

# **Report for 2002GA11B: Investigation of Chlorination and Ozonation of Antibiotics Detected in Georgia Waters**

- Water Resources Research Institute Reports:
  - Huang, Ching-Hua, 2003, "Investigation of Chlorination and Ozonation of Antibiotics Detected in Georgia Waters," Georgia Water Resources Institute, Georgia Tech, Atlanta, GA., 12p.
- Conference Proceedings:
  - Dodd, M. C. and Ching-Hua Huang, 2003, " Chemical Oxidation of Aquatic Antibiotic Microcontaminants by Free and Combined Chlorine, in proceedings of the AWWA Water Quality and Technology Conference, Philadelphia, PA.
- Articles in Refereed Scientific Journals:
  - Dodd, M. C. and Ching-Hua Huang, 2003, "Oxidation of Aquatic Antibiotic Microcontaminants by Free and Combined Chlorine. 1. Kinetics," Environ. Sci. Technol. 2003, in preparation.
  - Dodd, M. C. and Ching-Hua Huang, 2003, "Oxidation of Aquatic Antibiotic Microcontaminants by Free and Combined Chlorine. 2. Products and Reaction Pathways," Environ. Sci. Technol. 2003, in preparation.

**Report Follows:**

**Investigation of Chlorination and Ozonation of Antibiotics Detected in  
Georgia Waters**

Final Report

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## Executive Summary

This study investigates kinetics and degradation pathways for the reactions between sodium hypochlorite (free available chlorine – FAC) or chloramines (combined chlorine – CC) and substrate compounds representative of three commonly prescribed and environmentally prevalent classes of antibiotics: fluoroquinolones, sulfonamides, and dihydrofolate reductase (DHFR) inhibitors. Pseudo-first-order kinetics was observed for oxidation of the fluoroquinolone antibiotic enrofloxacin (EF), sulfonamide antibiotic sulfamethoxazole (SMX), and DHFR inhibitor trimethoprim (TMP) by FAC. Second-order rate constants for reactions involving EF, SMX, and TMP were calculated from observed pseudo-first-order constants, on the assumption that concentration of oxidant remained essentially constant throughout the monitoring periods of each kinetic experiment. EF, SMX, and TMP were directly oxidized by FAC at varying rates, where second-order rate constants for reaction with FAC at pH 7 were measured as  $4.80 \times 10^2 \text{ M}^{-1} \text{ s}^{-1}$ ,  $1.53 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$ , and  $4.74 \times 10^1 \text{ M}^{-1} \text{ s}^{-1}$ , respectively. In contrast, significantly lower second-order rate constants ( $9.6 \times 10^{-1} \text{ M}^{-1} \text{ s}^{-1}$ ,  $2.5 \times 10^{-1} \text{ M}^{-1} \text{ s}^{-1}$ , and  $6.0 \times 10^{-2} \text{ M}^{-1} \text{ s}^{-1}$  for EF, SMX, and TMP, respectively) were measured for reaction with CC at pH 7.

Solution pH exhibited a marked influence on reaction rates for all three antibiotic classes. Consideration of predominant antibiotic species and corresponding reaction rates at specific pH values allowed a preliminary determination of reactive sites within the antibiotics. Compounds representing the hypothesized reactive and non-reactive portions of each antibiotic class were utilized to verify the proposed location(s) of reactivity. LC/MS and  $^1\text{H}$ -NMR were used where applicable to identify reaction products and to assess product evolution during each reaction time course. Product characterization of CF reaction mixtures indicates the formation of a number of products, represented primarily by four major degradates corresponding to  $m/z$  263, 297, 306, and 340 (corresponding to full or partial dealkylation of the piperazine ring, and – in two cases – substitution of Cl on the quinolone structure's aromatic ring). The relatively rapid oxidation of SMX is accompanied by what appears to be a unique radical-chain cleavage of the S-N sulfonamide bond to yield 3-amino-5-methylisoxazole and an unknown product. S-N bond cleavage, combined with N-chlorination of the aniline functional group, also appears to lead to the formation of relatively stable dimers and a number of lower mass products. Chlorination of TMP yields primarily stable, multiply-halogenated products – with the parent compound undergoing relatively minor structural modification.

The results of this investigation indicate that representative members of these three antibiotic classes are substantially degraded under conditions simulating chlorination of water supplies during disinfection processes, yielding a wide variety of lower and higher mass degradates.

