Nitriles as main products from the oxidation of primary amines by Ferrate(VI): kinetics, mechanisms and toxicological implications for nitrogenous disinfection by-product control

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Abstract

Ferrate (Fe(VI)) is a novel water treatment oxidant that can be used for micropollutant abatement or disinfection byproduct mitigation. However, there are still knowledge gaps concerning the interaction between Fe(VI) and dissolved organic matter structures, notably primary amines. This study investigated the degradation kinetics and products of several aliphatic primary amines by Fe(VI). The primary amines showed appreciable reactivity toward Fe(VI) (2.7–68 $M^{-1}s^{-1}$ at pH 7–9), ranking as follows: benzylamine > phenethylamine > phenylpropylamine > methylamine ≈ propylamine. Nitriles were the main oxidation products of the primary amines, with molar yields of 61–103%. Minor products included aldehydes, carboxylic acids, nitroalkanes, nitrite, nitrate, and ammonia. The buffering conditions were important. Compared to phosphate, borate enhanced the reactivity of the amines and shifted the products from nitriles to carbonyls. An evaluation of the effect potency of some cyano compounds by an in vitro bioassay for oxidative stress response and cytotoxicity suggested that nitriles are unlikely to pose a significant threat because they were only toxic at high concentrations, acted as baseline toxicants and did not cause oxidative stress, unlike halonitroalkanes or halonitriles. The formation of nitriles is preferable to the formation of nitroalkanes arising from the ozonation of primary amines because nitroalkanes can further react during chlorination, forming halonitroalkanes.

Introduction

The fate of nitrogen-containing moieties during water treatment has been a research focus since they have been linked to the formation of disinfection byproducts (DBPs) that are far more potent than regulated trihalomethanes or haloacetic acids.[1, 2] Among these emerging DBPs, (halo)aldehydes and (halo)nitriles have been shown to be formed upon the chlorination of primary amines ($R\text{-}NH_3^+$/$R\text{-}NH_2$).[3-5] While dissolved organic nitrogen can range from 0.2 to several mgN L^{-1},[6-8] the fraction of primary amines is uncertain. Studies have reported the occurrence of amino acids and sugars[6, 9] or simple short-chain aliphatic structures,[10-15] but primary amines may also be contained in more complex and unidentified organic structures. Contaminants such as pharmaceuticals may also contain primary amines (e.g., some β-lactams, oseltamivir, gabapentin), which are a major reactive site during oxidative treatment.[16-18] Attention must therefore be paid to amine-containing moieties, especially as drinking water resources are increasingly impaired by nitrogen-rich inputs such as wastewater discharges, agricultural runoff, or algal blooms.[19]

Chlorination can be replaced or completed by other oxidative treatments that can mitigate the formation of regulated or emerging DBPs[20, 21] and enhance the degradation of micropollutants resistant to chlorine.[20-22] However, counterproductive outcomes can be obtained when using these alternative treatments. For instance, while preozonation can efficiently mitigate the formation of haloacetonitriles, the formation of halonitromethane is usually enhanced.[23-25] This adverse effect of pretreatment can be explained by the oxidation of primary amines to nitroalkanes during preozonation,[26] which can then be chlorinated during disinfection.[27] It is therefore important to understand the interactions between primary amines and oxidants.

Ferrate (Fe(VI)) has been considered as an alternative oxidant that can lead to efficient micropollutant abatement in wastewaters,[28, 29] be used as a preoxidation step to mitigate DBP formation in drinking water,[25, 30] and/or enhance coagulation.[31] In addition, Fe(VI) does not produce known harmful byproducts such as chlorinated organic compounds (from chlorine use), chlorite (from ClO_2 use), or any significant levels of bromate (from O_3 use in bromide-containing waters).[32-34] However, limited information is available on the reaction between Fe(VI) and primary amines. Previous studies suggested the primary amine as a main or secondary reaction site for Fe(VI).[16, 35-37] The proposed reaction pathways consistently involved C-N bond breakage and the simultaneous release of ammonia or hydroxylamine. However, these pathways sometimes lack strong analytical evidence or are incomplete, i.e., not all of the nitrogen is accounted for in the products. Oxidation of methylamine by Fe(VI) has been shown to form cyanate,[38] indicating that amines can retain the C-N bond. Products with a preserved C-N bond (e.g., amides, nitriles) might be formed from the Fe(VI) oxidation of other aliphatic amines with carbon chains longer than methylamine, but relevant information is currently unavailable.

This study aims to better understand the reaction between Fe(VI) and aliphatic primary amines by investigating the reaction kinetics and degradation products of several amines. The cytotoxicity and oxidative stress response of nitriles that are the main products were evaluated with the AREc32 bioassay for a comparative hazard assessment with other DBPs, such as halonitroalkanes and halonitriles.[39]

Materials and Methods

Standards and reagents. All chemicals and solvents (mostly the highest purity available) were purchased from various commercial suppliers and used as supplied and are shown in the Supporting Information (SI, Table S1). Potassium ferrate was purchased from Sigma-Aldrich

(K_2FeO_4, PN 723835, 60% w/w purity), which has been used in our previous studies.[40-42] Stock solutions of Fe(VI) (3–4 mM) were freshly prepared by dissolving potassium ferrate in pure water (pH ≈ 9.2), followed by rapid filtration through a 0.2 μm PVDF syringe filter (Whatman) and standardized spectrophotometrically (ε_{510} = 1150 $M^{-1}cm^{-1}$).[43]

