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Municipal Wastewater as a Year-Round Point Source of Neonicotinoid Insecticides that Persist in an Effluent-Dominated Stream

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ABSTRACT

Neonicotinoids in aquatic systems have been predominantly associated with agriculture, but some are increasingly being linked to municipal wastewater. Thus, the aim of this work was to understand the municipal wastewater contribution to neonicotinoids in a representative, characterized effluent-dominated temperate-region stream. Our approach was to quantify the spatiotemporal concentrations of imidacloprid, clothianidin, thiamethoxam, and transformation product imidacloprid urea: 0.1 km upstream, the municipal wastewater effluent, and 0.1 and 5.1 km downstream from the wastewater outfall (collected twice-monthly for one year under baseflow conditions). Quantified results demonstrated that wastewater effluent was a point-source of imidacloprid (consistently) and clothianidin (episodically), where chronic invertebrate exposure benchmarks were exceeded for imidacloprid (36/52 samples; 3/52 >acute exposure benchmark) and clothianidin (8/52 samples). Neonicotinoids persisted downstream where mass loads were not significantly different than those in the effluent. The combined analysis of neonicotinoid effluent concentrations, instream seasonality, and registered uses in Iowa all indicate imidacloprid, and seasonally clothianidin, were driven by wastewater effluent, whereas thiamethoxam and imidacloprid urea were primarily from upstream non-point sources (or potential in-stream transformation for imidacloprid urea). This is the first study to quantify neonicotinoid persistence in an effluent-dominated stream throughout the year—implicating wastewater effluent as a point-source for imidacloprid (year-round) and clothianidin (seasonal). These findings suggest possible overlooked neonicotinoid indoor human exposure routes with subsequent implications for instream ecotoxicological exposure.

Environmental Significance Statement

Neonicotinoids are the most widely-used insecticides in the world, yet little is known regarding their mass loads in municipal wastewater effluent throughout the year or their contributions to ecological exposure conditions in effluent dominated streams, particularly in temperate regions. Collecting twice-monthly samples at an effluent dominated stream in Iowa for 1 year, we discovered municipal wastewater effluent is a significant year-round point source of neonicotinoids—particularly imidacloprid and clothianidin. Frequent concentrations exceeded chronic benchmarks for invertebrates, and some episodic concentrations exceeded acute levels. Neonicotinoids from the wastewater treatment plant led to persistent ecotoxicological concentrations of concern 5 km downstream of the wastewater outfall. The neonicotinoid mass loads observed in the wastewater effluent also suggests that indoor neonicotinoid use is underappreciated.

INTRODUCTION

Neonicotinoids are the most-widely used insecticides in the world, with applications in agriculture, forestry, gardening, indoor/outdoor pest control, and pet treatments.[1–5] Due to their extensive use and hydrophilic nature (e.g., $\log K_{ow}$: -0.13–0.7),[6] the three most common neonicotinoids (imidacloprid, clothianidin, and thiamethoxam) have been detected in surface and groundwaters across the U.S., especially Midwestern streams, ranging between <1 ng/L to ~100 µg/L.[1,3,4,7–15] Additionally, neonicotinoids have been reported in surface waters internationally.[2,5,13,14,16–22] As neurotoxins, the prevalence of neonicotinoids can adversely impact aquatic and terrestrial ecosystems (e.g., insects, birds, fish).[1–5,13,14,23] Additionally, detection of

neonicotinoid transformation products in natural and engineered systems are of concern due to implications for human health.[5,12,24–27]

The presence of neonicotinoids in aquatic systems has been predominantly associated with agricultural activities[9,28] but are increasingly linked to urban sources (particularly imidacloprid), including stormwater runoff and wastewater effluent.[9,15,29,30] Studies examining wastewater as a source of neonicotinoids to receiving waters are limited and primarily focus on removal within a wastewater treatment plant (WWTP)[10,31–33] or assessing spatiotemporal trends along an effluent-impacted stream on a limited number of dates (e.g., two sampling dates, or proximal to a WWTP).[15,34] Imidacloprid, clothianidin, and thiamethoxam have each been detected in raw and treated wastewater, exhibiting no significant removal.[10,31,33] In treated wastewater effluent, imidacloprid has been reported between 20–387 ng/L, with presumed sources ascribed to pet treatments.[10,31,34,35] Clothianidin and thiamethoxam have been detected in treated wastewater at lower concentrations than imidacloprid (≤347 ng/L clothianidin and ≤~15.0 ng/L thiamethoxam).[31,32,35] None of these studies have quantified the spatiotemporal contributions of neonicotinoids from a WWTP to a stream reach over an extended time period, or analyzed wastewater for imidacloprid transformation products with known altered toxicological effects (e.g., imidacloprid urea).[5] Where municipal WWTPs operate on separated collection systems (i.e., not combined-sewers with stormwater influence), detection of neonicotinoids in wastewater also implicates extensive neonicotinoid use in and around homes/businesses.

Neonicotinoid inputs from wastewater are of increasing concern as WWTP effluent becomes a larger proportion of flows in receiving-waters.[36–39] Growing demand for freshwater has increased the prevalence of treated wastewater in environmental waters across the U.S.[36–42] Treated wastewater can significantly impact downstream water quality, particularly in effluent-

dominated streams where aquatic biota are chronically exposed to elevated concentrations of contaminants.[40–49] There is a critical knowledge gap regarding the contribution and persistence of neonicotinoids and transformation products from wastewater to effluent-dominated streams, spatiotemporal dynamics, and biotic exposure conditions. We hypothesized that wastewater could be a significant point source of neonicotinoids to an effluent dominated stream. Herein, we (1) quantified the prevalence of the three most environmentally prevalent neonicotinoids[5,9,15,28] (imidacloprid, clothianidin, thiamethoxam) and the photolysis/biotransformation transformation product imidacloprid urea (an environmentally stable, pharmacophore-altered transformation product)[5,12] in treated municipal wastewater and along the effluent-dominated receiving stream, (2) determined spatiotemporal trends in neonicotinoid concentration and mass loads to assess the impact of wastewater effluent on exposure conditions for instream aquatic biota, and (3) examined possible sources of neonicotinoids to the WWTP through analysis of registered uses of neonicotinoids.

METHODS

Study Site. Muddy Creek is an effluent-dominated stream in North Liberty, Iowa (USA), receiving treated wastewater from the North Liberty wastewater treatment plant (WWTP) and agricultural/stormwater runoff (**Figure S.1-S.2**). North Liberty is a rapidly growing community in east-central Iowa that operates a separated sewerage collection system (i.e., stormwater and wastewater not mixed). Muddy Creek was previously determined to be representative of an effluent-dominated stream research site, where effluent contributed 55–97% (median 91%) to streamflow during baseflow conditions.[49] Details regarding land use, the North Liberty WWTP, and effluent/streamflow conditions are provided in the SI (**Figures S.3–S.5**; **Table S.1, S.7**) and our prior study where we assessed stream conditions and spatiotemporal dynamics of

pharmaceuticals.[49] Four previously established sampling sites were chosen to investigate the impacts of wastewater effluent on Muddy Creek neonicotinoid concentrations: (1) 0.1 km upstream of the North Liberty WWTP (US1; USGS Station ID 05454050), (2) the North Liberty WWTP effluent/outfall (effluent; USGS Station ID 05454051), (3) 0.1 km downstream from the North Liberty WWTP outfall (DS1; USGS Station ID 05454051), and (4) 5.1 km downstream from the NL-WWTP outfall (DS2; USGS Station ID 05454090).

Sample Collection and Processing. Samples (1L) were collected in acid-washed, amber glass bottles with minimal headspace using the vertical centroid-of-flow method (described in Section 4.1.3A of the USGS National Field Manual for the Collection of Water-Quality Data)[50] roughly twice-monthly for one year (8/24/2018–8/29/2019, 18 dates) during baseflow conditions, in the same manner of our prior work at this stream studying pharmaceuticals.[49,51] This approach is commonly used in small, low-flow streams and in sampling wastewater effluent discharge and was shown to be a valid approach for this well-mixed stream (details in SI).[48–52] North Liberty WWTP effluent was collected at the point of discharge from the outfall pipe.[49] Baseflow conditions (**Figure S.3–S.4**) were targeted to characterize the impacts of wastewater-derived flow, rather than runoff conditions, and to aid in examining spatiotemporal trends in neonicotinoid concentrations by holding streamflow relatively constant. Samples were filtered, extracted, concentrated by solid phase extraction (SPE) with spiked isotopically-labeled imidacloprid-d_4 as a surrogate and analyzed for imidacloprid, clothianidin, thiamethoxam, and imidacloprid urea (see SI for details) using the methods we previously published.[12,53] Imidacloprid urea, rather than the mammalian toxic transformation product desnitro-imidacloprid, was chosen for analysis because it is more environmentally stable than desnitro-imidacloprid[5,12] and was present at higher concentrations than desnitro-imidacloprid (based on preliminary analyses and measurements at a nearby surface

water).[12] Stream bulk water quality parameters (e.g., pH, water temperature, specific conductivity, dissolved oxygen) were measured with a HACH HQ40D portable multimeter and are provided in the SI (**Table S.8**).

