# Supporting Information

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

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**Supporting information contains:** 28 pages including supporting methods, supporting results, supporting data analysis, 18 figures, and 17 tables, and supporting references.

## SUPPORTING METHODS

**Study site description.** Muddy Creek (Iowa, USA; Latitude 41°42'00", Longitude 91°33'46") has a drainage area of 22.5 $km^2$ and is comprised of agricultural and urban land use (**Figure S.1**, **Table S.1**). Flow in Muddy Creek is dominated by wastewater effluent discharged from the North Liberty Wastewater Treatment Plant (WWTP) which discharges to the Iowa River. North Liberty is the second-fastest growing city in Iowa and has an estimated population of 19,500.[1] The WWTP was built in 2008; it has a modern membrane bioreactor that removes particles >0.004 µm, and conducts biological nitrogen and phosphorus removal. The current wastewater discharge averages approximately 3.1 $ft^3/s$ (**Figure S.3**),[2] with discharge varying between 2 $ft^3/s$ to 7.2 $ft^3/s$ (median 2.9 $ft^3/s$, **Figure S.4**) at the approximate times of sampling throughout the 1 year sample collection period (stream flow measured at U.S. Geological Survey [USGS] gaging station 05454090, DS2).

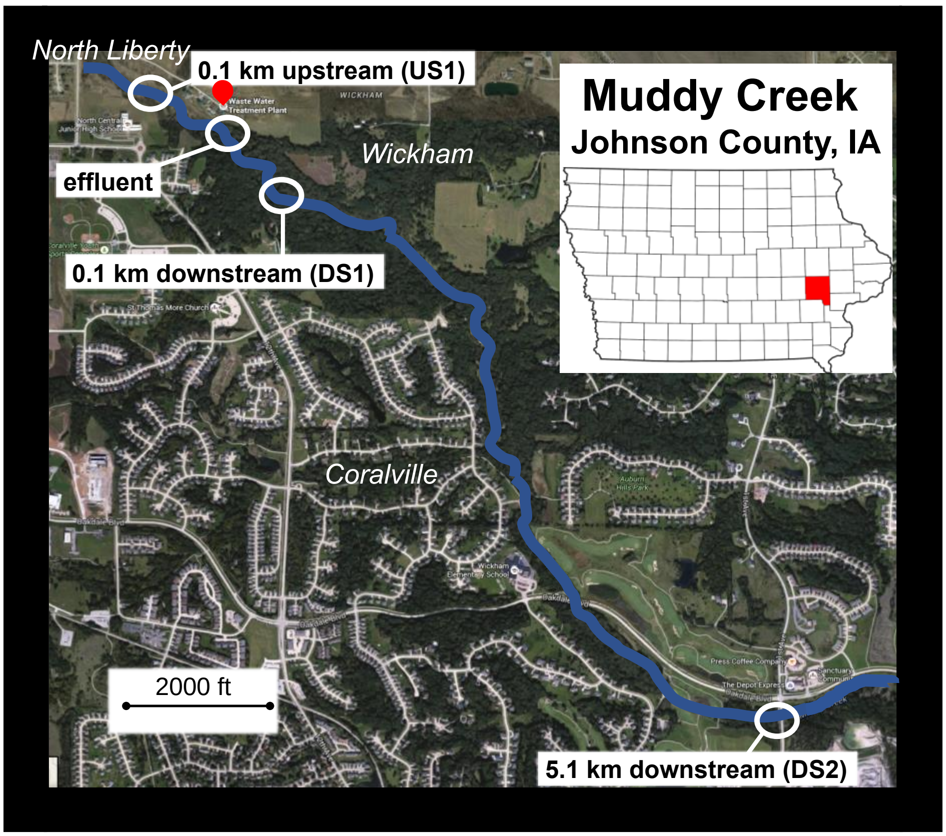


**Figure S.1**: Map of Muddy Creek in the cities of North Liberty, Coralville, and Wickham in Johnson County Iowa. Samples were collected 0.1 km upstream (US1), at the WWTP effluent outfall (effluent), 0.1 km downstream (DS1), and 5.1 km downstream (DS2). The red balloon (upper left-hand corner) indicates the location of the North Liberty WWTP.


**Figure S.2**: Photos of the sampling sites (A) 0.1 km upstream (US1), (B) the WWTP outfall (effluent), (C) 0.1 km downstream (DS1), and (D) 5.1 km downstream (DS2). Photos were taken by the authors on May 17th, 2018.

**Table S.1**: Land use information from Muddy Creek as determined by USGS National Landcover Database.(https://www.usgs.gov/centers/eros/science/national-land-cover-database?qt-science_center_objects=0#qt-science_center_objects)

| Station Name | Open Water | Developed | Barren Land (Rock/Sand/ Clay) | Forest | Shrub/ Scrub | Grassland/ Herbaceous | Pasture/ Hay | Cultivated Crops | Wetlands |
|---|---|---|---|---|---|---|---|---|---|
| US1 (05454050) | 0.13% | 72.49% | 0.07% | 1.69% | 0.00% | 1.61% | 2.82% | 20.72% | 0.44% |
| Effluent (05454051) | 0.13% | 72.50% | 0.07% | 1.70% | 0.00% | 1.61% | 2.81% | 20.69% | 0.46% |
| DS1 (05454052) | 0.13% | 72.32% | 0.07% | 2.0% | 0.00% | 1.60% | 2.86% | 20.52% | 0.47% |
| DS2 (05454090) | 0.40% | 59.97% | 0.04% | 12.36% | 0.02% | 2.38% | 7.06% | 17.45% | 0.31% |

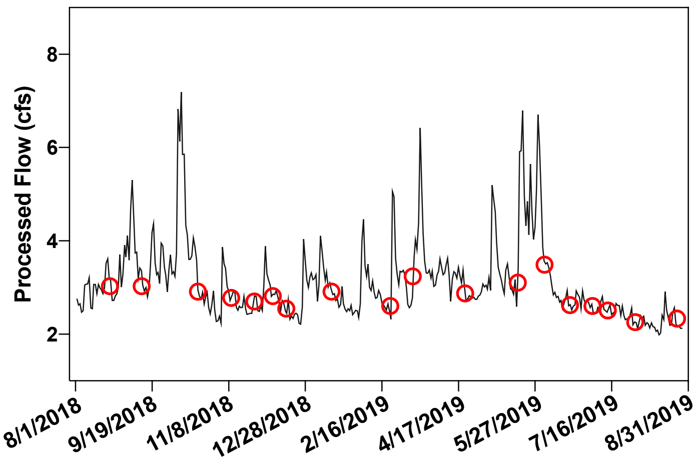


**Figure S.3**: Daily discharge from the North Liberty WWTP (cubic feet per second, cfs) during the sampling period (from 8/1/2018–9/1/2019) as provided by the WWTP. Red circles indicate sampling dates.

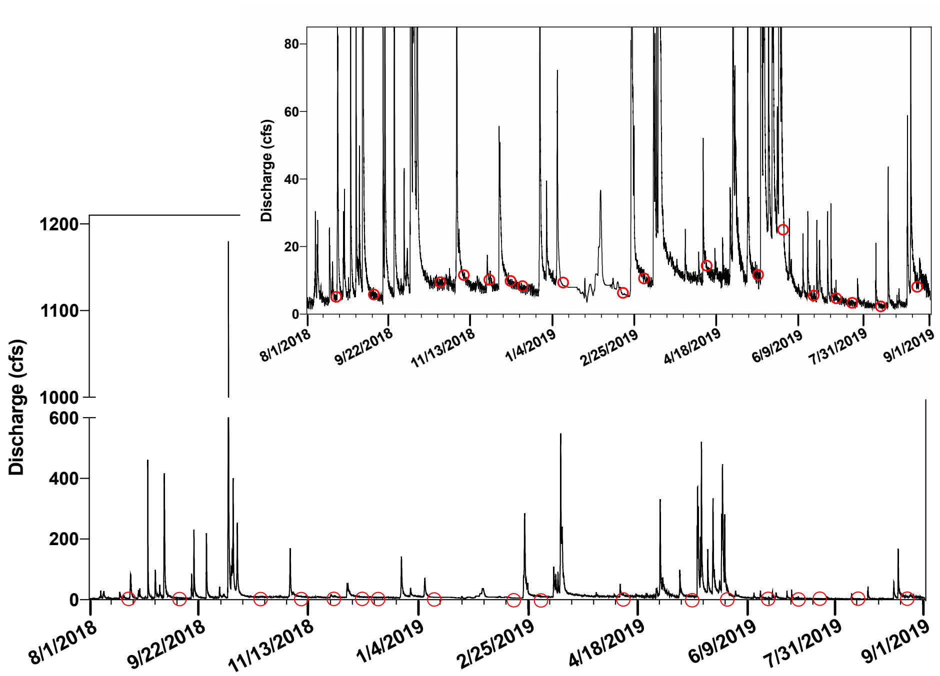


**Figure S.4:** Hydrograph of the discharge (cubic feet per second) in Muddy Creek 5.1 km downstream of the wastewater treatment plant outfall (downstream 2, DS2, USGS gaging station 05454090). Red circles indicate sampling dates (n=18). Samples were collected during baseflow (or predominantly baseflow) conditions. The inset is a zoomed-in image of the hydrograph.