Kinetic and stoichiometric experiments in buffered solutions. Kinetic studies were performed at room temperature (22±1 °C) in the presence of phosphate, borate, or carbonate buffer (5–20 mM) at pH 7–9. Excess Fe(VI) (10–15 times) was spiked into a 5–30 μM amine solution under rapid mixing. At predetermined sampling points, an aliquot was used to quantify Fe(VI). Another aliquot was quenched using thiosulfate ([thiosulfate] = 5 × $[Fe(VI)]_0$), filtered through a 0.22 μm PVDF syringe filter (Whatman), and kept for amine and product quantification within 10 h. Apparent second-order rate constants (k_{app}) for the reaction of amines with Fe(VI) could be determined using Eq. 1:[16, 40, 44]

$$\mathrm{Ln}\frac{[C]}{[C]_0} = -k_{app}\int_0^t [Fe(VI)]dt \qquad (1)$$

where [C] represents the amine concentration and the integral represents the Fe(VI) exposure during the reaction, calculated from the Fe(VI) decay using the trapezoidal integration method (an example is shown in Figure S1, SI). Plotting the natural logarithm of the relative amine decrease against Fe(VI) exposure therefore allows one to derive k_{app} (see Figure S1, SI). Kinetic experiments were also conducted at pH 9 in excess amine (10-25 times, pseudo first-order conditions) by monitoring Fe(VI) decay.

Product formation and stoichiometry experiments were conducted with higher amine concentrations (up to 100 μM) and Fe(VI):amine ratios ranging from 0.5 to 15. For product formation experiments, headspace-free conditions were ensured to limit the volatilization of some products (e.g., ammonia) until all Fe(VI) was consumed (between 1 h and 2 days).

Stoichiometry experiments were conducted at pH 9.4–9.8, at which Fe(VI) self-decay is slow ($k_{app} \leq 10^{-1}$ $M^{-1}s^{-1}$)[45] compared to its reactivity toward primary amines.

Real water experiments. The oxidation of primary amines by Fe(VI) (32−125 μM, 1.7−7 mgFe L^{-1}) was tested in a real river water matrix (see details in Text S1, SI). No buffer was used; hence, the pH varied between 8.5 and 9.5 after Fe(VI) addition (see Figure S2, SI).

Analytical methods. Fe(VI) was monitored spectrophotometrically at 415 nm after reaction with 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS).[46] For kinetic experiments conducted in borate buffer samples required filtration (0.22 μm PVDF syringe filter, Whatman) before reaction with ABTS to prevent interferences. Most of the phenyl-containing compounds were directly monitored by HPLC-UV (Ultimate 3000, Dionex) and after derivatization with 2,4-dinitrophenylhydrazine for aldehydes (see details in Text S2).[47] Propylamine, methylamine, cyanate, ammonia, nitrite, nitrate, and small carboxylic acids were monitored by ion chromatography (940 Professional IC Vario, Metrohm, see details in Text S2). Headspace-SPME followed by GC-MS was used to quantify propionitrile (see details in Text S2, SI).

Bioassays. The AREc32 bioassay indicative of oxidative stress response was performed according to Escher et al.,[48, 49] adapted to 384-well plate format by Neale et al.[50] Cell viability was quantified by live-cell imaging using an IncuCyte S3 microscope (Essen BioScience, Ann Arbor, Michigan, USA), where confluency was a measure of cell growth as previously described.[51] The concentration-response curves (given in SI, Figure S4) were evaluated at their low effect linear portion,[52] and inhibitory concentrations for 10% reduction of cell growth and viability (IC_{10}) as well as effect concentrations causing 50% higher induction of the antioxidant response element ARE, that is an induction ratio IR of 1.5 ($EC_{IR1.5}$).

Octanol-water partition constants ($\log K_{ow}$) of all test chemicals were retrieved from the Comptox Chemicals Dashboard,[53] and the liposome water partition constants ($\log K_{lipw}$) were

predicted with linear free solvation energy relationships[54] or with a logK_{ow}–logK_{lipw} quantitative structure-activity relationship (QSAR).[55] Two analytes contained carboxylic groups (1-cyanocyclohexaneacetic acid and cyanoacetic acid), which were deprotonated in the conditions used for bioassays (pH = 7.4), and the ionization-corrected liposome-water distribution ratio log$D_{lip/w}$(pH 7.4) was calculated, assuming that the charged species has a ten times lower affinity to the liposomes than the neutral species.

The $IC_{10,baseline\ toxicity}$ for baseline toxicity was predicted with a QSAR from Escher et al.,[51] and the toxic ratios TR = $IC_{10,baseline\ toxicity}/IC_{10}$ and specificity ratios $SR_{baseline}$ = $IC_{10,baseline\ toxicity}$/ $EC_{IR1.5}$ and $SR_{cytotoxicity}$ = $IC_{10}/EC_{IR1.5}$ were calculated according to Escher et al. (2020).[56]

Results and Discussion

Reaction stoichiometry and kinetics. Stoichiometry experiments showed a Fe(VI):amine stoichiometry of ~2.5:1 for all the tested amine compounds at pH 9.4–9.8 (Figure 1). At $[Fe(VI)]_0/[amine]_0$ ratios higher than 1.5, the stoichiometry tended to increase up to 3:1.

