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Neonicotinoid Insecticides in Surface Water, Groundwater, and Wastewater Across Land-Use Gradients and Potential Effects

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Abstract: Neonicotinoid insecticides cause adverse effects on nontarget organisms, but more information about their occurrence in surface and groundwater is needed across a range of land uses. Sixty-five sites in Minnesota, USA, representing rivers, streams, lakes, groundwater, and treated wastewater, were monitored via collection of 157 water samples to determine variability in spatiotemporal neonicotinoid concentrations. The data were used to assess relations to land use, hydrogeologic condition, and potential effects on aquatic life. Total neonicotinoid concentrations were highest in agricultural watersheds (median = 12 ng/L), followed by urban (2.9 ng/L) and undeveloped watersheds (1.9 ng/L). Clothianidin was most frequently detected in agricultural areas (detection frequency = 100%) and imidacloprid most often in urban waters (detection frequency = 97%). The seasonal trend of neonicotinoid concentrations in rivers, streams, and lakes showed that their highest concentrations coincided with spring planting and elevated streamflow. Consistently low neonicotinoid concentrations were found in shallow groundwater in agricultural regions (<1.2–16 ng/L, median = 1.4 ng/L). Treated municipal wastewater had the highest concentrations across all hydrologic compartments (12–48 ng/L, median = 19 ng/L), but neonicotinoid loads from rivers and streams (median = 4100 mg/d) were greater than in treated wastewater (700 mg/d). No samples exceeded acute aquatic-life benchmarks for individual neonicotinoids, whereas 10% of samples exceeded a chronic benchmark for neonicotinoid mixtures. Although 62% of samples contained 2 or more neonicotinoids, the observed concentrations suggest there were low acute and potential chronic risks to aquatic life. This the first study of its size in Minnesota and is critical to better understanding the drivers of wide-scale environmental contamination by neonicotinoids where urban, agricultural, and undeveloped lands are present. *Environ Toxicol Chem* 2021;40:1017–1033. © 2020 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

Keywords: Insecticides; Neonicotinoids; Pollution; Aquatic life; Environmental monitoring

INTRODUCTION

Neonicotinoids are the most widely used class of insecticides worldwide and make up >25% of the global insecticide market (Jeschke et al. 2011; Douglas and Tooker 2015; Nowak 2015). Neonicotinoids were introduced in the 1990s, and as of 2016, their use has grown to include >500 commercial and domestic products in the United States (Jeschke et al. 2011; Simon-Delso et al. 2015; Minnesota Department of Agriculture 2016; Sheets et al. 2016; Buszewski et al. 2019). Neonicotinoids are effective because they share a common mode of action toward many agricultural, urban, and household pests (e.g., aphids, coleopterans, whiteflies, mites; Matsuda et al. 2001) and were originally thought to be safer to wildlife and humans (Tomizawa and Casida 2005). Because neonicotinoids act nonselectively against most insects, there is a risk that nontarget organisms that consume plants or contact surfaces treated with neonicotinoids will be adversely affected (Bonmatin et al. 2015).

Neonicotinoids are water-soluble and exhibit slow dissipation rates (half-life = 0.5–0.7 yr; Schaafsma et al. 2016) in the environment (Goulson 2013; Hladik et al. 2014, 2017; Bonmatin et al. 2015; Todey et al. 2018; University of Hertfordshire 2020). Thus, they are relatively persistent, mobile, and readily transported from their point of application via surface and groundwater (Bonmatin et al. 2015). Neonicotinoids are also mobilized in the atmosphere by dust particles released from neonicotinoid-treated areas during

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planting (Bonmatin et al. 2015). This has caused neonicotinoid concentrations in air of >10 000 ng/m^3 during planting (Tapparo et al. 2012), with concentrations of approximately 100 ng/m^3 remaining at the field boundaries (Marzaro et al. 2011). Current research to investigate the spatiotemporal drivers of neonicotinoid occurrence have reported year-round detections in numerous surface and groundwaters in addition to water-treatment facilities in both agricultural and urban watersheds (Jeschke et al. 2011; Goulson 2013; Simon-Delso et al. 2015; Minnesota Department of Agriculture 2016; Sheets et al. 2016; Buszewski et al. 2019). In one study, Hladik et al. (2017) detected neonicotinoids in soil and water at corn and soybean fields more than 3 yr after neonicotinoid treatments had ended. Neonicotinoid concentrations in the environment are influenced by hydrogeology, land use, rates of transformation, and use patterns (Jeschke et al. 2011; Hladik and Kolpin 2015; Morrissey et al. 2015).

Most commercial neonicotinoid products (>99%) are composed of imidacloprid, clothianidin, acetamiprid, and thiamethoxam either individually or in mixtures (Hladik et al. 2014; Main et al. 2014; Sánchez-Bayo and Hyne 2014; Minnesota Department of Agriculture 2016). Neonicotinoids also undergo biotic and abiotic processes that produce transformation products which can be more toxic than the parent compounds (Lee Chao and Casida 1997; Goulson 2013; Simon-Delso et al. 2015; Aregahegn et al. 2017). Interactive effects between co-occurring pesticides or their transformation products in turn cause either synergistic or antagonistic effects depending on the compound and affected organism (Warne and Hawker 1995; Deneer 2000). It is therefore important to understand the distribution of neonicotinoids and their transformation products in aquatic systems.

The use of neonicotinoids in agricultural (seed coating, foliar spray, soil injection) and urbanized (home and garden sprays, nurseries, flea and tick medications, tree injections, bedbug treatments) areas has led to the contamination of nontarget ecosystems (Schulz 2004; Beketov and Liess 2008; Schäfer et al. 2012; Simon-Delso et al. 2015). Adverse effects against many nontarget pollinators (e.g., honeybees, Lepidoptera), insects (e.g., Ephemeroptera, Trichoptera), and plankton (e.g., copepods, *Daphnia*) have been reported with median lethal concentrations (LC50s) for acute exposure between 1 and 100 000 µg/L in a water body (US Environmental Protection Agency 2020a). Neonicotinoids also cause (sub) lethal effects on freshwater vertebrates and macrophytes but at much higher concentrations (acute LC50s ≥100 000 µg/L; Gibbons et al. 2015).

The state of Minnesota is an important location to study the potential for environmental contamination by neonicotinoids because it contains almost 20 000 km^2 of surface freshwater (8% by area; US Census Bureau 2010) and uses large amounts of neonicotinoid insecticides in both urban and agricultural settings. From 2006 to 2016, the estimated average use of imidacloprid, thiamethoxam, clothianidin, and acetamiprid in Minnesota was 86 000, 91 000, 14 000, and 1200 kg, respectively (US Department of Agriculture 2014; Wieben 2019). The purpose of the present study was to understand the occurrence of neonicotinoids in water bodies within various land-use (urban, agricultural, undeveloped) and hydrogeologic settings. Five neonicotinoids (imidacloprid, clothianidin, thiamethoxam, thiacloprid, acetamiprid) and 5 neonicotinoid transformation products (imidacloprid-olefin, imidacloprid-urea, desnitro-imidacloprid, thiacloprid-amide, acetamiprid-*N*-desmethyl) were studied in 11 rivers and streams, 11 lakes, 35 groundwater wells, and 8 wastewater-treatment plant (WWTP) effluents in Minnesota during the 2019 growing season. These data create a better understanding of the effects of watershed characteristics and seasonality on neonicotinoid concentrations. New data that document the baseline occurrence of neonicotinoids in natural and engineered waters are essential to determine their potential effects and to inform the development of safe use guidelines. This is the first study of its scale to assess the occurrence and effects of neonicotinoids across spatiotemporal and hydrogeologic gradients in Minnesota.

MATERIALS AND METHODS

Chemicals and reagents

Optima™-grade acetonitrile (99.9%), water, and formic acid (99.0%) were used (Fisher Scientific). The Chemical Abstracts Service number, manufacturer, and purity of all analytical standards are provided in Supplemental Data, Section S1. Stock solutions of each analyte were prepared in Optima-grade acetonitrile and stored at −20 °C.

Study area

A total of 157 samples were collected between April and October 2019 from a total of 65 surface water, groundwater, and WWTP sites in Minnesota, USA (Figure 1; Supplemental Data, Tables S2–S5). Samples were collected from rivers and streams ($n = 11$), lakes ($n = 11$), groundwater wells ($n = 35$), and WWTP effluents ($n = 8$), which represented 31 (48% of total watersheds) predominantly urban, 21 (32%) predominantly agricultural, and 13 (20%) predominantly undeveloped watersheds. Drainage areas ranged from 0.1 to 10 000 km^2. When possible, sample collection was coordinated with a public or private organization as part of an ongoing monitoring program. Complete descriptions of sampling locations and site-specific information are provided in Supplemental Data, Section S2.

Sample collection

All samples were collected into cleaned 1-L polycarbonate bottles (see Supplemental Data, Section S1), which were then placed on ice and immediately transported to the laboratory. All samples were filtered through a prebaked glass fiber filter (≤0.7 µm; MilliporeSigma; GF/F; 1 filter/sample). Samples were stored at 4 °C until extraction.