## Research Objectives

The proposed objective of this project was to determine the removal efficacy of chlorination and ozonation treatment processes for antibiotic compounds that had been commonly detected in Georgia waters with the aim of elucidating reaction kinetics and mechanisms. Studies were carried out to meet the research objective with a focus on oxidation of antibiotics by free chlorine and combined chlorine. Oxidation of antibiotics by ozone was not investigated in this study. The modification of the research objective was necessary based on several factors including new publications of ozonation of antibiotics by other research groups at the early stage of this project<sup>1</sup>, high level of complexity of the reactions between antibiotics and chlorine that warranted a more detailed investigation, and the relatively short period of one year for this project.

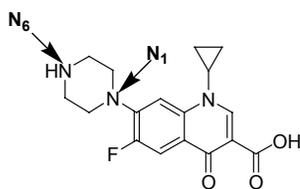
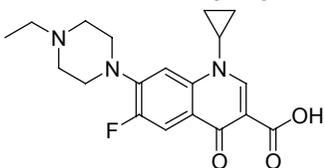
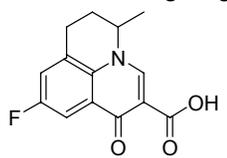
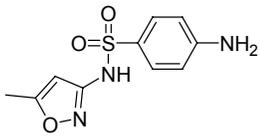
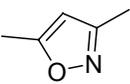
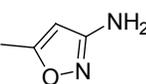
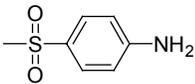
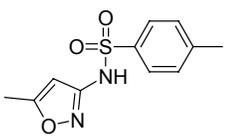
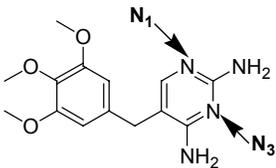
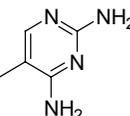
## Research Results

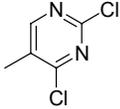
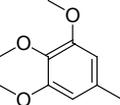
Significant progress has been made to meet the modified research objective. Results of this study have demonstrated that representative members of three common antibiotic classes are substantially degraded under conditions simulating chlorination of water supplies during disinfection processes. Reaction kinetics have been successfully described using a modified second-order kinetic model. Evaluation of the pH-dependencies of the reaction kinetics, kinetic results of an array of structurally-related compounds, and characterization of reaction products have elucidated the reactive sites of the antibiotic compounds and aided determination of reaction pathways. The research results are summarized in the following sections. This study has yielded several publications<sup>2-4</sup>, in which more details of the investigation can be found.

### Antibiotics and Related Compounds in this Study

A range of antibiotics including ciprofloxacin (CF), enrofloxacin (EF), flumequine (FLU), sulfamethoxazole (SMX) and trimethoprim (TMP) were examined in this study (see Table 1 for their structures). These antibiotics represent three commonly prescribed classes of modern antibiotics: fluoroquinolones, sulfonamides, and dihydrofolate reductase (DHFR) inhibitors. These three classes of antibiotics were also frequently detected in environmental occurrence studies in recent years<sup>5-7</sup>. The investigation was undertaken with the intent of quantifying reaction kinetics and clarifying reaction pathways involved in oxidative degradation of antibiotics by free available chlorine (FAC) and combined chlorine (CC) under conditions associated with chlorine-based municipal wastewater and drinking water disinfection processes. Structurally-related compounds (Table 1) that correspond to either the hypothesized reactive or inactive portions of each antibiotic class were also examined for their oxidation by FAC in order to verify the site(s) of reaction.

**Table 1. Selected Antibiotic Compounds and Associated Structures**

Compound	Structure	Molecular Weight	pK <sub>a</sub>
Ciprofloxacin (CF)		331.35	pK <sub>a1</sub> =6.43, pK <sub>a2</sub> =8.49 <sup>18</sup>
Enrofloxacin (EF)		359.39	pK <sub>a1</sub> =6.06, pK <sub>a2</sub> =7.7 <sup>18</sup>
Flumequine (FLU)		261.25	pK <sub>a1</sub> =6.42 <sup>19</sup>
Sulfamethoxazole (SMX)		253.28	pK <sub>a1</sub> =1.83, pK <sub>a2</sub> =5.57 <sup>20</sup>
3,5-dimethylisoxazole (DMI)		97.12	NA
3-amino-5-methylisoxazole (AMI)		98.1	pK <sub>a1</sub> =2.63 <sup>21</sup>
4-aminophenyl methyl sulfone (APMS)		171.22	pK <sub>a1</sub> =1.48 <sup>22</sup>
4-methyl-N-(5-methyl-isoxazol-3-yl)-benzenesulfonamide (MMIB)		252.29	pK <sub>a1</sub> =10.58 <sup>21</sup>
Trimethoprim (TMP)		290.32	pK <sub>a1</sub> =1.32 <sup>23</sup> , pK <sub>a2</sub> =7.45 <sup>24</sup>
2,4-diamino-5-methyl pyrimidine (DAMP)		124.14	pK <sub>a1</sub> =5.15, pK <sub>a2</sub> =7.54 <sup>21</sup>