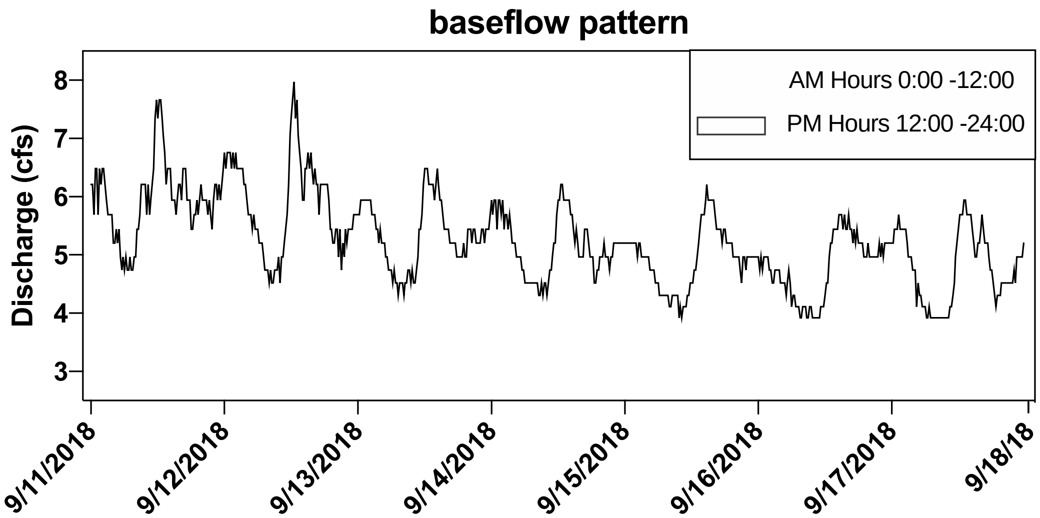


**Figure S.5**: Diurnal hydrograph from downstream 2 (DS2, USGS gaging station 05454090) showing baseflow conditions leading up to the sampling event on 9/17/2018. Sections in gray indicate morning hours (0:00–12:00) and white sections indicate afternoon hours (12:00–24:00), highlighting the diurnal impact of WWTP discharge to streamflow at the Muddy Creek study site.

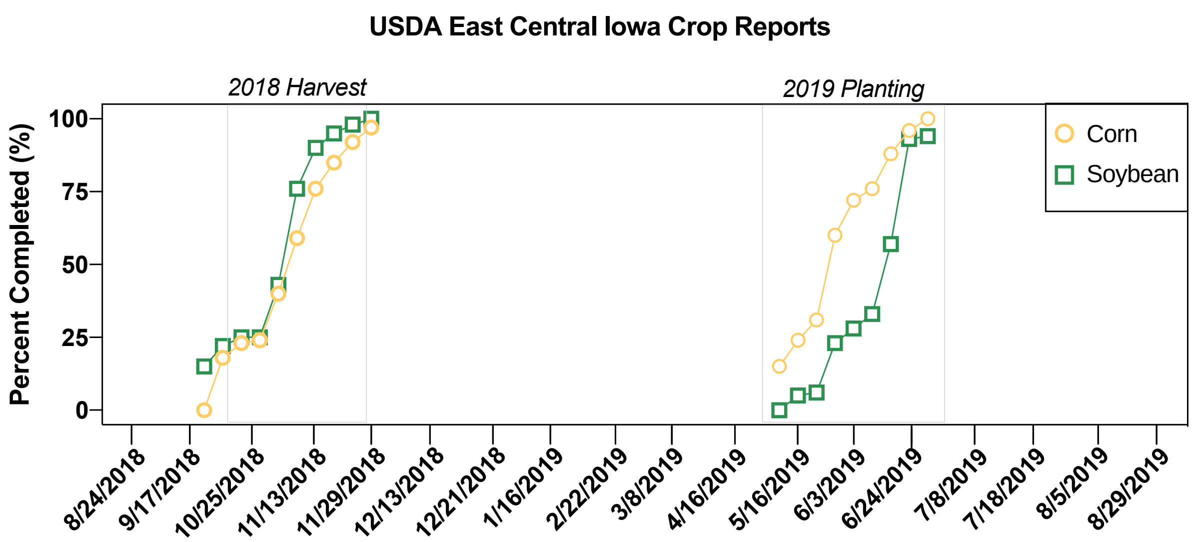


**Figure S.6**. Agricultural progress in East- Central Iowa overlaid on our Muddy Creek sampling period. Corn (yellow circles) and soybean (green squares) agriculture progress as reported by the USDA for the 2018 harvest and 2019 planting seasons.[3]

**Table S.2.** East-Central Iowa corn and soybean agriculture progress as reported by the USDA for the 2018 harvest and 2019 planting seasons.[3]

| Date | Corn (%) | Soybean (%) |
|---|---|---|
| **2018 Harvest** | | |
| 9/23/2018 | 0 | 15 |
| 9/30/2018 | 18 | 22 |
| 10/7/2018 | 23 | 25 |
| 10/14/2018 | 24 | 25 |
| 10/21/2018 | 40 | 43 |
| 10/28/2018 | 59 | 76 |
| 11/4/2018 | 76 | 90 |
| 11/11/2018 | 85 | 95 |
| 11/18/2018 | 92 | 98 |
| 11/25/2018 | 97 | 100 |
| **2019 Planting** | | |
| 4/28/2019 | 15 | 0 |
| 5/6/2019 | 24 | 5 |
| 5/13/2019 | 31 | 6 |
| 5/19/2019 | 60 | 23 |
| 5/26/2019 | 72 | 28 |
| 6/2/2019 | 76 | 33 |
| 6/10/2019 | 88 | 57 |
| 6/16/2019 | 96 | 93 |
| 6/23/2019 | 100 | 94 |

**Chemicals.** Analytical standards imidacloprid (Chemical Abstract Services Registry Number [CASRN] 138261-41-3), clothianidin (CASRN 210880-92-5), thiamethoxam (CASRN 153719-23-4) were Pestanal standards purchased from Sigma Aldrich. The metabolite imidacloprid urea (CASRN 120868-66-8) was of 99% purity and purchased from Crescent Chemical. Neonicotinoid internal standards were $d_4$-imidacloprid (Pestanal Sigma Aldrich; CASRN 1015855-75-0) and $d_3$-thiamethoxam (>98% CDN isotopes; CASRN 1294048-82-0). Solvents used for chromatography (water, acetonitrile, and formic acid) were of Optima Liquid Chromatograph Mass Spectrometer (LCMS) grade and purchased from Fisher Scientific (Fairlawn, NJ). Solvents used for solid phase extraction (acetone and dichloromethane) were, at a minimum, ACS grade and purchased from Fisher Scientific (Fairlawn, NJ).

**Bulk water-quality parameters.** Bulk water-quality parameters of the stream (e.g., pH, water temperature, specific conductivity, and dissolved oxygen) were measured with a HACH HQ40D portable multimeter (**Figures S.9–S.12**). Dissolved oxygen was measured with an Intellical™ LDO101 electrode, pH and water temperature were measured with an Intellical™ PHC101 electrode, and specific conductivity was measured with an Intellical™ CDC401 electrode. The HACH probe was only available for use for the 2019 sampling dates and, thus, only data for those sampling dates are provided.

**Solid Phase Extraction (SPE) method.** Parent neonicotinoids and imidacloprid urea were extracted from 1L water samples with Waters Oasis HLB LP extraction cartridges (500 mg, 6cc, PN: 186000115). SPE cartridges were conditioned by gravity with 5 mL dichloromethane followed by 5 mL acetone and 10 mL Optima LCMS water. 0.7 µm PES filtered water samples spiked with 10 µL $d_4$-imidacloprid were and pumped through the cartridge under vacuum at <10 mL $min^{-1}$. Cartridges were dried under vacuum until visibly dry. Parent neonicotinoids and imidacloprid urea were eluted (gravimetrically) with 10 mL of 1:1 dichloromethane:acetone solution. Eluents were evaporated under nitrogen (Praxair) on an Organomation OA Heat nitrogen evaporator on a heat level of three. Evaporated samples were reconstituted in a 1 mL solution of 1:1 acetonitrile:Optima LCMS water and spiked with 10 µL of $d_3$-thiamethoxam. Multiple blanks were used including solvent blanks and reconstitution blanks. SPE recoveries were previously reported and are provided in **Table S.3**. Field blanks were previously performed by transferring 1L Optima LCMS grade water into acid washed amber bottles at the sampling site (where tap water samples were previously collected) and revealed only trace levels of thiamethoxam (0.32 ng/L).[4]

**Table S.3: Solid Phase Extraction (SPE) Recovery:** Neonicotinoid and metabolite SPE recoveries as reported in Klarich Wong et al. 2019. Average of three replicates ± standard deviation. The three neonicotinoid parent compounds (imidacloprid, clothianidin, thiamethoxam) and imidacloprid urea were extracted with an Oasis HLB extraction cartridge (Part No. 186000115).

| **Compound** | **Recovery** (% ± standard deviation of 3 replicates) |
|---|---|
| Imidacloprid | 105 ± 4.7 |
| Clothianidin | 103 ± 5.0 |
| Thiamethoxam | 120 ± 14 |
| Imidacloprid Urea | 57 ± 19 |

**Table S.4. Mass Spectrometer Settings:** Agilent 6460 Triple quadrupole mass spectrometer instrumental settings are provided below. These settings were used for all LC-MS/MS analysis.

| **MS/MS Parameters** | |
|---|---|
| Gas Temperature (N2) | 300 °C |
| Gas Flow | 5 L/min |
| Nebulizer Pressure | 45 psi |
| Sheath Gas Temperature | 250 °C |
| Sheath Gas Flow | 11 L/min |
| Capillary Voltage (+)/(-) | 3500/3500 V |
| Nozzle Voltage (+)/(-) | 500/500 V |

**Table S.5. Multiple Reaction Monitoring Mode (MRM) Transitions:** Agilent 6460 Triple quadrupole mass spectrometer neonicotinoid and neonicotinoid metabolite mass transitions used for analysis. The quantitation ion (quant ion) was used for quantifying neonicotinoid and metabolite concentrations. The qualitative ion (qual ion) was used to verify compound identity. The collision energies for each MRM are provided in parentheses following the quantitative and qualitative ions.

| Compound | RT (min) | Parent Ion (m/z) | Quant Ion (m/z) (Collision Energy, V) | Qual Ion (m/z) (Collision Energy, V) | Fragment Voltage (V) | Dwell Time (ms) | Polarity | Accelerator Voltage (V) |
|---|---|---|---|---|---|---|---|---|
| **Thiamethoxam** | 4.8 | 392.03 | 211 (8) | 181 (20) | 63 | 200 | Positive | 4 |
| **$d_3$-Thiamethoxam** | 4.9 | 295.05 | 214 (8) | 131.9 (20) | 71 | 200 | Positive | 4 |
| **Imidacloprid Urea** | 5.1 | 212.06 | 128 (16) | 99.1 (16) | 76 | 200 | Positive | 4 |
| **Clothianidin** | 6.8 | 250.02 | 169.1 (8) | 131.9 (12) | 67 | 200 | Positive | 4 |
| **Imidacloprid** | 7.9 | 256.06 | 213 (8) | 175.1 (12) | 67 | 200 | Positive | 4 |
| **$d_4$-Imidacloprid** | 7.8 | 260.09 | 213 (12) | 179.1 (16) | 59 | 200 | Positive | 4 |

