Analysis and Modeling of Residual Compounds in Process Streams From U.S. Wastewater Treatment Plants

by

Bipin Prakash Chari

A Thesis Presented in Partial Fulfillment
of the Requirements for the Degree
Master of Science

Approved April 2012 by the
Graduate Supervisory Committee:

Rolf Halden, Chair
Paul Westerhoff
Peter Fox

ARIZONA STATE UNIVERSITY

May 2012

ABSTRACT

The presence of compounds such as pharmaceuticals and personal care products (PPCPs) in the environment is a cause for concern as they exhibit secondary effects on non-target organisms and are also indicative of incomplete removal by wastewater treatment plants (WWTPs) during water reclamation. Analytical methods and predictive models can help inform on the rates at which these contaminants enter the environment via biosolids use or wastewater effluent release to estimate the risk of adverse effects. The goals of this research project were to integrate the results obtained from the two different methods of risk assessment, (a) in silico modeling and (b) experimental analysis.

Using a previously published empirical model, influent and effluent concentration ranges were predicted for 10 sterols and validated with peer-reviewed literature. The in silico risk assessment analysis performed for sterols and hormones in biosolids concluded that hormones possess high leaching potentials and that particularly 17-α-ethinyl estradiol (EE2) can pose significant threat to fathead minnows (P. promelas) via leaching from terrestrial depositions of biosolids.

Six mega-composite biosolids samples representative of 94 WWTPs were analyzed for a suite of 120 PPCPs using the extended U.S. EPA Method 1694 protocol. Results indicated the presence of 26 previously unmonitored PPCPs in the samples with estimated annual release rates of 5-15 tons yr^{-1} via land application of biosolids. A mesocosm sampling analysis that was included in the study concluded that four compounds amitriptyline, paroxetine, propranolol and

sertraline warrant further monitoring due to their high release rates from land applied biosolids and their calculated extended half-lives in soils.

There is a growing interest in the scientific community towards the development of new analytical protocols for analyzing solid matrices such as biosolids for the presence of PPCPs and other established and emerging contaminants of concern. The two studies presented here are timely and an important addition to the increasing base of scientific articles regarding environmental release of PPCPs and exposure risks associated with biosolids land application. This research study emphasizes the need for coupling experimental results with predictive analytical modeling output in order to more fully assess the risks posed by compounds detected in biosolids.

ACKNOWLEDGMENTS

It isn’t very often that a disastrous phone interview between a professor and a prospective student gives rise to anything good. But that is my story and therein lies the paradox. I consider having Dr. Halden as academic advisor and being a member of the Halden group as one of the more surreal yet definitive moments in my academic career and my life until now. I thank Dr. Halden for making my stay here at Arizona State University a rewarding experience.

The past two years that I have spent at Arizona State University have been very eventful. I owe all of this and much more to my parents who have supported me throughout my life. As a student, I will always be in awe of the School of Sustainable Engineering and the Built Environment, the professors and the amazing classroom experience that they provide. I thank Dr. Westerhoff and Dr. Fox for being a part of my thesis committee and for helping me get settled in the graduate program.

A special mention goes to Mr. Arjunkrishna Venkatesan, Mr. Brian Goehner, Mr. Sudarshan Suresh, Mr. Shivaji R. Gaekwad, the Power Systems Engineering of ASU and all those who have inspired me along the way. Thank you all very much.

TABLE OF CONTENTS

CHAPTER Page

APPENDIX Page

LIST OF TABLES

LIST OF FIGURES

Figure

Page

PREFACE

As an independent project, a modeling analysis was performed using input data from the 2007 Targeted National Sewage Sludge Survey conducted by the7 U.S. Environmental Protection Agency (EPA). This *in silico* study utilized two empirical models that were derived independently. Obtained results from modeling of aqueous phase concentration ranges of anthropogenic organic compounds were organized in the form of a scientific paper and submitted to the peer-reviewed journal *Science of The Total Environment* in response to the editor's invitation to participate in the 2nd Annual SCARCE Special Issue. The paper is currently under review for publication. The findings have been included in the present research study under Chapter II.

In the month of August (2011), archived samples of municipal sewage sludge deemed fit for application on land (biosolids) and originally acquired by the U.S. EPA as part of the 2001 Targeted National Sewage Sludge Survey were re-analyzed using a mega-composite analysis approach and a new analytical protocol representing an expanded version of U.S. EPA Method 1694 (The new protocol currently is termed AXYS MLA-075). The results obtained were compiled and were used to report on newly detected organic trace contaminants in biosolids and to evaluate the reliability of national data generated using the mega composite sampling approach. In addition to the mega-composite sample analysis, archived soil-biosolids mixtures that were obtained as part of an outdoor mesocosm study conducted in 2010 were analyzed using the same protocol. The results were used to calculate the experimental half-lives for compounds that were

sequestered in biosolids and inform on their extended half-lives in soils. Findings were organized into a scientific paper submitted in the month of March 2012 to the peer-reviewed journal *Water Research*. This paper is currently under review for publication and has been included in this thesis as Chapter III.

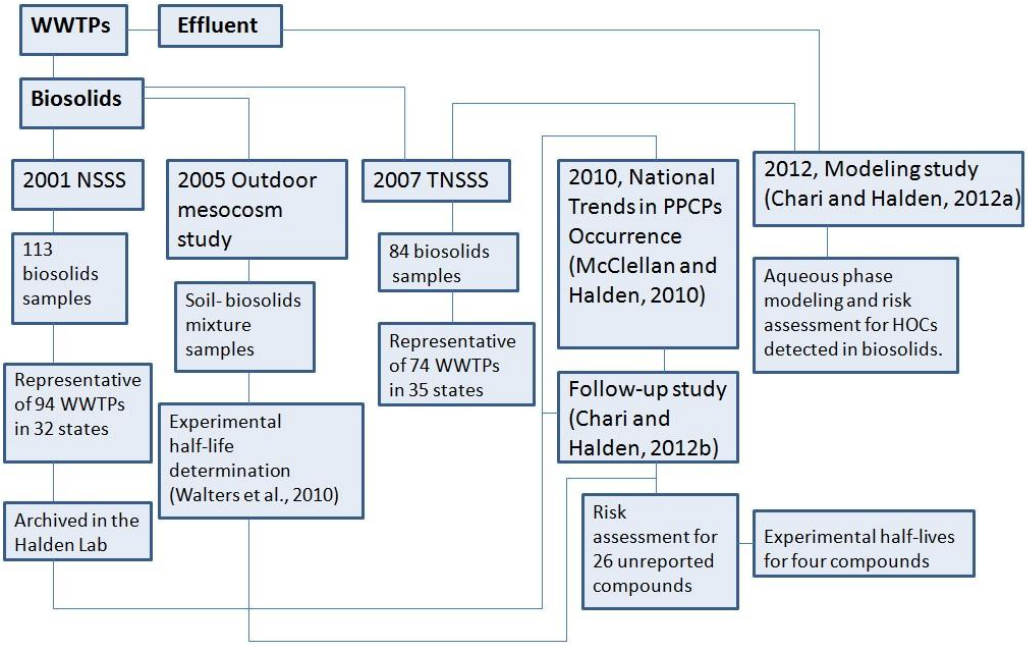


Figure 1. Research study flow chart

Chapter 1: INTRODUCTION

1.1 LITERATURE REVIEW

1.1.1 Wastewater treatment process

The National Stream Reconnaissance conducted during the years 1999 and 2000 by the U.S Geological Survey (USGS) served to identify and quantify a wide range of emerging contaminants in U.S surface waters (Kolpin et al 2002). Follow-up work on the behavior of organic compounds (OCs) during wastewater treatment firmly established a range of theoretically biodegradable compounds that are only removed incompletely and remain detectable in both treated effluent and biosolids, i.e., treated sewage sludge deemed fit for application on land (Ingrand et al 2003; Lorenzen et al 2004; Ying and Kookana 2005; Cicek et al 2007). When treated effluent and biosolids are used for beneficial purposes such as irrigation and soil amendment, there exists a threat of releasing the contained chemicals into the environment (Xia et al 2005; Kinney et al 2006; Wu et al 2010).

The primary aim of a wastewater treatment plant (WWTP) is to treat municipal discharges such that they meet the recommended microbiological and chemical quality guidelines before effluent release or reuse. This is achieved using a combination of physical, chemical and biological processes to remove solids, nutrients and contaminants from wastewater producing the effluent and sewage sludge as by-product. However, in recent times conventional WWTPs have come under scrutiny due to ineffective treatment and subsequent release of a

wide variety of chemical compounds via effluent discharge and land application of sewage sludge or biosolids.

The behavior of organic compounds during wastewater treatment is difficult to characterize due to the diversity of chemical structures and the complexity of the processes involved. Within the WWTPs, the influent gets segregated and transformed into two distinct process flows, organic-rich biosolids and the liquid effluent. Compounds undergo preferential partitioning between the aqueous phase and the sludge primarily based on their hydrophobicity, which typically is gauged by examining their *n*-octanol-water partition coefficient (K_{ow}) (Kinney et al 2006; Heidler and Halden 2008). Whereas hydrophobic compounds having large K_{OW} values (>10,000) partition almost completely into biosolids, compounds having lower K_{OW} values (<10,000) typically are removed by a combination of biodegradation and sorption processes (Khanal et al 2006). Since one of these processes results in the transformation of a chemical's mass (biodegradation) and the other merely implies a transfer of chemicals from the aqueous phase into the sorbed, solid phase, it is important to distinguish among the two when evaluating the fate of a chemical during municipal wastewater treatment.

1.1.2 Biosolids

Following the release of PPCPs into the environment, a multitude of exposure pathways have been found to exist between the source and human beings. An increasing number of research articles show that land application of

materials that contain sequestered chemicals, such as biosolids and feather-meal fertilizer could serve as an important mechanism for chemical re-entry into the environment (Chari and Halden 2012b; Love et al 2012). Biosolids is the solid, semi-solid or liquid organic material obtained as a by-product of municipal wastewater treatment. Following the Ocean Dumping Ban Act of 1988, recycling biosolids via land application has become the major means of disposal. The increase in production has resulted in a corresponding increase in the amount of biosolids used for beneficial purposes, from 36% in 1988 to approximately 55% in 2007 (USEPA 1992; NEBRA 2007). In recent years however, biosolids have been proved to behave as reservoirs for pollutants and land application can release pollutants into groundwater and waterways through leaching and runoff (Kinney et al 2006; Gottschall et al 2012; Chari and Halden 2012a).

1.1.3 Effluent dominated waters

An estimated 23% of WWTPs discharge effluents under conditions where instream dilution offered by the receiving waters is less than 10-fold. Depending on the season, the stream can be partially or fully dominated by the discharged effluent thus increasing the exposure to chemicals released via wastewater effluent discharge (Brooks et al 2003). Such a scenario occurs in perennial and ephemeral streams in arid and semi-arid regions around the world and especially the southwestern United States (Mladenov et al 2005; Brooks et al 2006). Study of aquatic ecosystems that are supported in part or completely by effluent flow is gaining interest owing to certain unique water quality characteristics inherent to

such streams and the ecotoxicological properties of the compounds released into the environment (Taylor 2002). One class of compounds that have long been scrutinized with regards to their occurrence in surface waters are phytosterols. Phytosterols occur naturally in the environment and also are major constituents of pulp and paper mill effluents. Sterol mixtures are known to be capable of inducing sexual and morphological changes in aquatic organisms (Nakari et al 2003; Honkanen et al 2004; Lopez et al 2011), and a large volume of literature shows that the products of microbially mediated breakdown of phytosterols, such as androstenedione and steroid hormones, possess endocrine disrupting properties at the ng/L range (Jenkins et al 2003; Orrego et al 2009).

1.1.4 Leaching potential of compounds

There have been several lab-scale and field-scale studies conducted that evaluate the leaching potential of compounds from land-applied biosolids to surface waters and groundwater. Although largely confined to pharmaceuticals and personal care products (PPCPs), studies conducted by Lapen et al (2008), Topp et al (2008) and Edwards et al (2009) confirmed that biosolids can act as a non-point sources of water contamination when sorbed compounds migrate from the site of application to tile drainage systems and eventually to surface waters. Key parameters that were identified to influence the transport of compounds from biosolids-amended fields were macroporous structures in the soil, soil texture, composition and moisture of the biosolids, and the method of biosolids application (e.g., surface spreading of solids vs. injection of the materials as a

slurry). A recent field study confirmed that hormones in land-applied biosolids are mobilized following strong rainfall events and that the total estrogen concentration in runoff exceeded thresholds for biological effects for time periods of >30 days post application (Yang et al 2012). The authors also stated that 35 days after application, biosolids-borne hormones were still detectable in the soil and did not complete degrade.

1.1.5 Targeted National Sewage Sludge Survey (TNSSS)

The TNSSS study and previous national sewage sludge surveys were conducted by the U.S Environmental Protection Agency (USEPA) under provisions of the Clean Water Act (CWA) to provide national estimates of compounds present in biosolids and their respective concentrations. Historically, biosolids have been analyzed for pathogens, heavy metals, poly aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-*p*-dioxins (PCDDs) as well as emerging contaminants, with an increasing focus on pharmaceuticals, sterols and hormones. Results from the first survey conducted in 1988 helped establish the Part 503 Biosolids Use and Disposal Regulations that led to a source reduction of dioxins and furans (USEPA 2002). The second study conducted in 2001 served to evaluate the reduced occurrences of dioxins and dioxin-like compounds in sludge samples (USEPA 2007). The most recent survey carried out in 2007 evaluated the occurrences of pharmaceuticals and personal care products (PPCPs) in 84 biosolids samples that were representative of 3,337 WWTPs across the nation. Seventy-two PPCPs and

25 steroids and hormones were detected in concentrations ranging from the parts-per-billion (ppb) to the parts-per-million (ppm) range by using USEPA Method 1694 (USEPA 2007).

As a part of TNSSS 2007 study and other CWA programs, the U.S. EPA developed and released an analytical protocol, EPA Method 1694. This protocol was originally designed to determine the concentration of 73 PPCPs in multi-media environmental samples by high performance liquid chromatography combined with tandem mass spectrometry (HPLC-MS/MS). It was based on analytical methods developed previously and those existing at the time and procedures developed at AXYS Analytical Services, a Canadian commercial laboratory. The analytical range of this protocol recently was expanded by AXYS to include a total of 120 PPCPs and the new, extended Method 1694 referred to as MLA-075 was used in the present study to analyze biosolids samples.

1.1.6 Mesocosm study

In 2005, a mesocosm study was conducted in Baltimore, Maryland to experimentally determine the half-lives of compounds sequestered in biosolids that are land-applied. The study was conducted under ambient outdoor conditions with no shelter or artificial irrigation. Biosolids and soil were mixed in the ratio 2:1 to facilitate detection of compounds three years after commencement. Six plastic containers were used of approximate dimensions of 30-80 x 30 x 25 cm featuring perforated bottoms to allow drainage of excess water. The leachate water was not included in the analysis. Containers filled with 100% soil showing

no background levels of PPCPs served as controls. Samples taken on the 57th, 115th, 520th, 859th and 995th day were analyzed for a suite of 120 PPCPs using the AXYS Method MLA - 075. Following a previous study conducted on the same samples by Walters et al. (2010), empirical half-lives were calculated by fitting the data to a first-order kinetics equation.

1.2 SCOPE OF STUDY

This research study is presented as

(i.) Study I, mega composite sampling approach undertaken to analyze archived biosolids samples for a suite of 120 PPCPs using the extended USEPA Method 1694, and

(ii.) Study II, modeling study undertaken to predict the aqueous phase concentrations for organic compounds in wastewater dominated streams and in run-off from biosolids amended soils.

The following chapters detail two original studies that I carried out related to biosolids and the TNSSS studies conducted by the U.S. EPA. The first study detailed in Chapter II describes the *in silico* approach undertaken to (i) identify potentially problematic organic compounds in biosolids, (ii) predict influent and effluent levels for hydrophobic organic compounds (HOCs) of emerging concern, and (iii) provide initial estimates of runoff concentrations, in this case four prominent hormones known to act as endocrine disruptors.

Chapter III details the second study, wherein mega-composite samples were constructed with archived biosolids samples and analyzed along with soil-biosolids mesocosm study samples using the extended U.S. EPA Method 1694. The results revealed (i) 26 previously non-reported compounds that were detected in ppb concentrations, (ii) the corresponding first estimates of nationwide release rates of these previously non-monitored compounds to soils, and (iii) estimates of half-lives of select compounds in soils amended with biosolids.

.

