Ozonation of organic compounds in water and wastewater: A critical review

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1. Introduction

Ozonation has been applied in drinking water treatment for disinfection and oxidation for decades. Currently, as its application area is being further extended to wastewater treatment and potable water reuse, ozonation is facing new challenges associated with higher concentrations and new types of dissolved organic matter as well as a larger range of organic contaminants. This critical review provides the background of the current developments in ozonation applications (Section 2), addresses new challenges associated with the impact of dissolved organic matter on ozonation performance (Section 3), and provides a critical compilation of kinetic and mechanistic information necessary to evaluate abatement of organic compounds during ozonation (Section 4). Furthermore, the abatement of antibiotic resistance is discussed (Section 5) and compared to the abatement of micropollutants and bromate formation.

2. Ozonation in drinking water and wastewater treatments

2.1. Drinking water

2.1.1. From disinfection to oxidation

Ozonation has been applied in drinking water for more than a century, mainly for disinfection purposes (Fig. 1) (von Sonntag and von Gunten, 2012). It was considered as an equivalent to chlorine and in some cases an ozone residual was maintained in the distribution system for groundwaters with low DOC concentrations and with high carbonate alkalinity, conditions which warrant a high ozone stability (Courbat et al., 1999; Hoigné, 1998). In addition to its outstanding capacity for the inactivation of microorganisms, such as viruses, bacteria, and protozoa (von Sonntag and von Gunten, 2012; Wolf et al., 2018), ozone was also applied at an early stage to oxidatively abate inorganic compounds (e.g., Fe(II), Mn(II), NO$_2^-$, HS$^-$) and to control taste and odor issues (Fig. 1) (von Gunten, 2003b). Only in the 1970s and 1980s, the mechanism of ozone decomposition in water was elucidated and it was demonstrated that hydroxyl radicals (•OH) are formed during this process (Bühler et al., 1984; Hoigné and Bader, 1975; Staehelin et al., 1984;
2.1.2. Disinfection byproducts formation and mitigation

In applications of chlorine, trihalomethanes (THMs) were reported as disinfection byproducts (DBPs) in the mid 1970s (Booij, 1974). However, it took much longer until DBPs were considered during ozonation processes (Hoigné, 1998; von Gunten, 2018). First the formation and removal (by biological post-filtration) of aldehydes, ketones and carboxylic acids was studied (Glaze et al., 1989; Hammes et al., 2006; Hammes et al., 2007; Nawrocki et al., 2003; Ramseier et al., 2011a; Swietlik et al., 2004; Swietlik et al., 2009; van der Kooij et al., 1989; Zürcher et al., 1978) and then the formation of trichloronitromethane (chloropicrin) during post-chlorination of ozonated waters was investigated (Fig. 1) (Hoigné and Bader, 1988; Thibaud et al., 1987). Even though bromate formation during ozonation of bromide-containing waters was already investigated in the early 1980s (Haag and Hoigné, 1983b), it only became an issue when it was found to be a possible human carcinogen in the early 1990s (Fig. 1) (WHO, 2017). The relatively stringent and quite universal drinking water standard for bromate (10 μg/L) often limits ozone applications for disinfection purposes. Therefore, several mitigation options were developed such as pH depression, ammonium addition, chlorine/ammonium or ammonium/-chlorine addition when disinfection is the main purpose and hydrogen peroxide addition when disinfection is not the primary target for ozonation (Buffe et al., 2004; Haag and Hoigné, 1983b; Krasner et al., 1993; Pinkernell and von Gunten, 2001; Song et al., 1996; Song et al., 1997; von Gunten and Hoigné, 1994; von Gunten and Oliveras, 1998; von Gunten, 2003a). An updated mechanism for bromate formation during ozonation is presented elsewhere (von Sonntag and von Gunten, 2012). With the discovery of the formation of N-nitrosamines during chlorination/-chlororamination in the early 2000s (Mitch and Sedlak, 2002; Mitch et al., 2003b; Najm and Trussell, 2001), pre-ozonation by ozone was found to be an interesting option for the mitigation of these undesired compounds (Chen and Valentine, 2008; Krasner et al., 2018; Lee et al., 2007a; McCurry et al., 2015; Shah et al., 2012). However, numerous studies also reported N-nitrosamine formation during ozonation of individual target compounds (Andrzejewski et al., 2008; Lim et al., 2016; Marti et al., 2015; Oya et al., 2008; Schmidt and Brauch, 2008), including a case of bromide-catalysis for dimethylsulfoxamide (Trogolo et al., 2015; von Gunten et al., 2010) and real water matrices (Fig. 1) (Gerrity et al., 2015; Krasner et al., 2013; Pisarenko et al., 2015). Finally, with the discovery of toxic iodo-DBPs, which can be formed during oxidative processes in presence of iodide (Dong et al., 2019; Li et al., 2020), pre-ozonation was recognized as an efficient mitigation strategy to minimize iodo-DBPs by oxidizing iodide quickly to iodate (Fig. 1) (Onias and von Gunten, 2019).

2.2. Wastewater

2.2.1. Disinfection

Similar to drinking water, ozonation has been implemented in wastewater as a disinfectant before its discharge to the receiving water bodies (Fig. 1) (Gerrity et al., 2012; Janex et al., 2000; Paraskeva and
Graham, 2002; Xu et al., 2002). However, wastewater disinfection requires quite high doses and it is expected that disinfection, including inactivation of ozone-resistant microorganisms such as protozoa, will lead to the formation of bromate above the drinking water standard of 10 µg/L (Lee et al., 2016b; Schindler Wildhaber et al., 2015; Zimmernann et al., 2011).

2.2.2. Oxidation of micropolutants for potable water reuse purposes (human health)

Due to issues related to water scarcity and water security, potable water reuse projects are increasing with > 25 full-scale systems in operation worldwide (Marron et al., 2019). Potable water reuse requires a multibarrier approach to guarantee microbial and chemical drinking water safety (Gerrity et al., 2013; Marron et al., 2019). Currently, most of the water reuse facilities rely on systems which treat municipal wastewater effluent by a sequence of membranes (micro- or ultrafiltration and reverse osmosis (RO)) followed by an advanced oxidation process (most commonly UV/H₂O₂) and a disinfection step for the distribution system (Marron et al., 2019). This scheme can also include a pre-ozonation for disinfection as the primary treatment step (Gerrity et al., 2013). Often, an additional cycle through a natural or managed system (reservoir, groundwater) is used to condition the water in terms of temperature, mineral content and to remove biodegradable organic compounds. This approach, which is known as indirect potable reuse, also has reasonable consumer acceptance. Recently, more direct potable reuse projects have been implemented due to the lack of suitable systems or regulations for the storage/recharge of the treated water (Steinle-Darling, 2015). Even though these potable reuse systems provide high-quality drinking water, the main drawback is the high waste stream from the concentrate of the RO (also containing antiscalants), which consists of about 20% of the treated water (Pérez-González et al., 2012). In coastal areas, this reject can be discharged to the ocean. However, for inland water reuse applications far from the ocean, such discharges are not possible and alternative treatment strategies have to be developed. Less tight membranes such as nanofiltration can ease this situation to a certain extent, but will still produce a significant waste stream and reject micropolutants to a smaller extent (Marron et al., 2019). Alternatively, treatment schemes involving activated carbon or ozonation combined with biological activated carbon (BAC) filtration were suggested, which reduce the problem of waste streams inherent to tight membrane-based systems significantly and can still provide a good drinking water quality, which was demonstrated in laboratory and pilot studies (Gerrity et al., 2011; Gerrity and Snyder, 2011; Gerrity et al., 2014; Hooper et al., 2020; Reungoat et al., 2012; Sundaram et al., 2020; Vaidya et al., 2019; Zhang et al., 2020). The first potable reuse system worldwide in Windhoek, Namibia comprised several treatment steps including the combination of ozonation with biological activated carbon filtration (Gerrity et al., 2013).

The assessment of ozone-based reuse systems was mainly based on the microbiological and the chemical quality related to micropolutants. Even though this approach seems quite promising, there are several critical points which need to be addressed more carefully for potable reuse systems: (i) Presence of ozone- and *OH-resistant compounds that are also not well removed during BAC treatment, (ii) formation of transformation products during ozonation of micropolutants, which are not abated during BAC treatment, (iii) formation of oxidation byproducts from dissolved organic matter (DOM) ozonation, which are not removed in BAC treatment, (iv) bromate formation during ozonation of bromide, which is often elevated in municipal wastewater effluent (see below), and (v) formation of precursors for disinfection byproduct during post-chlorination. Fig. 2 shows a summary of these different aspects.

Some of these issues are the same as for enhanced wastewater treatment for ecological reasons, however, in the context of potable water reuse, they become more critical:

(i) The occurrence of oxidant-resistant compounds such as per- and polyfluoroalkyl substances (PFAS) makes this class of toxic compounds a problem not only in drinking water resources but also in wastewater effluents (Crone et al., 2019; Gallen et al., 2018). Due to their resistance against oxidation, they are difficult to be abated by ozone and *OH (Lutze et al., 2018) and also limited removal efficiencies for activated carbon have been
(iii) Many of the oxygen-rich compounds formed during ozonation of DOM (e.g., aldehydes, ketones, carboxylic acids) are quite well biodegradable (Hammes et al., 2006; van der Kooij et al., 1989) (Fig. 2). In a recent study, α,β-unsaturated carbonyl compounds were detected after wastewater ozonation (Marron et al., 2020). Such electrophilic compounds are problematic because they can bind to DNA and proteins. Even though they are removed by about 90% in biological post-treatment, this class of compounds should be carefully monitored in potable water reuse systems.

(iv) So far in potable water reuse systems applying ozone, there is only limited information on bromate formation (Hooper et al., 2020). Based on the experiences from enhanced wastewater treatment, it can be expected that bromate will become a critical factor, especially if ozonation is applied to inactivate protozoa or bacterial spores (Morrison et al., submitted; Soltermann et al., 2016; Soltermann et al., 2017) (Fig. 2). However, if ozonation is applied for the inactivation of viruses or vegetative bacteria, significant disinfection credit can be achieved with limited bromate formation, because of the high second-order inactivation rate constants of such microorganisms (Hunt and Mariñas, 1999; Morrison et al., submitted; Voumard et al., submitted; Wolf et al., 2018).

(v) Whereas ozonation alone may lead to an increase of the DBP precursors, a combination of ozonation with biological treatment is effective in DBP precursor control (Chuang et al., 2019; Krasner, 2009; Sun et al., 2018) (Fig. 2). One case in point is nitromethane formation during ozonation of municipal wastewater effluent, which then leads to the formation of trichlororinitromethane during post-chlorination (Shi and McCurry, 2020). Nitromethane formed during wastewater ozonation was subsequently found to persist through membrane and advanced oxidation processes, but to be effectively removed by BAC (Shi et al., 2021). In addition to DBP concentrations, a toxicity-based approach has to be considered. It has been shown, that the combined effect of ozonation/BAC may lead to similar overall toxicity, because of a shift to more toxic DBPs (Chuang et al., 2019).

2.2.3. Oxidation of micropollutants to reduce their discharge to water bodies (ecosystem health)

With the development of the coupling of liquid chromatography with mass spectrometry, a new analytical window was established, which allowed the measurement of more polar compounds and led to the discovery of a wide range of (biologically active) organic micropollutants in municipal wastewater (Snyder, 2008; Ternes, 1998). These observations triggered environmental concerns and the ensuing ecological consequences were broadly investigated. Furthermore, the potential for abatement of micropollutants from municipal wastewaters by various strategies was explored, including membranes, activated carbon and chemical oxidation, which is the focus of this review (Fig. 1) (Joss et al., 2008; Ternes and Joss, 2006; Ternes et al., 2004). In these studies, several oxidation processes were investigated for enhanced wastewater treatment, including ferrate (VI) (Lee et al., 2009), chlorine dioxide (Huber et al., 2005b), UV/H2O2 and ozone (Huber et al., 2003; Huber et al., 2005a; Lee et al., 2013; Lee et al., 2016a; Ternes et al., 2003; Wert et al., 2009) and Fe-tetramido-macroyclic ligands (TAML)/H2O2 (Jans et al., 2021). Based on these studies and other information from literature, ozonation was selected as the oxidation process of choice for enhanced wastewater treatment in Switzerland and in other countries, because of the broad spectrum of micropollutants which can be abated during ozonation (Domenjoud et al., 2017; Eggen et al., 2014; Gerrity et al., 2012; Hölzler et al., 2009; Lee and von Gunten, 2010; Lee et al., 2013; Lee et al., 2014; Lee and von Gunten, 2016; Miklos et al., 2018; Ternes et al., 2003; Ternes et al., 2017; Zimmermann et al., 2011). This initiative triggered significant interest on the influence of oxidative treatment on biological effects of micropollutants and their transformation products (Prasse et al., 2015). It was shown that biologically active compounds such as estrogens, antibiotics, antivirals and pesticides lost their primary target effects during ozonation and other AOPs in laboratory studies (Dodd et al., 2009; Huber et al., 2004; Lange et al., 2006; Lee et al., 2008; Mestanka et al., 2011; Mestanka et al., 2012). During ozonation of real wastewaters in laboratory-, pilot- and full-scale studies, mixed results were obtained with various toxicological endpoints such as the fish early life stage toxicity test, the chironomid toxicity test, the Ceriodaphnia dubia toxicity test with mostly a reduction in toxicity but in some cases also an increase (Angelas et al., 2020; Escher et al., 2009; Magdeburg et al., 2014; Prasse et al., 2015; Stalter et al., 2010; Stalter et al., 2010b; Völker et al., 2019; von Gunten, 2018). However, biological post-treatment by sand or activated carbon filtration led mostly to a significant reduction of the toxicity, wherefore, typically, a biological post-treatment step is implemented after ozonation of wastewater (Bourgin et al., 2018; Chys et al., 2017b; Itzel et al., 2020; Knopp et al., 2016; Reungoat et al., 2012). This observation can most likely be explained by potentially (eco)toxic DBPs such as aldehydes and ketones formed during ozonation of wastewater DOM, which are often easily biodegradable (see above). However, potential for the formation of (eco) toxic transformation products from micropollutants exists as mentioned in Section 2.2.2 (Scholè, 2018). From a theoretical study and experimental results, it is expected that mainly transformation products from olefins and aromatic compounds are biodegradable, whereas products from nitrogen-containing compounds are expected to be mostly persistent (Bourgin et al., 2018; Hübner et al., 2015; Zucker et al., 2018), however, often such a simplified approach is not justified (Gulde et al., 2021b). An additional benefit of biological post-treatment is a significant abatement of N-nitrosodimethylamine (NDMA), which is already present in the raw wastewater and/or formed during ozonation (Bourgin et al., 2018; Krauss et al., 2009). To assess the feasibility of Ozonation of municipal wastewater effluents considering also toxicity, test procedures including chemical and toxicological methods have been developed and applied (Itzel et al., 2017; Schindler Wildhaber et al., 2015). Bromide levels in municipal wastewaters can be significantly higher than in drinking waters, occasionally reaching levels of tens of mg/L (Lee et al., 2013; Schindler Wildhaber et al., 2015; Soltermann et al., 2016). This may lead to significant bromate formation during ozonation of municipal wastewater, especially for specific oxygen doses > 0.5 gO2/gDOC, which are typical for enhanced wastewater treatment for micropollutant abatement (Soltermann et al., 2016; Soltermann et al., 2017). Under oxic conditions, which are prevalent after ozonation, bromate persists in biological post-treatment (see Fig. 2 and below).
3. Challenges in wastewater matrices and characterization of DOM by surrogate parameters

3.1. Water matrix: differences between drinking water and wastewater

Ozonation of secondary municipal wastewater effluents entails some challenges, which are not serious issues during drinking water treatment: (i) higher concentrations and different types of DOM, (ii) potential presence of nitrite, and (iii) higher and variable concentrations of bromide. (i) As a consequence of the higher DOM concentrations, higher concentrations of ozone and OH are consumed by reactive DOM sites and lead to higher concentrations of AOC/BDOC and low molecular weight oxygen-containing compounds (aldehydes, carboxylic acids) (Marron et al., 2020; Wert et al., 2007; Zimmermann et al., 2011). Because these compounds may lead to enhanced toxicity, a biological post-treatment is necessary for their removal (Prasse et al., 2015; Stalter et al., 2016b). Furthermore, the concentration of dissolved organic nitrogen is typically much higher in secondary municipal wastewater effluent (Chon et al., 2013; Parkin and McCarty, 1981; Pehlivanoglu-Mantas and Sedlak, 2006; Shon et al., 2006) and may lead to nitro and nitroso moieties (Pehlivanoglu-Mantas and Sedlak, 2008; Shi and McCurry, 2020) (see below).