Analytical Methods. All samples were analyzed by LC-MS/MS (Agilent 1260 Infinity liquid chromatograph and Agilent 6460 triple quadrupole mass spectrometer) and quantified in positive ionization multiple reaction monitoring mode (MRM) using our previously established methods.[11,12,53,54] Neonicotinoids were separated on an Agilent Zorbax eclipse plus C18 column (4.6 mm x 150 mm x 5 μm) with a Zorbax eclipse plus C18 guard column (4.6 mm x 12.5 mm x 5 μm). An injection volume of 20 μL was loaded onto the column preheated to 50 °C. The mobile phases contained 0.1% formic acid in (A) water (77.5%) and (B) acetonitrile (22.5%) with a flow rate of 0.8 mL min^{-1}. MS/MS operating settings are outlined in **Table S.4**. Two MRM transitions were monitored, a quantitative transition (for sample quantification) and a qualitative transition (for compound verification) are provided in **Table S.5** along with compound specific retention times and MRM settings. Peak analysis was conducted using Agilent MassHunter Qualitative Analysis software (version B.06.00). A five-point isotope-normalized (deuterated imidacloprid) external calibration curve was used to account for surrogate recovery and differential ionization during quantification and was linear throughout range. SPE lower limits of detection (LLD) were previously reported as follows: imidacloprid (0.428 ng/L), clothianidin (0.488 ng/L), thiamethoxam (0.081 ng/L), and imidacloprid urea (0.057 ng/L).[12,53] Additional details regarding chemicals, SPE, LLD, and mass spectrometry are provided in the SI (**Tables S.3–S.6**) and/or previously published works.[11,12,53,54]

Quality Assurance/Control and Data Analysis. QA/QC approaches (including method and field blanks) were previously reported.[12] Detected neonicotinoid concentrations spanned four orders of

magnitude, and followed a log-normal distribution (Shapiro-Wilks normality test, α=0.05) thus allowing parametric statistical analysis (e.g., t-tests). Spearman's rho correlation analyses were conducted at the 95% confidence level. Samples where neonicotinoid concentrations were <LLD were treated as ½LLD for statistical analyses (e.g., ratio matched-pairs t-tests), a valid approach when <LLD samples (i.e., left-censored results) comprise a small fraction of the data set.[55,56] All statistical analyses were conducted using Graphpad Prism 8 software via matched-pairs as appropriate, at the 95% confidence level.

RESULTS AND DISCUSSION

Wastewater effluent-derived neonicotinoids generate persistent instream exposure conditions of ecological concern. Municipal wastewater effluent was a significant, year-round point source of imidacloprid, which persisted through the 5.1 km study-reach. Although imidacloprid was present in all samples (from all sites), effluent concentrations were up to 240-fold higher than in the upstream (US1, p<0.0001). US1 imidacloprid concentrations (0.62–43.8 ng/L, **Figure 1, Table S.10** data separated by site, date) were consistent with those previously reported in agricultural and stormwater impacted surface waters of the United States (<2–42.7 ng/L),[11,15,28,29] while effluent concentrations (4.98–850 ng/L) were consistent with those previously reported in WWTP effluent (~20–387 ng/L)[10,31,33] as well as surface waters in China.[17–19] Imidacloprid attenuation occurred downstream (effluent to DS1 [p=0.0041], DS1 to DS2 [p=0.0132]; **Figure S.7, Table S.14**) where, due to effluent contributions, concentrations at DS1 and DS2 remained significantly greater than US1 (US1 vs. DS1: p=0.0005; US1 vs DS2: p=0.0096; **Table S.14**). Based on our previously study, we know that for Muddy Creek, stream specific conductivity is directly correlated with the wastewater effluent.[49,51] Here, imidacloprid concentrations were significantly correlated with stream specific conductance (Spearman

rho=0.518, p=0.001; **Figure S.12**), further demonstrating the significant contribution of wastewater effluent to downstream imidacloprid concentrations.[49–51] Nevertheless, imidacloprid concentrations downstream of the WWTP outfall may also be impacted by non-point sources (e.g., stormwater) within the 5 km stretch between the WWTP outfall and DS2.[49–51] The US EPA aquatic life benchmark (ALB, 30-day average exposure concentration) for chronic invertebrate exposure to imidacloprid[57] (10 ng/L) was exceeded in 22% (4/18) of US1 samples, 82% (14/17) of effluent, 76% (13/17) of DS1, and 39% (7/18) of DS2 samples. The acute invertebrate exposure ALB (385 ng/L) was exceeded twice in the effluent and DS1 (both on 7/8/2019, 8/29/2019) and once at DS2 (on 7/8/2019); on 7/8/2019, the acute ALB for imidacloprid was exceeded across the study-reach from wastewater outfall and downstream to DS2. Year-round ALB exceedances for imidacloprid in WWTP effluent and downstream of the WWTP outfall suggests exposure concerns for aquatic invertebrates and local foodwebs.[2,8,58]

The transformation product imidacloprid urea was detected in 94% of samples in this study (**Figure 1**) with concentrations significantly correlated with those of imidacloprid (all sites/dates, Spearman rho=0.362, p=0.0003; **Figure S.11**). In contrast to imidacloprid, however, imidacloprid urea concentrations were significantly higher (p=0.0117) in US1 (detected in 17/18 samples; 0.34–7.97 ng/L) compared to the effluent (detected in 14/17 samples; 0.18–1.78 ng/L, **Table S.11** data separated by site, date). Although the WWTP was not a significant contributor to instream imidacloprid urea concentrations, concentrations downstream of the WWTP outfall became progressively higher than those in the effluent (1.6-fold at DS1, p=0.0690 and 1.8-fold at DS2, p=0.0337, **Figure S.7, Table S.14**), suggesting possible instream formation of

imidacloprid urea and/or mixing with non-point sources.[5,29] Imidacloprid urea concentrations detected in Muddy Creek were similar to those reported in the nearby Iowa River from our prior work (0.1–0.66 ng/L)[12] and, to our knowledge, this is the first documentation of imidacloprid urea in wastewater effluent.

Episodic spikes in clothianidin concentration in the effluent suggests the WWTP as a point-source intermittently drove clothianidin concentrations in Muddy creek (**Figure 1**). Clothianidin was detected in 100% samples with US1 concentrations between 3.46–59.1 ng/L (**Table S.12;** data separated by site, date), consistent with those in agricultural and stormwater impacted local Midwestern and United States surface waters (7.82–257 ng/L).[8,11,15,28,59] Effluent clothianidin concentrations spanned 7.72–134 ng/L, similar to those previously reported in treated wastewater (<LLD–131 ng/L).[31] US1 clothianidin concentrations were also similar to surface water levels reported in China.[17–19] Although effluent clothianidin concentrations at times exceeded those at US1 (effluent>US1 9/17 sampling dates, up to 5.9-fold greater; **Table S.16**), concentrations were not significantly different between any site ($p>0.05$, **Table S.14**). Elevated concentrations of clothianidin in the effluent yielded ALB exceedances for chronic invertebrate exposure (ALB=50 ng/L clothianidin[57]) in 18% (3/17) of the effluent and DS1 samples (12/21/2019, 2/22/2019, 3/8/2019) and 11% (2/18) of DS2 samples (12/21/2019, 2/22/2019), compared to only one exceedance in US1 (3/8/2019).

