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# Pharmaceutical compounds in the wastewater process stream in Northwest Ohio

*Alison L. Spongberg*, Jason D. Witter*

*Department of Environmental Sciences, Mail Stop 604, University of Toledo, Toledo, Ohio 43606, United States*

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ABSTRACT

In order to add to the current state of knowledge regarding occurrence and fate of Pharmaceutical and Personal Care Products (PPCP's) in the environment, influent, effluent and biosolids from three wastewater treatment facilities in Northwest Ohio, USA, and a stream containing effluent discharge from a rural treatment facility were analyzed. The three WWTP facilities vary in size and in community served, but are all Class B facilities. One facility was sampled multiple times in order to assess temporal variability. Twenty compounds including several classes of antibiotics, acidic pharmaceuticals, and prescribed medications were analyzed using ultrasonication extraction, SPE cleanup and liquid chromatography–electrospray ionization tandem mass spectrometry. The highest number of compounds and the greatest concentrations were found in the influent from the largest and most industrial WWTP facility. Short-term temporal variability was minimal at this facility. Many compounds, such as clarithromycin, salicylic acid and gemfibrizol were found at concentrations more than one order of magnitude higher than found in the effluent samples. Effluent waters contained elevated levels of carbamazepine, clindamycin and sulfamethoxazole. Differences in composition and concentration of effluent waters between facilities existed. Biosolid samples from two different facilities were very similar in PPCP composition, although concentrations varied. Ciprofloxacin was found in biosolids at concentrations (up to 46 μg/kg dry mass) lower than values reported elsewhere. Diclofenac survived the WWTP process and was found to persist in stream water incorporating effluent discharge. The low variability within one plant, as compared to the variability found among different wastewater treatment plants locally and in the literature is likely due to differences in population, PPCP usage, plant operations and/or local environment. These data are presented here for comparison with this emerging set of environmental compounds of concern.

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## 1. Introduction

In July 2002, the National Research Council issued a report expressing concern over the practice of applying Class B Sewage sludge, commonly referred to as Class B biosolids, to agricultural fields in the United States (NRC, 2002). Currently around 67% of biosolids in the United States are land applied in the agricultural setting in order to gain the beneficial reuse of their nutrient content and provide a path for disposal other than incineration and land filling. Concern over the contribution and regulation of organic contaminants in wastewaters and biosolids are growing. Harrison et al. (2006) and Richardson and Ternes (2005) have documented the need for regulation other than for pathogens and some metals, such as over-the-counter and prescription pharmaceuticals and other antibiotic substances.

* *Corresponding author*. Tel.: +1 419 530 4091; fax: +1 419 530 4421.
*E-mail address:* aspongb@utnet.utoledo.edu (A.L. Spongberg).

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Research efforts by the scientific community to document occurrence and fate of this emerging class of contaminants have increased in the last 10 years. The development of methodologies needed for the analysis of these emerging contaminants, generally referred to as Pharmaceutical and Personal Care Products (PPCP's) are ongoing as new extraction materials are used, and detection and quantification limits are lowered by advances in analytical methodology and instrumentation. Mass spectrometry (single or dual) coupled with either gas or liquid chromatography is the preferred detection and quantitation technique today, dictated by thermal properties of the compounds or accessibility to instrumentation. Extraction and clean up is typically achieved using a combination of ultrasonication (USE) or accelerated solvent extraction and solid phase extraction (SPE) to isolate compounds of interest and reduce matrix effects. Kolpin et al. (2002) have presented methodology for detection of these compounds in water and cited their extensive occurrence in the U.S, and many researchers are following this trend by testing new matrices, such as biosolids (Golet et al., 2002; Göbel et al., 2005; Kinney et al., 2006a,b; Nakada et al., 2006), farm dust (Hamscher et al., 2003); plants (Boxall et al., 2006; Dolliver et al., 2007) and fish (Chu and Metcalfe 2007; Ramirez et al., 2007); adding new compounds of interest, including metabolites (Miao et al., 2005); and examining ecotoxicological effects of biosolid application (Jjemba 2002; Robinson et al., 2005). Several extensive review papers have documented analytical capabilities and the current state of the science and regulation, including occurrence data (Buchberger 2007; Jones-Lepp and Stevens 2007; Nikolaou et al., 2007).

With increased attention in research venues on these emerging contaminants, the availability of comparison data is also on the rise. Within the past several years researchers have begun to report the presence and concentrations of various PPCP compounds in the wastewater treatment process, surface waters and flora and fauna. In the wastewater stream the data are highly variable, and seem to be influenced by the level of treatment used at the plant, as well as the population being served by the facility. Thomas and Foster (2005) analyzed influent and effluent samples from three wastewater treatment plants (WWTP) and not only found much variability among treatment plants, but also within the European communities. Differences were attributed to factors such as location, socioeconomic status, pharmaceutical cost and other demographic data. A similar study comparing samples from Canadian WWTPs found overall lower concentrations compared to U.S. plants and related differences to hydraulic retention times and other treatment processes (Metcalfe et al., 2003a,b). Others have documented changes in PPCP wastewater stream (Kanda et al., 2003; Carballa et al., 2004; Castiglioni et al., 2006; Lishman et al., 2006). Table 1 gives concentrations for influents, effluents and biosolids for selected compounds for reference extracted from current literature.