2,4-dichloro-5-methylpyrimidine (DCMP)		163.00	NA
3,4,5-trimethoxytoluene (TMT)		182.22	NA

## Materials and Methods

**Chemical Reagents.** Sulfamethoxazole (SMX), ciprofloxacin (CF) hydrochloride, enrofloxacin (EF), and flumequine (FLU) were obtained from ICN Biomedicals (Irvine, California). Trimethoprim (TMP), 2,4-dichloro-5-methylpyrimidine (DCMP), 3,4,5-trimethoxytoluene (TMT), 3,5-dimethylisoxazole (DMI), 3-amino-5-methylisoxazole (AMI), and 4-aminophenyl methyl sulfone (APMS) were purchased from Sigma-Aldrich (St. Louis, MO). 2,4-diamino-5-methylpyrimidine (DAMP) was purchased from Daniels Fine Chemicals (Edmonton, Alberta, Canada). 4-methyl-N-(5-methyl-isoxazol-3-yl)-benzenesulfonamide (MMIB) was purchased from ASINEX (Moscow, Russia). All chemical standards were of reagent grade and were used without further purification. All reagent solutions (e.g., buffers, stocks, oxidants, quenching agents) were prepared using Barnstead Nanopure<sup>®</sup> water (Dubuque, IA). 100 mg/L stock solutions of all compounds (for use in kinetic experiments) were prepared in 10% methanol.

Aqueous sodium hypochlorite solutions (~7%) from Fisher Scientific (Pittsburgh, PA) were diluted to ~100 mg/L FAC for use in kinetic experiments. FAC stocks were periodically standardized iodometrically<sup>8</sup>. N,N-diethyl-*p*-phenylenediamine (DPD) was used through either DPD colorimetry or DPD-FAS titrimetry to measure free chlorine residual concentrations after completion of kinetic experiments<sup>8</sup>.

Pre-formed chloramine stocks were prepared at pH values of 4.5, 5, 6, 7, 8, and 9 in 0.1 M acetate (pH ≤ 5), 0.1 M phosphate (6 ≤ pH ≤ 8), and 0.025 M borate (pH 9) buffers, with modifications to the methods of Chapin<sup>9</sup>. Solutions of NH<sub>4</sub>Cl and FAC were combined at 25°C under completely-mixed conditions to produce ~100 mg/L of CC, at 2:1 NH<sub>4</sub>Cl:FAC molar ratios. CC stocks were prepared prior to each experiment, temporarily stored at <5°C, and used within 24 hours of generation. CC concentrations were standardized using DPD-FAS titrimetry.

**Reaction Kinetics Monitoring.** Temperature was maintained at 25°C in all experiments using a recirculating water bath connected to an acrylic water tank. Reaction solutions were partially immersed in the water tank and stirred with Teflon-coated stir bars using a 15-position magnetic stir-plate. 0.01 M acetate (pH 4, 4.5, 5), phosphate (pH 6, 7, 8), and borate (pH 9) buffers were used to maintain pH. Reactions were initiated by addition of appropriate volumes of FAC or CC stock (to achieve 10:1 oxidant:substrate ratio) to solutions containing 500 µg/L of substrate and 0.01 M buffer, under completely-mixed conditions, at 25°C. Reactions involving oxidation of SMX and TMP by CC were monitored by HPLC with ultraviolet detection immediately after sampling, without quenching. All other reactions were monitored by quenching 1-mL samples of each reaction solution with either NH<sub>4</sub>Cl/tris-hydroxymethyl aminomethane (THAM) or sodium thiosulfate (NH<sub>4</sub>Cl/THAM for SMX, TMP, APMS, and DAMP; Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> for all other

compounds) at appropriate time intervals and analyzing by HPLC with fluorescence or ultraviolet detection. An Agilent 1100 series HPLC system equipped with a 5  $\mu\text{m}$  particle-diameter, 4.6 mm  $\times$  250 mm Zorbax RX-C18 column, a fluorescence detector and a UV/Vis diode-array detector was used to monitor parent compounds and product formation during the oxidation reactions.