**Table S.6. Lower limits of detection (LLD).** The LLD was determined by methods previously reported and outlined by the Standards Method 1030 E Method Detection Level.[5] A standard containing imidacloprid (30 nM), clothianidin (20 nM), thiamethoxam (0.5 nM), and imidacloprid urea (1 nM each) were injected 7 consecutive times on the LC-MS/MS. The standard deviations of the measured concentrations were calculated for each compound and LLD calculated by 2*SD*1.654. Multiplying the standard deviation by 2*(t-statistic), from a cumulative normal distribution curve, reduces the probability of a type I error.

| Compound | LLD (ng/L) | Solid Phase Extraction LLD (ng/L) |
|---|---|---|
| **Thiamethoxam** | 81.2 | 0.081 |
| **Imidacloprid Urea** | 56.8 | 0.057 |
| **Clothianidin** | 488 | 0.488 |
| **Imidacloprid** | 428 | 0.428 |

## SUPPORTING RESULTS.

**Table S.7.** WWTP-outfall processed flows and downstream 2 (DS2) flows on each sampling day (n=18) between 8/24/2018–8/29/2019. Flows are reported as they were provided from the North Liberty WWTP and USGS gaging station 05454090. Estimated wastewater contributions were calculated as outfall flow divided by DS2 flow *100%.

| Sample Date collected between 9 AM–10 AM | Outfall Processed Flow (MGD) | Outfall Processed Flow (Liters day$^{-1}$) | DS 2 Flow (ft$^3$ s$^{-1}$) | DS 2 Flow (Liters day$^{-1}$) | Estimated % wastewater at DS2 |
|---|---|---|---|---|---|
| 8/24/2018 | 1.763 | 6,674,000 | 24.3 | 59,500,000 | 11.2 |
| 9/17/2018 | 1.933 | 7,317,000 | 3.92 | 9,590,000 | 76.3 |
| 10/25/2018 | 1.873 | 7,090,000 | 7.82 | 19,100,000 | 37.1 |
| 11/13/2018 | 1.661 | 6,288,000 | 6.81 | 16,700,000 | 37.7 |
| 11/29/2018 | 1.685 | 6,378,000 | 6.97 | 17,100,000 | 37.4 |
| 12/13/2018 | 1.758 | 6,655,000 | 7.42 | 18,200,000 | 36.7 |
| 12/21/2018 | 1.568 | 5,936,000 | 6.90 | 16,900,000 | 35.2 |
| 1/16/2019 | 1.855 | 7,022,000 | 7.95 | 19,500,000 | 36.1 |
| 2/22/2019 | 1.501 | 5,682,000 | 5.23 | 12,800,000 | 44.4 |
| 3/8/2019 | 1.797 | 6,802,000 | 8.30 | 20,300,000 | 33.5 |
| 4/16/2019 | 1.789 | 6,772,000 | 10.3 | 25,200,000 | 26.9 |
| 5/16/2019 | 2.204 | 8,343,000 | 10.0 | 24,500,000 | 34.1 |
| 6/3/2019 | 2.266 | 8,578,000 | 10.9 | 26,700,000 | 32.2 |
| 6/24/2019 | 1.845 | 6,984,000 | 6.76 | 16,500,000 | 42.2 |
| 7/8/2019 | 1.645 | 6,227,000 | 3.49 | 8,540,000 | 72.9 |
| 7/18/2019 | 1.606 | 6,079,000 | 3.55 | 8,690,000 | 70.0 |
| 8/5/2019 | 1.516 | 5,739,000 | 2.04 | 4,990,000 | 115 |
| 8/29/2019 | 1.406 | 5,322,000 | 7.66 | 18,700,000 | 28.4 |
| **minimum** | 1.406 | 5,322,000 | 2.04 | 4,990,000 | 11.2 |
| **median** | 1.761 | 6,664,500 | 7.20 | 17,650,000 | 36.9 |
| **maximum** | 2.266 | 8,578,000 | 24.3 | 59,500,000 | 115 |

**Table S.8.** Field water-chemistry parameters (pH, water temperature, water conductivity, and dissolved oxygen) measured at upstream (US1), WWTP effluent outfall, downstream 1 (DS1), and downstream 2 (DS2) between 1/16/2019–8/29/2019 as those were the dates when the Hach probes were available. Water chemistry was not measured on 6/24/2019 and is denoted as N/A.

| Sample location and Date collected between 9 AM–10 AM | pH | Water Temp (°C) | Conductivity (µS $cm^{-1}$) | DO (mg $L^{-1}$) |
|---|---|---|---|---|
| US1: 1/16/2019 | 7.58 | 2.5 | 228 | 11.58 |
| US1: 2/22/2019 | 8.01 | 1 | 1416 | 11.73 |
| US1: 3/8/2019 | 7.56 | 1.7 | 857 | 11.85 |
| US1: 4/16/2019 | 7.79 | 9.3 | 821 | 9.87 |
| US1: 5/16/2019 | 7.75 | 14.9 | 729 | 8.32 |
| US1: 6/3/2019 | 7.49 | 16.4 | 657 | 8.21 |
| US1: 6/24/2019 | **N/A** | **N/A** | **N/A** | **N/A** |
| US1: 7/8/2019 | 7.77 | 23.9 | 761 | 9.53 |
| US1: 7/18/2019 | 7.56 | 21.8 | 718 | 6.69 |
| US1: 8/5/2019 | 7.84 | 21.5 | 774 | 6.94 |
| US1: 8/29/2019 | 7.56 | 21.8 | 718 | 6.69 |
| Outfall: 1/16/2019 | 7.58 | 14.6 | 842 | 9.46 |
| Outfall:2/22/2019 | 7.93 | 12.7 | 1610 | 9.25 |
| Outfall:3/8/2019 | 7.8 | 12.5 | 1933 | 9.1 |
| Outfall:4/16/2019 | 7.64 | 13.7 | 1579 | 9.66 |
| Outfall:5/16/2019 | 7.6 | 16 | 1259 | 8.89 |
| Outfall:6/3/2019 | 7.58 | 16.7 | 1342 | 8.97 |
| Outfall:6/24/2019 | **N/A** | **N/A** | **N/A** | **N/A** |
| Outfall:7/8/2019 | 7.51 | 20.7 | 1840 | 8.11 |
| Outfall:7/18/2019 | 7.82 | 20.2 | 1782 | 8.69 |
| Outfall:8/5/2019 | 7.82 | 20.2 | 1782 | 8.69 |
| Outfall:8/29/2019 | 7.51 | 20.7 | 1840 | 8.11 |
| DS1: 1/16/2019 | 7.7 | 9.3 | 1556 | 10.46 |
| DS1: 2/22/2019 | 7.9 | 8.4 | 1754 | 10.15 |
| DS1: 3/8/2019 | 7.86 | 9.5 | 1543 | 10.68 |
| DS1: 4/16/2019 | 7.45 | 11.8 | 1112 | 9.86 |
| DS1: 5/16/2019 | 7.65 | 15.8 | 1259 | 8.78 |
| DS1: 6/3/2019 | 7.58 | 16.1 | 968 | 8.71 |
| DS1: 6/24/2019 | **N/A** | **N/A** | **N/A** | **N/A** |
| DS1: 7/8/2019 | 7.69 | 21.5 | 1658 | 8.47 |
| DS1: 7/18/2019 | 7.82 | 20.7 | 1459 | 8.17 |
| DS1: 8/5/2019 | 7.92 | 20.8 | 1643 | 8.34 |
| DS1: 8/29/2019 | 7.82 | 20.7 | 1459 | 8.17 |
| DS2: 1/16/2019 | 8.18 | 4 | 1179 | 12.56 |
| DS2: 2/22/2019 | 8.28 | 1.4 | 1487 | 12.58 |
| DS2: 3/8/2019 | 8.13 | 4.4 | 1332 | 12.86 |
| DS2: 4/16/2019 | 8.07 | 10.1 | 950 | 10.92 |
| DS2: 5/16/2019 | 8.05 | 15 | 1062 | 9.67 |
| DS2: 6/3/2019 | 7.64 | 18.8 | 804 | 9.11 |
| DS2: 6/24/2019 | **N/A** | **N/A** | **N/A** | **N/A** |
| DS2: 7/8/2019 | 8.06 | 22 | 1090 | 8.24 |
| DS2: 7/18/2019 | 8.05 | 21.3 | 8.17 | 8.11 |
| DS2: 8/5/2019 | 8.13 | 21.8 | 1192 | 8.1 |
| DS2: 8/29/2019 | 8.05 | 21.3 | 904 | 8.11 |

**Table S.9.** US EPA aquatic life benchmarks (ALBs) for chronic and acute invertebrate exposure.

| Neonicotinoid | Chronic | Acute |
|---|---|---|
| Imidacloprid | 10 ng/L | 385 ng/L |
| Clothianidin | 50 ng/L | 11,000 ng/L |
| Thiamethoxam | 740 ng/L | 17,500 ng/L |
| Imidacloprid Urea | N/A | >47,400 mg/L |

**Table S.10.** Muddy Creek imidacloprid concentrations (ng/L) determined by SPE. Standard error represents the error associated with the regression from the calibration curve. N/A denotes instances where a sample was not collected, or the sample bottle was broken.

