Mass Balance Assessment for Six Neonicotinoid Insecticides During Conventional Wastewater and Wetland Treatment: Nationwide Reconnaissance of U.S. Wastewater

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Summary
This supporting information contains 16 pages, including 6 tables, and 3 figures.
Table S1. Chemical Abstracts Service (CAS) Number and Molecular Design Limited (MDL) Number of Analytes

| analyte               | CAS no.      | MDL no.          |
|----------------------|--------------|------------------|
| imidacloprid         | 138261-41-3  | MFCD00468059     |
| acetamiprid          | 135410-20-7  | MFCD06201842     |
| acetamiprid-N-desmethyl | 190604-92-3  | MFCD08690484     |
| clothianidin         | 210880-92-5  | MFCD06200753     |
| thiacloprid          | 111988-49-9  | MFCD02101042     |
| thiamethoxam         | 153719-23-4  | MFCD03792862     |
| dinotefuran          | 165252-70-0  | MFCD06795001     |
| imidacloprid-$d_4$   | 1015855-75-0 | MFCD09037342     |
| acetamiprid-$d_3$    | N/A          | MFCD17019132     |
| clothianidin-$d_3$   | 1262776-24-8 | MFCD17019117     |

N/A, not available
Sampler programming

Seven portable automated samplers (6712 Full-Size Portable Sampler, Teledyne Isco, Lincoln, NE, USA) were programmed based on three-week average hourly-daily flow rate data.

24 subsamples were merged to get about 2.5 liters of daily flow-weighted composite sample. Samplers were programmed to draw 20 ml incremental samples for a given hour.

Volume of sample collected at hour $t = \frac{Q_t}{Q_{avg}} \times \frac{2500 \text{ ml}}{24}$

where, $Q_t =$ measured flowrate at $t$, $Q_{avg} =$ average daily flowrate over the course of three weeks

Figure S1. Flow diagram showing treatment processes for wastewater and sludge in the investigated activated sludge treatment plant. Numbers indicate the sampling locations used. At locations 1, 2, 3, 4, 5, 8, and 9 flow-weighted, 24-hour composite samples were collected using automated samplers. At locations 6 and 7, grab samples were collected. The boxes outlined in blue and brown color represent, respectively, the control volumes used to conduct mass balances on the wastewater treatment train and the engineered wetland located immediately downstream.
Isotope Dilution, Standard Addition, and Calibration

For imidacloprid, acetamiprid and clothianidin, the isotope dilution technique was utilized to determine losses during extraction and to compensate for potential ion suppression during LC-MS/MS detection. Deuterated isotopes (imidacloprid-\textit{d}_{4}, acetamiprid-\textit{d}_{3} and clothianidin-\textit{d}_{3}) were spiked before extraction at pre-determined, anticipated levels. A tri-deuterated isomer of acetamiprid (\textit{d}_{3}) was also used to enable quantification of acetamiprid-N-desmethyl. For imidacloprid, acetamiprid, acetamiprid-N-desmethyl, and clothianidin, calibration standards were prepared in clean matrix (water, methanol and formic acid mixtures (80/20/0.1, v/v/v)).

For thiacloprid, thiamethoxam, and dinotefuran, the method of standard addition was utilized to compensate for ion suppression during the LC-MS/MS detection.\textsuperscript{1} Matrix spike and matrix spike duplicates were performed in each sample matrix to determine the overall recovery of the analytes.

Quantification was performed using 8-point, linear calibration curves for each analyte in the specific concentration range of interest, and calibration curves with a coefficient of determination \(R^2 > 0.99\) were considered satisfactory.

Quality Assurance and Quality Control

Field, trip and instrument blanks, consisting of ultrapure reagent grade water placed in sampling containers, were analyzed; any resultant signals were subtracted from those obtained for study samples within the same analytical batch. For all analytes, field blank chromatograms showed no to <10% of the signal intensity obtained in chromatograms for actual samples.