Grab samples from rivers and streams were collected by wading or from a nearby structure with a dipper pole, weighted bucket, or Van Dorn sampler (Supplemental Data, Table S2). Sample collection began in May 2019, and one sample was

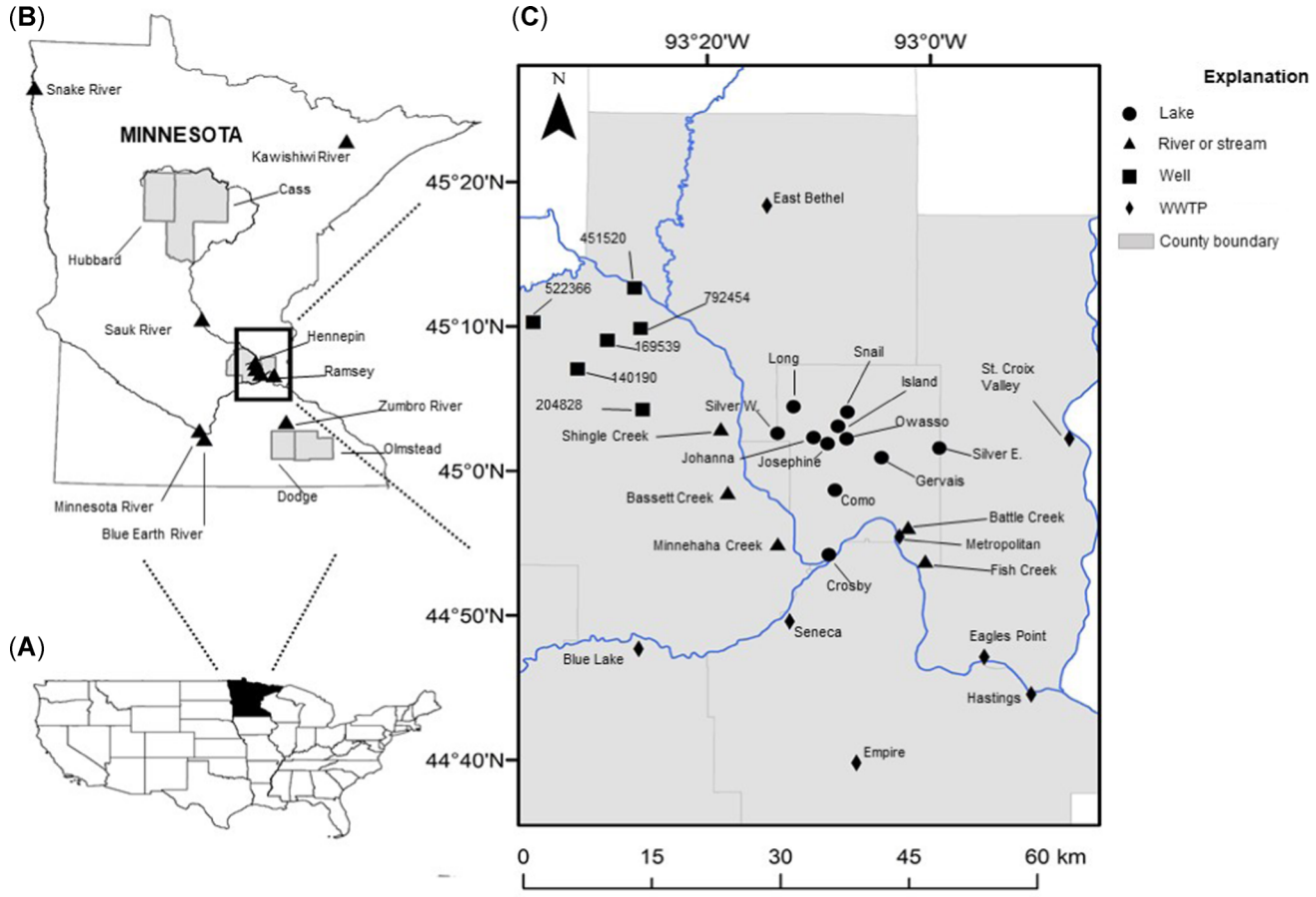

FIGURE 1: Sampling locations for 2019. Extent of locations (**A**) in the United States, (**B**) the state of Minnesota (shaded areas indicate counties where groundwater samples were collected), and (**C**) the Minneapolis–St. Paul metropolitan area (mainly Hennepin and Ramsey Counties). The state of Minnesota is represented by the shaded portion in the (**A**) US map. WWTP = wastewater-treatment plant.

collected in May, July, and August (3 total samples). The Blue Earth River ($n = 15$ total samples, predominately an agricultural watershed; Supplemental Data, Figure S1) and Shingle Creek ($n = 14$, predominately an urbanized watershed; Supplemental Data, Figure S1) were selected as high-frequency monitoring sites and sampled every 1 to 2 wk between April and October. When possible, samples were taken at or near active US Geological Survey gauging stations to provide streamflow information. The sampling of rivers and streams was performed in conjunction with the Minnesota Department of Agriculture, the Minnesota Pollution Control Agency, the International Water Institute, and the Vermillion Community College Watershed Science program.

All of the lakes are in moderate to highly urbanized portions of Ramsey County, MN, USA (Figure 1; Supplemental Data, Figures S1 and S2). Lakes were selected that were most likely to experience algal bloom formation based on historical observations from each lake. One 2-m-deep integrated surface water sample was collected from approximately the same location within each lake during June, July, and September, using a 2-m polyvinyl chloride (PVC) tube with a check valve on one end. This timing of sampling was designed to preclude, co-occur with, and follow the senescence of potentially harmful algal blooms. Como Lake was sampled differently because of its accessibility, urban landscape, and extensive monitoring data record. At Como Lake, samples were collected at approximately 2-wk intervals from June to October. The samples were collected from the same location in the lake with a Van Dorn sampler at the surface, 2 m depth, and just above the bottom (typically 4.2–4.9 m). Additional samples were collected from 7 of the lakes to assess the community composition of several planktonic taxa (details in Supplemental Data, Section S3). Lake sampling was coordinated with the Ramsey County Environmental Services, Lakes Management Division.

Groundwater samples were collected from wells in Cass ($n = 6$; August–September 2018), Hennepin ($n = 6$; May–June 2019), Hubbard ($n = 6$; May 2019), Dodge ($n = 6$; August 2019), and Olmstead ($n = 11$; September–October 2019) Counties (Figure 1). One sample was collected from each well. Samples were pumped from wells through a filter attached to the end of a 1-inch tube (PVC, Ultra Dynamic Products). The pump was operated for at least 5 min before sample collection to ensure stabilization of the groundwater chemistry. Well samples were obtained as part of the Minnesota Department of Natural Resources County Geologic Atlas program.

The WWTP effluents were sampled from the 8 WWTPs operated by the Minnesota Metropolitan Council Environmental Services. Samples were taken as a 24- to 48-h composites during 20 to 24 June 2019.

Sample preparation and analysis

Extraction of water samples. Samples were concentrated by solid-phase extraction (SPE) following previously established methods (Hladik and Calhoun 2012). Extractions were performed using Oasis HLB SPE cartridges (500 mg, 6 cc; Waters) that were conditioned with 5 mL dichloromethane, 5 mL acetone, and 10 mL water. Water samples were spiked with 10 ng of imidacloprid-d_4 and clothianidin-d_3 in acetonitrile as the surrogate standards and loaded onto HLB cartridges under vacuum flow at a rate of ≤10 mL/min. The cartridges were then dried completely under continued vacuum. The cartridges were eluted with 10 mL of a dichloromethane and acetone solution (50:50, v/v), and the eluent was evaporated to dryness under a gentle stream of nitrogen gas (99.99%; Matheson). The sample extracts were reconstituted in 1 mL of Optima-grade water and spiked with 50 ng of a 2.55 mg/L solution of caffeine-$^{13}C_3$ in acetonitrile as the internal standard. Acetonitrile was used to ensure that the final sample matrix matched the initial mobile phase during liquid chromatography (LC).

LC–tandem mass spectrometry. Neonicotinoids were measured by LC with tandem mass spectrometry (LC-MS/MS). Separations were performed by reverse-phase LC using a Dionex UltiMate 3000 RSLCnano Ultra Performance Liquid Chromatography system (Thermo Fisher Scientific) equipped with a Thermo Scientific Hypersil GOLD C18 column (100 × 2.1 mm, 1.9 µm particle size) maintained at 40 °C. A binary gradient of 0.1% formic acid in water and acetonitrile was used at a constant flow rate of 15 µL/min. The sample injection volume was 5 µL. The initial mobile phase was 10% acetonitrile and increased at a rate of 3% per minute for 13 min, followed by a ramp of 8% per minute for 5 min and then holding at 90% for 3 min. At 24 min, the mobile phase was abruptly decreased to 10% acetonitrile and held for 5 min before the next injection (29 min total analysis time). Detection and quantification were performed using a Thermo Scientific TSQ Vantage triple quadrupole MS in electrospray ionization–positive mode with full scan acquisition (m/z = 50–400, resolution = 70 000). The 2 most abundant ion transitions were simultaneously monitored via multiple reaction monitoring. The MS mass transitions, collision energies, and LC retention times for all compounds are provided in Supplemental Data, Section S4.

Quality assurance. The method detection limits (MDLs) were determined according to the protocol established by the US Environmental Protection Agency (2016). They are the minimum concentrations that can be reported with 99% confidence as being distinguishable from the method blanks. Seven replicate solutions containing 10 ng/L of each analyte were processed through the entire analytical procedure and analyzed by LC-MS/MS. The analyte-specific MDLs were calculated from Equation 1,

$$\mathrm{MDL}_i = t_{(n-1,1-\alpha=0.99)} S_i \quad (1)$$

where t is the t value of a single-tailed t test with (n – 1) degrees of freedom at the 99% confidence level and S is the standard deviation in neonicotinoid concentrations determined from the replicate injections for a given neonicotinoid (i). Measurements below the MDL were indicated by "<" followed by the corresponding MDL. The lower reporting limit (LRL) was set at twice the MDL. Neonicotinoid concentrations beyond the upper calibration range or between the LRL and MDL were considered estimated detections (indicated by "<" or ">" followed by the corresponding LRL) and included in the data analyses. The detection frequency of each analyte was defined as the proportion of calculated concentrations >MDL. For samples in which no neonicotinoids were observed >MDL, the entry was indicated with "<" and the corresponding MDL (see Supplemental Data, Table S8).

Quality assurance measures were based on method blanks, field blanks, equipment blanks, duplicate field samples, and recovery spikes into Optima-grade and field-collected water. Field blanks were obtained by exposing a bottle with Optima-grade water to the field atmosphere for at least 10 min. Equipment blanks were collected by processing Optima-grade water through the sampling equipment during sampling. A summary of MDLs and LRLs is shown in Supplemental Data, Table S8.