| Sample Date collected between 9 AM–10 AM | Upstream (US1) | Effluent | Downstream 1 (DS1) | Downstream 2 (DS2) |
|---|---|---|---|---|
| 8/24/2018 | 1.62 ±1.62 | 69.0 ± 1.75 | N/A | 61.7 ± 1.72 |
| 9/17/2018 | 43.8 ± 1.11 | 72.3 ± 1.12 | 67.3 ± 1.12 | 56.4 ± 1.11 |
| 10/25/2018 | 0.62 ± 26.8 | 4.98 ± 6.05 | 3.09 ± 8.12 | 2.13 ± 10.4 |
| 11/13/2018 | 1.63 ± 2.36 | 8.42 ± 1.70 | 4.94 ± 1.87 | 4.35 ± 1.91 |
| 11/29/2018 | 7.28 ± 1.74 | 104 ± 1.69 | 116 ± 1.72 | 9.55 ± 1.67 |
| 12/13/2018 | 1.80 ± 2.31 | 24.1 ± 1.52 | 5.72 ± 1.82 | 14.2 ± 1.58 |
| 12/21/2018 | 14.4 ± 1.58 | 75.4 ± 1.95 | 14.9 ± 1.58 | 7.02 ± 1.75 |
| 1/16/2019 | 5.58 ± 1.83 | 11.6 ± 1.62 | 16.3 ± 1.56 | 3.79 ± 1.97 |
| 2/22/2019 | 13.5 ± 1.59 | 25.9 ± 1.52 | 15.0 ± 1.58 | 6.14 ± 1.79 |
| 3/8/2019 | 6.55 ± 1.77 | 9.78 ± 1.66 | 11.3 ± 1.63 | 3.24 ± 2.03 |
| 4/16/2019 | 0.73 ± 2.84 | 22.2 ± 1.53 | 9.22 ±1.68 | 5.12 ± 1.85 |
| 5/16/2019 | 1.14 ± 2.56 | 22.1 ± 1.53 | 12.6 ± 1.61 | 7.24 ± 1.74 |
| 6/3/2019 | 6.22 ± 4.94 | 14.7 ± 3.77 | 10.0 ± 4.09 | 3.54 ± 6.69 |
| 6/24/2019 | 6.05 ± 5.00 | 26.1 ± 3.83 | 14.7 ± 3.77 | 7.81 ± 4.46 |
| 7/8/2019 | 3.57 ± 6.65 | 850 ± 31.1 | 601 ± 23.9 | 396 ± 17.5 |
| 7/18/2019 | 20.8 ± 3.73 | 17.8 ± 3.72 | 17.8 ± 2.95 | 74.2 ± 5.74 |
| 8/5/2019 | 0.94 ± 16.5 | N/A | 35.5 ± 4.13 | 19.9 ± 3.72 |
| 8/29/2019 | 6.24 ± 4.96 | 598 ± 23.5 | 427 ± 18.3 | 22.0 ± 3.74 |
| **minimum** | 0.62 | 4.98 | 3.09 | 2.13 |
| **median** | 5.82 | 24.1 | 14.9 | 7.53 |
| **maximum** | 43.8 | 850 | 601 | 396 |

**Table S.11.** Muddy Creek imidacloprid urea concentrations (ng/L) determined by SPE. Standard error represents the error associated with the regression from the calibration curve. N/A denotes instances where a sample was not collected, or the sample bottle was broken. ND indicates samples where imidacloprid urea was not detected.

| Sample Date collected between 9 AM–10 AM | Upstream (US1) | Effluent | Downstream 1 (DS1) | Downstream 2 (DS2) |
|---|---|---|---|---|
| 8/24/2018 | 7.97 ± 1.32 | 1.78 ±1.39 | N/A | 9.56 ± 1.33 |
| 9/17/2018 | 2.92 ± 1.18 | 0.75 ± 1.21 | 1.15 ± 1.20 | 1.08 ± 1.20 |
| 10/25/2018 | 0.49 ± 2.49 | ND | 0.27 ± 2.84 | 0.40 ± 2.60 |
| 11/13/2018 | 0.45 ± 1.90 | 0.18 ± 2.15 | 0.25 ± 2.07 | 0.55 ± 1.85 |
| 11/29/2018 | 1.16 ± 1.69 | 0.76 ± 1.77 | 2.83 ± 1.55 | 1.13 ± 1.69 |
| 12/13/2018 | 0.61 ± 1.82 | 0.52 ± 1.86 | 1.05 ± 1.71 | 0.47 ± 1.89 |
| 12/21/2018 | 0.67 ± 1.80 | ND | 0.78 ± 1.77 | 0.87 ± 1.75 |
| 1/16/2019 | 0.54 ± 1.85 | 0.51 ± 1.87 | 0.61 ± 1.82 | 0.74 ± 1.79 |
| 2/22/2019 | 0.60 ± 1.83 | ND | 1.19 ± 1.68 | 2.68 ± 1.55 |
| 3/8/2019 | ND | 0.49 ± 1.88 | 1.23 ± 1.67 | 0.97 ± 1.73 |
| 4/16/2019 | 0.45 ± 1.90 | 0.58 ± 1.84 | ND | 0.49 ± 1.87 |
| 5/16/2019 | 0.34 ± 1.97 | 0.64 ± 1.82 | 0.35 ± 1.96 | 0.53 ± 1.86 |
| 6/3/2019 | 1.69 ± 1.13 | 0.41 ± 1.19 | 0.47 ± 1.18 | 0.49 ± 1.18 |
| 6/24/2019 | 1.90 ± 1.12 | 0.55 ± 1.17 | 0.92 ± 1.15 | 0.93 ± 1.15 |
| 7/8/2019 | 3.55 ± 1.10 | 0.88 ± 1.15 | 1.39 ± 1.14 | 2.18 ± 1.12 |
| 7/18/2019 | 5.25 ± 1.09 | 0.81 ± 1.16 | 1.78 ± 1.12 | 5.31 ± 1.09 |
| 8/5/2019 | 3.97 ± 1.10 | N/A | 1.23 ± 1.14 | 1.47 ± 1.13 |
| 8/29/2019 | 6.06 ± 1.09 | 1.03 ± 1.15 | 1.89 ± 1.13 | 1.72 ± 1.13 |
| **minimum** | 0.34 | 0.18 | 0.25 | 0.4 |
| **median** | 1.16 | 0.61 | 1.10 | 0.95 |
| **maximum** | 7.97 | 1.78 | 2.83 | 9.56 |

**Table S.12.** Muddy Creek clothianidin concentrations (ng /L) determined by SPE. Standard error represents the error associated with the regression from the calibration curve. N/A denotes instances where a sample was not collected, or the sample bottle was broken.

| Sample Date collected between 9 AM–10 AM | Upstream (US1) | Effluent | Downstream 1 (DS1) | Downstream 2 (DS2) |
|---|---|---|---|---|
| 8/24/2018 | 4.32 ± 1.87 | 14.9 ± 1.59 | N/A | 13.6 ± 1.60 |
| 9/17/2018 | 14.0 ± 1.14 | 8.90 ± 1.15 | 9.71 ± 1.14 | 8.91 ± 1.15 |
| 10/25/2018 | 11.9 ± 2.61 | 8.40 ± 2.80 | 7.54 ± 2.88 | 6.77 ± 2.97 |
| 11/13/2018 | 13.2 ± 1.33 | 7.72 ± 1.40 | 7.07 ± 1.41 | 8.84 ± 1.38 |
| 11/29/2018 | 20.8 ± 1.30 | 27.2 ± 1.30 | 35.0 ± 1.30 | 22.2 ± 1.30 |
| 12/13/2018 | 18.2 ± 1.31 | 12.6 ± 1.34 | 16.5 ± 1.32 | 15.7 ± 1.32 |
| 12/21/2018 | 19.4 ± 1.31 | 134 ± 1.43 | 66.4 ± 1.34 | 54.4 ± 1.32 |
| 1/16/2019 | 18.7 ± 1.31 | 20.1 ± 1.30 | 28.3 ± 1.30 | 13.9 ± 1.33 |
| 2/22/2019 | 13.0 ± 1.34 | 77.7 ± 1.35 | 92.8 ± 1.38 | 61.2 ± 1.33 |
| 3/8/2019 | 59.1 ± 1.33 | 72.5 ± 1.35 | 58.8 ± 1.33 | 39.8 ± 1.30 |
| 4/16/2019 | 12.3 ± 1.34 | 15.8 ± 1.32 | 11.4 ± 1.35 | 10.7 ± 1.36 |
| 5/16/2019 | 29.9 ± 1.30 | 12.8 ± 1.34 | 17.5 ± 1.31 | 23.9 ± 1.30 |
| 6/3/2019 | 17.9 ± 1.15 | 4.90 ± 1.20 | 9.32 ± 1.16 | 8.57 ± 1.17 |
| 6/24/2019 | 20.0 ± 1.15 | 8.92 ± 1.17 | 11.2 ± 1.16 | 12.3 ± 1.16 |
| 7/8/2019 | 11.3 ± 1.16 | 9.20 ± 1.16 | 9.61 ± 1.16 | 8.95 ± 1.17 |
| 7/18/2019 | 5.58 ± 1.19 | 8.68 ± 1.17 | 8.44 ± 1.17 | 5.84 ± 1.19 |
| 8/5/2019 | 3.46 ± 1.22 | N/A | 7.13 ± 1.18 | 6.33 ± 1.18 |
| 8/29/2019 | 3.97 ± 1.21 | 12.0 ± 1.16 | 10.9 ± 1.16 | 6.09 ± 1.18 |
| **minimum** | 3.46 | 4.90 | 7.07 | 5.84 |
| **median** | 13.6 | 12.6 | 11.2 | 11.5 |
| **maximum** | 59.1 | 134 | 92.8 | 61.2 |

**Table S.13.** Muddy Creek thiamethoxam concentrations (ng/L) determined by SPE. Standard error represents the error associated with the regression from the calibration curve. N/A denotes instances where a sample was not collected, or the sample bottle was broken. ND indicates samples where thiamethoxam was not detected.