Debate over the environmental implications of these compounds on soils and adjacent aquatic ecosystems to which they are applied is a more recent concern. Hernando et al. (2006), show that adverse effects from many of these compounds on bacteria, invertebrate and algal populations can occur. Similar research by Carlsson et al. (2006), however do not support those findings. Doerr-MacEwen and Haight (2006) give an excellent summary of the diversity of scientific opinion on the severity of the problem. Based on the dichotomy of many findings and the relatively young methodology, consistent addition to the basic occurrence and concentration information, along with environmental health research and method development are needed.

In order to add to the current state of knowledge regarding occurrence and fate of PPCP's in the environment, the presence and concentrations of specific PPCP compounds were analyzed in the influent, effluent and biosolids from three WWTPs in Northwest Ohio, USA, and a stream containing effluent discharge from a rural treatment plant. These data are presented here for comparison with the current emerging set of environmental compounds of concern.

## 2. Methodology

Because methodology for these analyses is not standardized, detailed information of the procedures used in this study are given here. Table 2 gives the analytes included in this study

**Table 1 – Concentrations of selected PPCP compounds for influent, effluent and biosolids with references**

| | Influent | | Effluent | | Biosolid | |
|---|---|---|---|---|---|---|
| Compound | μg/L | Reference | μg/L | Reference | μg/kg | Reference |
| Caffeine | 63.2 | [12] | 0.002–9.9 | [6] [9] | 7.6–65 | [11] [12] |
| Carbamazepine | 0.043–1.9 | [3] [10] [12] | 2.3 | [3] | ND–258.1 | [11] [12] |
| Ciprofloxacin | 0.007–5.6 | [4] [5] [6] | 0.007–0.071 | [5] [16] | 200–4800 | [5] [16] |
| Clarithromycin | 0.160–0.263 | [4] [7] | 0.71 | – | – | – |
| Clindamycin | – | – | 1 | [6] | – | – |
| Diclofenac | – | – | 0.07–0.748 | [1] [2] | ND–183 | [11] |
| Gemfibrozil | 0.12–36.53 | [1] [2] | 2 | [15] | 121 | [15] |
| Salicylic acid | 0.1–27.8 | [1] [2] [3] | 0.01–59.6 | [1] [2] [3] | – | – |
| Sulfamethazine | 0.018–0.1 | [7] [8] | – | – | – | – |
| Sulfamethoxazole | 0.059–6 | [4] [5] [6] [9] | ND–0.304 | [5] [13] | – | – |

ND = not detected.

[1] Lishman et al., 2006 [2] Lee et al., 2005 [3] Metcalfe et al., 2003a,b [4] Segura et al., 2007 [5] Lindberg et al., 2005 [6] Batt et al., 2006 [7] Göbel et al., 2004 [8] Yang et al., 2005 [9] Kinney et al., 2006a,b [10] Benotti and Brownawell, 2007 [11] Nieto et al., 2007 [12] Miao et al., 2005 [13] Ashton et al., 2004 [14] Heberer et al., 2001 [15] Khan and Ongerth, 2002 [16] Golet et al., 2003.

**Table 2 – Ancillary information, instrumental parameters, methods and data validation for analytes examined in the current study**