Chapter 2: STUDY I

MODELING STUDY UNDERTAKEN TO PREDICT THE AQUEOUS PHASE CONCENTRATIONS FOR ORGANIC COMPOUNDS IN WASTEWATER DOMINATED STREAMS AND IN RUN-OFF FROM BIOSOLIDS AMENDED SOILS

2.1 INTRODUCTION

In the present study a validated empirical model leveraging mass balance approaches and partitioning theory (Deo and Halden 2009) was used to model the aqueous concentrations of 10 sterols based on their reported biosolids concentrations. The model was originally introduced as a screening tool to identify potentially problematic sewage constituents and to predict the behavior and extent of partitioning a given compound is likely to undergo during treatment in a real-world POTW (Heidler and Halden 2008; Deo and Halden 2010). The sole parameters required for operation of the model are the compound's concentration in biosolids ($C_{biosolids}$), its pH-adjusted *n*-octanol/water partitioning coefficient (D_{OW}) and a dimensionless curve-fitting parameter (p_{fit}) (Deo and Halden 2009; Weir et al 2010). Thus far, the model has been applied only to compounds within a limited range of log D_{OW} values of 4.9 – 6.4. The present study intended to expand the applicability of the model and to validate its performance using data available in the peer-reviewed literature.

A secondary objective of this paper was to also consider the potential for runoff and leaching of chemicals following application of biosolids on land for inexpensive disposal and for soil conditioning and fertilization. Langdon et al (2010) further utilized another model to estimate the toxicity posed by natural and synthetic hormones contained in biosolids using pore-water concentrations ($C_{porewater}$) as model input. This model adopted an equilibrium-based partitioning approach that required $C_{biosolids}$ and K_{OW} as primary input parameters in addition to general parameters discussed hereafter. In the present work, both models were combined to estimate the concentration in influent and effluent (C_{inf} and C_{eff}, respectively) for 10 sterols, and the run-off potential for four hormones that have been frequently reported in literature, estrone (E1), estradiol (E2), estriol (E3) and 17-β-ethinylestradiol (EE2).

2.2 EXPERIMENTAL METHODS

Aqueous phase concentrations were calculated for 14 hydrophobic organic compounds (HOCs) based on their $C_{biosolids}$, log D_{OW} and log K_{OW} values using two independent models.

2.2.1 EMPIRICAL MODEL I

The fraction of the total mass of a given compound entering a POTW ($f_{biosolids}$) was calculated using an established empirical model (Eq. 1) that previously had been validated for application in the log D_{OW} range of 4.9 – 6.4 (Deo and Halden, 2010). Prior studies also had identified a value of 1.76×10^{-6} as appropriate for the dimensionless fitting parameter, p_{fit}.

$$f_{biosolids} = p_{fit} \times \frac{D_{ow}}{(1+p_{fit} \times D_{ow})} \qquad \text{(Eq. 1)}$$

Using mass-balance approaches, the concentration of a chemical in biosolids, $C_{biosolids}$ can be determined as a function of the total concentration of the chemical entering the POTW, $C_{influent}$, the fraction amenable to sequestration in biosolids ($f_{biosolids}$) and the yield of biosolids per volume of raw wastewater treated (Y).

$$C_{biosolids} = C_{influent} \times f_{biosolids} \times \frac{1}{Y} \qquad \text{(Eq. 2)}$$

The value of *Y* was taken from the literature, 2.4×10^{-4} kg/L (Kinney et al., 2006). Eq. 2 was rearranged as shown in Eq. 3 to yield influent concentrations.

$$C_{influent} = \frac{C_{biosolids}}{f_{biosolids}} \times Y \qquad \text{(Eq. 3)}$$

At any time, the relation between total mass of the compound leaving a POTW, $C_{effluent}$ is dependent on its $C_{influent}$ and $C_{biosolids}$,values can be expressed as,

$$C_{influent} - C_{effluent} = C_{biosolids} \times Y \quad \text{(Eq. 4)}$$

The above relationship was rearranged to include Eq. 3 that enabled calculation of the effluent concentration ($C_{effluent}$).

$$C_{effluent} = \left(\frac{1}{f_{biosolids}} - 1\right) \times C_{biosolids} \times Y \quad \text{(Eq. 5)}$$

The removal efficiency of individual compounds and the model's accuracy was validated using the most recent results obtained from a study conducted on the removal of sterols in POTWs in Canada (Furtula et al., 2011). Removal efficiencies (Predicted vs. Observed) were matched for each compound by a paired *t*-test at the confidence level of $p = 0.01$.

2.2.2 EMPIRICAL MODEL II

The leaching potential of hormones was predicted based on $C_{biosolids}$ values reported in the recent Targeted National Sewage Sludge Survey (TNSSS) conducted by the U.S. EPA (USEPA 2009a). Model II was developed previously by another research group (Langdon et al 2010). It was specifically used for analyzing hormones, since these compounds are not as hydrophobic as the sterols, and previous models had a restrictive use with regards to compounds of low hydrophobicity.

The concentration of a given hormone in homogenized mixtures of soil and biosolids was calculated according to Eq. 4

$$C_{soil} = C_{biosolids} \times \frac{Mass_{biosolids}}{Mass_{soil}} \qquad \text{(Eq. 4)}$$

, where $C_{biosolids}$ and C_{soil} represent the mass of biosolids and soil, respectively, that were mixed together during application. For moist soils, the partitioning of a given compound between the solid and aqueous phases was estimated using Eq. 5,

$$\frac{M_b}{M_{soln}} = \frac{(K_d \times M_s)}{V_o} \qquad \text{(Eq. 5)}$$

, where M_b is the mass in µg of the compound associated with the solid phase at equilibrium, and M_{soln} in units of µg is the mass of the compound present in the dissolved state in the aqueous phase at equilibrium. Representative values for soil density, M_s, of 1.3 g cm^{-3} and for soil moisture content, V_o, of 0.22% were taken from the literature (De Lannoy et al 2006). The soil:biosolids mixing ratio was assumed to equal 25:1 based on recommendations by the U.S. EPA (McClellan and Halden 2010).

The pore-water concentration of a given compound at equilibrium was calculated using Eq. 6,

$$C_{porewater} = \frac{M_o}{\left[\left(\frac{M_b}{M_{soln}}\right) + 1\right]} \qquad \text{(Eq. 6)}$$

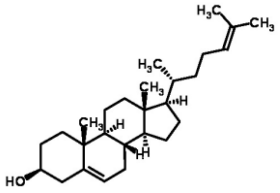
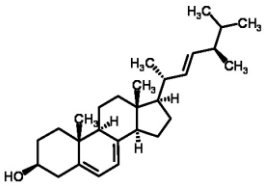
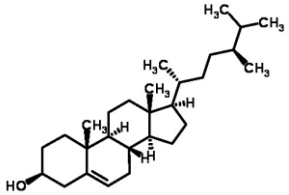
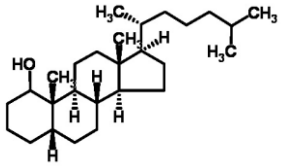
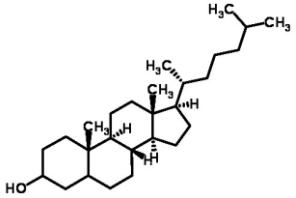
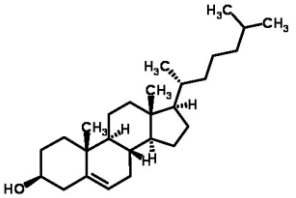
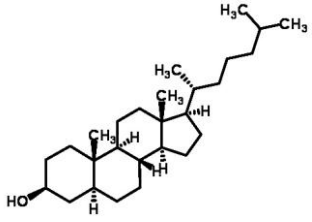
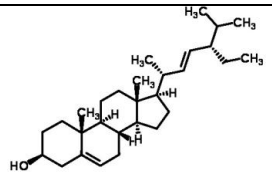
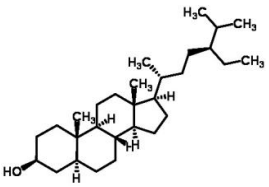
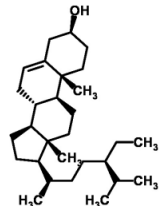
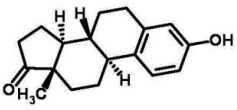
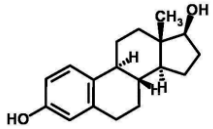
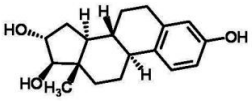
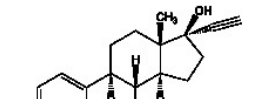
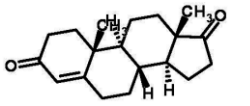
, where $C_{porewater}$ is the concentration of a compound in saturated soil (µg/L) and M_o is the mass of compound in 1 cm^3 of soil after equilibration (µg/1.3gm).

2.3 RESULTS

2.3.1 VALIDATION OF EMPIRICAL MODEL I

The present study served to expand the applicability of an existing empirical model and validate its output by comparing the predicted versus actual removal efficiencies (expressed in %) for the sterol compounds shown in Table 1. A paired *t*-test was performed for nine of the ten sterols considered here; ergosterol could not be included because the dataset by Furtula et al (2010) lacked information on this compound. Predicted values were found to match observed ones very closely, as indicated by a factor of 1.04 ± 0.04 that was very close to the ideal value of unity. Results from the *t*-test analysis confirmed the two datasets to be statistically indistinguishable at the 99% confidence interval.

Table 1. List of sterols that were analyzed in this study. $C_{biosolids}$ ($\mu g\ kg^{-1}$) values were taken from the TNSSS 2009 reports (U.S. EPA, 2009) and compound structures, log D_{ow} (sterols) and log K_{ow} (hormones) values and CAS numbers were taken from RSC online database and the literature (Ying et al., 2002). Half-life values reported are for aquatic environments and were estimated using the PBT Profiler of the U.S. EPA.

Desmosterol log D_{ow}: 9.27 CAS: 313-04-2 $C_{biosolids}$: 2230 (min) 94,400 (max) 14,816 (mean) $t_{1/2}$: 60 days Ergosterol log D_{ow}: 9.3 CAS: 57-87-4 $C_{biosolids}$: 2180 (min) 91,900 (max) 20,080 (mean) $t_{1/2}$: 60 days Campesterol log D_{ow}: 9.97 CAS: 474-62-4 $C_{biosolids}$: 2840 (min) 524,000 (max) 97,298 (mean) $t_{1/2}$: 60 days Coprostanol log D_{ow}: 10.06 CAS: 360-68-9 $C_{biosolids}$: 7720 (min) 43,700,000 (max) 2,795,254 (mean) $t_{1/2}$: 60 days Cholestanol log D_{ow}: 10.07 CAS: 80-97-7 $C_{biosolids}$: 3860 (min) 4,590,000 (max) 473,067 (mean) $t_{1/2}$: 60 days Cholesterol log D_{ow}: 9.62 CAS: 57-88-5 $C_{biosolids}$: 2,340 (min) 5,390,000 (max) 727,338 (mean) $t_{1/2}$: 60 days Epicoprostanol log D_{ow}: 10.06 CAS: 516-92-7 $C_{biosolids}$: 868 (min) 6,030,000 (max) 818,673 (mean) $t_{1/2}$: 60 days	      	Stigmasterol log D_{ow}: 10.07 CAS: 83-48-7 $C_{biosolids}$: 455 (min) 806,000 (max) 120,671 (mean) $t_{1/2}$: 60 days *β*-Stigmastanol log D_{ow}: 10.07 CAS: 83-45-4 $C_{biosolids}$: 3440 (min) 1,330,000 (max) 152,834 (mean) $t_{1/2}$: 60 days *β*-Sitosterol log D_{ow}: 10.73 CAS: 83-46-5 $C_{biosolids}$: 1210 (min) 1,640,000 (max) 302,123 (mean) $t_{1/2}$: 60 days Estrone (E1) log K_{ow}: 3.43 CAS: 53-16-7 $C_{biosolids}$: 19.70 (min) 965 (max) 109.34 (mean) $t_{1/2}$: 38 days 17-*β*-Estradiol (E2) log K_{ow}: 3.94 CAS: 50-28-2 $C_{biosolids}$: 16.20 (min) 355 (max) 35.57 (mean) $t_{1/2}$: 38 days Estriol (E3) log K_{ow}: 2.81 CAS: 50-27-1 $C_{biosolids}$: 5.80 (min) 232 (max) 38.85 (mean) $t_{1/2}$: 38 days 17-*α*-Ethinylestradiol (EE2) log K_{ow}: 4.15 CAS: 57-91-0 $C_{biosolids}$: 12.80 (min) 61.90 (max) 22.53 (mean) $t_{1/2}$: 38 days Androstenedione log K_{ow}: 2.75 CAS: 63-05-8 $C_{biosolids}$: 57.70 (min) 1520 (max) 328.44 (mean) $t_{1/2}$: 60 days	       

2.3.2 MODELED C_{INF} AND C_{EFF} VALUES

Modeling results for aqueous phase concentrations are shown in Figure 1. For a comparative analysis, the concentrations were plotted as logarithmic equivalents of the obtained values.

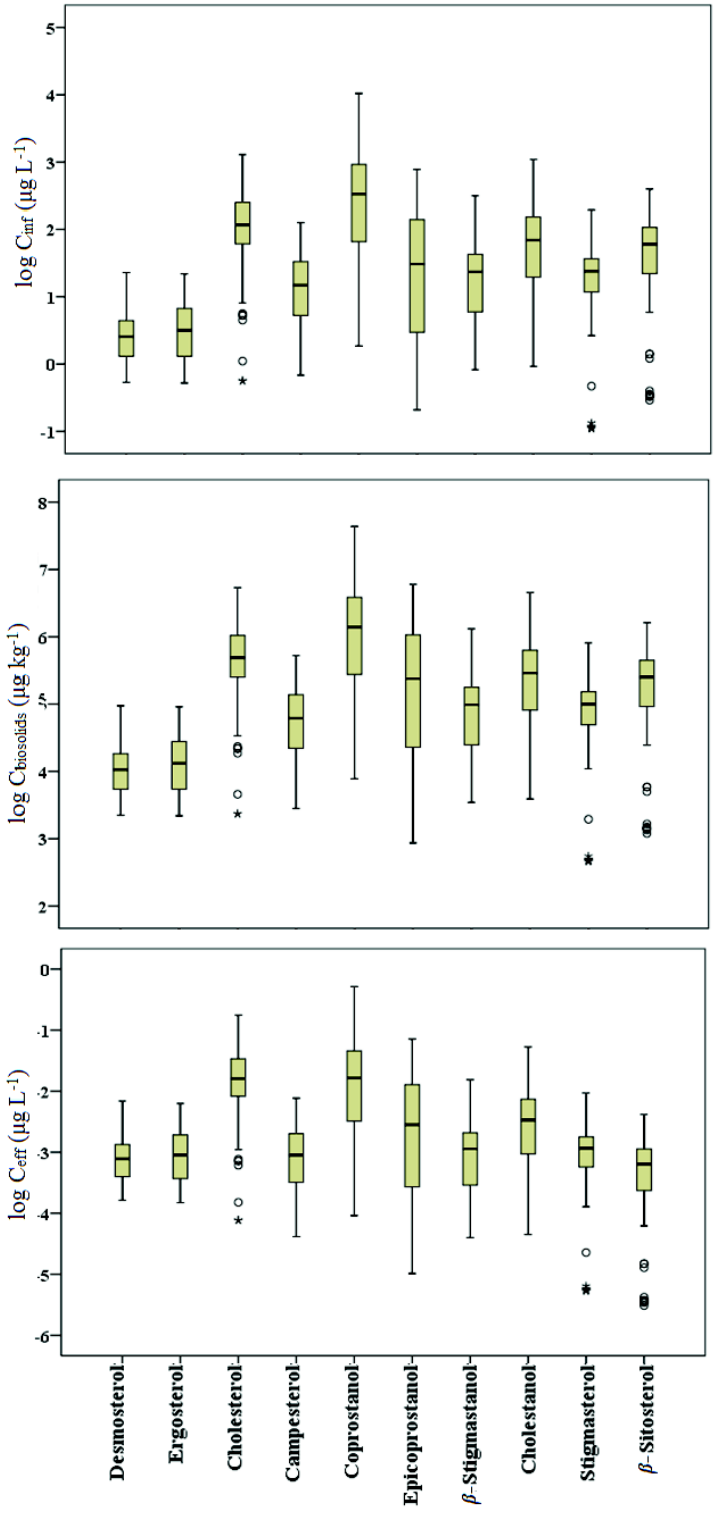

log Cinf (µg L-1)
log Cbiosolids (µg kg-1)
log Ceff (µg L-1)
Desmosterol
Ergosterol
Cholesterol
Campesterol
Coprostanol
Epicoprostanol
β-Stigmastanol
Cholestanol
Stigmasterol
β-Sitosterol

Figure 2. Predicted concentration ranges of 10 steroids, plotted on a logarithmic scale, in POTW influent (C_{inf}) and effluent (C_{eff}), calculated based on concentrations in biosolids ($C_{biosolids}$) reported in the peer-reviewed literature. Values that exceed or fall below the upper and lower quartiles by 1.5x appear as outlier symbols marked with the asterisk symbol (*). The greatest and smallest values excluding the outliers are denoted by the whiskers, whereas the box is delineated by the upper quartile (top of box), lower quartile (bottom of box) and the median value (center line).