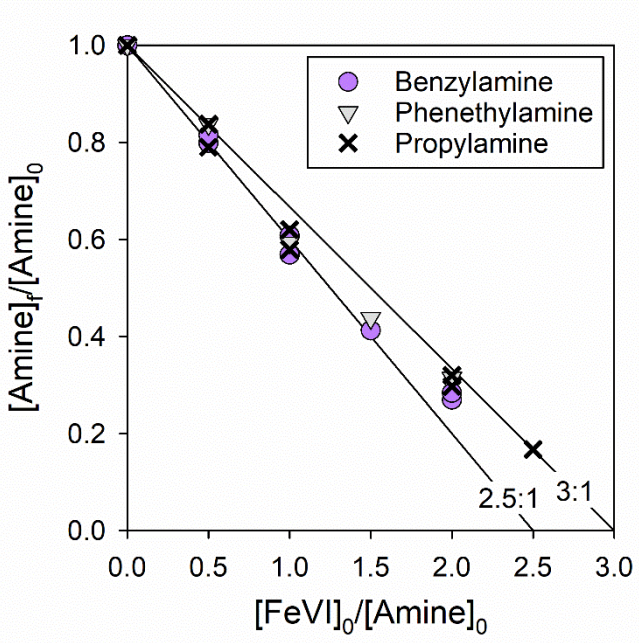


Figure 1. Apparent stoichiometry between Fe(VI) and aliphatic primary amines. Each symbol represents a single experiment. The lines represent the 2.5:1 and 3:1 Fe(VI):amine stoichiometries. $[Amine]_0$ = 40–80 µM, pH 9.4–9.8 (5 mM phosphate + 1 mM borate).

During the reaction of amines with excess Fe(VI), good linearity was observed between amine decay and Fe(VI) exposure (R^2 = 0.9397-0.9999, Table S2), suggesting that the reaction is first-order with respect to both amine and Fe(VI). From the slope of these linear plots, the second-order rate constants (k_{app}) were determined. The k_{app} could also be determined from the pseudo first-order k against amine concentration during the reaction of Fe(VI) with excess amine (Figure S5). The obtained k_{app} are summarized in Table 1.

At pH 8, the reactivity of the aliphatic primary amines in phosphate buffer showed the following order: benzylamine (20.6±0.6 $M^{-1}s^{-1}$) > phenethylamine (7.4±0.6 $M^{-1}s^{-1}$) > phenylpropylamine (5.0±0.3 $M^{-1}s^{-1}$) > propylamine/methylamine (2.7±0.4 $M^{-1}s^{-1}$). Increasing the pH from 8 to 9 significantly increased the reaction rate for all the amines by a factor of 1.8–2.8 while maintaining the same reactivity ranking (Table 1). A slight increase in the reaction rate was also observed when the pH was decreased from 8 to 7 for phenethylamine and benzylamine. The k_{app} of methylamine and propylamine were not measured at pH 7 due to the combined effect of limited analytical sensitivity and fast Fe(VI) self-decay compared to its reaction with methylamine and propylamine. In excess of amine, the k_{app} were 18.4±0.6 $M^{-1}s^{-1}$ and 42±3 $M^{-1}s^{-1}$ for methylamine and phenethylamine, respectively, at pH 9. The k_{app} determined in excess amine were thus 2.4-2.6-fold higher than the k_{app} determined in excess Fe(VI), which was consistent with the Fe(VI):amine stoichiometry of ~2.5:1 (Figure 1).

Table 1. Apparent second-order rate constants for the reaction of Fe(VI) with amine compounds at pH 7–9 (± 0.1). Rate constants determined in this study were measured in excess of Fe(VI), unless otherwise specified. All kinetic data are given in Table S2 (SI).

Compound	pK_a ($-NH_2/-NH_3^+$)	k_{app} ($M^{-1}s^{-1}$) in phosphate			k_{app} ($M^{-1}s^{-1}$) in borate	
		pH 7	pH 8	pH 9	pH 8	pH 9
Aliphatic primary amines						
Methylamine	10.6	ND [a]	2.7 ± 0.2 40 [b]	7.6 ± 0.7 18.4 ± 0.6 [c] 57 [b]	4.1 ± 0.2	11 ± 2
1-Propylamine	10.7	ND [a]	2.7 ± 0.4	6.7 ± 0.6	4.0 ± 0.6	8.0 ± 0.7
Benzylamine	9.3	25 ± 1	20.6 ± 0.6	38 ± 2	29.7 ± 0.9	68 ± 3
2-Phenethylamine	9.8	11 ± 1	7.4 ± 0.6	16.5 ± 0.8 42 ± 3 [c]	10.5 ± 0.4	28.9 ± 0.2
3-Phenylpropylamine	10.2	ND [a]	5.0 ± 0.3	9.7 ± 0.9	ND [b]	ND [b]
Aliphatic secondary amine						
Dimethylamine	10.7	9.2 [d] 352 [b]	6.8 [d] 140 [b]	6.1 [d] 57 [b]		
Aliphatic tertiary amine						
Trimethylamine	9.8	1.0 [d] 17 [b]	0.9 [d] 4.8 [b]	0.8 [d] 0.6 [b]		
Aromatic primary amine						
Aniline	6	6600 [e]	720 [e]	55 [e]	-	-

[a] ND: not determined, [b] k_{app} measured in pseudo first-order in amine and in undefined buffer (phosphate, borate or pyrophosphate) obtained from Carr,[38] [c] Determined in this study in pseudo first-order conditions in amine (see Figure S5, SI), [d] Calculated from species-specific constants determined in pseudo first-order in amine,[57] [e] k_{app} measured in pseudo first-order in aniline obtained from Lee et al.[28]