Precision was assessed by analyzing samples and duplicates, and calculating the corresponding relative percentage difference (RPD) value using the following equation:

\[
\text{RPD, } \% = \frac{C_{\text{sample}} - C_{\text{duplicate}}}{\frac{C_{\text{sample}} + C_{\text{duplicate}}}{2}} \times 100
\]  
(SE 1)

where, \(C_{\text{sample}}\) and \(C_{\text{duplicate}}\) are the detected concentrations in the original sample and its duplicate, respectively.

Reference:

1. Koester, C. J.; Beller, H. R.; and Halden, R. U. Analysis of Perchlorate in Groundwater by Electrospray Ionization Mass Spectrometry/Mass Spectrometry. \textit{Environ. Sci. Technol.}, \textbf{2000}, \textit{34}(9):1862-1864.
**Data Analysis and Reporting**

*Determination of Method Detection Limits and Reporting Limits.* Method detection limits (MDLs) were determined according to the EPA guidelines described in 40 CFR 136, Appendix B. Data are reported when peak areas were above the lowest concentration calibration standard prepared in clean matrix, when the peak had a signal-to-noise ratio of >3, and when the calculated concentration was equivalent or higher than the established MDL. Theoretical MDLs determined with this approach were verified by spiking authentic matrices and adjusting the MDL values upward as needed to account for matrix effects. In rare cases where blanks of analytical batches showed signals for analytes, the MDL was defined as 10 times the level of background detected in the blank. MDLs were used as reporting limits. The limit of quantitation (LOQ), was defined as 3 times the practical MDL. Data equal to or exceeding the reported LOQ values are considered more robust than those above the MDL but below the respective LOQ.

*Method Detection Limit Calculation.* The method detection limit (MDL) (the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the analyte) was determined by USEPA method. Seven replicate (n) spikes were prepared at an appropriately low concentration (about 1 to 5 times expected MDL) and processed through the entire analytical method. Following equation was used to determine MDL,

\[
\text{MDL} = \frac{s}{t_{(n-1,1-\alpha = 0.99)}} \times t_{(n-1,1-\alpha = 0.99)}
\]  

(Se 2)

where, \(s\) = standard deviation of measured concentrations of n spike determinations, \(n\) = number of replicate spike determinations = 7, \(\alpha\) = level of significance = 0.01, \(t\) = student’s \(t\) value at n-1 degrees of freedom and 1-\(\alpha\) confidence level = 3.14.

*Determination of Absolute and Relative Analyte Recovery.* Absolute recovery of analytes, expressed as a percentage, was determined by fortifying and analyzing representative environmental samples (influent, effluent, sludge) with authentic standards to obtain matrix spike and matrix spike duplicate information. For compounds for which isotope-labeled standards were available, relative recovery rates were calculated by adjusting the determined absolute recovery rate for non-ideal (not 100%) recovery of the respective labeled surrogate standard. In accordance with the isotope dilution method, only data reported for analytes featuring labeled surrogate standards were reported as normalized concentrations. All other data represent absolute concentrations determined.
**Statistical Data Analysis**

In this study, the error value in the average daily concentration was calculated as the standard deviation of measured concentrations obtained for daily samples and their respective replicates over the 5-day sampling period. The error value for the total mass during the 5-day sampling period was calculated using the maximum and minimum values obtained from two experimental replicates.

**Kathon CG/ICP**

In this study 600 mg/L of Kathon CG/ICP (purchased from Sigma-Aldrich Corp., St. Louis, MO, USA) preservative was added to wastewater to disinfect the samples. Kathon CG/ICP contains 1.15% 5-chloro-2-methyl-4-isothiazolin-3-one and 0.35% 2-methyl-4-isothiazolin-3-one as active ingredients.