(ii) In secondary wastewater effluents which are not or incompletely nitrified, elevated nitrite concentrations can be found. Since ozone reacts readily with nitrite \( k_{3} = 3.7 \times 10^{9} \text{ M}^{-1} \text{s}^{-1} \) (Hoigne et al., 1985), its presence leads to a stoichiometric consumption of ozone. Furthermore, it has been shown that nitro compounds can be formed as side reactions during ozonation of nitrite-containing waters (Hu et al., 2016; Manasfi et al., In prep.; Thibaud et al., 1987).

(iii) Similar to drinking waters, ozonation of bromide-containing wastewaters leads to bromate formation (Bourgin et al., 2018; Schindler Wildhaber et al., 2015; Wu et al., 2019). Compared to typical drinking waters, the bromide concentrations in secondary wastewater effluents are more variable and can also be significantly higher. This may be caused by discharge from industry, municipal incineration plants, fracking wastewaters, seawater intrusion, etc. (Dong et al., 2017; Harkness et al., 2015; Soltermann et al., 2016). Bromate is only removable under anaerobic conditions (van Ginkel et al., 2005), which are unlikely during biological post-treatment after ozonation. Nevertheless, there are several options to minimize bromate formation during ozonation of secondary municipal wastewater effluent, such as addition of hydrogen peroxide or a distributed dosing of ozone, each of which entails certain limitations and needs to be tested on a case-by-case basis (Merle et al., 2017; Soltermann et al., 2017).

3.2. Control of ozonation systems for enhanced municipal wastewater treatment

Compared to typical drinking water ozonation with ozone lifetimes in the range of tens of minutes, the lifetime of ozone in municipal wastewater effluents is much smaller (Acero and von Gunten, 2001; Buffle et al., 2006b). Therefore, ozone monitoring and control in drinking waters, which is based on direct ozone concentration and decay measurements (Kaiser et al., 2013), is not applicable to municipal wastewater, for which specialized equipment such as stopped-flow or quench-flow is needed to monitor kinetics (Buffle et al., 2006b).

Due to this shortcoming and the lack of fast monitoring systems for micropollutants and microorganisms, empirical methods have been developed, which correlate the abatement of microorganisms or micropollutants to surrogate parameters. It has been shown that the natural logarithm of the relative abatement of the UV absorption at 285 nm is correlated to the ozone exposure (Buffle et al., 2006a). Since the ozone exposure is the relevant parameter for disinfection and oxidation, this opens up the possibility to use UV absorption to assess the efficiency of wastewater ozonation. In addition to UV, changes in fluorescence have been tested as descriptors for micropollutant abatement during ozonation (Chys et al., 2017a; Park et al., 2017). A recently developed method to characterize the electron donor properties of the DOM is the electron donating capacity (EDC) (see below). Table 1 summarizes selected studies in which surrogate parameters have been applied to predict micropollutant abatement and inactivation of microorganisms. A detailed discussion of these proxies is provided in the sections below.

Table 1

| Parameter | Scale | Targets | Correlation coefficient $ R^2 $ | Reference |
|-----------|-------|---------|-------------------------------|-----------|
| UV (254 nm) | Pilot | 16 micropollutants, 2ecal indicator, bacteria, bromate | 0.56 – 0.95, 0.57 – 0.79 | (Bahr et al., 2007) |
| UV (254 nm) | Laboratory | 12 micropollutants | 0.91 | (Nanaboina and Korshin, 2010) |
| UV (254 and 366 nm) | Laboratory (3) | 24 micropollutants | Sigmoidal approach | (Wittmer et al., 2015) |
| UV (254 nm) | Pilot | 16 micropollutants | 0.28 – 0.92 | (Stap et al., 2016) |
| UV (254 nm) | Laboratory (3) | 2 viruses (MS2, coxsakievirus B5) | Logarithmic correlation | (Wolf et al., 2019) |
| UV (254 nm) | Laboratory (9) | 18 micropollutants, E. coli, MS2, R. subsilis spores | 0.50 – 0.83, 0.46 – 0.78 | (Gerrity et al., 2012) |
| UV (254 nm) | Laboratory (5) | 13 micropollutants | > 0.8 | (Park et al., 2017) |
| UV (254 nm) | Laboratory (1) | 9 micropollutants | No correlation, $ k_{32} $-based approach | (Chys et al., 2017a) |
| EDC or UV (254 nm) | Laboratory (2) | 6 micropollutants, bromate | 0.78 – 1.00, 0.82 – 0.83 | (Chon et al., 2015) |
| EDC and UV (255 nm) | Full-scale (1) | 22 micropollutants, bromate | No linear correlations | (Walpen et al., 2022) |

1 Number of wastewaters tested at this scale;
2 two surface waters and one wastewater;
3 lake water;
4 also predicted hydroxyl radical exposure based on $ \Delta $UV or $ \Delta $fluorescence;
5 One wastewater was collected 3 times;
6 Linear regression correlation coefficient for the $ \Delta $parameter versus the relative abatement of micropollutants or microorganisms.
formation of oxygenated disinfection byproducts (Hammes et al., 2007; Nawrocki et al., 2003; van der Kooij et al., 1989; Wert et al., 2007), (iv) abatement or formation of precursors for the formation of disinfection byproducts during post-disinfection (Fig. 2) (Hoigné and Bader, 1988; Hua and Reckhow, 2013; McCurry et al., 2016; Reckhow and Singer, 1984; Reckhow et al., 1986; Wert and Rosario-Ortiz, 2011), and (v) changes in photochemical and photophysical DOM properties (Leresche et al., 2019; Leresche et al., 2021).

To better understand the reactions of ozone with DOM, various DOM characterization methods have been developed with surrogate parameters (e.g., UV absorbance, fluorescence excitation-emission spectra, molecular weight distributions, relative intensities of different chemical group abundances and degrees of oxidation and to calculate the number of double bond equivalents (DBE) in the molecules.

HRMS has been applied to study the transformation of DOM by ozone in NOM isolates (Remucal et al., 2020; These and Reemtsma, 2005), solid phase extracts from surface waters (Phungsai et al., 2018; 2019), and wastewaters (Phungsai et al., 2016). Such studies typically find several thousand molecular features (i.e., mass spectral signals corresponding to putative molecules) and infer information about DOM composition and reactivity from trends in van Krevelen diagrams and DBE changes as a function of ozonation and/or advanced oxidation processes. The strongest effect of ozone is to increase the O:C ratio of molecular features, by up to 35% at 1.3 mg O3/mg C in DOM isolates (Remucal et al., 2020) and by a factor of 3.5 at 2.5 mg O3/mg C in Suwannee River Fulvic Acid (These and Reemtsma, 2005), indicating oxygen transfer reactions, in both the presence and absence of tert-butanol, pointing to a role for both ozone and *OH (Fig. 3) (Remucal et al., 2020). Double bond equivalents have been generally found to decrease slightly, but not as strongly as UV signals (Phungsai et al., 2018; Remucal et al., 2020) and H:C ratios are largely unchanged or slightly

3.2.2. Specific UV absorption - SUVA

One of the first methods to characterize changes in DOM upon ozonation is based on optical properties, in particular UV measurements at various wavelengths and a derived parameter, the specific UV absorbance (SUVA), which is the UV absorbance normalized to the dissolved organic carbon (DOC) concentration. The UV absorbance typically decreases during ozonation, due to bleaching of the DOM (Wenk et al., 2013). This effect is caused by the oxidation of activated aromatic systems, leading to moieties with lower UV absorbances. Other optical methods based on fluorescence measurements are also applied to characterize DOM during ozonation (Gerrity et al., 2012; Swietlik and Sikorska, 2004), which allows a qualitative classification of DOM moieties (McKay et al., 2018).

3.2.3. Electron donating capacity - EDC

Another tool for characterization of DOM is based on its EDC. This can be measured by electrochemical or spectrophotometric methods (Aeschbacher et al., 2011; Omby et al., 2018b; Walpen et al., 2020; Walpen et al., 2022), by the radical cation of 2,2-azino-bis(3-ethylbenzothiazole-6-sulphonate) (ABTS**), which can be produced electrochemically or by oxidation with chloride (Aeschbacher et al., 2010; Omby et al., 2018b). ABTS**, which absorbs light at various wavelengths in the visible range is reduced by reaction with electron donating moieties to ABTS, which is non-colored (Pinkernell et al., 1997). The EDC can be derived from the decoloration of ABTS** and allows a semi-quantitative determination of the availability of electrons, which can be abstracted by oxidants such as ozone, chloride and chloride dioxide (Wenk et al., 2013). EDC has also been shown in laboratory and full-scale experiments with a continuous online system to be a useful proxy for the abatement of micropollutants and the formation of bromate during ozonation of municipal wastewater effluents (Chon et al., 2015; Walpen et al., 2022). Furthermore, it has been shown that the combination of UV and EDC measurements can provide valuable insights into the DOM functional groups which are oxidized and formed.

Typically, a more efficient decrease of the EDC was observed compared to the UV absorbance (Omby et al., 2018a; Wenk et al., 2013). This can be interpreted by the oxidation of phenolic moieties leading to quinone-type and ring-opening products, which have a limited capacity to donate electrons to ABTS** but might still have UV absorption properties (Mvula and von Sonntag, 2003; Omby et al., 2018a; Ramseier and von Gunten, 2009; Tentscher et al., 2018; Wenk et al., 2013).

3.2.4. High Resolution Mass Spectrometry - HRMS

Recent increases in the accessibility of high resolution mass spectrometry (HRMS) instruments, including Fourier-transform ion cyclotron resonance (FT-ICR) and bench-top Orbitrap instruments with mass resolution of up to 1,000,000 have led to molecular-level characterization of natural organic matter (NOM). Rather than attempting to connect peaks on a mass spectrum to specific molecular structures, these efforts generally make empirical formula assignments, and draw conclusions based on those. Most commonly, H:C and O:C ratios are compared in van Krevelen diagrams (Andersson et al., 2020; Gonérior et al., 2014; Lavonen et al., 2015; Zhang et al., 2012) to infer functional group abundances and degrees of oxidation and to calculate the number of double bond equivalents (DBE) in the molecules.
increased (Phungsai et al., 2018; Remucal et al., 2020; These and Reemtsma, 2005), consistent with ozonation of phenol-type functional groups to highly oxygenated, lower molecular weight products (Fig. 3). Among partially abated compounds, those with lower H:C ratios (i.e., more aliphatic) favor reactions with ozone, while those with high H:C ratios (i.e., more aromatic) favor OH reactions (Remucal et al., 2020). Finally, OH has been shown to be a more potent initiator of decarboxylation reactions than ozone, with loss of CO₂ seen from ~3 – 5 × more molecular features ozonated under OH-promoting conditions than in the presence of a radical scavenger (Remucal et al., 2020).

3.2.5. Use of multiple characterisation methods

Because single methods are not suitable to fully characterize DOM, a combination of bulk techniques (e.g. UV, EDC) and HRMS can be applied to get further insights into DOM reactive moieties (Phungsai et al., 2016; Remucal et al., 2020). EDC and SUVA₂₅⁴ significantly decrease by ozone attack, possibly by fragmentation of phenolic groups to ring-opening products or oxidation of them to quinone-type structures (Fig. 4), while OH does not change EDC significantly. Ozone and OH both modestly increase the oxygen:carbon ratio of DOM, presumably through oxygen transfer and insertion reactions, respectively. Neither ozone nor OH substantially affect the hydrogen:carbon ratio (Fig. 4). Finally, the number of double bond equivalents is modestly decreased by both ozone and OH, likely due to loss of alkene and phenolic structures by reactions with ozone (Fig. 4), and loss of double bonds to OH through addition reactions. Overall, available evidence is consistent with the bulk of ozone-reactive sites in DOM being phenolic, and that they react analogously to phenol with ozone leading to oxygen addition and a decrease in molecular weight (Fig. 4). However, reactions between ozone and other functional groups, as well as reactions with OH, likely make some contribution to these bulk phenomena as well.

3.2.6. Chemical Derivatization

Chemical derivatization has long been used in environmental analytical chemistry, such as derivatization of charged haloacetic acids to neutral methyl esters for analysis via gas chromatography (Domino et al., 2003), and derivatization of small polar compounds such as dimethylamine to improve retention on hydrophobic stationary phases prior to liquid chromatography (Mitch et al., 2003a). However, most applications to date have been to aid in targeted quantification of known compounds. More recently, progress has been made in using derivatization techniques, often borrowed from the synthesis or biochemistry literature, to characterize DOM and its reactions with oxidants, including ozone, leading to unwanted byproducts.

In one example, a derivatization reaction was used to block specific ozone-reactive functional groups in water to establish the importance of compounds with those functional groups as DBP precursors. A reaction used synthetically to protect primary and secondary amines, addition of a tert-butoxycarbonyl group with di-tert-butyl-dicarbonate (Einhorn et al., 1991), was applied to DOM extracts prior to ozonation (McCurry et al., 2016). The resulting carbamates are much less reactive with ozone than the parent amine compounds, which was demonstrated with control experiments using model amines. Post-chlorination (after ozone) of derivatized DOM formed ~90% less chloropicrin than in controls, establishing primary and/or secondary amines as the principal chloropicrin precursors in water sequentially treated with ozone and chlorine. A similar approach has been applied for chlorine titrations with and without derivatization of amines to quantify primary and secondary amine moieties in DOM (Essaïed et al., 2022). In a more recent study, another derivatization reaction was applied to identify amines in water. Amines in DOM extracted by SPE were derivatized with either light
(ordinary) or heavy (deuterated) isotopes of formaldehyde prior to analysis with HRMS (Liu et al., 2019). The mass difference between the heavy and light isotopes of the resulting derivatives (4.025 Da) was used to pinpoint amines among the noise of other compounds in the DOM mass spectrum. Further, because primary amines were dimethylated, while secondary amines were monomethylated, information about amine order could also be extracted from the mass spectra. The authors used this technique to identify several dipeptides in water, which were then confirmed with analytical standards (Liu et al., 2019).

Derivatization was also applied to waters disinfected with chlorine, chloramines, or ozone to identify carbonyl-containing compounds. Suwannee River Fulvic Acid was derivatized with a hydrazine compound which was either partially deuterated or unlabelled, then analyzed via HRMS to search for peaks showing characteristic mass differences, as described above in (Liu et al., 2019). Prior to disinfection, most identified compounds in DOM (before oxidation) were poly-oxygenated with between five and ten double bond equivalents, and were proposed to be lignin-type structures (Liu et al., 2020). Ozonation led to a greater disappearance of putative lignin structures than chlorine or chloramines (Liu et al., 2020), consistent with selective reactivity toward activated aromatic compounds. Ozone increased the number of carbonyl-containing products relative to controls, whereas chlorine and chloramines led to their loss. Ozone tended to reduce the size of detected carbonyl compounds relative to controls, consistent with molecular fragmentation (e.g., via Criegee-type reactions) (Liu et al., 2020). In another study, phenolic moieties in DOM were derivatized with acetic anhydride to the phenylacetates. The latter have a very low reactivity another study, phenolic moieties in DOM were derivatized with acetic fragmentation (e.g., via Criegee-type reactions) (Liu et al., 2020). Ozonation led to their loss. Ozone tended to reduce the size of detected putative lignin structures than chlorine or chloramines (Liu et al., 2020), consistent with selective reactivity toward activated aromatic compounds. Ozone increased the number of carbonyl-containing products relative to controls, whereas chlorine and chloramines led to their loss. Ozone tended to reduce the size of detected carbonyl compounds relative to controls, consistent with molecular fragmentation (e.g., via Criegee-type reactions) (Liu et al., 2020). In another study, phenolic moieties in DOM were derivatized with acetic anhydride to the phenylacetates. The latter have a very low reactivity with chemical oxidants and this approach can be used to block phenolic moieties from reaction with e.g., ozone or chlorine dioxide (Houska et al., 2021). Even though this approach works well for synthetic chemicals, only part of the electron rich sites could be derivatized, potentially due to steric effects (Houska et al., 2021). Overall, derivatization shows promise as a means of reducing the complexity of analyzing reactions between DOM and oxidants by (i) allowing attribution of oxidation byproduct formation to specific precursor functional groups, regardless of the identity of individual precursor molecules, and (ii) adding characteristic functional group-specific tags to oxidation reaction precursors and products, to deconvolute their signals from analytical noise in complex matrices.

