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Reaction Pathways of the Diketonitrile Degradate of Isoxaflutole with Hypochlorite in Water

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Isoxaflutole (IXF; Balance) belongs to a new class of isoxazole herbicides. Isoxaflutole has a very short half-life in soil and rapidly degrades to a stable and phytotoxic degradate, diketonitrile (DKN). DKN was previously discovered to rapidly react with hypochlorite (OCl^-) in tap water, yielding the benzoic acid (BA) degradate as a major product, but the complete reaction pathway and mechanism have not been elucidated. Thus, the objectives of this work were to (1) determine the stoichiometry of the reaction between DKN and OCl^-; (2) identify products in addition to BA; and (3) propose a complete pathway and reaction mechanism for oxidation of DKN by OCl^-. Stoichiometry of the reaction showed a molar ratio of OCl^-/DKN of 2. In addition, two previously uncharacterized chlorinated intermediates were identified under conditions in which OCl^- was the limiting reactant. The proposed chemical structure of a chlorinated benzoyl intermediate was inferred from a series of HPLC/MS and HPLC/MS/MS experiments and the use of mass spectral simulation software. A chlorinated ketone intermediate was also identified using ion trap GC/MS. Two additional end products were also identified: cyclopropanecarboxylic acid (CPCA) and dichloroacetonitrile (DCAN). On the basis of the reaction stoichiometry, the structure of the chlorinated intermediates, and the identification of the products, two reaction pathways are proposed. Both pathways involve a two-step nucleophilic attack and oxidation of the diketone structure of DKN, leading to formation of BA, DCAN, and CPCA.

KEYWORDS: Isoxaflutole; diketonitrile degradate; benzoic acid degradate; hypochlorite; water treatment; cyclopropanecarboxylic acid; dichloroacetonitrile

INTRODUCTION

Balance [isoxaflutole, IFX: 5-cyclopropyl-4-(2-methanesulfonyl-4-trifluoromethyl-benzoyl)-isoxazole, CAS Registry No. 141112-29-0] belongs to a relatively new class of isoxazole herbicides (*1*). Isoxazoles control grass and broadleaf weeds at low application rates (30−100 g/ha) by inhibiting pigment biosynthesis, leading to a lack of chloroplast development (*2*). IXF is a proto-herbicide (i.e., it has no herbicidal activity) that spontaneously rearranges in the presence of water to the phytotoxic diketonitrile degradate [2-cyclopropyl-3-(2-methylsulfonyl-4-(trifluoromethyl)benzoyl)-3-oxopropanenitrile (DKN)] via opening of the isoxazole ring (**Figure 1**). DKN can then further degrade to a benzoic acid [2-methylsulfonyl-4-trifluoromethylbenzoic acid, (BA)] degradate. IXF was conditionally registered by the U.S. EPA in 1998 for use on corn in 17 states. The State of Michigan initially banned the use of IXF and has since refused to register it. Beginning in 2002, the State of Wisconsin agreed to register Balance, but very restrictive conditions were placed on its use. Reasons cited by state and federal agencies for restricting IXF use included designation of IXF as a probable human carcinogen, the high potential for contamination of surface and groundwaters by the parent and its two primary degradates (DKN and BA), and concerns about spray drift or contaminated irrigation water causing injury to nontarget crops and plants (*3*, *4*).

Recent studies support concerns about the transport of IXF degradates to surface and groundwaters (*5*−*8*). In a field lysimeter study using a sandy soil, Lin et al. (*6*) found significant levels of DKN and trace levels of BA in shallow groundwater within 1 day of application following about 27 mm of rainfall, but IXF was not detected. Twenty-five days after application, average relative losses of DKN to groundwater were 8.1% of the applied IXF as compared to losses of 4.4% for applied atrazine (*6*). From 1999 to 2002, DKN was detected during the spring and summer in Iowa streams at median levels ranging from about 1 to 40 ng/L (*5*, *7*). Peak DKN concentrations in the Iowa streams exceeded 100 ng/L in the spring of each year,

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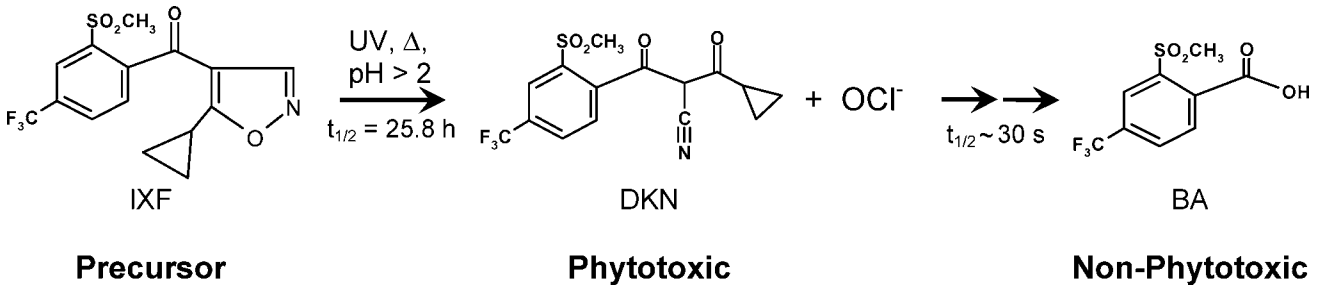