Reaction of an antibiotic or surrogate compound with chlorine oxidant can be described as a bimolecular reaction: Substrate + Oxidant  $\rightarrow$  Products. At least a 10-fold excess of chlorine oxidant with respect to substrate was always employed in the kinetic experiments in this study to ensure pseudo-first-order conditions. Therefore, using an approach similar to that used by von Gunten and Oliveras<sup>10</sup>, oxidant concentration was assumed to be constant and used to determine the second-order rate constant according to equation (1):

$$-\frac{d[\text{substrate}]_T}{dt} = k'_{app}[\text{substrate}]_T = k''_{app}[\text{oxidant}]_T[\text{substrate}]_T \quad (1)$$

where  $k'_{app}$  (in  $\text{S}^{-1}$ ) =  $k''_{app}[\text{oxidant}]_T$  or  $k''_{app}$  (in  $\text{M}^{-1}\text{S}^{-1}$ ) =  $\frac{k'_{app}}{[\text{oxidant}]_T}$

Kinetic experiments involving EF, SMX and TMP were conducted in triplicate, whereas those involving all other compounds were conducted in duplicate. 95% confidence limits were calculated and reported with all rate constants as error bars in relevant graphs.

**Product Characterization.** LC/MS was used to analyze unquenched reaction solutions of CF, SMX, and TMP. An Agilent 1100 series HPLC system equipped with a 4.1  $\mu\text{m}$  particle-diameter, 2.1 mm  $\times$  150 mm Zorbax SB-C18 column, a UV/Vis DAD, and an 1100 series quadrupole mass spectrometer was used in the characterization of reaction products. In order to identify peaks observed in HPLC chromatograms, LC/MS was also utilized to analyze appropriate fractions collected from samples initially resolved via HPLC. Fractions were collected (using an ISCO Foxy, Jr. fraction collector) for each major product peak. These dilute fractions were subsequently reconcentrated by evaporating under nitrogen gas at  $\sim 50^\circ\text{C}$  and analyzed by LC/MS to verify their identity.  $\text{H}^1$ -NMR was utilized to provide structural information for an unknown oxidation product of SMX. One-dimensional and 2-D COSY  $\text{H}^1$ -NMR spectra were obtained using a Bruker AMX 400 NMR instrument.

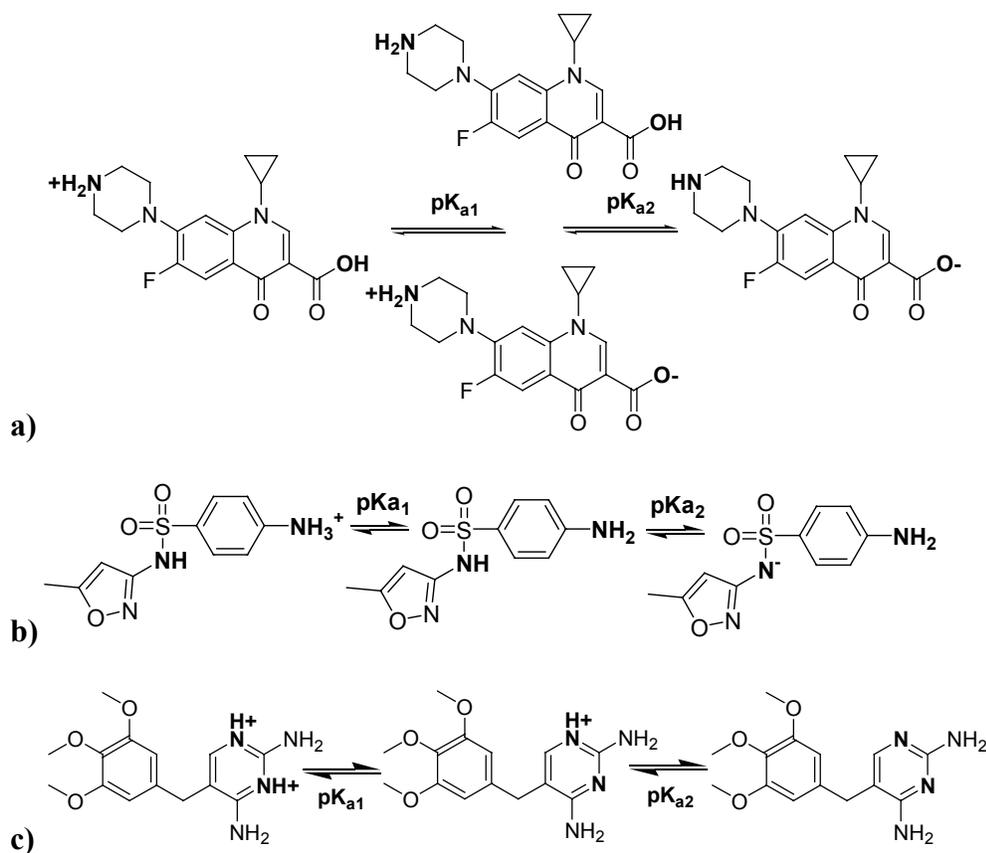
## Results and Discussion

**Kinetics of Reactions with Free Chlorine.** Solution pH strongly affects the speciation of free chlorine oxidant and antibiotic substrate, where relevant antibiotic speciation patterns are shown in Figure 1. Second-order rate equations incorporating pH-dependent speciation of both substrate and free chlorine oxidant have been utilized previously to model observed pH-dependent kinetics<sup>11,12</sup>. A similar kinetic model has been developed in this investigation to help explain variations in rate constants for degradation of antibiotics by FAC at different pH values. Six individual sub-reactions (comprised of the six possible combinations amongst the three antibiotic and two oxidant species) may be considered as contributing to the overall reactions between free chlorine

and the antibiotic compounds. These six sub-reactions can be incorporated into the second-order kinetic expression as shown in equation (1).