4. Ozone reaction kinetics and mechanisms

4.1. Introduction

4.1.1. General kinetic considerations

Understanding reaction kinetics is important for predicting micropollutant abatement efficiency during ozonation process (von Sonntag and von Gunten, 2012). The abatement kinetics for a micropollutant (MP) results from the concurrent oxidation by ozone and *OH (formed from ozone decomposition reactions) and can be formulated by the following integrated rate law (Eq. 1) (Lee and von Gunten, 2016):

\[
-Ln\left( \frac{[MP]_t}{[MP]_0} \right) = k_{O_3} \int_0^t [O_3] dt + k_{OH} \int_0^t [\cdot OH] dt
\]  

with \( k_{O_3} \) and \( k_{OH} \) as second-order rate constants for the reactions of micropollutant with ozone and *OH, respectively, and with \( \int_0^t [O_3] dt \) and \( \int_0^t [\cdot OH] dt \) as ozone and *OH exposures, respectively (Lee and von Gunten, 2016). The oxidant exposure is experimentally determined for a given water matrix and for a specific ozone dose. Matrix components such as DOM, nitrite, and carbonate significantly influence the stability of ozone and *OH and thereby their exposures. Ozone and *OH exposures have been determined for various types of wastewater (e.g., municipal wastewater, industrial wastewater, landfill leachate) and their correlations with the specific ozone doses (normalized by DOC concentration) has been determined (Lee and von Gunten, 2016; Wildhaber et al., 2015; Wolf et al., 2019). Based on this empirical model, the micropollutant abatement for a specific ozone dose can be predicted. Compounds with \( k_{O_3} \) higher than 10\(^3\) M\(^{-1}\) s\(^{-1}\) are considered to be well abated during ozonation for typical municipal wastewater, with > 80% abatement (Bourgin et al., 2018). To determine the fraction of a micropollutant reacting with ozone or *OH, respectively, the \( R_{ct} \) concept has been developed (Elovitz and von Gunten, 1999). It has been experimentally observed that the ratio of the *OH and ozone exposures (\( R_{ct} \)) is constant for most of the duration of ozone decomposition (Elovitz and von Gunten, 1999; Elovitz et al., 2000a,b; Eq. 2):

\[
R_{ct} = \frac{\int_0^t [\cdot OH] dt}{\int_0^t [O_3] dt} \Rightarrow \int_0^t [\cdot OH] dt = R_{ct} \int_0^t [O_3] dt
\]

Substituting \( \int_0^t [\cdot OH] dt \) in Eq. 1 by \( R_{ct} \int_0^t [O_3] dt \) yields a rate law for the abatement of a MP, which only depends on the ozone exposure and the \( R_{ct} \) (Eq. 3):

\[
-Ln\left( \frac{[MP]_t}{[MP]_0} \right) = k_{O_3} \int_0^t [O_3] dt + k_{OH}R_{ct} \int_0^t [O_3] dt = \int_0^t [O_3] dt (k_{O_3} + k_{OH}R_{ct})
\]

Based on Eq. 3, the fraction of the oxidation occurring by reacting with *OH can be calculated from Eq. 4 (Elovitz and von Gunten, 1999):

\[
f_{OH} = \frac{k_{OH}R_{ct}}{k_{OH}R_{ct} + k_{O_3}}
\]

The fraction of the MP reaction with *OH as a function of the \( R_{ct} \) and for selected values of \( k_{O_3} \) is shown in Fig. 5 (Elovitz and von Gunten, 1999).

**Fig. 5.** Fraction of a micropollutant (MP) reacting with *OH as a function of the \( R_{ct} \) according to Eq. 4. The curves are representative for second-order rate constants for the reaction with ozone (1-10\(^3\) M\(^{-1}\) s\(^{-1}\)). The second-order rate constant for the reaction with *OH is assumed to be \( 5 \times 10^3 \) M\(^{-1}\) s\(^{-1}\). The vertical lines show measured \( R_{ct} \) values for a karstic groundwater (Porentruy, Switzerland), a lake water (Zurich, Switzerland) and a river water (Sihl, Switzerland) (Elovitz et al., 2000b).
4.1.2. Determination of reaction kinetics

Second-order rate constants for the reactions of ozone with inorganic and organic compounds have been determined experimentally by directly monitoring the decrease of ozone or substrate over time under pseudo first-order conditions or by using competition kinetics methods (von Sonntag and von Gunten, 2012). To date, $k_{O3}$ for several hundred organic and inorganic compounds have been compiled (von Sonntag and von Gunten, 2012). Based on this wealth of information, regression models employing quantitative structure-activity relationships (QSAR) have been developed to predict unknown $k_{O3}$ for target compounds (Lee et al., 2015; Lee and von Gunten, 2012; Sudhakaran and Amy, 2013). Hammett and Taft constants are common molecular descriptors, which showed reliable prediction results for diverse ozone-reactive functional groups (Lee and von Gunten, 2012). However, empirical parameters such as Hammett and Taft constants are not always available. To overcome this limitation, quantum chemically computed molecular descriptors such as orbital energies or average local ionization energies have been assessed, showing promising results (see Fig. 8 and ensuing discussion) (Lee et al., 2017a). The current computed descriptors also have certain caveats (e.g., failing to account for steric effects) (Tentscher et al., 2019) and an effort for building better QSAR models should be continued. A multivariate model containing complementary quantum chemical variables may enhance the model performance. Additionally, ab initio calculations of barrier height ($\Delta G^\ddagger$) could be alternative QSAR variables, which have been successfully applied for predicting $k_{OH}$ (Minakata and Crittenden, 2011). However, for ozone reactions, ab initio calculations suffer from serious uncertainties of up to 5 orders of magnitude (Trogolo et al., 2015); the cause and possible solutions of this issue are worthy of further investigations.

As an electrophilic agent, ozone attacks moieties with high electron density, thus any modifications that alter the electron density of the moieties change their ozone reactivity. Typical examples are dissociating compounds. When protonated, a dissociating compound has a much lower $k_{O3}$ (than when deprotonated), because the electrons of the dissociating center are shared with the proton and less available for ozone (see e.g., aliphatic amines in Section 4.5). The protonation can also decrease the electron density inductively or by prohibiting resonance structures available to the deprotonated molecule (e.g., phenolic compounds in Section 4.3). Another example is compounds substituted by electron-donating/withdrawing groups. Typically, electron-donating groups such as alkyl groups increase $k_{O3}$, whereas electron-donating groups like carbonyl groups decrease $k_{O3}$ (e.g., olefins with various substituents in Section 4.2). An exception exists for heterocyclic compounds, some of which have higher $k_{O3}$ with the addition of carbonyl groups to the ring than without (Section 4.4). Substitution with electron-donating groups can result in an increased apparent $k_{O3}$ at circum-neutral pH ($k_{O3,app,pH7}$), for dissociating compounds bearing a $pK_a$ in the alkaline pH range (e.g., phenolic compounds in Section 4.3).

4.1.3. Transformation products and reaction mechanisms

Over the last few decades, many studies have investigated

| Reaction types and resulting products | Related functional groups |
|--------------------------------------|--------------------------|
| (i) C=C bond cleavage (Griegge mechanism) | • olefins  
• aromatic compounds  
• heterocyclic compounds |
| (ii) O-addition to ring | • aromatic compounds  
• heterocyclic compounds |
| (iii) O-addition to nitrogen | • aliphatic amines  
• aromatic compounds (anilines)  
• heterocyclic compounds |
| (iv) O-addition to sulfur | • organosulfur compounds |
| (v) N-C bond cleavage | • aliphatic amines |

Scheme 1. Conceptual scheme for the principal ozone reactions based on the target sites and the resulting products.
transformation products formed during ozonation and elucidated ozone reaction mechanisms. The principal ozone reaction mechanisms and the corresponding products are summarized in Scheme 1. The reaction type (i) is a carbon-carbon double bond cleavage based on the well-known Criegee mechanism (Criegee, 1975). An ozone molecule is added to a double bond to create an ozonide as a key intermediate, which subsequently degrades into carbonyl products. This type of reaction is also observed during the reaction of aromatic/heterocyclic compounds with ozone, opening the ring structure and forming aliphatic products with carbonyl groups. Aromatic and heterocyclic compounds also undergo reaction type (ii), leading to the oxygen-added ring products via radical intermediates (von Sonntag and von Gunten, 2012). Reaction types (iii) and (iv) show oxygen-transfer to the heteroatoms nitrogen and sulfur, respectively, resulting in the corresponding N-oxygenated and S-oxygenated products (von Sonntag and von Gunten, 2012). Nitrogen-containing compounds can also react with ozone via electron-transfer, forming an aminal radical cation and subsequently a dealkylated amine, as shown by reaction type (v) (von Sonntag and von Gunten, 2012). Each reaction type is also characterized by the formation of distinct reactive oxygen species. For example, hydrogen peroxide, singlet oxygen, and *OH are known as byproducts for the Criegee mechanism (i), O-addition to heteroatom (iii/iv), and N-C bond cleavage (v), respectively (Leitizzie and von Sonntag, 2009; Muñoz et al., 2001; von Gunten, 2003b). Thus, identifying/quantifying reactive oxygen species can provide additional evidence for certain reaction pathways. Alternatively, reaction mechanisms can be explored by quantum chemical computations. An increasing number of studies employs quantum chemical computations to corroborate experimental findings. Quantum chemical computations can predict initial reaction sites in a given molecular structure by computing molecular descriptors such as orbital energies or assess the thermodynamic feasibility of a proposed reaction mechanism by computing the Gibbs free energy (Tentscher et al., 2019). In addition, quantum chemical computations can provide insights related to unstable reaction intermediates, which escape experimental identification. A case in point is the formation of NDMA during ozonation of bromide- and dimethylsulfamide-containing waters, for which a detailed mechanism has been proposed based on experimental evidence and quantum chemical computations (Trogolo et al., 2015).

In Sections 4.2 to 4.9, ozone reaction kinetics and mechanisms are discussed on the basis of ozone-reactive functional groups. Direct ozone reactions are the main focus for most reaction mechanisms, as the role of *OH is minimal for the first generation transformation products for compounds with high ozone reactivity (Fig. 5). The majority of the recent findings in product analyses were obtained by HRMS. To discuss the HRMS results in a clear manner, the confidence level system (from level 1 to 5) defined by (Schymanski et al., 2014) is employed. Briefly, confidence level 1 indicates a confirmed structure with a reference standard, level 2 indicates a probable structure by e.g., a MS library match, level 3 provides some structural information by MS data and compound class, level 4 provides an unequivocal molecular formula and level 5 provides an exact mass.

4.2. Olefins (and alkynes)

Olefins are well-known ozone-reactive functional groups. In the 1970s, Rudolf Criegee revealed the key mechanism of the reaction of olefins with ozone, characterized by a cycloaddition of ozone to a carbon-carbon double bond to form carbonyl compounds as final products via ozonide as a cyclic intermediate (reaction type (i) in Scheme 1) (Criegee, 1975). On the basis of this landmark study, olefin-ozone reactions have been extensively studied in organic solvents, and later on, the knowledge has been transferred to aqueous solutions (von Sonntag and von Gunten, 2012). This section focuses on recent findings on $k_{O3}$ and summarizes briefly the olefin-ozone reaction mechanisms and ozonation products of aliphatic olefins. Cyclic olefins (or olefin moieties included in aromatic rings) are discussed in separate Sections 4.3 (Aromatic compounds) and 4.4 (Heterocyclic compounds).

4.2.1. Kinetics of Alkene-ozone reactions. Unsubstituted olefins react fast with ozone with $k_{O3}$ of approximately $10^5$ M$^{-1}$s$^{-1}$ (e.g., ethene: $k_{O3} = 1.8 \times 10^5$ M$^{-1}$s$^{-1}$ (Dowideit and von Sonntag, 1998)). In contrast, substituted olefins have a wide range of $k_{O3}$ from $10^3$ to $10^8$ M$^{-1}$s$^{-1}$ (Table S1, Supporting Information, SI), depending on the nature of the substituents (von Sonntag and von Gunten, 2012) (Fig. 6). Addition of electron-donating alkyl groups increases $k_{O3}$ (e.g., propene: $k_{O3} = 8.0 \times 10^5$ M$^{-1}$s$^{-1}$ (Dowideit and von Sonntag, 1998), but the magnitude of this effect is generally smaller than for electron-withdrawing groups as shown in Fig. 6 (Dowideit and von Sonntag, 1998). This is consistent with induction effects (Anslyn and Dougherty, 2006). Olefins with multiple electron-withdrawing substituents exhibit further decrease in reactivity (e.g., trichloroethene: $k_{O3} = 14 \times 10^5$ M$^{-1}$s$^{-1}$ (Dowideit and von Sonntag, 1998), tetrachloroethene: $k_{O3} < 0.1 \times 10^5$ M$^{-1}$s$^{-1}$ (Hoigne and Bader, 1983b)). Such additive effects of electron-withdrawing groups on $k_{O3}$ were also found in a study on polychloro-1,3-butadienes (e.g., tetrachloro-1,3-butadienes: $k_{O3} = 1.6 \times 10^4$ vs. 7.8 $\times 10^3$ M$^{-1}$s$^{-1}$, pentachloro-1,3-butadienes: $k_{O3} = 0.8$ and $10 \times 10^3$ M$^{-1}$s$^{-1}$, and hexachloro-1,3-butadiene: $k_{O3} < 0.1 \times 10^3$ M$^{-1}$s$^{-1}$ (Lee et al., 2017b)).

Differences in $k_{O3}$ also exist among the olefins substituted by the same number of electron-withdrawing groups but in different positions, i.e., stereoisomers. Examples are cis-1,2-dichloroethene ($k_{O3} = 5.4 \times 10^2$ M$^{-1}$s$^{-1}$) vs. trans-1,2-dichloroethene ($k_{O3} = 6.5 \times 10^3$ M$^{-1}$s$^{-1}$) (Dowideit and von Sonntag, 1998) and (E)-1,1,3,4-tetrachlorobutadiene ($k_{O3} = 1.1 \times 10^3$ M$^{-1}$s$^{-1}$) vs. (Z)-1,1,3,4-tetrachlorobutadiene ($k_{O3} = 4.0 \times 10^2$ M$^{-1}$s$^{-1}$) (Lee et al., 2017b). These significant differences in ozone reactivity between seemingly similar compounds may be explained by the geometry of the transition state in the rate-limiting step. Based on the reaction mechanism discussed in Section 4.2.2, the rate limiting step of the ozonolysis reaction likely requires the sp$^3$ carbon(s) in the double bond to rehybridize to sp$^2$, resulting in a decrease of the C-Cl or C-C bond angle from 120° (planar geometry) to 109.5° (tetrahedral geometry). This geometry brings the substituents in the cis position closer to each other, and if both substituents are bulky chlorines, rather than one chlorine and one methyl group, repulsion between these two chlorines in the transition state may potentially explain the lower $k_{O3}$ observed from the cis isomer of 1,2-dichloroethene.