Figure 1. Generalized reaction and kinetics for conversion of IXF to BA via DKN oxidation in the presence of hypochlorite (OCl^-) (based on ref *6*).

except 2001, and detection frequency of DKN reached 88% in June 2000. Concentration data for IXF, DKN, and BA in reservoirs from a five-state, multi-year study showed combined residue levels of the three compounds in the range of 5 to >300 ng/L (*8*). Given the results of the study by Lin et al. (*6*) and other studies that have demonstrated the short half-life of IXF in soils (*9*, *10*), it seems probable that only the two degradates were present in these reservoirs. Data from the same five-state study reported that average DKN concentrations in two Nebraska lakes were 277 ng/L (*5*). These recent reports demonstrate that DKN and BA may be transported to surface and groundwaters at significant levels. Therefore, in watersheds with considerable IXF usage, the high potential for off-site transport of these degradates indicates the strong likelihood that they will occur in source waters used by municipal water treatment facilities.

Chlorination continues to be widely employed for the disinfection of public water supplies (*11*). Chlorine added to water supplies as Cl_2(g), NaOCl, or $Ca(OCl)_2$ will result in hypochlorite (OCl^-) as the solution species responsible for disinfection. Hypochlorite is a strong oxidant capable of oxidizing natural and anthropogenic organic compounds present in raw (untreated) water (*11*−*17*). The oxidation of natural organic matter by OCl^- results in the formation of disinfection byproducts that are considered a public health threat by the U.S. EPA (*18*). In addition, some pesticides present in raw water may also be oxidized by OCl^- during the disinfection process (*12*, *14*, *15*, *17*). In some cases, chlorination can lead to pesticide oxidation products that are more toxic than the parent compound. For example, Zhang and Pehkonen (*15*) reported that the oxidation of diazinon by sodium hypochlorite resulted in the formation of the more toxic product, diazoxon. In other cases, oxidation of pesticides during chlorination apparently improves water quality by eliminating the known toxic forms of the pesticide, which may be either the parent compound or its degradates, from drinking water (*12*, *14*, *17*). The rapid oxidation of four sulfur containing *s*-triazines by HOCl resulted in the formation of hydroxylated *s*-triazines as the products via a three-step reaction involving oxidation of the sulfur atom (*14*). This reaction did not result in the formation of chlorinated byproducts, and the hydroxylated triazine products formed were less toxic than their corresponding parent compounds.

Lin et al. (*17*) observed rapid degradation of DKN in solutions prepared with tap water derived from a municipal source, and the primary agent responsible for DKN degradation in the tap water was shown to be OCl^-. In addition, they reported that the BA degradate was the primary product, with an additional unknown intermediate (molecular weight = 325 g) also detected (*17*). From this work, OCl^- was shown to be consumed by the reaction with DKN, and a general reaction describing the kinetics for the conversion IXF to DKN followed by DKN oxidation to BA was reported (**Figure 1**). This general reaction shows the relatively fast conversion of IXF to DKN followed by the extremely rapid oxidation of DKN by OCl^- to form BA. However, Lin et al. (*17*) did not elucidate the specific reaction pathway(s), its mechanism, or identify all possible products. Thus, the objectives of this work were to (i) determine the stoichiometry of the reaction between DKN and OCl^-; (ii) identify products in addition to BA; and (iii) propose a complete pathway and reaction mechanism for oxidation of DKN by OCl^-.

MATERIALS AND METHODS

Sample Preparation. Stock solutions of IXF, DKN, and BA were prepared from analytical standards of at least 95% purity acquired from Rhone-Poulenc Agro Co. (now Bayer CropScience, Research Triangle Park, NC) and initially dissolved in acetonitrile (ACN). Working solutions were prepared from the stock solutions and diluted in HPLC grade water for use in the various experiments. Hypochlorite solutions were prepared from fresh commercial grade NaOCl or reagent grade $Ca(OCl)_2$. All chemicals used for HPLC and GC analyses (described next) were HPLC grade. All other chemicals used were reagent grade.

Reaction Stoichiometry. The stoichiometry of the reaction between DKN and OCl^- was determined by iodometric back-titration (*19*, *20*). Solutions for the titration were prepared as follows: DKN, 0.56 mM; OCl^-, 6.12 mM; $Na_2S_2O_3 \cdot 5H_2O$, 1.99 mM; and KI, ~3 mM in 0.3 M H_2SO_4. All solutions were prepared using deionized−distilled water. A mixture containing 20 mL of DKN solution and 10 mL of OCl^- solution was allowed to react for 1 min, and 10 mL of KI was then added to react with the excess OCl^-. The triiodide (I_3^-) thus produced was then titrated to an endpoint (with the aid of a starch indicator) with thiosulfate.