Thiamethoxam was the least-frequently detected neonicotinoid (87%), with concentrations seemingly driven by non-point sources (**Figure 1**). Concentrations of thiamethoxam were correlated with clothianidin across all sampling sites/dates (Spearman rho=0.724, $p<0.0001$, **Figure S.11**), consistent with previous studies where co-occurrence was due to similar applications in agriculture and/or because clothianidin is a transformation product of thiamethoxam.[5,9,15,28]

Thiamethoxam was detected more frequently US1 (15/18 samples, 0.12–16.4 ng/L) than in the effluent (13/17 samples, 0.56–14.1 ng/L) (**Table S.13,** data separated by site, date). Thiamethoxam concentrations herein were within the range of those previously reported in Iowa surface waters (<2–190 ng/L)[11,15,28] and in treated wastewater effluent (<24 ng/L).[31,32] Thiamethoxam concentrations at Muddy Creek were lower in concentration and detection frequency than reported in surface waters in China.[17–19] Thiamethoxam concentrations were not significantly different ($p>0.05$, **Table S.14**) between sites, suggesting the WWTP effluent did not drive instream thiamethoxam concentrations. No samples exceeded the US EPA chronic ALB for invertebrate thiamethoxam exposure (ALB=740 ng/L).[57]

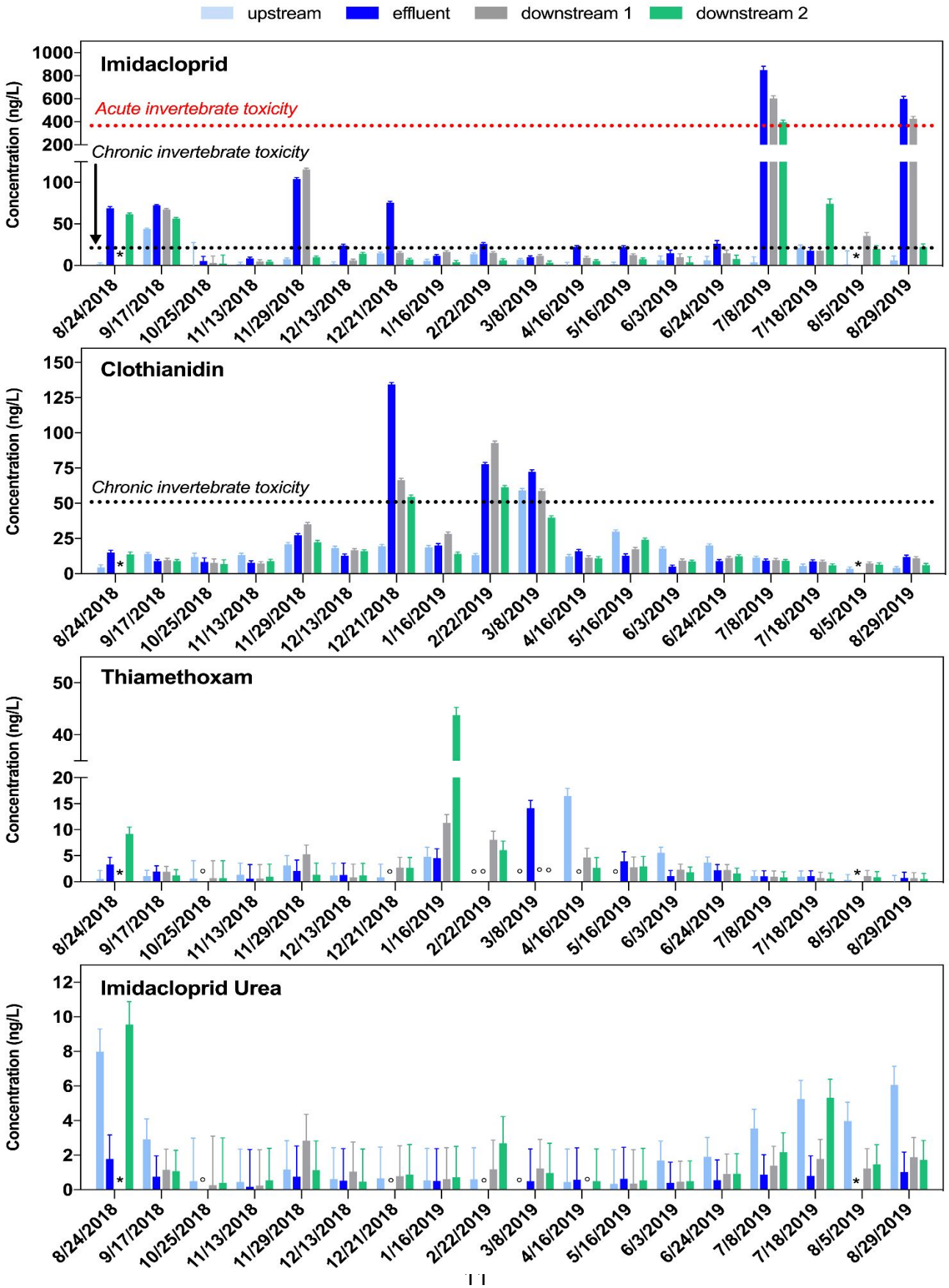
upstream
effluent
downstream 1
downstream 2
Imidacloprid
Acute invertebrate toxicity
Chronic invertebrate toxicity
Concentration (ng/L)
Clothianidin
Chronic invertebrate toxicity
Thiamethoxam
Imidacloprid Urea
8/24/2018
9/17/2018
10/25/2018
11/13/2018
11/29/2018
12/13/2018
12/21/2018
1/16/2019
2/22/2019
3/8/2019
4/16/2019
5/16/2019
6/3/2019
6/24/2019
7/8/2019
7/18/2019
8/5/2019
8/29/2019

Figure 1: Neonicotinoid concentrations (note different y-axis scales) throughout the sampling period (8/24/2018–8/29/2019) at each sampling location: upstream 1 (US1, light blue), wastewater treatment plant effluent (dark blue), downstream 1 (DS1, 0.1 km downstream of the effluent outfall; gray), and downstream 2 (DS2, 5.1 km downstream of the effluent outfall; green). Dotted black lines (---) indicate US EPA Aquatic Life Benchmarks for chronic invertebrate exposure (imidacloprid: 10 ng/L, clothianidin: 50 ng/L, thiamethoxam: 740 ng/L) and dotted red lines (---) indicate benchmarks for acute invertebrate exposure (imidacloprid: 385 ng/L). Note, such ALB values are not available for imidacloprid urea. Samples where a given neonicotinoid was not detected are indicated with a (°). The DS1 sample from 8/24/2018 and effluent sample from 8/5/2019 were not available for analysis and are indicated with a (*). Error bars represent the standard error associated with sample processing and analysis (i.e., composite enrichment, sample extraction, and analysis) using the same approach as our prior work.[11,12,53] Information regarding the east-central Iowa 2018 harvest and 2019 planting seasons of the corn and soybean are provided in the SI for reference.

Neonicotinoid mass loads persist instream. Although neonicotinoid concentrations are most important for assessing localized ecotoxicological exposure (*i.e.*, elevated concentrations impart toxic responses to aquatic biota), mass load analysis provides insight into neonicotinoid attenuation or flux downstream of the WWTP and the impacts of effluent on a watershed scale.[49] Neonicotinoid mass loads (calculated based on instantaneous grab samples extrapolated to a daily rate, see SI for details) from the WWTP effluent persisted downstream to DS2 (**Figure 2**). Imidacloprid mass loads were not significantly different between the outfall and DS2 ($p=0.6410$, **Table S.15-S.16**), indicating minimal mass load attenuation occurred within this 5.1 km stretch of the study reach. Interestingly, there was a significant increase in mass load at DS2 (compared to the WWTP outfall) for imidacloprid urea (2.8-fold, $p<0.0001$), clothianidin (2.2-fold, $p<0.0001$), and thiamethoxam (1.6-fold, $p=0.0182$; **Tables S.15-S.16**). These increases and relatively stable mass load of imidacloprid may reflect mixing of the effluent with the upstream flow and/or unmeasured non-point sources (e.g., stormwater). Additionally, instream transformation (e.g., biological, photolysis)[5] may contribute to the increased mass loads of imidacloprid urea and clothianidin (the latter of which is a known transformation product of thiamethoxam)[5,60] at DS2.

Muddy Creek contributions to neonicotinoid mass loads in the much-larger Iowa River are likely minor (estimated 4.40—3,380 mg/d at DS2 vs. 13,100—24,100 mg/d in the Iowa River[15]).

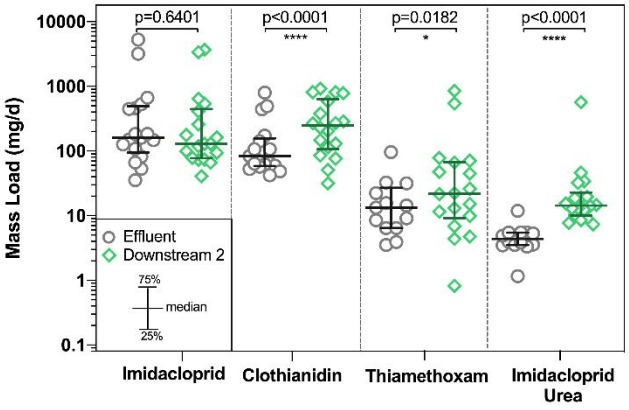

Figure 2: Calculated mass loads (mg/d) of each neonicotinoid in the effluent (gray circles) and at downstream 2 (DS2, green diamonds) for all sampling dates (8/24/2018–8/29/2019). Mass loads are determined from individual grab samples concentrations (representing an instantaneous measurement), the daily processed flow from the North Liberty WWTP and the flow rate at the DS2 gaging station (USGS) during time of sampling. The daily mass loads were calculated using flow rates and concentrations at each location for the day (assumed due to sampling under base flow conditions). A total of n=17 effluent and n=18 DS2 samples were used in statistical analysis. Of the mass loads used for statistics, thiamethoxam was not detected in n=4 effluent and n=1 DS2 samples, while imidacloprid urea was not detected in n=3 effluent samples. Where a neonicotinoid was not detected, the value of ½LLD was used in when calculating the mass load (omitted from figure). Data distribution (median and interquartile ranges) and p-values reflect all data. Note that imidacloprid urea is a transformation product of imidacloprid while clothianidin is sometimes a transformation product of thiamethoxam.