| Sample Date collected between 9 AM–10 AM | Upstream (US1) | Effluent | Downstream 1 (DS1) | Downstream 2 (DS2) |
|---|---|---|---|---|
| 8/24/2018 | 0.54 ± 1.60 | 3.34 ± 1.38 | N/A | 9.17 ± 1.34 |
| 9/17/2018 | 1.11 ± 1.09 | 1.96 ± 1.08 | 1.88 ± 1.08 | 1.21 ± 1.09 |
| 10/25/2018 | 0.63 ± 3.41 | ND | 0.65 ± 3.38 | 0.68 ± 3.34 |
| 11/13/2018 | 1.30 ± 2.30 | 0.56 ± 2.74 | 0.62 ± 2.67 | 0.91 ± 2.47 |
| 11/29/2018 | 3.11 ± 1.93 | 2.08 ± 2.09 | 5.27 ± 1.76 | 1.31 ± 2.30 |
| 12/13/2018 | 1.16 ± 2.35 | 1.28 ± 2.30 | 0.86 ± 2.50 | 1.19 ± 2.34 |
| 12/21/2018 | 0.83 ± 2.51 | ND | 2.73 ± 1.98 | 2.66 ± 2.00 |
| 1/16/2019 | 4.81 ± 1.79 | 4.52 ± 1.81 | 11.3 ± 1.56 | 43.8 ± 1.46 |
| 2/22/2019 | ND | ND | 8.07 ± 1.65 | 6.08 ± 1.71 |
| 3/8/2019 | ND | 14.1 ± 1.52 | ND | ND |
| 4/16/2019 | 16.4 ± 1.50 | ND | 4.62 ± 1.80 | 2.63 ± 2.00 |
| 5/16/2019 | ND | 3.89 ± 1.85 | 2.77 ± 1.98 | 2.90 ± 1.96 |
| 6/3/2019 | 5.56 ± 1.05 | 1.07 ± 1.06 | 2.32 ± 1.05 | 1.78 ± 1.06 |
| 6/24/2019 | 3.69 ± 1.05 | 2.23 ± 1.05 | 2.24 ± 1.05 | 1.57 ± 1.06 |
| 7/8/2019 | 1.05 ± 1.06 | 1.03 ± 1.06 | 1.00 ± 1.07 | 0.81 ± 1.07 |
| 7/18/2019 | 1.00 ± 1.07 | 1.06 ± 1.06 | 0.70 ± 1.06 | 0.55 ± 1.07 |
| 8/5/2019 | 0.31 ± 1.08 | N/A | 1.07 ± 1.06 | 0.88 ± 1.07 |
| 8/29/2019 | 0.12 ± 1.10 | 0.73 ± 1.07 | 0.67 ± 1.07 | 0.53 ± 1.08 |
| **minimum** | 0.12 | 0.56 | 0.62 | 0.53 |
| **median** | 1.11 | 1.96 | 2.06 | 1.31 |
| **maximum** | 16.4 | 14.1 | 11.3 | 43.8 |

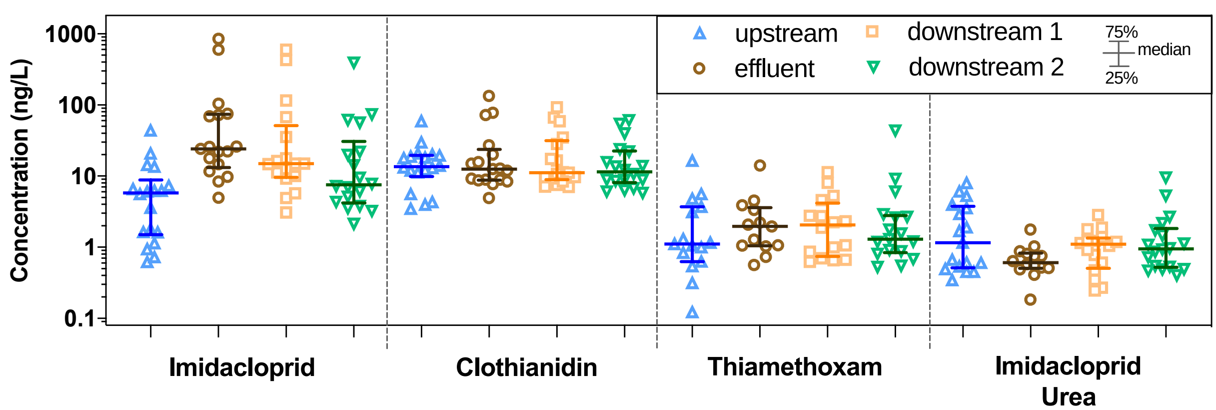


**Figure S.7**. Distributions of imidacloprid, clothianidin, thiamethoxam, and imidacloprid urea concentrations measured at the four sampling locations between 1/16/2019– 8/24/2019.

**Table S.14.** Ratio two-tailed paired t-tests of Muddy Creek neonicotinoid concentrations ($\alpha$=0.05). The neonicotinoid concentrations at each sampling site were log-normally distributed according to Shapiro-Wilks Normality test ($\alpha$= 0.05). Ratio paired t-tests were used to account for the wide distribution in concentrations analyzed at each site and thus, values of 0.5*LLD were used to replace non-detects to allow analysis of the full datasets for each neonicotinoid. Asterisks denote level of significance: p<0.05 (*), p<0.005 (**), p<0.0005 (***), p<0.0001 (****).

| Site A and Site B | Imidacloprid | Clothianidin | Thiamethoxam | Imidacloprid urea |
|---|---|---|---|---|
| Upstream 1 and Effluent | <0.0001**** | 0.5286 | 0.9657 | 0.0117* |
| Upstream 1 and Downstream 1 | 0.0005*** | 0.3887 | 0.1289 | 0.3854 |
| Upstream 1 and Downstream 2 | 0.0096* | 0.7718 | 0.1396 | 0.9459 |
| Effluent and Downstream 1 | 0.0041** | 0.5374 | 0.2484 | 0.0690 |
| Effluent and Downstream 2 | 0.0005*** | 0.2136 | 0.2770 | 0.0037** |
| Downstream 1 and Downstream 2 | 0.0132* | 0.0154* | 0.3704 | 0.1494 |

**Mass Load Calculations:** Mass loads (mg/d) were calculated for sampling sites where flow data were available (i.e., wastewater outfall and downstream 2 (DS2, USGS gaging station 05454090). Flow data from the wastewater treatment plant (WWTP) was provided by the North Liberty WWTP as the daily processed flow (million gallons per day, MGD) and converted to liters per day (**Table S.7**). Flow data from DS2 was obtained from the USGS National Water Information System (USGS gaging station 05454090, **Table S.7**) for the sampling dates at the approximate time of sampling (~9 AM). Instantaneous concentrations were converted to a daily mass load assuming the neonicotinoid concentration in treated wastewater effluent (and subsequently DS2) were relatively consistent throughout the day (a previous study showed relatively consistent concentrations of imidacloprid in WWTP effluent over 5 consecutive sampling days).[6] Considering mass load calculations were calculated on a per day scale and all sampling was done during baseflow conditions, DS2 flow rates were converted from cubic feet per second (cfs) to liters per day assuming flow rate was relatively consistent throughout the day (± 2 cfs).

**Table S.15.** Mass loads (mg/d) at the WWTP outfall (effluent) as determined by the instantaneous concentration and flow rate. N/A denotes instances where a sample was not collected, or the sample bottle was broken. N/A denotes instances where a sample was not collected, or the sample bottle was broken. ND indicates samples where imidacloprid urea was not detected.

| Sample Date collected between 9 AM–10 AM | Effluent Imidacloprid Mass Load (mg/d) | Effluent Clothianidin Mass Load (mg/d) | Effluent Thiamethoxam Mass Load (mg/d) | Effluent Imidacloprid Urea Mass Load (mg/d) |
|---|---|---|---|---|
| 8/24/2018 | 461 | 99.8 | 22.3 | 11.9 |
| 9/17/2018 | 529 | 65.1 | 14.3 | 5.51 |
| 10/25/2018 | 35.3 | 59.5 | ND | ND |
| 11/13/2018 | 53.0 | 48.6 | 3.53 | 1.16 |
| 11/29/2018 | 665 | 174 | 13.3 | 4.83 |
| 12/13/2018 | 161 | 83.5 | 8.51 | 3.47 |
| 12/21/2018 | 448 | 798 | ND | ND |
| 1/16/2019 | 81.7 | 141 | 31.7 | 3.58 |
| 2/22/2019 | 147 | 441 | ND | ND |
| 3/8/2019 | 66.5 | 493 | ND | 3.32 |
| 4/16/2019 | 150 | 107 | ND | 3.93 |
| 5/16/2019 | 184 | 107 | 32.5 | 5.31 |
| 6/3/2019 | 126 | 42.1 | 9.15 | 3.53 |
| 6/24/2019 | 182 | 62.3 | 15.6 | 3.87 |
| 7/8/2019 | 5290 | 57.3 | 6.42 | 5.49 |
| 7/18/2019 | 108 | 52.8 | 6.43 | 4.90 |
| 8/5/2019 | N/A | N/A | N/A | N/A |
| 8/29/2019 | 3180 | 63.8 | 3.90 | 5.49 |
| **minimum** | 35.3 | 42.1 | 3.53 | 1.16 |
| **median** | 161 | 83.5 | 11.2 | 4.38 |
| **maximum** | 5290 | 798 | 32.5 | 11.9 |

**Table S.16.** Mass loads (mg/d) at downstream 2 (DS2) as determined by the instantaneous concentration and flow rate. N/A denotes instances where a sample was not collected, or the sample bottle was broken. N/A denotes instances where a sample was not collected, or the sample bottle was broken. ND indicates samples where imidacloprid urea was not detected.

| Sample Date collected between 9 AM–10 AM | Downstream 2 (DS2) Imidacloprid Mass Load (mg/d) | Downstream 2 (DS2) Clothianidin Mass Load (mg/d) | Downstream 2 (DS2) Thiamethoxam Mass Load (mg/d) | Downstream 2 (DS2) Imidacloprid Urea Mass Load(mg/d) |
|---|---|---|---|---|
| 8/24/2018 | 3670 | 810 | 545 | 568 |
| 9/17/2018 | 541 | 85.5 | 11.6 | 10.4 |
| 10/25/2018 | 40.7 | 129 | 13.0 | 7.72 |
| 11/13/2018 | 72.5 | 147 | 15.2 | 9.11 |
| 11/29/2018 | 163 | 379 | 22.3 | 19.3 |
| 12/13/2018 | 258 | 286 | 21.6 | 8.52 |
| 12/21/2018 | 118 | 919 | 45.0 | 14.6 |
| 1/16/2019 | 73.7 | 271 | 852 | 14.1 |
| 2/22/2019 | 78.5 | 783 | 77.8 | 34.3 |
| 3/8/2019 | 65.8 | 809 | ND | 19.7 |
| 4/16/2019 | 129 | 269 | 66.4 | 12.4 |
| 5/16/2019 | 177 | 585 | 70.9 | 13.0 |
| 6/3/2019 | 94.4 | 228 | 47.5 | 13.1 |
| 6/24/2019 | 129 | 203 | 26.0 | 15.4 |
| 7/8/2019 | 3380 | 76.4 | 6.88 | 18.6 |
| 7/18/2019 | 645 | 50.7 | 4.77 | 46.1 |
| 8/5/2019 | 99.5 | 31.6 | 4.38 | 7.33 |
| 8/29/2019 | 413 | 114 | 9.91 | 32.3 |
| **minimum** | 40.7 | 31.6 | 4.38 | 7.33 |
| **median** | 129 | 249 | 22.3 | 14.4 |
| **maximum** | 3670 | 919 | 852 | 568 |