| Analyte | CAS number | Classification | MW[a] | Ionization mode | Precursor ion (m/z)[b] | Capillary [V][c] | Fragment ion | Collision energy [eV] | Methods | | | Instrumental detection limits (pg on column) | Method recovery [%] | | | Method detection limits | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Extraction | LC-MS/MS | Elution | | Total | Water | Biosolid | Water [μg/L] | Biosolid [μg/kg][d] |
| Caffeine | 58-08-2 | CNS stimulant | 194.2 | + | 195.2 | 50 | 138 | −16.0 | A | 1 | (a) | 10 | 86 | 44 | 196 | 0.00281 | 1.43489 |
| Carbamazepine | 298-46-4 | Antiepileptic | 236.3 | + | 237.1 | 41 | 194 | −16.0 | C | 6 | (c) | 3 | 49 | 52 | 94 | 0.00085 | 0.90042 |
| Chlortetracycline | 64-72-2 | Antibiotic | 515.0 | + | 479.0 | 55 | 444 | −18.0 | A | 2 | (a) | 55 | 105 | 89 | 118 | 0.00811 | 6.89079 |
| Cimetidine | 51481-61-9 | Antacid | 252.3 | + | 253.1 | 43 | 159 | −12.0 | C | 5 | (c) | 508 | 22 | 63 | 36 | 0.10485 | 294.90241 |
| Ciprofloxacin | 85721-33-1 | Antibiotic | 331.0 | + | 332.1 | 62 | 245 | −19.5 | B | 3 | (a) | 32 | 74 | 57 | 129 | 0.00728 | 5.62767 |
| Clarithromycin | 81103-11-9 | Antibiotic | 747.0 | + | 748.5 | 70 | 158 | −23.0 | B | 3 | (a) | 3 | 29 | 94 | 31 | 0.00042 | 1.39154 |
| Clindamycin | 18323-44-9 | Antibiotic | 461.5 | + | 425.2 | 70 | 126 | −22.0 | B | 3 | (a) | 1 | 53 | 51 | 105 | 0.00017 | 0.16079 |
| Clofibric acid | 882-09-7 | Cholesterol control | 214.7 | − | 213.1 | −35 | 127 | 12.5 | C | 4 | (b) | 13 | 41 | 56 | 73 | 0.00306 | 4.20561 |
| Cotinine | 486-56-6 | Metabolite of nicotine | 176.2 | + | 177.1 | 60 | 80 | −19.5 | B | 3 | (a) | 26 | 71 | 46 | 154 | 0.00722 | 4.67707 |
| Diclofenac | 15307-86-5 | Analgesic | 318.1 | − | 293.8 | −35 | 250 | 10.0 | C | 4 | (b) | 9 | 90 | 82 | 109 | 0.00149 | 1.36570 |
| Diltiazem | 42399-41-7 | Antihyper-tensive | 451.0 | + | 415.1 | 62 | 178 | −21.5 | C | 5 | (c) | 1 | 44 | 53 | 83 | 0.00022 | 0.26247 |
| Gemfibrozil | 25812-30-0 | Lipid regulator | 250.3 | − | 249.2 | −45 | 121 | 12.0 | C | 4 | (b) | 6 | 46 | 47 | 97 | 0.00153 | 1.56654 |
| Salicylic acid | 69-72-7 | Skin care product | 138.1 | − | 136.8 | −38 | 93 | 15.0 | C | 4 | (b) | 167 | 85 | 96 | 89 | 0.02257 | 25.46016 |
| Sulfadimethoxine | 122-11-2 | Antibiotic | 310.3 | + | 311.1 | 55 | 156 | −18.0 | A | 1 | (a) | 8 | 50 | 67 | 75 | 0.00154 | 2.04587 |
| Sulfamethazine | 57-68-1 | Antibiotic | 278.3 | + | 279.1 | 54 | 186 | −14.5 | A | 1 | (a) | 11 | 41 | 63 | 64 | 0.00231 | 3.58411 |
| Sulfamethizole | 144-82-1 | Antibiotic | 270.3 | + | 271.1 | 64 | 156 | −11.5 | A | 1 | (a) | 11 | 49 | 63 | 77 | 0.00225 | 2.91744 |
| Sulfamethoxazole | 723-46-6 | Antibiotic | 253.3 | + | 254.1 | 44 | 156 | −13.5 | A | 1 | (a) | 21 | 49 | 83 | 59 | 0.00331 | 5.65315 |
| Sulfathiazole | 72-14-0 | Antibiotic | 255.3 | + | 256.1 | 44 | 156 | −12.0 | A | 1 | (a) | 19 | 37 | 57 | 65 | 0.00436 | 6.74818 |
| Sulfisoxazole | 127-68-5 | Antibiotic | 267.3 | + | 268.1 | 50 | 156 | −11.5 | A | 1 | (a) | 15 | 47 | 59 | 79 | 0.00321 | 4.07695 |
| Tetracycline | 60-54-8 | Antibiotic | 444.4 | + | 445.1 | 55 | 410 | −20.5 | A | 2 | (a) | 40 | 69 | 68 | 101 | 0.00757 | 7.49156 |

Methods and calculations refer to descriptions given in the text.

[a] Molecular weight.

[b] Mass to charge ratio.

[c] Volts.

[d] Based on dry mass.

and references the following methods. Extraction methods were adapted from various sources (e.g. Sacher et al., 2001; Kolpin et al., 2002; Löffler and Ternes, 2003; Miao et al., 2002, 2004; Jones-Lepp, 2006), and LC-ESI-MS/MS methodology was optimized for our individual instrumentation. Each analyte and method combination was chosen based on best recovery using the procedure detailed in the following sections. Detailed methodological recovery for each analyte using each described method is beyond the scope of this paper, only data using optimal extractions and separation are presented.

### 2.1. Sample collection

Influent, effluent, and/or biosolid samples were collected using a grab sampler (Forestry Suppliers, Jackson MS) from three wastewater treatment plants and a stream receiving effluent from a rural plant. Not all sample types could be obtained from all plants. The rural site represents a 100,000 gal/day batch Class B WWTP that serves no industry and only an agricultural residential population. The suburban plant has a 3–4 million gallon per day capacity and treats influent from residential and limited industrial sources. The urban plant is a larger flow-through WWTP, handling close to 5 million gallons a day and has numerous industrial sources. Samples were collected in pre-washed 1 L amber glass bottles. Samples were transported back to the lab in ice-packed coolers until sample preparation began, typically no more than 2 h past sampling. Influent and effluent samples were filtered to 0.7 μm using methanol washed glass fiber filters (Fisher Scientific, Milford, MA). Biosolid samples were freeze-dried (Labconco, Kansas City, MO) for a period of 24 h. Processed samples were then stored at −5 °C. Extraction of analytes was performed within 72 h of sampling.

### 2.2. Compound extraction

#### 2.2.1. Aqueous extraction

Solid phase extraction (SPE) was used to concentrate analytes of interest and reduce matrix effects. A 12-port vacuum manifold with drying attachment and 12 large volume samplers (Supelco, St. Louis, MO) and Oasis Hydrophilic Lypophilic (HLB) SPE Cartridges, 6-mL volume and 500-mg bed mass (Waters, Milford, MA), were used. Three separate solid phase extraction methods (SPE) were used. Sample volumes for influent and effluent were either 500 or 1000 mL for each extraction and all samples were run in triplicate. Blanks and spiked samples were also included. Final extracts were filtered using 0.45-μm Teflon syringe filters, and stored at −5 °C until analysis by LC-ESI-MS/MS.