Seasonality in neonicotinoid concentrations. Neonicotinoid concentrations along the study-reach reveal seasonal trends in both non-point (upstream) and municipal (effluent) sources that impact downstream concentrations and mass loads (**Figure 3**).[9,28] Clothianidin concentrations were significantly higher during the cool-season (November-April, US1 water temperature ≤10°C) in the effluent (p=0.011), DS1 (p=0.0152), and DS2 (p=0.0085) compared to the warm-season[49] (May-October, US1 water temperature >10°C; **Figures S.8, S.10**). Elevated concentrations in effluent indicate there may be seasonal use of clothianidin within homes and/or businesses that result in down-the-drain transport (e.g., greenhouses); however, we cannot ascertain the direct cause of this phenomenon. Higher clothianidin concentration/mass loads downstream of the

WWTP in the cool season (**Figures S.8, S.9, 3B**) may be a combination of effluent derived, as well as groundwater leaching via subsurface transport and/or residue runoff from agricultural fields following fall harvest (**Figure S.6**).[61,62] Imidacloprid urea concentrations in US1 and effluent were higher in the warm season (**Figure S.8**), but mass loads in the effluent or DS2 exhibited no clear seasonality (**Figure 3, Figure S.9**).[5] There was no clear seasonality in imidacloprid or thiamethoxam concentrations (**Figures S.8**) or mass loads (**Figure S.9, Figure 3**).

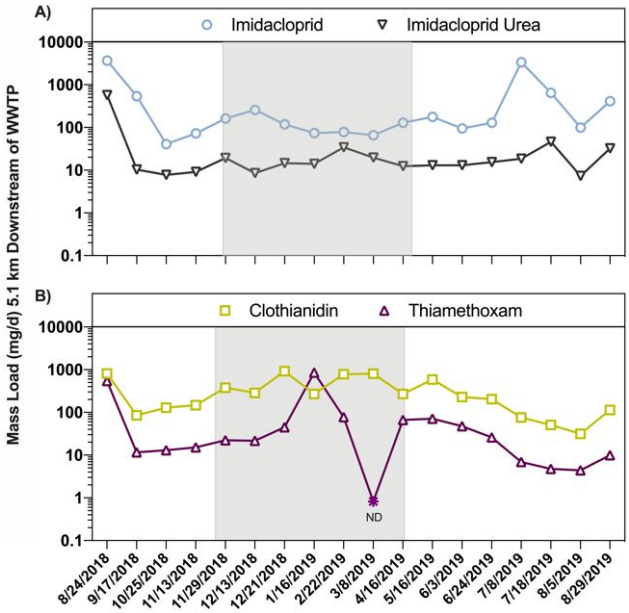

Figure 3: Calculated daily mass loads at the USGS Muddy Creek gaging downstream 2 (DS2), 5.1 km downstream of the WWTP effluent of: (A) imidacloprid (blue circles) and imidacloprid urea (gray inverted triangles) and (B) clothianidin (yellow squares) and thiamethoxam (purple triangles). The gray shaded region highlights the cool season (November-April) where upstream water temperatures were ≤10 °C. Thiamethoxam was not detected on 3/8/2019 and was plotted with the censored data calculated using the concentration of ½LLD, denoted as a purple asterisk and the letters ND (non-detect). Mass loads were calculated based on the assumption that instantaneous flow at downstream 2 (USGS gaging station 05454090) at the time of sampling was representative of the daily flow (as samples were taken during base flow conditions). The elevated mass loads on 8/24/2018 are in part due to a higher flow rate.

Analysis of possible neonicotinoid sources. We conducted an analysis of potential sources of neonicotinoids to the WWTP based on registered uses within the sewershed. Registered uses of the neonicotinoids imidacloprid, clothianidin, and thiamethoxam in products in Iowa can be

aggregated into five main use categories (agriculture, lawn/garden/forestry, indoor/outdoor pest control, pets, and 'other') based on their specified applications as provided by the Iowa Department of Agriculture and Land Stewardship Pesticide Bureau (**Figure 4,** see SI for further details).[63] Because a separated stormwater collection system is used in North Liberty, down-the-drain uses from households and businesses are likely the primary sources of neonicotinoids to the WWTP. Potential sources contributing neonicotinoids in treated wastewater could include source tap water, residues from food (i.e., excreted urine/feces, and in-sink washing and disposals), as well as pet insecticidal treatments.[5,10,35] We describe these potential contributing sources below.

Water and Food: We did not detect parent neonicotinoids above the LLD in the deep-groundwater used as drinking water for North Liberty (**Table S.17**), indicating the source water is unlikely a significant contributor to neonicotinoids in treated wastewater. Neonicotinoid contributions from washing produce and/or from excreted food residues[64] were estimated based on the median concentrations of each neonicotinoid detected in food residues reported by the UDSA Pesticide Data Program (PDP) from 2018 (**Figure S.14**). Assuming (1) everyone within the sewershed consumed the recommended 125 grams per serving of fruits and vegetables, (2) everyone consumed the North American Average of 5 servings of fruits and vegetables per day, and (3) and that all produce consumed contained the median residue concentrations for each neonicotinoid (**Figure S.14**, see SI for details), it is possible that neonicotinoid residues in food could account for much of the observed low-level effluent thiamethoxam mass loads. Nevertheless, food residues are unlikely to fully explain the mass loads of imidacloprid or clothianidin we observed in the North Liberty WWTP effluent (see SI for details), particularly during episodic spikes in effluent mass loads (i.e., when effluent mass loads were >2X the median effluent mass load).

Pet flea and tick treatments/preventatives: Flea and tick preventatives for pets have been implicated as a substantial source of imidacloprid and fipronil to WWTP effluent.[10,34,35,65] Imidacloprid is the only neonicotinoid included in this study registered for use as an insecticide for pets in Iowa.[63] Although flea and tick preventatives can be used on both dogs and cats, the contribution from cats is likely substantially less than dogs due to less grooming and we assume that indoor cats are less likely to be treated for ticks/fleas. To estimate the possible importance of pet flea and tick preventative products on the mass load of imidacloprid in the North Liberty WWTP, we used national statistics regarding pet ownership, preventative use among dog owners, and the products used in flea and tick prevention. We assumed (1) the average dog within the sewershed is medium size (20-55 pounds / 9-25 kg),[66] (2) 75% of the dogs are treated with a flea and tick preventative product,[31,67] (3) that 20% of the dogs treated with a flea and tick preventative use a product containing imidacloprid (~250–450 mg/dog/month),[63] and (4) that the imidacloprid applied to each dog is evenly leached from the dog throughout the time of use (e.g., imidacloprid transfer and rinsing via petting, laundering, or bathing).[10,34,35] Based on these assumptions, if just 1% of applied pet flea and tick preventative products containing imidacloprid were leached off dogs in the sewershed, this would yield an estimated mass load of 55–100 mg/d imidacloprid (full calculations in SI), and pet applications could account for a substantial portion of imidacloprid we measured in the WWTP effluent (which ranged between 35-5,290 mg/d, median 161 mg/d; **Figure 2, Table S.15**).[10] We used the 1% washoff value as lower-boundary estimate that is highly conservative (measurements of fipronil washoff from dogs are higher[65]); greater wash-off assumptions would increase estimated loads, but our goal was to see if a conservative estimate from pet products could explain imidacloprid loads to the WWTP. Even though imidacloprid concentrations due to pet flea and tick preventative products are likely to change between seasons

(some are recommended for year-round use), grooming events, dog demographics, etc., these products likely still account for a large percentage of the imidacloprid we observed in the treated wastewater.

