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Neonicotinoid Insecticide Hydrolysis and Photolysis: Rates and Residual Toxicity

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Abstract: Neonicotinoid insecticides are the most widely used class of insecticides worldwide. Concern has grown over their widespread environmental presence and potential unintended adverse effects. The present study examined hydrolysis and photolysis reaction rates of neonicotinoids and assessed any residual toxicity of reaction products. Hydrolysis rates were tested between pH 4 and 10 and found to be base-catalyzed. Experiments revealed a nonelementary rate law for hydrolysis, with the hydroxide concentration raised to a power of 0.55 ± 0.09, which has implications for accurate prediction of environmental half-lives. Divalent metal ions (Cu^{2+}, Ni^{2+}, Zn^{2+}) and minerals (kaolinite, goethite, TiO_2) had no effect on hydrolysis rates. The hydrolysis rate in a natural water, however, was slower than that predicted by buffered experiments. Nitenpyram, imidacloprid, thiamethoxam, and clothianidin reacted via direct photolysis in both ultrapure and natural waters, with average quantum yields of 0.024 ± 0.001, 0.0105 ± 0.0002, 0.0140 ± 0.0002, and 0.0101 ± 0.0001, respectively. Acetamiprid primarily underwent indirect photolysis by reaction with OH· ($1.7 \pm [0.2] \times 10^9\ M^{-1}\ s^{-1}$). For all compounds, the urea derivative was the most commonly detected product in both hydrolysis and photolysis experiments. Using mosquito (*Culex pipiens*) larvae, no residual toxicity of reaction products was observed. Results indicate long environmental half-lives for the tested neonicotinoids, which may help to explain their ubiquitous presence in environmental matrices. *Environ Toxicol Chem* 2018;37:2797–2809. © 2018 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals, Inc. on behalf of SETAC

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INTRODUCTION

Neonicotinoids (Figure 1) are a class of systemic insecticides widely used worldwide, with registration in over 120 countries for usage on more than 140 crops (Jeschke et al. 2010). Since their release in the 1990s as a replacement for carbamates and organophosphates, use has increased considerably, and neonicotinoids now account for a quarter of the world's insecticide use (Bass et al. 2015). Usage has spread beyond agriculture to home garden and lawn care, garden centers, and urban forestry to combat emerald ash borer (Cowles 2009; Cloyd and Bethke 2011).

Widespread use of neonicotinoids, perhaps unsurprisingly, has led to near ubiquitous environmental detection, including in surface water and groundwater (Hladik et al. 2014; Morrissey et al. 2015; Schaafsma et al. 2015). Detection in finished drinking water has also been reported in Iowa City, Iowa, USA (Klarich et al. 2017), and in Ontario, Canada (Sultana et al. 2018), with concentrations as high as 57.3 and 280 ng/L, respectively. Wastewater effluent frequently contains neonicotinoids, and traditional activated sludge treatment does little to remove them, resulting in an estimated 1000 to 3400 kg of neonicotinoids being discharged in effluent yearly (Peña et al. 2011; Sadaria et al. 2016). In soil, neonicotinoids have been detected at concentrations up to 20 μg/g and up to 3 yr after the last application (Goulson 2013; Jones et al. 2014; Schaafsma et al. 2015). This widespread detection indicates that neonicotinoids are environmentally persistent and effectively have slow abiotic degradation rates.

Previous work has shown long half-lives in water, with reported half-lives at pH 7 of >800 and >4000 d for thiamethoxam and imidacloprid, respectively (Zheng and Liu 1999; Karmakar et al. 2009). The full effect of pH on neonicotinoid degradation, however, is not understood because some work has shown degradation occurring at pH 4, whereas other work only reported degradation at basic pH values (Zheng and Liu 1999; Liu et al.

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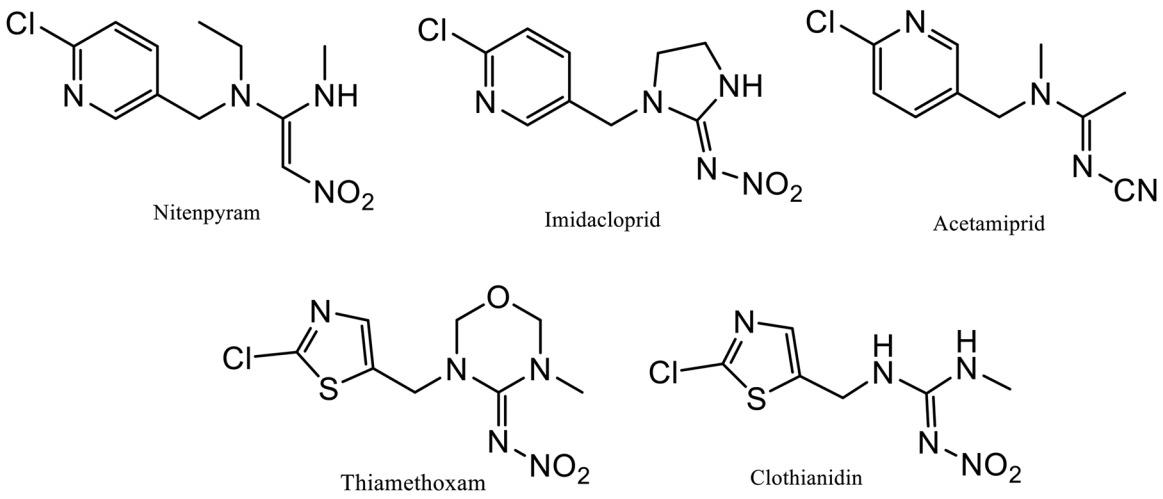

FIGURE 1: Selected neonicotinoid insecticides used in the present study.

2006; Bonmatin et al. 2015). In addition, the effect of the presence of metal ions and minerals, which have been shown to increase hydrolysis rates in other pesticides (Ketelaar et al. 1956; Smolen and Stone 1997), has not been explored for neonicotinoids. Direct photolysis has also been observed, with large variations in quantum yield between neonicotinoids and half-lives ranging from 12 min for imidacloprid to 42 h for thiacloprid (Lu et al. 2015). Indirect photolysis has been studied, with half-life estimates of 5 h to 19 d in aquatic reservoirs, indicating that hydroxyl radicals may play a role in neonicotinoid photolysis (Dell'Arciprete et al. 2009). The comparison of direct and indirect photolysis in the same study, however, has not been reported, and updated actinometry values (Laszakovits et al. 2017) require validation of previously reported quantum yields. Overall, accurate hydrolysis and photolysis rate constants would allow for increased accuracy in environmental fate modeling.

There has been growing concern over the impact of neonicotinoids on nontarget organisms. Detrimental effects have been observed at acute and subacute levels in honeybees (*Apis mellifera*), with neonicotinoids suspected of contributing to colony collapse disorder along with other problems such as decreased navigational ability and impaired learning (Henry et al. 2012; Gill et al. 2013). Although research has focused on honeybees, sublethal effects have been observed in aquatic arthropods, birds, and fish, including reproduction inhibition, delayed emergence, feeding inhabitation, and organ damage (Morrissey et al. 2015; Hladik et al. 2018). In addition, residual toxicity has been observed with several degradation products, such as the desnitro/guanidine and nitrosoguanidine derivatives of imidacloprid (Lee Chao and Casida 1997; Tomizawa and Casida 1999; Tomizawa et al. 2000).

Imidacloprid, clothianidin, and thiamethoxam (all nitroguanidines; Figure 1) account for >99% of total neonicotinoid usage in Minnesota, USA, and were thus selected for the present study. Acetamiprid (a cyanoamide) and nitenpyram (a nitromethylene) were also used, to allow for comparison of the 3 pharmacologically active groups currently used in neonicotinoids. The goals of the present study were to 1) understand the effects of pH, divalent metals (Cu^{2+}, Ni^{2+}, Zn^{2+}), and minerals (kaolinite, goethite, TiO_2) on hydrolysis of neonicotinoids; 2) measure photolysis rates; 3) identify reaction products; and 4) evaluate toxicity of hydrolysis and photolysis products.