HPLC/UV Analyses. HPLC/UV analyses were conducted as described by Lin et al. (*21*). The HPLC column was a Columbus C8 (4.6 mm × 100 mm; 5 μm) (Phenomenex, Torrance, CA) set to 40 °C. UV detection was 270 nm for IXF and BA and 300 nm for DKN. An isocratic mobile phase mixture of 0.1% H_3PO_4 (pH 2.2) and ACN at a ratio of 60% H_3PO_4/40% ACN was used for separating the compounds. Flow rate was 1 mL min^{-1}. Sample injection volume was 100 μL.

Mass Spectral Analyses. HPLC Tandem Mass Spectrometry. Initial HPLC tandem mass spectrometry analyses (HPLC/MS/MS) were conducted to confirm the presence of BA as described by Lin et al. (*17*). Additional HPLC/MS and HPLC/MS/MS analyses were performed on an aqueous sample mixture containing 4 mg/L DKN and 0.5 mg/L OCl^- to identify the unknown intermediate observed by Lin et al. (*17*) (**Figure 2**). A Thermo-Finnigan TSQ7000 triple quadrupole mass spectrometer with its associated LC system and Xcalibur software (Thermo Electron Corp., Waltham, MA) was used for all HPLC/MS and HPLC/MS/MS analyses. The HPLC column was a Columbus C8 (2 mm × 100 mm; 3 μm). The sample injection volume was 20 μL, and elution was isocratic using a 50:50 mixture of acetonitrile and water with 0.5% acetic acid. Eluting analytes were ionized by negative mode electrospray ionization to yield deprotonated pseudomolecular ions (i.e., $[M - H]^-$), which confirmed the presence of BA and DKN and also revealed the mass of the unknown intermediate (*17*). The precursor ions $[M - H]^-$ of the unknown intermediate (*m*/*z* 324 and *m*/*z* 326) were fragmented by collision-induced dissociation using argon at ~1

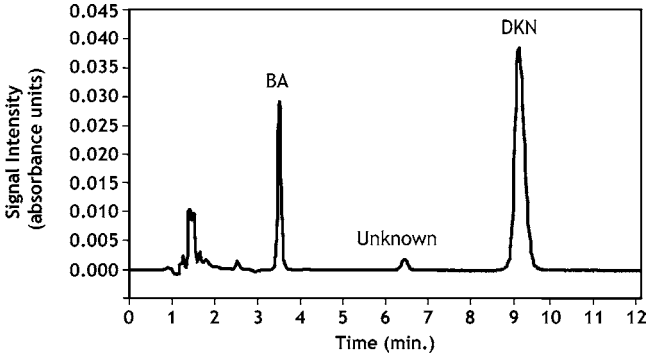

Figure 2. HPLC/UV chromatogram showing the presence of a stable unknown intermediate detected when OCl^- was the limiting reactant.

mTorr pressure as the collision gas to generate product ion spectra for structural determination. Interpretation of spectra and identification of the unknown were aided by isotope profiling based on the stable isotopes of Cl ($^{35}Cl/^{37}Cl = 3:1$), S ($^{32}S/^{34}S = 95:4.2$), and C ($^{12}C/^{13}C = 98.9:1.1$) and by the use of IsotopeViewer, Version 1.0, mass spectral simulation software (Thermo Electron Corp., Waltham, MA). Further analyses to identify products were done by direct infusion MS and MS/MS, wherein samples were infused (at 10 μL/min) directly into the electrospray source. An aqueous mixture containing 100 mg/L DKN and 20 mg/L OCl^- was prepared and allowed to react for several minutes before direct infusion MS analysis.

Gas Chromatography/Mass Spectrometry. Additional mass spectral analyses were conducted by ion trap gas chromatography/mass spectrometry (GC/MS) to identify possible intermediates or surmised product compounds that were not detected under the electrospray ionization conditions, described previously, or were poorly ionized and additional confirmation was required. To detect possible products and intermediates, a 50 mL solution containing a mixture of 50 mg/L DKN and 10 mg/L OCl^- was prepared in water. A 50 mL control solution was also prepared containing 10 mg/L OCl^- only. One purpose of this experiment was to trap and identify possible volatile compounds not detected by HPLC/MS/MS; therefore, 50 mL of ethyl acetate was added immediately after DKN addition to extract any nonpolar volatile products. Both samples (control and DKN spiked) were shaken intensively for 2 min in a separatory funnel. An emulsion formed in the DKN sample, which was removed by centrifugation at 600 rpm. No emulsion was observed in the control sample. Following liquid−liquid extraction, a 1 mL aliquot of solution was transferred to a chromatography vial and sealed with a Teflon-lined septum prior to analysis.