Mean relative recoveries of the surrogate standards were 94 ± 10% (imidacloprid-d_4) and 86 ± 17% (clothianidin-d_3); thus, the measured neonicotinoid concentrations were not recovery-corrected. Relative percentage differences of duplicate samples (n = 20 total samples) ranged from 1 to 57% with a mean (±standard deviation [SD]) of 15 ± 13%. Of the samples used for recovery spike experiments (n = 14 total samples), percentage recoveries ranged from 19 to 128% with a mean (±SD) of 75 ± 24% across all neonicotinoids. See Supplemental Data, Section S5 for a description of the procedures and calculations used for quality assurance.

Data analysis

Statistical comparisons. All data were analyzed using R, Ver 3.6.1 (R Development Core Team 2019) in RStudio, Ver 1.2.5033 (RStudio 2019). Data manipulations and descriptive statistics were determined with the dplyr (Ver 0.8.5), tidyr (Ver 1.0.2), psych (Ver 1.9.12.31), and stats (Ver 3.6.2) packages. Geospatial analyses (e.g., geolocation, watershed area calculations, land-use distributions) were performed in ArcGIS Desktop, Ver 10.6.1 (ESRI 2019) using data from the Minnesota Geospatial Commons and the 2016 National Land Cover Database (Yang et al. 2018).

Total neonicotinoid concentrations were defined as the sum of all neonicotinoid concentrations above the MDL in each sample. All measurements were included in the calculations.

Toxicity calculations. The pesticide toxicity index (PTI) for all samples was calculated following the method of Nowell et al. (2014). The PTI was calculated from the additive effects of neonicotinoid concentrations and the taxon-specific acute toxicity thresholds (Equation 2 and Supplemental Data, Section S6),

$$PTI_t = \sum_{i=1}^{n} E_i/TC_{i,t} \quad (2)$$

where n is the number of detected neonicotinoids, E is the concentration of neonicotinoid i, and $TC_{i,t}$ is the acute toxicity concentration of neonicotinoid i to the taxonomic group t. Values of $TC_{i,t}$ were obtained from the Ecotoxicology Knowledgebase (ECOTOX; US Environmental Protection Agency 2020a), the US Environmental Protection Agency's Office of Pesticide Programs (US Environmental Protection Agency 2020b), and the Pesticide Properties DataBase (University of Hertfordshire 2020). To determine the toxicity concentration, the average of all available acute LC50s, or median effect concentrations when LC50s were not available, specific to the mortality or immobility endpoints were calculated with data from ECOTOX, the Office of Pesticide Programs, and the Pesticide Properties DataBase. One toxicity concentration was calculated for each combination of taxon and neonicotinoid. A complete list of the $TC_{i,t}$ values used is provided in Supplemental Data, Table S9. For reference, the acute and chronic toxicity benchmarks for freshwater aquatic invertebrates, algae, and plants are provided in Supplemental Data, Table S10.

RESULTS

Overall detection frequencies

Measured neonicotinoid concentrations are summarized in Table 1. At least one neonicotinoid or neonicotinoid

TABLE 1: Summary of measured neonicotinoid concentrations in each type of water body[a]

		Imidacloprid		Clothianidin		Acetamiprid		Thiamethoxam		Total neonicotinoids
	n	DF (%)[b]	Median (ng/L)	DF (%)	Median (ng/L)	DF (%)	Median (ng/L)	DF (%)	Median (ng/L)	Maximum (ng/L)
Rivers and streams										
Battle Creek	3	100	2.3	67	0.46	33	<0.42	33	<0.12	8.4
Bassett Creek	3	100	5.0	100	0.81	33	<0.42	67	0.34	19
Fish Creek	3	100	11	100	2.2	33	<0.42	100	1.8	24
Minnesota River	3	100	1.6	100	10	33	<0.42	100	0.97	39
Kawishiwi River	4	25	<0.23	25	<0.42	25	<0.42	0	<0.12	2.0
Minnehaha Creek	4	75	2.5	75	1.5	50	0.74	75	0.45	8.1
Blue Earth River	14	100	1.3	100	17	14	<0.42	100	3.8	43
Shingle Creek	15	100	2.2	47	<0.42	27	<0.42	33	<0.12	21
Sauk River	4	100	1.5	75	4.6	75	1.5	100	1.3	13
Snake River	4	100	4.8	100	6.9	25	<0.42	75	2.1	32
Zumbro River	4	100	3.3	100	38	25	<0.42	100	8.0	140
Lakes										
Como	23	74	1.7	39	<0.42	4	<0.42	0	<0.12	13
Crosby	3	67	0.61	67	1.6	0	<0.42	33	<0.12	4.2
Gervais	3	100	3.5	33	<0.42	0	<0.42	33	<0.12	5.8
Island	3	33	<0.23	0	<0.42	0	<0.42	0	<0.12	0.65
Johanna	3	67	0.85	33	<0.42	0	<0.42	0	<0.12	2.6
Josephine	3	67	0.82	0	<0.42	33	<0.42	0	<0.12	4.3
Long	3	100	3.6	67	0.77	0	<0.42	0	<0.12	5.1
Owasso	3	67	0.88	67	0.73	33	<0.42	0	<0.12	4.6
Silver E.	3	67	0.45	0	<0.42	0	<0.42	33	<0.12	1.6
Silver W.	3	67	1.2	0	<0.42	0	<0.42	0	<0.12	1.7
Snail	3	67	0.65	67	0.48	0	<0.42	67	1.4	12
Wells										
Cass	6	50	<0.23	50	<0.42	17	<0.42	17	<0.12	3.4
Dodge	6	0	<0.23	0	<0.42	0	<0.42	0	<0.12	<0.12
Hennepin	6	83	0.27	83	1.2	67	1.1	83	0.20	15
Hubbard	6	83	0.25	100	1.2	100	1.4	100	0.22	3.1
Olmstead	11	9	<0.23	9	<0.42	55	0.44	64	0.38	15

WWTPs[c]	Imidacloprid Concentration (ng/L)	Clothianidin Concentration (ng/L)	Acetamiprid Concentration (ng/L)	Thiamethoxam Concentration (ng/L)	Acetamiprid-N-Desmethyl Concentration (ng/L)
Blue Lake	48	11	<0.42	<0.12	1.7
Eagles Point	24	2.9	0.48	<0.12	1.0
East Bethel	22	0.67	<0.42	<0.12	<0.48
Empire	13	<0.42	4.7	<0.12	1.5
Hastings	13	<0.42	0.64	0.44	1.0
Metropolitan	16	32	1.9	1.4	1.4
Seneca	29	5.2	<0.42	<0.12	1.8
St. Croix Valley	12	<0.42	0.43	<0.12	0.80

[a]Medians were calculated with all measurements from each sampling location.
[b]Detection frequencies are defined as the percentage of measurements greater than the method detection limit.
[c]Each entry is a single-effluent sample.
DF = detection frequency.

TABLE 2: Method detection limits, detection frequencies, and median concentrations of neonicotinoids in all samples ($n = 157$)

Compound	MDL (ng/L)	Detections >MDL (%)	Detections >LRL (%)	Median (ng/L)[a]
Acetamiprid	0.42	27	19	<0.42
Acetamiprid-*N*-desmethyl	0.48	5	4	<0.48
Clothianidin	0.42	55	40	0.52
Imidacloprid	0.23	75	62	1.3
Imidacloprid-olefin	1.7	0	0	<1.7
Imidacloprid-urea	1.5	0	0	<1.5
Desnitro-imidacloprid	0.71	0	0	<0.71
Thiacloprid	0.60	1	0	<0.60
Thiacloprid amide	0.65	1	1	<0.65
Thiamethoxam	0.12	43	9	<0.12
Total neonicotinoid concentration		86	62	3.0

[a]All measurements were included in the median calculations.
LRL = lower reporting limit; MDL = method detection limit.

transformation product (hereafter referred to as "neonicotinoids") was detected in 86% of all samples ($n = 157$); 62% contained 2 or more, 40% contained 3 or more, and 4 or more were detected in 18%. The maximum number of neonicotinoids in any sample was 5 ($n = 2$; Metropolitan WWTP and Blue Earth River, May); the median number of compounds detected per sample was 3. The most frequently detected compounds were imidacloprid (75%), clothianidin (55%), thiamethoxam (43%), and acetamiprid (27%). A significant, positive Spearman's rank correlation (defined as $p < 0.05$) was observed between the occurrence of thiamethoxam and clothianidin ($\rho = 0.870$, $p < 0.001$; Supplemental Data, Figure S3).

The median and maximum total neonicotinoid concentrations across all samples were 3.0 and 140 ng/L (Zumbro River, May), respectively. Individual neonicotinoid concentrations ranged from <MDL to 92 ng/L. There were no detections of thiacloprid or any imidacloprid transformation products (Table 2). Thiacloprid amide, a transformation product of thiacloprid, was measured >MDL (2.9 ng/L; Table 2) in only one sample.

Rivers and streams

Sixty-one samples were analyzed from 11 rivers and streams (Figures 2 and 3 and Table 1). Ninety-seven percent of all samples contained at least one neonicotinoid; the median number of co-occurring neonicotinoids was 2. Neonicotinoid concentrations were typically highest during May and decreased thereafter. Total neonicotinoid concentrations ranged from <0.12 to 140 ng/L (median = 9.3 ng/L). The watershed drainage areas were between 30 and 30 000 km^2 (Supplemental Data, Table S2) and included a mixture of predominantly urban ($n = 6$, including Shingle Creek) and agricultural ($n = 4$, including Blue Earth River) land use, with one predominantly undeveloped watershed of mixed-use forest (Kawishiwi River). The percentage of land dedicated to cultivated crops in agricultural watersheds ranged from 51% (Blue Earth River) to 95% (Snake River).