During method development, potential of interference of Kathon CG/ICP to detection was tested in deionized water, synthetic wastewater (made of peat moss) and wastewater. Results showed that Kathon CG/ICP did not interfere with the LC-MS/MS measurement of neonicotinoids.
Mass Balance Calculations

Mass balance for wetland was calculated by following equation.

\[ \dot{m}_{\text{lost}} = \Sigma (Q_{\text{WL,inf}} \times C_{\text{WL,inf}}) - \Sigma (Q_{\text{WL,eff}} \times C_{\text{WL,eff}}) \]  (SE 3)

where, \( \dot{m}_{\text{lost}} \) = mass input of neonicotinoids lost to transformation and accumulation during passage through wetland (g/day), \( Q_{\text{WL,inf}} \) = flowrate of influent entering wetland (L/day), \( C_{\text{WL,inf}} \) = concentration of neonicotinoids in influent entering wetland (g/L), \( Q_{\text{WL,eff}} \) = flowrate of effluent leaving wetland (L/day), \( C_{\text{WL,eff}} \) = concentration of neonicotinoids in effluent leaving wetland (g/L).

Mass balances for primary, secondary, and disinfection treatment were calculated using the following equations, respectively:

\[ \dot{m}_{\text{PT,transformed}} = \Sigma (Q_{\text{inf}} \times C_{\text{inf}}) - \Sigma (Q_{1'\text{eff}} \times C_{1'\text{eff}}) - \Sigma (Q_{\text{PS}} \times C_{\text{PS}}) \]  (SE 4)

where, \( \dot{m}_{\text{PT,transformed}} \) = mass input of neonicotinoids lost to transformation during primary treatment (g/day), \( Q_{1'\text{eff}} \) = flowrate of primary effluent leaving primary clarifier (L/day), \( C_{1'\text{eff}} \) = concentration of neonicotinoids in effluent leaving primary clarifier (g/L), \( Q_{\text{PS}} \) = flowrate of sludge leaving primary clarifier (L/day), \( C_{\text{PS}} \) = concentration of neonicotinoids in primary sludge (g/L) = \( C_{\text{PS,aq}} \) + (\( C_{\text{PS,particulates}} \times TSS_{\text{PS}} \)), \( C_{\text{PS,aq}} \) = concentration of neonicotinoids in aqueous phase of primary sludge (g/L), \( C_{\text{PS,particulates}} \) = concentration of neonicotinoids in sorbed phase of primary sludge (g/g-solids), \( TSS_{\text{PS}} \) = concentration of total suspended particles in primary sludge (g-solids/L).

\[ \dot{m}_{\text{ST,transformed}} = \Sigma (Q_{1'\text{eff}} \times C_{1'\text{eff}}) - \Sigma (Q_{2'\text{eff}} \times C_{2'\text{eff}}) - \Sigma (Q_{\text{WAS}} \times C_{\text{WAS}}) \]  (SE 5)

where, \( \dot{m}_{\text{ST,transformed}} \) = mass input of neonicotinoids lost to transformation during secondary treatment (g/day), \( Q_{2'\text{eff}} \) = flowrate of secondary effluent leaving secondary clarifier (L/day), \( C_{2'\text{eff}} \) = concentration of neonicotinoids in secondary effluent leaving secondary clarifier (g/L), \( Q_{\text{WAS}} \) = flowrate of waste activated sludge (L/day), \( C_{\text{WAS}} \) = concentration of neonicotinoids in waste activated sludge (g/L) = \( C_{\text{WAS,aq}} \) + (\( C_{\text{WAS,particulates}} \times TSS_{\text{WAS}} \)), \( C_{\text{WAS,aq}} \) = concentration of neonicotinoids in aqueous phase of waste activated sludge (g/L), \( C_{\text{WAS,particulates}} \) = concentration of neonicotinoids in sorbed phase of waste activated sludge (g/g-solids), \( TSS_{\text{WAS}} \) = concentration of total suspended particles in waste activated sludge (g-solids/L).

