Fate and transformation of quinoxaline *N'N'*-dioxide antibacterial agents during chlorination

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Abstract

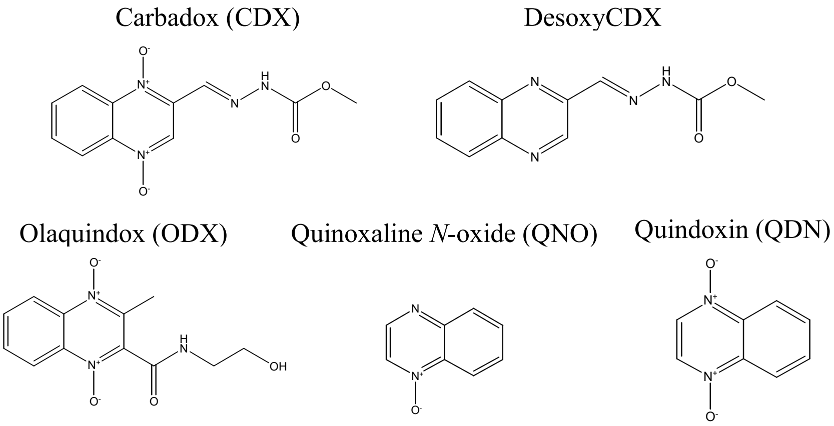
Quinoxaline *N'N'*-dioxides are an important group of veterinary antibiotics whose fate in the environment can be greatly altered by potential reactions during chlorination of wastewater and drinking waters. Their presence is of particular concern due to their potential mutagenic, carcinogenic, and embryotoxic effects. Experiments show that quinoxaline *N'N'*-dioxides such as carbadox and its metabolite, desoxycarbadox, are highly susceptible to oxidation with chlorine and chloramines, in which the reaction rate of carbadox with free chlorine is extremely rapid and highly pH-dependent. No reactions were found to occur for a series of structurally-related compounds that resemble portions of this parent compound, which suggest that carbadox's side chain, rather than the aromatic *N'N'*-dioxide moiety, holds especially high reactivity to chlorine and chloramines oxidants. Several dominant oxidation by-products of carbadox and desoxycarbadox have been preliminarily identified. Further studies will elucidate the transformation pathways and evaluate reaction kinetics in real water matrices.

Keywords: Quinoxaline N'N'-dioxides, carbadox, desoxycarbadox, olaquindox, veterinary antibiotics, oxidation, chlorine disinfection.

1 Introduction

Quinoxaline *N'N'*-dioxides (QNOs) are antibacterial agents primarily used in animal husbandry to promote growth and prevent dysentery and bacterial enterities in young swine. Two compounds that are most widely used from this class are carbadox (CDX) (methyl-3-(2-quinoxalinylmethylene) carbazate *N,N'*-dioxide) and olaquindox (ODX) (2-methyl-3-(*N*-(2-hydroxyethyl)carbamoyl)-

quinoxaline *N,N'*-dioxide) (see Figure 1). When QNOs such as these are consumed by the animal, they are absorbed through the gastro-intestinal tract and are found to accumulate as well as rapidly metabolize within liver and kidney tissues [1-5]. CDX and ODX follow a similar two step metabolic transformation in which reduction of the N-oxide groups forming mono- and desoxy-compounds is followed by side-chain cleavage to form quinoxaline-2-carboxylic acid (QCA) [3] and 3-methyl-quinoxaline-2-carboxylic acid (MQCA) [6], respectively. Under current regulations, QCA and MQCA are designated as market residues to monitor CDX and ODX use in animals raised for human consumption [7,8].


Carbadox (CDX)

DesoxyCDX

Olaquindox (ODX)

Quinoxaline *N*-oxide (QNO)

Quindoxin (QDN)

Figure 1: QNOs and structurally-related compounds investigated in this study.

On the other hand, little attention has been paid regarding the level of residual and reduced QNOs that may be excreted by animals and concentrated in their wastes [3,5]. Such wastes may end up in manure pits or be spread on agricultural fields, and the combination of these sources along with contamination by spilt feeds, may lead to low concentrations of QNOs making their way into soil and water systems. Both the environmental and human health risks of low level contamination are of increasing concern due to studies indicating that CDX, ODX, and desoxyCDX (see Figure 1) harbour a variety of mutagenic, carcinogenic, and embryotoxic effects [3, 9-11]. Further concerns have also led the European Union to ban the use of CDX and ODX in animal feeds in 1998 [12].