Clothianidin was the most frequently detected neonicotinoid at both agricultural (100% detection, median concentration = 15 ng/L) and urban (100%, 0.71 ng/L) sampling locations (Table 1). The median concentrations of imidacloprid, however, were greater than those of clothianidin in urban-affected watersheds (2.6 ng/L). In all streams, thiamethoxam

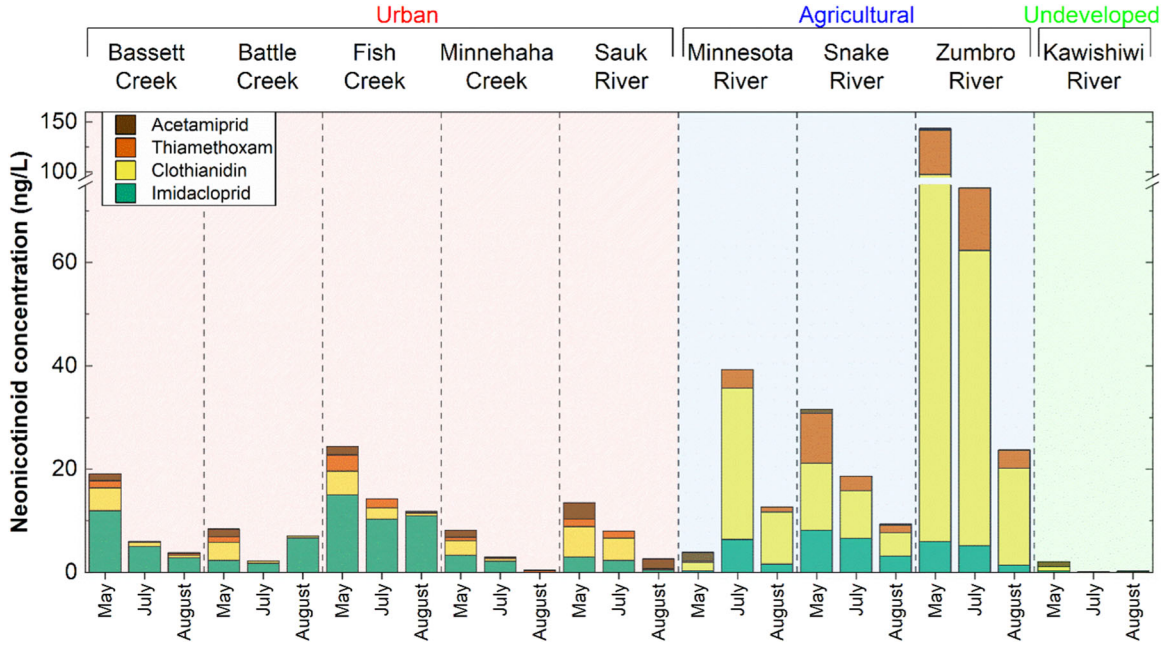

FIGURE 2: Neonicotinoid concentrations in rivers and streams, excluding Shingle Creek and Blue Earth River (see Figure 3), during 2019. Colored panels indicate the prevailing land use (red = urban, blue = agricultural, green = undeveloped) of the surrounding watershed. Each column represents one sample.

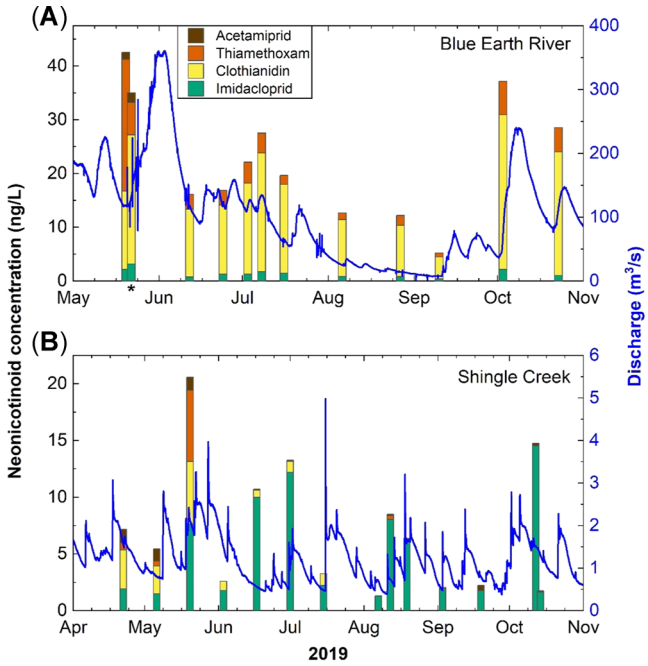

FIGURE 3: Neonicotinoid concentration (left axis, columns) and instantaneous streamflow (right axis, solid line) in (**A**) Blue Earth River (agricultural watershed) and (**B**) Shingle Creek (urban watershed). *Sample in which thiacloprid amide was detected (2.9 ng/L).

(69%, 0.76 ng/L) and acetamiprid (30%, <0.42 ng/L) were detected less frequently and at lower concentrations (Table 1). The highest concentration of an individual neonicotinoid was clothianidin (92 ng/L, Zumbro River). The median number of co-occurring neonicotinoids in all samples was 3. Only one sample from rivers and streams in the present study contained a neonicotinoid transformation product (thiacloprid amide, 2.9 ng/L) > MDL (Blue Earth River, asterisk in Figure 3A). Because it was not detected in any quality assurance and control samples, the result was likely not caused by sample contamination or an erroneous detection.

Instantaneous neonicotinoid yields (Supplemental Data, Figure S4) were calculated for each of the rivers and streams where continuous streamflow measurements were available (calculations in Supplemental Data, Section S7). Clothianidin had the highest median yield (20 mg/km^2/d) at agricultural locations, and all other neonicotinoids were <4.0 mg/km^2/d. In urban watersheds, clothianidin (4.2 mg/km^2/d) and imidacloprid (3.4 mg/km^2/d) had the largest median yields, whereas all other compounds were ≤1.1 mg/km^2/d.

The increased frequency and total number of sampling events at Blue Earth River (Figure 3A) and Shingle Creek (Figure 3B) were used to investigate the temporal variation in neonicotinoids and mitigate potential bias caused by infrequent sampling (Stehle et al. 2013; Stehle and Schulz 2015; Spycher et al. 2018; Wolfram et al. 2019). The characteristic decrease in total neonicotinoid concentration (see Figure 2) was observed throughout the season. The increased temporal resolution revealed one or more additional neonicotinoid spikes at each sampling location during July and October (Figure 3). A review of stream gauge data (US Geological Survey 2020) and weather records (National Oceanic and Atmospheric Administration 2020) showed that these spikes corresponded with precipitation-induced pulses in streamflow (Figure 3, solid line), which could have increased the neonicotinoid load. The relative paucity of concentration measurements in relation to streamflow, however, made it

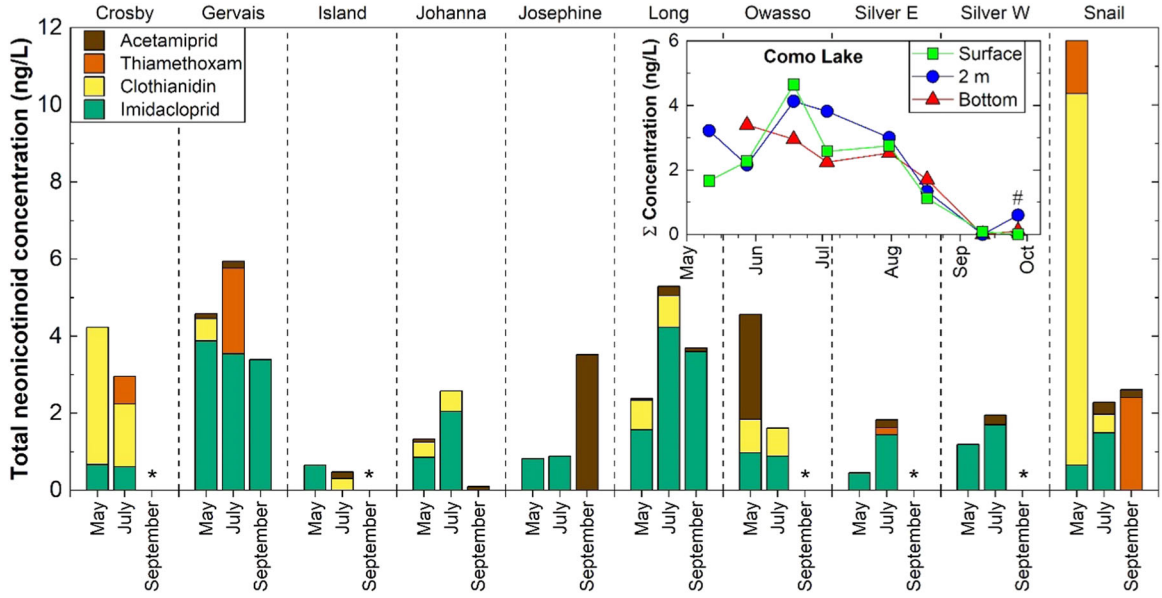

FIGURE 4: Distribution of neonicotinoid concentrations at 10 lakes in Ramsey County, Minnesota, USA, during 2019. Water samples were integrated over the top 2 m of each lake. (Inset) Total neonicotinoid concentrations in Como Lake at 3 depths over time. Total concentrations were mainly composed of imidacloprid. *No neonicotinoids detected. #The acetamiprid transformation product acetamiprid-*N*-desmethyl was detected in one bottom water sample at a concentration of 13 ng/L (Oct 2019) in Como Lake (inset). Because this is the only such detection in all lake samples and owing to its high concentration relative to acetamiprid (<0.42 ng/L), it is not included in the summed concentration.

difficult to confidently conclude that a significant correlation does exist between concentration and streamflow.