Besides the induction effects from electron-donating/withdrawing groups, steric effects from bulky substituents can also affect $k_{O3}$. An example is cis-1,2-dichloroethene ($k_{O3} = 5.4 \times 10^2$ M$^{-1}$s$^{-1}$ (Dowideit and von Sonntag, 1998)) vs. endrin ($k_{O3} < 0.02 \times 10^2$ M$^{-1}$s$^{-1}$ (Yao and Haag, 1991)). Endrin also has a cis-1,2-dichloroethene moiety, but the ozone attack is hindered by the presence of the neighboring chlorinated carbons, resulting in a much lower $k_{O3}$ (Yao and Haag, 1991).
4.2.1.2. Kinetics of Alkyne-ozone reactions. Alkynes can react with ozone through a similar reaction mechanism as olefins, but with generally lower $k_{O3}$. For example, the $k_{O3}$ for the reaction of 1-ethinyl-1-cyclohexanol with ozone ($k_{O3} = 2.0 \times 10^2 \text{M}^{-1} \text{s}^{-1}$ (Huber et al., 2004)) is much lower than for buten-3-ol ($k_{O3} = 7.9 \times 10^4 \text{M}^{-1} \text{s}^{-1}$ (Dowideit and von Sonntag, 1998)), both of which feature the reactive moiety geminal to a substituted alcohol. To date, there is lack of information on ozonation kinetics of alkynes to make further generalizations about their reactivity.

4.2.2. Reaction mechanisms

The predominant pathway for the reaction of olefins with ozone is through pathway (i) in Scheme 2, in which the first zwitterion (A) formed in aqueous solutions (von Sonntag and von Gunten, 2012) leads to an ozonide (B), which subsequently forms a second zwitterion (C), or decomposes via a concerted reaction to the third zwitterion (D) and a carbonyl product. Zwitterion (D) is the branching point where aqueous reactions deviate from reactions in organic solvents. In aprotic solvents, the zwitterion (D) recombines with the carbonyl product to produce a Criegee ozonide (E) (Criegee, 1975). In water, the zwitterion D rapidly hydrates to $\alpha$-hydroxyalkylhydroperoxide (F), which reversibly dissociates to hydrogen peroxide and the second carbonyl product. Complete mass balances were achieved by the corresponding carbonyl products for the reactions of ozone with simple olefins like ethene and propene (Dowideit and von Sonntag, 1998).

There are minor pathways leading to the formation of partial cleavage products from zwitterion (A), such as a carbonyl product (G), a glycol (H) and an epoxide (I), all of which contain the two initial olefin carbons still linked by a single bond after ozonation. Partial cleavage products were observed in early studies in organic solvents, typically for sterically-hindered compounds like 3,3-dimethyl-2-tert-butyl-butene and 3,3-dimethyl-2-isopropyl-butene in methylene chloride (Bailey and Lane, 1967) and 1,1,2-trichloro-1,2,2-trifluoroethene in dichloromethane (Agopovich and Gillies, 1980; Keay and Hamilton, 1976). Dichloroacetaldehyde was identified as a minor product from ozonating dichloroethane (Scheme 2 (ii)) (Dowideit and von Sonntag, 1998). Glycol and carbonyl compounds (with two carbon atoms linked) were quantified in aqueous ozone reactions from tetramethylethene, dichloroethenes, and trichloroethene, with yields $<15\%$ (Dowideit and von Sonntag, 1998) (Scheme 2 (iii)). Epoxides were suggested as intermediates in aqueous ozone reactions mostly for olefin moieties in aromatic/heteroaromatic compounds, but with limited experimental

| $k_{O3}$ for substituted olefins (M$^{-1}$ s$^{-1}$) |
|-----------------------------------------------|
| (i) ethenes                                  |
| ethene                                       |
| $1.8 \times 10^5$                            |
| vinyl chloride                               |
| $1.4 \times 10^4$                            |
| trans-1,2-dichloroethene                     |
| $6.5 \times 10^3$                            |
| cis-1,2-dichloroethene                       |
| $5.4 \times 10^2$                            |
| trichloroethene                              |
| 14                                            |
| tetrachloroethene                            |
| $<0.1$                                        |
| (ii) propenes                                |
| propene                                      |
| $8.0 \times 10^5$                            |
| 1,1,-dichloropropene                         |
| $2.6 \times 10^3$                            |
| (iii) butenes and butadienes                 |
| buten-3-ol                                   |
| $7.9 \times 10^4$                            |
| (E)-1,1,3,4-tetrachlorobutadiene             |
| $1.1 \times 10^3$                            |
| (Z)-1,1,3,4-tetrachlorobutadiene             |
| $4.0 \times 10^2$                            |
| 1,1,2,4,4-pentachlorobutadiene               |
| 10                                            |
| hexachlorobutadiene                          |
| $<0.1$                                        |

Fig. 6. Effects of substitution by chlorine and hydroxyl groups on $k_{O3}$ for (i) ethene, (ii) propene, and (iii) butene and butadiene derivatives (Dowideit and von Sonntag, 1998; Lee et al., 2017b).
4.2.3. Micropollutants

Many ozone-reactive micropollutants in water feature olefin functional groups. Some important taste and odor compounds in drinking water contain an olefin moiety. They can be quickly inactivated during ozonation, because of their high $k_{O3}$ for the reactions with ozone (Table S1, SI). Examples are 3-hexen-1-ol ($k_{O3} = 5.4 \times 10^{5}$ M$^{-1}$s$^{-1}$), β-ionone ($k_{O3} = 1.6 \times 10^{5}$ M$^{-1}$s$^{-1}$), 2,6-nonadienal ($k_{O3} = 8.7 \times 10^{5}$ M$^{-1}$s$^{-1}$) and 1-penten-3-ol ($k_{O3} = 5.9 \times 10^{4}$ M$^{-1}$s$^{-1}$) (Peter and von Gunten, 2007).

Microcystins are a class of compounds with multiple ozone-reactive sites. The olefin moieties are the main ozone reaction sites for most microcystins except the ones with tyrosine and tryptophan substituents (MC-YR and MC-LW, respectively) (Kim and Lee, 2019; Onstad et al., 2007). As a result, primary ozonation products of MC-YR and MC-LW are likely to contain undamaged olefins and to retain toxicity for small specific ozone doses (note that the conjugated olefins of the base structure of microcystins are responsible for hepatotoxicity) (Kim and Lee, 2019). See Section 4.9 for more discussions on compounds with multiple reaction sites.

The ozone reactions of natural olefinic compounds have also been investigated in the aqueous phase of the atmosphere such as clouds and fogs. Examples are β-caryophyllonic acid ($k_{O3,app} = 4.8 \times 10^{5}$ M$^{-1}$s$^{-1}$ at pH 2 (Witkowski et al., 2019)), limononic acid ($k_{O3,app} = 4.2 \times 10^{5}$ M$^{-1}$s$^{-1}$ at pH 2 (Witkowski et al., 2018)), and α-terpineol ($k_{O3} = 9.9 \times 10^{6}$ M$^{-1}$s$^{-1}$ in the absence of radical scavenger (Leviss et al., 2016)).

Full mineralization of micropollutants is typically not achieved during ozonation. Olefin-containing micropollutants react with ozone leading to the formation of transformation products through pathways listed in Scheme 2. The chemical structures of the major transformation products from ozone reactions of selected olefin-type micropollutants are provided in Table S2 (SI). Ozonation of micropollutants containing an olefin moiety such as imazalil (Genena et al., 2011), octinoxate (Hopkins et al., 2017), tamoxifen and toremifene (Knoop et al., 2018), and carbamazepine (Hübner et al., 2014; McDowell et al., 2005) follow the predominant pathway (Scheme 2 (i)) because there is no steric hindrance on the olefin group. This leads to the formation of expected carbonyl compounds such as aldehydes and ketones. Keto-limononic acid was semi-quantified by LC-MS with a surrogate standard (30% yield), after ozonation of limononic acid (Witkowski et al., 2019). For ozonation of α-terpineol, more than 90% of a primary aldehyde product was expected based on the yields of the subsequent products (trans- and cis-lactol by rearrangement of the aldehyde) quantified by NMR (Leviss et al., 2016).

For micropollutants containing olefin moieties that are less accessible, hindering formation of the initial ozonide, such as carotenoids (Henry et al., 2000), acyclovir (Prasse et al., 2012), acetamidoantipyrine (Favier et al., 2015), carbamazepine (Hübner et al., 2014) and tetracycline (Khan et al., 2010), partial cleavage products such as glycol or epoxides have been suggested to be formed (Scheme 2 (iv)) (Table S2, SI). However, these reports were based on confidence level 2b (by diagnostic evidence), and there is no direct confirmation with reference
standards of epoxide formation in aqueous ozone reactions.

Alkyne-containing micropollutants react with ozone through a mechanism analogous to Criegee ozonation of alkenes. Without any bulky substituent groups nearby, complete cleavage products, including ketones and carboxylic acids are formed (Huber et al., 2004).

4.3. Aromatic compounds

4.3.1. Kinetics

Aromatic compounds react with ozone with a large range of second-order rate constants \(k_{O3,app} \approx 0.1 \text{ to } \sim 10^6 \text{ M}^{-1}\text{s}^{-1}\) (basic structures and recently reported examples in Table S3, SI), depending on the substituents. Unsubstituted arenes react with ozone slowly (e.g., benzene: \(k_{O3} = 2 \text{ M}^{-1}\text{s}^{-1}\) (Hoigne and Bader, 1983b)). Electron-donating alkyl substituents modestly increase \(k_{O3}\) (e.g., \(14 \text{ M}^{-1}\text{s}^{-1}\) for toluene (Hoigne and Bader, 1983b)). As with olefins, electron-withdrawing substituents reduce \(k_{O3}\) (e.g., \(0.57 \text{ M}^{-1}\text{s}^{-1}\) for 1,3-dichlorobenzene (Hoigne and Bader, 1983b)). Ring substituents featuring lone pairs of electrons which can delocalize into the ring somewhat increase \(k_{O3}\) (e.g., \(2.9 \times 10^2 \text{ M}^{-1}\text{s}^{-1}\) for methoxybenzene) (Hoigne and Bader, 1983b)). One exceptional class are polycyclic aromatic hydrocarbons, which have significantly higher \(k_{O3}\) than benzene or alkylbenzenes (e.g., \(3 \times 10^3 \text{ M}^{-1}\text{s}^{-1}\) for naphthalene (Hoigne and Bader, 1983b)), for reasons which have never been fully elucidated.

Phenol and phenol-derived compounds have high \(k_{O3}\) (Table S3, SI). Even in a wastewater matrix with strong scavenging of ozone, phenolic compounds can be abated to a large extent by ozone at relatively low specific ozone doses (Chedeville et al., 2009; Lee and von Gunten, 2010). The apparent \(k_{O3}\) for the reaction of phenol with ozone increases with increasing pH because of greater electron density of phenolate than phenol, due to the delocalization of phenolate electron lone pair into the ring. The \(k_{O3}\) is \(1.3 \times 10^3 \text{ M}^{-1}\text{s}^{-1}\) for phenol, which is 6 orders of magnitude lower than for phenolate (\(1.4 \times 10^9 \text{ M}^{-1}\text{s}^{-1}\)) (Hoigne and Bader, 1983a). This corresponds to a half-life of \(\sim 12\) s at pH 12 and \(\sim 10\) s at pH 12 for an ozone concentration of 2 mg/L.

The pK\(_a\) of phenol and phenol derivatives is a critical parameter for determining \(k_{O3,app}\), the apparent second-order rate constant at a given pH. It is influenced by electron-withdrawing or donating groups on the ring. For example, substitution by halogen or nitro groups decreases the pK\(_a\) of the hydroxyl group (e.g., the pK\(_a\) of dibromo-phenol is 6.67 (Serjeant and Dempsey, 1979) compared to 9.98 for phenol (Gross and Bader, 1983a)). This corresponds to a half-life of \(\sim 12\) s at pH 12 and \(\sim 10\) s at pH 6.5, but under acidic conditions. Aniline (pK\(_a\) = 9.2) and 4-nitrophenol (pK\(_a\) = 7.2) as a function of pH (Fig. 7). For substituted phenols with electron-donating substituents, the net effect can be an increased reactivity at neutral pH, while at low or high pH the \(k_{O3,app}\) are lower. A similar trend in \(k_{O3,app}\) at neutral pH is observed for substituted nitrogen-containing compounds, discussed in Section 4.6 (Fig. 12).

The ozone reactivity of anilines is also influenced by pK\(_a\) and given pH conditions. Aniline (pK\(_a\) of conjugate acid = 4.6) reacts readily with ozone (\(k_{O3,app} = 1.4 \times 10^4 \text{ M}^{-1}\text{s}^{-1}\) at pH 6.5), but under acidic conditions the positively charged anilinium has a significantly lower ozone reactivity (\(k_{O3,app} = 5.9 \times 10^4 \text{ M}^{-1}\text{s}^{-1}\) at pH 1.5) (Hoigne and Bader, 1983a; Pierpoint et al., 2001), because the nitrogen lone pair is unable to delocalize into the ring when protonated. Substituted anilines have a range of \(k_{O3}\) values depending on the ring substituents (Table S3), within approximately two orders of magnitude (Pierpoint et al., 2001; Tekle-Röttinger et al., 2016c).

QSAR methods have been applied to predict \(k_{O3}\) for aromatic compounds based on empirically determined Hammett and Taft constants (Lee and von Gunten, 2012) and quantum chemically computed energies of the highest occupied molecular orbital (\(E_{HOMO}\)) (Lee et al., 2015; Tentscher et al., 2019). Other tested descriptors include the number of bonds, dipole moment, partial charge distribution, and 3D configuration of the molecule (Cheng et al., 2018; Huang et al., 2020; Jiang et al., 2010; Zhu et al., 2014; Zhu et al., 2015).

In Fig. 8, \(k_{O3}\) for aromatic compounds are plotted as a function of the \(E_{HOMO}\) (Tentscher et al., 2019). It shows an overall good correlation for various types of aromatic compounds, suggesting \(E_{HOMO}\) as a promising quantum molecular descriptor for aromatic ring systems. Generally, for aromatic compounds the predicted \(k_{O3}\) based on both \(E_{HOMO}\) and Hammett and Taft constants agree well with the experimentally determined \(k_{O3,app}\) for aromatic compounds at a given pH. Even though the \(k_{O3}\) values for phenol and phenolate are higher than for their substituted analogues, the \(k_{O3,app}\) for the substituted phenols are higher at neutral pH (see also insert).
determined $k_{O_3}$ within a factor of 4 (Lee et al., 2015; Lee and von Gunten, 2012).

4.3.2. Reaction mechanisms

The three major pathways for oxidation of aromatic compounds by ozone are oxygen addition, electron transfer and the Criegee-type mechanism (Scheme 3). Of these, the most common reaction is oxygen addition to the ring (reaction type (ii) in Scheme 1 and pathway (i) in Scheme 3). For activated aromatic compounds with high $k_{O_3}$ (e.g., phenols), ozone adduct formation occurs in ortho/para position, which has an elevated electron density. The ozone adduct can then lose singlet oxygen and form a hydroxylated product (Scheme 3 (i)) (Mvula and von Sonntag, 2003). Hydroquinones and catechol are the major products through this pathway for phenolate (Mvula and von Sonntag, 2003; Ramseier and von Gunten, 2009; Tentscher et al., 2018). For aniline, the major products are $p$-hydroxyaniline and $o$-hydroxyaniline, with the sum of all hydroxyanilines accounting for ~40% of the initial aniline (Tekle-Röttering et al., 2016c).

The second pathway is electron transfer (Scheme 3 (ii)). It is unclear if this occurs via an ozone adduct formation or a direct electron transfer. Phenolate reacts with ozone to phenoxyl radical that can further react to benzoquinone (Ramseier and von Gunten, 2009). The coupling product (4,4-dihydroxybiphenyl), is an artefact of high phenol concentrations in laboratory experiments. Similar dimerization products were hypothesized from aniline such as 2- amino-5-anilino-benzochinon-anil and 2,5-dianilino-p-benzoquinon-imin, which are only expected at high initial concentrations of aniline (Tekle-Röttering et al., 2016c).