\[ \dot{m}_{\text{DT,transformed}} = \Sigma (Q_{2'\text{eff}} \times C_{2'\text{eff}}) - \Sigma (Q_{\text{eff}} \times C_{\text{eff}}) \]  (SE 6)

where, \( \dot{m}_{\text{DT,transformed}} \) = mass input of neonicotinoids lost to transformation during disinfection treatment (g/day).
Table S2. Mass Spectrometric Parameters for the Detection of Neonicotinoids and Isotope-Labeled Surrogate Standards

| analyte                  | \(Q_1\) (m/z) | \(Q_3\) (m/z) | \(Q_3'\) (m/z) | \(t_R\) (min) | DP (V) | CE (V) | EP (V) | CXP (V) |
|--------------------------|----------------|----------------|----------------|---------------|--------|--------|--------|---------|
| acetamiprid              | 223.1          | 126.0          | 99.0           | 7.95          | 56     | 31     | 15     | 6       |
| clothianidin             | 250.0          | 169.0          | 132.0          | 7.70          | 50     | 30     | 8      | 8       |
| dinotefuran              | 203.0          | 129.3          | 113.1          | 6.06          | 50     | 30     | 15     | 8       |
| imidacloprid             | 256.0          | 175.1          | 209.2          | 7.50          | 50     | 30     | 10     | 8       |
| thiacloprid              | 253.0          | 126.0          | 73.1           | 8.27          | 50     | 30     | 15     | 12      |
| thiamethoxam             | 292.0          | 211.1          | 181.0          | 7.01          | 50     | 30     | 8      | 8       |
| acetamiprid-N-desmethyl  | 211.1          | 128.0          | 149.0          | 8.06          | 61     | \(27^p,13^s\) | 8 | 22 |

| internal standards       |                |                |                |               |        |        |        |         |
|--------------------------|----------------|----------------|----------------|---------------|--------|--------|--------|---------|
| imidacloprid-\(d_4\)     | 260.1          | 213.1          | 179.2          | 7.50          | 76     | 25^p,33^s | 6 | 14^p, 8^s |
| acetamiprid-\(d_3\)      | 226.0          | 125.9          | 99.0           | 7.95          | 61     | 31^p,55^s | 15 | 10^p, 8^s |
| clothianidin-\(d_3\)     | 252.8          | 171.9          | 131.9          | 7.70          | 56     | 19^p,25^s | 10 | 16^p,10^s |

\(Q_1\) mass-to-charge ratio (m/z) of precursor ion; \(Q_3\) m/z of most abundant fragment ion; \(Q_3'\) m/z of second most abundant fragment ion; \(t_R\) retention time; DP declustering potential; CE collision energy; EP entrance potential; CXP collision cell exit potential; \(^p\) quantifier ions; and \(^s\) qualification ions.
Table S3. Daily Concentrations of Detected Compounds in Treatment Streams

|          | imidacloprid | clothianidin |
|----------|--------------|--------------|
|          | concentration, %RPD | concentration, %RPD |
|          | ng/L         | ng/L         |
| day      |              |              |
| primary influent |              |              |
| 1        | 63.4 ± 0.3 1% | 666.4 ± 15.3 5% |
| 2        | 51.4 ± 1.1 4% | 53.2 ± 4.8 18% |
| 3        | 64.7 ± 8.2 25% | 18.0 ± 9.2 102% |
| 4        | 44.5 ± 1.5 7% | 11.0 ± 0.6 10% |
| 5        | 49.5 ± 1.3 5% | BDL (< 0.9 ng/L) |
| primary effluent |              |              |
| 1        | 59.6 ± 19.2 62% | 382.0 ± 14.0 7% |
| 2        | 58.6 ± 13.7 19% | 396.8 ± 47.3 24% |
| 3        | 63.9 ± 17.5 45% | 27.0 ± 5.6 41% |
| 4        | 53.0 ± 7.3 23% | 13.0 ± 3.7 57% |
| 5        | 57.1 ± 10.4 32% | BDL (< 0.9 ng/L) |
| secondary effluent |              |              |
| 1        | 43.1 ± 2.4 11% | 66.5 ± 1.5 5% |
| 2        | 50.9 ± 8.8 34% | 441.9 ± 28.9 13% |
| 3        | 53.8 ± 2.9 11% | 128.4 ± 5.3 8% |
| 4        | 52.0 ± 7.8 30% | 19.7 ± 0.7 8% |
| 5        | 43.1 ± 4.4 20% | BDL (< 0.9 ng/L) |
| tertiary effluent |              |              |
| 1        | 45.0 ± 7.0 31% | 45.3 ± 8.3 37% |
| 2        | 52.1 ± 10.9 42% | 374.2 ± 4.9 3% |
| 3        | 49.6 ± 8.8 36% | 140.9 ± 3.8 5% |
|          | 1       | 2       | 3       | 4       | 5       | 1       | 2       | 3       | 4       | 5       |
|----------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
|          | 47.8 ± 3.2 | 46.8 ± 2.7 | 42.1 ± 1.9 | 48.9 ± 1.9 | 48.7 ± 0.3 | 54.4 ± 3.4 | 46.8 ± 2.7 | 42.1 ± 1.9 | 48.9 ± 1.9 | 48.7 ± 0.3 |
|          | 13%     | 12%     | 9%      | 8%      | 1%      | 12%     | 12%     | 9%      | 8%      | 1%      |
|          | 23.1 ± 4.0 | 313.7 ± 27.5 | 201.3 ± 18.3 | 56.6 ± 0.6 | 22.3 ± 2.1 | 30.0 ± 0.5 | 313.7 ± 27.5 | 201.3 ± 18.3 | 56.6 ± 0.6 | 22.3 ± 2.1 |
|          | 34%     | 18%     | 18%     | 2%      | 19%     | 3%      | 18%     | 18%     | 2%      | 19%     |