The top panel shows concentrations estimated for raw sewage entering the POTWs. The middle panel shows the model input values, i.e., the concentrations of analytes in biosolids on a dry weight basis. The bottom panel of Figure 1 shows the range of effluent concentrations returned by the model. An examination of the top and bottom panels of Figure 1and the information presented in Table 2 reveals that the predicted removal efficiencies for all sterols were uniformly high at 99.9% and thus were in good agreement with those (86.4 to 99.1%) determined experimentally by Furtula et al (2011). Similarly, values reported by the U.S. EPA for C_{inf} and C_{eff} detected at POTWs (USEPA 2009b) were of the same magnitude as those predicted here and depicted in Figure 1.

Table 2. Modeled influent, effluent concentrations and removal efficiency for 10 sterols. Removals calculated at POTWs were obtained from Furtula et al (2011)

Compound	Concentration in influent (ng L^{-1})			Concentration in effluent (ng L^{-1})			% Removal Predicted	% Removal Observed
	$C_{influent\ (Min)}$	$C_{influent}$ (Max)	$C_{influent}$ (Mean)	$C_{eff.}$ (Min)	$C_{eff.}$ (Max)	$C_{eff.}$ (Mean)		
Desmosterol	5.35E+02	2.27E+04	3.56E+03	0.163	6.9	1.1	99.9	93.1
Ergosterol	5.23E+02	2.21E+04	4.82E+03	0.149	6.3	1.4	99.9	-
Cholesterol	5.62E+02	1.29E+06	1.75E+05	0.077	176.3	23.8	99.9	98.7
Campesterol	6.82E+02	1.26E+05	2.34E+04	0.041	7.7	1.4	99.9	98
Coprostanol	1.85E+03	1.05E+07	6.71E+05	0.092	519	33.2	99.9	99.1
Epicoprostanol	2.08E+02	7.82E+05	1.06E+05	0.01	71.6	9.7	99.9	94.3
β-Stigmastanol	8.26E+02	3.19E+05	3.67E+04	0.04	15.4	1.8	99.9	96.7
Cholestanol	9.26E+02	1.10E+06	1.14E+05	0.045	53.3	5.5	99.9	98.7
Stigmasterol	1.09E+02	1.93E+05	2.90E+04	0.005	9.4	1.4	99.9	86.4
β-Sitosterol	2.90E+02	3.94E+05	7.25E+04	0.003	4.2	0.8	99.9	97.7

2.3.3 MODELED $C_{POREWATER}$ VALUES

Porewater concentrations (shown in Table 3) indicate the migration potential of compounds applied on soils in biosolids. Chemicals present in porewater are available for leaching into ground and surface water during rainfall events. Modeled $C_{porewater}$ values for hormones, shown in Table 3, were in the parts-per-trillion range, owing to the low $C_{biosolids}$ values.

Table 3. Modeled porewater concentrations for four hormones.

Compound	$C_{porewater}$ (ng L^{-1}) Minimum	$C_{porewater}$ (ng L^{-1}) Maximum	$C_{porewater}$ (ng L^{-1}) Mean	Aquatic toxicity (ng L^{-1})
Estrone (E1)	0.00	55.14	5.80	15[a]; 50[b]
17-β-Estradiol (E2)	0.49	10.71	1.07	5[c]
Estriol (E3)	0.00	28.82	4.60	10[e]
17-α-Ethinylestradiol (EE2)	0.00	0.70	0.55	0.32[d]
Androstenedione*	0.00	204	41	-

* Androstenedione is an androgen that was selected for modeling purposes only.

a. Increased aggressiveness of fathead minnows (*P. promelas*) as seen during a 21-day study by Dammann et al (2011).

b. Diminished egg production in *P. promelas* as seen during a 21-day study by Dammann et al (2011).

c. Caused vitellogenin induction in adult male Zebra fish (*D. rerio*) as reported by Brion et al (2004).

d. Decreased male sex characteristics and reduced egg fertilization success observed in adult male *P. promelas* were reported by Parrott and Blunt (2004).

e. Altered sex ratio seen in Japanese Medaka fish (*O. latipes*) as reported by Metcalfe et al (2001).

In order to assess the hazard posed to aquatic organisms by the flushable mass fraction of compounds present in the aqueous phase (i.e., porewater), toxicity values taken from the literature were plotted alongside modeled values.

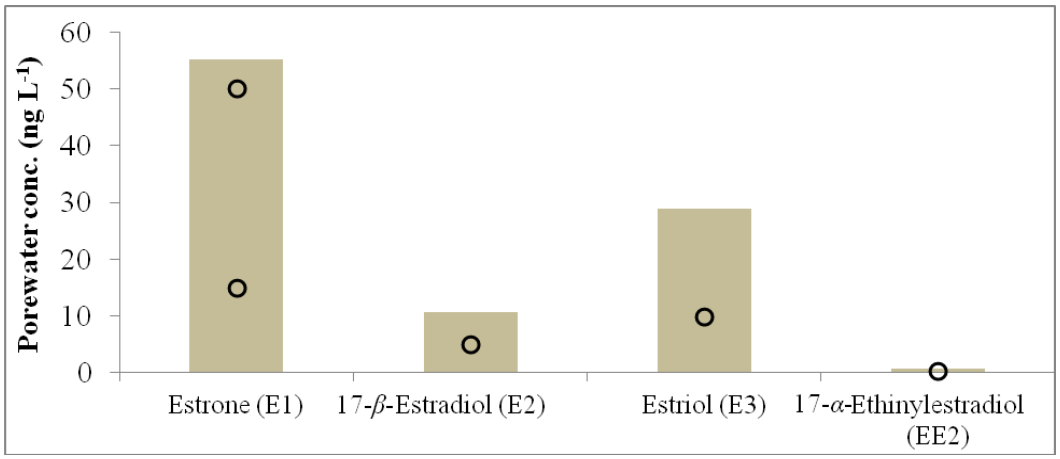


Figure 3. Predicted $C_{porewater}$ range based on $C_{biosolids}$ values for 4 hormones. Circles represent aquatic toxicity threshold values that were reported in literature.

As can be seen in Figure 2, the expected range of porewater concentrations of all 4 analytes considered bracketed the toxicity values for sensitive aquatic species including fathead minnows (*P. promelas*) (Dammann et al 2011), Zebra fish (*D. rerio*) (Brion et al 2004) and Japanese Medaka fish (*O. latipes*) (Metcalfe et al 2001). Additional information on calculated minima, means and maxima is presented in Table 3 along with specific information on observed adverse outcomes (Table 3, footnotes).

2.4 DISCUSSION

2.4.1 STEROLS

As shown in Table 2, the predicted removal efficiencies for all sterols were >99% which resulted in the extremely low concentrations in the effluent (Figure 2). These predictions matched closely the empirical removal efficiencies observed at treatment plants, as reported by Furtula et al (2010). Of the compounds that were analyzed, four were grouped as phytosterols (campesterol, β-sitosterol, stigmasterol and β-stigmastanol), five were associated with cholesterol as either precursors or breakdown products (cholesterol, desmosterol, cholestanol, coprostanol and epicoprostanol), and one was a fungal cell-wall component (ergosterol).

Phytosterols are naturally occurring compounds that are known to be present in high concentrations in paper mill effluents and have been detected at the parts-per-billion range downstream of rivers receiving effluent from mills. These compounds have been linked to androgenic effects in aquatic organisms caused either directly or as breakdown products of the parental sterols (Ellis et al 2003; Jenkins et al 2003; Orrego et al 2009). Similar to phytosterols, cholesterol and related compounds have long been suspected of causing androgenic effects or giving rise to compounds that have androgenic effects (Ellis et al 2003; Jenkins et al 2003). More specifically, androstenedione, a microbial transformation product of phytosterols and cholesterol, is a known androgen (Jenkins et al 2004). In the present study, androstenedione (not presented in Fig. 2) was found to have highest

runoff potential among the hormones analyzed, owing to its low hydrophobicity (log K_{OW} = 2.75) (See Table 1, Table 3). The $C_{porewater}$ value calculated for androstenedione was 204 ng L^{-1} and nearly four times greater than that of the hormone estrone (E1), which had the highest migration potential amongst the four hormones analyzed. Considering that both phytosterols and cholesterol related compounds were detected in mg kg^{-1} range, the concentration of androstenedione that could migrate/leach to aqueous phase could be potentially higher than the values reported here. EPA's PBT Profiler software suggests aquatic half-life values for the sterols considered here on the order of 60 days (Table 1).

2.4.2 HORMONES

A recent study ranked hormones among the most studied compounds with regards to their presence in the environment, and the compounds estrone (E1) and 17-*β*-estradiol (E2) being the most thoroughly investigated (Miège et al 2009). The list of hormones analyzed in this study comprises both naturally occurring (E1, E2, E3) and synthetic (EE2) ones. While E1, E2 and E3 are excreted by humans naturally, EE2 is excreted only by women taking contraceptive pills. All of these hormones have been detected in the natural environment in the ppb range, especially in surface waters and all are known to cause a variety of secondary effects in aquatic organisms, including reversal of sex-ratios and delayed fertilization (Brion et al 2004; Parrott and Blunt 2004; Schäfers et al 2007; Dammann et al 2011).

While adequate details on the presence of E3 in the environment could not be gathered, the hazard posed by the remaining four hormones was assessed. The range of $C_{porewater}$ predicted for E1, E2, E3 and EE2 was noted to encompass the values at which these compounds exert adverse effects on fathead minnows and zebra fish (see Table 3 and Figure 2). EE2 was found to have the smallest Lowest Observed Effect Concentration (LOEC) value amongst the hormones and was determined to possess the highest toxicity, whereas E1 was found to have the highest leaching potential, due to its relatively high concentration in biosolids and comparatively low hydrophobicity (log K_{OW} 3.43). Yang et al (2012) suggested that both increased rainfall and increased hormone load applied in biosolids on land can trigger elevated runoff concentrations. The mass of hormones contained in runoff and leachate also is expected to vary based on the temporal precipitation profile, with intermittent rains potentially leading to the largest amount of mass released due to the extended equilibration time. However, the present model does not take into consideration variations in rainfall and assumes constant soil moisture content.

2.4.3 ENVIRONMENTAL IMPLICATIONS

The present study served to predict influent and effluent concentration ranges for 10 sterols and investigate the implications of four hormones detected in biosolids samples as reported in the 2009 TNSSS study. The results presented in this study are representative of effluent discharges that receive little or no dilution via surface waters, and the here presented empirical model serves to function as a

screening tool that can be used to calculate the theoretical removal rates and the aqueous phase concentrations based on reported $C_{biosolids}$ values for compounds with log D_{ow} values in the range of 4.9 to 10.

Also calculated in this study were the migration potentials of four hormones included in the TNSSS. Modeled $C_{porewater}$ values indicated that the migration potential of the hormones were in the descending order of E1>E3>E2>EE2; EE2 was found to have a problematic hazard potential since the compound had the smallest LOEC value. The run-off predictions presented here and the order of migration potentials were found to agree with those presented by Yang et al. (2012). In conjunction with these results, a medaka assay conducted by Metcalfe et al (2001) revealed the relative estrogenic potentials of these four hormones were EE2>E2≈ E1>E3. Conventional POTWs are faced with the challenge of treating a continuous load of organic compounds, including phytosterols and hormones that possess endocrine disrupting properties. Findings from this study suggest that although sterols are removed from wastewaters at high efficiencies they tend to accumulate in biosolids, where they may be subject to microbial conversion to metabolites that possess relatively greater endocrine disrupting potential than the parent compounds. Compounds sequestered in biosolids and subsequently applied on land potentially can migrate into groundwater and surface waters and thus can pose a significant hazard to aquatic organisms, as shown by the results of this study. However, it is important to note that the mass fraction of compounds present in porewater is very small when compared to the

amount of chemical sorbed onto particles, and that equilibrium concentrations present in porewater will be diluted significantly during rainfall events. Thus, a hazard assessment based on equilibrium porewater concentrations has to be interpreted as a worst-case scenario. In reality, flushed porwater volumes will be diluted significantly by rainwater, thereby lowering due to dilution the risk of harmful exposures. Results of this modeling study suggest that although hydrophobic compounds readily partition into biosolids, they also are expected to be bioavailable at levels sufficiently high to cause endocrine disruption in sensitive aquatic species upon leaching from field soils amended with biosolids.

Chapter 3: STUDY II

MEGA COMPOSITE SAMPLING APPROACH UNDERTAKEN TO ANALYZE ARCHIVED BIOSOLIDS SAMPLES FOR A SUITE OF 120 PPCPS USING THE EXTENDED USEPA METHOD 1694

3.1 INTRODUCTION

The purpose of the present study was to examine the occurrence of previously unmonitored pharmaceuticals in archived biosolids samples and to critically evaluate the mega composite sampling approach that – for matters of convenience, speed and cost-effectiveness – relies on a very limited number of measurements to create estimates of national inventories of chemicals in biosolids, but whose reproducibility from an experimental perspective is as of yet unknown. Building on the list of 72 previously reported compounds (McClellan and Halden 2010), an additional 48 compounds were monitored in this work using a newly introduced analytical method (AXYS Method MLA-075) that extends the analyte range of U.S. EPA Method 1694 without changing any of the attributes inherent to the originally reported protocol. Study results reveal the identities of 26 newly reported PPCPs in biosolids, yield U.S mass inventories for the latter, and provide evidence for the reproducible preparation and analysis of large biosolids composite samples constructed from 94 WWTPs across the U.S.

3.2 EXPERIMENTAL METHODS

3.2.1 SAMPLING PROCEDURE

This study utilized 113 biosolids samples obtained by the U.S. Environmental Protection Agency (EPA) for the 2001 National Sewage Sludge Survey (NSSS). These samples make up a small fraction of the U.S National Biosolids Repository, maintained at the Biodesign Institute at Arizona State University in the laboratory of Dr. Halden. During the 10-year period between sample acquisition and analysis, samples were stored at -20^{o}C. Along with the NSSS samples, soil-biosolids mixtures from an outdoor mesocosm study conducted in Baltimore, Maryland were also analyzed as a part of this study to experimentally determine the half-lives of the extended list of PPCPs. Details about the design of the mesocosm studies have been provided previously (Walters et al., 2010).

3.2.2 COMPOSITE SAMPLE PREPARATION

Of the 113 NSSS samples three were excluded from analysis as the containers were broken or compromised (McClellan and Halden 2010). Five groups were created with the remaining 110 samples, by weighing out approximately one g of dry weight from each sample and pooling it to obtain five composite samples each containing solids from between 21 and 24 individual samples. A split sample of composite 1 was prepared to serve as blind duplicate. All procedural steps were identical to those described previously for the initial mega composite study (McClellan and Halden 2010; Appendix A).

3.2.3 SAMPLE ANALYSIS

Samples were analyzed by AXYS Analytical Services (2045 Mills Road West, Sydney, British Columbia, V8L 358) according to AXYS Method MLA – 075, a modification of the USEPA Method 1694. All analytes were separated by liquid chromatography and detected by tandem mass spectrometry. Analytes were quantified using isotope dilution technique or internal standard quantification with linear regression calibration. More detailed information on the analysis method is available in Appendices A and B.

3.2.4 QUALITY ASSURANCE

Before sample analyses were performed, several tests were carried out to ensure system and laboratory performance. A verification of calibration accuracy was performed using calibration standard solution with native and labeled analytes. The retention times of both the native and labeled compounds were required to be within ± 15 s of the respective retention times determined during initial calibration. Throughout the analysis precision and recovery were ensured. Lab blanks were analyzed prior to each sample analysis. Analysis of duplicate samples was performed by the lab for each batch consisting of seven to 20 samples. In addition to these, a blind duplicate was included in the sample set to evaluate analysis precision according to the following formula.

$$RPD\ (\%) = \left(\frac{C_{sample} - C_{duplicate}}{\frac{C_{sample} + C_{duplicate}}{2}} \right) X\ 100 \qquad \text{Equation 1}$$

3.2.5 REPRODUCIBILITY OF RESULTS

To gauge the integrity of the samples and efficiency of the analytical method, a statistical comparison was carried out between the present and previous datasets obtained for composites created from the same archived individual biosolids samples. Data from the study by McClellan and Halden (2010) and from the present study were compared statistically using a paired *t*-test approach and scatter-plot correlation analysis.