$$\frac{d[\text{substrate}]_T}{dt} = -k_{00}[\text{HOCl}][\text{H}_2\text{A}] - k_{10}[\text{OCl}^-][\text{H}_2\text{A}] - k_{01}[\text{HOCl}][\text{HA}] - k_{11}[\text{OCl}^-][\text{HA}] - k_{02}[\text{HOCl}][\text{A}] - k_{12}[\text{OCl}^-][\text{A}] \quad (1)$$

where  $k_{00-12}$  represent the specific second-order rate constants corresponding to each combination of reactant species.



**Figure 1.** Speciation patterns of: a) CF, b) SMX, and c) TMP

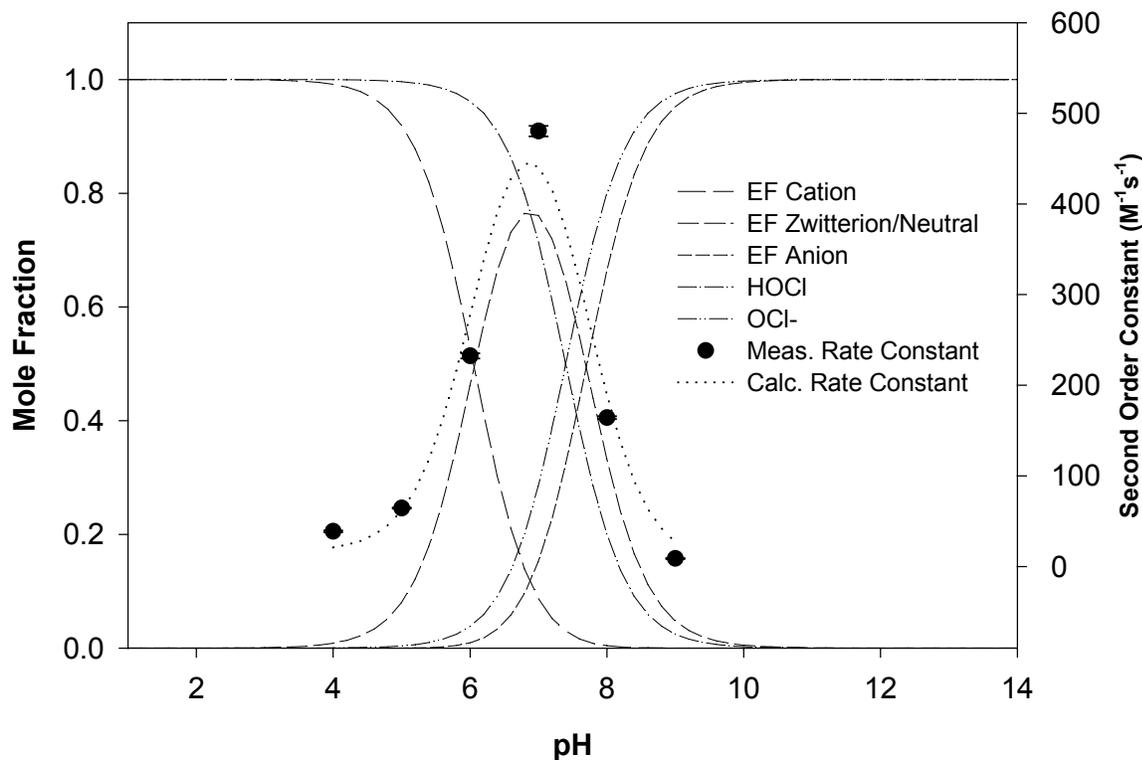
Application of principal component analysis (PCA)<sup>13</sup> to the linear systems formulated with this equation allows simplification to yield less complex models for each antibiotic compound. The revised models for oxidation of EF, SMX, and TMP by FAC can be represented by equations (2), (3), and (4), respectively:

$$\frac{d[\text{EF}]_T}{dt} = -k_{00}\alpha_0[\text{ox.}]_T\alpha'_0[\text{sub.}]_T - k_{01}\alpha_0[\text{ox.}]_T\alpha'_1[\text{sub.}]_T - k_{02}\alpha_0[\text{ox.}]_T\alpha'_2[\text{sub.}]_T \quad (2)$$

$$\frac{d[\text{SMX}]_{\text{T}}}{dt} = -k_{00}\alpha_0[\text{ox.}]_{\text{T}}\alpha'_0[\text{sub.}]_{\text{T}} - k_{01}\alpha_0[\text{ox.}]_{\text{T}}\alpha'_1[\text{sub.}]_{\text{T}} \quad (3)$$

$$\frac{d[\text{TMP}]_{\text{T}}}{dt} = -k_{00}\alpha_0[\text{ox.}]_{\text{T}}\alpha'_0[\text{sub.}]_{\text{T}} - k_{01}\alpha_0[\text{ox.}]_{\text{T}}\alpha'_1[\text{sub.}]_{\text{T}} - k_{02}\alpha_0[\text{ox.}]_{\text{T}}\alpha'_2[\text{sub.}]_{\text{T}} - k_{12}\alpha_1[\text{ox.}]_{\text{T}}\alpha'_2[\text{sub.}]_{\text{T}} \quad (4)$$

The apparent rate constants,  $k_{ij}$ , for reaction between hypochlorous acid/hypochlorite and each individual substrate species can be calculated from experimental data using a matrix algebra routine written in MATLAB. Using equations (2), (3), and (4), the overall apparent second-order rate constant,  $k''_{app}$ , can be determined from the reaction-specific constants,  $k_{ij}$ , for EF, SMX, or TMP at any pH value within the range considered. An example of measured second-order rate constants and fitted curves based on the simplified models is shown in Figure 2, for reaction of EF with FAC.



**Figure 2.** pH-dependency of apparent second order rate constants for reaction of FAC with EF

The second-order pH-dependency model serves as a useful qualitative tool in evaluating the importance of each antibiotic and oxidant species in the overall oxidation reactions. This can in turn aid in identification of the sites of free chlorine attack on the antibiotics. Experimental data suggest that attack of EF, SMX, and TMP by free chlorine is tied to protonation and

deprotonation of basic or acidic nitrogen groups within each compound, as well as of the oxidant species.

Oxidation of EF appears to occur at the N<sub>6</sub> nitrogen of the compound's piperazine ring (Table 1), as evidenced by the decrease in reactivity with FAC for pH below ~7, which corresponds to a decrease in neutral/Zwitter ionic EF and an increase in cationic (protonated) EF. Attack of SMX appears to occur at the compound's amido nitrogen, as shown by a decrease in reactivity below pH ~5.6, corresponding to an increase in concentration of the neutral SMX relative to the deprotonated anionic SMX. However, additional experimental data is necessary to verify this seemingly unlikely mechanism. TMP appears to undergo oxidative attack on the compound's diaminopyrimidine moiety, as indicated by a drop in reactivity below pH ~7.7, corresponding to an increase in concentration of monoprotonated TMP relative to neutral TMP. However, TMP also appears to undergo attack at another site, as indicated by the sharp increase in reactivity for pH < 5. This additional site of oxidative attack on the TMP structure is likely located on its trimethoxy moiety, which could be expected to exhibit higher susceptibility to FAC oxidation with increasing acidity<sup>14</sup>. The validity of these conclusions has been further investigated through the use of various antibiotic substructures and structural surrogates in additional oxidation experiments, in conjunction with structural characterization of reaction products, as will be discussed below.

**Kinetics of Reactions with Combined Chlorine.** Reactions between CC and EF, SMX, or TMP were extremely slow compared to those with FAC, as typified by measured second-order rate constants of  $9.6 \times 10^{-1} \text{ M}^{-1}\text{s}^{-1}$ ,  $2.5 \times 10^{-1} \text{ M}^{-1}\text{s}^{-1}$ , and  $6.0 \times 10^{-2} \text{ M}^{-1}\text{s}^{-1}$  for reaction of EF, SMX, and TMP, respectively, with CC at pH 7. In contrast, measured reaction rate constants for reaction of EF, SMX, and TMP with FAC were  $4.80 \times 10^2 \text{ M}^{-1}\text{s}^{-1}$ ,  $1.53 \times 10^3 \text{ M}^{-1}\text{s}^{-1}$ , and  $4.74 \times 10^1 \text{ M}^{-1}\text{s}^{-1}$ , respectively, at pH 7. In fact, TMP exhibited discernible reactivity toward chloramines only at pH 7, after nearly 40 hours of reaction time.