MATERIALS AND METHODS

Chemicals

Analytical-grade neonicotinoids were used in all experiments. Imidacloprid (99.5%), acetamiprid (99.5%), thiamethoxam (99.5%), and clothianidin (99.5%) were purchased from Chem Service. Nitenpyram (99.9%) was purchased from Fluka Analytical. Solvents (methanol, acetonitrile; high-performance liquid chromatography [HPLC] grade) were purchased from Sigma-Aldrich. Ultrapure water (18.2 MΩ · cm) was obtained using a Milli-Q Academic system (Millipore). Buffers were made using American Chemical Society (ACS)–grade chemicals. Sodium acetate (99.5%) was purchased from BDH Chemicals, 3-(*N*-morpholino)propanesulfonic acid (MOPS; 99.5%) was purchased from Sigma-Aldrich, sodium tetraborate (assayed purity 102.2%) was purchased from Fisher Chemicals, and potassium phosphate monobasic (>99.0%) and sodium phosphate dibasic (>99.0%) were purchased from J.T. Baker. Acetic acid (ACS-grade; 99.9%) was purchased from BDH Chemicals. Zinc (II) chloride (>98%) and nickel (II) chloride (>99.9%) were purchased from Sigma-Aldrich, and copper (II) chloride (99%) was purchased from Acros Organics. Titanium dioxide type P25 (>99.5%) was purchased from Acros Organics, kaolinite type KGa-1b was purchased from the Clay Mineral Society, and goethite was synthesized and characterized by Jeanette Voelz in the University of Minnesota Department of Chemistry. The compounds *p*-nitroanisole (PNA; 98%) and pyridine (>99.0%) were purchased from Sigma-Aldrich. Sodium nitrate (99.2%) was purchased from Fisher Chemical, and *p*-chlorobenzoic acid (*p*CBA; 99%) was purchased from Acros Organics.

Buffer solutions

To determine the hydrolysis rates over a range of pH values, buffer solutions were prepared at pH 4.0, 6.3, 7.0, 8.0, and 10.0, with the exception that a pH 9.0 buffer was used for thiamethoxam instead of pH 10.0, because of rapid degradation

of thiamethoxam at pH 10.0. Acetate was used as a buffer for pH 4.0; MOPS was used for pH 6.3, 7.0, and 8.0 buffers; and sodium tetraborate (i.e., borate) was used for pH 9.0 and 10.0 experiments. The acetate buffer was prepared by dissolving 60 mg of sodium acetate in 500 mL of Milli-Q water, then titrating with acetic acid until pH 4 was reached; MOPS (1.046 g) was dissolved in 500 mL of Milli-Q water, then titrated with 1 M NaOH or 1 M HCl until the desired pH was achieved. Sodium tetraborate (1.906 g) was dissolved in 500 mL of Milli-Q water and titrated with 1 M NaOH until the desired pH was reached.

Hydrolysis

To determine hydrolysis rates at different pH values, buffer solutions were prepared at pH 4.0, 6.3, 7.0, 8.0, and 9.0/10.0. Reactors at each pH were dosed with a methanolic stock solution of the desired neonicotinoid to achieve an initial concentration of 1 μM. Reactors were stored in foil-wrapped glass scintillation vials in cabinets to prevent photolysis. Degradation was monitored for up to 150 d.

Reactors containing metal ions and minerals were also studied. To determine if metal ions had an effect on neonicotinoid degradation, copper (II) chloride, nickel (II) chloride, and zinc (II) chloride were added to reactors at 1 mM (pH 4.0 and 6.3) or 0.1 mM (pH 8.0 and 10.0) and spiked with neonicotinoids to a concentration of 1 μM using the same buffers as baseline experiments. Although equilibrium calculations indicate that precipitation could occur for all 3 metals at pH 10 and for copper at pH 8, no formation of solids was observed during the experiments. For reactors containing minerals, kaolinite, goethite, and titanium dioxide were added (1 g/L) to the reactors and stirred for 18 to 24 h before adding neonicotinoids (10 μM). Reactors were constantly stirred on a 16-position analog stir-plate (Scilogex) using a 1/8 × 1/2″ PTFE disposable stir bar (Fisher Scientific). Regular samples were taken (250 μL) and filtered through a 13-mm PTFE syringe-tip filter (pore size 0.2 μm; Fisher Scientific) before analysis.

A comparison to hydrolysis rates in a natural water was also performed. Mississippi River water was collected from the University of Minnesota Boathouse (Minneapolis, MN, USA) dock, prefiltered with combusted glass-fiber filters (Millipore; 0.7 μm), filter-sterilized with nitrocellulose membrane filters (Millipore; 0.22 μm), and stored at 4 °C until used. Two separate Mississippi River water samples were collected, on 12 July 2017 and on 3 November 2017. Characterization of each sample is found in Supplemental Data, Table S1. Conductivity was measured using a model 72 Engineered Systems and Design conductivity meter, and pH was measured with a WTW 340i pH meter fitted with a Sensorex S200C probe. Dissolved organic carbon was measured with a Shimadzu TOC-L analyzer operated in nonpurgeable organic carbon mode. Samples were dosed with neonicotinoids to an initial concentration of 10 μM and monitored for 150 d.

Reactors containing metals and minerals were compared to baseline studies using a *t* test to compare slopes of kinetic regression lines, based on a method published by Howell (2011). The null hypothesis was that the slopes are equal; thus, if a *p* value ≥0.05 was calculated, the null hypothesis could not be rejected, and there was not considered to be a statistical difference between tested slopes.

Photolysis

Photolysis experiments were performed in both natural sunlight as well as simulated sunlight in an Atlas Suntest CPS+ solar simulator with a xenon arc lamp fitted with a 290-nm cutoff filter. Natural sunlight experiments were conducted on the roof of the Department of Mechanical Engineering Building, University of Minnesota–Twin Cities campus (44°58′30.6″N, 93°14′01.1″W). A solar spectrum for this location was generated using the Natural Renewable Energy Laboratory Simple Model of the Atmospheric Radiative Transfer of Sunshine model (Ver 2.9.5). To determine the relative importance of direct and indirect photolysis, solutions were prepared in ultrapure water (Milli-Q) and Mississippi River water by dosing neonicotinoids using an aqueous stock solution, resulting in a 10 μM contaminant concentration. Pyridine–PNA actinometers were run to allow determination of quantum yield, using 5 μM PNA and variable concentrations of pyridine, because of differences in neonicotinoid reactivity. Data were analyzed using methods prescribed by Leifer (1988) with the recent update to the PNA quantum yield relationship (Laszakovits et al. 2017). Details of the equations used to calculate quantum yields and screening factors are provided in the Supplemental Data.

After initial experiments were performed, further tests were run to determine photolysis in nitrate-amended waters (10 mg/L as N, added as sodium nitrate). Experiments were performed in the solar simulator. Experiments were run in parallel in triplicate with neonicotinoid added to each of the following: Milli-Q water, Mississippi River water, and Mississippi River water amended with nitrate. A *p*CBA probe (5 μM, $k_{pCBA,HO\cdot} = 5 \times 10^9\ M^{-1}\ s^{-1}$; Westerhoff et al. 1999) was used to determine steady-state hydroxyl radical concentrations.

Results from nitrate experiments were analyzed by comparing rate constants of Milli-Q samples, Mississippi River water samples, and nitrate-amended Mississippi River water samples and calculating second-order rate constants using hydroxyl radical concentrations obtained from *p*CBA probes. Bimolecular rate constants of neonicotinoids with hydroxyl radicals ($k_{A,HO\cdot}$) were derived from the linear regression of natural log-normalized concentrations of neonicotinoids (*A*) versus *p*CBA, shown in Equation 1, where $k_{pCBA,HO\cdot}$ is the bimolecular rate constant of *p*CBA reaction with hydroxyl radicals.

$$\ln\left(\frac{[A]}{[A]_0}\right) = \frac{k_{A,HO\cdot}}{k_{p,CBA,HO\cdot}} \ln\left(\frac{[pCBA]}{[pCBA]_0}\right) \quad (1)$$

Analytical methods

Light absorbance of each neonicotinoid was measured from 200 to 800 nm using a Shimadzu UV-1601PC spectrophotometer with 1-cm quartz cuvettes. Neonicotinoid, *p*CBA, and PNA concentrations were measured using HPLC on an Agilent 1200 system equipped with a diode-array detector. All compounds were detected using an Ascentis Supelco RP-Amide C-16 column (15 cm × 4.6 mm, 5 μm); specific method information

for each compound is provided in Supplemental Data, Table S2.