3.2.6 MODELING OF POREWATER AND EQUILIBRIUM SOIL CONCENTRATION

In order to inform environmental risk assessments for the compounds newly detected in biosolids, the soil concentrations following land application were calculated following a previously established approach (McClellan and Halden 2010). Calculations took into account a soil-biosolids mixing ratio of 25:1. Bulk densities of soil and biosolids used in these calculations were assumed to be 1.3 g cm^{-3} and 1.6 g cm^{-3} respectively, and an average soil moisture content of 22% (v/v) was assumed as reported earlier by others (De Lannoy et al 2006). Organic carbon fractions of soil and biosolids were assumed to equal 0.4 (Causarano et al 2008; USEPA 2007). Calculations involved the two equations below:

$$C_{porewater} = \frac{\frac{m_{biosolids} \times C_{biosolids}}{m_{soil+biosolids}}}{\frac{f_{porewater} \times \rho_{porewater}}{m_{soil+biosolids} + K_{OC} \times f_{OC}}} \qquad \text{Equation 2}$$

$$C_{soil} = \frac{m_{biosolids} \times C_{biosolids}}{m_{soil+biosolids}} - \frac{f_{porewater} \times \rho_{porewater}}{m_{soil+biosolids}} \times C_{porewater} \qquad \text{Equation 3}$$

, where m is the dry mass in kg m^{-3} of the solids, C is the concentration in μg kg^{-1}, ρ is the density in kg m^{-3} and $f_{porewater}$ and f_{OC} are the dimensionless fractions, respectively, of the pore-water and organic carbon in the soil/biosolids mixture, respectively.

3.2.7 DRUG USAGE AND ECOTOXICITY DATA

Information on drug sales and uses were obtained from Internet sources (http://www.rxlist.com) and from the IMS Health database (2009). Ecotoxicity and half-life data were predicted using the PBT Profiler software provided online by the U.S EPA as described previously (McClellan and Halden 2010).

3.2.8 MODELING OF ANNUAL LOADING TO AGRICULTURAL SOIL

Annual loading of PPCPs to agricultural soils was calculated for a biosolids production rate of 7.2 million dry tons per year, of which 55% is land applied (NEBRA 2007) using a previously established approach (McClellan and Halden 2010).

3.2.9 EXPERIMENTAL CALCULATION OF HALF-LIFE

As a part of this study, archived mesocosm samples that contained soil:biosolids mixtures were analyzed using the same analytical procedure described previously (Walters et al 2010) in order to calculate the half-lives of the sequestered compounds.

3.3 RESULTS

3.3.1 DATA QUALITY ASSURANCE

No detections above the method detection limit were observed in the lab blanks for any of the analytes; hence, measured concentrations of all analytes were accepted. An On-going Precision and Recovery (OPR) procedure was carried out for each target analyte as part of the Quality Assurance/Quality Control (QA/QC) protocol. A reference matrix containing known background levels of target analytes was spiked with an aliquot of spiking solution while the test samples were spiked with surrogate standards. The recovery range obtained from the test samples was compared with the reference matrix recovery to establish method detection limits and the analyte's recovery rate. The average recovery rate for a subset of 26 compounds that were quantified using internal standards was 103% with recoveries ranging from 47-357%. Individual recoveries for all these compounds were noted to be within the method's lower and upper control limits with the notable exception of desmethyldiltiazem (357%) whose recovery exceeded the method's upper control limit range of 350%. The values reported for this compound thus may represent an over-estimation (Table 4). Recovery rates for two compounds were close to the method's lower control limit, amlodipine (47 vs. 45-130%) and alprazolam (73 vs. 70-130%). A significant analytical cross-interference was seen between hydrocodone and codeine compounds. An algebraic correction was performed for both compounds that enabled detection

and correction of false positive occurrences. Values for hydrocodone represent approximate concentrations with the interferences taken into account. Duplicate analyses revealed a 15% relative percent difference (RPD) for all compounds detected consistently in each sample and its corresponding duplicate.

Table 4. Results for 26 PPCPs monitored in five composite samples representing 94 U.S. wastewater treatment plants. Compounds observed inconsistently are marked with an asterisk (*) and the number of positive detections (n<5) is shown in parentheses.

#	Compound	MDL µg kg^{-1}	Mean ± Std. Dev. µg kg^{-1}	Recovery %	Use
1	10-Hydroxy-amitriptyline	0.4	14.4 ± 5.7	97.4	Metabolite
2	Amitriptyline	0.6	275.4 ±92.8	78.8	Antidepressant
3	Amlodipine *	4.2	ND (n=3)	47	Ca^{2+} Channel blocker
4	Alprazolam*	0.7	ND (n=1)	72.9	Anxiolytic
5	Atenolol*	1.7	ND (n=1)	94.9	β Blocker
6	Atorvastatin*	1.4	ND (n=4)	79.7	Antilipidemic
7	Benzoylecgonine*	0.3	ND (n=2)	92.1	Metabolite
8	Benztropine	0.5	2.9 ± 0.1	101	Anticholinergic
9	Cocaine	0.1	3.6 ± 3	97.6	Illicit drug
10	DEET	0.6	7.4 ± 2.8	112	Insect repellant
11	Desmethyl-Diltiazem	0.3	7.4 ± 6.1	357	Metabolite
12	Furosemide*	104	ND (n=2)	92.6	Diuretic
13	Glyburide*	15.6	ND (n=2)	104	Antidiabetic
14	Hydrocodone*	4.5	ND (n=4)	83.8	Narcotic analgesic
15	Metoprolol	4.5	24.5 ± 10.1	93.4	β Blocker
16	Norfluoxetine	2	42 ± 25.1	100	Metabolite
17	Norverapamil	0.3	458 ± 169.4	82.7	Metabolite
18	Oxycodone*	1.2	ND (n=3)	108	Narcotic analgesic
19	Paroxetine	7.1	61.6 ± 21.7	105	Anti-depressant
20	Promethazine	0.6	22 ± 6.2	108	Antihistamine

21	Propoxyphene	1.2	50 ± 23.2	88.7	Narcotic analgesic
22	Propranolol	2.5	107.4 ± 36	91.2	β-Blocker
23	Sertraline	0.5	458 ± 168.3	74	Anti-depressant
24	Triamterene	0.5	430.4 ± 139.9	81.2	Diuretic
25	Valsartan*	9.2	ND (n=4)	107	Antihypertensive
26	Verapamil	0.3	551.4 ± 226.2	92.2	Ca^{2+} Channel blocker

*More comprehensive information on each compound is available in Appendix B.

3.3.2 STUDY REPRESENTATIVENESS AND SAMPLE INTEGRITY

The results of this study are conservative with respect to the concentration of analytes in biosolids from the perspective of storage time, as prolonged storage of samples may have allowed for degradation of labile compounds and sample pooling may have diluted the concentrations of some analytes to levels below the detection limit.

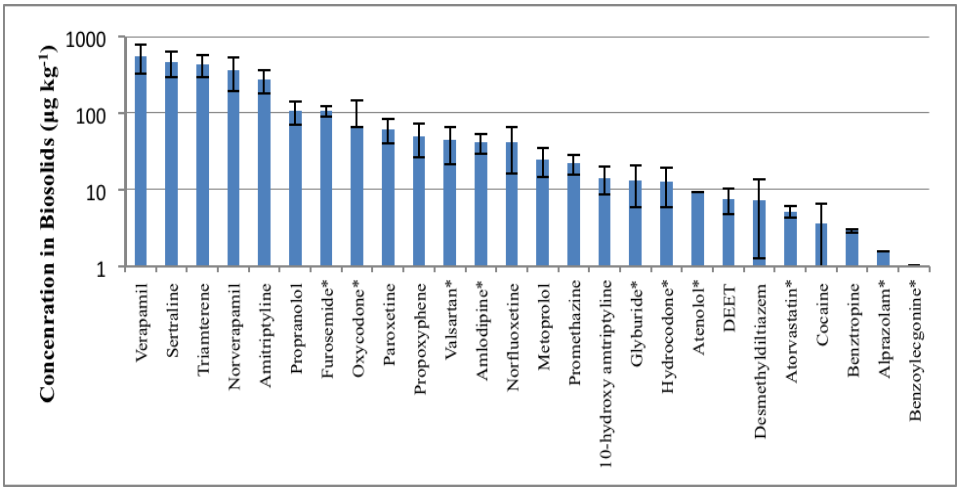


Figure 4. Rank order of mean concentrations of 26 previously unmonitored PPCPs that were detected for the first time in composites of 110 U.S biosolids samples from the 2001 NSSS. Error bars depict ± one standard deviation (n=5), and asterisks (*) indicate compounds that were detected inconsistently.

3.3.3 OCCURRENCE OF PPCPS IN BIOSOLIDS

Of the 120 pharmaceuticals tested for, 59 compounds were detected in at least one composite sample (Refer to Figure 4). The mean concentration of the sum of all PPCPs detected in the five composite samples was approximately 58.7 ± 19.8 mg/kg. Four compounds previously reported to occur in the ppm range in these samples were detected again at similar levels; these included triclocarban, triclosan, ciprofloxacin and ofloxacin. Combined, these four analytes contributed about 85% of the total mass of all PPCPs detected.

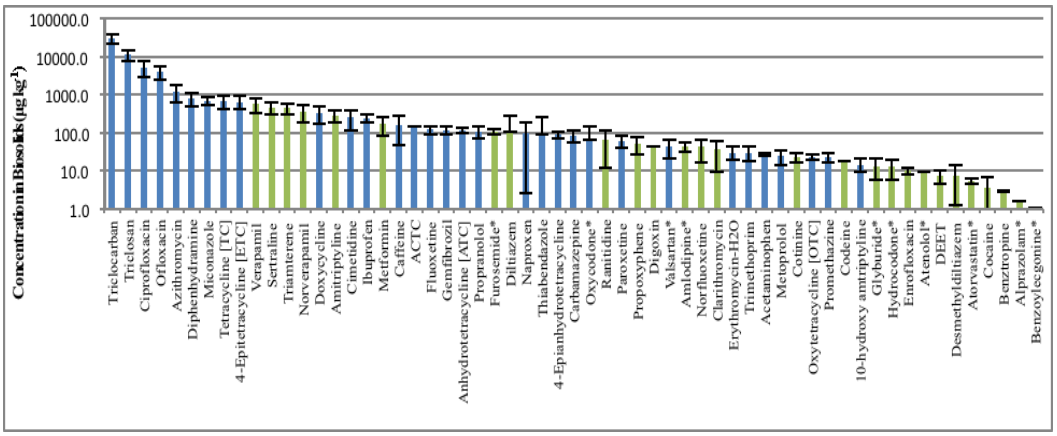
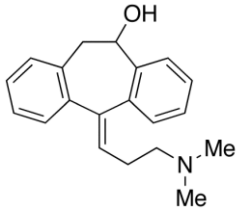
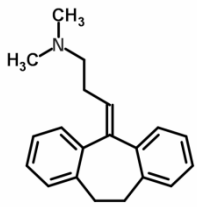
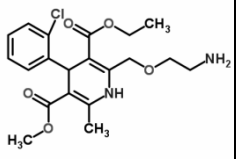
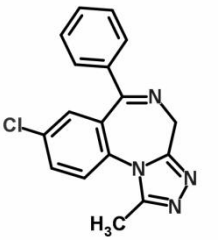
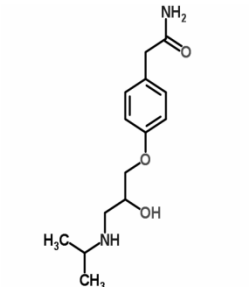
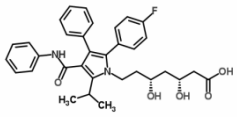
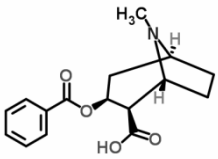
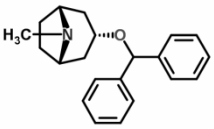
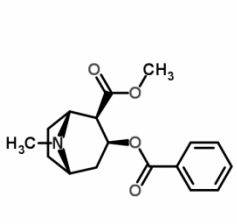
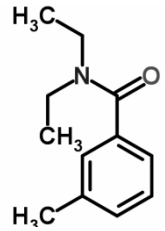
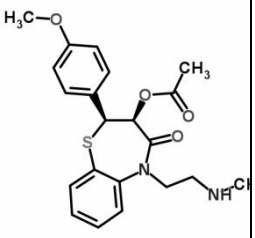
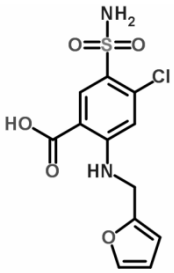


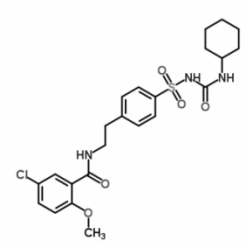
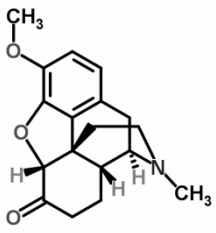
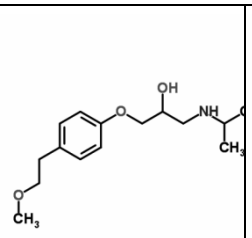
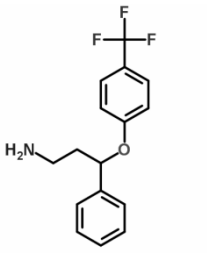
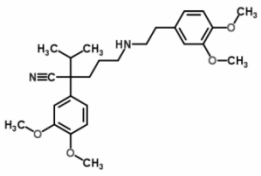
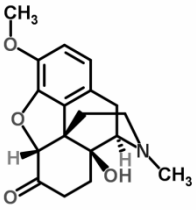
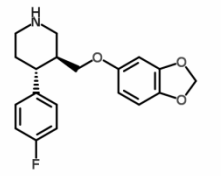
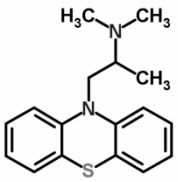
Figure 5. Rank order of mean concentrations of 59 PPCPs that were detected in composites of 110 U.S biosolids samples archived from the 2001 NSSS. Error bars depict ± one standard deviation (n=5), and compounds marked with (*) indicate inconsistent detection.

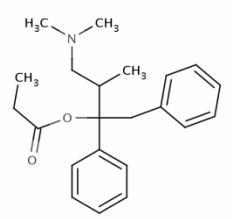
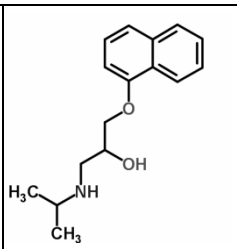
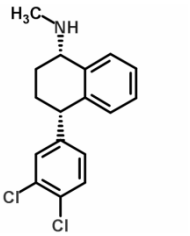
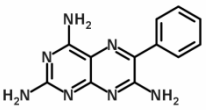
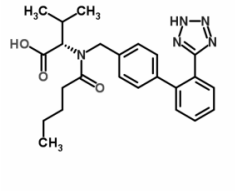
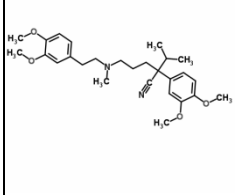
Overall, 26 unreported pharmaceuticals were detected in at least one of the composite samples (Figure 4; Table 5). In the following section these compounds have been grouped as major and minor contaminants based on the detected concentrations and frequency of detection in the samples.

Table 5. List of 26 PPCPs detected as part of this analytical study. The number of positive occurrences in the five samples analyzed are marked beside the mean concentrations; for compounds that were inconsistently detected the maximum concentration is reported. The rank of each compound is based on the number of prescriptions dispensed for the year 2009 (IMS Health, 2009). The half-life and EC_{50} values were estimated using PBT Profiler software. Structure and chemical properties of each compound were taken from the Royal Society of Chemistry database.