2.2.1.1. *Method A (adapted from Miao et al., 2004; Kolpin et al., 2002).* SPE cartridges were conditioned with 6-mL acetone and methanol, respectively, and equilibrated with 6 mL 50 mM $Na_2EDTA$. Sample aliquot was adjusted to pH 3, using $H_2SO_4$, and 0.5 g/L $Na_2EDTA$ was added. Sample was loaded at 10 mL/min. After vacuum drying, the cartridge was eluted with 3×2 mL methanol, then evaporated to 300 μL under a gentle stream of nitrogen, and reconstituted in 1 mL of acetonitrile.

2.2.1.2. *Method B (adapted from Miao et al., 2004; Kolpin et al., 2002).* Cartridges were conditioned with 6 mL acetone and methanol, respectively, and equilibrated with 6 mL of Nanopure water adjusted to pH 6. Sample aliquot was adjusted to pH 6 using $H_2SO_4$, and was loaded at 10 mL/min. After vacuum drying, the cartridge was eluted with 3×2 mL methanol, then evaporated to 300 μL under a gentle stream of nitrogen, and reconstituted in 1 mL of acetonitrile.

2.2.1.3. *Method C (adapted from Jones-Lepp, 2006).* Cartridges were conditioned with 5 mL methanol and equilibrated with 2×5 mL Nanopure water. Sample aliquot was adjusted to pH 2, using $H_2SO_4$, and loaded at 10 mL/min. After vacuum drying, the cartridge was eluted with 3×2 mL 1% acetic acid in methanol, then evaporated to 300 μL under a gentle stream of nitrogen, and reconstituted in 1 mL of acetonitrile.

#### 2.2.2. Sludge extraction (adapted from Göbel et al., 2005; Ternes et al., 2005)

Five hundred milligrams of ground freeze-dried biosolid were extracted using Sequential Ultrasonic Extraction (USE) for each aqueous extraction. Samples were first extracted using 2×3 mL methanol, then 3 mL acetone for 5 min each in an ultrasonic bath. After each 3 mL extraction the sample was centrifuged to separate suspended material, and supernatants combined and filtered using a 13-mm 0.45-μm PTFE syringe filter. Extracts were evaporated under a gentle stream of nitrogen to a volume of 200 μL, then combined with 150 mL of groundwater containing concentrations of analytes of interest less than detectable limits. The aqueous extract sample was further extracted and concentrated using the three SPE methods described previously.

### 2.3. Analytical methods

The LC-ESI-MS/MS chromatographic analysis was conducted on a Varian 1200 L Triple Quad Mass Spectrometer equipped with an ESI interface, two Prostar 210 solvent delivery modules and a Prostar 430 Autosampler (Varian Inc., Palo Alto, CA). Nitrogen was used as the nebulizing gas in both negative and positive ionization modes. Nitrogen and air were used as drying gases in positive and negative ionization modes, respectively. For collision-induced dissociation argon was used at a pressure of 2.0 mTorr. Varian Workstation 6.8 software was used to control the system and quantify data.

Optimization of parameters and analysis of fragment ions for each compound was conducted using analytical standards dissolved in HPLC grade methanol, at concentrations ranging between 0.5 and 1 mg/L, and directly injected at a continuous flow of 20 μL/min using a mechanical syringe pump (Harvard Apparatus, Hollisten, MA). Shield, ion transfer capillary, nebulizing needle, detector voltages and drying gas temperature were optimized for each analyte. The optimum MS fragment parent to product ion transition with the highest intensity was chosen to quantify each analyte.

Six chromatographic separation methods were used, grouping analytes based on similar optimized conditions (except for capillary voltage which could be adjusted individually) and best separation on several analytical columns. Instrumental parameters, major fragment ions and collision

energies used for fragmentation, and extraction and instrumental methodology references matching the text are given for each analyte in Table 2.

Three gradient elutions were used in conjunction with the six separation methods. Elution gradients and solvents were optimized for separation and signal to noise ratio for each analytical column and analyte combination. Elution (a) held for 2 min at 5% solvent B, ramped to 100% B in 8 min, held for 2 min, returned to 5% B in 1 min, and finally equilibrated for 2 min at original conditions. Elution (b) held at 5% solvent B for 3 min, ramped to 100% B in 7 min, held at 100% for 10 min, returned to 5% B in 1 min, and held at initial conditions for 4 min. Elution (c) held at 5% solvent B for 2 min, ramped to 100% B in 26 min, held at 100% for 2 min, returned to 5% B in 1 min, and held at initial conditions for 2 min.

#### 2.3.1. *Method 1*

Positive ionization mode. Separation using a Lichrosphere 5-μm RP-18 (150×3 mm) and Chromsep Guard SS, packing matched, (10×2 mm) (Varian Inc., Walnut Creek, CA). Drying gas temperature was 350 °C, detector 1430 V, nebulizing needle 4200 V, and shield 200 V. 0.1% formic acid in deionized water as mobile phase A, and 0.1% formic acid in acetonitrile as mobile phase B at 0.2 mL/min. Injection volume was 20 μL. Gradient Elution (a).