Lakes

Eleven small, shallow lakes (≤1.5 km^2 surface area, 39–100% littoral) with small drainage areas (0.5–53 km^2; Supplemental Data, Table S3) were sampled from urbanized portions (44–91% urban land use) of Ramsey County, MN, USA (Figure 4 and Table 1). Imidacloprid was the most frequently detected neonicotinoid (72% detection, median = 1.64 ng/L) in all lakes. The other neonicotinoids were detected less frequently (≤36%) and at lower concentrations (Table 1). Almost all samples (98%) contained one or more neonicotinoid; the median number of co-occurring neonicotinoids was 3.

Almost all of the lakes had higher concentrations of neonicotinoids in the early part of the year (May–July) than in the later months (September and October; Figure 4). Five of the lakes had no observable neonicotinoid concentrations >MDL in September. Como Lake was sampled on 8 separate dates ($n = 23$ total samples; Figure 4, inset) and showed a similar trend in which the highest total neonicotinoid concentrations were in mid-June and decreased thereafter. No significant correlations of concentrations with sampling depth at Como Lake (determined by Spearman rank analysis) were found (Supplemental Data, Figures S5 and S6). Como Lake is a shallow lake with a prominent flow-through nature, which maintains regular mixing throughout the entire lake.

Ten zooplankton and 38 phytoplankton taxa (Supplemental Data, Table S6) in 7 of the lakes were analyzed to determine whether the presence of neonicotinoids affected their taxonomic distributions. Many of these species constitute the foundation of important aquatic food webs and can be an indicator of overall aquatic health (Hladik et al. 2018b). The most abundant phytoplankton taxa were *Cryptomonas* (73% detection, median 269 cells/mL), *Oscillatoria* (61%, 3700 cells/mL), and *Anabaena* (58%, 430 cells/mL). The most common zooplankton were nauplii (100%, 13 counts/L) and cyclopoids (98%, 10 counts/L). There were no observable linear relationships or significant Spearman's rank correlations between total neonicotinoid concentrations with phytoplankton (Supplemental Data, Figure S7) or zooplankton (Supplemental Data, Figure S8) populations over the entire sampling period (April–November).

Groundwater

The median and maximum total neonicotinoid concentrations in groundwater wells were 1.4 and 16 ng/L, respectively (Figure 5). At least one neonicotinoid was detected in 74% of wells, 2 or more were detected in 49%, and 2 were detected in 26%. The median number of co-occurring neonicotinoids was 2. By county, the median total neonicotinoid concentrations were 3.2 ng/L (Hennepin), <0.12 ng/L (Dodge), 1.3 ng/L (Olmstead), 3.0 ng/L (Hubbard), and 1.3 ng/L (Cass; Table 1). No thiacloprid or any of the neonicotinoid transformation products were detected in any of the groundwater samples.

The predominant type of land use surrounding each sampling location is indicated by the background color in Figure 5. Except for Hennepin County, all wells in each county represented the same type of land use: agricultural (Olmstead and Dodge) or undeveloped (Cass and Hubbard). Because drainage areas are not easily determined for groundwater, land use was estimated in the 1-km radius zone surrounding each well. The wells in Hennepin County included both predominantly urban ($n = 2$) and agricultural ($n = 4$) watersheds. The depth of wells ranged from 11 to 195 m below ground

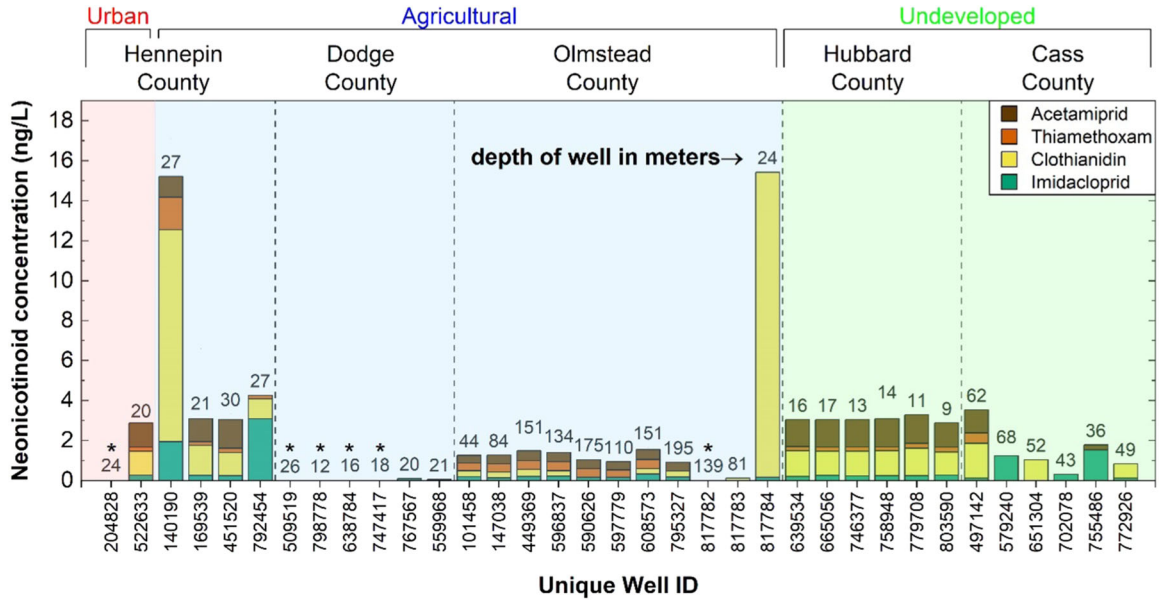

FIGURE 5: Distribution of neonicotinoid concentrations in groundwater wells. Bottom-axis labels show the unique well ID used in the Minnesota Well Index. Upper-column labels indicate the depth of each well in meters. *No neonicotinoids detected.

level, as shown by the labels above the columns in Figure 5 (see Supplemental Data, Table S4).

Hennepin County had the highest concentrations of clothianidin (83% detection, median = 1.2 ng/L; Table 1) and acetamiprid (100%, median = 1.1 ng/L). Imidacloprid and thiamethoxam were also frequently detected (83 and 67%, respectively).

No neonicotinoids were detected >MDL in any of the samples from Dodge County (86–95% agricultural land use). Olmstead County (49–88% agricultural land use) is directly adjacent to Dodge County and had low neonicotinoid concentrations (median total neonicotinoid concentration 0.88 ng/L; Table 1).

In predominately undeveloped Hubbard County (all wells were 79–95% undeveloped land use), clothianidin, thiamethoxam, and acetamiprid were detected >MDL in every sample and imidacloprid was detected in 83% of samples (Table 1). No neonicotinoids were detected in the quality assurance and control samples from Hubbard County, which indicated that the measurements were not attributable to experimental error. Cass County is also largely undeveloped (70–96% undeveloped land use). In the Cass County wells, imidacloprid (50% detection, median 0.22 ng/L) and clothianidin (50%, 0.36 ng/L) were frequently detected. No thiacloprid or any of the neonicotinoid transformation products were detected in any of the groundwater samples.

WWTPs

Sewersheds (i.e., collection areas) for the sampled WWTPs range from 45 km^2 (St. Croix Valley) to 1900 km^2 (Metropolitan), with a median area of 230 km^2 (Supplemental Data, Table S5). Five of the sewersheds have predominately urban land use (45–73%), 2 have predominately agricultural land use (59–64%), and 1 has mostly undeveloped land use (52%). Two or more neonicotinoids were detected in every sample; 7 of the samples contained 3 neonicotinoids, and 1 sample contained 5 (Table 1 and Figure 6).

Imidacloprid was detected in 100% of samples at concentrations of 12 to 48 ng/L (median 19 ng/L; Figure 6A and Table 1). The acetamiprid transformation product acetamiprid-*N*-desmethyl was also frequently detected (88% detection, median 1.4 ng/L). Clothianidin was the next most abundant neonicotinoid (63% detection, median 1.8 ng/L), followed by acetamiprid (63%, 0.45 ng/L) and thiamethoxam (25%, <0.12 ng/L). Total neonicotinoid concentrations (median = 26 ng/L, maximum = 61 ng/L) at WWTPs were higher than those in rivers, lakes, or groundwater (Table 1).

Instantaneous neonicotinoid loads were calculated from the total neonicotinoid concentration and mean discharge at each WWTP (Figure 6B, left axis; see Supplemental Data, Equation S3). Loads were highest at the Metropolitan (34 g/d), Blue Lake (6.2 g/d), and Seneca (3.0 g/d) WWTPs and lowest at the East Bethel (0.04 g/d) and Hastings (0.10 g/d) WWTPs. Positive Spearman rank-order correlations were determined for neonicotinoid loads with sewershed area ($\rho = 0.905$, $p = 0.002$; Supplemental Data, Figure S9A) and the population served ($\rho = 0.995$, $p < 0.001$; Supplemental Data, Figure S9B). The calculated loads were normalized to the population served by each WWTP to calculate the mean per capita neonicotinoid load to each plant (Supplemental Data, Equation S5). The values ranged from 3.4 µg/person/d (East Bethel) to 21 µg/person/d (Blue Lake) as total neonicotinoid concentration (Figure 6B, right axis). Per capita loads were positively correlated with the sewershed area of each WWTP ($\rho = 0.714$, $p = 0.05$; Supplemental Data, Figure S9C).