Initially, mass spectra were obtained for the DKN and control samples and standards of DKN and BA. DKN and BA standards were 10 mg/L in ethyl acetate. Final spectra containing possible products were obtained by subtracting the control spectrum from the DKN sample spectrum. In addition, ions considered unique to the reaction of DKN and OCl^- were identified from a combination of the background subtracted spectrum and the spectra from the DKN and BA standards. Using these spectra, reconstructed ion chromatograms were screened for ions of common mass and retention time, and these ions were eliminated as possible reaction products. Only unique ions present in the background subtracted spectrum, and not present in the standard spectra, were considered reaction products. The GC was a Varian 3400 with a Saturn 2000 ion trap MS detector (Varian, Harbor City, CA). An HP-1 (Agilent Technologies, Palo Alto, CA) capillary fused-silica column (0.33 μm film thickness; 12 m × 0.2 mm i.d.) was used with He as the carrier gas at a flow rate of 1 mL/min to separate the analytes. A split/splitless injector was used in splitless mode with an injector temperature of 200 °C and an injection volume of 1 μL. A multistep temperature program with a total run time of 27 min was used to separate the analytes. MS conditions were transfer line temperature, 250 °C; trap temperature, 250 °C; electron impact energy, 70 eV; electron multiplier voltage, −2000 V; mass scan range, 32−400; and detection mode, full range.

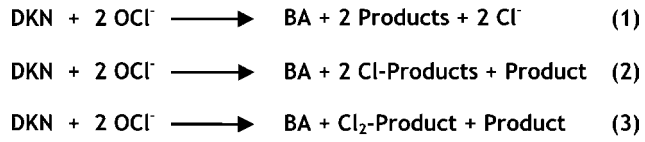

Figure 3. Stoichiometry and three potential fundamental reactions proposed for DKN oxidation by hypochlorite (OCl^-).

In a separate experiment, ion trap GC/MS analysis was also conducted to confirm the presence of dichloroacetonitrile ($CHCl_2CN$, DCAN). The experimental setup included triplicate samples containing 66.6 mg/L (2.78 μmol total) DKN mixed with 53.3 mg/L (5.57 μmol total) OCl^-, added as $Ca(OCl)_2$, in a total of 15 mL of aqueous solution. Blanks of the same volume included one sample each of HPLC grade water, 66.6 mg/L DKN only, and 53.3 mg/L OCl^- only. All samples were prepared in 20 mL screw cap Pyrex test tubes with PTFE-lined septa. The DKN and OCl^- samples were allowed to react for a minimum of 5 min at room temperature (~25 °C). Extraction of DCAN from the samples was accomplished using EPA method 551.1 (*22*). After the reaction between DKN and OCl^- was complete, all samples were acidified to pH ~4.5 with approximately 1 g of a phosphate buffer containing 1% Na_2HPO_4 and 99% KH_2PO_4 (w/w) to prevent hydrolysis of DCAN. A 3 mL aliquot of methyl-*tert*-butyl-ether (MTBE) was added to each sample using a syringe and needle. To minimize volatilization losses of DCAN and MTBE, the septa were pierced with the needle to deliver the MTBE. The samples were mixed by hand in an end-over-end fashion for 4 min. To remove the emulsion that formed (only observed in the DKN and OCl^- samples) after the MTBE extraction step, approximately 6 g of anhydrous Na_2SO_4 was added to each sample, and the samples were mixed end-over-end until all Na_2SO_4 was dissolved. A 100 μL aliquot of the MTBE layer was removed and placed in chromatography vials with septa-lined screw caps for GC/MS analysis. GC/MS analysis was performed using the following temperature program: 32 °C held for 4 min to 175 °C at 50 °C per minute and hold 175 °C for 2 min for a total time of 9.5 min. The injector temperature was 175 °C. MS conditions were mass scan range, 74−114, and detection mode, selected ion storage (m/z 82 + 84 + 86). All other GC/MS conditions were as noted previously. Confirmation of DCAN was based on retention times established from a DCAN standard (98% purity, Acros Organics, Morris Plains, NJ) and the detection of diagnostic chlorinated ions (m/z 82 + 84 + 86) (*23*).

RESULTS AND DISCUSSION

Stoichiometry and Fundamental Reactions. Results of the iodometric titration showed that the average molar ratio of OCl^-/DKN was 2.06 ± 0.01. From this result, we concluded that the reaction stoichiometry was essentially 2 mol of OCl^- reacting with 1 mol of DKN. Assuming that 1 mol of BA was formed per mol of completely oxidized DKN (*17*), three fundamental reactions were presumed (**Figure 3**). Reaction 1 would produce Cl^- while reactions 2 and 3 reflect the possibility of forming chlorinated byproducts. Chloride was not observed by direct infusion MS analysis. Thus, reaction 1 was eliminated from further consideration, and additional studies focused on the identification of chlorinated and non-chlorinated products, as well as an intermediate product observed by Lin et al. (*17*) (**Figure 2**), for discerning the pathway(s) for the reaction between OCl^- and DKN.