#### 2.3.2. *Method 2*

Positive ionization mode. Separation using a Genesis C18 3-μm (150×2.1 mm), packing-matched Finesse Guard (10×2.1) (Grace Vydac, Deerfield, IL). Drying gas temperature was 275 °C, detector 1700 V, nebulizing needle 4500 V, and shield 275 V. Mobile phases were the same as in Method 1 with flow rate at 0.2 mL/min. Injection volume was 20 μL. Gradient Elution (a).

#### 2.3.3. *Method 3*

Positive ionization mode. Separation using the same column and mobile phases as in Method 1. Drying gas temperature was 350 °C, detector 1700 V, nebulizing needle 3900 V, and shield 275 V. Flow rate was 0.2 mL/min. Injection volume was 20 μL. Gradient Elution (a).

#### 2.3.4. *Method 4*

Negative ionization mode. Separation using a Genesis C18 3 μm (150×2.1 mm), packing-matched Finesse Guard (10×2.1) (Grace, Deerfield, IL). Drying gas temperature was 400 °C, detector 1780 V, nebulizing needle −3800 V, and shield −600 V. 0.1% ammonium acetate in deionized water as mobile phase A, and 40:60 methanol:acetonitrile mix as mobile phase B at 0.2 mL/min. Injection volume was 20 μL. Gradient Elution (b).

#### 2.3.5. *Method 5*

Positive ionization mode. Separation using a Luna C8(2) 3 μm 100 A (100×4.6 mm) and Security Guard C8 (4×2 mm) (Phenomenex, Torrance, CA). Drying gas temperature was 350 °C, detector 1330 V, nebulizing needle 3600 V, and shield 200 V. Mobile phases same as in Method 1 with flow rate at 0.2 mL/min. Injection volume was 20 μL. Gradient elution (c).

#### 2.3.6. *Method 6*

Positive ionization mode. Separation using a Nova-Pak C18 4 μm (150×3.9 mm), packing matched, Waters Sentry Guard (Waters, Milford, MA). Drying gas temperature was 350 °C, detector 1330 V, nebulizing needle 3600 V, and shield 200 V. Mobile phases same as in Method 1 with flow rate at 0.2 mL/min. Injection volume was 20 μL. Gradient elution (c).

### 2.4. *Quantification and method validation*

Seven point calibration curves and the most abundant product ion for each analyte of interest were used for quantification. Calibration standards represented a concentration range of 0.002 to 1.000 mg/L. All calibration curves provided a linear relationship greater or equal to $r^2$>0.99. Retention time and presence of product ions and abundance ratios similar to the extracted and optimization standards were used for confirmation. Groundwater was used as a method blank for the solid phase extraction. Samples of groundwater were spiked with known amounts of standard for each analyte and were extracted and analyzed using the appropriate methods described previously. Groundwater collected from a carbonate bedrock aquifer containing no measurable traces of PPCP's of interest was chosen as the recovery matrix, since a clean matrix similar to influent and effluent was not feasible in the expected concentration range. The groundwater provided matrix components such as organic matter and trace elements that typically affect ionization efficiency and overall recovery. Due to the expense of the analyses each data point represents multiple LC analysis of one sample, extracted in triplicate from a composite sample collected for each matrix and point in time. Data for the blank is not presented here since no concentrations above the instrumental detection limits were ever detected.

In order to assess method recovery from water using the described methodology, a 500 mL aliquot of groundwater was spiked with a known amount of analyte before solid phase extraction, so the final concentration in 1 mL of extract with 100% recovery would be 200 μg/L. Recovery for the biosolids extraction was achieved by extracting biosolids then spiking the diluted extract with a known amount of standard before the clean up step. The biosolid was previously analyzed for PPCP's, and these values were used to accommodate for the background concentrations and decide on spiking concentrations. After extraction, clean up, and analysis by LC-ESI-MS/MS recovery samples were compared against standards prepared in methanol at the expected concentration at 100% recovery. Recovery values are given in Table 2.

Instrumental detection limits were set as the concentration at which the RMS signal to noise ratio was no lower than 3. Each analyte was prepared in methanol at concentrations ranging from 0.002 to 1.000 mg/L and injected using the appropriate LC-ESI-MS/MS. Instrumental detection limits (IDL) in picograms (pg) on column and method limits of quantitation are given in Table 2. Method limits of quantitation (LOQ) are calculated here by dividing the IDL in μg by the injection volume 0.020 mL, multiplying by the average extract volume of 1.3 mL, dividing by the sample volume of 0.5 L for water or

mass of 0.0005 kg for biosolid, and dividing by the appropriate matrix recovery value.

## 3. Results and discussion

Concentrations for three sampling dates (9/6/2006, 12/4/2006 and 3/2/2007) found in influent, effluent and biosolids for twenty PPCP compounds for samples collected at the Urban wastewater treatment plant are presented in Table 3. For reference Table 1 also contains concentration ranges for selected compounds discussed here. Sixteen analytes were detected above the method LOQ's at least once in the influent. Sulfamethizole, sulfathiazole and cimetidine were not detected during this study at the urban WWTP. Carbamazepine and clindamycin were detected in every sample regardless of matrix or sampling date. Generally no indication of higher PPCP presence during a particular sampling event or any variation due to general climatic changes can be inferred for influent during the three sampling periods, although ciprofloxacin and salicylic acid were at least one order of magnitude higher in the first sampling, while cotinine and clarithromycin were higher in the third sampling. Many compounds, especially sulfadimethoxine, were detected only once and were observed near the method detection limit, allowing analytical variability to confound these results. The majority of the other compounds detected were at least two times the method detection limit. A long-term study at this location may accentuate differences due to changes in PPCP usage before entrance into the wastewater treatment process. Seasonal differences in influent and effluent concentrations for diclofenac and other PPCP compounds have been documented elsewhere (Vieno et al., 2005), and will be considered in the future.