The k_{app} of primary amines at pH 8 are similar to those of secondary amines (6.8 $M^{-1}s^{-1}$ for dimethylamine) and higher than that of tertiary amines (0.9 $M^{-1}s^{-1}$ for trimethylamine) but much lower than that of aniline as an aromatic amine (720 $M^{-1}s^{-1}$) (Table 1).[28, 57] Notably, the k_{app} determined in excess methylamine was significantly lower than previously reported (18.4 vs 57 $M^{-1}s^{-1}$ at pH 9, Table 1).[38] However, significantly lower reaction constants for

dimethylamine and trimethylamine have also previously been found compared to the same study reporting methylamine kinetic constants (Table 1).[38, 57]

The pH dependency of k_{app} in the studied range is unusual compared to other organic moieties, in particular secondary and tertiary amines, the reactivity of which decreases with increasing pH due to the decreasing fraction of the more reactive $HFeO_4^-$.[57] Species-specific rate constants derived from the speciation of the reactants, which have been successfully determined for the reaction of many organic compounds with Fe(VI),[28, 29, 57] could not be calculated in the case of primary amines. The lack of compliance of amine-containing compounds to models derived from amine/Fe(VI) speciation has already been observed,[37, 58] and investigations are currently underway to elucidate the case of primary amines [in preparation].[59]

Phosphate is commonly used for its good buffering capacity at near-neutral pH and for its complexing properties that prevent optical interferences due to Fe(III) precipitation when directly monitoring Fe(VI) decay.[60] However, phosphate may complex high-valent iron intermediates (i.e., Fe(V) and Fe(IV)) that otherwise could contribute to the oxidation, leading to an inhibition of the Fe(VI) oxidation efficiency.[61] Kinetics in excess of Fe(VI) were also conducted in borate buffer (in the absence of phosphate) at pH 8 and 9 (Table 1). An increase in reaction rate by a factor of 1.2–1.8 was observed when using borate instead of phosphate (Table 1). Recently, experimental evidence and kinetic modeling of the reaction between Fe(VI) and ABTS provided by Huang et al. suggested that phosphate prevented Fe(V) and Fe(IV) from reacting, leading to an oxidation capacity of Fe(VI) approximately 1.6–1.9 times higher in borate than in phosphate at pH 7.[61] A similar inhibitory effect of phosphate on the oxidation of primary amines with high-valent iron intermediates may occur and explain the higher k_{app} observed in borate than phosphate. Carbonate, another buffer more relevant to real water matrices, was also tested on benzylamine at pH 9 (10 mM carbonate) and led to a k_{app} of 41±1

$M^{-1}s^{-1}$, similar to that found in phosphate (38±2 $M^{-1}s^{-1}$) but lower than that found in borate (68±3 $M^{-1}s^{-1}$), suggesting an inhibitory effect of carbonate on high-valent iron intermediate reactivity. The kinetic differences observed in borate and phosphate demonstrate that buffering conditions used for kinetic measurement must be carefully chosen when studying Fe(VI).

Reaction products and yields. The product distribution is shown in Figure 2 (see also Figure S6 for data) for benzylamine and in Figures S7–S11 (SI) for phenethylamine, propylamine, and methylamine. Upon Fe(VI) oxidation, benzylamine was found to form benzonitrile as a major product in phosphate (Figure 2). Minor products included carbonyls (benzaldehyde and benzoic acid), nitromethylbenzene, nitrite, nitrate and ammonia (Figure 2). The % molar yield (=Δ[product]/Δ[benzylamine]×100) of benzonitrile was > 90% at pH 9 but decreased to 63% at pH 7. Concomitantly, carbonyls increased with decreasing pH (from 5% at pH 9 to 24% at pH 7), and some nitromethylbenzene was also formed at pH 7 (9%). Following the same trend as the carbonyls, inorganic nitrogen yields increased with decreasing pH (from 2% at pH 9 to 25% at pH 7). Compared to phosphate, nitrile formation greatly decreased in borate at pH 8 (from 85% to 39%), while the carbonyl yields increased (from 14% to 41%). Inorganic nitrogen yields also increased to a similar extent (from 16% to 43%), while a significant fraction was not characterized (20%). At pH 9, no significant difference was observed between phosphate and borate (≥ 89% nitrile, Figure S6, SI).