Finally, aromatic compounds can react through a Criegee-type mechanism (Scheme 3 (iii)), producing ring opening products such as muconic acid (Ramseier and von Gunten, 2009). Similar products were also observed from the ozone reaction with anisole, 1,2-dimethoxybenzene and 1,3,5-trimethoxybenzene (Mvula et al., 2009). 2-Pyridine-carboxylic acid is a Criegee-type product detected from ozone reactions with aniline (Sarasa et al., 2002), however it cannot be confirmed that ozone is responsible for its formation, because $^\cdot$OH was not scavenged in this study.

For aniline-type compounds, the sites of ozone attack can also be the nitrogen, producing nitrosobenzene, nitrobenzene, azobenzene and azoxybenzene as products (Chan and Larson, 1991; Sarasa et al., 2002), but these reactions are minor based on the observed low yields (< 8%) (Tekle-Röttering et al., 2016c). Ozonation of nitrogen-containing compounds will be further discussed in Sections 4.5 and 4.6.

Ring substitution can affect the final products from the reactions of phenol with ozone. In a systematic study of phenol substituent effects on

![Scheme 3. Ozone reaction pathways for phenolate and aniline. (i) Hydroxylation, (ii) electron transfer with formation of an ozonide radical, (iii) Criegee ozonide formation leading to ring opening. (Mvula and von Sonntag, 2003; Mvula et al., 2009; Ramseier and von Gunten, 2009; Tekle-Röttering et al., 2016c)](image-url)
product distribution, para-substituted catechols were found to be the most common products at pH 7, quantified from ozonation of para-substituted phenols (Tentscher et al., 2018), but with no obvious trend in their yields with respect to substitution. Somewhat surprisingly, many para-substituted phenols formed 1,4-benzoquinone, indicating loss of the substituent group during ozonation (Tentscher et al., 2018). Quinones have been previously observed as products of chlorophenol ozonation (Hong and Zeng, 2002; Qiu et al., 2004; Tentscher et al., 2018; Utsumi et al., 1998), and the dechlorination achieved by ozone (Tentscher et al., 2018) may increase the biodegradability (Adams et al., 1997; Hong and Zeng, 2002; Pi et al., 2007; Qiu et al., 2004; Utsumi et al., 1998). For two para-alkylated phenols, the corresponding 4-hydroxy-4-alkylcyclohexadienone was observed, but not for any other phenols, possibly due to the poor leaving group properties of the alkyl substituents preventing further transformation to benzoquinones. Hydroquinones were only detected as ozonation products from 4-methoxyphenol (mequinol) and 4-formylphenol, and were attributed to reactions other than direct attack by ozone (Tentscher et al., 2018).

4.4. Heterocyclic compounds

Heterocyclic compounds are an important class of functional groups in micropollutants as well as in DOM. They usually consist of five-membered, six-membered, or fused rings and include at least one heteroatom (e.g., N, O, S) in the ring. For N-containing heterocyclic compounds, only unsaturated compounds are discussed in this section. The reactions of N-containing saturated heterocyclic compounds are similar to aliphatic amines, their acyclic analogues (Tekle-Röttering et al., 2016a). Aromatic rings without heteroatoms are discussed in the Section 4.3.

4.4.1. Kinetics

Heterocyclic compounds are characterized by extraordinarily diverse chemical properties and therefore undergo a wide range of reaction types: some react readily with electrophiles while others do not (Joule and Mills, 2012). Their reactivity with ozone varies widely, with reported $k_{O3}$ from $< 0.1$ M$^{-1}$s$^{-1}$ to $\sim 10^{9}$ M$^{-1}$s$^{-1}$ (Table S5, SI).

4.4.1.1. N-containing heterocyclic compounds. Six-membered N-containing heterocyclic compounds like pyridine and pyrazine react slowly with ozone with $k_{O3} \leq 3$ M$^{-1}$s$^{-1}$ (Tekle-Röttering et al., 2016b), whereas five-membered rings such as pyrrole and imidazole react much faster with ozone with $k_{O3} > 10^{5}$ M$^{-1}$s$^{-1}$ (Tekle-Röttering et al., 2020). The surprisingly large difference in $k_{O3}$ between six- and five-membered heterocycles seems attributable to the electron distribution in the rings. In pyridine, resonance withdraws electron density from carbons to the nitrogen (Fig. 9). As a result, pyridine carbons become $\pi$-deficient and thus less reactive towards ozone. Resonance in pyrrole works in the opposite way, pushing the electron density from the nitrogen towards carbons, making carbon-carbon double bonds more susceptible to an electrophilic ozone attack. Heterocycles consist often of complex structures with diverse substituents. Effects of substituents on $k_{O3}$ for selected heterocyclic compounds are shown in Fig. 10. (i) Pyrimidine and (ii) purine derivatives: the addition of a carbonyl or an amino group to the pyrimidine or purine significantly increases $k_{O3}$. All derivatives have higher $k_{O3}$ than the simple heterocycles, by up to 7 orders of magnitude (pyrimidine vs. 2,4-

![Fig. 9. Resonance structures of pyridine, pyrrole, and 1H-pyridin-2-one.](https://example.com/fig9.png)
| Heterocycle                  | Rate Constant, M⁻¹ s⁻¹ |
|-----------------------------|-------------------------|
| (i) pyrimidine derivatives  |                         |
| pyrimidine                  | 0.07                    |
| uracil                      | 6.5 × 10²                |
| cytosine                    | 1.4 × 10³                |
| 2,4-diamino-5-methylpyrimidine | 1.3 × 10⁶               |
| (ii) purine derivatives     |                         |
| purine                      | 7.9                     |
| adenine                     | 12                      |
| caffeine                    | 6.5 × 10²                |
| 2’-deoxyguanosine           | 1.9 × 10⁴                |
| carboxy-acyclovir            | 1.8 × 10⁴                |
| (iii) pyrrole/diazole       |                         |
| pyrrole                     | 1.4 × 10⁶                |
| maleimide                   | 4.2 × 10³                |
| imidazole                   | 2.4 × 10⁵                |
| creatinine                  | 2                       |
| pyrazole                    | 56                      |
| phenacone                   | 6.6 × 10⁴                |

Fig. 10. Effects of carbonyl and amino group substitution on second-order rate constants for the reactions of ozone with (i) pyrimidine, (ii) purine, and (iii) pyrrole/diazole derivatives. Arrows indicate the ozone attack sites based on identified products in presence of tert-butanol, for the compounds with product information. References: ¹(Tekle-Rottering et al., 2016b), ²(Theruvathu et al., 2001), ³(Dodd et al., 2006), ⁴(Legube et al., 1987), ⁵(Broseus et al., 2009), ⁶(Prasse et al., 2012), ⁷(Tekle-Rottering et al., 2020), ⁸(Hoigné and Bader, 1983a), and ⁹(Favier et al., 2015).

diamine 5-methylpyrimidine). (iii) Pyrrole/diazole derivatives: unlike pyrimidines and purines, pyrrole and imidazole derivatives with a carbonyl or amino group show lower \( k_{O3} \) (imidazole vs. creatinine). For pyrazole derivatives, \( k_{O3} \) seems to increase by a carbonyl group (pyrrole vs. phenazone) as for pyrimidines and purines, but the benzene ring on a nitrogen of phenazone may also contribute to the increase in \( k_{O3} \). To confirm this, it is necessary to compare pyrazole with pyrazole derivatives substituted only by a carbonyl group, however, kinetic information of such derivatives is currently not available. The higher \( k_{O3} \) of uracil than for pyrimidine is especially interesting, as the substitution by an electron-withdrawing carbonyl group is expected to reduce the ozone reactivity. One way to explain these unusual cases with carbonyl substituents is by their resonance structures (Fig. 9). While pyridine lacks in electron density in the ring as discussed above, a 1H-pyridin-2-one (pyridine with a carbonyl substituent) develops a higher negative charge on the ring by resonance, suggesting a possibly higher \( k_{O3} \).

4.4.1.2. O-containing heterocyclic compounds. Generally, there is little information on the ozone reactivity of heterocyclic compounds containing heteroatoms other than nitrogen. Furans, five-membered heterocyclic rings containing oxygen, show high reactivity towards ozone with \( k_{O3,app,ph} \) > 10⁴ M⁻¹ s⁻¹ (Table S5, SI) (Jeon et al., 2016; Zoumpouli et al., 2021). Depending on the type of substituents (e.g., methyl, carbonyl groups) and their locations on the ring (e.g., α-, β-carbons), the \( k_{O3,app,ph} \) varied from 8.5 × 10⁴ M⁻¹ s⁻¹ for furan-2,5-dicarboxylic acid to 3.2 × 10⁶ M⁻¹ s⁻¹ for 3-(2-furyl)propanoic acid (Zoumpouli et al., 2021).

4.4.2. Reaction mechanisms

Heterocyclic compounds react with ozone by various types of mechanisms, mostly by reaction type (i) in Scheme 1, which opens the ring by carbon-carbon double bond cleavage (Criegee-type mechanism). As a result, ring-opened primary products with two carbonyl groups on each end are formed. The ring-opened products undergo subsequent reactions, sub-categorized as (i-a), (i-b), and (i-c) in Scheme 4. They are subject to further transformation by ozone to form secondary products with typically a shorter chain length (i-a). Examples of such secondary products are quinolinic acid from quinoline with 65% yield (Andreozzi et al., 1992b) and glyoxal from pyrrole with 5% yield (Tekle-Rottering et al., 2020). In a rarer case, ring-opened products are fully fragmented without further oxidation (i-b). A case in point is imidazole, which is transformed to formamide, cyanate, and formate, with 100% yields, respectively, by a single ozone attack (Tekle-Rottering et al., 2020).

Ring-opened products are also susceptible to an intramolecular cyclization (i-c). This results in the formation of products with smaller ring size as the parent heterocyclic compounds. For example, hydantoins with a five-membered ring were identified after the reactions of ozone with six-membered heterocyclic compounds like uracil (Matsui et al., 1990) and thymine (Flyunt et al., 2002), at yields of ~30%. Although it is unclear whether these products resulted from direct ozone reactions (because of lack of information on the use of radical scavengers), similar ring products with reduced size were also identified in more controlled experiments (in the presence of tert-butanol) for 2-hydroxypryridine (Andreozzi et al., 1992a) and thymidine (confidence level 3) (Funke et al., 2021). The reaction type (i) was also suggested for reactions of furans with ozone, based on the formation of ring-opened products, α,β-unsaturated dicarbonyl compounds (Zoumpouli et al., 2021). However, the products were identified in absence of radical scavengers. Therefore, experimental evidence under more controlled conditions is required to confirm the proposed mechanism of the furan-ozone reaction.

Some heterocyclic compounds react with ozone via reaction type (ii) (Scheme 1 and Scheme 4), in analogy to typical reactions of aromatic compounds with ozone (Section 4.3.2). In this case, oxygen-added ring products with the same ring structure as the parent heterocycle are formed. The formation of maleimide (34% yield) from the reaction of

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Pyrrrole with ozone supports an oxygen-addition mechanism (Tekle-Røttering et al., 2020). Another ring product structurally close to maleimide (called TP2 in the original study) was also identified but with unknown yield. One heterocyclic compound can react through multiple reaction types, as for pyrrrole, which reacts with ozone via reaction types (i) and (ii) in Scheme 4 (Tekle-Røttering et al., 2020).

Ozone attack on the heteroatom instead of a carbon-carbon double bond is rarely reported during ozonation of heterocyclic compounds (Scheme 4, (iii)). Pyridine and pyridazine are transformed to N-oxides with yields of ≤ 93% (Andreozzi et al., 1991; Tekle-Røttering et al., 2016b). This N-oxide formation is similar to reactions of tertiary amines with ozone, as illustrated in reaction type (iii) in Scheme 1. Nevertheless, high yields of N-oxides from heterocycles are unlikely under realistic ozonation conditions, because of the low ozone reactivity of pyridine and pyridazine (kO3 ~ 2 M⁻¹s⁻¹ for both (Tekle-Røttering et al., 2016b)). Such ozone-refractory compounds are mainly oxidized by •OH (Fig. 5), which consequently leads to a different spectrum of transformation products. During ozonation in absence of tert-butanol (•OH reaction possible), pyridine was transformed to ring-opened products like formamido(oxo)acetic acid and oxamic acid (Andreozzi et al., 1991).

4.4.3. Micropollutants

Micropollutants often contain heterocyclic moieties, which are the

Scheme 4. Ozone reactions with heterocyclic compounds categorized based on the resulting transformation products. References: a (Andreozzi et al., 1992b), b (Tekle-Røttering et al., 2020), c (Flyunt et al., 2002), d (Andreozzi et al., 1991; Tekle-Røttering et al., 2016b).
main site of ozone attack. Acesulfame is attacked on the carbon-carbon double bond in the ring to form ring-opened products susceptible to further oxidation, as described in (i-a) in Scheme 4. The resulting products are a hydrated form of a carbonyl product and carboxylic acid, with ~50% and 80% yields, respectively (Scheurer et al., 2012). Phenazon derivatives (Favier et al., 2015) and benzo-triazole (Mawhinney et al., 2012) were also reported to form ring-opened products upon ozonation, with sufficient MS evidences (confidence level 2) but with unknown yields. Furosemide and ranitidine, both of which feature a furan moiety, were also suggested to be oxidized at a carbon-carbon double bond of furan to form the corresponding ring-opened products based on MS results (confidence level 2) (Zoumpouli et al., 2021). Caffeine was found to be oxidized by ozone to form five- and six-membered ring products with 20–30% yields, resulting from the intramolecular cyclization of an initial ring-opened product ((i-c) in Scheme 4) (Kolonko et al., 1979). In this study, however, ozonation experiments were conducted in absence of *OH scavengers and thus an impact of *OH cannot be excluded (Kolonko et al., 1979). Carboxy-acyclovir, containing a purine moiety, was attacked by ozone at the carbon-carbon double bond shared by the fused six- and five-membered rings (Prasse et al., 2012). The resulting ozonide intermediate underwent rearrangement of the ring and hydrolyzed to form a final product containing a five-membered ring with 100% yield (similar to (i-c) in Scheme 4) (Prasse et al., 2012). Zidovudine, a thymidine derivative, reacted with ozone at the carbon-carbon double bond of the thymine moiety. Ring-opened products via (i-a) as well as ring products by rearrangement via (i-c) were proposed by MS analyses (confidence level 3) (Funke et al., 2021). Lamotrigine, containing a 1,2,4-triazine moiety, was oxidized by ozone at the nitrogen to form a N-oxide (iii) in Scheme 4), which underwent subsequent oxidation (Bollmann et al., 2016).

4.5. Aliphatic amines

4.5.1. Kinetics

Aliphatic amines are present in natural waters as part of the dissolved organic nitrogen pool (Westerhoff and Mash, 2002). They are also common functional groups of micropollutants (Bourgin et al., 2018). The lone electron pair on the nitrogen of aliphatic amines is susceptible to an electrophilic attack by ozone. As a result, aliphatic amines in the neutral form react fast with ozone with a rate constant of 10^5 M^-1 s^-1 (von Sonntag and von Gunten, 2012). The reason why the electron withdrawing effect by halogens is not as pronounced as expected is not clear. In the case of dimethylsulfamide (DMS), the effect of halogenation is even more pronounced. For the reactions of DMS and Br-DMS with ozone, k_{DMS,app} = 26 M^-1 s^-1 (Haag and Hoigné, 1983a) and k_{Br-DMS,app} = 40 M^-1 s^-1 (Haag and Hoigné, 1983a), in comparison to ammonia (k_{NH_3} = 20 M^-1 s^-1 (Hoigné and Bader, 1983a)).

Scheme 5. Reactions of aliphatic amines with ozone.