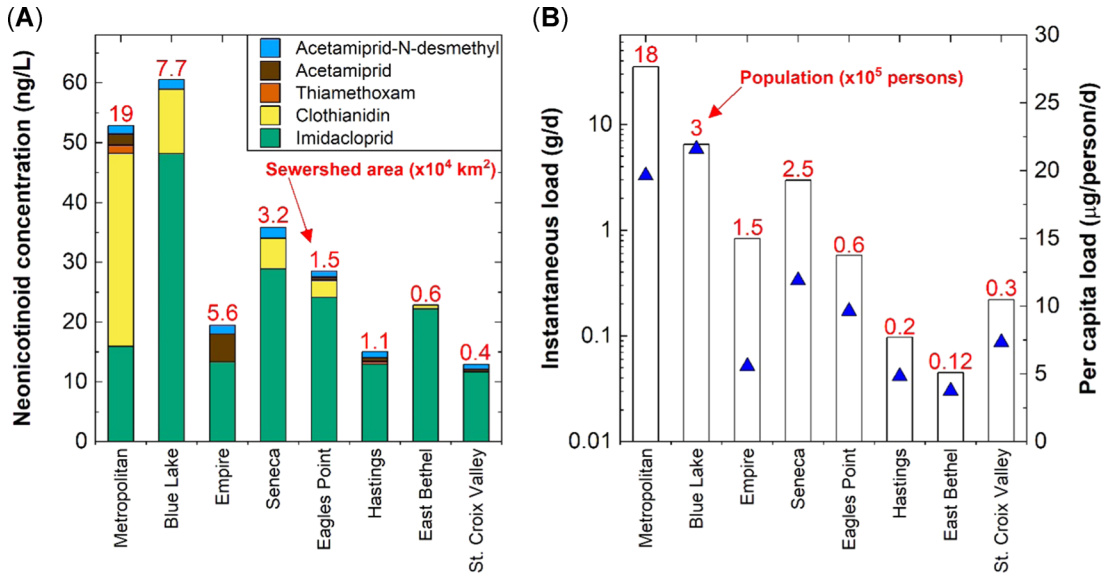

FIGURE 6: Occurrence of neonicotinoids in wastewater-treatment plant (WWTP) effluents. (**A**) Neonicotinoid concentrations (*y*-axis, columns) and sewershed area (top-column labels) of each WWTP. (**B**) Instantaneous (left *y*-axis, columns) and per capita (right *y*-axis, triangles) neonicotinoid loads. Upper-column labels indicate the population served by each WWTP. The data in both panels are arranged by decreasing sewershed area, from left to right.

PTIs

For all samples, PTIs were determined based on the observed neonicotinoid concentrations and the taxon-specific toxicity concentration for a variety of aquatic life (Equation 2). The indexes are derived from LC50s, which ranged from 0.123 µg/L (imidacloprid, mayfly; Roessink et al. 2013) to >90 000 µg/L (thiamethoxam, *Lemna gibba*; University of Hertfordshire 2020) for the individual neonicotinoids detected in the present study. Values of PTIs were between 10^{-9} and 10^{-5}. The median taxon-specific PTIs across all samples were $10^{-5.9}$ (*Daphnia*), $10^{-6.0}$ (crustaceans), $10^{-6.2}$ (mollusks), $10^{-7.9}$ (fishes), and $10^{-8.8}$ (macrophytes). A summary of the PTI values is provided in Supplemental Data, Table S9. For reference, the acute and chronic toxicity benchmarks for neonicotinoids to freshwater invertebrates, fish, and aquatic plants are shown in Supplemental Data, Table S10.

DISCUSSION

Effects of land use and watershed characteristics

In lakes and streams, the neonicotinoid concentrations and detection frequencies were related to the type of land use within each watershed (Figure 7 and Table 3), the drainage area (Supplemental Data, Section S2), and flow conditions (Supplemental Data, Section S9).

Most neonicotinoid products currently registered for non-agricultural uses (pet products, lawn and garden sprays, tree injections) contain imidacloprid; most agricultural products (seed coatings, foliar sprays) consist primarily of clothianidin or thiamethoxam (Jeschke et al. 2011; Minnesota Department of Agriculture 2016). Imidacloprid (73% detection, median concentration = 1.6 ng/L) and clothianidin (62%, 15 ng/L) were the most frequently detected neonicotinoids in rivers and streams draining agricultural watersheds. The relationships between imidacloprid with urban land use and clothianidin with agricultural land use are presented in Supplemental Data, Figure S10A to D. Clothianidin is also formed from the transformation of thiamethoxam in the environment (Nauen et al. 2003). The national use of thiamethoxam during the last decade has exceeded imidacloprid (US Department of Agriculture 2014; Wieben 2019); thus, its lower detection frequency in the present study could be due to its degradation to clothianidin.

A similar study by Hladik and Kolpin (2015) determined a significant relationship between clothianidin and agricultural land use ($\rho = 0.465$, $p = 0.003$) with measurements from agricultural streams ($n = 38$) in Iowa, USA.

The wide use of neonicotinoids in seed coatings (>90% of corn and 44–50% of soybeans in the United States [Douglas and Tooker 2015; Simon-Delso et al. 2015]) and their poor incorporation into the targeted crop (2–20% of coating is absorbed by the crop [Nuyttens et al. 2013; Sánchez-Bayo 2014]) contribute to their frequent observation in agricultural streams. Neonicotinoids are also deposited on pesticide-coated dust particles that are emitted during seed drilling, which can be transported short distances in the atmosphere to nearby off-field sites (Nuyttens et al. 2013; Bonmatin et al. 2015). The median total neonicotinoid concentration and the median number of neonicotinoids detected in each sample were higher in agricultural compared to urban or undeveloped watersheds (Table 3). There was a generally positive relationship between total neonicotinoid concentrations and agricultural land use (Supplemental Data, Figure S10B). In 2016, it was estimated that neonicotinoids were applied to >98% of the total area of insecticide-treated cropland in North America (540 000 km^2), and in Minnesota alone, the combined agricultural sales of neonicotinoids exceeded 45 000 kg (Minnesota Department of Agriculture 2016).

The predominant land uses surrounding the East Bethel WWTP (52% undeveloped, 31% agricultural, 17% urban) and well 792454 (42% undeveloped, 37% agricultural, and 21% urban) indicate that the classification of "predominantly undeveloped" does not preclude the possibility that much of the surrounding landscape is agriculturally or urban-impacted. The highest total neonicotinoid concentrations at undeveloped locations occurred at the East Bethel WWTP (23 ng/L) and in Hennepin County well 792454 (4.3 ng/L). Moreover, at least one neonicotinoid >MDL was detected in 15 of 17 samples from undeveloped areas. These observations emphasize that the broader watershed should be considered, in addition to the immediate surrounding landscape, when performing landscape analyses.

In the present study, stronger relationships between drainage area and neonicotinoid concentrations were observed for WWTPs, rivers, and streams than for lakes. The drainage area for WWTPs was defined as the land area served by each plant, whereas the drainage area for rivers, streams, and lakes was the total land area that drains to each sampling location. Significant, positive correlations occurred between drainage areas and total neonicotinoid concentrations ($\rho = 0.444$, $p < 0.001$; Supplemental Data, Figure S10E) and the number of neonicotinoids detected ($\rho = 0.428$, $p = 0.002$; Supplemental Data, Figure S10F).

Surface runoff following storm events increases streamflow and can increase the movement of neonicotinoids to the receiving water bodies, especially after the planting of crops in agricultural areas (Krupke et al. 2012; Hladik et al. 2014; Hladik and Kolpin 2015). The relationships between individual and total neonicotinoid concentrations with streamflow measurements of the present study revealed significant correlations between total neonicotinoid concentrations and streamflow from rivers and streams (Supplemental Data, Figure S11), discharge from WWTPs (Supplemental Data, Figure S9D), and drainage from Como Lake (Supplemental Data, Figure S5).

Como Lake is a heavily engineered system, controlled by several stormwater inlets and one common outlet. Previous data (B. Belden, Capitol Region Watershed District, St. Paul, MN, USA, personal communication) have shown that the intensive management of Como Lake, in combination with its small size, creates short hydraulic residence times and allows for it to be considered as a continuously flowing system. For example, the 0.2-m^3/s spikes in discharge from Como Lake create an estimated residence time of <20 h following large rain events (Supplemental Data, Figure S5). This causes an

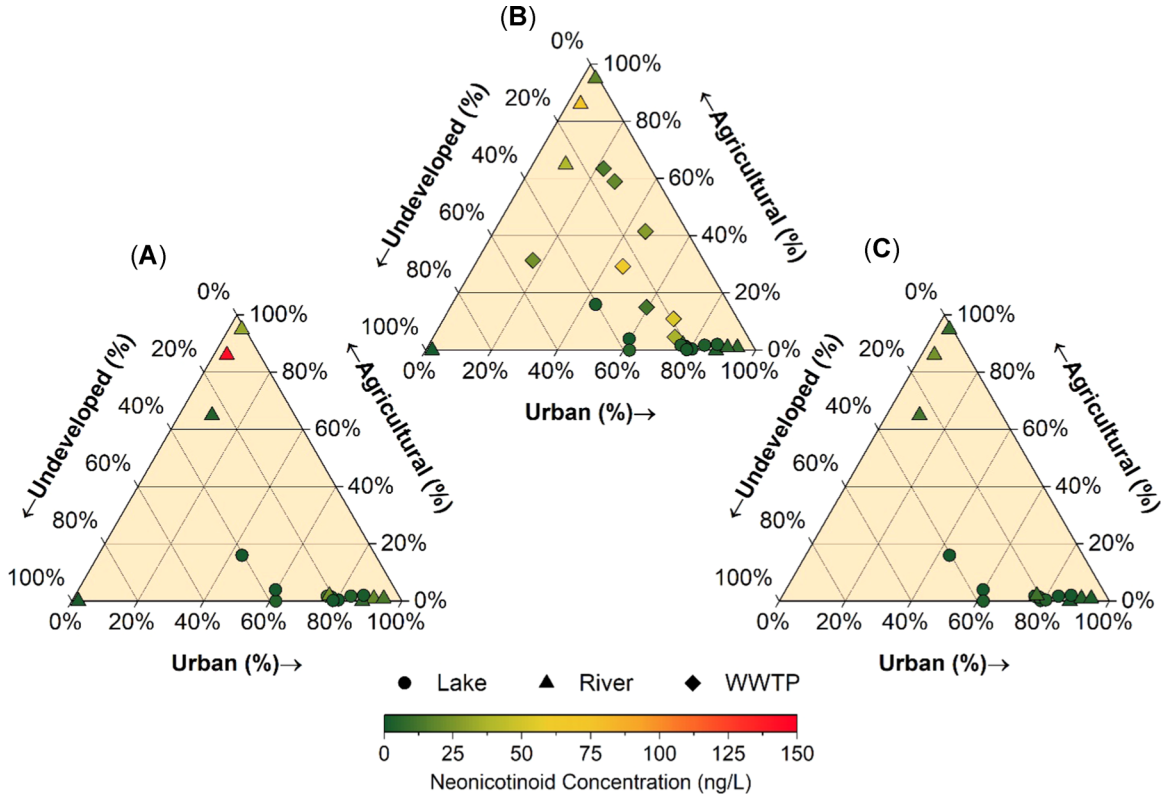

FIGURE 7: Neonicotinoid concentrations as a function of land use and seasonality. Measurements were made during the (**A**) early (May), (**B**) middle (July), and (**C**) late (August–September) 2019 growing season. Triangle vertices correspond to a 100% agricultural (top), undeveloped (bottom left), or urban (bottom right) land use. The color scale indicates the total neonicotinoid concentration in each sample. The type of water body is represented by the symbol shape. Wastewater-treatment plants are shown only for (**B**) because all samples were taken during the mid-growing season (20–24 June 2019). WWTP = wastewater-treatment plant.

exchange of approximately all of the total lake volume over a 24-h period. The rapid turnover prohibits well-developed stratification, which is observed in the relatively consistent neonicotinoid concentrations at the 3 depths (Figure 4, inset).

