

## Degradation of Isoxaflutole (Balance) Herbicide by Hypochlorite in Tap Water

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Chlorine has been widely employed for the disinfection of drinking water. Additionally, it has the capacity to oxidize many organic compounds in water. Isoxaflutole (Balance; IXF) belongs to a new class of isoxazole herbicides. Isoxaflutole has a very short soil half-life and rapidly degrades to a stable and phytotoxic metabolite, diketonitrile (DKN). Further degradation of DKN produces a nonbiologically active benzoic acid (BA) metabolite. In experiments using high-performance liquid chromatography-UV spectroscopy (HPLC-UV) and HPLC tandem mass spectrometry (HPLC-MS/MS), DKN was found to rapidly react with hypochlorite in tap water, yielding the BA metabolite as the major end product. One milligram per liter (19  $\mu\text{M}$ ) of hypochlorite residue in tap water was able to completely oxidize up to 1600  $\mu\text{g/L}$  (4.45  $\mu\text{mol/L}$ ) of DKN. In tap water, the disappearance of IXF was much more rapid than in DI water. As soon as the IXF is hydrolyzed to DKN, the DKN quickly reacts with the  $\text{OCl}^-$  to form nonphytotoxic BA. As a result, the herbicide solutions prepared with tap water at 500  $\mu\text{g/L}$  will no longer possess any herbicidal activity after 48 h of storage. However, in agronomic settings, highly concentrated tank solutions (600–800 mg/L) may be prepared with tap water since the conversion of IXF to BA would represent <5% of the herbicide; therefore, any impact on the herbicide efficacy would be negligible. Results of this study show that current chlorination disinfection protocols in municipal water systems would completely eliminate the phytotoxic form of this new herbicide, DKN, from drinking water supplies; yet, farmers can use chlorinated tap water without significant loss of efficacy.

**KEYWORDS:** Isoxaflutole; hypochlorite, disinfection, herbicide; metabolites; chromatography; diketonitrile; benzoic acid metabolite, HPLC-MS/MS; HPLC-UV

### INTRODUCTION

Chlorination has been widely employed for disinfection purposes during municipal water treatment (1). Chlorine is usually applied in the form of  $\text{Cl}_2$  gas, chlorine dioxide ( $\text{ClO}_2$ ), sodium hypochlorite ( $\text{NaOCl}$ ), or calcium hypochlorite [ $\text{Ca}(\text{OCl})_2$ ]. When the chlorine is used in the gaseous form, hydrolysis and ionization occur. Hypochlorous acid ( $\text{HOCl}$ ) and hypochlorite ions ( $\text{OCl}^-$ ) are the products of this reaction and serve as major oxidants during disinfection. The ratio of  $\text{HOCl}$  to  $\text{OCl}^-$  in water depends on both pH and temperature (1). When  $\text{NaOCl}$  and  $\text{Ca}(\text{OCl})_2$  are used,  $\text{OCl}^-$  is the major ionization product. During the chlorination process at a treatment facility, chlorine is added to achieve a target level of 2–5 mg/L for

disinfection. In water supply systems, free residual chlorine is maintained at 0.5–1 mg/L for chlorination effectiveness (1).

Both  $\text{HOCl}$  and  $\text{OCl}^-$  have the capacity for oxidation of organic pesticides in water (2, 3). Methoxyl, phenolic, and ketonic structural groups are more sensitive to chlorine than alkyl or carboxyl groups (4). However, high concentrations of chlorine and long contact times are required for significant levels of oxidation to occur (4). For instance, it requires 38.5, 29.5, and 11.0 mg/L chlorine at contact times of 15, 16, and 180 min, respectively, to reduce the concentration of rotenone from 100 to 5  $\mu\text{g/L}$  (5). Most chlorinated hydrocarbons, e.g., toxaphene, are inert to oxidation by chlorine at 1–5 mg/L (5). Diazinon, a phosphorothioate, is one of the few pesticides shown to be oxidized by chlorine at low concentrations. At pH 7.0, the degradation half-life of diazinon at a chlorine concentration of 0.5 mg/L is just 16 min (6). The oxidation of pesticides by chlorine usually follows second-order kinetics (2, 6).