Compound			
10-hydroxy-amitriptyline  CAS: 1159-82-6 $logK_{ow}$: 2.66 $logD_{ow}$: 1.50 Half-life: N.A Soil loading: 32-80 kg yr^{-1} EC_{50}: N.A	Amitriptyline  CAS: 50-48-6 $logK_{ow}$: 4.92 $logD_{ow}$: 2.65 Half-life: 120 d^{-1} Soil loading: 570-1402 kg yr^{-1} EC_{50}: 500 µg L^{-1} Rank: 143	Amlodipine *  CAS: 88150-42-9 $logK_{ow}$: 3.0 $logD_{ow}$: 1.50 Half-life:75 d^{-1} Soil loading: 113-205 kg yr^{-1} EC_{50}: 45 µg L^{-1} Rank: 15	Alprazolam*  CAS: 28981-97-7 $logK_{ow}$: 2.12 $logD_{ow}$: 1.92 Half-life: 75 d^{-1} Soil loading: 6 kg yr^{-1} EC_{50}: 10^4 µg L^{-1} Rank: 39

Atenolol*  CAS: 29122-68-7 $logK_{ow}$: 0.16 $logD_{ow}$: -1.76 Half-life: 75 d^{-1} Soil loading: 36.6 kg yr^{-1} EC_{50}: 8.1 x 10^3 µg L^{-1} Rank: 53	Atorvastatin*  CAS: 134523-00-5 $logK_{ow}$: 3.85 $logD_{ow}$: 0.74 Half-life: N.A Soil loading: 16-25 kg yr^{-1} EC_{50}: 10^4 µg L^{-1} Rank: 2	Benzoylecgonine*  CAS: 519-09-5 $logK_{ow}$: -1.32 $logD_{ow}$: -0.24 Half-life: 30 d^{-1} Soil loading: 4 kg yr^{-1} EC_{50}: 6.5 x 10^5 µg L^{-1}	Benztropine  CAS: 86-13-5 $logK_{ow}$: 4.28 $logD_{ow}$: 1.21 Half-life: 75 d^{-1} Soil loading: 11-12 kg yr^{-1} EC_{50}: 48 µg L^{-1} Rank: -
Cocaine  CAS: 50-36-2 $logK_{ow}$: 2.30 $logD_{ow}$: 1.51 Half-life: 75 d^{-1} Soil loading: 7-36 kg yr^{-1} EC_{50}: 740 µg L^{-1}	DEET  CAS: 134-62-3 $logK_{ow}$: 2.18 $logD_{ow}$: 2.42 Half-life: 75 d^{-1} Soil loading: 19-45 kg yr^{-1} EC_{50}: 91 µg L^{-1}	Desmethyl-Diltiazem  CAS: 86408-45-9 $logK_{ow}$: 2.58 $logD_{ow}$: 2.07 Half-life: N.A Soil loading: 8-69 kg yr^{-1} EC_{50}: N.A	Furosemide*  CAS: 54-31-9 $logK_{ow}$: 2.03 $logD_{ow}$: -0.82 Half-life:120 d^{-1} Soil loading: 356-483 kg yr^{-1} EC_{50}: 1400 µg L^{-1} Rank: 98
Glyburide*	Hydrocodone*	Metoprolol	Norfluoxetine

			
CAS: 10238-21-8 $logK_{ow}$: 4.79 $logD_{ow}$: 1.14 Half-life: 360 d^{-1} Soil loading: 23-82 kg yr^{-1} EC_{50}: 2 µg L^{-1} Rank: 149	CAS: 126-29-1 $logK_{ow}$: 2.16 $logD_{ow}$: 0.68 Half-life: N.A Soil loading: 24-86 kg yr^{-1} EC_{50}: 730 µg L^{-1} Rank: 1	CAS: 51384-51-1 $logK_{ow}$: 1.88 $logD_{ow}$: 0.68 Half-life: 75 d^{-1} Soil loading: 46-150 kg yr^{-1} EC_{50}: 1400 µg L^{-1} Rank: 170	CAS: 83891-03-6 $logK_{ow}$: 4.18 $logD_{ow}$: 2.12 Half-life: N.A Soil loading: 8-329 kg yr^{-1} EC_{50}: 300 µg L^{-1}
Norverapamil	Oxycodone*	Paroxetine	Promethazine
			
CAS: 67018-85-3 $logK_{ow}$: 4.59 $logD_{ow}$: 1.35 Half-life: N.A Soil loading: 499-2336 kg yr^{-1} EC_{50}: N.A	CAS: 76-42-6 $logK_{ow}$: 0.66 $logD_{ow}$: 1.21 MDL: 1.2 µg kg^{-1} Mean ± Std. dev: nd (n=3) Recovery: 108 % Half-life: 360 d^{-1} Soil loading: 43-622 kg yr^{-1} EC_{50}: 5400 µg L^{-1} Rank: 33	CAS: 61869-08-7 $logK_{ow}$: 2.57 $logD_{ow}$: 1.48 Half-life: N.A Soil loading: 122-356 kg yr^{-1} EC_{50}: 140 µg L^{-1} Rank: 127	CAS: 60-87-7 $logK_{ow}$: 4.81 $logD_{ow}$: 3.40 Half-life: 120 d^{-1} Soil loading: 60-117 kg yr^{-1} EC_{50}: 34 µg L^{-1} Rank: 113
Propoxyphene	Propranolol	Sertraline	Triamterene

			
CAS: 469-62-5 $logK_{ow}$: 4.18 $logD_{ow}$: 2.32 Half-life: 120 d^{-1} Soil loading: 64-304 kg yr^{-1} EC_{50}: 15 µg L^{-1} Rank: 79	CAS: 525-66-6 $logK_{ow}$: 3.48 $logD_{ow}$: 0.79 Half-life: 30 d^{-1} Soil loading: 231-578 kg yr^{-1} EC_{50}: 360 µg L^{-1} Rank: -	CAS: 79617-96-2 $logK_{ow}$: 5.29 $logD_{ow}$: 3.04 Half-life: N.A Soil loading: 883-2519 kg yr^{-1} EC_{50}: 4.3 x 10^4 µg L^{-1} Rank: 28	CAS: 396-01-0 $logK_{ow}$: 0.98 $logD_{ow}$: 1.12 Half-life: 75 d^{-1} Soil loading: 1030-2309 kg yr^{-1} EC_{50}: 4600 µg L^{-1} Rank: 106
Valsartan*	Verapamil		
			
CAS: 137862-53-4 $logK_{ow}$: 3.65 $logD_{ow}$: 0.20 Half-life: N.A Soil loading: 95-254 kg yr^{-1} EC_{50}: N.A Rank: 30	CAS: 52-53-9 $logK_{ow}$: 3.79 $logD_{ow}$: 2.46 Half-life: 360 d^{-1} Soil loading: 586-2665 kg yr^{-1} EC_{50}: 1.3 µg L^{-1} Rank: 198		

The majority of these compounds have not been reported previously in the NSSS nor were they detected in U.S biosolids samples, although several were detected previously in sludges from other parts of the world (Ternes et al 2007).

3.3.4 REPRODUCIBILITY OF RESULTS

When comparing the present list of detected analytes to those reported previously by McClellan and Halden (2010), 30 analytes were found to be common to both studies, whereas eight compounds that had been detected previously were uniformly not detected in any of the samples during the present study. A comparison of mean concentrations of compounds detected in this study and those reported in 2010 (Figure 6) shows good agreement between the two. A paired *t*-test conducted on both the log-transformed and original datasets showed the results to be statistically indistinguishable at the 99% confidence level. Mean concentrations of compounds were within a factor of about 1.3 between previously obtained and current data.

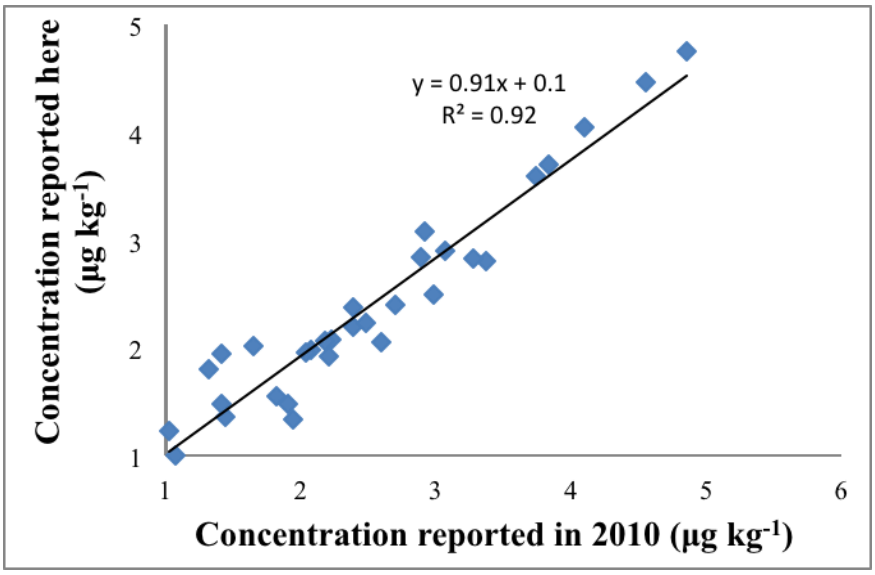


Figure 6. Log-log scatterplot comparing mean concentrations from the present study to those reported previously for 30 compounds commonly detected. Both datasets represent results obtained from analysis of composites created two years apart from the same group of archived samples.

3.3.5 EXPERIMENTAL HALF-LIFE DETERMINATION

Analytical results from the mesocosm samples revealed consistent first-order loss rates for four compounds, namely amitriptyline, paroxetine, propranolol and sertraline (Figure 7).

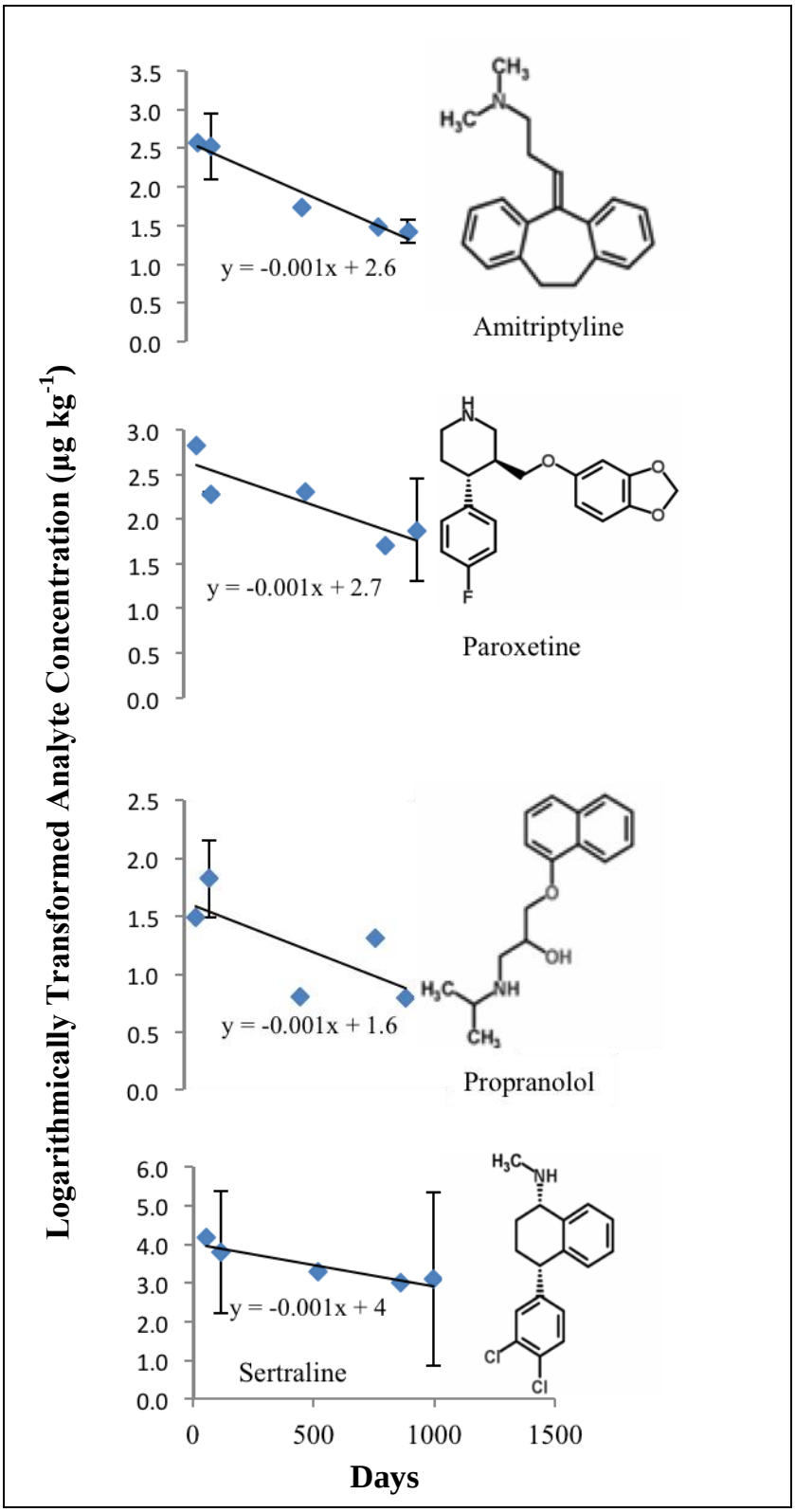

Logarithmically Transformed Analyte Concentration (µg kg-1)
y = -0.001x + 2.6
Amitriptyline
y = -0.001x + 2.7
Paroxetine
y = -0.001x + 1.6
Propranolol
y = -0.001x + 4
Sertraline
Days

Figure 7. Decreasing concentrations of compounds plotted as natural logarithms vs. time (common x-axis). Compound structures were obtained from the database of the Royal Society of Chemistry.

Compound-specific half-lives in biosolids-amended soils were calculated by fitting the experimental data to first-order kinetics, yielding values ranging from 533 to 866 days (Table 6).

Table 6. Half-lives determined experimentally in mesocosm experiments versus, shown in parentheses, the corresponding values estimated using the PBT Profiler of the U.S. EPA.

Compound	Calculated half-life, $days^{-1}$	Predicted loading to U.S. soils, $kg\ yr^{-1}$
Amitriptyline	533 (120)	570 - 1402
Paroxetine	770 (NA)	122 - 356
Propranolol	866 (30)	231 - 578
Sertraline	630 (NA)	883- 2519

3.4 DISCUSSION

3.4.1 STUDY LIMITATIONS

For the purpose of this study, 110 archived biosolids samples were pooled together to create five megacomposite samples. This technique effectively reduced the number of samples required to estimate the mean concentrations of drug residues in U.S. sludges. This approach is economically attractive but does not allow for extrapolating obtained results to individual treatment plants and operating conditions. Compounds not detected in this study may still occur at detectable concentrations in some of the individual samples from specific plants because the pooling of samples can dilute out low-level analytes that occur infrequently. Nevertheless, this technique was found to be suitable for identifying contaminants and their average concentrations in a large sample set in an economical and efficient fashion.

It is important to recall that separate batches of composite samples were prepared and analyzed for both studies. Since labile compounds are eliminated during the wastewater treatment process, more refractory compounds tend to persist and accumulate in sludge. Such compounds are fairly resilient to environmental stress and tend to have extended half-lives in the sequestering matrix. The closeness of results between the two analysis campaigns (Figure 6) indicates that the sample integrity had been preserved over the years during storage and that good reproducibility can be achieved with the composite sample approach.

3.4.2 NEWLY REPORTED PPCPS IN BIOSOLIDS

3.4.2.1 MAJOR CONTAMINANTS

Calcium channel blockers and metabolites. Two parent compounds (amlodipine, verapamil) and two metabolites (norverapamil, desmethyldiltiazem) were detected in the biosolids samples. Amlodipine was detected at a maximum concentration of 51.7 μg kg^{-1} and verapamil was detected in all samples at a mean concentration of 551.4 ± 226.2 μg kg^{-1}. The metabolites norverapamil and desmethyldiltiazem were uniformly detected in all samples at mean concentrations of 360.2 ± 169.4 μg kg^{-1} and 7.4 ± 6.1 μg kg^{-1}, respectively. Calcium channel blockers have been reported previously in the μg L^{-1} to ng L^{-1} range in wastewater effluents and in surface waters (Batt et al 2008; Nagarnaik et al 2010). All four compounds lacked data for establishing a comparative analysis, as they have not been reported in any major study.

Antidepressants and metabolites. Three antidepressants (paroxetine, sertraline, amitriptyline) and two metabolites (10-hydroxy-amitriptyline, norfluoxetine) were detected as well. Paroxetine, sertraline and norfluoxetine were uniformly detected in all samples at mean concentrations of, respectively, 61.6 ± 21.7, 458 ± 168.3 and 41.6 ± 25.1 μg kg^{-1}. Paroxetine, sertraline, norverapamil and 10-hydroxy-amitriptyline have been reported to occur in surface waters across the U.S in the ng L^{-1} range (Schultz and Furlong 2008; Wu et al 2009, Batt et al 2008) and interestingly, in the low to high μg L^{-1} range in fish tissues (Chu and Metcalfe 2007; Ramirez et al 2009). Radjenovic et al (2009) reported paroxetine in biosolids at a concentration of 40.7 ± 13.0 μg kg^{-1}, similar to the mean

concentration reported here. While in this study the highest detected concentration of sertraline was 636 μg kg^{-1}, Barron (2009) reported levels as high as 22 mg kg^{-1} in biosolids samples from Sweden and Norway; they also reported values for amitriptyline and its metabolite similar to those presented here (275.4 ± 92.8 and 14.4 ± 5.7 μg kg^{-1}, repectively). The latter two compounds have been detected in aquatic matrices (Kasprzyk-Hordern et al 2008; Batt et al 2008).