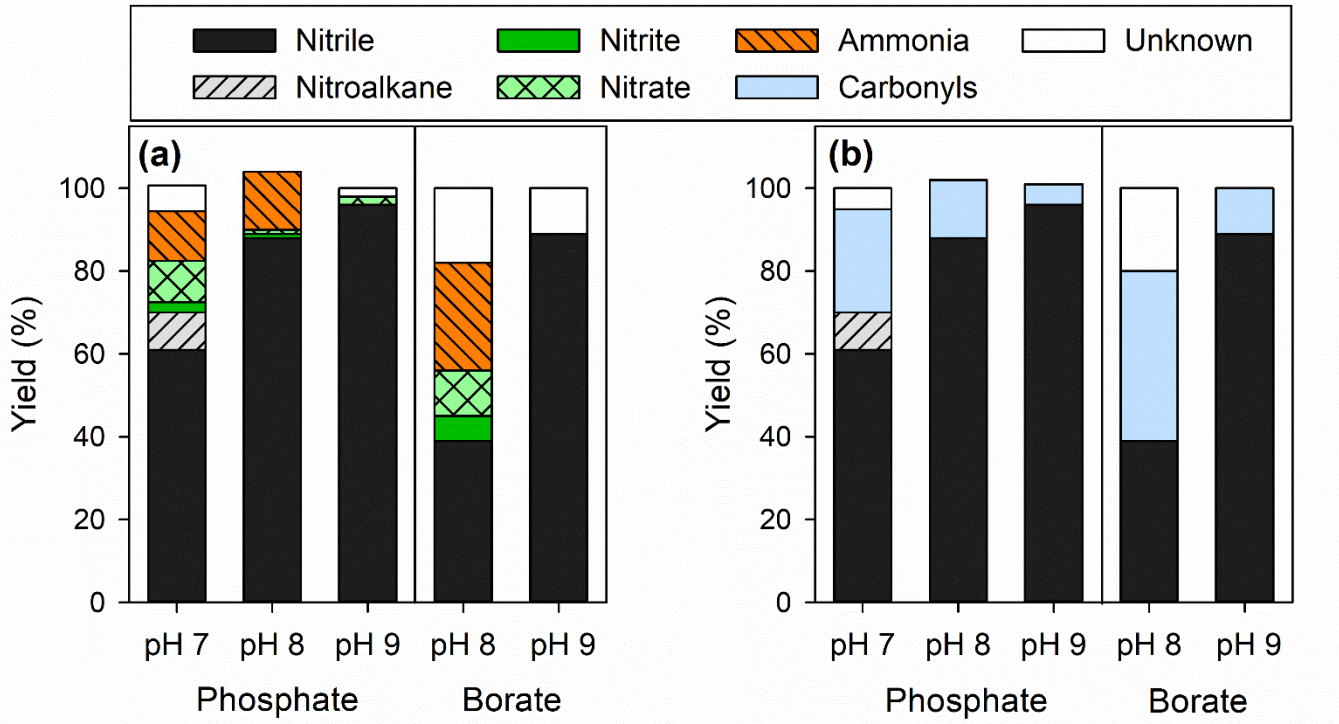


Figure 2. Molar yields of nitrogen-containing (a) and benzene-containing (b) products from the oxidation of benzylamine by Fe(VI). Nitrile and nitroalkane correspond to benzonitrile and nitromethylbenzene, respectively, while "carbonyls" refer to the sum of benzaldehyde and benzoic acid. The yields determinations are shown in Figure S6. Experimental conditions: $[Fe(VI)]_0$ = 20–480 µM, pH 7–9 (10 mM phosphate or borate), duplicate experiments.

Nitrile was also the main product for phenethylamine and propylamine (Table 2, Figures S7–S9), and carbonyls, nitroalkane, nitrite, and nitrate were also formed from phenethylamine at yields consistent with those observed for benzylamine (Figure S7). The oxidation of methylamine in phosphate did not lead to cyanide but to cyanate (> 90% yield at pH 8 and 9, Figure S10a, SI). At pH 7, although methylamine could not be accurately quantified due to interference from potassium (originating from Fe(VI)), the products shifted toward nitrite and nitrate, which represented 38% of the cyanate production (Figure S11). Formaldehyde was

formed along with nitrite/nitrate, representing 24% of the cyanate production (Figure S11). Borate greatly decreased cyanate yields (from 98% to 60% at pH 8) while increasing nitrite/nitrate formation (from 2% to 27%) (Figure S10b, SI).

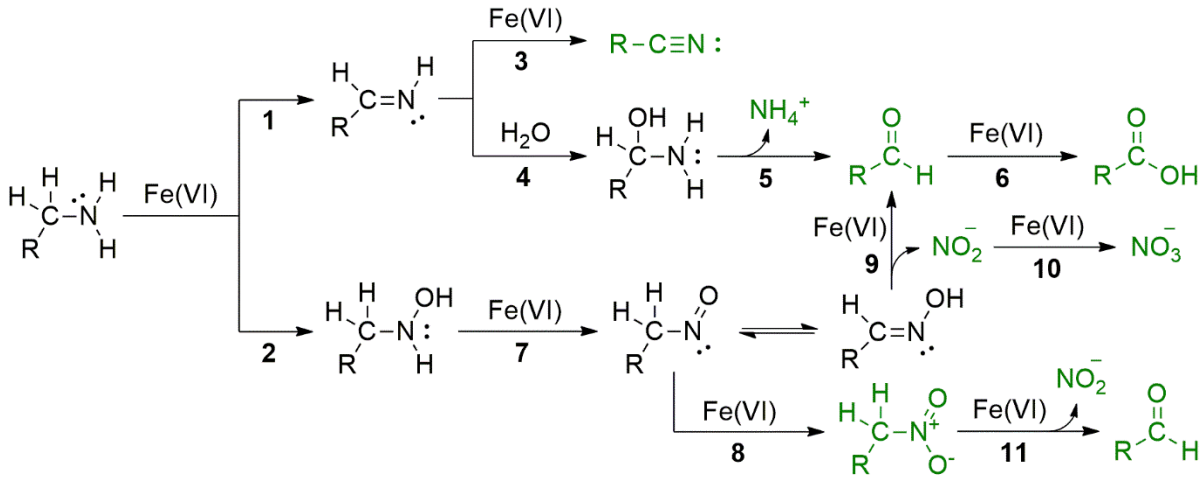
Table 2. Molar yields of nitriles from the oxidation of primary amines. All yields were relative to the amine consumption and were measured in phosphate or in borate (shown within brackets). Data are shown in SI (Figures S6, S8 and S9).