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**Table 1.** Concentrations of DKN and BA Metabolites Analyzed by HPLC-UV for DKN Spiked in Four Water Treatments at 200  $\mu\text{g/L}$  ( $n = 4$ )<sup>a</sup>

treatments	DKN	SD	BA	SD
DI water	198.4 (198.3)	10.5 (15.7)	0.0 (0.0)	0.0 (0.0)
dechlorinated tap water 1 (chemically neutralized)	203.1	1.7	0.0	0.0
dechlorinated tap water 2 (activated carbon)	205.0	1.5	0.0	0.0
chlorinated DI water	0.0	0.0	187.8	14.1
tap water	0.0 (0.0)	0.0 (0.0)	168.9 (134.91)	12.1 (22.53)

<sup>a</sup> SD = standard deviation; validation from HPLC-MS/MS analysis is shown in parentheses.

Balance (isoxaflutole; IXF; 5-cyclopropyl-4-(2-methylsulfonyl-4-trifluoromethyl-benzoyl)-5-cyclopropyl isoxazole, CAS Registry No. 141112-29-0) represents a new class of herbicides, referred to as isoxazole. IXF itself does not possess any herbicidal activity until it is converted to its ring-cleaved cyano diketone metabolite (7, 8), diketone nitrile (DKN). Further degradation of DKN produces a nonbiologically active benzoic acid (BA) metabolite (8). IXF is sensitive to hydrolysis and light and has a very short half-life in soil (14–24 h) (9, 10). DKN has been reported as the predominant form of this herbicide under field conditions (10).

In the process of developing a method for measuring Balance and its metabolites in water, it was observed that rapid degradation of DKN occurred in solutions prepared with tap water derived from a municipal water source. The objectives of this study are to identify the agent(s) responsible for DKN degradation in tap water and to evaluate its (their) implication and impact on drinking water safety and herbicidal efficacy.

## MATERIALS AND METHODS

**Sample Preparation.** *Degradation of DKN in Chlorinated Tap Water.* Five replicates of distilled deionized (DI) water and tap water acquired from the City of Columbia, Missouri, were fortified with DKN to make 200  $\mu\text{g/L}$  solutions. A 100  $\mu\text{L}$  volume of each water sample was immediately injected into a high-performance liquid chromatography system coupled with a UV detector (HPLC-UV). To confirm the results of HPLC-UV, the same water samples were also analyzed by HPLC tandem mass spectrometry (HPLC-MS/MS). Solutions with various DKN concentrations (100–4000  $\mu\text{g/L}$ ; 0.28–11.14  $\mu\text{mol/L}$ ) were also prepared in tap water to evaluate the degradation capacity of tap water.

*Identification and Confirmation of Hypochlorite ( $\text{OCl}^-$ ) as the Reactant in Tap Water.* A concentration of approximately 1 mg/L of total chlorine was detected in the tap water samples using a HACH colorimetric test kit (HACH no. 2060400). To confirm that hypochlorite was the agent degrading DKN, samples of DKN were prepared using three water treatments: DI water, tap water, and chlorinated DI water. Chlorinated DI water was prepared by adding NaOCl to achieve a final concentration of 1 mg/L. Additionally, dechlorinated tap water samples were produced by two separate methods. The first involved the neutralization of hypochlorite by adding approximately 2 mL of solution containing 10 mM sodium thiosulfate ( $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ ) and 5 mM sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) to 500 mL of tap water. The second method used activated carbon (Culligan) to remove the hypochlorite. Four replicates of each water treatment were prepared. All samples were fortified to 200  $\mu\text{g/L}$  with DKN and injected into the HPLC-UV system.