In recent years, a growing number of studies have assessed the fate and transformation of QNOs in different environmental matrices. CDX and desoxyCDX have shown significant sorption to soils and clays, in which sorption correlated well with organic carbon content in soils and was enhanced in both soils and clays by the presence of K^+ ions [14]. In contrast, ODX was found to weakly sorb to sands and manure [14, 15]. QNOs and quinoline *N*-oxides have also been reported to be highly susceptible towards manganese-oxide facilitated oxidation [16]. By way of this reaction, the parent compound undergoes deoxygenation of the N-oxide moiety and hydroxylation at the neighbouring α-

C, while manganese oxide is simultaneously reduced to Mn^{+2} [16]. Photolysis of quinoxaline *N'N'*-dioxide (quindoxin) (see Figure 1) has also been well documented in past literature, where irradiation at 366 nm at neutral pH conditions leads to formation of quinoxalin-2-one-4-oxide via an oxaziridine intermediate [17-19]. Additional QNOs have also been shown to undergo photolysis when using organic solvents [18]. For desoxyCDX, one study in 1984 found that its concentration in pork tissues decreased after two hours of exposure to indirect incandescent light [20]. Such analyses suggest that subsequent photolytic transformation of CDX, ODX, and their metabolites when exposed to natural light is likely to occur. In addition, given their biologically active nature, QNOs may also be susceptible towards biodegradation, especially under aerobic conditions, as demonstrated by rapid degradation of ODX (half life of 4-8 days) in an aerobic reactor spiked with lake sediment [21].

Several occurrence studies within Canada and the United States have attempted to measure residual levels of CDX and ODX in order to assess their overall recalcitrance in the environment [22, 23]. These studies failed to detect concentrations above the maximum detection limits (MDL) set for either compound in all tested streams and wastewater effluents. Given the extensive use of CDX and ODX in animal farms, these results suggest that multiple processes must strongly influence their rapid removal and transformation.

One type of water treatment process that is likely important in affecting the fate of QNOs is the use of free and combined chlorine for disinfection purposes. Prior studies have indicated that antibiotics from a range of classes are readily transformed by chlorine oxidants such as free chlorine and monochloramine in laboratory controlled systems [24, 25] as well as in full-scale treatment plants (26). Mineralization of these antibiotics, however, is seldom achieved in reactions with chlorine species. Instead, a range of lower- and higher-molecular weight and chlorinated by-products are generated (25). Up to date research on chemical oxidation of antibiotics under water disinfection conditions has been conducted with only a limited number of structural classes, and to this date, the potential chemical oxidation and by-product formation of QNOs have not yet been fully understood.

The overall objective of this study is to elucidate the transformation of representative QNOs, such as CDX, desoxyCDX, and ODX by reaction with free and combined chlorine under typical water disinfection conditions. Kinetics of these reactions will be evaluated along with identification of intermediates and oxidation by-products. Structurally related compounds are used to facilitate identification of reactive functional groups and further evaluate reaction mechanisms and pathways. In addition, the impact of changing water quality parameters such as pH and NOM concentrations are also assessed.

2 Materials and methods

2.1 Chemicals and reagent preparation

CDX and ODX were purchased from Sigma (St. Louis, MO) and ICN Biomedicals, Inc. (Irvine, CA), respectively. DesoxyCDX, quindoxin (QDX), and quinoxaline N-oxide (QXO) (see Figure 1) were synthesized following methods described previously [16]. NaOCl solutions at were obtained at ~5% in water from Fisher Scientific (Pittsburgh, PA). All the chemicals used were of reagent or HPLC grade, and were used directly without further purification. All the reagent solutions were prepared using Mili-Q water (18.2 MΩ-cm resistivity) produced from a Millipore water purification system.

Except for desoxyCDX, all QNO stock solutions were prepared at 25 or 50 mg/L in 50/50% (v/v) MeOH/H_2O. DesoxyCDX solutions were prepared at 100 mg/L in 50/50% (v/v) MeOH/H_2O. Free available chlorine (FAC) concentrations were monitored using the standard iodometric titration method (Standard Methods, 1998), and stock solutions were prepared at 100 mg/L, 3.1 g/L, or 3.6 g/L as Cl_2. Combined chlorine was prepared by adjusting the pH of pre-formed NH_2Cl stock solutions, which were initially synthesized by slowly adding FAC to NH_4Cl solutions (pH > 9.5) at a molar ratio of 1: 0.7 ([NH_4Cl] : [FAC]). NH_2Cl concentrations were monitored using the standard DPD/FAS titrimetric method (Standard Methods, 1998), and stock solutions were prepared at 1000-1100 mg/L as Cl_2.

2.2 Experimental setup

For monitoring slow kinetic rates and oxidation by-products, batch reactors were designed using 20 or 25 mL screw-cap amber glass vials with Teflon septa. Magnetic stirrers were used to maintain continuously mixing conditions, and reactors were placed in a water bath to control temperature at 22 and 25°C. Reaction mixtures were spiked with QNO stock solution to obtain a target initial concentration of 0.01-0.10 mM and buffered using acetate (pH 4 -5), phosphate (pH 6-8) and borate (pH 9) at 10-50 mM to adjust pH. Reactions were initiated when FAC or combined chlorine were added in either excess or equimolar amounts compared to the target compound. Sample aliquots were then periodically taken and instantaneously quenched upon collection with excess $Na_2S_2O_3$ to prevent further reaction.