Underappreciated sources: Clothianidin is not registered for use in Iowa as pet treatments and preventatives,[63] and inputs from washed produce and/or excreted food residues are unlikely to fully explain effluent clothianidin mass loads based on our above estimates (mass estimate calculations in SI, **Figures S.13–S.14**)—particularly the episodic spikes we measured. Thus, other registered products containing neonicotinoids (e.g., indoor pest control for bed bugs, treatment of wall voids/baseboards/windows via monthly pest control programs; indoor/outdoor plants/flowers/lawns, and wood structures/playgrounds; **Figures 4**)[63] likely contribute to the presence of not only clothianidin (see SI for details), but also imidacloprid and thiamethoxam in wastewater via direct transport to drains (i.e., indoor spraying) and indirect transfer to skin or clothing that is subsequently washed down-the-drain. The presence of thiamethoxam in some lawn, garden, and indoor application products could also contribute a portion of the clothianidin mass loads due to thiamethoxam-to-clothianidin transformation. North Liberty is a rapidly growing commuter suburb in where many multi-resident buildings have routine insecticide spraying maintenance programs; therefore, use of indoor neonicotinoid spraying might account for portions of the neonicotinoid mass loads at the North Liberty WWTP effluent and be a potentially underappreciated route of human exposure to neonicotinoids.[5,35,63] Non-occupational exposure to pesticides is important for exposure assessment (e.g., as established by the US EPA).[68]

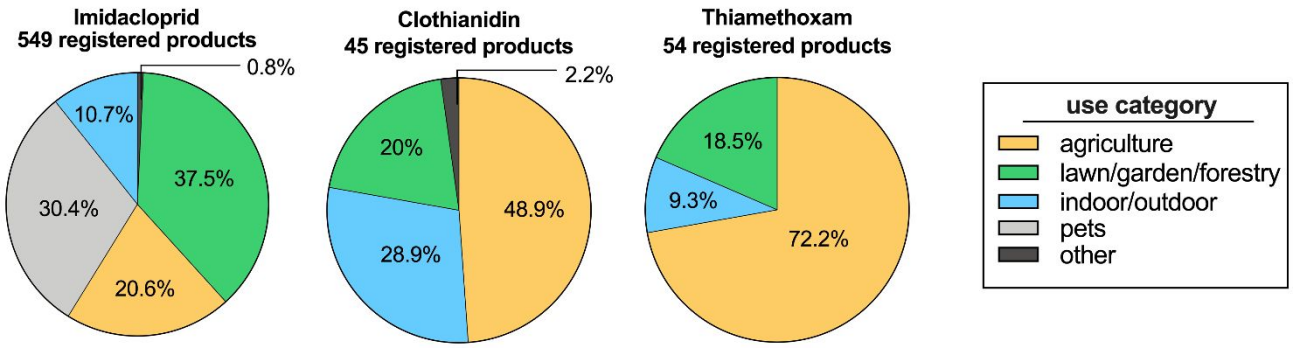

Figure 4: Distribution in uses of products registered in the State of Iowa that contain imidacloprid, clothianidin, or thiamethoxam (data obtained from the Iowa Department of Agriculture and Land Stewardship Pesticide Bureau.[63]) Use was divided into five categories: agriculture (seed and foliage treatment), lawn and garden (sod, turf, and ornamental trees, shrubs, flowers, forest trees), indoors and outdoors of buildings (homes, restaurants, institutions, businesses, barns), pets (topical preventatives, treated collars, shampoo for cats and dogs), and other (manufacturing or unspecified uses).

Conclusions. Although pesticides have been shown to drive ecological stream health more than other trace organic contaminants,[69] they are often neglected for study in effluent-dominated streams where studies are often focused on pharmaceuticals. We demonstrate, for the first time, that municipal wastewater effluent is a year-round point source of neonicotinoids to a wastewater effluent-dominated stream where neonicotinoid mass loads persist >5 km downstream of the WWTP outfall. The neonicotinoid concentrations discharged into and persisting within Muddy Creek likely generate a localized ecotoxicological exposure concern for organisms within the reach (e.g., aquatic invertebrates and their consumers). Because Muddy Creek is a representative study-reach,[49] we anticipate elevated neonicotinoid concentrations in small, effluent dominated streams is likely commonplace and could lead to chronic or acute toxic responses in aquatic biota, thereby impacting the local aquatic and terrestrial ecosystem.[8,58] The results presented in our study contrast prior work in agriculturally-impacted wetlands where clothianidin dominated and all measurements were below EPA chronic toxicity benchmarks; here, imidacloprid levels were the highest and chronic—and some acute—concentrations were recorded.[70] Effluent dominated

streams are becoming increasingly common in temperate regions due to population growth, climate change, and pressures on water resources;[36,37,42] thus, understanding loading and dynamics of emerging pesticides is critical.

Establishing that municipal wastewater effluent from a separated collection system (i.e., no stormwater) is a point-source of imidacloprid and clothianidin to the effluent-dominated stream allows us to evaluate underappreciated sources and potential exposure routes of neonicotinoids. The mass loads of imidacloprid and clothianidin we observed are not likely fully explained by food residues. Thus, it is possible other previously overlooked indoor/home and/or outdoor uses and exposure routes for humans to neonicotinoids occur (e.g., registered uses in Iowa include agriculture, pets, gardening/horticulture, indoor and outdoor pest control). Additional research should consider focus on indoor sources of/potential exposure to neonicotinoid insecticides, as well as subsequent impacts to effluent-dominated streams/ecosystems.

SUPPORTING INFORMATION. Additional method details, statistical analysis, quality assurance / control, additional detailed data / results / analysis in figures and tables.

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NOTES. The authors declare no competing financial interest.

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REFERENCES.

(1) Morrissey, C. A.; Mineau, P.; Devries, J. H.; Sanchez-Bayo, F.; Liess, M.; Cavallaro, M. C.; Liber, K. Neonicotinoid Contamination of Global Surface Waters and Associated Risk to Aquatic Invertebrates: A Review. *Environ. Int.* **2015**, *74*, 291–303. https://doi.org/10.1016/j.envint.2014.10.024.

(2) Hladik, M. L.; Main, A. R.; Goulson, D. Environmental Risks and Challenges Associated with Neonicotinoid Insecticides. *Environ. Sci. Technol.* **2018**, *52* (6), 3329–3335. https://doi.org/10.1021/acs.est.7b06388.

(3) Bass, C.; Denholm, I.; Williamson, M. S.; Nauen, R. The Global Status of Insect Resistance to Neonicotinoid Insecticides. *Pestic. Biochem. Physiol.* **2015**, *121*, 78–87. https://doi.org/10.1016/j.pestbp.2015.04.004.

(4) Van Dijk, T. C.; Van Staalduinen, M. A.; Van der Sluijs, J. P.; Maxim, L.; Sluijs, J. P. Van der; Krupke, C. H.; Hunt, G. J.; Eitzer, B. D.; Andino, G.; Given, K.; Buckingham, S. D.; Lapied, B.; Corronc, H. Le; Grolleau, F.; Sattelle, D. B.; Matsuda, K.; Buckingham, S. D.; Kleier, D.; Rauh, J. J.; Sattelle, D. B.; Tomizawa, M.; Yamamoto, I.; Deglise, P.; Grunewald, B.; Gauthier, M.; Sardo, A. M.; Soares, A.; Kreutzweiser, D. P.; Good, K. P.; Chartrand, D. T.; Scarr, T. A.; Thompson, D. G.; Sanchez-Bayo, F.; Goka, K.; Feng, S.; Kong, Z.; Wang, X.; Zhao, L.; Peng, P.; Sanchez-Bayo, F.; Beketov, M. A.; Schäfer, R. B.; Marwitz, A.; Paschke, A.; Liess, M.; Mohr, S.; Berghahn, R.; Schmiediche, R.; Hübner, V.; Loth, S.; Hayasaka, D.; Korenaga, T.; Sánchez-Bayo, F.; Goka, K.; Alexander, A. C.; Culp, J. M.; Liber, K.; Cessna, A. J.; Alaux, C.; Brunet, J.-L.; Dussaubat, C.; Mondet, F.; Tchamitchan, S.; Pettis, J. S.; VanEngelsdorp, D.; Johnson, J.; Dively, G.; Vidau, C.;