Compound	Nitrile		
	pH 7	pH 8	pH 9
1-Propylamine	ND	95% (ND)	100% (103%)
Benzylamine	63%	85% (39%)	97% (89%)
2-Phenethylamine	61%	84% (43%)	91% (88%)

ND: Not determined

Degradation pathways and mechanisms of primary amines by Fe(VI). Based on the identified products and the stoichiometry, a general reaction scheme is proposed for the reaction of primary amines with Fe(VI) (Scheme 1). In the first step, Fe(VI) reacts with the primary amine to form either an imine (1) or a hydroxylamine (2). The imine can either further react with Fe(VI) to form a nitrile (3) or be hydrolyzed to an aldehyde (4 and 5), releasing ammonia.[4] The aldehyde can be further oxidized to a carboxylic acid (6) (see Figure S12, $k_{app} \approx 14\ M^{-1}s^{-1}$ for benzaldehyde at pH 8 and in phosphate). The hydroxylamine is quickly oxidized to a nitroso (7), which can be further oxidized to a nitroalkane (8).[62, 63] The nitroso is in equilibrium with its aldehyde-oxime tautomer,[64] which is readily oxidized to aldehyde (9),

releasing nitrite (see Figure S13, SI). The latter can be further oxidized to nitrate (10).[65] The nitroalkane can also be further oxidized to aldehyde (11) (Figure S14, SI).



Scheme 1. Proposed degradation pathways and mechanisms of primary amines by Fe(VI). Structures in green represents products quantified in this work.

The formation of imine has previously been suggested for the oxidation of primary amines by chlorine and permanganate.[4, 66] However, for both oxidants, imine is not further oxidized to nitrile but only hydrolyzed to aldehyde. In the case of chlorine, nitrile can be formed, but it proceeds via a chlorinated imine pathway.[3, 4] The dominance of nitrile products at pH > 8 observed here suggests that the imine is very reactive toward Fe(VI) (or Fe(V)/Fe(IV) formed from Fe(VI) decay), although the reaction pathway is unknown.

The case of methylamine is probably unique among the primary amines due to the lack of substitution on the amine's α-carbon. Carr previously suggested the formation of an imine followed by oxygen transfer to form formamide during Fe(VI) oxidation of methylamine, where formamide is further oxidized to cyanate.[38] However, no cyanate formation was

observed from the reaction of formamide (40–80 µM) and Fe(VI) (80–480 µM) at pH 7 and 9 (10 mM phosphate). Instead, the formation of cyanide via pathways (1) and (3) may occur, followed by further oxidation to cyanate.[67] At pH 8 and 9, cyanide is rapidly oxidized by Fe(VI) (k_{app} = 315–605 $M^{-1}s^{-1}$)[67] and does not accumulate.[68] The alternative pathway involving N-hydroxylation (2) may also occur for methylamine, leading to the formation of nitrite/nitrate (nitromethane was not monitored) and formaldehyde observed at pH 7 (Figure S11a, SI). Cyanate may also be oxidized by Fe(VI) to nitrite/nitrate,[67] but experiments conducted at pH 7 showed no abatement of cyanate (80 µM) by Fe(VI) (160–480 µM) (Figure S11b, SI), dismissing this reaction as a source of nitrite/nitrate at this pH.

In the absence of phosphate, the shift in product formation observed at pH 8 (Figure 2 and Figures S7 and S10, SI) suggests that high-valent iron intermediates and/or precipitated iron oxide influence the reaction pathways.

Products from amine oxidation in real water. The oxidation of amines by 31–125 µM Fe(VI) (1.7–7 mgFe L^{-1}) was tested in a real surface water matrix (its characteristics are given in Text S1, SI). A Fe(VI) dose of 63 µM (3.5 mgFe L^{-1}) could oxidize 35, 42, and 72% of methylamine, phenethylamine, and benzylamine, respectively (Figure S15, SI). Figure 3 shows that in real water, the oxidation of primary amines also led mainly to nitrile with a molar yield of ~80% (or cyanate for methylamine), consistent with the results observed in pure water. In practice, Fe(VI) doses significantly higher than 3.5 mgFe L^{-1} can be used,[69] which is expected to oxidize a significant fraction of primary amines to nitriles. It is currently unknown whether the formation of non-halogenated nitriles is of concern.

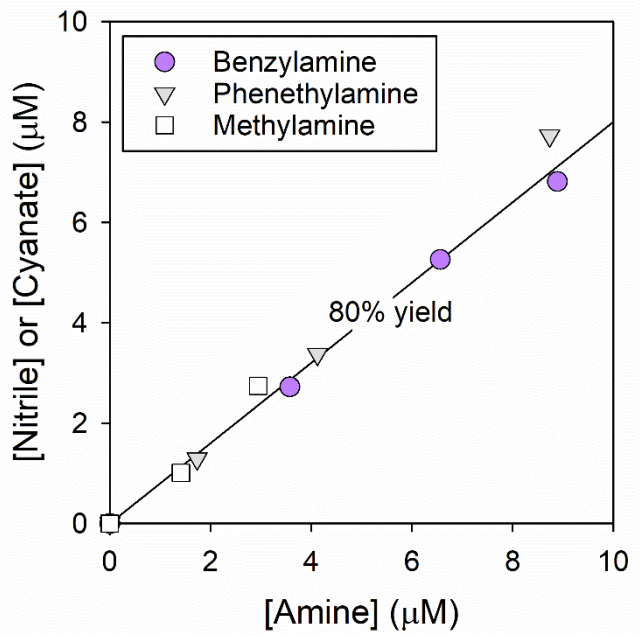


Figure 3. Yields of nitrile (or cyanate) from benzylamine, phenethylamine and methylamine in river water. Experimental conditions: $[Amine]_0$ = 10 μM, $[Fe(VI)]_0$ = 31–125 μM, pH = 8 before Fe(VI) addition and 8.5-9.5 after Fe(VI) addition. Water characteristics are given in Text S1 (SI).