Identification of Chlorinated Intermediates. As previously discussed, Lin et al. (*17*) observed a stable unknown compound in HPLC/UV chromatograms under conditions in which OCl^- was the limiting reactant (**Figure 2**). Initial HPLC/MS analysis of this intermediate product indicated that its molecular weight was ~325 g and that its retention time and UV absorbance suggested an aromatic compound of intermediate polarity to that of DKN and BA. Given that Cl^- was not released into solution

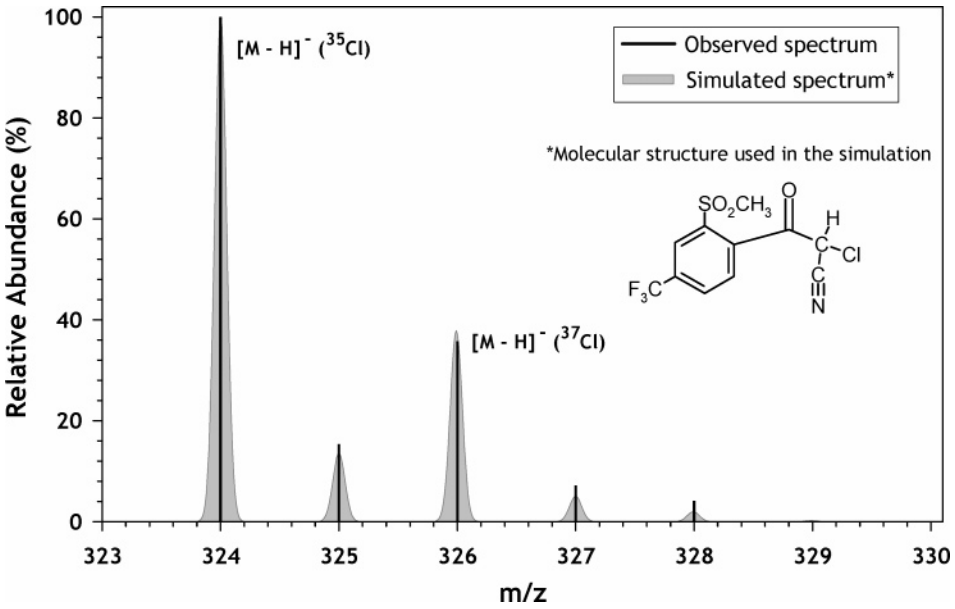

Figure 4. HPLC/MS precursor ion spectrum of the chlorinated benzoyl intermediate (CTOP) and a simulated spectrum based on the hypothesized molecular structure shown. Note: the simulated spectrum was based on the deprotonated pseudomolecular ion of CTOP.

by the reaction, we assumed that the intermediate was chlorinated. The identification of this unknown was considered key to elucidation of one possible reaction pathway. A three-step process was used to identify this unknown: (i) precursor ion mass spectra (HPLC/MS) confirmed the molecular weight and identified molecular ions containing Cl isotopes; (ii) product ion mass spectra (HPLC/MS/MS) of precursor ions were used to obtain additional structural information; and (iii) using a probable structure, mass spectral simulation software was used to predict the distribution and abundance of ions in the precursor ion spectra for comparison against the sample spectra.

The precursor ion spectrum produced a deprotonated base peak of *m*/*z* 324 that was tentatively assumed to contain ^{35}Cl and a corresponding deprotonated ion of *m*/*z* 326 that presumably contained ^{37}Cl (**Figure 4**). Using the base peak mass, the unknown had a molecular weight of 325 g, confirming the Lin et al. value (*17*). The distribution and abundance of precursor ions in the spectrum (**Figure 4**) supported our assumption that the unknown product was chlorinated. For example, the ratio of relative abundances between *m*/*z* 324 and *m*/*z* 326 was 2.8:1, close to the theoretical ratio of 3:1 for the stable isotopes of Cl. Next, HPLC/MS/MS analyses were conducted to obtain product ion spectra from the two presumed chlorinated precursor ions (*m*/*z* 324 and 326) observed in **Figure 4**. Product ion spectra of these precursor ions showed corresponding fragmentation patterns and relative ion intensities (**Figure 5A**,**B**). Chlorinated product ions, based on tentative fragment identification (**Figure 5A**,**B**), showed the expected offset of two atomic mass units associated with the stable chlorine isotopes for the ion pairs *m*/*z* 306/308, *m*/*z* 74/76, and *m*/*z* 35/37. In addition, a non-chlorinated ion, *m*/*z* 249, was also observed in both product ion spectra. From this information, a probable structure was developed based on a chlorinated compound with a molecular weight of 325 g and the assumption that the oxidation of DKN occurred via nucleophilic attack of the β-ketone (i.e., the carbonyl adjacent to the cyclopropyl group). This structure was then entered into the simulation software, and the predicted and observed precursor ion spectra were then compared (**Figure 4**). As can be seen from **Figure 4**, the simulated and observed spectra matched very well in terms of both ion masses and their relative abundances. The combination of precursor and product ion spectra, coupled with the high degree of conformity between the observed and the simulated precursor spectra, confirmed