Ultrahigh pressure liquid chromatography–tandem mass spectrometry (UHPLC-MS/MS) was used to identify neonicotinoid hydrolysis and photolysis degradation reaction products. Aliquots were taken from samples generated for toxicology experiments (see *Toxicology* section) and analyzed at the University of Minnesota's Masonic Cancer Center on a Thermo Fisher UltiMate 3000 UHPLC system paired to a Thermo Fisher Linear Trap Quadrupole Orbitrap Velos UHPLC-MS/MS using a C18 nanoflow column with a gradient method (see Supplemental Data, Table S3). The mass spectrometer was run in positive mode and set to analyze for 33 min, with 6 scans, all from 80.0 to 400 (*m/z*) and using a collision energy of 35 eV. Scan 1 had a resolution of 60 000, and scan 2 was set to collect the parent neonicotinoids. Scans 3 to 6 had a resolution of 15 000 and isolated the first, second, third, and fourth most abundant peaks (not including the parent) from scan 1, with a peak exclusion area set to 500. Mass spectrometric data were analyzed using Thermo Fisher Compound Discoverer 2.1 software. Untargeted environmental analyses and targeted environmental analyses of expected degradation products were run to identify products. Products were first compared using exact mass, with MS2 data and database identification used to verify product structure by comparing to literature data when available.

Toxicology

Samples for parent compound toxicity tests were prepared by dosing methanol stock solution to a 10-mL volumetric flask so that the final concentration was 50 μM, filling with Milli-Q ultrapure water and mixing well. Concentration was verified using HPLC. Hydrolysis samples (baseline, with metals, and with minerals) containing products were prepared by creating a 50-μM parent solution at pH 10.0 and monitoring until the neonicotinoid concentration decreased to 10 μM. Samples were filtered through a 0.2-μm syringe tip filter and neutralized to pH 7 using metals-grade concentrated HCl. Photolysis samples were prepared by reacting a 50-μM aqueous solution in an Atlas CPS+ solar simulator and monitoring using HPLC until the concentration of the parent compound was 10 μM, yielding samples with an approximate 4:1 ratio of reaction products to parent compound. Samples were stored at –20 °C.

Toxicity experiments were performed using mosquito (*Culex pipiens*) fourth instar larvae. Larvae were placed in distilled water and distributed into vials (5 larvae in each of 3 replicate vials), volumes were adjusted to 9.0 ± 0.1 mL, and 1 mL of test solution was added to each vial, giving final parent neonicotinoid concentrations from 0.1 to 1.0 μM. Control vials received 1 mL of distilled water. After 20 h, larvae that exhibited movement were scored as alive. All calculated values of 0 and 100% are based on averages from 3 vials from 2 separate experiments. Median lethal concentration (LC50) values were then calculated by plotting response (percentage) versus dose (concentration) and determining the point at which 50% of larvae died.

RESULTS AND DISCUSSION

Hydrolysis

Baseline hydrolysis. Neonicotinoid baseline hydrolysis reactors were monitored and sampled for 50 to 150 d. Pseudo-first-order rate constants were calculated using linear regression of natural log concentration versus time for all reactors; results are given in Table 1 and Figure 2. In pH 4.0, 6.33, and 7.0 samples for all neonicotinoids, little to no degradation was observed, with half-lives calculated to be over 1000 d for most compounds. Significant error is present in calculations for reactors below pH 8.0. In many cases, the 95% confidence interval is the same order of magnitude as the calculated pseudo-first-order rate constant.

Baseline imidacloprid results are similar to previously reported hydrolysis studies, in which imidacloprid was only observed to react at pH values >9 (Zheng and Liu 1999; Liu et al. 2006). Thiamethoxam hydrolysis kinetics at high pH were similar to previously reported work (Liqing et al. 2006; Karmakar et al. 2009; Klarich et al. 2017). Karmakar et al. (2009) observed significantly larger k_{obs} for thiamethoxam in phosphate-buffered solutions at pH 4.0 (100 times larger) and pH 7.0 (10 times larger) than was observed in the present study. Klarich et al. (2017), however, saw no hydrolysis at pH 7, consistent with the present results.

Hydrolysis in the presence of metal ions. Neonicotinoid reactors containing 1 mM (pH 4.0, 6.3) and 0.1 mM (pH 8.0, 10.0) divalent metal ions were monitored for 50 to 150 d, depending on the rate of reaction. Pseudo-first-order rate constants were calculated using linear regression of the natural log of concentration versus time; results are given in Table 1. Similar to baseline reactors, little to no degradation was observed at pH 4.0 and 6.3 (see Supplemental Data, Figure S1), with broad intervals at the 95% confidence level. At pH 8.0 and 10.0 (Supplemental Data, Figure S2), metals do not appear to have an effect on degradation rate. Calculated *p* values from slope tests are given in Supplemental Data, Table S4.

Determination of reaction order with [OH^-]. To account for the variation in pH, hydrolysis reactions were assumed to be second order because the rate of degradation increased as the concentration of hydroxide ion increased. Thus, second-order rate constants could be calculated by dividing the observed, pseudo-first-order rate constant by the measured values of [OH^-] in each experiment (which were ±0.05 units from the target value), giving a rate constant with units of per molar per day. Propagation of error was performed using the standard deviation of results from the pseudo-first-order linear regression. Error was calculated by dividing 95% confidence interval by [OH^-].

Calculated second-order rate constants (see Supplemental Data, Table S5) indicate that the hydrolysis reaction that the neonicotinoids undergo is, in fact, not a second-order elementary reaction. From pH 4.0 to 10.0, calculated second-order rate constants vary by 5 to 6 orders of magnitude (e.g., for clothianidin, the calculated rate constants range from 3.0 $[\pm 1.1] \times 10^{-6}\ M^{-1}$

TABLE 1: Calculated pseudo-first-order rate constants for hydrolysis of neonicotinoids (per day)[a]