Toxicity evaluation of nitrile-containing products. In recent years, the potency of low molecular weight halogenated DBPs has been intensively studied using in vitro bioassays to better understand the risks that they may induce.[1, 39, 70] In particular, the oxidative stress response has been a commonly observed effect, with 98% of 45 investigated halogenated DBPs activating this defense mechanism.[39] The potency of non-halogenated byproducts such as the nitriles formed in the present work has not yet been studied using these novel bioanalytical tools. The inhibitory concentrations for cytotoxicity (IC_{10}) and effective concentrations for the oxidative stress response ($EC_{IR1.5}$) of a group of non-halogenated nitriles are shown in Table 3 and Figure 4. Because of the volatility and low medium/air partition coefficients of the nitrile-containing structures detected in this work, only phenylacetonitrile and cyanate (although not a nitrile) as well as 12 other nitrile-containing compounds (Table 3) could be included in these bioassays, which are not amenable to too volatile chemicals.[51]

IC_{10} could be derived for 11 of 14 compounds. 3-Cyanopropionic acid and 3-oxobutyronitrile precipitated before causing cytotoxicity, and cyanoacetic acid did not show any cytotoxicity up to 0.12 M (Table 3). Figure 4 shows the negative logarithm of IC_{10} (open diamonds) plotted against their liposome/water partition coefficient ($\log K_{lip/w}$). The QSAR line (plain line in Figure 4) represents the baseline toxicity, which is the minimum toxicity that every compound is expected to elicit.[51] If the measured $\log(1/IC_{10})$ of the tested compounds exceeds their QSAR-predicted $\log(1/IC_{10,baseline}$ toxicity) by a one log-unit (Toxic Ratio, TR > 10), they are considered specifically acting or reactive toxicants that may induce toxicity via specific pathways.[56] This threshold, represented by a dotted line in Figure 4, was not exceeded by any non-halogenated nitrile, suggesting that cytotoxicity was merely caused by baseline toxicity.

Table 3. Cytotoxicity (IC_{10}) and activation of the oxidative stress response ($EC_{IR1.5}$) by non-halogenated nitrile-containing compounds in the AREc32 bioassay.

#	Chemical	IC_{10} (M)	$EC_{IR1.5}$ (M)
1	1-cyanocyclohexaneacetic acid	$1.4 \pm 0.4 \times 10^{-3}$	$4.3 \pm 0.9 \times 10^{-3}$
2	2-hydroxy(2-phenyl)acetonitrile	$1.3 \pm 0.1 \times 10^{-3}$	$8 \pm 1 \times 10^{-4}$
3	3-chlorophenylacetonitrile	$3.7 \pm 0.3 \times 10^{-4}$	no activation up to IC_{10}
4	3-cyanopropionic acid	no cytotoxicity up to 0.05 M*	$2.6 \pm 0.1 \times 10^{-3}$
5	3-hydroxypropionitrile	$2.8 \pm 0.3 \times 10^{-2}$	no activation up to IC_{10}
6	3-methoxypropionitrile	$1.2 \pm 0.3 \times 10^{-2}$	no activation up to IC_{10}
7	3-oxobutyronitrile	no cytotoxicity up to 3×10^{-4} M*	$7.8 \pm 0.4 \times 10^{-4}$
8	4-hydroxybenzonitrile	$2.8 \pm 0.3 \times 10^{-4}$	no activation up to IC_{10}
9	4-hydroxybenzylcyanide	$2.2 \pm 0.2 \times 10^{-3}$	$1.4 \pm 0.2 \times 10^{-3}$

10	2-phenylacetonitrile	$2.4 \pm 0.3 \times 10^{-3}$	no activation up to IC_{10}
11	sodium cyanate	$3.2 \pm 0.3 \times 10^{-3}$	$1.7 \pm 0.1 \times 10^{-3}$
12	cyanoacetic acid	no cytotoxicity up to 0.6 M	$9.5 \pm 0.6 \times 10^{-3}$
13	hydroxyacetonitrile	$6 \pm 1 \times 10^{-3}$	no activation up to IC_{10}
14	potassium cyanide	$1.6 \pm 0.2 \times 10^{-3}$	no activation up to IC_{10}

* Higher concentrations led to precipitation

50% the tested compounds activated the oxidative stress response in the human MCF7 cell-based AREc32 bioassay (Table 3), with $EC_{IR1.5}$ ranging between 7.8 x 10^{-4} and 9.5 x 10^{-3} M. Both oxidative stress response and cytotoxicity were observed in only 4 compounds: cyanate, 1-cyanocyclohexaneacetic acid, 2-hydroxy(2-phenyl)acetonitrile and 4-hydroxybenzylcyanide (Table 3). Oxidative stress response occurred with very low specificity, $SR_{baseline}$ and $SR_{cytotoxicity}$ both being ≤ 10, i.e., the oxidative stress response was not activated at concentrations significantly lower than the cytotoxicity (Table S4). The only exceptions were 3-cyanopropionic acid and 3-oxobutyronitrile, which had $SR_{baseline}$ values of 12.7 and 43.5, respectively (Table S4, SI). By comparison, halomethanes (excluding iodo-) as well as tri- and dichloroacetics also exhibited low $SR_{baseline}$ (≤ 10), while haloacetonitriles, haloacetamides (excluding trichloro- and dichloro-), haloketones and halonitromethanes led to an $SR_{baseline}$ of 10^2–10^4 (Table S5 and Figure S16, SI).[39] Overall, these results suggest that the formation of non-halogenated nitriles is unlikely to pose a significant threat because they are of low potency (high IC and EC values) and are not specifically acting for cytotoxicity and oxidative stress response unlike many other halogenated DBPs. However, more toxicological studies, such as genotoxicity or studies on the impact of metabolic activation, are needed to further evaluate the potency of non-halogenated nitriles in water.