*Effects of Tap Water on the Fate of Balance (IXF).* Two replicates of DI and tap water samples were fortified to 250 and 500  $\mu\text{g/L}$  with an analytical standard and the commercial formulation of IXF (water dispersible granule end-use product with 75% IXF). Samples were transferred to transparent glass vials under laboratory light intensity of approximately 10  $\mu\text{einsteins/m}^2/\text{s}$  of visible radiation (400–700 nm) and room temperature (25  $^\circ\text{C}$ ). Samples were evaluated by time series sampling over a 60 h period.

**Analysis Performed by HPLC-UV.** Analyses of IXF, DKN, and BA were performed by octyl ( $\text{C}_8$ ) reverse phase HPLC using a Beckman

HPLC system (San Ramon, CA) equipped with a UV detector. The analytical column was a Columbus  $\text{C}_8$  (4.6 mm  $\times$  100 mm, 5  $\mu\text{m}$ ) silica-based column (Phenomenex, Torrance, CA). The column temperature was set at 40  $^\circ\text{C}$ . These compounds were detected and quantified by their UV absorbance at 270 nm for IXF and BA and 300 nm for DKN. The mobile phase consisted of 0.1% phosphoric acid ( $\text{H}_3\text{PO}_4$ ) buffer (pH 2.2) and 100% acetonitrile (ACN) solution mixed to give an isocratic mixture of 40% ACN:60%  $\text{H}_3\text{PO}_4$  buffer. The flow rate was 1.0 mL/min. The injection volume was 100  $\mu\text{L}$ . Retention times were 3.3 min for BA, 7.6 min for DKN, and 12.8 min for IXF. The concentration of each analyte was determined from an external standard calibration curve. Limits of quantitation (LOQ) were 10  $\mu\text{g/L}$  for IXF and DKN and 25  $\mu\text{g/L}$  for BA.

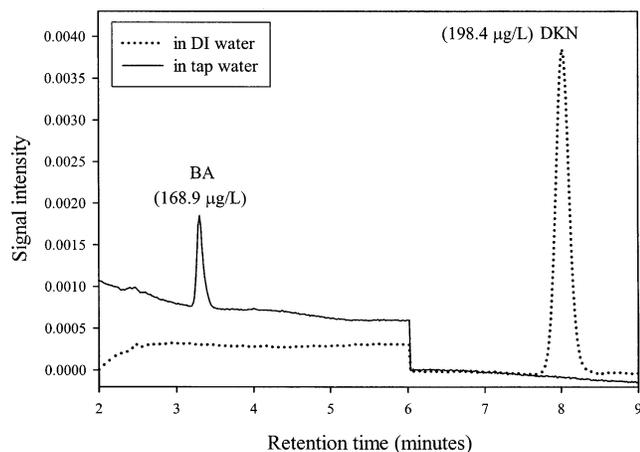
**Analysis Performed by HPLC-MS/MS.** IXF, DKN, and BA were separated and quantified using a Shimadzu LC-10AT HPLC system coupled to a Perkin-Elmer Sciex 365 LC-MS/MS system with TurboIonSpray atmospheric pressure ionization (API) ion source. The analytical column for HPLC was a  $\text{C}_8$  Luna (2 mm  $\times$  30 mm, 3  $\mu\text{m}$ ) silica-based column (Phenomenex). The sample injection volume was 10  $\mu\text{L}$ . The mobile phases were as follows: A, DI water with 1.5% of acetic acid ( $\text{CH}_3\text{COOH}$ ) containing 1 mM ammonium acetate ( $\text{NH}_4\text{-OAc}$ ), and B, 90% ACN:10% DI water containing 1.5% of  $\text{CH}_3\text{COOH}$ .

For MS/MS, the TurboIonSpray API ion source was operated in the negative ion mode. Multireaction monitoring (MRM) was used for detection and quantification of IXF, DKN, and BA (11). The parent molecular ions  $[\text{M} - \text{H}]^-$  (IXF,  $m/z$  358; DKN,  $m/z$  358; BA,  $m/z$  267) were isolated by the first MS filter. After fragmentation, the prominent fragment daughter ion  $m/z$  79 was used to detect and quantify IXF and DKN, and the  $m/z$  159 daughter ion was used to quantify BA. External calibration curves were developed using standard samples to relate known concentrations to daughter ion intensities. Quantitation of DKN and BA was achieved using external standard calibrations.