4.5.2. Reaction mechanisms

Ozonation of tertiary amines has been studied extensively. N-oxides were identified as major products with high yields (> 90%) for simple tertiary amines with short alkyl chains and micropollutants containing a tertiary amine moiety (von Sonntag and von Gunten, 2012) (Scheme 5 and reaction type (iii) in Scheme 1).

Besides N-oxides, dealkylated products were also identified from the reaction of tertiary amines with ozone, but as minor products with yields ≤ 10% (Lange et al., 2006; Lim et al., 2019; Zimmermann et al., 2012). While N-oxides are products formed via an oxygen-transfer pathway,
dealkylated products result from an electron-transfer pathway involving an amine radical cation as a reaction intermediate (von Sonntag and von Gunten, 2012) (reaction type (v) in Scheme 1).

The mechanisms of the reactions of primary and secondary amines with ozone have also been investigated (de Vera et al., 2017; Essaïed et al., 2022; Lim et al., 2019; McCurry et al., 2016; Shi and McCurry, 2020; Tekle-Röttering et al., 2016a). Nitroalkanes were identified as major products (yields up to 100%) from simple primary and secondary amines (Lim et al., 2019; McCurry et al., 2016) (Scheme 5). A potentially undesirable outcome associated with the formation of nitroalkanes is discussed in Section 2.2.2.

Hydroxylamines were suggested as ozonation products of secondary amines (Benner and Ternes, 2009a; Benner and Ternes, 2009b; Tekle-Röttering et al., 2016a; von Gunten, 2003b), in analogy to N-oxides as ozonation products of tertiary amines. However, recent studies identified hydroxylamines not as end products but as reaction intermediates that further react with ozone to eventually form nitroalkanes (Scheme 5; see also Section 4.6). This occurs especially under neutral pH conditions, where the ozone reactivity of hydroxylamines is typically much higher than the ozone reactivity of amines, because of the lower pKa of protonated hydroxylamines compared to protonated amines. Besides hydroxylamines, nitroxides, nitrones, and nitrosoalkanes have been suggested as other reaction intermediates, but only the formation of nitrate was confirmed by an analytical standard during ozonation of a secondary amine (Lim et al., 2019).

Nitrate is also a final product for ozonation of aliphatic amines along with carbonyl products originating from the carbonaceous part of the amine compounds. It was shown that the yields of nitrate were highly variable for primary and secondary amines with a 20-fold molar ozone excess (17-100%) (de Vera et al., 2017; Essaïed et al., 2022). Amino acids showed consistently high yields of nitrate under similar ozonation conditions (Essaïed et al., 2022). Under these extreme conditions, nitroalkanes and N-oxides were possibly further oxidized to nitrate. Certain primary amines form nitrate more efficiently. Glycine was transformed into nitrate with a > 80% yield with a 5-fold ozone excess (Berger et al., 1999). This is in contrast to ethylamine, forming nitroethane as the only product under similar ozonation conditions (Lim et al., 2019). Nitrate formation during continuous ozonation was used as a surrogate for the potential formation nitro and/or nitroso compounds from DOM. It could be shown that the nitrate formation potential during ozonation was in the range of 13-45% of the dissolved organic nitrogen for 6 different DOM sources (Essaïed et al., 2022).

Overall, all types of aliphatic amines (primary, secondary, and tertiary) are transformed into products containing N-O bonds (nitroalkanes, N-oxides, and nitrate) with high yields. This indicates that aliphatic amines react with ozone mainly via oxygen-transfer pathways (reaction type (iii) in Scheme 1). Additional evidence is provided by high yields of singlet oxygen (a bp product of the oxygen-transfer pathway) and quantum chemically computed Gibbs free energy favoring an oxygen-transfer over an electron-transfer pathway (Lim et al., 2019). However, the reaction pathway can be shifted to some extent under real ozonation conditions where °O2H is formed as a secondary oxidant and plays a role in oxidizing aliphatic amines (Fig. 5), due to the relatively low kO3,app at neutral pH. In this case, aliphatic amines undergo hydrogen atom abstraction to form N-centered radicals that are subsequently degraded into dealkylated products and ultimately ammonia (reaction type (v) in Scheme 1) (de Vera et al., 2017; Le Lacheur and Glaze, 1996).

4.5.3. Micropollutants

The formation of N-oxides during ozonation has been reported for many pharmaceuticals containing a tertiary amine moiety such as clarithromycin, tramadol, venlafaxine, tamoxifen, and toremifene (Knoop et al., 2018; Lange et al., 2006; Lester et al., 2013; Zimmermann et al., 2012). Pharmaceuticals containing a tertiary amine moiety in a saturated heterocyclic form, such as cetirizine (containing piperazine), fexofenadine (piperidine), sulpiride (pyrrolidine), and amisulpride (pyrrolidine), were also transformed into N-oxides upon ozonation (Bollmann et al., 2016; Borowska et al., 2016). The formation of N-oxides has been also observed in pilot- and full-scale wastewater treatment plants with ozonation of secondary wastewater effluent (Bourgin et al., 2018; Merel et al., 2017; Zucker et al., 2018). Once formed, N-oxides are persistent in the treated water. They are not biodegradable during typical aerobic biological post-treatments after ozonation (Bourgin et al., 2018; Hüblner et al., 2015; Knoop et al., 2016; Zucker et al., 2018).

In contrast to the ample evidence of the N-oxide formation, the formation of hydroxylamines from the oxidation of micropolllutants containing secondary/primary amines by ozone has been reported to a lesser extent (e.g., propanolol (Benner and Ternes, 2009a)). This is probably because of the further oxidation of hydroxylamines, ultimately forming nitro compounds, as is shown for sitagliptin, a pharmaceutical containing a primary amine moiety (Hermes et al., 2020). The corresponding nitro product of sitagliptin was also detected in pilot-scale and full-scale ozonation process (Gulde et al., 2021a; Hermes et al., 2020).

4.6. Other aliphatic nitrogen-containing compounds

4.6.1. Kinetics

Various derivatives are related to aliphatic amines by substitution of the nitrogen with heteroatoms (e.g., O, N, S). The kO3 of these compounds are in the range from < 1 to ~106 M-1 s-1. Currently available information on kO3 is summarized in Fig. 11, categorized by characteristic chemical bonds found in the functional groups. Differences in pKa values result in a different trend in kO3,app as a function of the pH for substituted nitrogen-containing compounds in comparison to aliphatic amines (Fig. 12).

(i) N-O bond: compounds containing N-O bonds, e.g., N-oxides, hydroxylamines, nitroxides, and nitroalkanes, are common ozonation products of aliphatic amines (Section 4.5.2). They show a wide range of kO3, from slow-reacting N-oxides and nitroalkanes (kO3,app,pH7 < 10 M-1 s-1) to fast-reacting nitrates and hydroxylamines (kO3,app,pH7 > 105 M-1 s-1). It should be noted that the reported kO3 for the fexofenadine N-oxide is associated with other moieties (Borowska et al., 2016) and therefore kO3 of the N-oxide moiety would be even lower than 6 M-1 s-1. Hydroxylamines are especially reactive because of their pKa values, 3 – 5 units lower than the pKa of the amine analogous (Table S6, SI). Therefore, unlike aliphatic amines, hydroxylamines remain highly reactive towards ozone at pH 7 where they are dominantly present in the neutral form (Fig. 12).

(ii) N-N bond: hydrazines and hydrazides react fast with ozone with high kO3. Similarly to hydroxylamines, their protonated forms have a lower pKa than protonated aliphatic amines (Table S6, SI), resulting in high kO3,app,pH7 (Fig. 12). Diaminozide reacts fast with ozone over a wide pH-range (kO3 ≥ 106 M-1 s-1 for pH 3 – 9), due to its low pKa of 3.7.

(iii) N-N=O bond: nitroamines and nitrosoamines react very slowly with ozone with most kO3 ≤ 0.5 M-1 s-1 (Mestankova et al., 2014).

(iv) N-C(=N)-N bond: The nitrogen of guanidine derivatives is present in the protonated form under most pH conditions because of the high pKa (e.g., 13.6 for guanidine (Table S6, SI)). As a consequence, their kO3 are likely to be low, as shown for metformin (1.2 M-1 s-1) and creatine (0.5 M-1 s-1) (Hoigné and Bader, 1983a; Jin et al., 2012).
$k_{O3}$ for $N$-containing compounds other than amines (M$^{-1}$ s$^{-1}$)

(i) N-O bond (N-oxides, hydroxylamines, nitrones, nitroalkanes)

- fexofenadine N-oxide$^a$  
  $k_{O3, app, pH7} = 6$
- pyridine N-oxide$^b$  
  $k_{O3, app, pH7} = 0.8$
- $N,N$-diethylhydroxylamine$^c$  
  $k_{O3, app, pH7} = 6.8 \times 10^5$
- $N$-ethylthiouronium$^d$  
  $k_{O3, app, pH7} = 1.9 \times 10^3$
- nitroethane$^e$  
  $k_{O3, app, pH7} = 3.4$

(ii) N-N bond (hydrazines, hydrazones, diazenes)

- 1,1-dimethylhydrazine$^d$  
  $k_{O3, app, pH7} = 1.1 \times 10^6$
- daminozide$^d$  
  $k_{O3, app, pH7} = 5.0 \times 10^5$
- azobenzene$^g$  
  $k_{O3} = 2.2 \times 10^2$

(iii) N-N=O bond (nitrosamines, nitroamines)

- nitrosamines$^f$  
  $k_{O3} = 0.04 \text{ to } 8.5$
- hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)$^h$  
  $k_{O3, app, pH2} \approx 0.97$
- creatine$^l$  
  $k_{O3, app, pH2} \approx 0.5$
- metformin$^l$  
  $k_{O3, app, pH7} = 1.2$

(iv) N-C(=N)-N bond (guanidines)

- N(4)-acetyl-sulfamethoxazole$^k$  
  $k_{O3, app, pH7} = 2.5 \times 10^2$
- N,N-dimethylsulfamide$^l$  
  $k_{O3, app, pH7} \approx 20$
- acesulfame$^n$  
  $k_{O3, app, pH7} = 88$

(v) N-S(=O)=O bond (sulfonamides, sulfamides, sulfamates)

- hydrochlorothiazide$^a$  
  $k_{O3, app, pH7} = 8.5 \times 10^4$
- N(4)-acetyl-sulfamethoxazole$^k$  
  $k_{O3, app, pH7} = 2.5 \times 10^2$
- N,N-dimethylsulfamide$^l$  
  $k_{O3, app, pH7} \approx 20$
- acesulfame$^n$  
  $k_{O3, app, pH7} = 88$

References:

- $a$ (Borowska et al., 2016), $b$ (Tekle-Rötterting et al., 2016b)
- $c$ (Lim et al., 2019), $d$ (Lim et al., 2016), $e$ (Yao and Haag, 1991), $f$ (Lee et al., 2007b), $g$ (Mes-
  tankova et al., 2014), $h$ (Chen et al., 2008), $i$ (Hoigné and Bader, 1983a), $j$ (Jin et al., 2012), $k$ (Dodd et al., 2006), $l$ (von Gunten et al., 2010), and $m$ (Kaiser et al., 2013).

(v) N-S(=O)=O bond: The nitrogen adjacent to sulfur in sulfon-
  amides, sulfa-mides, and sulfa-mates undergoes acid-base specia-
  tion from the neutral to the deprotonated, anionic form (Table S6, SI) 
and therefore $k_{O3}$ strongly depends on the pH. $k_{O3}$ of the 
deprotonated forms are $\sim 3 \times 10^5$ M$^{-1}$ s$^{-1}$ for hydrochlorothia-
  zide (Borowska et al., 2016), 2.6 $\times 10^2$ M$^{-1}$ s$^{-1}$ for $N$
(4)-acetyl-sulfamethoxazole (Dodd et al., 2006), $> 10^3$ M$^{-1}$ s$^{-1}$ for 
$N,N$-dimethylsulfamide (von Gunten et al., 2010), and 88 M$^{-1}$ s$^{-1}$
for acesulfame (Kaiser et al., 2013). Only hydrochlorothiazide 
with a $pK_a \approx 7.0$ remains ozone-reactive at neutral pH with $k_{O3, app, pH7} = 8.5 \times 10^4$ M$^{-1}$ s$^{-1}$, while the other compounds show low 
to moderate $k_{O3, app, pH7}$. Fig. 11. Second-order rate constants $k_{O3}$ for the reactions of ozone with selected $N$-containing compounds: (i) N-O bond, (ii) N-N bond, (iii) N-N=O bond, (iv) N-C (=N)-N bond, and (v) N-S(=O)=O bond. Arrows indicate the sites of ozone attack. Structures of major species at given pH are depicted based on the $pK_a$ summarized in Table S6, SI, except hydrochlorothiazide (shown as deprotonated species) for which the major species at the given pH is unclear because of its ill-defined $pK_a$.
4.6.2. Reaction mechanisms

Depending on the substituents, the reactions of aliphatic amine derivatives with ozone differ significantly. Some are attacked by ozone on the nitrogen, e.g., 1,1-dimethylhydrazine, whereas others shift the main attack site to an α-carbon, e.g., nitroethane (Section 4.8.1). The reactions for which nitrogen is the main ozone-reactive site are summarized in Scheme 6. Systematic investigations on product formation and reaction mechanisms for this group of compounds are scarce. Because of the lack of information with model compounds, some of the mechanisms are discussed for micropollutants.

(i) Compounds with N-O bond: hydroxylamines are quantitatively transformed to nitroalkanes upon ozonation, forming nitrones as reaction intermediates (Lim et al., 2019; Shi and McCurry, 2020). This supports that hydroxylamines are likely involved in the reactions of primary/secondary amines with ozone, which also form nitroalkanes as final products.

(ii) Compounds with an N-N bond: hydrazine/hydrazide-ozone reactions have been studied mainly with regard to the formation of N-nitrosodimethylamine (NDMA), a probable human carcinogen. Upon ozonation, one of the two nitrogen atoms is directly attacked by ozone and forms a new N–O bond. NDMA was quantified as final product with high molar yields for 1,1-dimethylhydrazine and dimaminozide (Lim et al., 2016), and industrial chemicals containing semicarbazides (Kosaka et al., 2009; Kosaka et al., 2014). Hydrazides are also found in heterocycles (Section 4.4), e.g., phenozone-type pharmaceuticals (Favier et al., 2015). In this case, however, the main ozone attack site is not the hydrazide-nitrogen but an endocyclic double bond.

(iii) and (iv) Compounds with N=N–O and N=C(=N)-N bonds: There is little information on the direct ozone reactions with nitroamines, nitroamines, and guanidines, mostly because of their low ozone reactivity, which will promote an *OH oxidation under realistic ozonation conditions (Fig. 5).

(iv) Compounds with a N-S(=O)=O bond: Typically, substitution by electron-withdrawing groups reduces the ozone reactivity of the nitrogen. In certain cases, if the electron-withdrawing effect is strong enough that the substituted nitrogen becomes acidic, the deprotonated nitrogen is the favorable target site for ozone. This was observed for hydrochlorothiazide of which the sulfonamide-nitrogen was oxidized by ozone via electron transfer and transformed into chlorothiazide as a primary product (Borowska et al., 2016). In contrast, N(4)-acetyl-sulamethoxazole, with the aniline-nitrogen deactivated by an amide, is likely oxidized by ozone on the isoazole or the aniline ring, rather than on the sulfonamide (Dodd et al., 2006). Acesulfame, containing a nitrogen substituted by both sulfanyl and amide groups, reacts with ozone mainly via an oxidation on the endocyclic double bond (Scheuer et al., 2012). The presence of the second substitution group or other ozone-reactive moieties seems a determining factor for assessing the sulfonamide-ozone reaction. The reaction of N,N-dimethylsulfamide with ozone was studied in detail because of its potential to form NDMA (Schmidt and Brauch, 2008; Trogolo et al., 2015; von Gunten et al., 2010). The ozone reaction is slow and does not lead to NDMA formation. However, in presence of bromide, N,N-dimethylsulfamide (DMS) reacts fast with hypobromous acid (HOBBr) \( k_{\text{HOBBr,DMS}} = 8 \times 10^6 \text{ M}^{-1} \text{s}^{-1} \) with the deprotonated form of N,N-dimethylsulfamide (Heeb et al., 2017)) to a bromamine. HOBBr is formed as a secondary oxidant from the reaction of bromide with ozone (Haag and Hoigne, 1983b). This intermediate is then further transformed to NDMA with a yield of about 50% (von Gunten et al., 2010; see above for kinetic assessment).