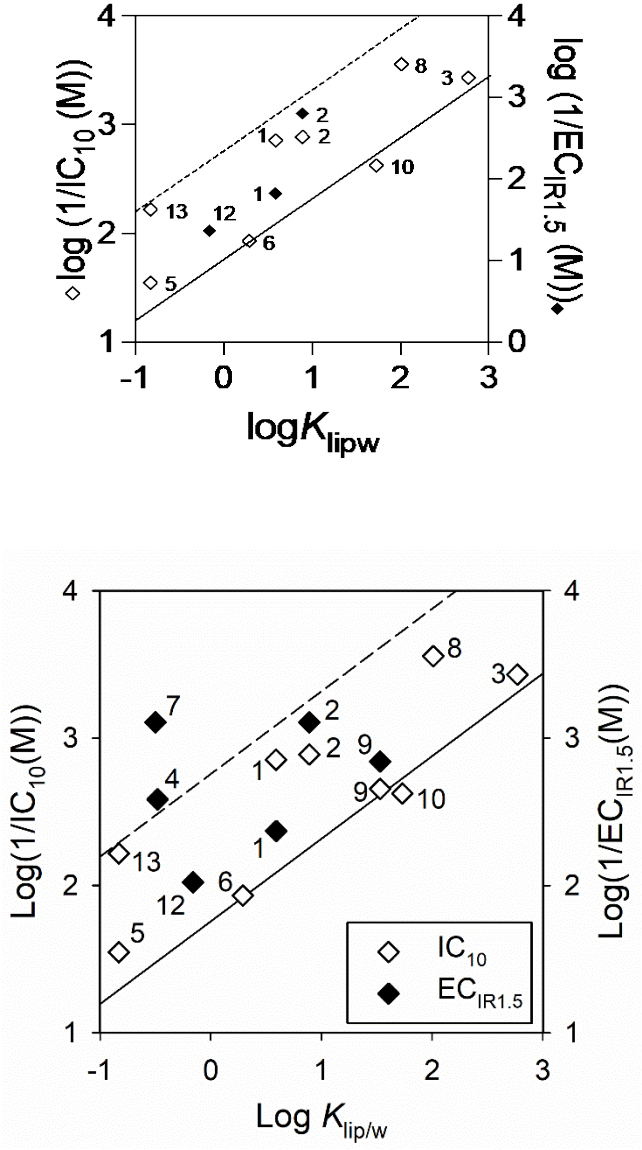


Figure 4. 10% inhibitory concentration for cell viability (IC_{10}) and activation of oxidative stress response ($EC_{IR1.5}$) compared to the liposome/water partition coefficient ($K_{lip/w}$) for nitrile-containing structures. Cyanate and cyanide are not shown as they are inorganic and have no $K_{lip/w}$ defined. The plain line corresponds to the QSAR of baseline toxicants and the dotted line corresponds to a toxic ratio of 10. Raw data and chemical names associated with the numbers are given in Table 3 and Table S3 (SI).

Practical implications. The oxidation of aliphatic primary amines by Fe(VI) is slower than that of phenols or anilines but within the same order as that of olefins or secondary

amines.[44] Aliphatic primary amines, therefore, need to be considered main or secondary reactive sites. In contrast to previously proposed pathways, the oxidation of primary amines by Fe(VI) does not necessarily lead to C–N breakage bonds. Except the shortest amine-containing structure (methylamine), the formation of high yields of nitrile was observed for all studied compounds. A complete examination of product formation from benzylamine and phenethylamine also showed that minor products included aldehydes, carboxylic acids, nitroalkanes, ammonia, and nitrite/nitrate, formed at increasing yields with decreasing pH to 7. Cytotoxicity and oxidative stress response bioassays conducted on 14 different nitrile-containing compounds suggested that the formation of non-halogenated nitriles is unlikely to be a major concern. Furthermore, during an eventual post-disinfection, nitriles would not be reactive toward chlorine or chloramine;[3] thus, Fe(VI) preoxidation should not enhance halonitrile formation, which are known potent DBPs.[1, 39] Some nitroalkanes may be produced at pH 7, but their yields (< 20%) should be low in comparison to O_3 preoxidation, which is known to produce 100% nitroalkanes from primary amines.[26, 71] While the potency of nitroalkanes is unknown, post-chlorination would lead to the formation of very potent halonitroalkanes due to the acidic nature of carbon α to the nitro group.[27] By comparison, if ClO_2 is used as a preoxidant, primary amines should remain intact and be available to react with chlorine during disinfection, forming organic chloramines. These chloramines can be stable, and their toxicity is currently largely unknown.[3, 72] Primary amines are notably present in amino acids, which, due to the presence of a carboxylic acid group, form products other than nitriles [in preparation].[59] They are also likely to be contained in complex NOM structures, and their occurrence is uncertain, as there is no analytical method to discriminate between the different forms of organic nitrogen. The contribution of primary amine transformation products to the potency of treated waters is therefore unclear, but based on the present work, Fe(VI) may be a better option than O_3 or chlorine for their oxidation.

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