**Reactions of Substructures and Structural Surrogates with FAC.** Additional kinetic studies – in which substructures or structural surrogates of each antibiotic were subjected to application of FAC – were performed in order to verify the locations of proposed reaction sites. FLU, DMI, MMIB, and DCMP (Table 1) were all unreactive toward FAC. Oxidation studies involving fluoroquinolones and their substructure FLU – which represents a fluoroquinolone lacking the characteristic piperazine ring – show that the reactive portion of the fluoroquinolone antibiotics is located on the piperazine ring, presumably at the N<sub>6</sub> amino nitrogen, as indicated by kinetic results for EF. Oxidation studies involving DMI, coupled with those involving APMS (which contains the aniline amino-nitrogen but no sulfonyl amido-nitrogen) and MMIB (which contains the sulfonyl amido-nitrogen, but not the aniline amino-nitrogen), show that direct oxidative attack of the SMX structure occurs on its amino-nitrogen. However, as noted in the pH-dependency of apparent kinetic rate constants for reaction of SMX with FAC, overall oxidation of the SMX structure is further modulated by protonation of the compound's amido nitrogen via an effect that is not completely clear at the current stage. Oxidation studies involving TMP and its substructures verify that reaction rates are influenced primarily by speciation of the 2,4-diaminopyrimidinyl moiety for pH ≥ 5, while interactions with the 3,4,5-trimethoxybenzyl moiety predominate for pH < 5. This indicates that oxidative attack of the TMP structure occurs

primarily on the 3,4,5-trimethoxybenzyl ring under highly acidic conditions, and on the 2,4-diaminopyrimidine moiety for mildly acidic to basic conditions.

### Product Characterization and Proposed Degradation Pathways

**Ciprofloxacin.** CF was chosen as a representative FQ in product characterization studies as it is essentially a template common to all other FQs (Table 1). LC/MS analyses of oxidation products generated by reaction of CF with FAC revealed a number of transient products, which appear to be N-chlorinated intermediates: m/z 352 (1 Cl), 366 (1 Cl), 374 (2 Cl), and 408 (3 Cl). The number of chlorines assigned to each compound was based on relative abundance of Cl isotopic peaks. The intermediates with m/z 366, 374, and 408 exhibited spontaneous rearrangement to stable products corresponding to m/z 306, 263 and 297, respectively, over a time-span of several hours. The products with m/z 306 and 263 correspond to partial dealkylation (removal of a single ethylene group) and complete dealkylation (removal of both ethylene groups and the amino group) of the piperazine ring of CF, respectively. The m/z 297 product corresponds to a chlorinated analogue of m/z 263, in which one chlorine substitution occurs on the quinolone ring. Temporal distribution of these products indicated that m/z 297 is likely a terminus in the degradation of CF and other fluoroquinolones. Products identified by LC/MS for the reactions between CC and CF were the same as those detected for reactions involving FAC. Thus, similar degradation pathways are expected to apply in the case of FQ oxidation by CC.

**Sulfamethoxazole.** Mass chromatograms corresponding to oxidation products of SMX yielded a number of distinct, stable peaks, corresponding primarily to lower mass degradates (e.g., m/z 99 and 190) and higher mass dimers (e.g., m/z 501 and 503). Two transient peaks, both corresponding to m/z 288, appear to be a N-chloro intermediate and a transient ring-chlorinated SMX molecule. A prominent, early-eluting peak in HPLC and LC/MS chromatograms was identified as 3-amino-5-methylisoxazole (AMI) through comparison to a pure standard. Another prominent product peak detected by way of its UV absorption in kinetic experiments failed to ionize in either APCI or ESI modes during LC/MS analyses.  $H^1$ -NMR analysis of this unknown product revealed that cleavage at the sulfonyl amido nitrogen of SMX had taken place, indicating that this particular site somehow participated in the chlorination reaction. Additionally, generation of AMI was detected at roughly the same reaction time as the unknown product throughout the reaction time course, indicating that cleavage of the SMX structure yields both AMI and the unknown contemporaneously. Despite the above evidence, lack of a definite m/z value for the unknown product prevents identification of its chemical structure at this point.

Taking into account all available kinetic data and product characterization results, the reaction between FAC and SMX can be envisioned as one in which initial attack of the SMX structure occurs at its amino-nitrogen<sup>15</sup>, followed by subsequent attack of sulfonamide S-N bonds – possibly by aminium radicals generated through initial oxidation of amino-nitrogens – to yield a number of products resulting from cleavage of the sulfonamide S-N bond. Support for generation of aminium radicals in the absence of metal or photolytic chain initiators can be found in studies conducted on oxidation of amines (via hypochlorous acid and chlorine dioxide) by the U.S. Army Edgewood Arsenal Research Laboratories<sup>16</sup>. Oxidation product distributions generated by reaction of SMX with CC are very similar to those observed for reaction of SMX with FAC.