		Baseline	Copper[b]	Nickel[b]	Zinc[b]	Baseline (stir-plate)[c]	Kaolinite[d]	Goethite[d]	TiO_2[d]
Compound	pH	21.5 °C				28.0 °C			
Nitenpyram	4.0	$8.5(\pm 2.5)\times 10^{-4}$	$6.2(\pm 1.7)\times 10^{-4}$	$7.1(\pm 1.5)\times 10^{-4}$	$7.5(\pm 2.9)\times 10^{-4}$	—	—	—	—
	6.3	$1.4(\pm 0.4)\times 10^{-3}$	$1.1(\pm 0.7)\times 10^{-3}$	$8.4(\pm 3.5)\times 10^{-4}$	$1.4(\pm 0.5)\times 10^{-3}$	—	—	—	—
	7.0	$1.3(\pm 0.2)\times 10^{-3}$	—	—	—	—	—	—	—
	8.0	$3.4(\pm 0.4)\times 10^{-3}$	$1.5(\pm 0.3)\times 10^{-3}$	$2.5(\pm 0.6)\times 10^{-3}$	$1.7(\pm 0.4)\times 10^{-3}$	$1.8(\pm 0.4)\times 10^{-3}$	$3.0(\pm 0.3)\times 10^{-3}$	$2.1(\pm 0.4)\times 10^{-3}$	$5.0(\pm 0.5)\times 10^{-3}$
	10.0	$5.1(\pm 0.1)\times 10^{-2}$	$4.8(\pm 0.3)\times 10^{-2}$	$5.0(\pm 0.3)\times 10^{-2}$	$5.0(\pm 0.4)\times 10^{-2}$	$10(\pm 0.1)\times 10^{-2}$	$9.8(\pm 1.0)\times 10^{-2}$	$12(\pm 1.5)\times 10^{-2}$	$12\pm 2.4)\times 10^{-2}$
Imidacloprid	4.0	$4.3(\pm 1.6)\times 10^{-4}$	$2.9(\pm 0.9)\times 10^{-4}$	$3.0(\pm 0.7)\times 10^{-4}$	$3.6(\pm 2.7)\times 10^{-4}$	—	—	—	—
	6.3	$5.4(\pm 1.0)\times 10^{-4}$	$6.9(\pm 4.3)\times 10^{-4}$	$5.1(\pm 3.1)\times 10^{-4}$	$5.4(\pm 4.4)\times 10^{-4}$	—	—	—	—
	7.0	$4.2(\pm 1.4)\times 10^{-4}$	—	—	—	—	—	—	—
	8.0	$7.9(\pm 0.9)\times 10^{-4}$	$9.9(\pm 5.2)\times 10^{-4}$	$1.3(\pm 0.5)\times 10^{-3}$	$9.7(\pm 5.6)\times 10^{-4}$	$1.6(\pm 1.6)\times 10^{-4}$	$9.9(\pm 5.6)\times 10^{-4}$	$4.8(\pm 1.9)\times 10^{-4}$	$1.6(\pm(0.5)\times 10^{-3}$
	10.0	$1.8(\pm 0.1)\times 10^{-2}$	$1.5(\pm 0.1)\times 10^{-2}$	$1.5(\pm\times 10^{-2}$	$1.5(\pm 0.1)\times 10^{-2}$	$3.6(\pm 0.1)\times 10^{-2}$	$3.9(\pm 0.2)\times 10^{-2}$	$4.0(\pm 0.1)\times 10^{-2}$	$4.0(\pm(0.1)\times 10^{-2}$
Acetamiprid	4.0	$4.0(\pm 3.3)\times 10^{-4}$	$5.2(\pm 2.6)\times 10^{-4}$	$5.9(\pm 2.0)\times 10^{-4}$	$2.5(\pm 1.6)\times 10^{-4}$	—	—	—	—
	6.3	$4.2(\pm 4.2)\times 10^{-4}$	$7.5(\pm 2.0)\times 10^{-4}$	$5.9(\pm 2.3)\times 10^{-4}$	$6.6(\pm 1.6)\times 10^{-4}$	—	—	—	—
	7.0	$1.0(\pm 0.4)\times 10^{-3}$	—	—	—	—	—	—	—
	8.0	$1.5(\pm 0.2)\times 10^{-3}$	$2.0(\pm 0.5)\times 10^{-3}$	$2.2(\pm 0.6)\times 10^{-3}$	$1.9(\pm 0.5)\times 10^{-3}$	$2.1(\pm 1.6)\times 10^{-4}$	$7.1(\pm 4.6)\times 10^{-4}$	$5.5(\pm 1.6)\times 10^{-4}$	$1.8(\pm 0.2)\times 10^{-3}$
	10.0	$2.8(\pm 0.1)\times 10^{-2}$	$2.6(\pm 0.1)\times 10^{-2}$	$2.6(\pm 0.1)\times 10^{-2}$	$2.5(\pm 0.1)\times 10^{-2}$	$4.6(\pm 0.1)\times 10^{-2}$	$5.1(\pm 0.1)\times 10^{-2}$	$6.4(\pm 0.2)\times 10^{-2}$	$6.3(\pm 0.2)\times 10^{-2}$
Thiamethoxam	4.0	$2.0(\pm 1.8)\times 10^{-4}$	$3.4(\pm 7.4)\times 10^{-4}$	$2.9(\pm 1.1)\times 10^{-4}$	$2.8(\pm 2.1)\times 10^{-4}$	—	—	—	—
	6.3	$5.7(\pm 1.7)\times 10^{-4}$	$6.3(\pm 82)\times 10^{-4}$	$2.4(\pm 1.8)\times 10^{-3}$	$3.1(\pm 2.6)\times 10^{-3}$	—	—	—	—
	7.0	$1.0(\pm 0.3)\times 10^{-3}$	—	—	—	—	—	—	—
	8.0	$5.4(\pm 0.2)\times 10^{-3}$	$3.4(\pm 0.9)\times 10^{-3}$	$4.0(\pm 1.1)\times 10^{-3}$	$4.6(\pm 1.2)\times 10^{-3}$	$6.8(\pm 0.7)\times 10^{-3}$	$8.5(\pm 0.5)\times 10^{-3}$	$10(\pm 0.3)\times 10^{-3}$	$11(\pm 0.4)\times 10^{-3}$
	9.0	$5.8(\pm 0.1)\times 10^{-2}$	$5.3(\pm 0.8)\times 10^{-2}$	$5.2(\pm 0.1)\times 10^{-2}$	$5.2(\pm 0.1)\times 10^{-2}$	$9.5(\pm 0.1)\times 10^{-2}$	$10(\pm 0.6)\times 10^{-2}$	$17(\pm 0.7)\times 10^{-2}$	$16(\pm 0.5)\times 10^{-2}$
Clothianidin	4.0	$3.4(\pm 1.2)\times 10^{-4}$	$7.0(\pm 11)\times 10^{-5}$	$1.0(\pm 2.0)\times 10^{-4}$	$3.2(\pm 3.3)\times 10^{-4}$	—	—	—	—
	6.3	$4.1(\pm 0.5)\times 10^{-4}$	$2.8(\pm 2.6)\times 10^{-4}$	$2.3(\pm 2.1)\times 10^{-4}$	$2.9(\pm 1.4)\times 10^{-4}$	—	—	—	—
	7.0	$4.3(\pm 2.1)\times 10^{-4}$	—	—	—	—	—	—	—
	8.0	$4.6(\pm 1.2)\times 10^{-4}$	$5.3(\pm 0.1)\times 10^{-4}$	$8.8(\pm 0.1)\times 10^{-4}$	$8.1(\pm 11)\times 10^{-4}$	$6.0(\pm 20)\times 10^{-6}$	$1.1(\pm 0.5)\times 10^{-3}$	$7.0(\pm 5.0)\times 10^{-4}$	$2.0(\pm 48)\times 10^{-5}$
	10.0	$5.2(\pm 0.4)\times 10^{-3}$	$5.1(\pm 0.6)\times 10^{-3}$	$4.9(\pm 0.6)\times 10^{-3}$	$5.1(\pm 0.7)\times 10^{-3}$	$1.0(\pm 0.1)\times 10^{-2}$	$1.2(\pm 0.2)\times 10^{-2}$	$1.2(\pm 0.1)\times 10^{-2}$	$1.2(\pm 0.1)\times 10^{-2}$

[a] Errors are the 95% confidence intervals.
[b] Copper, nickel, and zinc columns represent observed rate constants of neonicotinoids in the presence of each metal cation at 21.5 °C.
[c] Baseline (stir-plate) gives observed rate constant of baseline reactors run on the stir-plate used for mineral reactions.
[d] Kaolinite, goethite, and TiO_2 columns give observed rate constants in the presence of each mineral at 28 °C.

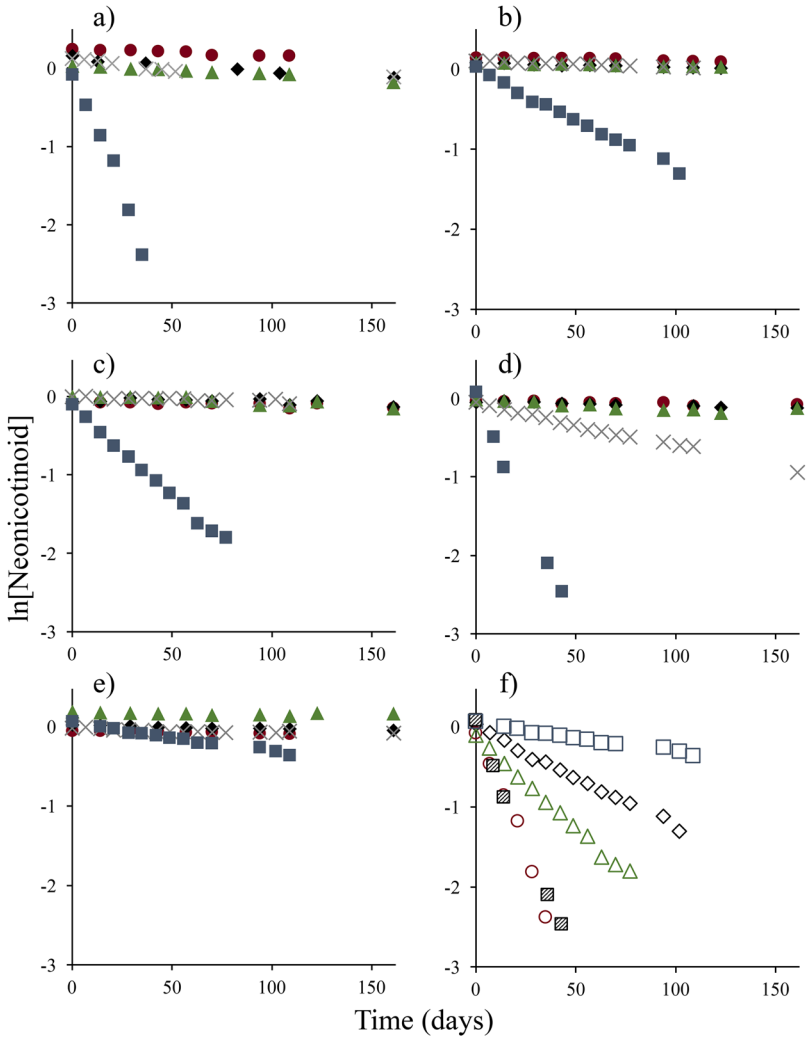

FIGURE 2: Baseline hydrolysis of neonicotinoid insecticides at pH 4, 6.33, 7, 8, and 10: (**a**) nitenpyram, (**b**) imidacloprid, (**c**) acetamiprid, (**d**) thiamethoxam, (**e**) clothianidin, (**f**) pH 10 (pH 9 for thiamethoxam) hydrolysis results. Legend graphs: (**a–e**) ● pH 4, ♦ pH 6.33, ▲ pH 7, X pH 8, ■ pH 10; (**f**) ○ nitenpyram pH 10, ◊ imidacloprid pH 10, △ acetamiprid pH 10, ▨ thiamethoxam pH 9, □ clothianidin pH 10.

d^{-1} at pH 4 to 58 M^{-1} d^{-1} at pH 10), indicating that the assumed reaction order is incorrect and the reaction with OH^- is not elementary.