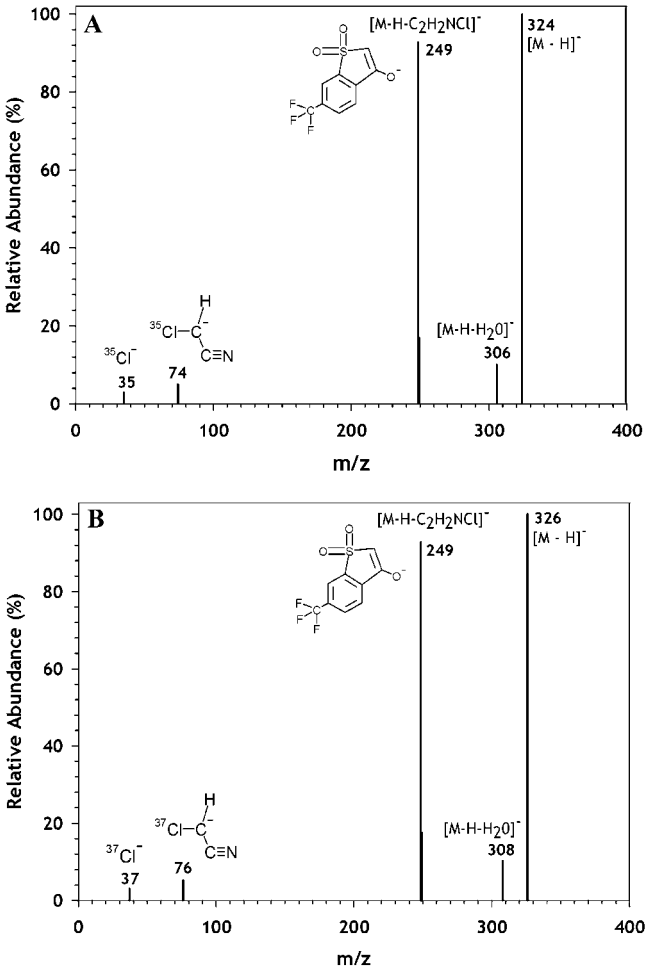

Figure 5. HPLC/MS/MS product ion spectra of the chlorinated benzoyl intermediate (CTOP). (**A**) Product ion spectra from precursor ion *m*/*z* 324. (**B**) Product ion spectra from precursor ion *m*/*z* 326.

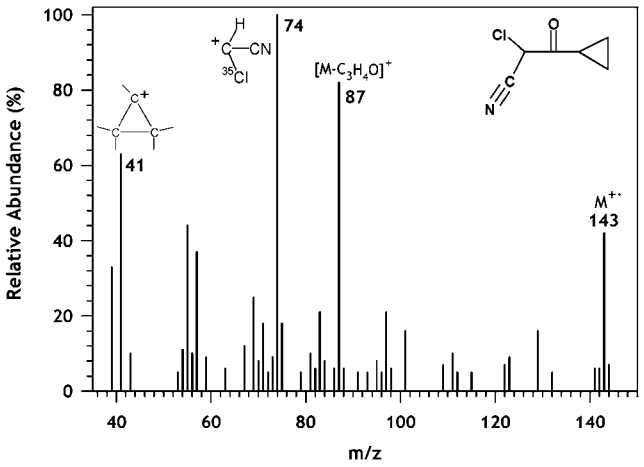

Figure 6. GC/MS spectrum of the chlorinated ketone intermediate (CCOP) based on tentatively identified diagnostic ions from the *m*/*z* 143 nominal mass ion.

the identity of this chlorinated benzoyl intermediate (see structure in **Figure 4**) as 2-chloro-3-[2-(methylsulfonyl)-4-(trifluoromethyl)phenyl]-3-oxopropanenitrile (CTOP).

An additional chlorinated intermediate was also surmised based on the likelihood that the initial nucleophilic attack could also occur at the benzoyl carbonyl (i.e., the carbonyl adjacent to the aromatic ring) of DKN. This reaction would presumably result in a chlorinated ketone compound with a nominal mass of 143 g (**Figure 6**) and directly results in the formation of the BA degradate. The anticipated intermediate would not be expected to undergo facile deprotonation via electrospray and was not observed in such experiments; however, GC/MS analysis showed the presence of a product (*m*/*z* 143) at a retention time of 20.68 min. Background subtracted spectrum of this ion resulted in several tentatively identified product ions consistent with the chlorinated ketone structure shown in **Figure 6**. On the basis of this spectrum, the chlorinated ketone intermediate was tentatively confirmed as 2-chloro-3-cyclopropyl-3-oxopropanenitrile (CCOP).