Quantifying the ultimate persistence and transport of PPCP compounds in the end products, here being effluent and biosolids, of the wastewater treatment process is very important, but does have limitations. Biosolid concentration data can only be used qualitatively since they are not in a linear treatment cycle. Sludge separated from influent at this and many treatment facilities are temporal composites of solid (sludge) and liquid (typically effluent) adjusted by plant operators to meet specific criteria for production of biosolid (such as dewatering, aeration and added input from the waste cycle). Effluent volume is approximately equal to the influent volume at each of the WWTP facilities studied here, although samples collected at the influent intake, then collected later at the effluent outtake may reflect a temporal lag and volumetric input differences changing quickly. The dynamic nature of the process and need to fully describe and understand the process increase the difficulty in calculating a mass balance, though several trends can be observed for specific compounds using several samplings over time as was done in this study.

In the urban wastewater treatment plant concentrations of caffeine, salicylic acid and gemfibrozil consistently decreased by an order of magnitude or more in the effluent. Cotinine (a metabolite of nicotine) was present from 0.04–1.58 μg/L in the influent, but was not detected any further in the effluent or biosolid. However, several of the antibiotics were found with elevated levels in the effluent with respect to the influent, indicating enrichment during treatment. In particular, carbamazepine, clindamycin, diclofenac and sulfamethoxazole increased by a factor of two to three in most cases. A comparative study on three WWTP effluents in Canada by Miao et al. (2002) compares well to the concentrations of diclofenac and gemfibrozil, and non-detection of clofibric acid

**Table 3 – PPCP concentrations for influent, effluent and biosolid samples from an urban wastewater treatment plant in Northwest Ohio**

| | Influent | | | Effluent | | | Biosolid | | |
|---|---|---|---|---|---|---|---|---|---|
| Compound | 9/6/06 | 12/4/06 | 3/2/07 | 9/6/06 | 12/4/06 | 3/2/07 | 9/6/06 | 12/4/06 | 3/2/07 |
| Caffeine | 2.4488 | 4.8658 | 2.7698 | 0.0039 | 0.0122 | 0.0231 | <LOQ | 5.2051 | 4.5241 |
| Carbamazepine | 0.0393 | 0.0509 | 0.0248 | 0.0759 | 0.1112 | 0.0337 | 4.7569 | 12.8581 | 5.8025 |
| Chlortetracycline | <LOQ | <LOQ | 0.0159 | <LOQ | <LOQ | <LOQ | 12.7913 | <LOQ | 14.7471 |
| Cimetidine | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ |
| Ciprofloxacin | 0.3772 | 0.0114 | 0.0635 | 0.0674 | 0.0088 | 0.1099 | 46.3619 | 22.6412 | <LOQ |
| Clarithromycin | <LOQ | 0.1057 | 0.7242 | <LOQ | 0.0702 | 0.6106 | <LOQ | 1.5740 | 30.2402 |
| Clindamycin | 0.0133 | 0.0101 | 0.0068 | 0.0223 | 0.0325 | 0.0149 | 4.3734 | 15.4159 | 3.7175 |
| Clofibric acid | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | 8.0524 | <LOQ | <LOQ |
| Cotinine | 0.1969 | 0.0412 | 1.5811 | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ |
| Diclofenac | 0.0095 | 0.0139 | <LOQ | 0.0083 | 0.0317 | 0.1771 | 10.4481 | 23.0953 | <LOQ |
| Diltiazem | <LOQ | 0.0691 | 0.0405 | <LOQ | 0.1073 | 0.0939 | <LOQ | 2.8213 | 12.8216 |
| Gemfibrozil | 0.1818 | 0.4502 | 0.4513 | <LOQ | 0.0421 | 0.0835 | 3.4083 | 2.4746 | <LOQ |
| Salicylic acid | 8.0361 | 0.4339 | 0.6368 | 0.0472 | 0.0252 | <LOQ | 96.3040 | 252.8671 | <LOQ |
| Sulfadimethoxine | 0.0026 | <LOQ | <LOQ | <LOQ | <LOQ | 0.0019 | 2.6397 | <LOQ | 8.1467 |
| Sulfamethazine | 0.0269 | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | 26.6575 | <LOQ | 10.9979 |
| Sulfamethizole | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ |
| Sulfamethoxazole | 0.2610 | 0.1587 | 0.0135 | 0.4724 | 0.2737 | 0.0794 | <LOQ | <LOQ | <LOQ |
| Sulfathiazole | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ |
| Sulfisoxazole | 0.0221 | <LOQ | <LOQ | <LOQ | <LOQ | 0.0119 | 21.9198 | <LOQ | 9.1383 |
| Tetracycline | 0.0389 | <LOQ | 0.0293 | 0.0344 | <LOQ | 0.0310 | 15.7569 | <LOQ | <LOQ |

<LOQ = below method limit of quantification.
Units are in μg/L for influent and effluent, and μg/kg dry mass for biosolid.