**Trimethoprim.** Reaction mixtures obtained from the oxidation of TMP by FAC at  $\text{pH} \geq 5$  yield considerably more complex chromatograms than reactions involving SMX. One stable product was identified as containing four Cl atoms (with molecular ion of 445), indicating that Cl substitution can occur in numerous locations on the TMP structure. Fragmentation of this compound was minimal. Unstable products which appeared to be intermediates exhibited mass spectra corresponding to:  $m/z$  377 (2 Cl) and  $m/z$  411 (3 Cl). A fragment ion peak with  $m/z$  181 in TMP product mass spectra apparently corresponds to the 3,4,5-trimethoxytoluene fragment of the molecule, cleaved from the parent ion during LC/MS analysis, at the aliphatic carbon bridging it to the 2,4-diaminopyrimidine moiety. The parent TMP does not yield such a fragment upon ionization, indicating that chlorine somehow sensitizes TMP to fragmentation at the aliphatic carbon. Experiments conducted at  $\text{pH} < 5$  also yielded relatively complex mixtures of halogenated products, for which LC/MS analyses indicated very low abundances of fragment ions exhibiting  $m/z$  181, but significant quantities of ions with  $m/z$  215 or 249 (which represent mono- and dichlorinated 3,4,5-trimethoxytoluene fragments, respectively).

TMP oxidation by FAC at  $\text{pH} \geq 5$  can be envisioned as proceeding through initial attack on TMP at one of its exocyclic amino nitrogens to yield an iminoquinone methide intermediate<sup>17</sup> (in which the antibiotic's aliphatic carbon participates in resonance with the aromatic heterocyclic ring of the 2,4-diaminopyrimidine), followed by subsequent Cl attack on the TMP structure's aliphatic carbon, then by Cl substitution at various positions on the 2,4-diaminopyrimidine moiety to yield singly- and multiply-halogenated products. TMP oxidation at  $\text{pH} < 5$  appears to proceed primarily through direct attack of the TMP structure's 3,4,5-trimethoxy moiety to yield singly- and multiply-halogenated products, where Cl substitution occurs on the benzene ring of the 3,4,5-trimethoxy moiety. As mentioned earlier, TMP did not exhibit any appreciable degradation in the presence of CC, even after 40 hours.

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## Scientific and Educational Contributions

Results of this study indicate that representative members of three environmentally relevant antibiotic classes - fluoroquinolones, sulfonamides, and dihydrofolate reductase (DHFR) inhibitors - are substantially degraded under conditions simulating chlorination of water supplies during disinfection processes, yielding a wide variety of lower and higher mass degradates. These results are important in facilitating a better risk assessment for these compounds in the aquatic environment. Results of this study also indicate that evaluation of the toxicity of the chlorination products of antibiotics is necessary. The mechanistic understanding of the reactions between chlorine and these three antibiotics classes provides a critical basis for predicting the fate of related antibiotics and pollutants in the chlorination disinfection processes.

This project has provided significant training for a master student in experimental, problem solving and communication skills. A master thesis has been completed from this study and received an outstanding master thesis award within the School of Civil and Environmental Engineering of Georgia Tech. In addition, two journal publications, one conference proceedings, and at least two conference presentations as listed below are also products of this study, providing avenues of sharing the research results with scientific community and water industry.

Products of this investigation:

### *Publications:*

1. Dodd, M. C. "Chemical Oxidation of Aquatic Antibiotic Microcontaminants by Free and Combined Chlorine" **2003**, MS thesis, School of Civil and Environmental Engineering, Georgia Institute of Technology, Atlanta, Georgia.
2. Dodd, M. C.; Huang, C.-H. "Oxidation of Aquatic Antibiotic Microcontaminants by Free and Combined Chlorine. 1. Kinetics", *Environ. Sci. Technol.* **2003**, in preparation.
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4. Dodd, M. C.; Huang, C.-H. " Chemical Oxidation of Aquatic Antibiotic Microcontaminants by Free and Combined Chlorine", proceedings of the AWWA Water Quality and Technology Conference, November 2-5, **2003**, Philadelphia, PA.

### *Conference Presentations:*

1. Huang, C.-H. "Factors Affecting the Concentrations of Antibacterial Agents Released to the Aquatic Environment", AWWA Georgia/South Carolina Conference, September 25-26, **2003**, Savannah, GA.
2. Dodd, M. C.; Huang, C.-H. " Chemical Oxidation of Aquatic Antibiotic Microcontaminants by Free and Combined Chlorine", AWWA Water Quality and Technology Conference, November 2-5, **2003**, Philadelphia, PA.