Diogon, M.; Aufauvre, J.; Fontbonee, R.; Vigès, B.; Beketov, M. A.; Liess, M.; Tennekes, H. A.; Sanchez-Bayo, F.; Tennekes, H. A.; Sanchez-Bayo, F.; Jeschke, P.; Nauen, R.; Jeschke, P.; Nauen, R.; Schindler, M.; Elbert, A.; Sur, R.; Stork, A.; Tišler, T.; Jemec, A.; Mozetič, B.; Trebše, P.; Haith, D. A.; D, Q. T.; Jorgenson, B. C.; Wissel-Tyson, C.; Watanabe, H.; Young, T. M.; Felsot, A. S.; Cone, W.; Yu, J.; Ruppert, J. R.; Gustafson, D. I.; Gupta, S.; Gajbhiye, V.; Kalpana, T.; Agnihotri, N. P.; Selim, H. M.; Jeong, C. Y.; Elbana, T. A.; Miranda, G. R. B.; Raetano, C. G.; Silva, E.; Daam, M. A.; Cerejeira, M. A.; Suchail, S.; Debrauwer, L.; Belzunces, L. P.; Hayasaka, D.; Korenaga, T.; Suzuki, K.; Sanchez-Bayo, F.; Goka, K.; Starner, K.; Goh, K. S.; Hill, B. A.; Collier, T. K.; Cresswell, J. E.; Desneux, N.; VanEngelsdorp, D.; Maxim, L.; Sluijs, J. P. Van der; Liess, M.; Beketov, M. A.; Overmyer, J. P.; Mason, B. N.; Armbrust, K. L.; Beketov, M. A.; Liess, M.; Bonada, N.; Zamora-Munoz, C.; Rieradevalla, M.; Prat, N.; Stuijfzand, S. C.; Engels, S.; Ammelrooy, E. Van; Jonker, M.; Szczepaniec, A.; Creary, S. F.; Laskowski, K. L.; Nyrop, J. P.; Raupp, M. J.; Zeng, C.-X.; Wang, J.-J.; Hayasaka, D.; Korenaga, T.; Suzuki, K.; Saito, F.; Sánchez-Bayo, F.; Sanchez-Bayo, F.; Goka, K.; Englert, D.; Bundschuh, M.; Schulz, R.; Kreutzweiser, D. P.; Good, K. P.; Chartrand, D. T.; Scarr, T. A.; Thompson, D. G.; Pestana, J. L. T.; Loureiro, S.; Baird, D. J.; Soares, A.; Wijngaarden, R. P. A. Van; Brock, T. C. M.; Brink, P. J. Van Den; Chen, X. D.; Culbert, E.; Hebert, V.; Stark, J. D.; Key, P.; Chung, K.; Siewicki, T.; Fulton, M.; Loureiro, S.; Svendsen, C.; Ferreira, A. L. G.; Pinheiro, C.; Ribeiro, F.; Wu, G.; Jiang, S.; Miyata, T.; Iwasa, T.; Motoyama, N.; Ambrose, J. T.; Roe, R. M. Macro-Invertebrate Decline in Surface Water Polluted with Imidacloprid. *PLoS One* **2013**, *8* (5), e62374–e62374. https://doi.org/10.1371/journal.pone.0062374.

(5) Thompson, D. A.; Lehmler, H.-J.; Kolpin, D. W.; Hladik, M. L.; Vargo, J. D.; Schilling, K. E.; LeFevre, G. H.; Peeples, T. L.; Poch, M. C.; LaDuca, L. E.; Cwiertny, D. M.; Field, R. W. A Critical Review on the Potential Impacts of Neonicotinoid Insecticide Use: Current Knowledge of Environmental Fate, Toxicity, and Implications for Human Health. *Environ. Sci. Process. Impacts* **2020**, *22* (6), 1315–1346. https://doi.org/10.1039/C9EM00586B.

(6) PubChem Open Chemistry Database https://pubchem.ncbi.nlm.nih.gov/.

(7) Van Metre, P. C.; Alvarez, D. A.; Mahler, B. J.; Nowell, L.; Sandstrom, M.; Moran, P. Complex Mixtures of Pesticides in Midwest U.S. Streams Indicated by POCIS Time-Integrating Samplers. *Environ. Pollut.* **2017**, *220*, 431–440. https://doi.org/https://doi.org/10.1016/j.envpol.2016.09.085.

(8) Nowell, L. H.; Moran, P. W.; Schmidt, T. S.; Norman, J. E.; Nakagaki, N.; Shoda, M. E.; Mahler, B. J.; Van Metre, P. C.; Stone, W. W.; Sandstrom, M. W.; Hladik, M. L. Complex Mixtures of Dissolved Pesticides Show Potential Aquatic Toxicity in a Synoptic Study of Midwestern U.S. Streams. *Sci. Total Environ.* **2018**, *613–614*, 1469–1488. https://doi.org/10.1016/j.scitotenv.2017.06.156.

(9) Hladik, M. L.; Corsi, S. R.; Kolpin, D. W.; Baldwin, A. K.; Blackwell, B. R.; Cavallin, J. E. Year-Round Presence of Neonicotinoid Insecticides in Tributaries to the Great Lakes, USA. *Environ. Pollut.* **2018**, *235*, 1022–1029. https://doi.org/10.1016/j.envpol.2018.01.013.

(10) Sadaria, A. M.; Sutton, R.; Moran, K. D.; Teerlink, J.; Brown, J. V.; Halden, R. U. Passage of Fiproles and Imidacloprid from Urban Pest Control Uses through Wastewater Treatment

Plants in Northern California. *Environ. Toxicol. Chem.* **2016**. https://doi.org/10.1002/etc.3673.

(11) Klarich, K. L.; Pflug, N. C.; DeWald, E. M.; Hladik, M. L.; Kolpin, D. W.; Cwiertny, D. M.; LeFevre, G. H. Occurrence of Neonicotinoid Insecticides in Finished Drinking Water and Fate during Drinking Water Treatment. *Environ. Sci. Technol. Lett.* **2017**, *4* (5), 168–173. https://doi.org/10.1021/acs.estlett.7b00081.

(12) Klarich Wong, K. L.; Webb, D. T.; Nagorzanski, M. R.; Kolpin, D. W.; Hladik, M. L.; Cwiertny, D. M.; Lefevre, G. H. Chlorinated Byproducts of Neonicotinoids and Their Metabolites: An Unrecognized Human Exposure Potential? *Environ. Sci. Technol. Lett.* **2019**, *6* (2). https://doi.org/10.1021/acs.estlett.8b00706.

(13) Bonmatin, J.-M.-M.; Giorio, C.; Girolami, V.; Goulson, D.; Kreutzweiser, D. P.; Krupke, C.; Liess, M.; Long, E.; Marzaro, M.; Mitchell, E. A. D.; Noome, D. A.; Simon-Delso, N.; Tapparo, A. Environmental Fate and Exposure; Neonicotinoids and Fipronil. *Environ. Sci. Pollut. Res.* **2015**, *22* (1), 35–67. https://doi.org/10.1007/s11356-014-3332-7.

(14) Goulson, D. An Overview of the Environmental Risks Posed by Neonicotinoid Insecticides. *J. Appl. Ecol.* **2013**, *50* (4), 977–987. https://doi.org/10.1111/1365-2664.12111.

(15) Hladik, M. L.; Kolpin, D. W. First National-Scale Reconnaissance of Neonicotinoid Insecticides in Streams across the USA. *Environ. Chem.* **2016**, *13* (1), 12–20. https://doi.org/10.1016/j.scitotenv.2017.09.097.

(16) Struger, J.; Grabuski, J.; Cagampan, S.; Sverko, E.; McGoldrick, D.; Marvin, C. H. Factors Influencing the Occurrence and Distribution of Neonicotinoid Insecticides in Surface Waters of Southern Ontario, Canada. *Chemosphere* **2017**, *169*, 516–523. https://doi.org/10.1016/j.chemosphere.2016.11.036.

(17) Zhang, C.; Tian, D.; Yi, X. H.; Zhang, T.; Ruan, J.; Wu, R.; Chen, C.; Huang, M.; Ying, G. G. Occurrence, Distribution and Seasonal Variation of Five Neonicotinoid Insecticides in Surface Water and Sediment of the Pearl Rivers, South China. *Chemosphere* **2019**, *217*, 437–446. https://doi.org/10.1016/j.chemosphere.2018.11.024.

(18) Yi, X.; Zhang, C.; Liu, H.; Wu, R.; Tian, D.; Ruan, J.; Zhang, T.; Huang, M.; Ying, G. Occurrence and Distribution of Neonicotinoid Insecticides in Surface Water and Sediment of the Guangzhou Section of the Pearl River, South China. *Environ. Pollut.* **2019**, *251*, 892–900. https://doi.org/10.1016/j.envpol.2019.05.062.

(19) Zhang, C.; Yi, X.; Chen, C.; Tian, D.; Liu, H.; Xie, L.; Zhu, X.; Huang, M.; Ying, G. G. Contamination of Neonicotinoid Insecticides in Soil-Water-Sediment Systems of the Urban and Rural Areas in a Rapidly Developing Region: Guangzhou, South China. *Environ. Int.* **2020**, *139*, 105719. https://doi.org/10.1016/j.envint.2020.105719.

(20) Mahai, G.; Wan, Y.; Xia, W.; Wang, A.; Shi, L.; Qian, X.; He, Z.; Xu, S. A Nationwide Study of Occurrence and Exposure Assessment of Neonicotinoid Insecticides and Their Metabolites in Drinking Water of China. *Water Res.* **2021**, *189*, 116630. https://doi.org/10.1016/j.watres.2020.116630.

(21) Rico, A.; Arenas-Sánchez, A.; Pasqualini, J.; García-Astillero, A.; Cherta, L.; Nozal, L.;

Vighi, M. Effects of Imidacloprid and a Neonicotinoid Mixture on Aquatic Invertebrate Communities under Mediterranean Conditions. *Aquat. Toxicol.* **2018**, *204*, 130–143. https://doi.org/10.1016/j.aquatox.2018.09.004.

(22) Sultana, T.; Murray, C.; Kleywegt, S.; Metcalfe, C. D. Neonicotinoid Pesticides in Drinking Water in Agricultural Regions of Southern Ontario, Canada. *Chemosphere* **2018**, *202*, 506–513. https://doi.org/10.1016/j.chemosphere.2018.02.108.