Diuretics. Triamterene was uniformly detected in all samples at a mean concentration of 430.4 ± 139.9 μg kg^{-1}, whereas furosemide was found at a maximum level of 122 μg kg^{-1}. Furosemide has been reported to have a highly variable removal range (8-54%) during wastewater treatment including nearly zero removal in winter (Castiglioni et al 2006). The most recent study conducted on the behavior of pharmaceuticals during conventional wastewater treatment reported a maximum detected value of 75 μg kg^{-1} in biosolids and calculated a removal range of 30-80% for furosemide (Jelic et al 2011). There were no reports on the occurrence of triamterene in biosolids.

***β*-Blockers.** Metoprolol and propranolol were uniformly detected in all samples at mean concentrations of 24.5 ± 10.1 and 107.4 ± 36 μg kg^{-1}, respectively, whereas atenolol was detected at a maximum concentration of 9.25 μg kg^{-1}. Metoprolol has been observed previously in biosolids at 35 ± 7 μg kg^{-1} (Barron 2009). Apart from this, there have been several published studies conducted on metoprolol and its presence in aquatic matrices. These studies indicated a range of removal efficiencies exhibited by WWTPs, from 0 – 80% (Jelic et al 2011). Propranolol

also has been detected in aquatic and solid matrices alike. Levels in the low ppb range were reported in WWTP process streams and in surface waters (Bendz et al 2005; Scheurer et al 2010). Concentrations found in Norwegian biosolids (101 ±3 μg kg^{-1}) mirrored the range reported here, whereas Radjenović et al 2009 observed Propranol at lower concentrations of 26.2 ± 10.7 μg kg^{-1} in sludge samples from Spain. Atenolol has been detected previously in sludge at levels similar to those reported here (Barron 2009; Jelic et al 2011); it is one of the most frequently tested for pharmaceuticals in leachates from biosolids (Lapen et al 2008; Topp et al 2008).

(Narcotic) Analgesics. Propoxyphene was detected in all samples at mean concentration of 49.9 ± 23.2 μg kg^{-1}, whereas hydrocodone and oxycodone were detected at maximum concentrations of 21.7 μg kg^{-1} and 157 μg kg^{-1}, respectively. Historically, all three drugs have been associated with abuse, and are frequently detected by U.S forensics labs (Daughton 2011). Both hydrocodone and oxycodone have been detected previously in surface waters and wastewater streams, most frequently in the low to high ppb range in surface waters of the U.S. and other nations (Hummel et al 2006; Batt et al 2008; Chiaia et al 2008; Phillips et al 2010) although no published studies were found with regards to analyses of solid matrices.

3.4.2.2 MINOR CONTAMINANTS

MISCELLANEOUS COMPOUNDS

DEET (*N*,*N*-diethyl-meta-toluamide) has been reported in aquatic matrices including surface waters (Kolpin et al 2002), landfill leachates (Eggen et al 2010), WWTP streams (Trenholm et al 2006; Bartelt-Hunt et al 2009) and in U.S groundwater (Barnes et al 2004). In the present study, DEET was uniformly detected in all biosolids samples at a mean concentration of 7.4 ± 2.8 μg kg^{-1}. Howard et al (2010) listed promethazine and benztropine as high production volume (HPV) pharmaceuticals that have not been detected in the environment. In the present study both pharmaceuticals were uniformly detected in all samples at mean concentration of 21.9 ± 6.2 μg kg^{-1} and 2.9 ± 0.1 μg kg^{-1}.

The maximum detected concentrations of valsartan, glyburide, alprazolam, and atorvastatin were 64.1, 20.6, 1.56, and 6.2 μg kg^{-1}. Atorvastatin has previously been detected in WWTP process streams and in biosolids at concentrations of 20 – 46 ppb (Miao and Metcalfe 2003; Batt et al 2008; Jelic et al 2011).

Cocaine and metabolite. Testing of environmental matrices (most commonly aquatic) for the presence of illicit drugs has recently gained impetus. The presence of cocaine and benzoylecgonine (metabolite) has been reported in wastewater streams and surface waters across Europe (Castiglioni et al 2006; Ventura et al 2007; Kasprzyk-Hordern et al 2008; Postigo et al 2010; Gonzáles-Mariño et al 2010) and in U.S WWTP influent (Chiaia et al 2008). Here, we report uniform detection of cocaine in all biosolids composites analyzed. To our knowledge, this

is the first study to report the presence of cocaine in U.S biosolids; however, the mean concentration found was low at 3.6 ± 3 μg kg^{-1}. Benzoylecgonine was detected at even lower levels, never exceeding 1.05 μg kg^{-1}

These results provide some of the first documented occurrences of select compounds in biosolids. For a more detailed analysis of worldwide occurrences of these and other seldom monitored compounds, readers should refer to Appendix C.

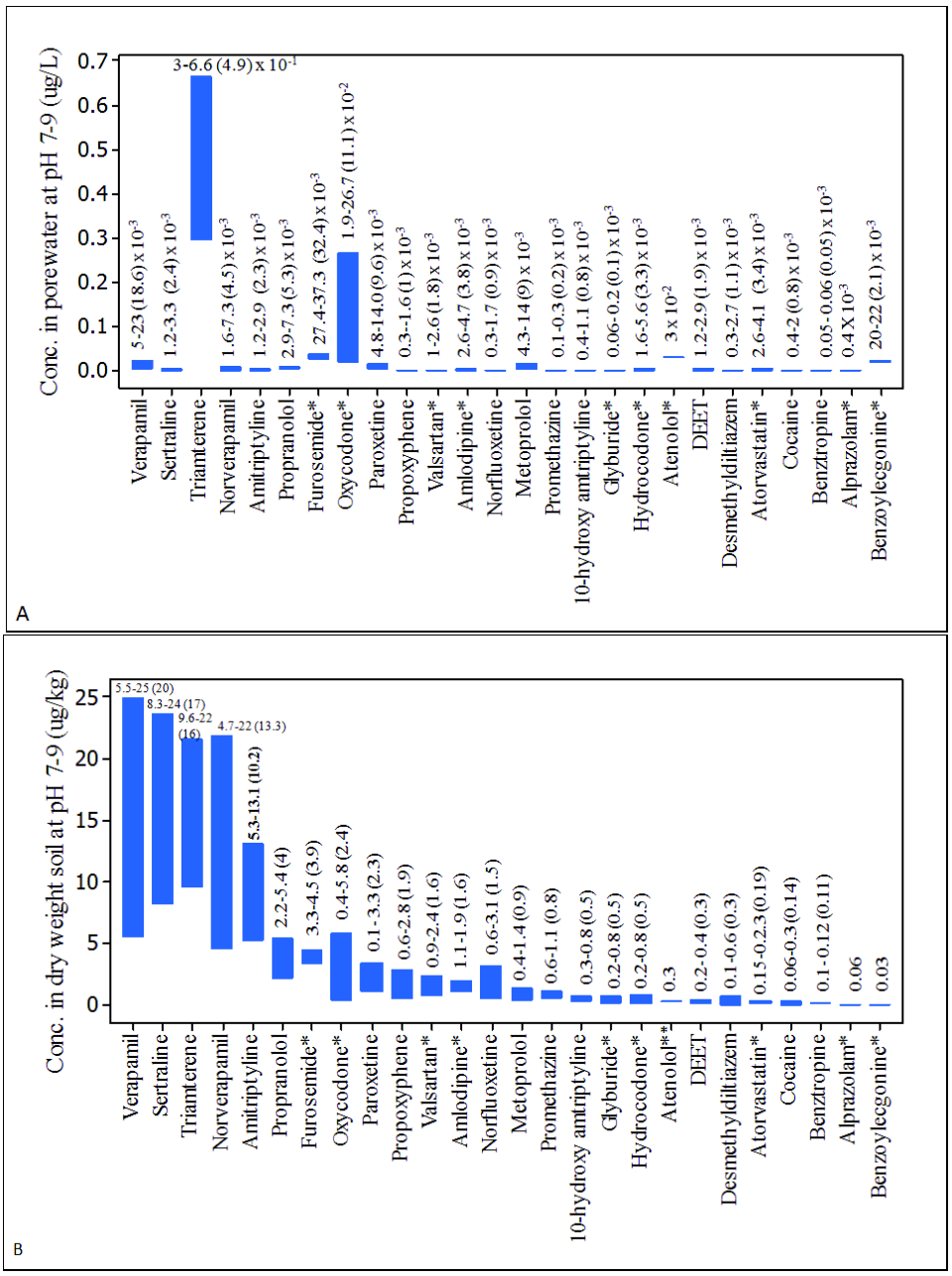


Figure 8. Predicted porewater concentration range (A) and equilibirum concentration range in soils-biosolids mixtures (B) for 26 PPCPs at environmentally relevant pH range 7-9. Concentration range for each compound

has been printed with corresponding mean values in paranthesis. Compounds marked with (*) were inconsistently detected.

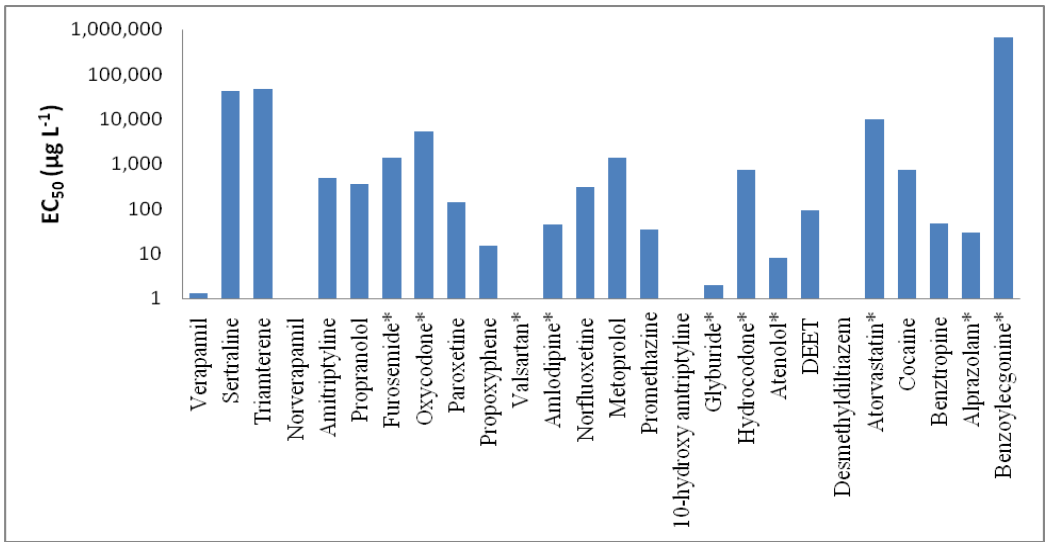


Figure 9. Predicted EC_{50} values for 22 compounds. Values for some compounds could not be calculated and may represent potential hazard to aquatic organisms. Compounds marked with (*) indicate inconsistent detection.

3.4.3 SOIL/POREWATER EQUILIBRIA AND HALF-LIFE CALCULATION

In order to determine if the compounds detected in biosolids potentially pose a threat to aquatic organisms, toxicity values were estimated using the PBT Profiler of the U.S. EPA. From a comparison of the modeled porewater concentrations for these compounds with predicted toxicity data (Fig. 7 (A.) vs. Fig. 8), it was concluded that the presence of these compounds in biosolids at the detected concentrations posed no threat to aquatic organisms in nearby surface waters. The EC_{50} values were at least one order of magnitude higher than the porewater concentrations calculated for these compounds. Since we followed EPA-

recommended soil-biosolids mixing ratio of 25:1 when predicting analyte levels in surface water impacted by porewater leaching, there is a possibility that these compounds may be toxic to organisms in situations where mixing ratios may differ (e.g. applications in forests).

Results from the PBT Profiler were also significant when estimating the persistence of these compounds in the environment. It was noted that all compounds had half-life values of $\geq$30 days. Upon correlating these findings with their respective log K_{OW} values, it was found that the tendency to accumulate in solids was in part due to the high hydrophobicity of some compounds, whereas forces other than hydrophobic interactions are presumed to govern partitioning of compounds such as cocaine and oxycodone. In mesocosm studies containing soil-biosolids mixtures, Walters et al (2010) experimentally showed that the half-lives of compounds applied to soils in the form of biosolids were much greater than half-lives predicted by fate models and laboratory studies using addition of neat chemicals to soils. By leveraging the archived soil/biosolids samples from the aforementioned mesocosm study, we were able to compute half-lives for four compounds whose loss over time followed first-order kinetics. These analytes were amitriptyline, paroxetine, propranolol and sertraline (Figure 7).

3.4.4 RISK ASSESSMENTS AND DATA GAPS

Of all PPCPs detected in this study, three groups of compounds that have been noted for their pharmacodynamic effects on humans were found to occur uniformly in the high ppb range either as parent compounds or as metabolites.

These were antidepressants (n =5), β-blockers (n =2) and narcotic analgesics (n =3). Owing to an absence of terrestrial field studies, experimental and modeled values had to be relied upon as best estimates for potential hazards caused by the presence of PPCPs in the environment. Studies have demonstrated the ability of lipophillic compounds to undergo bioaccumulation and biomagnification within terrestrial food chains (Higgins et al 2007; Kinney et al 2008). Given the magnitude of annual loading reported in this study and the lack of bioaccumulation studies for these newly detected PPCPs, further work in this area appears to be warranted.

Of particular significance were the calculated half-lives for the compounds amitriptyline, paroxetine, propranolol and sertraline (Table 5). While values were absent for paroxetine and sertraline, the experimental $t_{1/2}$ value was noted to exceed the predicted $t_{1/2}$ by at least a factor of 4 for amitriptyline and by 29 for propranolol. Correlating the experimental $t_{1/2}$ value with the individual annual loading rate proved crucial in gauging the magnitude of potential exposure to these pharmaceuticals.

Apart from this, sequestered compounds tend to occur as mixtures and not discreetly in biosolids and soils. Thus, greater emphasis should be placed on the effects of pharmaceutical mixtures on soil-dwelling organisms. Although effort has gone into studying the effects of antibiotics on soil microorganisms and the development of resistant species, the environmental pressures exerted by these compounds especially in solid matrices have not been thoroughly investigated.

This lack of information prevents the completion of a comprehensive risk assessment for all compounds.

Chapter 4: CONCLUSION

The here presented research study aims at highlighting the *in silico* and experimental approaches that allow for assessing the risks posed by compounds that are sequestered in biosolids samples. Conclusions for the two independently performed studies are as follows.

4.1 STUDY I

The expansion, validation and application of mathematical modeling performed in this work provided new estimates on the concentration range of ten sterols and four hormones in environmental compartments. A hazard assessment revealed that the hormones E1, E2 and E3 feature high migratory potentials in soil, with E1 and E3 projected to yield the highest porewater concentrations, and EE2 posing a potential threat to fathead minnows (*P. promelas*). This work underscored the utility of modeling for assessing the potential impact of endocrine disrupting compounds sequestered in biosolids during wastewater treatment.

4.2 STUDY II

Twenty-six compounds were newly detected in archived biosolids samples, and are predicted to enter terrestrial environments in the U.S. through biosolids application at a combined rate of 5-15 tons yr^{-1}. The majority of these compounds have not been extensively investigated with regards to occurrence and effects in the environment and exposure pathways to humans. This study further demonstrated that consistent results can be obtained when analyzing archived biosolids from national sampling campaigns by using a mega composite

approach. This implies that long-term storage of samples in the freezer at temperatures of -20 degrees Celsius or less does not significantly impact the analytes examined here, and that the mixing of composite samples from thawed slurries, although being challenging, can be performed such that consistent results are obtained. This finding is noteworthy as this mega composite sampling approach could help to dramatically reduce the cost of environmental monitoring on the regional and national scale. While it has been established that mean concentrations of several PPCPs in U.S biosolids have remained fairly constant over the years, the detection of a set of new compounds in biosolids warrants analysis of more recent biosolids samples in order to establish a trend, with regards to both their occurrence and concentrations detected.