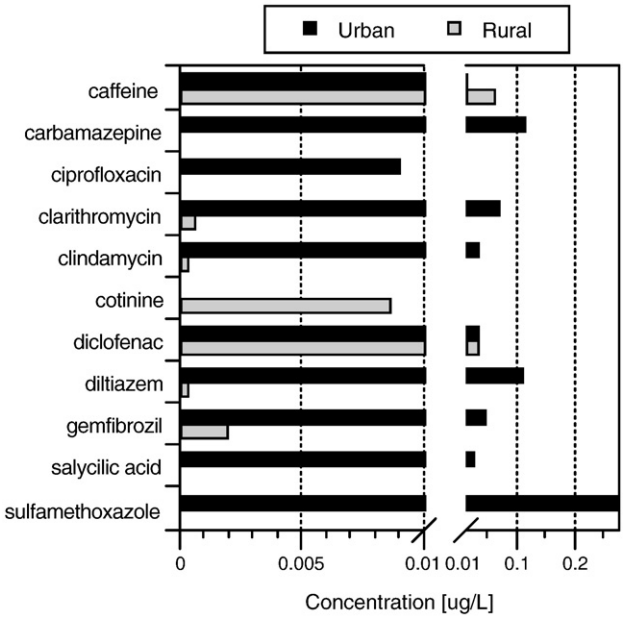


**Fig. 1 – Comparison of effluent concentrations from a rural and an urban WWTP. Both effluents collected on 12/4/2006.**

found here. Brown et al. (2006) found ciprofloxacin concentrations from below the detection limits to more than two orders of magnitude higher when compared to the current Ohio study.

All of the compounds found in effluent were also found in the biosolid at least once, except sulfamethoxazole. Only carbazamepine and clindamycin were detected during all three samplings. Ciprofloxacin, clarithromycin, diclofenac, salicylic acid, sulfamethazine and sulfisoxazole all had concentrations higher than 20 μg/kg. Although the highest measured concentration of ciprofloxacin was 46 μg/L, relatively low compared to other findings, an effect on some algal and microbial communities at concentrations within the range of these findings has been documented (Robinson et al., 2005; Maul et al., 2006). The persistence of these compounds in biosolid that are applied to the agricultural landscape need to be researched further in order to understand the future implications.

Variability among WWTP's from different countries and cities is expected since lifestyles, medical costs and philosophies can vary widely. While several antibiotics were not detected in New York effluent waters (Batt et al., 2006) they were detected well above the detection limits in the Northwest Ohio samples. Data from a WWTP in Virginia (Thomas and Foster, 2005), with a range of 0.013–0.056 μg/L, were similar to this study for the analyzed compounds. In order to compare effluent composition on a local scale (less than 50 km) concurrent samples were collected on 12/4/2006 from a rural and an urban wastewater treatment plant in Northwest Ohio. Data for the eleven compounds found to be above method LOQ's at both sites for the sampling time were compared, and are given in Fig. 1. Effluent samples still show significant concentrations of several of the analyzed compounds, although only seven out of the eleven compounds found in the urban effluent were detected. Diclofenac is very similar in concentration to the urban counterpart, whereas cotinine and caffeine nearly double. Other compounds found in the urban plant are present but are more than two orders of magnitude smaller in concentration, while others are unique to the urban plant. These data show that service area of a wastewater treatment plant does not necessarily determine effluent composition, since some compounds are similar in concentration although distinct differences exist in the area and possibly treatment efficiency. Further study at this plant is planned, since new residential construction and a new treatment facility have been constructed since this study.

Similar to the effluent comparison, biosolid samples from the urban and a suburban WWTP were analyzed for PPCP composition. Data is given in Fig. 2 and are strikingly similar to each other. All but two compounds, tetracycline and clarithromycin, were detected in both biosolids. Salicylic acid and ciprofloxacin were found at two times or greater concentration in the urban biosolid. Elevated concentrations in the suburban sample of carbamazepine (used in the treatment of seizures) and gemfibrozil (used to lower lipid levels) were found, at 21 and 17 μg/kg respectively. Both treatment plants produce Class B biosolids, however, the suburban site yields a much higher solids content than the urban site (12–20% versus 2–5%).

The increased concentration within both the effluent and biosolids and relative persistence of many other compounds would indicate that the wastewater treatment process is not effective in breaking down these compounds. Khan and Ongerth (2002), using modeling, estimated that many PPCP compounds, including gemfibrozil and carbazamepine are likely to enter the environment via liquid biosolids incorporation into soil. Metabolites have been reported for many PPCP compounds and may prove to be important environmentally since many retain similar active properties as the parent