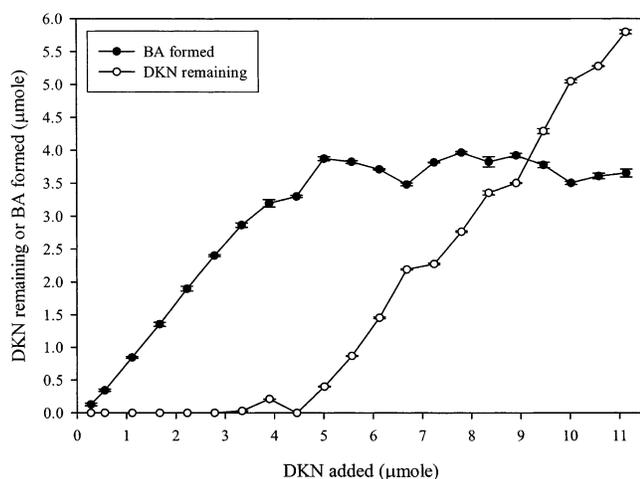
## RESULTS AND DISCUSSION

**Transformation of DKN in Tap Water.** Analysis of results derived from both HPLC-UV and HPLC-MS/MS analysis suggested a rapid transformation of DKN to BA in tap water. Samples containing 200  $\mu\text{g/L}$  DKN prepared in tap water showed that virtually all of the DKN was converted to BA in less than 1 min (Table 1 and Figure 1). A similar finding was also observed with the tap water collected from Lawrence, KS (unpublished data). In addition, DKN was completely degraded in tap water at concentrations up to 1600  $\mu\text{g/L}$  (4.45  $\mu\text{mol/L}$ ) (Figure 2). At DKN concentrations ranging from 1600 to 4000  $\mu\text{g/L}$  (4.45–11.14  $\mu\text{mol/L}$ ) in tap water, 10–50% of DKN remained (Figure 2). This finding suggested that hypochlorite was consumed by the reaction. During this experiment, another product was identified when the fortified DKN concentrations were greater than 1600  $\mu\text{g/L}$  (4.45  $\mu\text{mol/L}$ ). This product has a molecular weight of 325 g, and its polarity is between DKN and BA, based on its retention time of 7.8 min. This product may explain the lack of mass balance in this study (Figure 2).

To determine if hypochlorite was the agent responsible for DKN transformation, treatments of DI water, DI water with hypochlorite, and dechlorinated tap water were tested for their ability to convert DKN to BA. In the DI water with hypochlorite



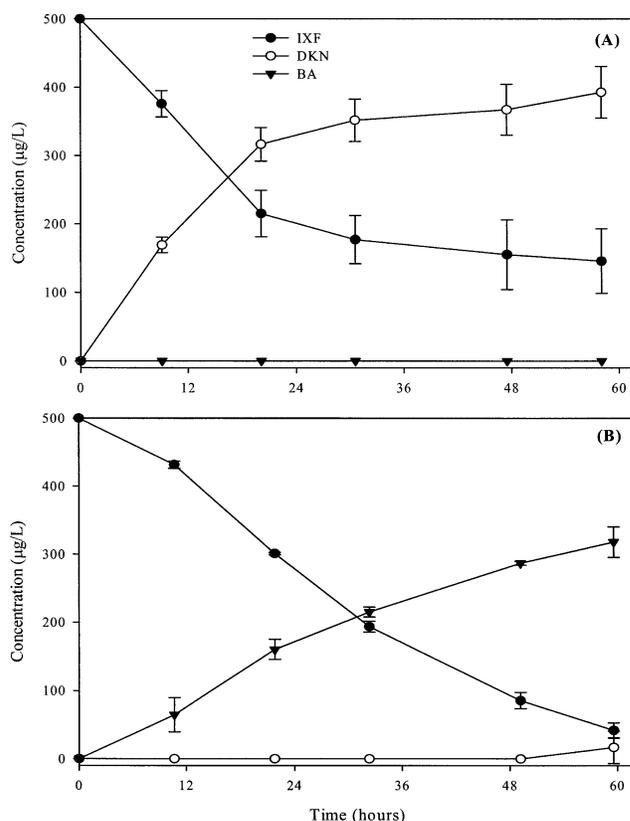
**Figure 1.** Overlaid HPLC-UV chromatograms of DKN spiked in distilled, DI water and tap water at 200  $\mu\text{g/L}$ . The UV detection wavelength was 300 nm for DKN and 270 nm for BA.