4.3 FUTURE RESEARCH NEEDS

Toxicity data that have been published in the peer-reviewed literature are helpful resources when assessing the risks posed by xenobiotics in the environment. Although field data are preferred and more credible for use in risk analyses, predictive data from the PBT Profiler of the U.S. EPA can serve as a substitute. A similar situation was experienced with both research studies presented here and predictive data were used in place of experimental data owing to incomplete information, non-uniformity of test species or the complete lack of half-life data. Over the years since its inception, results from the U.S. EPA supervised TNSSS have played a vital role in changing the perception associated with beneficial reuse of biosolids. These results have also contributed to source control and

exposure management of chemicals sequestered in biosolids. However, a major improvement to this program would be to improve the frequency at which it is conducted, so that the scientific community can keep track of the ever increasing number of chemicals that are discharged into sewers by the public and industries.

REFERENCES

Barnes, Kimberlee, Christenson, Scott, Kolpin, Dana, Focazio, Michael, Furlong, Edward, Zaugg, Steven, Meyer, Michael, Barber, Larry. "Pharmaceuticals and other organic wastewater contaminants within a leachate plume downgradient of a municipal landfill". *Ground Water Monitoring and Remediation* 24(2004); 119–26.

Barron Leon. "Occurrence and fate of pharmaceuticals and personal care products within sewage sludge and sludge-enriched soils: Summary of Findings". http://www.epa.ie/downloads/pubs/research/waste/STRIVE_34_PPCPs_Summary_Findings.pdf

Bartelt-Hunt, Shannon, Snow, Daniel, Damon, Teyona, Shockley, Johnette, Hoagland, Kyle, "The occurrence of illicit and therapeutic pharmaceuticals in wastewater effluent and surface waters in Nebraska". *Environmental Pollution* 157(2009); 786–791.

Batt, Angela, Kostich, Mitch, Lazorchak, James, "Analysis of Ecologically Relevant Pharmaceuticals in Wastewater and Surface Water Using Selective Solid-Phase Extraction and UPLC-MS/MS". *Analytical Chemistry* 80(2008); 5021–5030.

Bendz, David, Paxeus, Nicklas, Ginn, Timothy, Loge, Frank, "Occurrence and fate of pharmaceutically active compounds in the environment, a case study: Hoje River in Sweden". *Journal of Hazardous Materials* 122(2005); 195–204.

Bound, Jonathan, Voulvoulis, Nikolaos, "Household disposal of pharmaceuticals as a pathway for aquatic contamination in the United Kingdom". *Environmental Health Perspectives* 113(2005); 1705–1711.

Brion, Tyler, Palazzi, Laillet, Porcher, Garric and Flammarion. "Impacts of 17β-estradiol, including environmentally relevant concentrations, on reproduction after exposure during embryolarval-Juvenile- and adult-life stages in zebrafish (Danio rerio)". *Aquatic Toxicology* 68(2004); 193– 217.

Brooks, Bryan, Chambliss, Kevin, Stanley, Jacob, Ramirez, Alejandro, Banks, Kenneth, Johnson, Robert, Lewis, Russell. "Determination of select antidepressants in fish from an effluent-dominated stream". *Environmental Toxicology and Chemistry* 24(2005); 464–9.

Brooks, Bryan, Foran, Christy, Richards, Sean, Weston, James, Turner, Philip, Stanley, Jacob, Solomon, Keith, Slattery, Marc, LaPoint, Thomas. "Aquatic ecotoxicology of fluoxetine". *Toxicology Letters* 142(2003); 169–83.

Brooks, Bryan, Riley Timothy, Taylor, Ritchie. “Water quality of effluent-dominated ecosystems: ecotoxicological, hydrological, and management considerations”. *Hydrobiologia* 556(2006); 365–79.

Castiglioni, Sara, Baganti, Renzo, Fanelli, Roberto, Pomati, Francesco, Calamari, David, Zuccato, Ettore. “Removal of pharmaceuticals in sewage treatment plants in Italy”. *Environmental Science & Technology* 40(2006); 357–63.

Causarano, Hector, Franzluebbers, Alan, Shaw, Joey, Reeves, Wayne, Raper, Randy, Wood, Wesley. “Soil organic carbon fractions and aggregation in the Southern Piedmont and Coastal Plain”. *Soil Science Society of America Journal* 72(2008); 221–230.

Chari, Bipin, and Halden, Rolf. “Predicting the concentration range of unmonitored chemicals in wastewater-dominated streams and in run-off from biosolids-amended soils”. *Science of The Total Environment* (submitted).

Chari, Bipin, and Halden, Rolf. **“**Validation of Mega Composite Sampling and Nationwide Mass Inventories for 26 Previously Unmonitored Contaminants in Archived Biosolids from the U.S National Biosolids Repository”. *Water Research* (submitted).

Chiaia, Aurea, Banta-Green, Caleb, Field, Jennifer. “Eliminating solid phase extraction with large-volume injection LC/MS/MS: analysis of illicit and legal drugs and human urine indicators in U.S. wastewaters”. *Environmental Science and Technology* 42(2008); 8841-8848.

Chu, Shaogang, and Metcalfe, Chris, “Analysis of paroxetine, fluoxetine, and norfluoxetine in fish tissues using pressurized liquid extraction, mixed mode solid phase extraction cleanup and liquid chromatography– tandem mass spectrometry”. *Journal of Chromatography A* 1163(2007); 112– 118.

Cicek Nazim, Londry Kathleen, Oleszkiewicz Jan, Wong, Denny, Lee, Yoomin. “Removal of Selected Natural and Synthetic Estrogenic Compounds in a Canadian Full-Scale Municipal Wastewater Treatment Plant”. *Water Environ Research* 79(2007); 795-800.

Dammann, Shappell, Bartell, and Schoenfuss. “Comparing biological effects and potencies of estrone and 17β-estradiol in mature fathead minnows, *Pimephales promelas*”. *Aquatic Toxicology* 105(2011); 559-568.

Daughton, Christian. “Illicit drugs: contaminants in the environment and utility in forensic epidemiology”. *Reviews of Environmental Contamination and Toxicology* 210(2011); 59-110.

De Lannoy, Gabrielle, Verhoest, Niko, Houser, Paul, Gish, Timothy, Meirvenne, Marc Van. "Spatial and temporal characteristics of soil moisture in an intensively monitored agricultural field (OPE3)". *Journal of Hydrology* 331(2006); 719–730.

Deo, Randhir and Halden, Rolf. "Empirical model for predicting concentrations of refractory hydrophobic organic compounds in digested sludge". *Environmental Chemistry* 6(2009); 544–550.

Deo Randhir and Halden, Rolf. "*In silico* screening for unmonitored, potentially problematic high production volume (HPV) chemicals prone to sequestration in biosolids". *Journal of Environmental Monitoring* 12(2010); 1840-1845.

Edwards, Topp, Edward, Metcalfe, Chris, Li, Gotschall, Bolton, Curnoe, Payne, Beck, Kleywegt and Lapen. "Pharmaceutical and personal care products in tile drainage following surface spreading and injection of dewatered municipal biosolids to an agricultural field". *Science of the Total Environment* 407(2009); 4220–30.

Eggen, Trine, Moeder, Monika and Arukwe, Augustine. "Municipal landfill leachates: A significant source for new and emerging pollutants". *Science of The Total Environment* 408(2010), 5147-5157.

Furtula, Vesna, Liu, Johnny, Chambers, Patricia, Osachoff, Heather, Kennedy, Chris, Harkness, Joanne. "Sewage treatment plants efficiencies in removal of sterols and sterol ratios as indicators of fecal contamination sources". *Water Air Soil Pollution* 2011. (accepted for publication August 2011).

Gobel, Anke, Thomsen, Angela, McArdell, Christa, Joss, Adriano, Giger, Walter. "Occurrence and sorption behavior of sulfonamides, macrolides, and trimethoprim in activated sludge treatment". *Environmental Science & Technology* 39(2005); 3981–3989.

Gonzáles-Mariño, Iria, Quintana, Jose, Rodriguez, Isaac and Cela, Rafael. "Determination of drugs of abuse in water by solid-phase extraction, derivatisation and gas chromatography–ion trap-tandem mass spectrometry". *Journal of Chromatography A* 1217(2010); 1748–1760.

Gottschall, Topp, Edward, Metcalfe, Chris, Edwards, Payne, Kleywegt, Russell, Lapen."Pharmaceutical and personal care products in groundwater, subsurface drainage, soil, and wheat grain, following a high single application of municipal biosolids to a field". *Chemosphere*. Jan 31 2012.. [Epub ahead of print].

Halling-Sorensen, Bent, Nielsen, Soren, Lanzky, P.F., Ingerslev, Flemming, Lutzhoft, H.C.H., Jorgensen, Sven. "Occurrence, fate and effects of pharmaceutical substances in the environment - a review". *Chemosphere* 36(1998); 357–394.

Heidler, Jochen, Halden, Rolf. “Meta-analysis of mass balances examining chemical fate during wastewater treatment”. *Environmental Science and Technology* 42(2008); 6324–32.

Heidler, Jochen, Halden, Rolf. “Mass balance assessment of triclosan removal during conventional sewage treatment”. *Chemosphere* 66(2007); 362–369.

Hernando, Mezcua, Fernández-Alba, and Barceló, Damia. “Environmental risk assessment of pharmaceutical residues in wastewater effluents, surface waters and sediments”. *Talanta* 69(2006); 334–342.

Higgins, Christopher, McLeod, Pamela, MacManus-Spencer, Laura, Luthy, Richard. “Bioaccumulation of perfluorochemicals in sediments by the aquatic oligochaete Lumbriculus variegatus”. *Environmental Science & Technology* 41(2007); 4600–4606.

Honkanen, Kostamo, Kukkonen. “Toxicity of a Phytosterol Mixture to Grayling (*Thymallus thymallus*) during Early Developmental Stages”. *Archives of Environmental Contamination and Toxicology* 48(2005); 391-396.

Howard, Philip, Muir, Derek. “Identifying New Persistent and Bioaccumulative Organics Among Chemicals in Commerce”. *Environmental Science & Technology* 44(2010); 2277-2285.

Huerta-Fontela, Maria, Galceran, Maria, Ventura, Francesc, “Ultraperformance liquid chromatography–tandem mass spectrometry analysis of stimulatory drugs of abuse in wastewater and surface waters”. *Analytical Chemistry* 79(2007); 3821–3829.

Hummel, Daniela, Löffler, Dirk, Fink, Guido, Ternes, Thomas, “Simultaneous determination of psychoactive drugs and their metabolites in aqueous matrices by liquid chromatography mass spectrometry”. *Environmental Science & Technology* 40(2006); 7321–7328.

IMS Health, 2009. http://www.rxlist.com/script/main/hp.asp

Ingrand, Valerie, Herry, Gaela, Beausse, Johanne, de Roubin, Marie-Renee. “Analysis of steroid hormones in effluents of wastewater treatment plants by liquid chromatography-tandem mass spectrometry”. *J Chromatography A* 1020(2003); 99-104.

Jelic, Aleksandra, Gros, Meritxell, Ginebreda, Antoni, Cespedes-Sánchez, Raquel, Ventura, Francesc, Petrovic, Mira, Barcelo, Damia, “Occurrence, partition and removal of pharmaceuticals in sewage water and sludge during wastewater treatment”. *Water Research* 45(2011); 1165– 1176.

Jenkins, Ronald, Wilson, Elizabeth, Angus Robert, Howell, Mike and Kirk, Marion. "Androstenedione and progesterone in the sediment of a river receiving paper mill effluent". *Toxicology Sciences* 73(2003); 53–9.

Jenkins, Ronald, Wilson, Elizabeth, Angus, Robert, Howell, Mike, Kirk, Marion, Moore, Ray, Nance, Marione and Brown, Amber. "Production of Androgens by Microbial Transformation of Progesterone *in Vitro*: A Model for Androgen Production in Rivers Receiving Paper Mill Effluent". *Environmental Health Perspectives* 112(2004);1508-1511.

Jones-Lepp, Tammy and Stevens, Rick. "Pharmaceuticals and personal care products in biosolids/sewage sludge: the interface between analytical chemistry and regulation". *Analytical and Bioanalytical Chemistry* 387(2007); 1173–83.

Kasprzyk-Hordern, Barbara, Dinsdale, Richard, Guwy, Alan. "The occurrence of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs in surface water in South Wales, UK". *Water Research* 42(2008); 3498–3518.

Kinney, Chad, Furlong, Edward, Zaugg, Steven, Burkhardt, Mark, Werner, Stephen, Cahill, Jeffery and Jorgenstein, Gretchen. "Survey of organic wastewater contaminants in biosolids destined for land application". *Environmental Science and Technology* 40(2006);7207–15.

Kinney, Chad, Furlong, Edward, Kolpin, Dana, Burkhardt, Mark, Zaugg, Steven, Werner, Stephen, Bossio, Joseph, and Benotti, Mark. "Bioaccumulation of pharmaceuticals and other anthropogenic waste indicators in earthworms from agricultural soil amended with biosolid or swine manure". *Environmental Science & Technology* 42(2008); 1863-1870.

Kolpin, Dana, Furlong, Edward, Meyer, Michael, Thurman, Michael and Zaugg, Steven. "Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999–2000: a national reconnaissance". *Environmental Science & Technology* 36(2002); 1202 –1211.

Langdon, Kate, Warne, Michael Kookana, Rai. "Aquatic hazard assessment for pharmaceuticals, personal care products and endocrine disrupting compounds from biosolids amended land". *Integrated Environmental Assessment and Management*. 6(2010); 663–76.

Lapen, Topp, Edward, Metcalfe, Chris, Lic, Edwards, Gottschall, Bolton, Curnoed, Payne and Beck. "Pharmaceutical and personal care products in tile drainage following land application of municipal biosolids". *Science of The Total Environment* 399(2008); 52–59.

López, Chamorro, Silva, Bay-Schmith and Vidal. Chronic "Effects of *Pinus radiata* and *Eucalyptus globulus* Kraft Mill Effluents and Phytosterols on

Daphnia magna". *Bulletin of Environmental Contamination and Toxicology* 87(2011); 633–637.

Lorenzen, Angela, Hendel, John, Conn, Kenneth, Bittman, Shabtai, Kwabiah, Allan, Lazarovitz, George, Masse, Daniel, McAllister, Tim and Topp, Edward. "Survey of hormone activities in municipal biosolids and animal manures". *Environmental Toxicology* 19(2004); 216–25.

Love, Halden, Rolf, Davis, Nachman. "Feather Meal: A Previously Unrecognized Route for Reentry into the Food Supply of Multiple Pharmaceuticals and Personal Care Products (PPCPs)". *Environmental Science and Technology* March 2012 (accepted for publication).

Maurer, Escher, Richle, Schaffner, and Alder. "Elimination of beta-blockers in sewage treatment plants". *Water Research* 41(2007); 1614–1622.

McClellan, Kristin and Halden, Rolf. "Pharmaceuticals and personal care products in archived U.S. biosolids from the 2001 EPA national sewage sludge survey". Water Research 44(2010); 658-668.

Metcalfe, Chris, Metcalfe, Tracy, Kiparissis, Yiannis, Koenig, Brenda, Khan, Colin, Hughes, Richard, Croley, Timothy, March, Raymond and Potter, Thomas. "Estrogenic potency of chemicals detected in sewage treatment plant effluents as determined by in vivo assays with Japanese medaka (*Oryzias latipes*)". *Environmental Toxicology and Chemistry* 20(2001); 297–308.

Miao, Xiu-Sheng and Metcalfe, Chris, Determination of pharmaceuticals in aqueous samples using positive- and negative-voltage switching microbore liquid chromatography/electrospray ionization tandem mass spectrometry. *Journal of Mass Spectrometry* 38(2003); 27–34.

Miege, Choubert, Ribeiro, Eusebe and Coquery. "Fate of pharmaceuticals and personal care products in wastewater treatment plants — conception of a database and first results". *Environmental Pollution* 157(2009); 1721–6.

Mladenov, Natalie, Strzepek and Serumola. "Water quality assessment and modeling of an effluent-dominated stream, the Notwane River, Botswana". Environmental Monitoring and Assessment 109(2005); 97–121.

Nagarnaik, Pranav, Batt, Angela, and Boulanger, Bryan. "Concentrations and mass loadings of cardiovascular pharmaceuticals in healthcare facility wastewaters". *Journal of Environmental Monitoring* 12(2010); 2112-2119.

Nakari, Tarja and Erkomaa, Kirsti. "Effects of phytosterols on zebrafish reproduction in multigeneration test". *Environmental Pollution* 123(2003); 267–273.

North East Biosolids and Residuals Association, 2007. A National Biosolids Regulation, Quality, End Use & Disposal Survey.