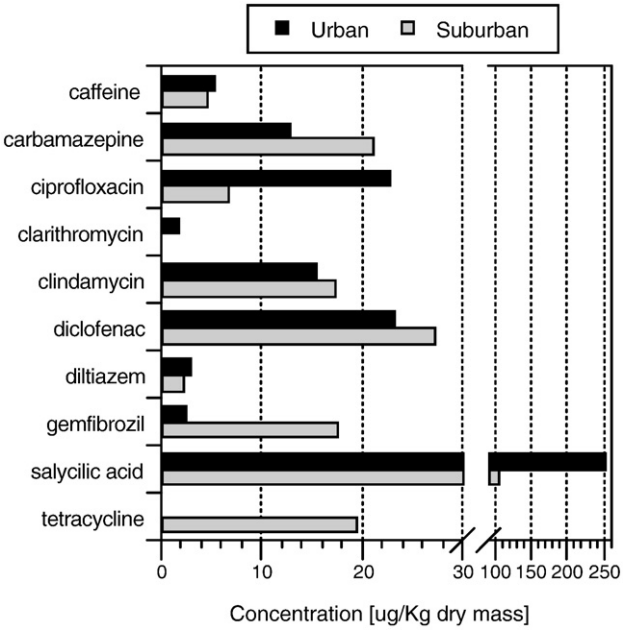


**Fig. 2 – Comparison of biosolid concentrations from a suburban and an urban WWTP.**

**Table 4 – Analysis of samples collected on 12/4/2006 in a stream fed by effluent and agricultural runoff**

| Compound | Upstream | WWTP effluent | 5 m downstream | 50 m downstream | Reported in literature | Reference |
|---|---|---|---|---|---|---|
| Caffeine | 0.0267 | 0.0615 | 0.3197 | 0.0366 | 0.2–6 | [1] [2] [3] |
| Carbamazepine | <LOQ | <LOQ | 0.0135 | <LOQ | 0.00014–0.00024 | [4] |
| Clarithromycin | 0.0014 | 0.0006 | 0.0090 | 0.0049 | 0.0750 | [5] |
| Clindamycin | 0.0003 | 0.0003 | 0.0013 | 0.0010 | 0.0900 | [1] |
| Cotinine | <LOQ | 0.0086 | <LOQ | <LOQ | 0.036–0.9 | [2] [3] |
| Diclofenac | 0.0245 | 0.0304 | <LOQ | 0.0319 | 0.002–0.599 | [6] [7] |
| Diltiazem | <LOQ | 0.0003 | 0.0052 | 0.0003 | | |
| Gemfibrozil | <LOQ | 0.0019 | <LOQ | 0.0058 | 0.79 | [2] |
| Salycilic acid | 0.0470 | <LOQ | <LOQ | <LOQ | | |
| Sulfadimethoxine | 0.0047 | <LOQ | <LOQ | <LOQ | 0.003–0.06 | [2] [3] |

[1] Batt et al., 2006 [2] Kolpin et al., 2002 [3] Haggard et al., 2006 [4] Pederson et al., 2005 [5] McArdell et al., 2003 [6] Lindqvist et al., 2005 [7] Ashton et al., 2004.
Units are in μg/L. Concentrations reported in the literature are also included.

compound. Miao et al. (2005) have shown the presence of carbamazepine metabolites in treated effluents using UV irradiation and resulting surface waters in Canada. Analytical limitations and lack of metabolite compound standards used for quantification increase the difficulty of metabolite analysis, but further research is needed.

In order to assess the impact of effluent discharge into the environment samples were taken upstream and downstream of a WWTP effluent discharge point. Unfortunately the urban WWTP effluent is discharged directly into Lake Erie, however, the rural effluent discharges into a small local ditch with minimal flow from which samples could be obtained. Data for the eleven PPCP compounds detected in the discharge stream 5 and 50 m downstream and 1 point upstream, the effluent discharge spout and concentrations found by others are given in Table 4.

Eight out of the eleven compounds analyzed were found upstream of the discharge point, although half the compounds showed an increase in concentration 5 m downstream of the discharge point. Carbamazepine increased to detectable limits, and than dissipated again by the second downstream point. Cotinine was detected in the effluent, but was not detected any further. Caffeine concentration was elevated after the discharge point, but remained comparable to upstream values further downstream. Diclofenac, which concentrated in the effluent and biosolids at the urban plant, was present at similar concentrations at the final downstream sampling point. Lindqvist et al. (2005) found that even 90 km downstream of a WWTP discharging 0.040 μg/L of diclofenac, the compound was still present at detectable concentrations. Sacher et al. (2001) in Germany, and Hilton and Thomas (2003) in the United Kingdom also found diclofenac and carbamazepine in groundwaters at concentrations as high as 590 and 900 μg/L.

At 50 m downstream the concentrations had dropped considerably for most compounds. However, measurable concentrations of diltiazem (an anti-hypertensive drug) downstream, but not upstream of the discharge point indicates an introduction to the environment at this location. Clarithromycin, and several other compounds were also found at higher concentrations 50 m downstream compared to upstream values. Salicylic acid and sulfadimethoxine were detected upstream of the plant at concentration near the LOQ, but dissipated below detection limits downstream. This preliminary data show that effluents from small rural WWTP's can introduce and increase concentrations of many PPCP compounds into environmentally sensitive areas. A further temporal and spatial study is proposed to identify long-term trends and discriminate sampling, upstream and natural environmental bias. Partitioning in the stream sediments and biota are also important for further understanding.

## 4. Summary

Within this paper we report a rigorous geochemical analysis of twenty pharmaceutical compounds in influent, effluent, biosolid and stream samples from Northwest, Ohio. These data are quantified and compared to several of the current studies that also report such data. The relatively small short-term temporal variability for many compounds within one plant is interesting, compared to the high degree of concentration and occurrence variability among plants. This likely reflects the differences in populations, WWTP operations, and the effects of the local environment to preserve or degrade these compounds. The variability of compound concentrations downstream from an effluent discharge point indicates that environmental release is possible. As more data on these compounds are reported, a better understanding of environmental fate, transport, and importance can be obtained.

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