Hydrolysis reactions can occur because of the reaction of a compound with H^+, H_2O, or OH^-. Because the reaction at pH 4 for all neonicotinoids is slower than all higher pH reactors, it was assumed that there were no hydrolysis reactions occurring attributable to catalysis by H^+; thus, the rate of reaction observed at pH 4 was assumed to be the baseline rate of hydrolysis reaction with respect to H_2O. The observed rate constant is then assumed to be a sum of the rate attributable to hydrolysis from water and the rate attributable to base-catalyzed hydrolysis. Because hydrolysis does increase with increasing concentration of hydroxide, the concentration of hydroxide was assumed to be part of the overall rate expression but expressed to some unknown power of n. The exponent n is calculated by graphing the log of $k_{obs} - k_{pH\,4}$ versus the –pOH of each reactor run at higher than pH 4.0 and calculating the regression line of the resulting scatterplot. Plots are given in Figure 3.

$$\text{rate} = k_{H_2O}[\text{Neonic}] + k_{OH^-}[\text{Neonic}][OH^-]^n = k_{obs}[\text{Neonic}] \quad (2)$$

$$k_{obs} = k_{H_2O} + k_{OH^-}[OH^-]^n \quad (3)$$

$$\text{Assume } k_{H_2O} = k_{pH\,4} \quad (4)$$

$$k_{obs} = k_{pH\,4} = k_{OH^-}[OH^-]^n \quad (5)$$

$$\log(k_{obs} - k_{pH\,4}) = n \times -pOH + \log(k_{OH^-}) \quad (6)$$

Calculated reaction orders range from 0.50 ± 0.105 (clothianidin) to 0.67 ± 0.183 (thiamethoxam), with imidacloprid (0.52 ± 0.121), acetamiprid (0.62 ± 0.125), and nitenpyram (0.60 ± 0.121) in the middle. Errors are 95% confidence intervals. Because of the relative similarity between the calculated reaction orders, a slope test was performed to compare each of the log–log regression lines to determine if there was a significant

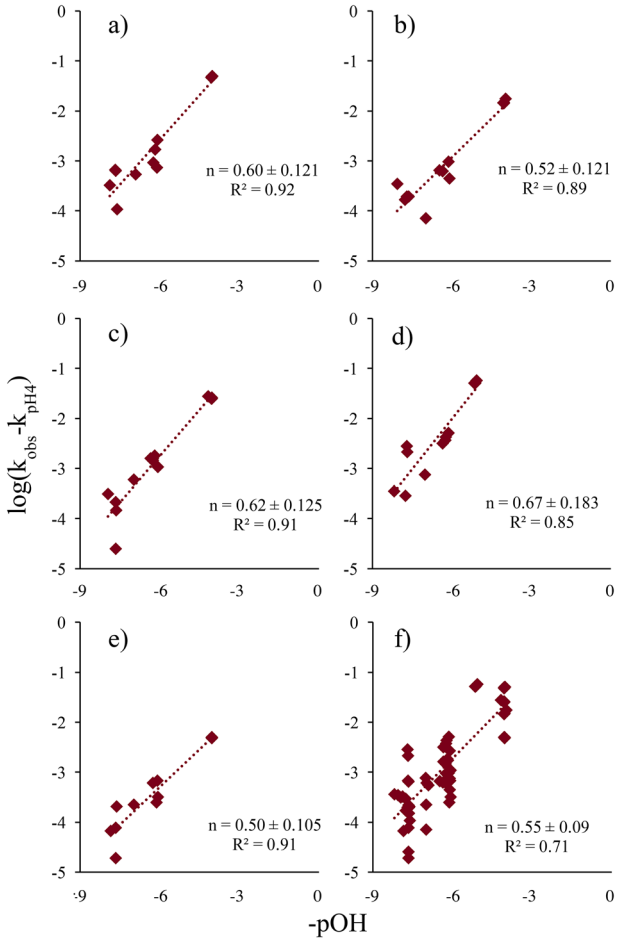

FIGURE 3: Log–log plot of hydroxide concentration and the difference between k_{obs} and $k_{pH\,4}$. The resulting slope is the approximate value of *n*, the exponent for [OH^-] in the nonelementary reaction of neonicotinoid hydrolysis: (**a**) nitenpyram, (**b**) imidacloprid, (**c**) acetamiprid, (**d**) thiamethoxam, (**e**) clothianidin, and (**f**) all neonicotinoids combined. All data points were combined to estimate the value of *n* after slope testing revealed no statistical significance between the slopes of each of the individual neonicotinoids.

difference between individual neonicotinoid reaction orders. Calculated *p* values (see Supplemental Data, Table S6) show that there is not a statistically significant difference between each of the calculated slopes, with *p* values ranging from 0.09 to 0.83, indicating highly correlated slopes. All data points were placed in a single plot to provide a comprehensive estimate of the value of *n*. Linear regression of the resulting plot returned a slope of 0.55 ± 0.09. Hydroxide rate constants were then calculated for all experiments and are given in Table 2. The nonelementary rate expression indicates that the hydrolysis mechanism is likely not the straightforward process previously depicted (e.g., Zheng and Liu 1999; Karmakar et al. 2009) but rather one where reversible, preequilibrium steps occur and where OH^- is involved in multiple steps. Further work would be necessary to determine the elementary reaction steps that occur leading to the observed approximately 0.5 power dependence on [OH^-].

When hydroxide rate constants are compared at the 95% confidence interval, rate constants do not differ between baseline and metal-containing solutions, with the exception of the acetamiprid pH 10.0 baseline reactor and metal reactors, in which the metals slightly decrease the rate of reaction. Thus, these results indicate that divalent metal cations in solution do not change the rate of hydrolysis of neonicotinoids.

Hydrolysis in the presence of minerals. Reactors containing minerals (kaolinite, goethite, or titanium dioxide) were monitored for up to 100 d, depending on the speed of the reaction. Placement of a box over the stir plate to reduce the possibility of light contamination created the possibility of a slightly increased rate of reaction because other work has shown that the neonicotinoid hydrolysis reaction rate increases with temperature (Zheng and Liu 1999; Liqing et al. 2006). To account for the potential effect of temperature and the potential effect of stirring mineral reactors constantly whereas previous reactors had not been stirred, new baseline reactors were run along with mineral reactors. Pseudo-first-order rate constants were calculated for all reactions and are given in Table 1. Reaction kinetics are shown in Supplemental Data, Figure S3, and the slopes tests comparisons are given in Supplemental Data, Table S7. At pH 10, the faster reaction rates (2.1–2.5 times increase) compared to original baseline and metals experiments is attributed to the increased temperature. When accounting for the actual [OH^-] in each experiment (which again varied ±0.05 units from the target