**wetland influent**

|          | 1       | 2       | 3       | 4       | 5       | 1       | 2       | 3       | 4       | 5       |
|----------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
|          | 42.3 ± 9.0 | 39.4 ± 11.4 | 37.4 ± 5.6 | 38.2 ± 5.9 | 49.9 ± 14.6 | 42.3 ± 9.0 | 39.4 ± 11.4 | 37.4 ± 5.6 | 38.2 ± 5.9 | 49.9 ± 14.6 |
|          | 43%     | 58%     | 30%     | 31%     | 58%     | 43%     | 58%     | 30%     | 31%     | 58%     |
|          | 9.6 ± 0.5 | 19.6 ± 0.0 | 61.0 ± 2.0 | 123.4 ± 1.0 | 133.0 ± 3.1 | 9.6 ± 0.5 | 19.6 ± 0.0 | 61.0 ± 2.0 | 123.4 ± 1.0 | 133.0 ± 3.1 |
|          | 10%     | 0%      | 7%      | 2%      | 5%      | 10%     | 0%      | 7%      | 2%      | 5%      |

**wetland effluent**

|          | 1       | 2       | 3       | 4       | 5       | 1       | 2       | 3       | 4       | 5       |
|----------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
|          | 26.5    | 29.9    | 33.9    | 29.9    | 33.3    | 61.9    | 452.3   | 62.2    | BDL (< 0.9 ng/L) | BDL (< 0.9 ng/L) |
|          |         |         |         |         |         |         |         |         |         |         |
|          |         |         |         |         |         |         |         |         |         |         |