All lakes in the present study are located in Ramsey County, Minnesota, USA (area = 440 km^2, <44% urban; Figure 1; Supplemental Data, Figure S1); thus, it was difficult to determine the effects of watershed characteristics on neonicotinoid occurrence. Nevertheless, many of the important factors influencing neonicotinoid occurrence in surface and groundwater (e.g., discharge, land use, drainage area) described in the present study and elsewhere (Hladik et al. 2014; Hladik and Kolpin 2015) were also true for lakes. Imidacloprid was detected most frequently and at higher concentrations than the other compounds (72% detection, median = 1.2 ng/L). These results are still significant because as of 2016 no

TABLE 3: Summary statistics of neonicotinoid concentrations by predominant land use for the 65 river, stream, lake, and WWTP sites and for each hydrologic compartment

	n	Median number of neonicotinoids detected	Median total concentration (ng/L)	Maximum total concentration (ng/L; site)	Maximum individual concentration (ng/L; compound)
Land use					
Agricultural	48	3	12	140; Zumbro River	92; Clothianidin[a]
Urban	92	2	2.9	61; Blue Lake WWTP	48; Imidacloprid[b]
Undeveloped	17	2	1.9	3.5; Kawishiwi River	1.8; Clothianidin[c]
Compartment					
Rivers and streams	61	3	11	140; Zumbro River	92; Clothianidin[a]
Lakes	53	1	2.4	12; Snail Lake	9.6; Clothianidin[d]
Wells	35	1	2.2	15; Well ID 140190	15; Clothianidin[c]
WWTPs	8	3	26	61; Blue Lake WWTP	48; Imidacloprid[b]

[a]Zumbro River (May).
[b]Blue Lake WWTP.
[c]Cass County Well ID 497142.
[d]Snail Lake (July).
WWTP = wastewater-treatment plant.

neonicotinoids had been detected in any of Minnesota's lakes (Minnesota Department of Agriculture 2016).

Median neonicotinoid concentrations in groundwater (Figure 5 and Table 3) were less than the other hydrologic compartments (Figures 2–4 and 6 and Table 3). Hydrogeologic variables, such as groundwater recharge rates, water flow paths, and groundwater age, especially in southeastern Minnesota (Barry et al. 2020), influence the movement and occurrence of neonicotinoids. For example, despite the abundance of agriculture in Dodge and Olmstead Counties, much of the shallow subsurface in the region contains highly degraded karst features, including sinkholes, and allows rapid infiltration to groundwater (Steenberg 2019). Water, and accompanying neonicotinoids can therefore quickly exchange with the shallow system. A hydrogeologic survey of the region conducted by Barry et al. (2020) showed many highly fractured aquifer materials with groundwater flow rates of 1.6 to 4.8 km/d. The well with the highest total neonicotinoid concentration (817784; Figure 3) was located in this shallow subsurface (depth to bedrock 2.1 m; Supplemental Data, Table S4). The water in deeper wells, which were completed below a confining layer, had much lower or no neonicotinoids present. The depths of each well and of the confining bedrock layer are provided in Supplemental Data, Table S4. The wells in Cass, Hubbard, and Hennepin Counties are completed in glacial till aquifers. These wells have relatively consistent, low levels of multiple neonicotinoids present. It is not known why one well in Hennepin County had a substantially higher total neonicotinoid concentration (140190; Figure 3), but it is possibly due to a combination of locally high use, sandy soils, and poor well construction.

Because the wells in the present study were groundwater-monitoring wells and not drinking-water wells, the measured concentrations likely do not pose a significant human health risk. Neonicotinoids have been detected, however, in private wells used for potable water (Huseth and Groves 2014; Bradford et al. 2018) and in finished drinking water sourced from surface water (Klarich et al. 2017; Klarich Wong et al. 2019). The present study does not rule out the possibility that neonicotinoids in groundwater can pose a health risk, especially in regions which rely heavily on groundwater for irrigation and potable water use.

Temporal patterns in neonicotinoid concentrations

The temporal analysis of neonicotinoids in rivers and streams showed that the occurrence of neonicotinoids is influenced by their period of use before and during the growing season (Figures 2 and 3). The elevated early-season (May) concentrations, at most sites, both urban and agricultural, were attributed to runoff from spring storms following their application, which could have mobilized both the current year's neonicotinoids and those remaining from previous seasons (Thurman et al. 2002; Hladik et al. 2014, 2018a). In agricultural watersheds, the subsequent decreases in concentrations reflect the fact that most neonicotinoids are administered as seed coatings and applied during planting. Some fraction of compounds not incorporated into the target crop are removed from the surrounding landscape. In urban environments, neonicotinoids are more often applied toward the middle of summer when domestic and commercial uses increase (e.g., home pest control, foliar garden sprays, pet medications).

The exception to these seasonal trends in rivers and streams was at the Minnesota River near Judson, which had a lower total neonicotinoid concentration in May (3.9 ng/L), followed by a higher concentration in July (39 ng/L). The low concentration was likely caused by delayed planting in response to an abnormally wet spring in southwestern Minnesota during 2019 (National Oceanic and Atmospheric Administration 2020). In addition, the sample collection in July was preceded by a rain event (~2 cm), which caused increased streamflow (National Oceanic and Atmospheric Administration 2020). Rain events are directly linked to elevated runoff and streamflow, which promotes neonicotinoid mobilization. In the present study, positive relationships between stream streamflow and median total neonicotinoid concentrations were observed in rivers and streams ($\rho = 0.444$, $p < 0.001$; Supplemental Data, Figure S11).

The number of neonicotinoids detected in each sample did not change (by Spearman rank correlations; Supplemental Data, Figure S12) throughout the season. There were also no substantial changes in the relative ratios of individual neonicotinoids. For example, the ratio of imidacloprid to clothianidin in agricultural and urban watersheds over the entire growing season were (mean ± SD) 0.35 ± 0.54 and 4.1 ± 4.9, respectively.

As observed in rivers and streams (Figures 2 and 3), neonicotinoid concentrations in lakes generally decreased throughout the season (Figure 4) but to a lesser extent. This was attributed to the typical application timeline of pesticides in different watersheds. For example, the larger decrease of neonicotinoid concentrations in rivers and streams indicates the influence of agriculture, in which neonicotinoids are primarily applied as seed coatings only during planting. The more consistent neonicotinoid concentrations in lakes, which were all in urbanized watersheds, likely occurred from use on lawns, gardens, and trees applied throughout the growing season. As a result, urban watersheds are more likely to experience low-level chronic neonicotinoid exposure throughout the growing season, whereas agricultural watersheds are more likely to experience higher concentration spikes at the start of the growing season, followed by steady declines.

Potential toxicity and ecosystem impacts

The aquatic-life benchmarks for neonicotinoid exposure to freshwater invertebrates (e.g., midges, scuds, daphnids) range from 10 to >20 000 000 ng/L (chronic) and from 385 to >114 500 000 ng/L (acute; US Environmental Protection Agency 2020b). No neonicotinoids in the present study were detected above their lowest acute benchmark, and only 10% of all samples contained one or more compound that exceeded the

minimum chronic benchmark (Supplemental Data, Table S10). Surface water sampling conducted by the Minnesota Department of Agriculture in 2014 found no measurements that exceeded any benchmark value in any sample (Minnesota Department of Agriculture 2016). Although these data suggest that the neonicotinoid concentrations in the present study will likely not cause acute toxicity to aquatic life, previous research has shown that even if neonicotinoid concentrations do not exceed benchmark values, the health of aquatic communities could still be negatively affected (e.g., paralysis, impaired mobility, reduced fertility; Gibbons et al. 2015; Schepker et al. 2020). This highlights the importance of the potential sublethal chronic effects that contribute to aquatic health metrics (Morrissey et al. 2015; Cavallaro et al. 2018).

The acute risks posed by (co)occurring neonicotinoids were further evaluated by calculating the PTI (Nowell et al. 2014) of all samples (Supplemental Data, Table S11). Currently available aquatic-life benchmarks (University of Hertfordshire 2020; US Environmental Protection Agency 2020a, 2020b) indicate that toxicity thresholds for neonicotinoids to most aquatic taxa are exceeded when PTIs are greater than approximately 0.4 (Supplemental Data, Figure S13). Median PTIs in the present study ranged from 10^{-9} to 10^{-5}; individual taxon-specific PTIs decreased in the order of *Daphnia* ($10^{-5.9}$), crustaceans ($10^{-6.0}$), mollusks ($10^{-6.2}$), fishes ($10^{-7.9}$), and macrophytes ($10^{-8.8}$). This suggests that there was a low acute risk to aquatic life from all observed concentrations of neonicotinoid mixtures.