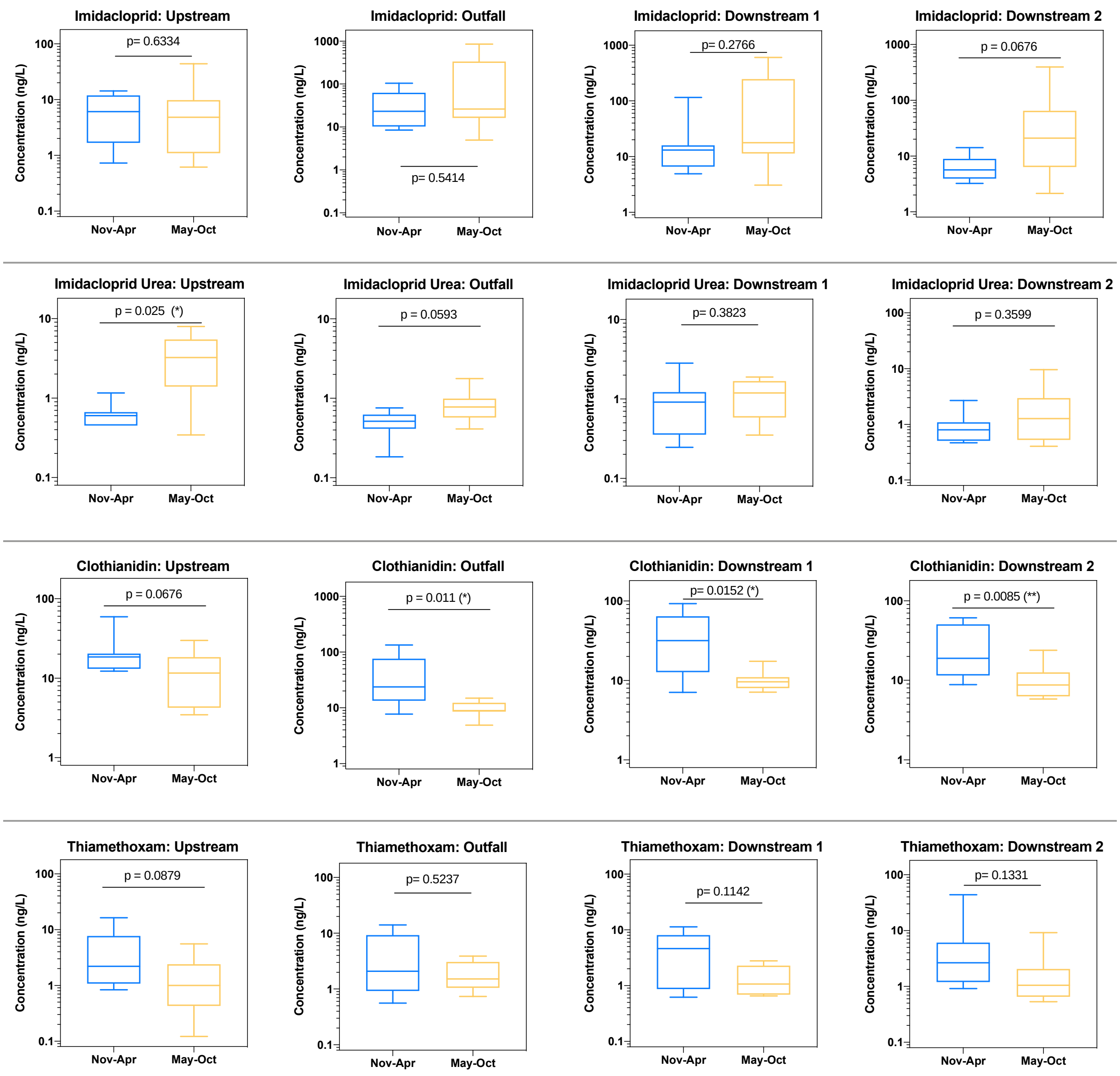


**Figure S.8**. Concentration distributions of imidacloprid (top row), imidacloprid urea (second row), clothianidin (third row), and thiamethoxam (bottom row) at each sampling location in the cool season (November–April, water temperature below 10 °C at US1) and warm season (May–October, water temperature above 10 °C at US1). Statistical comparisons are based on a Mann-Whitney unpaired nonparametric t-test ($\alpha$=0.05).

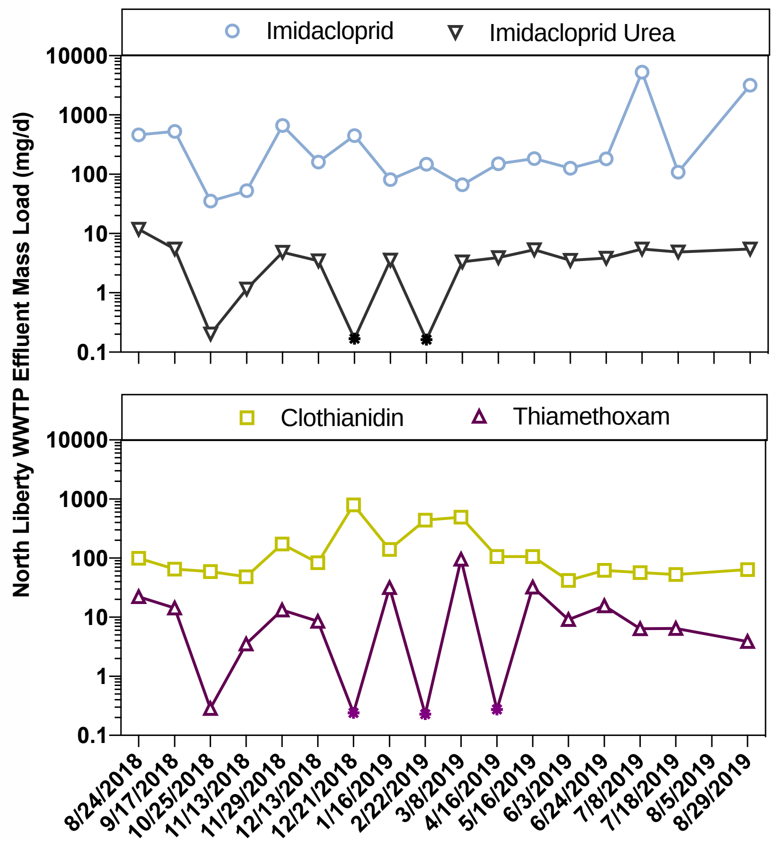


**Figure S.9**. Mass load of imidacloprid (blue circles), imidacloprid urea (black inverted triangles), clothianidin (yellow squares), and thiamethoxam (purple triangles) in the wastewater effluent. Instances where a neonicotinoid was not detected (imidacloprid urea and thiamethoxam) are indicated with an asterisk and were calculated with the censored data using the LLD/2. Mass loads in the effluent are relatively constant except for increases in clothianidin and thiamethoxam in the cooler months.

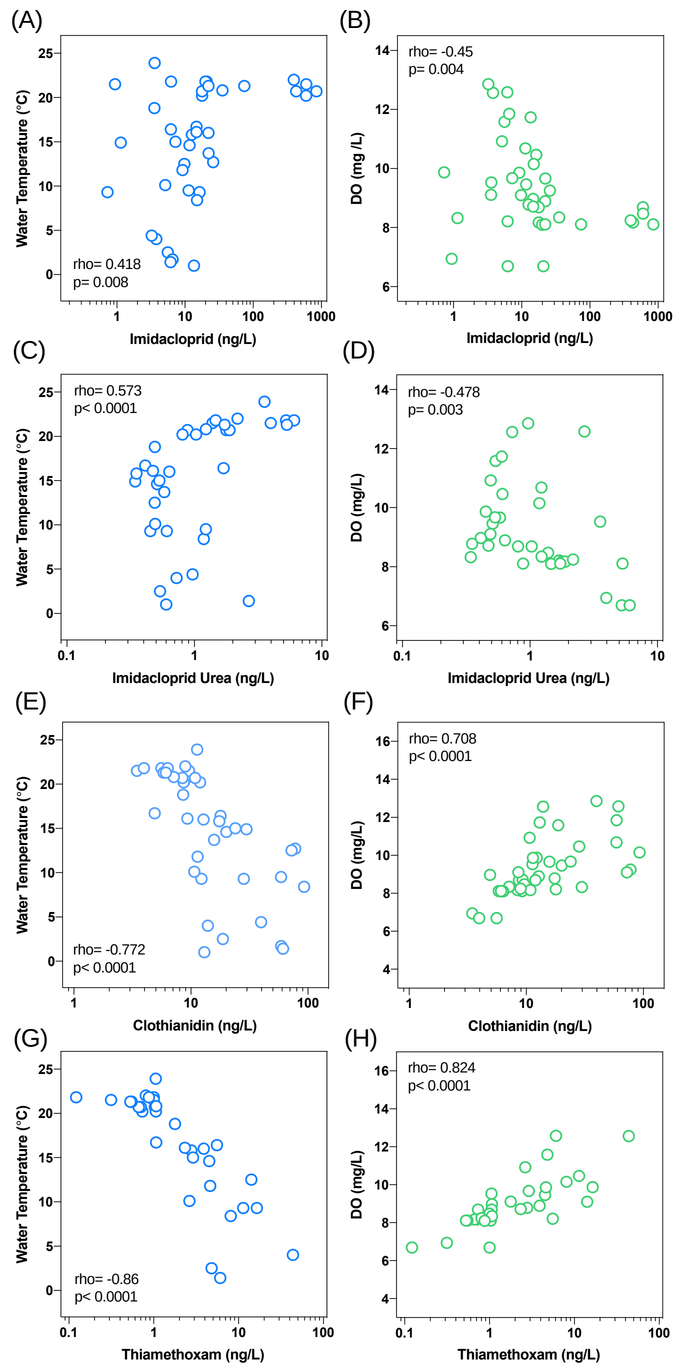


**Figure S.10**. Correlations between water temperature and dissolved oxygen and the concentrations of imidacloprid (A-B), imidacloprid urea (C-D), clothianidin (E-F), and thiamethoxam (G-H) at all sites for dates when bulk water-quality parameters for the stream were measured (between 1/16/2019 and 8/24/2019). Imidacloprid and imidacloprid urea are positively correlated with water temperature and negatively correlated with dissolved oxygen, suggesting seasonal effects where concentrations are higher during the warm season. Clothianidin and thiamethoxam concentrations are negatively correlated with water temperature and positively correlated with dissolved oxygen, suggesting seasonal effects where concentrations are greatest in the cool season.