Identification of Products. On the basis of the identification of the intermediates and the previous work by Lin et al. (*17*), three products were assumed from the reaction of DKN with OCl^-, and the formation of all three products was confirmed. As previously reported by Lin et al. (*17*), the BA degradate was confirmed as a product by HPLC/MS/MS analyses. In these experiments, a sample of the reaction mixture containing DKN and OCl^- was used to compare against an authentic BA standard to obtain three points of identification: retention time, precursor ion mass ($[M - H]^-$, *m*/*z* 267), and diagnostic product ion ($[M - H - COO - SO_2]^-$, *m*/*z* 159). All three points of identification confirmed that BA was a product. Another product, cyclopropanecarboxylic acid (CPCA), was confirmed by detection of the pseudomolecular ion ($[M - H]^-$, *m*/*z* 85) using direct infusion MS of a sample aliquot from a DKN and OCl^- reaction mixture. Both BA and CPCA were also observed as products in an analogous reaction in which DKN was oxidized by dimethyldioxirane (*24*). Mass balance of the proposed reaction pathways (see following discussion) and the absence of free chloride in the direct infusion MS experiment led to the assumption that a third product, DCAN, was also formed. DCAN was identified in all three samples containing DKN and OCl^- using ion trap GC/MS. Retention times of three replicate sample reaction mixtures matched to within 0.02 min, and the diagnostic chlorinated ions were detected in all three samples. In addition, no peaks with retention times corresponding to DCAN were detected in any of the sample blanks. The possible reaction of OCl^- with the sulfonyl group was also considered but ruled out for two reasons. First, nucleophilic attack of the sulfonyl would not yield the observed products. Second, no intermediates consistent with this reaction pathway were detected by direct infusion MS.

Reaction Mechanism and Degradation Pathways. The identification of the chlorinated intermediates provided important insight to the reaction pathways and mechanism (**Figure 7**). Given that OCl^- is a strong nucleophile and that a carbonyl carbon is an electrophilic center (σ^+), nucleophilic attack and oxidation of the carbonyl carbons was the probable reaction mechanism. Elucidation of the chemical structure of the two chlorinated intermediates and confirmation of the three final products supported this as the reaction mechanism. The intermediates also confirmed that two pathways exist, depending upon which carbonyl moiety initially reacted with OCl^-. These pathways are analogous, with both involving successive two-step nucleophilic attack and oxidation of the diketone structure. Pathway 1 shows a two-step nucleophilic attack beginning with the β-ketone, which initially results in the formation of CPCA and the intermediate, CTOP. Subsequent nucleophilic attack at the benzoyl ketone of CTOP produces BA and the chlorinated byproduct, DCAN. Pathway 1 was based on (i) structural identification and confirmation of CTOP; (ii) confirmation of the products; and (iii) the reaction stoichiometry. Pathway 2 shows the alternate reaction sequence in which the nucleophilic attack begins with the benzoyl ketone. This pathway initially results in the formation of BA and the intermediate, CCOP. Further reaction of CCOP produces CPCA and DCAN. Thus, both pathways result in the production of the same three products: BA, CPCA, and DCAN. Pathway 2 was based on (i) confirmation of CCOP; (ii) confirmation of the products; and (iii) the reaction stoichiometry.

Implications for Drinking Water Safety and Environmental Contamination. From the perspective of drinking water safety, these results, and those of Lin et al. (*17*), showed that standard chlorine disinfection will eliminate DKN, at ambient concentrations in raw water, from drinking water supplies. The level of hypochlorite employed by water treatment facilities is typically in the range of 2−5 mg/L to achieve disinfection (*11*). Assuming an ambient DKN concentration of 100 ng/L in raw water and an OCl^- concentration of 2 mg/L used for water treatment, this would result in a molar ratio of OCl^-/DKN of 139 068. Since the reaction stoichiometry is 2 mol of OCl^- to 1 mol of DKN, then OCl^- would be in excess by 69 534 times that needed to completely oxidize DKN. This excess, even in the presence of other organic C in the raw water, should be more than sufficient to completely oxidize DKN. Additionally, given the anticipated levels of DKN in raw water and the high levels of OCl^- used for drinking water disinfection, neither of the chlorinated intermediates (CTOP and CCOP) would be expected to be present in finished drinking water.

Of the three products formed, two (CPCA and DCAN) are known human toxins (*25*−*28*). CPCA is a liver toxin that inhibits mitochondrial fatty acid oxidation leading to glycogen depletion, hepatic microvesicular steatosis, cell death, and liver failure (*27*, *28*). Reported toxic effects of CPCA (*27*) occurred at concentrations that were approximately 3 orders of magnitude higher than those anticipated in water supplies based on ambient DKN concentrations reported to date (*8*). DCAN is a known mutagen that induces DNA strand breaks in cultured human lymphoblastic cells (*25*) and DNA damage in HeLa S3 cells