(23) Pisa, L. W.; Amaral-Rogers, V.; Belzunces, L. P.; Bonmatin, J. M.; Downs, C. A.; Goulson, D.; Kreutzweiser, D. P.; Krupke, C.; Liess, M.; McField, M.; Morrissey, C. A.; Noome, D. A.; Settele, J.; Simon-Delso, N.; Stark, J. D.; Van der Sluijs, J. P.; Van Dyck, H.; Wiemers, M. Effects of Neonicotinoids and Fipronil on Non-Target Invertebrates. *Environ. Sci. Pollut. Res.* **2015**, *22* (1), 68–102. https://doi.org/10.1007/s11356-014-3471-x.

(24) Tomizawa, M.; Casida, J. E. Selective Toxicity of Neonicotinoids Attributable to Specificity of Insect and Mammalian Nicotinic Receptors. *Annu. Rev. Entomol.* **2003**, *48* (1), 339–364. https://doi.org/10.1146/annurev.ento.48.091801.112731.

(25) Tomizawa, M.; Casida, J. E. Imidacloprid, Thiacloprid, and Their Imine Derivatives Up-Regulate the A4β2 Nicotinic Acetylcholine Receptor in M10 Cells. *Toxicol. Appl. Pharmacol.* **2000**, *169* (1), 114–120. https://doi.org/http://dx.doi.org/10.1006/taap.2000.9057.

(26) Tomizawa, M.; Zhang, N.; Durkin, K. A.; Olmstead, M. M.; Casida, J. E. The Neonicotinoid Electronegative Pharmacophore Plays the Crucial Role in the High Affinity and Selectivity for the Drosophila Nicotinic Receptor: An Anomaly for the Nicotinoid Cation--Pi Interaction Model. *Biochemistry* **2003**, *42* (25), 7819–7827. https://doi.org/10.1021/bi0300130.

(27) Tomizawa, M.; Casida, J. E. Neonicotinoid Insecticide Toxicology: Mechanisms of Selective Action. *Annu. Rev. Pharmacol. Toxicol.* **2005**, *45* (1), 247–268. https://doi.org/10.1146/annurev.pharmtox.45.120403.095930.

(28) Hladik, M. L.; Kolpin, D. W.; Kuivila, K. M. Widespread Occurrence of Neonicotinoid Insecticides in Streams in a High Corn and Soybean Producing Region, USA. *Environ. Pollut.* **2014**, *193*, 189–196. https://doi.org/10.1016/j.envpol.2014.06.033.

(29) Masoner, J. R.; Kolpin, D. W.; Cozzarelli, I. M.; Barber, L. B.; Burden, D. S.; Foreman, W. T.; Forshay, K. J.; Furlong, E. T.; Groves, J. F.; Hladik, M. L.; Hopton, M. E.; Jaeschke, J. B.; Keefe, S. H.; Krabbenhoft, D. P.; Lowrance, R.; Romanok, K. M.; Rus, D. L.; Selbig, W. R.; Williams, B. H.; Bradley, P. M. Urban Stormwater: An Overlooked Pathway of Extensive Mixed Contaminants to Surface and Groundwaters in the United States. *Environ. Sci. Technol.* **2019**, *53* (17), 10070–10081. https://doi.org/10.1021/acs.est.9b02867.

(30) Burant, A.; Selbig, W.; Furlong, E. T.; Higgins, C. P. Trace Organic Contaminants in Urban Runoff: Associations with Urban Land-Use. *Environ. Pollut.* **2018**, *242*, 2068–2077. https://doi.org/10.1016/J.ENVPOL.2018.06.066.

(31) Sadaria, A. M.; Supowit, S. D.; Halden, R. U. Mass Balance Assessment for Six Neonicotinoid Insecticides During Conventional Wastewater and Wetland Treatment:

Nationwide Reconnaissance in U.S. Wastewater. *Environ. Sci. Technol.* **2016**, *50* (12), 6199–6206. https://doi.org/10.1021/acs.est.6b01032.

(32) Iancu, V. I.; Radu, G. L. Occurrence of Neonicotinoids in Waste Water from the Bucharest Treatment Plant. *Anal. Methods* **2018**, *10* (23), 2691–2700. https://doi.org/10.1039/c8ay00510a.

(33) Hope, B. K.; Pillsbury, L.; Boling, B. A State-Wide Survey in Oregon (USA) of Trace Metals and Organic Chemicals in Municipal Effluent. *Sci. Total Environ.* **2012**, *417–418*, 263–272. https://doi.org/10.1016/j.scitotenv.2011.12.028.

(34) Perkins, R.; Whitehead, M.; Civil, W.; Goulson, D. Potential Role of Veterinary Flea Products in Widespread Pesticide Contamination of English Rivers. *Sci. Total Environ.* **2020**, 143560. https://doi.org/10.1016/j.scitotenv.2020.143560.

(35) Sutton, R.; Xie, Y.; Moran, K. D.; Teerlink, J. Occurrence and Sources of Pesticides to Urban Wastewater and the Environment. In *ACS Symposium Series*; American Chemical Society, 2019; Vol. 1308, pp 63–88. https://doi.org/10.1021/bk-2019-1308.ch005.

(36) Rice, J.; Wutich, A.; Westerhoff, P. Assessment of de Facto Wastewater Reuse across the U.S.: Trends between 1980 and 2008. *Environ. Sci. Technol.* **2013**, *47* (19), 11099–11105. https://doi.org/10.1021/es402792s.

(37) Rice, J.; Westerhoff, P. Spatial and Temporal Variation in de Facto Wastewater Reuse in Drinking Water Systems across the U.S.A. *Environ. Sci. Technol.* **2015**, *49* (2), 982–989. https://doi.org/10.1021/es5048057.

(38) Bischel, H. N.; Lawrence, J. E.; Halaburka, B. J.; Plumlee, M. H.; Bawazir, A. S.; King, J. P.; McCray, J. E.; Resh, V. H.; Luthy, R. G. Renewing Urban Streams with Recycled Water for Streamflow Augmentation: Hydrologic, Water Quality, and Ecosystem Services Management. *Environ. Eng. Sci.* **2013**, *30* (8), 455–479. https://doi.org/10.1089/ees.2012.0201.

(39) Hubbard, L. E.; Keefe, S. H.; Kolpin, D. W.; Barber, L. B.; Duris, J. W.; Hutchinson, K. J.; Bradley, P. M. Understanding the Hydrologic Impacts of Wastewater Treatment Plant Discharge to Shallow Groundwater: Before and after Plant Shutdown. *Environ. Sci. Water Res. Technol.* **2016**, *2* (5), 864–874. https://doi.org/10.1039/c6ew00128a.

(40) Brooks, B. W.; Riley, T. M.; Taylor, R. D. Water Quality of Effluent-Dominated Ecosystems: Ecotoxicological, Hydrological, and Management Considerations. *Hydrobiologia*. Springer February 2006, pp 365–379. https://doi.org/10.1007/s10750-004-0189-7.

(41) Halaburka, B. J.; Lawrence, J. E.; Bischel, H. N.; Hsiao, J.; Plumlee, M. H.; Resh, V. H.; Luthy, R. G. Economic and Ecological Costs and Benefits of Streamflow Augmentation Using Recycled Water in a California Coastal Stream. *Environ. Sci. Technol.* **2013**, *47* (19), 10735–10743. https://doi.org/10.1021/es305011z.

(42) Rice, J.; Via, S. H.; Westerhoff, P. Extent and Impacts of Unplanned Wastewater Reuse in US Rivers. *J. Am. Water Works Assoc.* **2015**, *107* (11), E571–E581. https://doi.org/10.5942/jawwa.2015.107.0178.

(43) Schultz, M. M.; Furlong, E. T.; Kolpin, D. W.; Werner, S. L.; Schoenfuss, H. L.; Barber, L. B.; Blazer, V. S.; Norris, D. O.; Vajda, A. M. Antidepressant Pharmaceuticals in Two U.S. Effluent-Impacted Streams: Occurrence and Fate in Water and Sediment, and Selective Uptake in Fish Neural Tissue. *Environ. Sci. Technol.* **2010**, *44* (6), 1918–1925. https://doi.org/10.1021/es9022706.

(44) Barber, L. B.; Keefe, S. H.; Brown, G. K.; Furlong, E. T.; Gray, J. L.; Kolpin, D. W.; Meyer, M. T.; Sandstrom, M. W.; Zaugg, S. D. Persistence and Potential Effects of Complex Organic Contaminant Mixtures in Wastewater-Impacted Streams. *Environ. Sci. Technol.* **2013**, *47* (5), 2177–2188. https://doi.org/10.1021/es303720g.