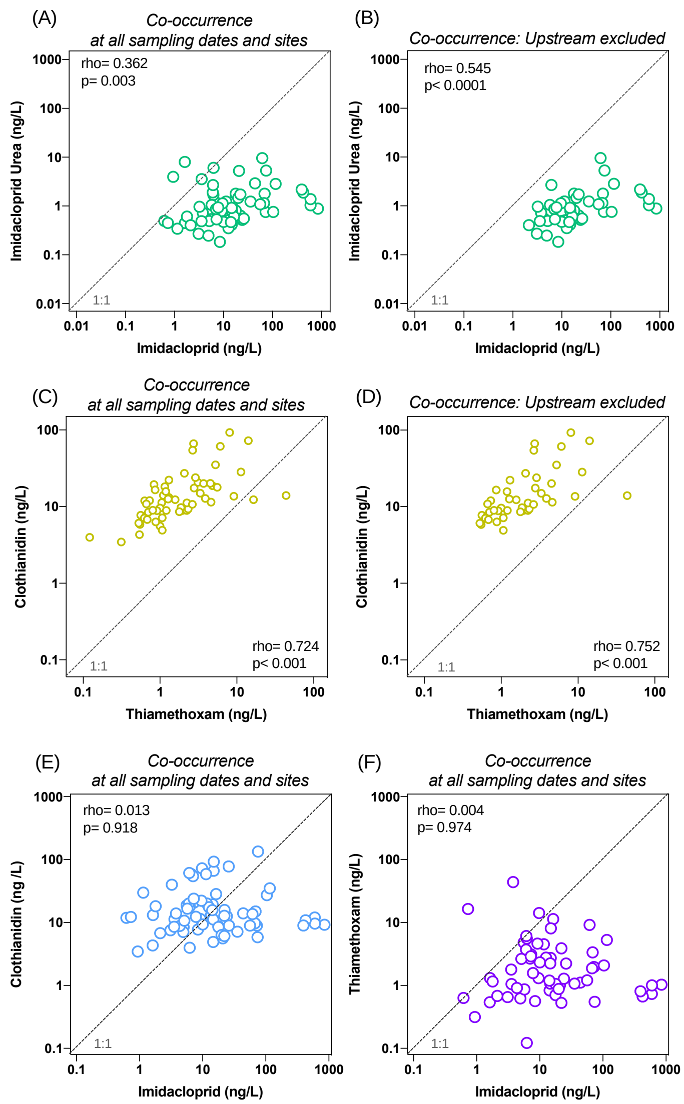


**Figure S.11.** Correlations between imidacloprid and imidacloprid urea concentrations at all sampling sites and dates (A) and excluding those measured upstream (B). Correlations between clothianidin and thiamethoxam concentrations at all sampling sites and dates (C) and excluding those measured upstream (D). Correlation between imidacloprid with clothianidin (E) and thiamethoxam (F) concentrations at all sampling sites. Non-parametric two-tailed spearman correlations ($\alpha$=0.05) indicate that imidacloprid and imidacloprid urea are significantly correlated with one another in all samples. However, if the upstream data is omitted, the correlation becomes stronger (rho= 0.545 compared to 0.362) indicating the effluent is a significant source of imidacloprid and, in effect, indirectly impacts downstream concentrations of imidacloprid urea. Clothianidin and thiamethoxam are strongly and significantly correlated (rho>0.7) at all sampling sites, which does not substantially change when the upstream concentrations are omitted, indicating that the upstream and outfall are roughly equivalent sources for clothianidin and thiamethoxam in Muddy Creek. There was no significant correlation between imidacloprid and clothianidin or thiamethoxam.

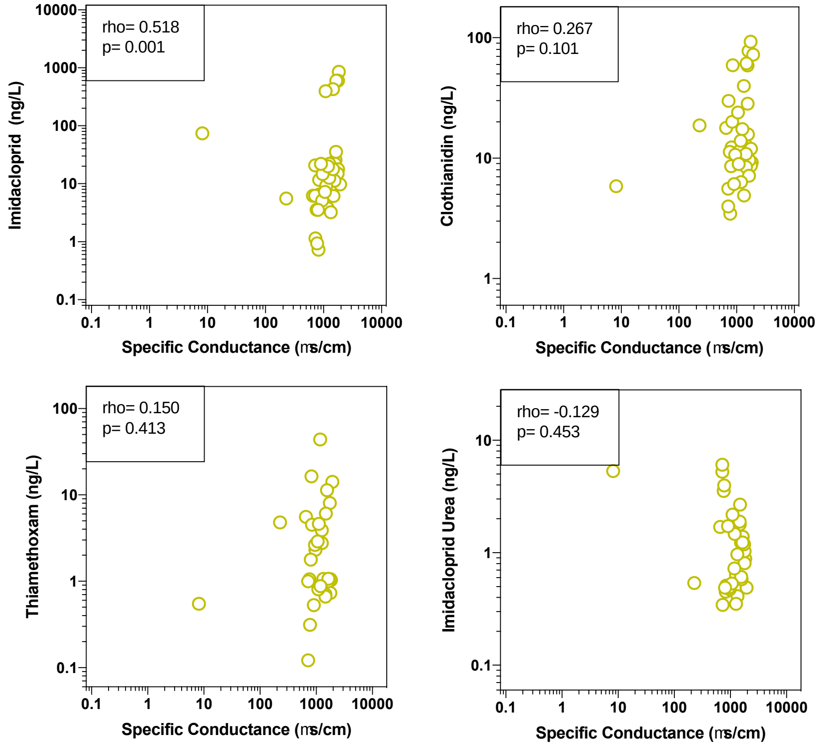


**Figure S.12**. Correlations between neonicotinoid concentration and specific conductance (μs/cm) at all sampling sites when specific conductance was measured in the stream (1/16/2019–8/24/2019). Spearman correlation coefficients and significance of the correlation are provided ($\alpha$=0.05). Imidacloprid was the only one of the four neonicotinoid compounds where concentration was significantly correlated with specific conductance.

***Data Analysis:* Possible Sources**

**North Liberty Drinking Water:** To assess the potential contribution of source/tap water to neonicotinoid concentrations at the North Liberty WWTP, two 1-L samples of source water were collected at the North Liberty drinking water treatment plant (DWTP, raw water prior to any treatment) and analyzed for imidacloprid, clothianidin, thiamethoxam, and imidacloprid urea. The North Liberty DWTP obtains its source water from deep groundwater wells tapping the Jordan and Silurian aquifers. Samples were analyzed by SPE as described above and are reported in **Table S.23**. None of the parent neonicotinoids (imidacloprid, clothianidin, or thiamethoxam) were detected in North Liberty source water above their LLDs indicating that source/tap water is not a significant contributor to neonicotinoid concentrations in the North Liberty WWTP effluent. Imidacloprid urea was detected in one of the source water samples (at 0.37 ng/L) and thus, source could possibly be a contributor to imidacloprid urea in North Liberty WWTP effluent.

**Table S.17.** Concentrations of imidacloprid, clothianidin, thiamethoxam, and the metabolite imidacloprid urea in North Liberty source water. Duplicate samples were collected at the North Liberty drinking water treatment plant at 8 AM in acid washed 1 L amber bottles.

| | Imidacloprid | Clothianidin | Thiamethoxam | Imidacloprid urea |
|---|---|---|---|---|
| North Liberty source water A | <LLD | <LLD | ND | 0.370 |
| North Liberty source water B | <LLD | ND | ND | ND |

**Registered neonicotinoid products in Iowa:** Lists of products registered for use in Iowa that contain the neonicotinoids imidacloprid, clothianidin, and thiamethoxam were obtained from the Iowa Department of Agriculture and Land Stewardship Pesticide Bureau.[7] Products containing imidacloprid, clothianidin, and thiamethoxam were divided into five use categories: agriculture (seed and foliage treatment), lawn and garden (sod, turf, trees, shrubs, flowers), interior and exterior buildings uses (homes, restaurants, institutions, businesses, barns), pets (topical preventatives, treated collars, shampoo for cats and dogs), and other (manufacturing and unspecified uses). Imidacloprid was present in 549 products in Iowa with greatest uses in lawn/garden care (37.5%), pets (30.4%), and agriculture (20.5%). Thiamethoxam was present in fewer products (54 products), with uses primarily in agriculture (72.2%) and lawn/garden care (18.5%). Clothianidin was in 45 products and used primarily in agriculture (48.9%), indoors and outdoors of buildings (28.9%), and lawn and garden care (20%). A summary is shown in the main manuscript Figure 4.