**Figure 2.** Amount of BA produced and DKN remaining in 1 L of tap water solution at spiked DKN levels ranging from 0.28 to 11.14  $\mu\text{mol}$  (100–4000  $\mu\text{g}$ ). Error bars represent one standard deviation ( $n = 3$ ).

treatment, DKN was rapidly converted to BA. However, in DI water and dechlorinated tap water treatments, DKN remained intact (**Table 1**). This finding strongly suggested that hypochlorite ( $\text{OCl}^-$ ) was the chemical agent in tap water responsible for the transformation of DKN to BA. Estimates of DKN in surface water using the pesticide root zone model indicate a maximum concentration about 2  $\mu\text{g/L}$  (12). Therefore, from the perspective of drinking water safety, the results presented here imply that chlorine disinfection can easily eliminate the phytotoxic form of Balance, i.e., DKN, in drinking water systems.

The reaction kinetics reported here for DKN degradation by hypochlorite were much faster than those reported in other studies (4, 13). High concentrations of hypochlorite and long contact times are usually required for significant levels of oxidation of many herbicides to occur (4). For example, it requires 38.5, 29.5, and 11.0 mg/L chlorine at contact times of 15, 16, and 180 min, respectively, to reduce the concentration of rotenone from 100 to 5  $\mu\text{g/L}$  (5). Most chlorinated hydrocarbons, e.g., toxaphene, are inert to oxidation by chlorine at 1–5 mg/L (2, 5). Previous research has shown that transformation kinetics of DKN to BA by other chemical or microbial oxidants, such as dimethyldioxirane, lignin peroxidase, and manganese-dependent peroxidase, occurred much more slowly



**Figure 3.** Change in concentrations of IXF, DKN, and BA metabolite over time. Sample solution was prepared using commercial IXF spiked in (A) DI water (pH 6.82) and (B) tap water (pH 8.36) at 500  $\mu\text{g/L}$ . Degradation of IXF in panel A follows second-order kinetics ( $k_2 = 2 \times 10^{-6} \text{ L } \mu\text{g}^{-1} \text{ min}^{-1}$ ;  $t_{1/2} = 16.7 \text{ h}$ ); degradation of IXF in panel B follows first-order kinetics ( $k_1 = 4.5 \times 10^{-4} \text{ min}^{-1}$ ;  $t_{1/2} = 25.8 \text{ h}$ ).

than the reaction kinetics that we observed for hypochlorite oxidation of DKN (13). The completed transformation of DKN required more than 24 h for dimethyldioxirane and more than 15 days for the oxidative enzymes. Mougin et al. (13) proposed a hypothesis in which two successive oxidation reactions were responsible for the transformation of DKN to BA by the chemical oxidant dimethyldioxirane or by oxidative enzymes. In their work, two major intermediates were identified after a reaction time of 5 min, an unstable epoxide and a lactone. The epoxide is rapidly converted to the lactone. All lactone is subsequently cleaved to BA and cyclopropanoic acid with a release of cyanide.

A detailed reaction pathway for DKN oxidation to BA by  $\text{OCl}^-$  is currently under investigation. The reaction pathway will require identification and confirmation of the observed unknown product and other possible products such as cyclopropanoic acid, chlorinated hydrocarbons (i.e., chlorination byproducts), acetonitrile, and/or release of the cyanide anion. The complete reaction pathway is important to fully understanding the human health implications of DKN oxidation in chlorinated drinking water.