TABLE 2: Hydroxide rate constants (k_{OH} in $M^{-0.55}\,d^{-1}$) for neonicotinoid hydrolysis reactions at 21.5 °C[a]

		pH		
Compound	Experiment	6.3	8.0	10.0
Nitenpyram	Baseline	10.3 ± 6.2	5.6 ± 0.9	8.0 ± 0.1
	Copper	6.5 ± 13.4	1.6 ± 0.7	7.7 ± 0.4
	Nickel	1.6 ± 5.1	4.1 ± 1.3	8.0 ± 0.5
	Zinc	9.7 ± 8.3	2.4 ± 1.1	7.8 ± 0.6
	Average[b]		6.1 ± 0.9	
Imidacloprid	Baseline	8.0 ± 1.5	1.7 ± 0.2	2.6 ± 0.1
	Copper	17.4 ± 10.9[c]	3.4 ± 1.8	2.4 ± 0.2
	Nickel	9.0 ± 5.5	2.9 ± 1.1	2.5 ± 0.1
	Zinc	8.6 ± 7.0	2.9 ± 1.7	2.4 ± 0.1
	Average[b]		4.2 ± 0.5	
Acetamiprid	Baseline	0.4 ± 5.8	2.3 ± 0.5	5.3 ± 0.2
	Copper	6.8 ± 4.4	4.7 ± 1.5	4.2 ± 0.2
	Nickel	2.2 ± 3.4	4.3 ± 1.4	4.2 ± 0.2
	Zinc	3.4 ± 2.4	3.8 ± 1.4	4.1 ± 0.2
	Average[b]		3.8 ± 0.5	
Thiamethoxam	Baseline	5.0 ± 2.9	11.5 ± 0.5	33.8 ± 0.8
	Copper	10.5 ± 242.6[d]	9.3 ± 2.5	33.4 ± 0.5
	Nickel	33.2 ± 27.9	9.6 ± 2.9	32.7 ± 0.7
	Zinc	44.8 ± 42.0	10.9 ± 2.9	33.7 ± 0.6
	Average[b]		23.4 ± 2.3	
Clothianidin	Baseline	3.0 ± 0.7	0.5 ± 0.3	0.8 ± 0.1
	Copper	1.3 ± 5.0	0.7 ± 2.1	0.8 ± 0.1
	Nickel	0.3 ± 3.2	1.4 ± 2.4	0.8 ± 0.1
	Zinc	1.2 ± 2.1	1.6 ± 3.0	0.8 ± 0.1
	Average[b]		1.1 ± 0.5	

[a] Errors are the 95% confidence intervals.
[b] Average rate constants were calculated using rate constants from baseline and metal experiments at pH 6.33, 8, and 10.
[c] Imidacloprid pH 6.33 copper was excluded as an outlier because it had an outsized effect on the mean.
[d] Thiamethoxam pH 6.33 copper was excluded as an outlier because of the large error associated with the value.

value) and using 0.55 for n and pH 4 baseline results for k_{H_2O} (Supplemental Data, Table S8), the calculated hydroxide rate constants indicate that pH may be responsible for any observed variations in reaction rates between mineral and baseline experiments as determined by the slope test (Supplemental Data, Table S7). Thus, minerals likely do not have an impact on neonicotinoid hydrolysis rates.

Hydrolysis in Mississippi River water. Samples of Mississippi River water were monitored for 150 d. Pseudo-first-order rate constants were calculated as ln[Neonicotinoid] versus time for experiments in Mississippi River water and are given in Table 3. Kinetic data are given in Supplemental Data, Figure S4. The pH of the Mississippi River water was 8.3; thus, pseudo-first-order rate constants were expected to be faster than hydrolysis rates at pH 8.0. This was observed for nitenpyram, where the pseudo-first-order rate constant is marginally larger than the average pseudo-first order at pH 8.0. Thiamethoxam pseudo-first-order rate constants were the same, whereas clothianidin, imidacloprid, and acetamiprid pseudo-first-order rate constants were slower.

Comparison of hydroxide rate constants, which accounts for comparison across several pH values, indicates that every neonicotinoid reacts 45 to 90% slower in Mississippi River water, accounting for the pH of Mississippi River water. No explanation is currently available to account for the changes in reaction rates, although a buffer effect from carbonate is one possibility. These results do indicate that hydrolysis degradation rates may be slower in natural water bodies than predicted by laboratory tests performed in less complicated matrices.

Photolysis

Kinetic data for photolysis experiments in natural sunlight and in a solar simulator are given in Figure 4. Calculated quantum yields are given in Table 4. In the solar simulator, calculated quantum yields for nitenpyram, imidacloprid, thiamethoxam, and clothianidin in Milli-Q water are all larger by 8 to 25% than quantum yields in Mississippi River water after adjusting for screening, indicating that indirect photolysis does not play a part neonicotinoid photodegradation. Results were similar in natural sunlight experiments, with similar quantum yields calculated between natural sunlight and solar simulator experiments, though calculated quantum yields were lower for thiamethoxam and clothianidin. In natural sunlight, once adjusted for screening, thiamethoxam Milli-Q results were lower than Mississippi River water quantum yields. A one-tailed paired t test comparing the 2 means gave a value of 0.12, indicating that at the 95% confidence interval the 2 quantum yields cannot be distinguished. Thus, thiamethoxam is likely to follow the same behavior as imidacloprid, nitenpyram, and clothianidin, which photolyze only because of direct photolysis.

Calculated quantum yields in the present study are similar to previously reported values. For imidacloprid, quantum yields of 0.0092 (Lu et al. 2015; medium-pressure mercury lamp) and 0.0055 (von Gunten 2012; natural sunlight, 47°N latitude, Zurich, Switzerland) have previously been reported, as compared with the quantum yields calculated in the present study, which ranged from 0.0089 to 0.0119. Quantum yields of thiamethoxam (0.0130–0.0167) are between previously reported quantum yields of 0.019 (Lu et al. 2015) and 0.013 (European Comission 2006). Similarly, with clothianidin, quantum yields of 0.0073 (von Gunten 2012) and 0.013 (Lu et al. 2015) have been reported, which are in the range of those calculated in the present study (0.0080–0.0133). The differences between the present values and those previously reported could be attributable to differences in the light sources (i.e., there is wavelength dependence of quantum yield) or because we have used the updated values for the PNA actinometer (Laszakovits et al. 2017). The updated actinometer values should give 29% lower quantum yields, which is the effect seen for thiamethoxam and clothianidin in the natural sunlight experiments and in the solar simulator with Mississippi River water when comparing with values of Lu et al. (2015). It is not clear why the same effect is not observed for imidacloprid.

Acetamiprid samples were originally studied in the solar simulator, where results after 3 h of exposure gave an estimated half-life of >100 h. Although experiments were conducted on the rooftop of the University of Minnesota Mechanical Engineering building, exposure to sunlight for >1 mo yielded little to no degradation of acetamiprid in Mississippi River water samples or Milli-Q samples, indicating that direct photolysis was not an important environmental degradation pathway. These indicate a much longer half-life than reports in the literature, where Lu et al. (2015) found acetamiprid to have a half-life of 26 h with a quantum yield of 0.0022 ± 0.0003.

TABLE 3: Calculated pseudo-first-order and hydroxide rate constants for hydrolysis reactions in Mississippi River water (MRW) hydrolysis experiments at 21.5 °C[a]

Compound	$k_{obs,\ MRW}$[b] (d^{-1})	$k_{avg,\ pH\ 8}$[c] (d^{-1})	$k_{OH-,\ MRW}$[d] ($M^{-0.55}\ d^{-1}$)	$k_{OH-,\ avg}$[e] ($M^{-0.55}\ d^{-1}$)
Nitenpyram	$3.4 \pm (1.2) \times 10^{-3}$	$2.3 \pm (0.2) \times 10^{-3}$	3.5 ± 1.6	6.1 ± 0.9
Imidacloprid	$6.5 \pm (2.7) \times 10^{-4}$	$1.0 \pm (0.2) \times 10^{-3}$	0.4 ± 0.4	5.3 ± 0.7
Acetamiprid	$3.5 \pm (2.2) \times 10^{-4}$	$1.9 \pm (0.2) \times 10^{-3}$	0.1 ± 0.3	3.8 ± 0.5
Thiamethoxam	$4.4 \pm (0.5) \times 10^{-3}$	$4.4 \pm (0.4) \times 10^{-3}$	5.5 ± 0.7	22 ± 8
Clothianidin	$6.4 \pm (4.4) \times 10^{-4}$	$6.7 \pm (4.2) \times 10^{-4}$	0.6 ± 0.6	1.1 ± 0.5

[a] Errors are the 95% confidence intervals.
[b] Pseudo-first-order rate constant for hydrolysis reactions in Mississippi River water (pH 8.3).
[c] Averaged pseudo-first-order rate constants at pH 8.0.
[d] Hydroxide rate constant for Mississippi River water hydrolysis experiments.
[e] Average hydroxide rate constant across all pH values.