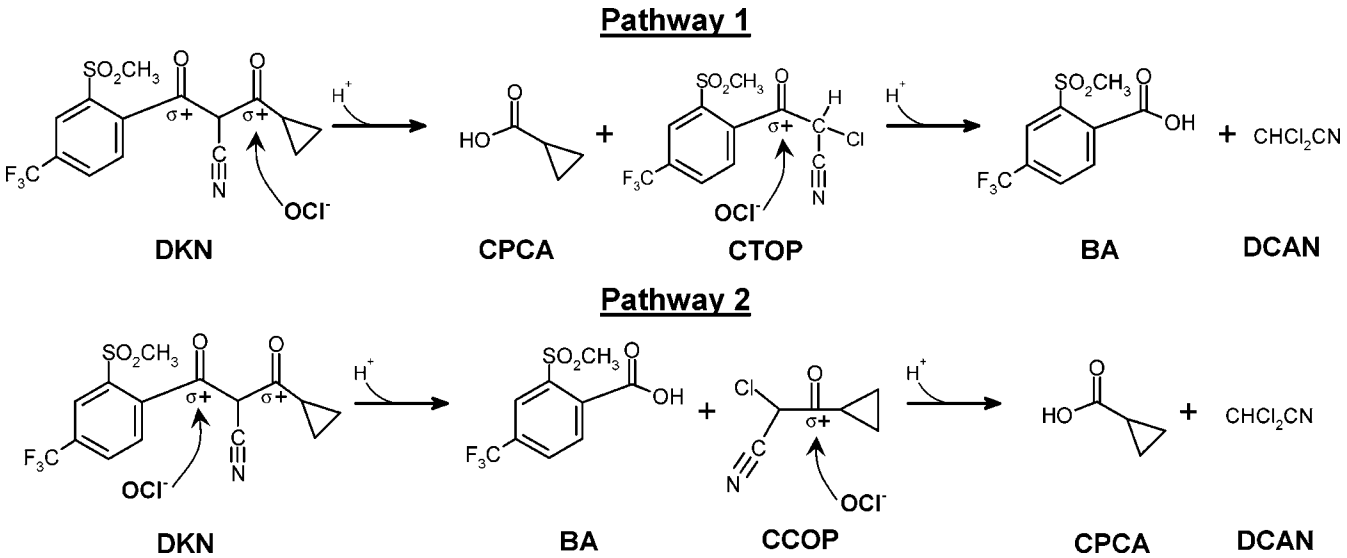

Figure 7. Pathways for reaction of DKN with hypochlorite. Pathway 1 shows a two-step nucleophilic attack beginning with the β-ketone of DKN. Pathway 2 shows the alternate two-step reaction sequence in which the nucleophilic attack initially occurs at the benzoyl ketone of DKN. For the purpose of achieving mass balance for the reactions, the existing pathways are shown as occurring under acidic conditions, but they may also occur under basic conditions according to the following general reaction: $R + OCl^- + H_2O \rightleftharpoons P + OH^-$, where R = reactant and P = product.

(*26*). Using the comet assay, Muller-Pillet et al. (*26*) reported that significant DNA damage was observed for DCAN at concentrations as low as 1 μM (110 μg/L). This concentration is near the target DCAN concentration of 90 μg/L recommended by the World Health Organization for drinking water (*23*), but it was 1−2 orders of magnitude greater than DCAN concentrations reported in drinking water supplies (*29*, *30*). With respect to BA, the U.S. EPA (*31*) reported no adverse health effects of BA in a 28-day subchronic toxicity study using male and female rats. Given ambient DKN concentrations reported in raw water, the levels of DCAN and CPCA would be insufficient to induce toxic effects. However, DCAN and CPCA may also be formed from naturally occurring precursor compounds (*29*, *32*), and the presence of DKN in addition to these natural precursors in raw water may, in some cases, result in toxic levels of these compounds being formed.

The potential for environmental contamination of surface or groundwaters by the chlorinated intermediates and the final products also exists for situations in which chlorinated tap water is used to prepare IXF solutions for field applications. In this scenario, IXF initially hydrolyzes to DKN in the presence of water, and DKN subsequently reacts with the OCl^- present in the spray mixture. Since OCl^- would be the limiting reactant in this case, both chlorinated intermediates and some of the end products could be formed in the mixing tank. However, Lin et al. (*17*) reported that the half-life for conversion of IXF to DKN in the presence of OCl^- was 25.8 h (**Figure 1**); thus, significant formation of the chlorinated intermediates and end products would only occur if the spray mixture was not immediately applied. Moreover, less than 5% of the DKN would be consumed in the reaction given the low concentrations of OCl^- typically present in tap water (*17*). Although the amount of intermediates and end products formed would be low, repeated use of IXF on the same field could lead to significant application of these compounds to soil, creating the potential for off-site hydrologic and aerial transport. The potential toxic effects of the chlorinated intermediates to humans and wildlife is currently unknown. Therefore, it is recommended that farmers not use chlorinated water when preparing IXF solutions, but if this cannot be avoided, then application should occur immediately after preparing spray solutions to prevent the potential introduction of the chlorinated intermediates and final products into the environment.

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