**Effect of Tap Water on Fate of IXF.** In DI water, both the chemical standard and the commercial formulation of IXF rapidly transformed to the more stable DKN metabolite and no BA was detected (**Figure 3A**). This reaction followed second-order kinetics ( $k_2 = 2 \times 10^{-6} \text{ L } \mu\text{g}^{-1} \text{ min}^{-1}$ ) with a half-life ( $t_{1/2}$ ) of 16.7 h (**Figure 3A**). However, in tap water, IXF was transformed to BA without any detectable levels of DKN formed

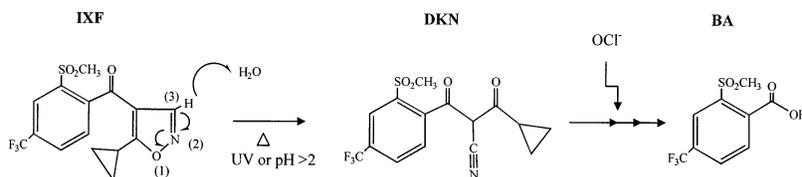


Figure 4. Transformation of IXF to DKN and subsequent DKN oxidation by hypochlorite in aqueous solutions.

during the first 48 h (Figure 3B). This reaction showed first-order kinetics ( $k_1 = 4.5 \times 10^{-4} \text{ min}^{-1}$ ) with a  $t_{1/2}$  of 25.8 h (Figure 3B). The conversion of IXF to BA was apparently due to the reaction of DKN with  $\text{OCl}^-$  in tap water. This contributed to the observed differences in reaction order and half-life between the DI and the tap water treatments. In the presence of tap water, IXF transformation to BA was a two step process. First, IXF was hydrolyzed to DKN, and then, DKN was immediately oxidized by  $\text{OCl}^-$  to form nonphytotoxic BA. In addition, the disappearance of IXF was more rapid after 30 h in the presence of  $\text{OCl}^-$ , suggesting that hydrolysis to DKN was the rate-limiting step (Figure 3A,B).

IXF has been reported to be extremely sensitive to light and high temperatures (9, 10), conditions that accelerate its transformation to DKN. Thus, light or high temperature will increase the transformation rate of IXF to DKN and subsequent conversion of DKN to BA in chlorinated tap water (Figure 4). This may have a significant impact on studies in which IXF was prepared with chlorinated tap water at relatively dilute concentrations ( $<5000 \mu\text{g/L}$ ). In a greenhouse bioassay experiment with selected forage grass species, IXF efficacy was significantly reduced when it was prepared in tap water derived from the same municipal water system used in this study. When a herbicide solution of  $250 \mu\text{g/L}$  was prepared in municipal tap water, the growth inhibition rate in smooth brome grass was reduced from 32.5 to 7.5%. In orchard grass, the growth inhibition rate was reduced from 34.3 to 17.3% at the same application rate. However, in agronomic settings, highly concentrated tank solutions ( $600\text{--}800 \text{ mg/L}$ ) may be prepared with tap water since the conversion of IXF to BA would represent  $<5\%$  of the herbicide. Therefore, any impact on the herbicidal efficacy would be negligible.

## CONCLUSION

From the perspective of drinking water safety, the results presented here indicate that chlorine disinfection can easily eliminate the phytotoxic form of Balance (i.e., DKN) in drinking water systems. In large-scale agronomic settings, farmers may use chlorinated tap water in preparing Balance for field spraying without significant loss of efficacy. However, for small scale applications, such as greenhouse bioassay or research experiments, in which application concentrations are relatively dilute ( $<5000 \mu\text{g/L}$ ), the oxidation of DKN by hypochlorite in tap water may significantly reduce efficacy. Future work will focus on the detailed transformation pathway for the reaction of DKN with hypochlorite, including reaction mechanisms and identification of stable products.

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