There was a strong influence of watershed characteristics on PTIs in all types of water bodies (Supplemental Data, Table S11). Significant positive correlations were found between agricultural land use and all taxon-specific PTIs. Except for crustaceans, positive correlations were observed for all PTIs with drainage area (Supplemental Data, Table S12). Despite lower concentrations in smaller watersheds, however, a meta-analysis of insecticide concentrations in US surface waters ($n = 4391$; Wolfram et al. 2019) concluded that the risk posed to aquatic organisms by insecticides was disproportionally greater in small watersheds. This was true even when organisms in smaller watersheds were exposed to equal or lesser concentrations than in larger watersheds (Schulz 2004). Several zooplankton and phytoplankton taxa were enumerated in 7 of the lakes from April to November (Supplemental Data, Figures S7 and S8). These data showed no observable linear relationships or significant Spearman rank correlations between total neonicotinoid concentrations and plankton concentrations.

To evaluate the combined environmental and biological factors that influence plankton abundance, a multivariate statistical analysis described by Pereira et al. (2018) was used to more accurately determine the potential responses of planktonic taxa to neonicotinoids beyond simple linear relationships. Any meaningful interpretations of these data, however, could not be made because of the limited spatiotemporal resolution of the samples containing synchronous measurements of plankton population sizes and neonicotinoid concentrations (i.e., 3 discrete sampling events for 10 lakes in Ramsey County during 1 yr). Therefore, the results of the present study were limited to qualitative observations.

Only the parent neonicotinoids (imidacloprid, clothianidin, acetamiprid, thiamethoxam, thiacloprid) were considered in the calculations of PTI because toxicity benchmarks have not been established for many of the transformation products, and there was limited detection of transformation products. It is possible that the transformation products were degraded quickly relative to the parent compounds or that transformation products were present that were not included in the LC-MS/MS analytical method. Additional research is needed to address the knowledge gap in the concentrations and fate of neonicotinoid transformation products (Pietrzak et al. 2020; Thompson et al. 2020). The benchmarks that do exist for the transformation products provide conflicting evidence for their relative toxicities. Some studies (Suchail et al. 2004; Casida 2011) suggest that neonicotinoid transformation products possess toxicities equal to or greater than the parent compounds, whereas others indicate that toxicities are several orders of magnitude lower (Morrissey et al. 2015; US Environmental Protection Agency 2020b).

Despite these uncertainties, the common mode of action for all neonicotinoids (Matsuda et al. 2001) suggests that the transformation products could cause harm to nontarget organisms. It is also unclear how co-occurring neonicotinoids impact aquatic organisms. For example, neonicotinoid mixtures have been shown to cause interactions, but the effects have been both synergistic and antagonistic (Warne and Hawker 1995; Deneer 2000; Maloney et al. 2017, 2018). Because neonicotinoid-based products often contain mixtures of neonicotinoids or other pesticides (Hladik et al. 2014; Main et al. 2014; Sánchez-Bayo and Hyne 2014), future research should address this gap in knowledge.

Sources to WWTPs

Because the primary source of water to the WWTPs in the present study was through municipal wastewater-collection networks, it was expected that any surface and groundwater contributions of neonicotinoids would be minimal. The WWTPs had among the highest total (13–61 ng/L) and individual (e.g., imidacloprid = 12–48 ng/L) neonicotinoid concentrations. This is consistent with reports of elevated neonicotinoid concentrations at other WWTPs. In a study of 13 US WWTPs, year-round detections of imidacloprid occurred at every facility, with mean influent and effluent concentrations of 60.5 and 58.5 ng/L, respectively. This equates to an overall approximately 3% removal of imidacloprid during the WWTP treatment process (Sadaria et al. 2016). Poor removal has also been observed at WWTPs in the United States, China, and Europe (Heeb et al. 2012; Hope et al. 2012; Campo et al. 2013; Masia et al. 2013; Qi et al. 2015). It is estimated that 1000 to 3400 kg of imidacloprid are released from WWTPs in the United States each year (Sadaria et al. 2016). In the present study, total neonicotinoid loads from rivers and streams (median = 4.1 g/d) were greater than those from WWTPs (0.70 g/d). This does indicate, however, that discharge from WWTPs constitutes a significant portion of the neonicotinoid load in rivers and streams.

The type of treatment used at a WWTP can also influence the transformation of neonicotinoids. The transformation product acetamiprid-*N*-desmethyl was detected at all WWTPs except East Bethel (Figure 6A). East Bethel is the only WWTP that employs advanced membrane filtration coupled to ultraviolet irradiation for disinfection before discharging the effluent to an underlying aquifer. All of the other WWTPs disinfect by chlorination/dechlorination or ultraviolet irradiation and discharge the effluents to surface water. The stricter disinfection process at East Bethel is used because of the different environment receiving WWTP discharge. Because many homes rely on aquifers for home water demand, more stringent effluent regulations are required to ensure that contaminant limits are met. Similar ranges of acetamiprid-*N*-desmethyl concentrations in WWTP effluents to those in the present study have been reported elsewhere (Sadaria et al. 2016). Because this compound was not detected in the influent, the authors speculated that it was formed during the treatment process.

Because imidacloprid is the main neonicotinoid used for nonagricultural purposes (Jeschke and Nauen 2005), it was not surprising that it was the most frequently detected in the largely urban WWTP effluents (Figure 6; median = 19 ng/L, maximum = 48 ng/L). It is more difficult to explain the consistent occurrence of clothianidin (median = 1.8 ng/L, maximum = 32 ng/L). The concentration of clothianidin strongly correlates with the sewershed area ($\rho = 0.960$, $p < 0.001$; Supplemental Data, Figure S9E). The larger the sewershed, the more likely it is that the sewer system receives inputs from agricultural land. For example, despite primarily urban land use surrounding the Blue Lake WWTP, the sewershed still contains approximately 30% agricultural land.

In the Minneapolis–St. Paul Metropolitan Area, the sewershed areas are positively correlated with the population served by the WWTP ($\rho = 0.960$, $p < 0.001$). The instantaneous neonicotinoid loads and size of population served were used to determine per capita neonicotinoid yields at each WWTP (Figure 6B). The Metropolitan and East Bethel WWTPs had the largest and smallest neonicotinoid loads and serve the largest and smallest populations, respectively. There is a significant correlation between neonicotinoid loads and population size ($\rho = 0.995$, $p < 0.001$; Supplemental Data, Figure S9B). This is reflected in the per capita yields (Figure 6B). There are currently no guidelines to regulate the discharge of neonicotinoids from WWTPs, but the measured concentrations were still substantially below the acute aquatic-life benchmarks for neonicotinoids (385–>114 500 000 ng/L; University of Hertfordshire 2020; US Environmental Protection Agency 2020a, 2020b). Chronic effects, however, could be possible when comparing the detected concentrations in wastewater to the relevant benchmarks (Supplemental Data, Table S10), especially in effluent-dominated systems.

Several nonagricultural sources introduce neonicotinoids to WWTPs including residues on foods and pets treated with neonicotinoid-containing medications (Chen et al. 2014; Lu et al. 2018). In a survey of 2 US congressional cafeterias in Washington, DC, neonicotinoid residues were detected on 100% of the fruit and vegetable samples and, in some cases, exceeded safe consumption levels (Supplemental Data, Table S13; Lu et al. 2018). A connected study of Chinese markets detected at least one neonicotinoid in 91% of all samples at similar concentrations to those found in the United States (Lu et al. 2018). Tomatoes, apples, and melons contained the highest neonicotinoid concentrations. For all tested produce, total neonicotinoid concentrations ranged from 0.01 to 100 µg/kg. Pets that have been administered flea and tick–treatment products containing neonicotinoids also release neonicotinoids to wastewater-collection systems. Craig et al. (2005) showed that when a commercial flea-control medication was given to dogs at the recommended dose, neonicotinoids were detected on gloves worn by individuals who came into contact with the dogs for up to 1 mo after application at residual concentrations near 100 µg/kg of glove.

CONCLUSIONS

The neonicotinoid concentrations observed in surface and groundwater in Minnesota were similar to those measured in other studies in the midwestern United States. Neonicotinoids were detected the most frequently and at the highest concentrations at agricultural locations, followed by urban and undeveloped regions. Clothianidin was closely associated with agricultural land cover, whereas imidacloprid was more closely associated with urban land use. The type of water body is an important driver of neonicotinoid occurrence. There were consistently high concentrations in WWTP effluents and significantly lower concentrations in the other hydrologic compartments. Low neonicotinoid concentrations in wells in agricultural regions suggest that their occurrence is likely influenced by one or many hydrogeologic variables. Total neonicotinoid concentrations were correlated to drainage area and streamflow. Neonicotinoid concentrations were typically highest in the spring and early summer following agricultural and urban use and during elevated streamflow conditions following rain events. Because sampling was conducted during only one growing season, these data do not capture the annual variability in environmental conditions such as changing weather patterns, pesticide regulations, and application rates. Further research is needed to improve the spatiotemporal resolution of these results. No samples exceeded acute aquatic-life benchmarks of mortality or immobility, whereas 10% of samples exceeded a chronic benchmark for neonicotinoid mixtures. This suggests that direct toxic effects to nontarget organisms are not expected, but chronic effects warrant further evaluation. Neonicotinoid occurrence in aquatic systems is expected to continue because of their wide-scale use and their relative persistence and high mobility in the environment. Further research and monitoring of neonicotinoid insecticides in water bodies in Minnesota and beyond are therefore needed to better understand their fate and transport in the environment and predict any potential adverse effects of their continued use.

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This article has earned an Open Data/Materials badge for making publicly available the digitally shareable data necessary to reproduce the reported results. The data are available at http://hdl.handle.net/11299/214867. Learn more about the Open Practices badges from the Center for Open Science (https://osf.io/tvyxz/wiki).

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