimental setting B

Table C.6 Average of determined peak area counts for CBZ and its ozonation products in duplicate samples of each run in experimental setting B.

Average of duplicates	Avg. conc. (ng/L)	Average peak area					
	CBZ	CBZ	BQM	BQD	CBZ-EP	CBZ-DiOH	CBZ-OH
CBZ T1-Run1	<MDL	3.60E+04	1.50E+05	3.90E+04	No Peak	No Peak	No Peak
CBZ T1-Run2	<MDL	2.18E+05	4.85E+05	1.10E+05	No Peak	No Peak	No Peak
CBZ T2-Run1	<MDL	2.40E+04	1.50E+04	2.60E+03	No Peak	No Peak	No Peak
CBZ T2-Run2	<MDL	1.96E+05	4.93E+04	1.37E+04	No Peak	No Peak	No Peak
CBZ T3-Run1	<MDL	6.20E+04	No peak	No peak	No Peak	No Peak	No Peak
CBZ T3-Run2	<MDL	1.35E+05	6.45E+03	1.58E+03	No Peak	No Peak	No Peak

Experimental setting C

Table C.7 Detected peak area counts for carbamazepine and its ozonation by-products in duplicate samples of run1 of experimental setting C.

Sample	Conc. (ng/L)	Peak area					
	CBZ	CBZ	BQM	BQD	CBZ-EP	CBZ-DiOH	CBZ-OH
CBZ T1	< MDL	1.05E+04	4.81E+03	No Peak	No Peak	No Peak	No Peak
CBZ T1	< MDL	9.06E+03	7.30E+03	No Peak	No Peak	No Peak	No Peak
CBZ T2	< MDL	7.79E+03	2.18E+03	No Peak	No Peak	No Peak	No Peak
CBZ T2	< MDL	5.14E+03	4.15E+03	No Peak	No Peak	No Peak	No Peak
CBZ T3	196	6.88E+05	2.30E+03	No Peak	No Peak	No Peak	No Peak
CBZ T3	71	4.25E+05	1.89E+03	No Peak	No Peak	No Peak	No Peak

Table C.8 Detected peak area counts for carbamazepine and its ozonation by-products in duplicate samples of run2 of experimental setting C.

Sample	Conc. (ng/L)	Peak area					
	CBZ	CBZ	BQM	BQD	CBZ-EP	CBZ-DiOH	CBZ-OH
CBZ T1	< MDL	9.02E+04	3.39E+03	No Peak	No Peak	No Peak	No Peak
CBZ T1	< MDL	6.82E+04	2.40E+03	No Peak	No Peak	No Peak	No Peak
CBZ T2	< MDL	5.66E+04	3.73E+03	No Peak	No Peak	No Peak	No Peak
CBZ T2	< MDL	5.27E+04	3.15E+03	No Peak	No Peak	No Peak	No Peak
CBZ T3	< MDL	4.36E+04	5.46E+03	No Peak	No Peak	No Peak	No Peak
CBZ T3	< MDL	3.55E+04	2.46E+03	No Peak	No Peak	No Peak	No Peak

Summary Table for Experimental setting C

Table C.9 Average of determined peak area counts for carbamazepine and its ozonation products in duplicate samples of each run in experimental setting C.

Average of duplicates	**Average conc. (ng/L)**	**Average peak area**					
	CBZ	CBZ	BQM	BQD	CBZ-EP	CBZ-DiOH	CBZ-OH
CBZ T1-Run1	<MDL	9.80E+03	6.10E+03	No Peak	No Peak	No Peak	No Peak
CBZ T1-Run2	<MDL	7.92E+04	2.90E+03	No Peak	No Peak	No Peak	No Peak
CBZ T2-Run1	<MDL	6.50E+03	3.20E+03	No Peak	No Peak	No Peak	No Peak
CBZ T2-Run2	<MDL	5.47E+04	3.44E+03	No Peak	No Peak	No Peak	No Peak
CBZ T3-Run1	133	5.60E+05	2.10E+03	No Peak	No Peak	No Peak	No Peak
CBZ T3-Run2	<MDL	3.96E+04	3.96E+03	No Peak	No Peak	No Peak	No Peak

Experimental setting D

Table C.10 Detected peak area counts for carbamazepine and its ozonation by-products in duplicate samples of run1 of experimental setting D.

Sample	**Conc. (ng/L)**	**Peak area**					
	CBZ	CBZ	BQM	BQD	CBZ-EP	CBZ-DiOH	CBZ-OH
CBZ T1	< MDL	6.48E+04	4.82E+05	9.89E+02	No Peak	No Peak	No Peak
CBZ T1	< MDL	5.02E+04	4.87E+05	9.88E+02	No Peak	No Peak	No Peak
CBZ T2	< MDL	4.55E+04	5.53E+04	5.21E+02	No Peak	No Peak	No Peak
CBZ T2	< MDL	1.45E+05	4.32E+04	2.06E+03	No Peak	No Peak	No Peak
CBZ T3	< MDL	1.38E+05	5.10E+03	1.19E+03	No Peak	No Peak	No Peak
CBZ T3	< MDL	9.43E+04	7.79E+03	1.27E+03	No Peak	No Peak	No Peak

Table C.11 Detected peak area counts for carbamazepine and its ozonation by-products in duplicate samples of run2 of experimental setting D.

Sample	**Conc. (ng/L)**	**Peak area**					
	CBZ	CBZ	BQM	BQD	CBZ-EP	CBZ-DiOH	CBZ-OH
CBZ T1	< MDL	1.28E+04	4.35E+03	2.3E+02	No Peak	No Peak	No Peak
CBZ T1	< MDL	1.13E+04	4.08E+03	3.9E+02	No Peak	No Peak	No Peak
CBZ T2	< MDL	8.35E+03	2.29E+03	3.8E+02	No Peak	No Peak	No Peak
CBZ T2	< MDL	1.41E+04	4.40E+03	3.7E+02	No Peak	No Peak	No Peak
CBZ T3	< MDL	2.17E+04	1.35E+03	2.2E+02	No Peak	No Peak	No Peak
CBZ T3	< MDL	2.05E+04	1.88E+03	1.5E+02	No Peak	No Peak	No Peak

Summary Table for Experimental setting D

Table C.12 Average of determined peak area counts for carbamazepine and its ozonation products in duplicate samples of each run in experimental setting D.

Avg. of duplicates	**Avg. conc. (ng/L)**	**Avg. peak area**					
	CBZ	CBZ	BQM	BQD	CBZ-EP	CBZ-DiOH	CBZ-OH
CBZ T1-Run1	<MDL	5.75E+04	4.85E+05	9.89E+02	No Peak	No Peak	No Peak
CBZ T1-Run2	<MDL	1.21E+04	4.22E+03	3.10E+02	No Peak	No Peak	No Peak
CBZ T2-Run1	<MDL	9.53E+04	4.93E+04	1.29E+03	No Peak	No Peak	No Peak
CBZ T2-Run2	<MDL	1.12E+04	3.35E+03	3.80E+02	No Peak	No Peak	No Peak
CBZ T3-Run1	<MDL	1.16E+05	6.45E+03	1.23E+03	No Peak	No Peak	No Peak
CBZ T3-Run2	<MDL	2.11E+04	1.61E+03	1.90E+02	No Peak	No Peak	No Peak

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