**primary sludge**

|          | 1       | 2       | 3       | 4       | 5       | 1       | 2       | 3       | 4       | 5       |
|----------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
|          | 16.8 ± 3.0 | 20.8 ± 3.4 | 31.3 ± 2.2 | 21.9 ± 2.0 | 20.8 ± 1.7 | 16.8 ± 3.0 | 20.8 ± 3.4 | 31.3 ± 2.2 | 21.9 ± 2.0 | 20.8 ± 1.7 |
|          | 36%     | 32%     | 14%     | 19%     | 16%     | 36%     | 32%     | 14%     | 19%     | 16%     |
|          | 7.5 ± 2.6 | 194.3 ± 67.5 | 36.0 ± 3.8 | BDL (< 0.9 ng/L) | BDL (< 0.9 ng/L) | 7.5 ± 2.6 | 194.3 ± 67.5 | 36.0 ± 3.8 | BDL (< 0.9 ng/L) | BDL (< 0.9 ng/L) |
|          | 69%     | 70%     | 21%     | 19%     |         | 69%     | 70%     | 21%     | 19%     |         |
|       | acetamiprid concentration, ng/L | acetamiprid-N-desmethyl concentration, ng/L | %RPD | %RPD |
|-------|---------------------------------|----------------------------------------------|------|------|
|       | day                             |                                              |      |      |
|       |                                 | acetamiprid-N-desmethyl                      |      |      |
|       |                                 | concentration, %RPD                         |      |      |
|       |                                 |                                              |      |      |
| primary influent | 1 | 4.3 ± 0.2 | 11% | 1.1 ± 0.2 | 40% |
|       | 2 | 4.7 ± 0.3 | 12% | 1.6 ± 0.3 | 37% |
|       | 3 | 3.2 ± 0.1 | 8%  | 1.2 ± 0.1 | 13% |
|       | 4 | 3.0 ± 0.3 | 19% | 1.3 ± 0.2 | 28% |
|       | 5 | 3.1 ± 0.7 | 41% | 1.2 ± 0.1 | 16% |
| primary effluent | 1 | 3.8 ± 0.3 | 17% | 1.9 ± 0.3 | 30% |
|       | 2 | 4.2 ± 0.2 | 11% | 2.3 ± 0.1 | 5%  |
|       | 3 | 3.0 ± 0.3 | 23% | 1.8 ± 0.1 | 12% |
|       | 4 | 3.0 ± 0.0 | 0%  | 1.4 ± 0.0 | 1%  |
|       | 5 | 3.0 ± 0.0 | 2%  | 1.4 ± 0.1 | 8%  |
| secondary effluent | 1 | 1.9 ± 0.3 | 30% | 2.0 ± 0.2 | 20% |
|       | 2 | 2.3 ± 0.1 | 5%  | 2.1 ± 0.0 | 3%  |
|       | 3 | 1.8 ± 0.1 | 12% | 1.7 ± 0.3 | 39% |
|       | 4 | 1.4 ± 0.0 | 1%  | 1.4 ± 0.4 | 58% |
|       | 5 | 1.4 ± 0.1 | 8%  | 1.2 ± 0.1 | 16% |
| tertiary effluent | 1 | 2.0 ± 0.2 | 20% | 1.2 ± 0.1 | 16% |
|       | 2 | 2.1 ± 0.0 | 3%  | 1.2 ± 0.2 | 34% |
|       | 3 | 1.7 ± 0.3 | 39% | 1.3 ± 0.5 | 77% |
|       | 4 | 1.4 ± 0.4 | 58% | 1.1 ± 0.3 | 61% |

BDL (< 0.6 ng/L)
|                  | 5 | 1.1 ± 0.2 | 40% | 1.6 ± 0.2 | 29% |
|------------------|---|-----------|-----|-----------|-----|
| wetland influent | 1 | 2.0 ± 0.0 | 3%  | 1.7 ± 0.1 | 10% |
|                  | 2 | 2.5 ± 0.4 | 33% | 1.4 ± 0.5 | 67% |
|                  | 3 | 2.4 ± 0.5 | 46% | 1.6 ± 0.1 | 7%  |
|                  | 4 | 1.8 ± 0.6 | 62% | 1.1 ± 0.2 | 34% |
|                  | 5 | 1.8 ± 0.4 | 41% | 1.3 ± 0.1 | 13% |
| wetland effluent | 1 | 1.8 ± 0.1 | 6%  | 1.6 ± 0.0 | 6%  |
|                  | 2 | 2.0 ± 0.1 | 8%  | 1.3 ± 0.1 | 15% |
|                  | 3 | 2.0 ± 0.3 | 26% | 1.5 ± 0.0 | 5%  |
|                  | 4 | 1.9 ± 0.1 | 9%  | 1.5 ± 0.0 | 2%  |
|                  | 5 | 2.3 ± 0.2 | 18% | 2.0 ± 0.5 | 49% |
| primary sludge  | 1 | 0.6       |     |           |     |
|                  | 2 | 1.3       |     |           |     |
|                  | 3 | 1.8       |     | BDL (< 0.6 ng/L) | |
|                  | 4 | 0.8       |     |           |     |
|                  | 5 | 0.5       |     |           |     |
| waste activated | 1 | 2.0 ± 1.7 | 172%|           |     |
| sludge          | 2 | 1.2 ± 0.5 | 73% |           |     |
|                  | 3 | 1.8 ± 1.5 | 166%| BDL (< 0.6 ng/L) | |
|                  | 4 | 0.9 ± 0.8 | 173%|           |     |
|                  | 5 | 1.4 ± 1.2 | 165%|           |     |
Table S4. Average Flow Rate Over 5-day Sampling Period in Process Streams of the Wastewater Treatment Plant and the Constructed Wetland. Error Values Shown Represent Standard Deviations (SDs)