4.7. Organosulfur compounds

4.7.1. Kinetics

Ozonation of sulfur has been observed for at least 75 years, such as the reaction of aqueous 2-chloroethyl sulfide with ozone to produce the corresponding sulfoxide (Price and Bullitt, 1947). Sulfur compounds can exist in oxidation states between (-II) and (VI) (Fig. 13). S(VI) compounds such as sulfate do not react with ozone (von Sonntag and von Gunten, 2012). Sulfur-containing functional groups in oxidation states < VI (with the exception of sulfones and analogues) react with ozone, with generally higher \( k_{\text{O3}} \) with increasing sulfur electron density. Sulfoxides react sparingly with ozone (e.g., dimethylsulfoxide: \( k_{\text{O3}} = 8 \times 10^6 \text{ M}^{-1} \text{s}^{-1} \) (Pryor et al., 1984), and compounds with sulfur bonded to carbonyls are moderately reactive (e.g., molinate: \( k_{\text{O3}} = 5 \times 10^6 \text{ M}^{-1} \text{s}^{-1} \); S-Ethyl-N, N-dipropylthiocarbamate: \( k_{\text{O3}} = 5 \times 10^6 \text{ M}^{-1} \text{s}^{-1} \) (Chen et al., 2008). However, sulfuruous functional groups in lower oxidation states such as thioles, thioethers, and disulfides react readily with ozone.

Thiols and thioethers react very fast with ozone (cysteine: \( k_{\text{O3}} = 4 \times 10^6 \text{ M}^{-1} \text{s}^{-1} \); methionine: \( k_{\text{O3}} = 4 \times 10^6 \text{ M}^{-1} \text{s}^{-1} \) (Pryor et al., 1984). Thiocyclic acid was found to be completely oxidized within 9 minutes of exposure to 12 mg/L ozone (Gilbert and Hoffmann-Glew, 1990), but *OH was not suppressed, so the \( k_{\text{O3}} \) cannot be determined.

Reactions with disulfides are generally fast (e.g., bis(2-hydroxyethyl)
Sulfur groups | Structures | Oxidation state of S | Typical \( k_{O_3} \) | Examples
---|---|---|---|---
sulfones | \( \text{O} \) | +2 | low | dimethylsulfoxide \( k_{O_3} = 8 \, \text{M}^{-1}\text{s}^{-1} \)
sulfoxides | \( \text{O} \) | 0 | low | molinate \( k_{O_3} = 500 \, \text{M}^{-1}\text{s}^{-1} \)
thiocarbamates | \( \text{O} \) | 0 | moderate | cystine \( k_{O_3,\text{app},\text{pH}3} = 1 \times 10^5 \, \text{M}^{-1}\text{s}^{-1} \)
disulfides | \( \text{R} \) | -1 | high | cysteine \( k_{O_3} = 4 \times 10^4 \, \text{M}^{-1}\text{s}^{-1} \)
thiols | \( \text{R} \) | -2 | high | methionine \( k_{O_3} = 4 \times 10^4 \, \text{M}^{-1}\text{s}^{-1} \)

Fig. 13. Typical species-specific second-order rate constants for the reactions of organosulfur functional groups with ozone. References: (Chen et al., 2008; Hoigné and Bader, 1983a; Muñoz et al., 2001; Pryor et al., 1984)

Disulfide: \( k_{O_3} = 1.7 \times 10^5 \, \text{M}^{-1}\text{s}^{-1} \); trans-1,2-dithiane-4,5-diol: \( k_{O_3} = 2.1 \times 10^6 \, \text{M}^{-1}\text{s}^{-1} \) (Muñoz et al., 2001), with final products being S-alkyl sulfinites (Muñoz et al., 2001). Cystine is an unusual outlier as a slower-reacting disulfide (\( k_{O_3} = 1 \times 10^5 \, \text{M}^{-1}\text{s}^{-1} \) (Hoigné and Bader, 1983a)), a difference for which no explanation has been proposed.

Thioether-containing \( \beta \)-lactam antibiotics react quickly with ozone. Penicillin and cephalexin were both observed to react quickly with ozone at pH 7 with high \( k_{O_3,\text{app},\text{pH}7} \) of \( 4.8 \times 10^3 \, \text{M}^{-1}\text{s}^{-1} \) and \( 8.7 \times 10^4 \, \text{M}^{-1}\text{s}^{-1} \), respectively (Dodd et al., 2006). Penicillin has no other clearly oxidizable functional groups, so the \( k_{O_3,\text{app}} \) was interpreted as the species-specific \( k_{O_3} \) for the reaction with the thioether, but cephalexin also features olefin and primary amine functional groups, complicating the kinetic interpretation. Lincomycin and methicillin also react quickly at their thioether sites with \( k_{O_3,\text{app},\text{pH}7} \) of \( 6.75 \times 10^5 \, \text{M}^{-1}\text{s}^{-1} \) and \( 8.1 \times 10^4 \, \text{M}^{-1}\text{s}^{-1} \), respectively (Qiang et al., 2004; Jin et al., 2012). The thioether of ranitidine was reported to be highly reactive with ozone, with a species-specific \( k_{O_3} \) of \( 2.0 \times 10^5 \, \text{M}^{-1}\text{s}^{-1} \) (Jeon et al., 2016).

Data on ozone reactions with aromatic sulfur are scarce. Benzothiazole and analogues were reportedly abated during ozonation of synthetic wastewater (Derozo et al., 2011). However, no studies to date have reported species-specific \( k_{O_3} \) or products for benzothiazole reactions with ozone or \(^1\text{OH} \). Therefore, it cannot be determined whether the site of ozone attack was at the sulfur, the nitrogen, or an aromatic carbon site. Increased abatement rates at pH 8.5 compared to 5.8 suggested that \(^1\text{OH} \) may have been responsible for the benzothiazole degradation, implying a low second-order rate constant for the reaction with ozone. One study measured second-order rate constants for benzothiazole reactions with ozone and \(^1\text{OH} \) and found them to be \( k_{O_3} = 2.3 \, \text{M}^{-1}\text{s}^{-1} \) and \( k_{\text{OH}} = 6 \times 10^9 \, \text{M}^{-1}\text{s}^{-1} \), respectively (Valdes et al., 2004). The measurements were made in a solution with continuous ozone sparging, during which the concentration of ozone and \( ^1\text{OH} \) may be somewhat unstable. Nevertheless, these measurements are useful as preliminary estimates. After ozonation of secondary municipal wastewater at ~0.6 mg O\(_2\)/mg DOC, the benzothiazole concentration was found to decrease by 70% in one study (Hollender et al., 2009), but in another after ozonation at 0.81 mg O\(_2\)/mg DOC, benzothiazole was observed to be abated by only 7% (Margot et al., 2013), and the reason for the discrepancy is unclear.

4.7.2. Reaction Mechanisms

Information on the mechanisms and products of sulfur reactions with ozone are relatively scarce, but available evidence points to oxygen transfer reactions as the dominant pathway, with sulfoxides being the most commonly-reported product functional groups (reaction type (iv) in Scheme 1). Ozonation of methionine was found to convert the thioether to the corresponding sulfoxide as the quantitative product (Muñoz et al., 2001). Ozonation of thioglycolic acid was reported to first lead to a dimerization to the corresponding thioether, thiodiglycolic acid, although no mechanism was proposed (Gilbert and Hoffmann-Glew, 1990). This thioether was then reported to undergo a series of further ozonation reactions, first to the sulfoxide, then the corresponding sulfone, then to the sulfonic acid. Finally at high pH, fragmentation and further oxidation produced sulfate and oxalic acid (Gilbert and Hoffmann-Glew, 1990). Because no \(^1\text{OH} \) scavenger was used, involvement of \(^1\text{OH} \) in the fragmentation reaction seems likely. The thioether of penicillin G was reported to be ozonated quantitatively to the corresponding sulfoxide, while for cephalexin the Criegee product is also observed from alkene scission in addition to oxidation of the thioether (Dodd et al., 2010; von Sonntag and von Gunten, 2012).

Ozonation of reduced sulfur seems to occur generally via stepwise oxygen transfer reactions, increasing the oxidation state by +2 at each step (Scheme 1 (iv)). However, it is currently unknown why some reduced sulfur compounds appear to stop at the sulfoxide as a stable product, while others proceed further in the presence of ozone to the
sulfone, sulfonic acid, and possibly sulfate. In experiments with \(^{1}OH\) scavenging, direct ozone oxidation beyond sulfones (to sulfonic acids and sulfate) has not yet been conclusively established. The role of ozone versus \(^{1}OH\) in breaking C-S bonds to fragment sulfones is also unclear at present, however, \(^{1}OH\) involvement seems likely, as sulfones have no obvious available sites for ozone reactions.

### 4.8. Other functional groups

While the majority of research on ozonation of organic compounds has focused on electron rich moieties such as olefins, activated aromatic compounds, reduced nitrogen and reduced sulfur, ozone may attack other functional groups as well. Comparatively less is known about these reactions, but ozone has been shown to oxidize carbanions produced by the deprotonation of relatively strong carbon acids, to react fast with a \(\beta\)-diketone, and to directly functionalize certain carbon-hydrogen bonds by hydride transfer or other mechanisms.

#### 4.8.1. Carbanions

Despite a lack of obviously oxidizable functional groups (i.e., no carbon-carbon double bonds, no activated aromatic moieties, no lone pairs on N or S), some alkyl compounds with electron-withdrawing substituents have been observed to react with ozone. However, these reactions are typically slow and under realistic ozonation conditions, the reactions with \(^{1}OH\) will dominate (Fig. 5).

Nitroethane was reported to have a \(k_{O3,app} = 3.4 \text{ M}^{-1}\text{s}^{-1}\) at pH 7 (Lim et al., 2019). Ozonation of the amino acids serine (Le Lacheur and Glaze, 1996) and glycine (Berger et al., 1999; de Vera et al., 2017; McCurry et al., 2016) and other amino acids and amines (Essaiedi et al., 2022), produces nitrate upon exposure to excess ozone, potentially implicating further oxidation of an oxime (de Vera et al., 2017; Lim et al., 2019) or nitro intermediate (McCurry et al., 2016), the latter of which lacking any clear ozone attack sites.

One explanation for these reactions is an ozone attack of a carbanion conjugate base of electron-deficient structures such as nitroalkanes \((pK_a < 14)\) (Anslyn and Dougherty, 2006). Aqueous oxidation of carbanions has been reported for chlorine (Orvik, 1980), and has been proposed to explain halonitromethane formation from nitromethane (McCurry et al., 2016; Orvik, 1980; Shi and McCurry, 2020), and halonitroalkane formation from the \(\epsilon\)-nitro analogue of lysine during chlorination (McCurry et al., 2016). Less is known about carbanion reactions with ozone, but carbanion intermediates have been proposed in the synthesis literature as sites for electrophilic ozone attack. The carbanion resonance structure of nitrones was invoked to explain electrophilic attack of ozone on nitroene carbon (Erickson and Myszkiewicz, 1965), leading to aldehydes and \(\epsilon\)-nitroso compounds. Ozone reactions with deprotonated secondary nitro compounds to ketones were rationalized by invoking a nitronate structure (McMurry et al., 1974), which is in resonance with the corresponding nitroalkyl anion (Scheme 7). Deprotonation of a malonate derivative to the corresponding carbanion and subsequent carbanion oxidation was proposed to explain the hydroxylation of the central (acidic) carbon by ozone (White and Egger, 1984). Kinetic data on carbanion oxidation are scarce. Based on reported \(k\) for the reaction with chlorine, the deprotonation of nitromethane \((pK_a = 10.2)\) to nitromethyl anion is the rate-limiting step below pH \(\sim 11.5\), rather than oxidation of the corresponding carbanion (Orvik, 1980). It is unclear why deprotonation is the rate limiting step during neutral oxidation of nitromethane, in contrast to other weak acids in which the conjugate base is more reactive (e.g., phenol), for which the reaction can be successfully modeled assuming base in equilibrium with acid. Regardless, it appears likely that deprotonation of nitromethane will also be rate-limiting at neutral pH for the reaction with ozone, given that ozone reactions generally proceed with higher \(k_{O3}\) than reactions with chlorine (Deborde and von Gunten, 2008; von Gunten, 2003b).

The species-specific second order rate constant for HOCI reacting with the nitromethane anion was reported to be \(9.5 \times 10^2 \text{ M}^{-1}\text{s}^{-1}\) (Orvik, 1980), which is likely a lower bound for its reaction rate constant with ozone. To date, the only reported \(k_{O3}\) for carbanions are for nitroethane \((k_{O3,app} = 3.4 \text{ M}^{-1}\text{s}^{-1}\) at pH 7; (Lim et al., 2019)) and malonate \((k_{O3} = 7 \pm 2 \text{ M}^{-1}\text{s}^{-1};\) (Hoigné and Bader, 1983a)), wherefore determining structure-activity relationships is not possible. However, it is likely that as with phenols/phenolates or amines, carbanions and their conjugate acids will be subject to a tradeoff, in which increasing electron withdrawing substituents will decrease the \(pK_a\) and shift speciation from the unreactive neutral acid to the reactive base, while also decreasing electron density at the nucleophilic site (Scheme S1, SI).

To date, the net effect of these competing phenomena is not clear and should be investigated further. However, in each of these pathways, ozone would be competing against \(^{1}OH\) for reactive sites, and ozone reactions will rarely be more important than \(^{1}OH\) reactions, because at \(R_t\) values typical of water and wastewater \((10^{-8} - 10^{-9})\) (von Sonntag and von Gunten, 2012), ozone reactions with an apparent second-order rate constant below \(~5 - 50\) \(\text{M}^{-1}\text{s}^{-1}\) will be less important than \(^{1}OH\) reactions with the same compound (Fig. 5).

#### 4.8.2. \(\beta\)-Diketones

Despite the presence of electron-withdrawing carbonyl groups, simple \(\beta\)-diketones (e.g., acetylacetone, dimerdone) exhibit generally high reactivities towards various oxidants such as chlorine, chlorine dioxide, and permanganate (Deborde and von Gunten, 2008; Hoigné and Bader, 1994; Jäky et al., 2006). Acetylacetone shows very high reactivity towards ozone with \(k_{O3,app,\epsilon-Diketone} = 5.1 \times 10^5 \text{ M}^{-1}\text{s}^{-1}\) (Houska et al., 2021). The reason for the unusually high reactivity for \(\beta\)-diketones is not entirely clear. Like the nitroalkanes discussed in the previous

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**Scheme 7.** Potential pathway for the formation of nitrate from the ozonation of nitroalkanes.
section, β-diketones are present in different forms in aqueous solution due to tautomerization (keto and enol form) and dissociation (enol and enolate), with the enolate featuring a carbanion resonance structure (Scheme 8). The relative contributions of these different forms to ozone reactivity should be carefully assessed. Because β-diketones are common moieties of DOM, understanding the kinetics and mechanisms of their reactions with ozone is highly relevant to predict potential oxidation byproducts and warrants further investigation.

4.8.3. Hydrocarbons

For compounds with no ozone-reactive moieties, limited ozone reactivity has still been observed at the site of carbon-hydrogen bonds. Four possible mechanisms have been proposed, including H-abstraction, direct electron transfer, hydride transfer, and insertion (von Sonntag and von Gunten, 2012). The most ozone-reactive compound in this category, formate, has been reported to have a species-specific \( k_{O3}\) of 0.1 M\(^{-1}\)s\(^{-1}\) for the protonated form of simple aliphatic amines (von Sonntag and von Gunten, 2012), the \( k_{O3}\) of the protonated form is still very high, indicating the shift of the reaction site from the amine moiety to the olefin, the second reactive moiety. In this sense, the species-specific \( k_{O3}\) for the neutral and protonated forms of formate can be considered the moiety-specific \( k_{O3}\) for the amine and olefin moieties, respectively.