To assess rapid kinetics, a SFA-20 Hi-Tech Scientific Rapid Kinetics Stopped-Flow system was used in which two pneumatically-driven syringes instantaneously pushed target compound and oxidant into a flow-through cell (400 µL dead volume). Reaction kinetics were monitored using a Jasco FP-6300 spectrofluorometer where fluorescent intensity decrease of the QNO compound was measured as a function of time.

2.3 Analysis of QNOs and oxidation products in batch experiments

In the batch experiments, the loss of parent QNOs was monitored using a reverse-phase HPLC system with a Zorbax RX-C18 column and dioded-array UV/Vis detector (1100, Agilent Technology). Oxidation products were examined using a HPLC/DAD/MS system with a Zorbax SB-C18 column under electrospray ionization conditions and positive ion mode (1100/1100MSD, Agilent Technology).

3 Results and discussion

Results obtained thus far indicate that CDX and desoxyCDX are highly reactive with free available chlorine (FAC) and combined chlorine in both reagent water and environmentally-relevant sample matrices. The reaction rate of CDX with FAC is extremely rapid and highly pH-dependent. The pseudo-first order rate constants (k'_{app}) for CDX in the presence of excess FAC (10×) ([CDX]:[FAC]= 0.01:0.1 mM) for pH 4 and 5 are 3.60×10^{-3} s^{-1} and 3.58×10^{-2} s^{-1}, respectively (Figure 2). For desoxyCDX in excess FAC (10×) ([CDX]:[FAC]= 0.05:0.5 mM), the k'_{app} at pH ~ 4 is 6.60×10^{-3} s^{-1} (Figure 2). Both CDX and desoxyCDX, at pHs ≥ 6 and pH ~7, respectively, degraded completely in less than 10 seconds under excess chlorine (10×) conditions and could not be monitored in batch experiments. Stopped flow experiments are currently being conducted to evaluate such kinetics.

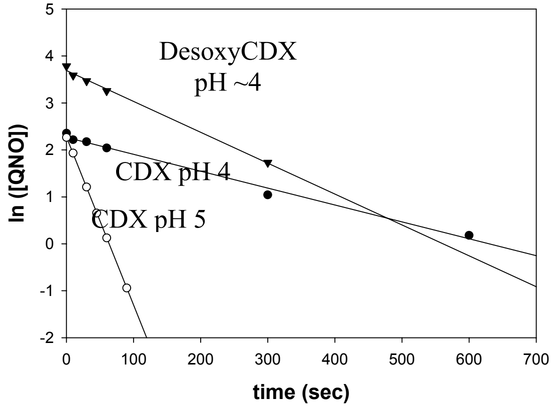


Figure 2: CDX and desoxyCDX pseudo-first order decay in the presence of excess FAC at varying pH.

Structurally-related compounds, olaquindox, quindoxin and quinoxaline-*N*-oxide (Figure 1), did not react with excess amount of FAC under experimental conditions tested. These results indicate that the carbazate side chain, rather than the aromatic *N'N'*-dioxide moiety, of CDX is the reactive site to chlorine oxidant species. This conclusion is also in agreement with the high reactivity of desoxyCDX to FAC, which contains the same carbazate side chain but does not contain the *N*-oxide functional groups. Further studies will closely compare the kinetic rate constants of CDX and desoxyCDX oxidation under various conditions to assess the potential effect of *N*-oxide functional groups on the reactivity of the carbazate side chain.

In the presence of excess free chlorine, CDX forms a number of intermediates and by-products under pH conditions ranging from 6 to 9. Preliminary evidence also suggests that desoxyCDX forms different by-products than CDX. While molecular weight and fragmentation patterns have been obtained for these various products from LC/MS analyses, structural identification of the products is not yet possible. The preliminary results indicate that the major products are not chlorinated based on the lack of chlorine isotope peaks. NMR analysis will be conducted in the future to obtain exact structural information for the products.

On the basis of the very rapid reaction kinetics of CDX and desoxyCDX with chlorine ($t_{1/2}$ < 10s) and monochloramine ($t_{1/2}$ = 15m) in reagent water, it is likely that substantial transformation of CDX and desoxyCDX by these chlorine oxidants will occur during residence times typical of wastewater (5-30 minutes) and drinking water clearwells (1-24 hours). Further studies will be conducted in a range of real water matrices to assess the effect of water quality parameters on the reaction kinetics and pathways. In the end, it is expected that this research will help foster a better understanding of how current chlorine-based water treatment processes affect the fate of quinoxaline *N'N'*-dioxide antibiotics and related emerging contaminants. Information gleaned from this work will facilitate water utilities to optimize chlorine-based disinfection processes for inactivation of pathogens as well as removal of trace organic contaminants.

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