| process stream                  | flow rate (MLD) |
|---------------------------------|------------------|
| wastewater treatment plant       |                  |
| influent                        | 243.8 ± 4.1      |
| primary effluent                | 241.9 ± 4.1      |
| secondary effluent              | 240.2 ± 3.8      |
| disinfection effluent           | 240.2 ± 3.8      |
| engineered wetland              |                  |
| influent                        | 283.6 ± 7.6      |
| effluent                        | 247.2 ± 14.6     |
Partitioning Coefficients for Neonicotinoids in Sludge and Linear Relationship with $K_{OW}$

Partitioning coefficients for neonicotinoids in sludge are tabulated in Table S5. A linear relationship between partitioning coefficient $K_D$ and $n$-octanol water partition coefficient ($K_{OW}$) was obtained as shown in Figure S2.

**Table S5. $K_D$ values for sludge**

| analyte         | Log $K_{OW}$ | $K_D$ for sludge, L/Kg | log $K_D$ for sludge |
|-----------------|--------------|------------------------|----------------------|
| acetamiprid     | 0.80         | 21.12                  | 1.32                 |
| clothianidin    | 0.91         | 15.99                  | 1.20                 |
| dinotefuran     | -0.55        | 2.17                   | 0.34                 |
| imidacloprid    | 0.57         | 15.68                  | 1.20                 |
| thiacloprid     | 1.26         | 28.28                  | 1.45                 |
| thiamethoxam    | -0.13        | 2.36                   | 0.37                 |

**Figure S2.** Linear relationship between log $K_D$ and log $K_{OW}$

$y = 0.69x + 0.65$

$R^2 = 0.93$
Table S6. Estimation of sorbed concentration onto sludge particulate

|                  | PS            | WAS           |
|------------------|---------------|---------------|
| **imidacloprid** |               |               |
| average daily aqueous concentration in decant, ng/L | 22.3 ± 5.7    | 30.7 ± 3.1    |
| average daily predicted sorbed concentration, ng/kg | 481.4 ± 46.9  | 349.9 ± 89.7  |
| total mass, grams/5 days | 0.6             | 0.2           |
| mass in sludge/ influent mass | 0.9%                | 0.3%          |
| **acetamiprid** |               |               |
| average daily aqueous concentration in decant, ng/L | 1.0 ± 0.7      | 1.5 ± 1.4     |
| average daily predicted sorbed concentration, ng/kg | 20.8 ± 11.7    | 31.4 ± 28.7   |
| total mass, grams/5 days | 0.02                | 0.02 ± 0.01   |
| mass in sludge/ influent mass | 0.4%                | 0.4%          |
| **clothianidin** |               |               |
| average daily aqueous concentration in decant, ng/L | 115.3 ± 190.9  | 47.6 ± 84.8   |
| average daily predicted sorbed concentration, ng/kg | 1843.6 ± 3053.2 | 760.7 ± 1356.3 |
| total mass, grams/5 days | 2.2                     | 0.8 ± 0.2     |
| mass in sludge/ influent mass | 1.2%                | 0.5%          |

PS, primary sludge; WAS, waste activated sludge
Figure S3. Mass and concentrations of imidacloprid and acetamiprid in engineered wetland streams, implying persistence to treatment.