An example is tamoxifen (contains a tertiary amine and an olefin) of which species-specific \( k_{O3}\) for the neutral and the protonated forms are 3.2 \( \times \) 10\(^{8}\) M\(^{-1}\)s\(^{-1}\) and 1.6 \( \times \) 10\(^{8}\) M\(^{-1}\)s\(^{-1}\), respectively (Knoop et al., 2018). In contrast to the typical \( k_{O3}\) of < 0.1 M\(^{-1}\)s\(^{-1}\) for the protonated form of simple aliphatic amines (von Sonntag and von Gunten, 2012), the \( k_{O3}\) of the protonated tamoxifen is still very high, indicating the shift of the reaction site from the amine moiety to the olefin, the second reactive moiety. In this sense, the species-specific \( k_{O3}\) for the neutral and protonated forms of tamoxifen can be considered the moiety-specific \( k_{O3}\) for the amine and olefin moieties, respectively.

By combining the moiety-specific \( k_{O3}\) of the two reaction sites (amine and olefin) with the degree of protonation of the amine moiety, the fraction of ozone attacking at the amine moiety of tamoxifen at a specific pH can be expressed as (Eq. 6):

\[
\text{fraction of ozone attack at amine} = \frac{k_{O3,\text{amine}} \cdot (1 - a) + k_{O3,\text{amine-NH}} \cdot (a)}{k_{O3,\text{amine-NH}} \cdot (1 - a) + k_{O3,\text{amine-NH}} \cdot (a) + k_{O3,\text{olefin}}}
\]

where \( a = \frac{[H^+]}{[H^+] + [OH^-]} \) is the degree of protonation of the amine moiety of tamoxifen (R\(_2\)NH\(^{+}\) ↔ R\(_2\)N\(^{+}\)) based on the reported \( \text{pK}_a = 9.49 \) (Knoop et al., 2018). \( k_{O3,\text{amine-NH}} \) is the moiety-specific \( k_{O3}\) for the amine moiety (neutral form), i.e., 3.2 \( \times \) 10\(^{8}\) M\(^{-1}\)s\(^{-1}\) (Knoop et al., 2018). \( k_{O3,\text{olefin}} \) is the moiety-specific \( k_{O3}\) for the olefin moiety, approximated by the reported species-specific \( k_{O3}\) for the protonated tamoxifen, i.e., 1.6 \( \times \) 10\(^{8}\) M\(^{-1}\)s\(^{-1}\) (Knoop et al., 2018). \( k_{O3,\text{amine-NH-}} \) is the moiety-specific \( k_{O3}\) for the amine moiety as the protonated form, arbitrarily given 0.1 M\(^{-1}\)s\(^{-1}\) according to the typical \( k_{O3}\) for protonated amines.

Fig. 14 shows the calculated % ozone attack at the amine moiety for selected pharmaceuticals containing multiple reaction sites based on Eq. 6, by extending the approximation to other secondary reaction sites (aromatic and sulfur moieties).

Depending on the difference of moiety-specific \( k_{O3}\), the main reaction site at a specific pH can differ significantly (Fig. 14). For tamoxifen, the moiety-specific \( k_{O3}\) for the neutral tertiary amine is about four orders of magnitude higher than for the olefin (3.2 \( \times \) 10\(^{8}\) M\(^{-1}\)s\(^{-1}\) and 1.6 \( \times \) 10\(^{8}\) M\(^{-1}\)s\(^{-1}\), respectively (Knoop et al., 2018). This results in 98 % of ozone attack at the tertiary amine at pH 7, despite the predominance of the protonated form of the amine (tamoxifen \( \text{pK}_a = 9.5\)). In contrast, for nortriptyline with a comparable \( \text{pK}_a \) of 10.2, the difference in the moiety-specific \( k_{O3}\) is smaller (\( k_{O3} = 4.3 \times 10^{8}\) M\(^{-1}\)s\(^{-1}\) for the neutral secondary amine and 2.1 \( \times \) 10\(^{8}\) M\(^{-1}\)s\(^{-1}\) for the olefin (Benitez et al., 2013)). As a consequence, at pH 7, nortriptyline is oxidized by ozone mostly at the olefin moiety.

The \( \text{pK}_a \) of amine moieties also plays a role in determining the main reaction site for a given pH. The \( k_{O3}\) of the thiazine moiety of cephalexin is as high as the \( k_{O3}\) of the primary amine moiety (8.2 \( \times \) 10\(^8\) M\(^{-1}\)s\(^{-1}\) and 9.3 \( \times \) 10\(^8\) M\(^{-1}\)s\(^{-1}\), respectively (Dodd et al., 2006); see the structure in Figure S1, S2). At pH 7, cephalexin is attacked by ozone at the amine moiety with a moderate proportion (33%), because of a \( \text{pK}_a \) of 7.1, more acidic than typical primary amines due to the presence of a carbonyl as a neighboring group. At higher pH, the thiazine and the amino groups

#### Scheme 8
Different forms of acetylacetone by tautomerization, acid-base dissociation, and resonance, and the reported \( k_{O3}\text{app},pH7\) for acetylacetone. References are (Moriyasu et al., 1986), (Yudinoff, 1945), (Houska et al., 2021).
contribute similarly to the oxidation of cephalixin by ozone, due to the similar $K_a$ for the two moieties.

Many heterocyclic moieties contain an ionizable nitrogen as well as a carbon-carbon double bond within a cyclic structure. Therefore, identifying the main ozone reaction site within a heterocyclic moiety can be challenging. Regioselectivity prediction by quantum chemical computations could be especially useful in these cases. Computed molecular descriptors such as average local ionization energies, orbital energies, and bond dissociation energies can describe where the ozone reaction would preferentially take place within a molecule (Tentscher et al., 2019) and have been applied recently in an ozone pathway prediction system (Lee et al., 2017a) and for studying the ozone reaction for selected heterocyclic compounds (Chen et al., 2019; Yao et al., 2020).

5. Kinetics of the reaction of ozone with macromolecules: Antibiotic resistance gene (ARG) inactivation and DNA oxidation

Some macromolecules (e.g., DOM) are highly heterogeneous and therefore their reactions with ozone are largely assessed in terms of the effect on bulk parameters (e.g., specific UV absorbance, EDC, etc.) or elemental ratios (see Section 3.2). However, because biomolecules (e.g., proteins, nucleic acids) are often macromolecules consisting of known sequences of discrete subunits (e.g., amino acids, nucleobases), they present an opportunity to predict the kinetics of macromolecule in ozonation as the aggregation of reactions with individual subunits, for which reactivities with ozone are generally known. One example is the reaction of ozone with antibiotic resistance genes, discussed below.

5.1. Ozonation of Antibiotic Resistant Bacteria

Concerns about the potential for wastewater treatment facilities to act as breeding grounds for antibiotic resistant bacteria (Dodd, 2012; Pruden et al., 2013; Rizzo et al., 2013; Zarei-Baygi et al., 2019) have led to interest in the use of ozone, and other disinfectants, for antibiotic resistance control. Antibiotic resistance inactivation presents a special challenge for chemical disinfectants. For ordinary microorganisms the outcome is agnostic of the means of action (i.e., inactivating a bacterium by oxidizing its cell membrane proteins and/or its DNA both achieve the same outcome). However, for antibiotic resistant bacteria, inactivating the organism does not guarantee loss of the potential for antibiotic resistance, as multiple mechanisms of antibiotic resistance gene (ARG) horizontal transfer exist, including uptake by non-resistant organisms of ARGs from dead cells, particularly if their cell membranes are lysed during disinfection (Dodd, 2012). Additionally, several studies have indicated the possibility for antibiotic resistance ‘rebound’ after disinfection, in which the absolute and/or relative abundance of antibiotic resistant bacteria (ARB) increases after disinfection (Czekalski et al., 2016; Jakovides et al., 2019). Finally, the disinfection process may select for ARBs over non-resistant populations (Alexander et al., 2016). Therefore, in addition to the interest in ARB inactivation, attention has more recently been focused on inactivation of the ARGs themselves, which is the primary subject of this critical review.

As with most microorganisms, ozone rapidly inactivates antibiotic resistant bacteria (Pak et al., 2016), achieving for instance 3 – 4-log inactivation of sulfonamide-resistant E. coli J53 after application of 0.08 mg/L ozone in buffered deionized water (Czekalski et al., 2016). ARG inactivation in secondary wastewater effluent is also highly effective. ~2-log inactivation of trimethoprim- or sulfamethoxazole-resistant E. coli was achieved with specific ozone doses as low as 0.25 mg O₃/mg DOC in secondary effluent, and was unaffected by the addition of hydrogen peroxide (Jakovides et al., 2019).

5.2. Ozonation of Antibiotic Resistance Genes

Inactivation of ARGs by ozone has been widely measured, including in clean systems (Czekalski et al., 2016; He et al., 2019), secondary wastewater effluent (Alexander et al., 2016; Ben et al., 2017; Czekalski et al., 2016; Jakovides et al., 2019; Lamba and Ahammad, 2017; Zheng et al., 2017; Zhuang et al., 2015), and have been applied recently in an ozone pathway prediction system (Lee et al., 2017a) and for studying the ozone reaction for selected heterocyclic compounds (Chen et al., 2019; Yao et al., 2020).

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\[ k_{\text{O, DNA}} = mk_a + nk_G + qk_C + pk_T \]  

where \( m, n, q, \) and \( p \) are the sums of adenine (A), guanine (G), cytosine (C), and thymine (T) bases per strand of DNA, and \( k_a, k_G, k_C, \) and \( k_T \) are the respective second-order rate constants for their reactions with ozone.

Before calculating the overall predicted rate of DNA oxidation, the acid-base speciation of nucleobases must be accounted for by converting species-specific \( k_{O_3} \) to pH-dependent \( k_{O_3, app} \) for each nucleobase, as shown in Table S7 (SI). Computing this overall predicted \( k_{O_3} \) from the shortest (266 bp) ampiclon in (He et al., 2019), leads to an overall expected \( k_{O_3} \) for the reaction between ozone and the ampiclon of \( 6.49 \times 10^6 \) M\(^{-1}\)s\(^{-1}\) and a specific (per nucleobase) \( k_{O_3} \) of \( 2.40 \times 10^6 \) M\(^{-1}\)s\(^{-1}\) (Table S8, SI), approximately 370 times higher than that observed by (He et al., 2019), and 59 times higher than observed with calf DNA (Theruvathu et al., 2001).

The reasons for this discrepancy between the predicted and observed \( k_{O_3} \) for DNA in clean systems are not immediately clear, but several possibilities exist. One option is analytical artifacts: DNA abatement during disinfection is commonly monitored by qPCR (McKinney and Pruden, 2012; Yoon et al., 2017), but a single modification at certain sites may be insufficient to eliminate or drastically reduce amplification efficiency (Sikorsky et al., 2004; 2007), leading to underprediction of the extent of DNA damage. Additionally, different amplification efficiencies of damaged DNA are observed when using different polymerase reagent kits (Hajibabaei et al., 2005), and certain polymerase reagents are specifically designed to amplify damaged DNA (Robertson et al., 2014). Another possibility is tertiary structure leading to reduced reactivity due to steric shielding of ozone targets from the bulk solution, as is observed during protein oxidation, in which some oxidizable residues may be buried in the hydrophobic core of the protein and unavailable for oxidation reactions (Choe et al., 2015; Jensen et al., 2012; Lundeen and McNeill, 2013; Sivey et al., 2013). Finally, one possibility is to explain the dramatically lower \( k_{O_3} \) observed for DNA than would be predicted based on the sum of the constituent nucleobases is an increase in the \( pK_a \) of nucleobases, due to a combination of hydrogen bonding between bases and pi-stacking interactions. These interactions have been reported to increase the \( pK_a \) of protonated nucleobases by up to 3–4 orders of magnitude, which in some cases (A, C), could convert the majority of the bases from their neutral or negatively-charged reactive forms to their charged forms, which are nearly inert (Tang et al., 2007; Wilcox et al., 2011). However, performing the same calculation as in Table S7 (SI) but assuming that the \( pK_a \) of all nucleobases is \( 1 \) units higher produces only a modest decrease in calculated \( k_{O_3, app} \). Therefore, this \( pK_a \) shift alone is unlikely to explain the discrepancy between calculated and observed DNA oxidation. The reason that a dramatic \( pK_a \) shift fails to produce a major change in outcome is likely that overall reactivity appears dominated by thymine (Table S7, SI), and the protonated form of thymine is still quite reactive with ozone (\( k_{O_3} = 4.2 \times 10^6 \) M\(^{-1}\)s\(^{-1}\)).

In contrast to ozonation of ARBs in clean systems or of extracted DNA, inactivation of antibiotic resistance genes in real wastewater effluent and sludge is relatively ineffective (Alexander et al., 2016; Ben et al., 2017; Cengiz et al., 2010; Iakovides et al., 2019; Lamba and Ahammad, 2017; Pei et al., 2016; Zheng et al., 2017; Zhuang et al., 2015). ARB and ARG inactivation generally require comparable doses as ~80% abatement of Group I and Group II microorganisms identified by (Lee et al., 2013) as being highly (\( k_{O_3, app, pH7} > 10^5 \) M\(^{-1}\)s\(^{-1}\)) and moderately (10 M\(^{-1}\)s\(^{-1} < k_{O_3, app, pH7} < 10^5 \) M\(^{-1}\)s\(^{-1}\)) reactive with ozone (Fig. 15). In Fig. 15, Group II is further divided into Group IIA (10\(^3\) M\(^{-1}\)s\(^{-1} < k_{O_3, app, pH7} < 10^5 \) M\(^{-1}\)s\(^{-1}\)) and Group IIB (10 M\(^{-1}\)s\(^{-1} < k_{O_3, app, pH7} < 10^6 \) M\(^{-1}\)s\(^{-1}\)) using micropollutant abatement data from Bourgin et al. (2018). Ozone doses are often constrained in practice by ozone formation, which strongly depends on the specific ozone doses (Soltermann et al., 2017). Specific ozone doses shown to achieve 2-log inactivation of ARB (~0.4 mg O\(_3\)/mg DOC) (Czekalski et al., 2016) are generally at or below the threshold for moderate bromate formation (Soltermann et al., 2017), while specific ozone doses to achieve 2-log abatement of ARGs (Alexander et al., 2016; Czekalski et al., 2016) are in the ozone dose range with moderate to high bromate yields (Fig. 15).

Reconciling the paradox of effective ARG inactivation in clean systems or with extracted DNA with relatively ineffective inactivation in practical systems will require further studies. Possible contributors to this discrepancy include the location of the ARGs (chromosomal vs. plasmid, intracellular vs. extracellular) (Dodd, 2012), and issues potentially related to the lower reactivity of DNA than one would predict as discussed above.

6. Conclusions

Due to a significant knowledge gain in the ozone chemistry in aqueous solution and shifts in application of ozonation processes, this critical review highlights the most important aspects of the ozone chemistry and applications as follows:

- A paradigm shift in the application of ozone for impaired water qualities (enhanced wastewater treatment, water reuse) needs a new focus on higher levels and different types of dissolved organic matter (DOM) than for typical drinking water applications. Furthermore, bromate formation has to be carefully monitored due to sometimes higher and variable levels of bromide.
- Several bulk parameters (UV absorbance, electron donating capacity) and high resolution mass spectrometry can be applied to elucidate changes in DOM properties and/or used as proxies to monitor and control ozonation processes for micropollutant abatement.

![Fig. 15. Specific ozone doses in wastewater effluent required for 2-log inactivation of ARBs and ARGs, and specific ozone dose ranges required for ~80% abatement of the four categories of micropollutants (MPs) discussed in (Lee et al., 2013)](image-url)
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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2022.118053.

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