Orrego, Rodrigo, Guchardi, John, Hernandez, Victor, Krause, Rachelle, Roti, Lucia, Armour, Jeffrey, Ganeshakumar, Mathumai and Holdway, Douglas. "Pulp and paper mill effluent treatments have differential endocrine disrupting effects on rainbow trout". Environmental Toxicology and Chemistry 28(2009); 181–8.

Parrott and Blunt. "Life-cycle exposure of fathead minnows (Pimephales promelas) to an ethinylestradiol concentration below 1 ng/L reduces egg fertilization success and demasculinizes males". *Environmental Toxicology* 20(2005); 131–41.

Phillips, Patrick, Smith, Steven, Kolpin, Dana, Zaugg, Steven, Buxton, Herbert, Furlong, Edward, Esposito, Kathleen and Stinson, Beverly. "Pharmaceutical formulation facilities as sources of opioids and other pharmaceuticals to wastewater-treatment-plant effluents". *Environmental Science & Technology* 44(2010), 4910–6.

Postigo, Cristina., Alda, López de, Jose, Maria and Barceló, Damia, "Drugs of abuse and their metabolites in the Ebro River basin: Occurrence in sewage and surface water, sewage treatment plants removal efficiency, and collective drug usage estimation". *Environment International* 36(2010); 75-84.

Radjenovic, Jelena, Petrovic, Mira and Barcelo, Damia, "Fate and distribution of pharmaceuticals inwastewater and sewage sludge of the conventional activated sludge (CAS) and advanced membrane bioreactor (MBR) treatment". *Water Research* 43(2009); 831–41.

Ramirez, Alejandro, Brain, Richard, Usenko, Sascha, Mottaleb, Mohammad, O'Donnell, John, Stahl, Leanne, Wathen, John, Snyder, Blaine, Pitt, Jennifer, Perez-Hurtado, Pilar, Dobbins, Laura, Brooks, Bryan, Chambliss, Kevin, "Occurrence of pharmaceuticals and personal care products in fish: Results of a national pilot study in the United States". *Environmental Toxicology and Chemistry* 28(2009); 2587-2597.

Richards, Sean and Cole, Shaun. "A toxicity and hazard assessment of fourteen pharmaceuticals to Xenopus laevis larvae". *Ecotoxicology* 15(2006); 647–56.

Khanal, Samir, Xie, Bin, Thompson, Michael, Sung, Shihwu, Ong, Say-Kee, Van Leeuwent. "Fate, transport, and biodegradation of natural estrogens in the environment and engineered systems". *Environmental Science and Technology* 40(2006); 6537–46.

Schäfers, Teigeler, Wenzel, Maack, Fenske and Segner. "Concentration- and time-dependent effects of the synthetic estrogen, 17 alpha-ethinylestradiol, on

reproductive capabilities of the zebrafish, Danio rerio". *Journal of Toxicology and Environmental Health Part A* 70(2007); 768–79.

Scheurer, Marco, Ramil, Maria, Metcalfe, Chris, Groh, Stefanie and Ternes, Thomas. "The challenge of analyzing beta-blocker drugs in sludge and wastewater". *Analytical and Bioanalytical Chemistry* 396(2010); 845-856.

Schultz, Melissa and Furlong, Edward. "Trace analysis of antidepressant pharmaceuticals and their select degradates in environmental aquatic matrices by LC/ESI/MS/MS". *Analytical Chemistry* 80(2008); 1756– 1762.

Schultz, Melissa, Higgins, Christopher, Huset, Carin, Luthy, Richard, Barofsky, Douglas and Field, Jennifer. "Fluorochemical Mass Flows in a Municipal Wastewater Treatment Facility". *Environmental Science & Technology* 40 (1991); 7350–7357.

Taylor, Ritchie. "Water Quality Aspects of an Intermittent Stream and Backwaters in an Urban North Texas Watershed". (Ph.D. Dissertation, University of North Texas, 2002).

Ternes, Thomas. "Occurrence of Drugs in German Sewage Treatment Plants and Rivers". *Water Research* 32(1998); 3245-3260.

Ternes, Thomas, Bonerz, Matthias, Herrmann, Nadine, Teiser, Bernhard, Andersen, Henrik. "Irrigation of treated wastewater in Braunschweig, Germany: an option to remove pharmaceuticals and musk fragrances". *Chemosphere* 66(2007); 894–904.

Topp, Edward, Monteiro, Sara, Beck, Andrew, Coelho, Bonnie, Boxall, Alistair, Duenk, Peter, Kleywegt, Sonya, Lapen, David, Payne, Michael, Sabourin, Lyne, Li, Hongxia and Metcalfe, Chris, "Runoff of pharmaceuticals and personal care products following application of biosolids to an agricultural field". *Science of The Total Environment* 396(2008); 52–59.

USEPA, 2007. Method 1694: Pharmaceuticals and Personal Care Products in Water, Soil, Sediment, and Biosolids by HPLC/MS/ MS Washington, D.C. EPA-821-R-08–002.

USEPA. 1992. Sewage sludge use and disposal rule (40 CFR Part 503) – Fact Sheet. EPA-882-F-92-002. Office of Water, Fact Sheet WH-556. U.S. Environmental Protection Agency, Washington, DC

USEPA. Occurrence of contaminants of emerging concern in wastewater from nine publicly owned treatment works. 2009b; Office of Water, Washington, DC, U.S. Environmental Protection Agency.

USEPA. Targeted National Sewage Sludge Survey Statistical Analysis Report. 2009a; EPA-822-R-08-018. Washington, DC:Office of Water, U.S. Environmental Protection Agency.

Walters, Evelyn, McClellan, Kristin and Halden, Rolf. "Occurrence and loss over three years of 72 pharmaceuticals and personal care products from biosolids-soil mixtures in outdoor mesocosms". *Water Research* 44(2010); 6011–6020.

Weir, Alex, Moiles, William, Brockman, Brian, Mattick, Carolyn, McClellan, Kristin, Gerwe, Lisa, Deo, Randhir and Halden, Rolf. Concentrations of Hydrophobic Organic Pollutants in U.S Wastewater Treatment Plants and in Receiving Surface Waters Modeled from EPA Biosolids Monitoring Data. In: Halden, R. U. (Ed.), ACS Book Series Vol. 1048. Contaminants of Emerging Concern: Ecotoxicological and Human Health Considerations. American Chemical Society (ACS) Book Series. Pages 421-436. Oxford University Press, New York, NY. ISBN13: 9780841224964; eISBN: 9780841224971; DOI: 10.1021/bk-2010-1048

Williams, Richard and Cook, Jon. "Exposure to pharmaceuticals present in the environment". *Drug Information Journal* 41 (2007); 133–141.

Wu, Chenxi, Spongberg, Alison, Witter, Jason, Fang, Min, Czajkowski, Kevin and Ames, April. "Dissipation and leaching potential of selected pharmaceutically active compounds in soils amended with biosolids". *Archives of Environmental Contamination and Toxicology* 59(2010); 343–51.

Wu, Chenxi, Spongberg, Alison, Witter, Jason, Fang, Min, Ames, April and Czajkowlski, Kevin. "Detection of Pharmaceuticals and Personal Care Products in Agricultural Soils Receiving Biosolids Application". *CLEAN-Soil Air Water* 8(2010); 230-237.

Wu, Chenxi, Spongberg, Alison, Witter, Jason and Czajkowlski, Kevin. "Occurrence of selected pharmaceuticals in an agricultural landscape, western Lake Erie basin". *Water Research* 43(2009); 3407–3416.

Xia, Kia, Bhandari, Alok, Das, Keshav and Pillar, Greg. "Occurrence and fate of pharmaceuticals and personal care products (PPCPs) in biosolids". *Journal of Environmental Quality* 34(2005); 91–104.

Yang, Yun-Ya, Gray, James, Furlong, Edward, Davis, Jessica, ReVello, Rhiannon and Borch, Thomas. "Steroid Hormone Runoff from Agricultural Test Plots Applied with Municipal Biosolids". *Environmental Science and Technology*. 2012 Jan 30. [Epub ahead of print].

Ying, Guang-Guo and Kookana, Rai. “Sorption and degradation of estrogen-like-endocrine disrupting chemicals in soil”. Environmental Toxicology and Chemistry 24(2005); 2640–5.

APPENDIX A

SAMPLING PROCEDURE

and

SAMPLE ANALYSIS

The 2001 NSSS aimed at analyzing sewage sludge that was intended for land disposal for dioxin and dioxin-like compounds. During a 7 week sampling period between February and March 2001, 94 wastewater treatment plants were randomly selected from a pool of 174 facilities that had been sampled during the 1989 NSSS. These WWTPs were representative of 32 states and the District of Columbia. Eighty-nine facilities were noted to have a single system for treating and processing their sludge and hence one final sludge sample was collected from each of these facilities. Five facilities had two systems for treating their sludge material and therefore one sample per treatment process was taken. A total of 99 product samples were collected from the 94 wastewater treatment plants and for quality control testing and field duplicates were collected from 15% of treatment plants. The total number of samples that were analyzed was 113.

Composite sludges were divided into two aliquots of up to 1 g dry solids, and adjusted for pH with phosphate buffer and ammonium hydroxide, respectively, prior to acid and base extraction. Acidic and base fractions were spiked with stable isotope-labeled surrogate standards of the target analytes. Both fractions were sonicated and extracted three times with a phosphate buffer/acetonitrile solution for the

acid fraction, and with an ammonium hydroxide/acetonitrile solution for the base fraction. Then, both fractions were concentrated to remove acetonitrile and re-diluted with reagent water. For acid extraction Na4EDTA was added for stabilization. Both the acid fraction and the base fraction were cleaned using a

solid-phase extraction (SPE) hydrophilic-lipophilic balance (HLB) 20ccm cartridges containing 1 g of resin. The acid fraction was washed with reagent water to remove EDTA, and the analytes were eluted
with methanol. In addition, triclocarban and triclosan were eluted with a mixture of equal parts of acetone and methanol. The base fraction was eluted with methanol followed by 2% formic acid. After extraction, both fractions were concentrated under a nitrogen atmosphere and reconstituted in methanol. Internal standards were added to both fractions just prior to analysis.

For the purpose of compound detection, the 120 analytes were divided into five groups. All analytes were separated by liquid chromatography and detected by tandem mass spectrometry. Groups 1,2,3 and 5 were extracted under acidic conditions at pH 2. Groups 1,2, 4 and 5 were analyzed in positive electrospray ionization (ESI) mode, with Group 2 being specific to tetracyclines. Group 3 was analyzed in negative ESI mode. Group 4 was extracted under basic conditions at pH 10 and analyzed in positive ESI mode.

APPENDIX B

TABLE 7

List of 120 analytes that were detected for using the MLA-075 method. Compounds printed in bold indicate new analytes.

List 1 (Acid extraction, positive ESI)	Acetaminophen Ampicillin[1] Azithromycin Caffeine Carbadox Carbamazepine Cefotaxime	Ciprofloxacin Clarithromycin Clinafloxacin Cloxacillin Dehydroni-fedipine Digoxigenin Digoxin	Diltiazem 1,7-Dimethyl-xanthine Diphen-hydramine Enrofloxacin Erythromycin Flumequine Fluoxetine	Lincomycin Lomefloxacin Miconazole Norfloxacin Norgestimate Ofloxacin Ormetoprim
	Oxacillin Oxolinic acid Penicillin G Penicillin V Roxithromycin Sarafloxacin Sulfachloro-pyridazine	Sulfadiazine Sulfadimethoxine Sulfamerazine Sulfamethazine Sulfamethizole Sulfamethoxazole Sulfanilamide	Sulfathiazole Thiabendazole Trimethoprim Tylosin Virginiamycin	

List 2 (Tetracyclines, positive ESI)	Anhydrochlortetracycline (ACTC) Anhydrotetracycline (ATC) Chlortetracycline (CTC) Demeclocycline Doxycycline 4-Epianhydrochlortetracycline (EACTC) 4-Epianhydrotetracycline (EATC)	4-Epichlortetracycline (ECTC) 4-Epioxytetracycline (EOTC) 4-Epitetracycline (ETC) Isochlortetracycline (ICTC) Minocycline Oxytetracycline (OTC) Tetracycline (TC)
List 3 (Acid extraction, negative ESI)	**Bisphenol A** **Furosemide** Gemfibrozil **Glipizide** **Glyburide** **Hydrochlorothiazide** **2-hydroxy-ibuprofen**	Ibuprofen Naproxen Triclocarban Triclosan Warfarin
List 4 (Base extraction, positive ESI)	Albuterol **Amphetamine** **Atenolol** **Atorvastatin** Cimetidine Clonidine Codeine	Cotinine Enalapril **Hydrocodone** Metformin **Oxycodone** Ranitidine **Triamterene**

List 5 (Acid Extraction, positive ESI)	**Alprazolam** **Amitriptyline** **Amlodipine** **Benzoylecgonine** **Benztropine** **Betamethasone** **Cocaine**	**DEET (N,N-diethyl-m-toluamide)** **Desmethyl-diltiazem** **Diazepam** Fluocinonide **Fluticasone propionate** **Hydrocortisone** **10-hydroxy-amitriptyline**	**Meprobamate** **Methyl-prednisolone** **Metoprolol** **Norfluoxetine** **Norverapamil** **Paroxetine** **Prednisolone**	**Prednisone** **Promethazine** **Propoxy-phene** **Propranolol** **Sertraline** **Simvastatin** **Theophylline** **Trenbolone** **Trenbolone-acetate** **Valsartan** **Verapamil**

[1] Due to instability accuracy of Ampicillin data is unknown.

APPENDIX C

TABLE 8

World-wide occurrences of the newly detected 26 PPCPs in the present study

#	Compound class	Detection matrix				Source
		Wastewater		Surface water	Biosolids	
		Influent	Effluent			
1	**Calcium channel blockers**					
a.	Amlodipine	√				Nagarnaik et al., 2010
b.	Verapamil		√			Batt et al., 2008
2	**Diuretics**					
a.	Furosemide	√	√	√		Castiglioni et al., 2006
		√	√		√	Jelic et al., 2011
b.	Triamterene		√	√		Batt et al., 2008
3	**Selective serotonin reuptake inhibitors**					
a.	Paroxetine		√			Batt et al., 2008
			√	√		Schultz and Furlong 2008
				√		Wu et al., 2009
			√			Radjenović et al., 2009
b.	Sertraline		√	√		Schultz and Furlong 2008
					√	Barron, 2009
			√			Batt et al., 2008
4	**Metabolites**					
a.	Norverapamil			√		Batt et al., 2008

b.	Norfluoxetine			√		Schultz and Furlong 2008; Kolpin et al., 2002
c.	10-hydroxy-amitriptyline			√		Batt et al., 2008
d.	Desmethyldiltiazem		√	√		Batt et al., 2008
e.	Benzoylecgonine			√		Kasprzyk-Hordern et al.,2008
		√	√			Quintana et al., 2010
		√	√	√		Ventura et al., 2007; Postigo et al., 2010
		√				Field et al., 2008
		√	√			Castiglioni et al., 2006
5	**Tricyclic antidepressants**					
a.	Amitriptyline			√		Kasprzyk-Hordern et al.,2008
					√	Batt et al., 2008
6	**Drugs of abuse**					
a.	Cocaine	√	√			Ventura et al., 2007; Quintana et al., 2010
					√	Kasprzyk-Hordern et al., 2008; Postigo 2010; Quintana 2010
		√				Field et al., 2008

7	**Beta blockers**					
a.	Metoprolol	√	√			Ternes et al., 2007;
						Scheurer et al., 2010
		√	√	√		Jelic et al., 2011
			√	√		Hernando et al., 2005
					√	Barron, 2009
b.	Propranolol	√	√			Bendz et al., 2005;
						Maurer et al., 2007;
						Scheurer et al., 2010
					√	Radjenović et al., 2009;
						Barron, 2009
			√	√		Batt et al., 2008
c.	Atenolol		√	√		Batt et al., 2008
8	**Analgesics**					
a.	Oxycodone	√				Ternes et al., 2006;
						Chiaia et al., 2008
			√			Phillips et al., 2010
b.	Propoxyphene		√			Batt et al., 2008
c.	Hydrocodone		√	√		Batt et al., 2008
		√				Chiaia et al., 2008
9	**Ungrouped chemicals**					
a.	DEET	√	√			Bartelt-Hunt et al., 2008
				√		Trenholm et al., 2006
b.	Promethazine		√			Batt et al., 2008

c.	Valsartan		√	√		Batt et al., 2008
d.	Glyburide		√	√		Batt et al., 2008
e.	Alprazolam		√	√		Batt et al., 2008
f.	Benztropine					
g.	Atorvastatin	√	√		√	Jelic et al., 2011
			√			Metcalfe et al., 2003; Batt et al., 2008

*This is not a comprehensive list.