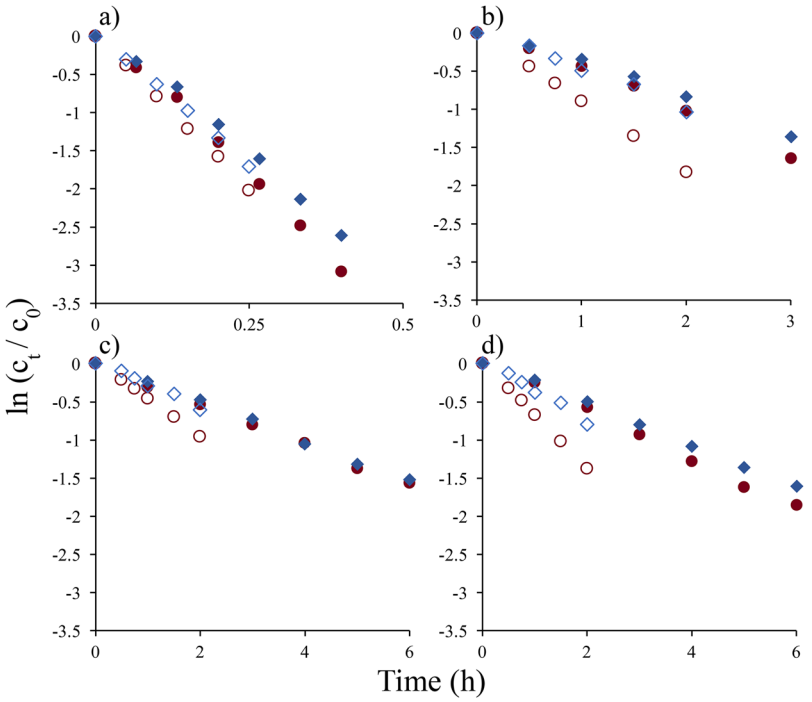

FIGURE 4: Photolysis of neonicotinoid insecticides in Milli-Q and Mississippi River water in natural and simulated sunlight: (**a**) nitenpyram, (**b**) imidacloprid, (**c**) thiamethoxam, (**d**) clothianidin. ● Milli-Q, natural sunlight; ♦ MRW, natural sunlight; ◊ MRW, solar simulator; ○ Milli-Q, solar simulator.

From the quantum yields calculated, indirect photolysis does not initially appear to be important. However, Mississippi River water generally contains lower levels of nitrate, a hydroxyl radical sensitizer, than could potentially be present in other waters, such as agricultural runoff. Further experiments were conducted using imidacloprid, acetamiprid, thiamethoxam, and clothianidin to study the effect of high concentrations of hydroxyl radicals using nitrate-amended Mississippi River water (10 mg/L as N). Nitenpyram was not used in nitrate experiments because direct photolysis is rapid.

First-order rate constants were calculated using linear regression of ln[C] versus time (see Supplemental Data, Figure S5). At a hydroxyl radical concentration of 2×10^{-15} M, as determined by the *p*CBA, imidacloprid, thiamethoxam, and clothianidin showed no increased degradation, indicating that hydroxyl radicals do not play a part in their photolysis. In acetamiprid experiments, with a hydroxyl radical concentration of $2.8 \pm (0.1) \times 10^{-15}$ M, hydroxyl radicals approximately doubled photolysis rates over 36 h in the solar simulator. A bimolecular rate constant of $1.7 (\pm 0.2) \times 10^{9} = M^{-1} s^{-1}$ was calculated for acetamiprid degradation by hydroxyl radicals.

Toxicity studies

Hydrolysis reaction products for toxicity tests were generated for nitenpyram, imidacloprid, acetamiprid, and thiamethoxam, including samples amended with metal ions and minerals. No hydrolysis products were generated for clothianidin because of the long degradation rate, even at pH 10.0. Similarly, photolysis products were produced for nitenpyram, imidacloprid, thiamethoxam, and clothianidin, but no products were produced for acetamiprid given its long half-life in simulated and natural sunlight experiments.

TABLE 4: Calculated average quantum yields for neonicotinoid insecticides in natural and simulated sunlight[a]

Light source		Nitenpyram	Imidacloprid	Thiamethoxam	Clothianidin
Solar simulator	Milli-Q	0.025 ± 0.001	0.0119 ± 0.0001	0.0167 ± 0.0002	0.0133 ± 0.0001
	MRW[b]	0.023 ± 0.001	0.0089 ± 0.0001	0.0136 ± 0.0001	0.0099 ± 0.0001
Natural sunlight	Milli-Q	0.025 ± 0.001	0.0115 ± 0.0005	0.0127 ± 0.0003	0.0091 ± 0.0002
	MRW[b]	0.024 ± 0.001	0.0100 ± 0.0005	0.0130 ± 0.0003	0.0080 ± 0.0001
Average		0.024 ± 0.001	0.0105 ± 0.0002	0.0140 ± 0.0002	0.0101 ± 0.0001
Literature	Lu et al. (2015)	—	0.0092 ± 0.0005	0.019 ± 0.001	0.013 ± 0.001
	Other Work	—	0.0055[c]	0.013[d]	0.0073[c]

[a] Errors are the 95% confidence interval.
[b] Mississippi River water samples were adjusted for screening by dividing by calculated screening factors, leading to an increase of 4 to 5%.
[c] von Gunten (2012).
[d] European Comission (2006).
MRW = Mississippi River water.

Solutions with reaction products contained approximately 20% parent compound and approximately 80% products. Testing was performed so that the concentration of parent neonicotinoid added to mosquito tests was the same in all exposures. Thus, if products exhibited toxicity, the LC50 values of tests with product present would be smaller relative to values for the parent neonicotinoids, whereas if products did not exhibit toxicity, the LC50 values would remain unchanged or increase. Calculated LC50 values are given in Table 5. The results indicate that there is no residual toxicity associated with products from hydrolysis or photolysis reactions to mosquito larvae. Although other studies have also shown lower toxicity of the urea derivatives (Simon-Delso et al. 2015) to insects, structural modifications of neonicotinoids are known to lead to binding to other receptors (Lee Chao and Casida 1997; Tomizawa and Casida 1999; Tomizawa et al. 2000). Thus, other relevant endpoints and organisms would need to be tested to confirm that no undesired toxic effects remain.

Product identification

All structures of identified compounds and MS/MS data are available in the Supplemental Data; UHPLC-MS/MS studies were only run in positive mode. It is possible there are reaction products which could be detected in negative mode. In addition, products were not preconcentrated prior to analysis, so it is possible that additional compounds could have been detected if this procedure was performed. Two hydrolysis products of nitenpyram were identified, with substitution of the $=CHNO_2$ functional group for $=O$ with an exact mass of 227.0825 (nitenpyram − urea), and removal of $–NHCH_3$ and subsequent substitution with an oxygen, as either an alcohol or a ketone, giving an exact mass of 257.0567. Exact mass and MS/MS data were used to identify products. Because there was not enough product generated to use nuclear magnetic resonance spectroscopy to determine which structural isomer of the nitenpyram degradation product (257) was produced, it is assumed that both structural isomers were generated. The nitenpyram product with exact mass 257.0567 has previously been identified in the literature (Noestheden et al. 2016), as has nitenpyram-urea. Photolysis samples also generated 2 reaction products, the urea derivative as well as a product with exact mass 211.0876, where the pharmacological moiety is removed entirely and replaced with a double bond from the carbon to the exterior nitrogen. The structure of the product with mass 211 was obtained by comparing MS/MS data with the available literature (Noestheden et al. 2016).

For imidacloprid hydrolysis and photolysis experiments, only imidacloprid-urea was observed, with exact mass 211.05124. Fragmentation patterns of imidacloprid-urea were collected from MS/MS results, yielding the same fragmentation pattern as previous work (Zheng and Liu 1999). Compound Discoverer matched MS2 fragmentation patterns to databases, resulting in positive identification of imidacloprid-urea. No variation was observed with hydrolysis products from metal ion or mineral experiments.

For acetamiprid, product testing was performed only for hydrolysis samples. As previously discussed, acetamiprid did not undergo any photolysis in an environmentally relevant time frame, and no samples could be generated for toxicity studies or reaction product identification. The urea derivative of acetamiprid was the only product observed. Exact mass was used to initially identify the product, and MS2 results were compared for all baseline, metal, and mineral studies, yielding the same fragmentation pattern. The observed product matches the expected hydrolysis product (Si et al. 2016).

The urea derivative of thiamethoxam was the only hydrolysis or photolysis product identified through UHPLC-MS/MS. Identification was performed using exact mass. Results did not vary between baseline hydrolysis, metal, and mineral experiments; and MS2 fragmentation patterns for the urea derivative of thiamethoxam matched each other, as did the MS2 for the photolysis sample.