(45) Gao, H.; Lavergne, J. M.; Carpenter, C. M. G.; Desai, R.; Zhang, X.; Gray, K.; Helbling, D. E.; Wells, G. F. Exploring Co-Occurrence Patterns between Organic Micropollutants and Bacterial Community Structure in a Mixed-Use Watershed. *Environ. Sci. Process. Impacts* **2019**, *21* (5), 867–880. https://doi.org/10.1039/c8em00588e.

(46) Grabicova, K.; Grabic, R.; Fedorova, G.; Fick, J.; Cerveny, D.; Kolarova, J.; Turek, J.; Zlabek, V.; Randak, T. Bioaccumulation of Psychoactive Pharmaceuticals in Fish in an Effluent Dominated Stream. *Water Res.* **2017**, *124*, 654–662. https://doi.org/10.1016/j.watres.2017.08.018.

(47) Karakurt, S.; Schmid, L.; Hübner, U.; Drewes, J. E. Dynamics of Wastewater Effluent Contributions in Streams and Impacts on Drinking Water Supply via Riverbank Filtration in Germany - A National Reconnaissance. *Environ. Sci. Technol.* **2019**, *53* (11), 6154–6161. https://doi.org/10.1021/acs.est.8b07216.

(48) Bradley, P. M.; Barber, L. B.; Duris, J. W.; Foreman, W. T.; Furlong, E. T.; Hubbard, L. E.; Hutchinson, K. J.; Keefe, S. H.; Kolpin, D. W. Riverbank Filtration Potential of Pharmaceuticals in a Wastewater-Impacted Stream. *Environ. Pollut.* **2014**, *193*, 173–180. https://doi.org/10.1016/j.envpol.2014.06.028.

(49) Zhi, H.; Kolpin, D. W.; Klaper, R. D.; Iwanowicz, L. R.; Meppelink, S. M.; LeFevre, G. H. Occurrence and Spatiotemporal Dynamics of Pharmaceuticals in a Temperate-Region Wastewater Effluent-Dominated Stream: Variable Inputs and Differential Attenuation Yield Evolving Complex Exposure Mixtures. *Environ. Sci. Technol.* **2020**, *54* (20), 12967–12978. https://doi.org/10.1021/acs.est.0c02328.

(50) U.S. Geological Survey. National Field Manual for the Collection of Water-Quality Data, Chapter A4, Collection of Water Samples. In *Version 2, Techniques of Water-Resources Investigations 09-A4*; Reston, VA, 2006. https://doi.org/10.3133/twri09A4.

(51) Meppelink, S. M.; Kolpin, D. W.; Lane, R. F.; Iwanowicz, L. R.; Zhi, H.; LeFevre, G. H. *Water-Quality Data for a Pharmaceutical Study at Muddy Creek in North Liberty and Coralville, Iowa, 2017-2018: U.S. Geological Survey Data Release*; 2020. https://doi.org/10.5066/P9WOD2XB.

(52) Bradley, P. M.; Journey, C. A.; Romanok, K. M.; Barber, L. B.; Buxton, H. T.; Foreman, W. T.; Furlong, E. T.; Glassmeyer, S. T.; Hladik, M. L.; Iwanowicz, L. R.; Jones, D. K.; Kolpin, D. W.; Kuivila, K. M.; Loftin, K. A.; Mills, M. A.; Meyer, M. T.; Orlando, J. L.; Reilly, T. J.; Smalling, K. L.; Villeneuve, D. L. Expanded Target-Chemical Analysis

Reveals Extensive Mixed-Organic-Contaminant Exposure in U.S. Streams. *Environ. Sci. Technol.* **2017**, *51* (9), 4792–4802. https://doi.org/10.1021/acs.est.7b00012.

(53) Webb, D. T.; Nagorzanski, M. R.; Powers, M. M.; Cwiertny, D. M.; Hladik, M. L.; LeFevre, G. H. Differences in Neonicotinoid and Metabolite Sorption to Activated Carbon Are Driven by Alterations to the Insecticidal Pharmacophore. *Environ. Sci. Technol.* **2020**, acs.est.0c04187. https://doi.org/10.1021/acs.est.0c04187.

(54) Muerdter, C. P.; Lefevre, G. H. Synergistic Lemna Duckweed and Microbial Transformation of Imidacloprid and Thiacloprid Neonicotinoids. *Environ. Sci. Technol. Lett.* **2019**, *6* (12), 761–767. https://doi.org/10.1021/acs.estlett.9b00638.

(55) U.S. Environmental Protection Agency. *Practical Methods for Data Analysis, EPA QA/G-9, QA00 Update*; Washington, D.C., 2000.

(56) Helsel, D. R.; Hirsch, R. M.; Ryberg, K. R.; Archfield, S. A.; Gilroy, E. J. *Techniques of Water-Resources Investigations, Book 4, Chapter A3, Version 1.1*; USGS Numbered Series: Reston, VA, 2002. https://doi.org/https://doi.org/10.3133/twri04A3.

(57) United States Environmental Protection Agency. Aquatic Life Benchmarks and Ecological Risk Assessments for Registered Pesticides https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/aquatic-life-benchmarks-and-ecological-risk#ref_4 (accessed May 14, 2020).

(58) Miller, J. L.; Schmidt, T. S.; van Metre, P. C.; Mahler, B. J.; Sandstrom, M. W.; Nowell, L. H.; Carlisle, D. M.; Moran, P. W. Common Insecticide Disrupts Aquatic Communities: A Mesocosm-to-Field Ecological Risk Assessment of Fipronil and Its Degradates in U.S. Streams. *Sci. Adv.* **2020**, *6* (43), eabc1299. https://doi.org/10.1126/sciadv.abc1299.

(59) Hou, F.; Tian, Z.; Peter, K. T.; Wu, C.; Gipe, A. D.; Zhao, H.; Alegria, E. A.; Liu, F.; Kolodziej, E. P. Quantification of Organic Contaminants in Urban Stormwater by Isotope Dilution and Liquid Chromatography-Tandem Mass Spectrometry. *Anal. Bioanal. Chem.* **2019**, *411* (29), 7791–7806. https://doi.org/10.1007/s00216-019-02177-3.

(60) Nauen, R.; Ebbinghaus-Kintscher, U.; Salgado, V. L.; Kaussmann, M. Thiamethoxam Is a Neonicotinoid Precursor Converted to Clothianidin in Insects and Plants. *Pestic. Biochem. Physiol.* **2003**, *76* (2), 55–69. https://doi.org/10.1016/S0048-3575(03)00065-8.

(61) Hladik, M. L.; Bradbury, S.; Schulte, L. A.; Helmers, M.; Witte, C.; Kolpin, D. W.; Garrett, J. D.; Harris, M. Neonicotinoid Insecticide Removal by Prairie Strips in Row-Cropped Watersheds with Historical Seed Coating Use. *Agric. Ecosyst. Environ.* **2017**, *241*, 160–167. https://doi.org/https://doi.org/10.1016/j.agee.2017.03.015.

(62) Bradford, B. Z.; Huseth, A. S.; Groves, R. L. Widespread Detections of Neonicotinoid Contaminants in Central Wisconsin Groundwater. *PLoS One* **2018**, *13* (10), e0201753. https://doi.org/10.1371/journal.pone.0201753.

(63) Iowa Department of Agriculture and Land Stewardship. Pesticide Product Registration http://www.kellysolutions.com/ia/pesticideindex.asp.

(64) USDA Agricultural Marketing Service. Pesticide Data Program Database

https://apps.ams.usda.gov/pdp.

(65) Teerlink, J.; Hernandez, J.; Budd, R. Fipronil Washoff to Municipal Wastewater from Dogs Treated with Spot-on Products. *Sci. Total Environ.* **2017**, *599–600*, 960–966. https://doi.org/10.1016/j.scitotenv.2017.04.219.

(66) American Kennel Club. 2018 Most Popular Dog Breeds https://www.akc.org/most-popular-breeds/2018-full-list/.

(67) Puro, G. *2015 Packaged Facts: Pet Medications in the US, 4th Edition*.

(68) Standard Operating Procedures for Residential Pesticide Exposure Assessment | Pesticide Science and Assessing Pesticide Risks | US EPA https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/standard-operating-procedures-residential-pesticide (accessed Apr 8, 2021).

(69) Munz, N. A.; Burdon, F. J.; de Zwart, D.; Junghans, M.; Melo, L.; Reyes, M.; Schönenberger, U.; Singer, H. P.; Spycher, B.; Hollender, J.; Stamm, C. Pesticides Drive Risk of Micropollutants in Wastewater-Impacted Streams during Low Flow Conditions. *Water Res.* https://doi.org/http://dx.doi.org/10.1016/j.watres.2016.11.001.

(70) Schepker, T. J.; Webb, E. B.; Tillitt, D.; LaGrange, T. Neonicotinoid Insecticide Concentrations in Agricultural Wetlands and Associations with Aquatic Invertebrate Communities. *Agric. Ecosyst. Environ.* **2020**, *287*, 106678. https://doi.org/10.1016/j.agee.2019.106678.