**Estimated food residue contributions:** Neonicotinoid mass loads related to the consumption of residues in food was estimated based on the residue concentrations detected in produce reported in the USDA Pesticide Data Program from 2018 (**Figure S.14**). Median food residue concentrations were 0.010 ppm imidacloprid, 0.007 ppm clothianidin, and 0.015 ppm thiamethoxam, and reflect concentrations from washed, fresh weight produce that was homogenized and extracted by QuEChRS methods.[8] Between 1994-2018, clothianidin and thiamethoxam were detected in similar types of produce, including spinach, kale, sweet bell peppers, watermelon, potatoes, cherries, tomatoes, hot peppers, and cherry tomatoes.[9] Assuming everyone in North Liberty, Iowa (US Census Bureau 2019 estimate: 19,501)[1] consumed the recommended 125 grams of fruits and vegetables per serving[10] and the average North American consumes about 5 servings of fruits and vegetables per day (each of which contained the median neonicotinoid concentrations),[10] it was estimated that 122 mg imidacloprid, 85 mg clothianidin, and 183 mg thiamethoxam would enter the WWTP each day. Studies assessing neonicotinoid metabolism in mice showed that 87% imidacloprid, 36% clothianidin, and 63% thiamethoxam was metabolized.[11] Thus, the estimated food residue daily mass loads for imidacloprid, clothianidin, and thiamethoxam entering the WWTP were adjusted to reflect possible human metabolism, including the transformation of 12% of thiamethoxam into clothianidin—an assumption we based on Thompson et al[11]—leaving 16 mg/d imidacloprid, 77 mg/d clothianidin, and 50 mg/d thiamethoxam. The mass loads entering the WWTP were adjusted downward by 25% to account for estimated removal within the WWTP (based on reports in the literature),[6] resulting in treated North Liberty WWTP effluent mass loads of 12 mg/d imidacloprid, 57 mg/d clothianidin, and 37 mg/d thiamethoxam.

The calculated food residue mass load for imidacloprid (12 mg/d) likely does not account for the full mass loads calculated for the North Liberty WWTP (median mass load of 161 mg/d) based on our measured concentrations. The calculated food residue mass load for clothianidin (57 mg/d) could account for some of the mass load observed in the North Liberty WWTP (median mass load of 83.5 mg/d but is unlikely to account for the whole clothianidin mass load leaving the WWTP—particularly in the context of episodic higher spike levels observed. Other registered products containing clothianidin are thus thought to be significant contributors to the clothianidin mass load in the North Liberty WWTP (e.g., pest control products for indoor and outdoor building, and lawn and garden applications), especially on the occasions where there are spikes in clothianidin mass loads (mass loads >2x the median level). Indeed, we would expect the contributions from food residue to remain stable through time, whereas episodic spikes in concentration in clothianidin are more likely to be attributed to other pest control applications. For thiamethoxam, the calculated food residue mass load estimate of 47 mg/d was nearly 4-fold higher than that of the North Liberty WWTP effluent mass loads (median mass load of 11 mg/d and maximum mass load of 32.5 mg/d, based on measured concentrations and flow values from the WWTP). 72% of the products containing thiamethoxam in Iowa are registered for use in agricultural practices, therefore it is likely that the thiamethoxam detected in the North Liberty WWTP effluent is from food residues (**Figure S.13, S.14**). However, considering the estimated food residue mass load is 4-fold higher than that calculated for the WWTP effluent, it is unlikely that all the produce consumed within North Liberty contains the full residue concentrations of thiamethoxam or other removal processes occur.

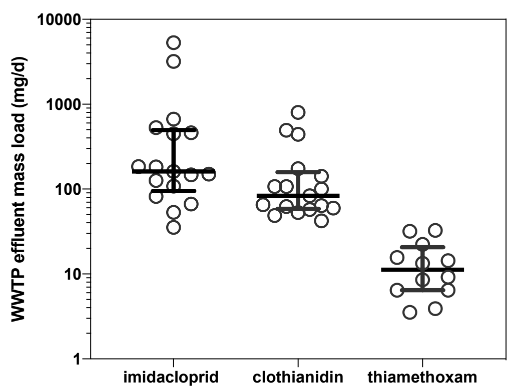


**Figure S.13.** Distribution of imidacloprid, clothianidin, and thiamethoxam mass loads (mg/d) calculated for the North Liberty WWTP. Dark lines indicate the median mass loads and the interquartile ranges. Median values were used to compare effluent mass loads (clothianidin [83.5 mg/d], thiamethoxam [11.2 mg/d], and imidacloprid [161 mg/d]) to those estimated for food residues (clothianidin [78.2 mg/d], thiamethoxam [44.4 mg/d], and imidacloprid [13.1 mg/d]).

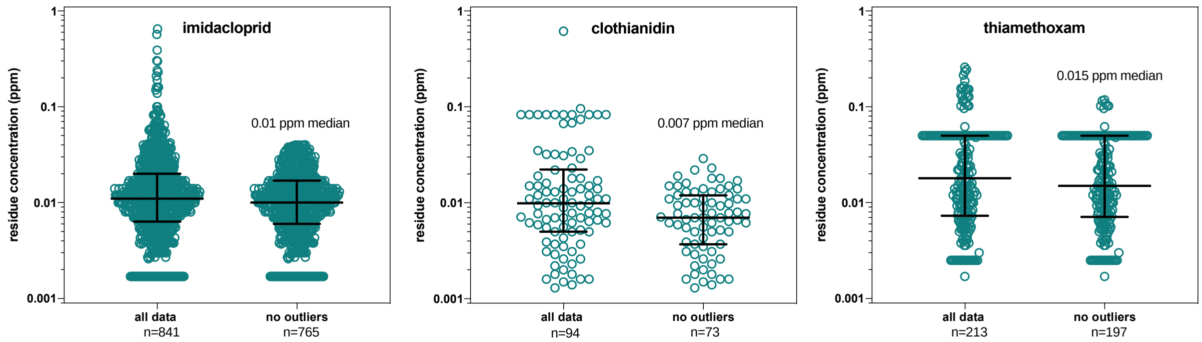


**Figure S.14.** Distribution of imidacloprid, clothianidin, and thiamethoxam residues for 2018 as reported by the USDA Pesticide Data Program. Dark lines indicate the median mass loads and the interquartile ranges. Data were filtered (right side of each plot) to remove statistical outliers using the ROUT Method[12] at the 1% false discovery rate where median food residue concentrations were 0.010 ppm imidacloprid, 0.007 ppm clothianidin, and 0.015 ppm thiamethoxam. These median values were used to estimate food residue contributions to effluent mass load in this study.[9]

**Estimated pet preventative contributions:** Imidacloprid is the only neonicotinoid, included in this study, to be registered for use as an insecticide for flea and tick prevention for pets in Iowa.[7] 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 that indoor cats are less likely to be treated for ticks/fleas. The average sized dog in the U.S. is approximately 20-55 lbs (medium sized),[13] to which imidacloprid pet preventatives are applied via a monthly topical treatment or 8-month collar (supplying ~250–450 mg/dog/month). The monthly imidacloprid concentration was converted to a daily dose washed down the drain, assuming the imidacloprid in the preventative evenly dissipated throughout the month following bathing, washing linens in contact with treated pets, petting of dogs and washing hands, etc.[14] The total number of dogs in North Liberty, Iowa, was estimated using population data from the US Census Bureau (2019, total population of 19,500, 2.56 people per household)[1] and population statistics provided by the American Veterinary Medical Association (AVMA, see below).[15]

Number of households:

(total population/ average number of people per household)

Number of dogs in the community:

(Number of households)*(0.614)

where the factor of 0.614 is the product of the average U.S. percentage of households owning dogs and the average number of pets per household.[15] Previous studies have estimated that 75% of pet owners use some kind of insect preventative, and this value was used here to estimate the number of dogs in North Liberty treated with flea and tick preventative.[6,16] Of all the products registered for flea and tick preventatives in Iowa, 20.2% contain imidacloprid.[7] Thus, we estimated that of the dogs within North Liberty (4,677 dogs), 75% of them use a preventative (3,258 dogs). Assuming 20% of the dogs treated with preventative are treated with a preventative containing imidacloprid (as would be representative of the market-share of the pesticide), imidacloprid would be used on an estimated 658 dogs. The estimated daily mass load of imidacloprid in the WWTP effluent was calculated by multiplying the number of imidacloprid treated dogs by the concentration of imidacloprid estimated from each dog per day (whether that be from human contact and subsequent washing down the sink, washing bedding / pet linens, or washing the dog themselves).[14,17,18] Thus, the maximum North Liberty WWTP daily mass load of imidacloprid due to flea and tick preventatives was estimated to be 5,480–9,970 mg/d (average ~7,000 mg/d) which corresponds to 100% of the applied imidacloprid being evenly washed off the dog/bedding/owners hands throughout the month (a very conservative estimate). More realistically, substantially less than 100% of the applied imidacloprid would be washed down a drain. Assuming 10% of the imidacloprid enters the drain, the daily mass load to the WWTP would range between 550-1,000 mg/d (700 mg/d average); assuming 25% wash off would range between 1,400-2,500 mg/d (1,800 mg/d average); assuming 50% wash off would the mass load would be between 2,700-5,000 mg/d (3,500 mg/d average).

**Estimated indoor pest control contributions for Clothianidin:** Clothianidin is registered for use in Iowa as either an agricultural insecticide (i.e., seed treatment, spray) and for indoor/outdoor dwelling pest control (e.g., cockroaches, bed bugs, ants, beetles, flies).[7] Clothianidin-containing indoor/outdoor dwelling pest control products are composed of 0.01–23% clothianidin. One product that is used for insect indoor/domestic prevention of multiple insects (including ants, aphids, beetles, centipedes, cockroaches, fleas, flies, and silverfish) is a spray that contains 3.2% clothianidin. According to mixing instructions, the final concentration of clothianidin, ready for use, would be 0.25 g/L for a 1,000 square foot application for routine 90-day residue (i.e., maintenance program), or 0.011 mg/ft$^2$/day. If the entire clothianidin mass loads on the dates where clothianidin was most concentrated (174 [11/29/2018], 798 [12/21/2018], 441 [2/22/2019], and 493 mg/d [3/8/2019]) were completely due to indoor pest control spraying inadvertently being washed down the drain (by drift, washing rugs or towels, or touching /washing contaminated surfaces), these mass loads would correspond to ~16-75 "doses" of clothianidin (equivalent to treating ~16,000–75,000 ft$^2$). North Liberty is a rapidly growing commuter suburb in East-Central Iowa where many multi-resident buildings have routine insecticide spraying maintenance programs, therefore, use of indoor clothianidin spraying might account for portions of the clothianidin mass loads at the North Liberty WWTP. These programs could also be using products containing imidacloprid and thiamethoxam, contributing to their mass loads in wastewater effluent and human exposure.

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