Clothianidin-urea was the only observed hydrolysis and photolysis product by UHPLC-MS/MS. Initial identification was performed using exact mass; additional identification was performed by comparing MS2 data to the literature. The MS2 fragmentation gave peaks at 132 and 113, matching literature MS2 fragmentation data (Žabar et al. 2012). Results did not vary

TABLE 5: Median lethal concentration values for tested neonicotinoid insecticides[a]

LC50 (μM)	Nitenpyram	Imidacloprid	Acetamiprid	Thiamethoxam	Clothianidin
Parent	0.3	0.15	0.4	0.6	0.15
Photolysis 1	0.3	0.15	—	0.7	0.15
Photolysis 2	0.4	0.15	—	0.7	0.15
MRW[b]	0.4	0.2	—	0.6	0.15
Baseline Hydrolysis	0.4	0.2	0.5	1.0	—
Ni^{2+}[c]	0.5	0.2	0.4	0.9	—
Cu^{2+}[c]	0.4	0.3	0.4	0.8	—
Zn^{2+}[c]	0.5	0.2	0.6	0.8	—
Kaolinite[d]	0.5	0.2	0.6	0.8	—
Goethite[d]	0.3	0.3	0.3	0.9	—
TiO_2[d]	0.3	0.3	0.4	0.8	—

[a] Reaction products were tested by exposing mosquitoes to a 20% parent, 80% product solution. LC50 values were normalized to parent concentrations and not total concentration of products + parent.
[b] The Mississippi River water samples were photolysis samples exposed to light in Mississippi River water.
[c] Metal samples contained 0.1 mM of metal ions.
[d] Minerals were filtered out of samples prior to testing.
LC50 = median lethal concentration; MRW = Mississippi River water.

between baseline hydrolysis, metal, and mineral experiments, as with photolysis experiments.

Implications for environmental fate of neonicotinoids

Previous work had shown that neonicotinoid hydrolysis rates increased with increasing pH, indicating pH dependence; however, some results had indicated faster hydrolysis at acidic pH values (Zheng and Liu 1999; Liqing et al. 2006; Karmakar et al. 2009). Results of the present study indicate that neonicotinoids hydrolyze only under base-catalyzed conditions. Furthermore, these results indicate that in an environmentally relevant pH range (5–8.5) hydrolysis is unlikely to contribute meaningfully to degradation in the environment. This is backed by results from Mississippi River water experiments. At pH 8.3, in Mississippi River water, observed half-lives ranged widely, with significant error present. Expected environmental hydrolysis half-lives are 140 to 180 d for thiamethoxam, 150 to 320 d for nitenpyram, 800 to 1800 d for imidacloprid, 600 to 3500 d for acetamiprid, and 1200 to 5300 d for clothianidin. These half-lives will be longer at lower temperatures. This helps to explain the widespread detection of neonicotinoids in surface waters globally.

It is also of critical importance that the reaction order of OH^- is approximately 0.5 and not the 1.0 expected for an elementary reaction. Thus, second-order rate constants for base-catalyzed hydrolysis measured at a single pH value and assuming a reaction order of 1.0 will lead to incorrect values of half-life if extrapolated to other pH values. For example, the baseline imidacloprid pH 10 k_{obs} was 0.018 d^{-1}, which gave a second-order rate constant of 550 M^{-1} d^{-1}. Using this value to calculate a pseudo-first-order rate constant at pH 8 gives a value of 0.00055 d^{-1}, with a predicted half-life of 790 d. Using a reaction order of 0.5 and the same k_{obs} of 0.018 d^{-1}, however, gives a hydroxide rate constant of 2.6 $M^{-0.55}$ d^{-1} (see Table 2). The predicted pseudo-first-order rate constant at pH 8.0 is then 0.0013 d^{-1}, which gives a half-life of 530 d. Assuming a second-order elementary reaction will yield inaccurate estimates for extrapolated rate constants and half-lives.

As shown in the present study and in previous work (Lu et al. 2015), several neonicotinoids do undergo direct photolysis, with nitenpyram reacting very quickly in sunlight. These experiments, however, do not necessarily take into account the change in solar intensity throughout the day or seasonally. To estimate photolysis half-lives in the environment, integrated solar irradiances (L_λ) for 40°N at midsummer obtained from Leifer (1988), quantum yields calculated from natural sunlight Mississippi River water samples, and calculated molar absorptivity values were used to estimate photolysis rate constants (k_{dcE}) using Equation 7, where ϕ_{dc} is the calculated quantum yield, ε_λ is the molar absorptivity, and L_λ is the irradiance.

$$k_{dcE} = \phi_{dc} \Sigma_\lambda \epsilon_\lambda L_\lambda \quad (7)$$

Estimated near-surface environmental direct photolysis half-lives are 9 mins for nitenpyram, 45 min for imidacloprid, 90 min for clothianidin, and 120 min for thiamethoxam. These values are likely overestimates, given that midsummer clear days would give maximum rates. At 45°N, where the quantum yields were calculated, exposure on a midsummer day gave half-lives of 14 min for nitenpyram, 140 min for imidacloprid, 250 min for clothianidin, and 260 min for thiamethoxam.

The indirect photolysis half-life of acetamiprid is calculated by assuming a hydroxyl radical concentration of 1×10^{-16} M, assuming 7 h of sunlight per day and using the bimolecular rate constant calculated in the present study, leading to an estimated environmental half-life of 131 d. Overall, photolysis is not expected to contribute significantly to environmental degradation of acetamiprid.

Furthermore, these values are only relevant in near-surface conditions. Neonicotinoids have been shown to only break down in the top 8 cm of a water body (Lu et al. 2015). In any lake or larger river, such as the Mississippi River, environmental half-lives will be much longer. For example, if near-surface photolysis is expected to occur in the top 10 cm of a water body such as the Mississippi River, which is approximately 3 m deep, assuming a well-mixed system, the observed half-life would be 30 times the experimental half-life. At 45°N, environmental half-lives would increase to 2.9 d for imidacloprid, 5.5 d for thiamethoxam, and 5.2 d for clothianidin. In addition, experiments were conducted in filter-sterilized water. Although some lakes are pristine, many lakes and rivers, particularly in agricultural areas, are much more sediment-impaired and have higher turbidity than observed in laboratory experiments. This would lead to more light screening and scattering and thus longer degradation half-lives, which helps to explain the widespread detection of neonicotinoids in the natural water bodies.

The observed reaction product of most reactions results in the removal of the pharmacologically active moiety (–NO_2/–CN), with formation of the urea derivative of each compound. It appears the urea derivative of each neonicotinoid is the major hydrolysis and photolysis reaction product, but the limitations in our detection method need to be taken into account. The formation of the same products also implies that a photo-hydration reaction occurs during photolysis.

Results from toxicity tests further confirm literature results, which have generally concluded that urea derivatives do not have residual toxicity to the nicotinic receptor channels but that some may target other receptors (Simon-Delso et al. 2015). *Culex pipiens* larvae have previously been studied when exposed to thiacloprid, with a 14-d LC50 at 0.02 μM and a 5-d LC50 at 0.04 μM observed (Larissa et al. 2017). Experiments with *Aedes* sp., which is in the same family (Culicidae) as *Culex pipiens*, found 48-h LC50 values of 0.23 μM for thiamethoxam, 0.16 μM for imidacloprid, 0.11 μM for clothianidin, and 0.71 μM for acetamiprid (Raby et al. 2018), which are similar to results found in the present study. Assessment of other potential toxicological endpoints may still be needed.

CONCLUSIONS

Neonicotinoids, although widely used, have come under more scrutiny because of their observed environmental persistence,

near ubiquitous environmental presence, and impact on nontarget organisms (namely *Apis mellifera*). The present study has shown that neonicotinoids undergo base-catalyzed hydrolysis and that the reaction is nonelementary, with the hydroxide concentration raised to a power of 0.55 in the rate law. Furthermore, divalent metal cations and minerals were not observed to change hydrolysis rates. Direct photolysis was observed for nitenpyram, imidacloprid, thiamethoxam, and clothianidin, with quantum yields of 0.025 ± 0.001, 0.0119 ± 0.0001, 0.0167 ± 0.0002, and 0.0133 ± 0.0001, respectively. Acetamiprid degraded very slowly via direct photolysis but was found to undergo indirect photolysis because of reaction with OH· with a bimolecular rate constant of $1.7 \pm (0.2 \times 10^9)\ M^{-1}\ s^{-1}$. The urea derivative was the most commonly detected product, but in experiments using mosquitoes (*Culex pipiens*), no residual toxicity was observed. Results from experimental work indicate long environmental half-lives for the tested neonicotinoids, which may help to explain their observed persistence in environmental matrices.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI:10.1002/etc.4256

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Data Accessibility—Data are available in the Supplemental Data section, in the Data Repository for the University of Minnesota, (https://doi.org/10.13020/D6XQ2S) and on request to the authors (arnol032@umn.edu).

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