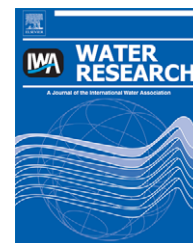


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Pharmaceuticals and personal care products in archived U.S. biosolids from the 2001 EPA national sewage sludge survey

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ABSTRACT

In response to the U.S. National Academies' call for a better assessment of chemical pollutants contained in the approximately 7 million dry tons of digested municipal sludge produced annually in the United States, the mean concentration of 72 pharmaceuticals and personal care products (PPCP) were determined in 110 biosolids samples collected by the U.S. Environmental Protection Agency (EPA) in its 2001 National Sewage Sludge Survey. Composite samples of archived biosolids, collected at 94 U.S. wastewater treatment plants from 32 states and the District of Columbia, were analyzed by liquid chromatography tandem mass spectrometry using EPA Method 1694. Thirty-eight (54%) of the 72 analytes were detected in at least one composite sample at concentrations ranging from 0.002 to 48 mg kg⁻¹ dry weight. Triclocarban and triclosan were the most abundant analytes with mean concentrations of 36 ± 8 and 12.6 ± 3.8 mg kg⁻¹ (n = 5), respectively, accounting for 65% of the total PPCP mass found. The loading to U.S. soils from nationwide biosolids recycling was estimated at 210–250 metric tons per year for the sum of the 72 PPCPs investigated. The results of this nationwide reconnaissance of PPCPs in archived U.S. biosolids mirror in contaminant occurrences, frequencies and concentrations, those reported by the U.S. EPA for samples collected in 2006/2007. This demonstrates that PPCP releases in U.S. biosolids have been ongoing for many years and the most abundant PPCPs appear to show limited fluctuations in mass over time when assessed on a nationwide basis. The here demonstrated use of five mega composite samples holds promise for conducting cost-effective, routine monitoring on a regional and national basis.

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1. Introduction

Pharmaceuticals and personal care products (PPCPs) are common contaminants of the environment, and have been detected in surface water (Kolpin et al., 2002; Cahill et al., 2004; Moldovan, 2006; Roberts and Thomas, 2006; Tamtam et al., 2008), groundwater (Heberer et al., 2000; Lindsey et al.,

2001; Fick et al., 2009), and drinking water (Stackelberg et al., 2004; Loraine and Pettigrove, 2006; Loos et al., 2007; Focazio et al., 2008), as well as in agricultural soils subject to land application of digested municipal sludge (Kinney et al., 2008; Kupper et al., 2004; Wu et al., 2009), also known as biosolids. Wastewater treatment plants were identified as one possible source for surface water contamination. Over-the-counter

Abbreviations: DL, Detection limit; EPA, Environmental protection agency; NEBRA, North east biosolids and residuals association; NSSS, National sewage sludge survey; PPCP, Pharmaceuticals and personal care products; RPD, Relative percent difference; TNSSS, Targeted national sewage sludge survey.

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and prescription drugs enter the wastewater via excretion of urine and feces containing parental drugs and their conjugates as well as other metabolites, or from disposal of unwanted or expired medications (Halling-Sorensen et al., 1998; Fent et al., 2006). Similarly, chemical constituents of personal care products may be directly disposed of into domestic wastewater. Removal of PPCPs during municipal wastewater treatment is rarely complete, thereby creating a pathway for entry of these compounds into aquatic environments via wastewater reclamation (Halling-Sorensen et al., 1998; Ternes, 1998; Daughton and Ternes, 1999; Hirsch et al., 1999) and into terrestrial environments via land application of biosolids (Ternes et al., 2004a).

Of the more than 7 million tons of sewage sludge produced in the United States in 2004, about 50% was applied to land as fertilizer or soil amendment, and 45% was disposed of in landfills or as landfill cover (NEBRA, 2007). Terrestrial environments can offer effective biological, physical, and chemical attenuation mechanisms for manmade pollutants. However, they also can act as a source term for chemical migration into surface and groundwater from biosolids runoff and leachate.

Pharmaceutical compounds are designed to be biologically active and therefore may have effects on non-target organisms even at trace concentrations extant in terrestrial and aquatic environments. While acute toxic effects of pharmaceuticals on non-target organisms have been investigated for some compounds, chronic toxicity and potential subtle environmental effects are only scarcely known (Fent et al., 2006). Also insufficiently investigated is the effect of mixtures of pharmaceuticals on aquatic organisms, although biochemical interactions of drugs in humans are well known. Of additional concern is the possible uptake of contaminants into food crops grown on agricultural fields that were fertilized with biosolids (Kumar et al., 2005; Dolliver et al., 2007). Currently, no regulation exists in the United States for PPCPs contained in biosolids, and a need for more information on the occurrence of and risk from these compounds has been noted by the National Research Council of the National Academies of the United States (National Research Council, 2002). In the past, analytical methods were limited, especially for trace analyses of complex environmental samples. In 2007, the release of U.S. EPA method 1694 (USEPA, 2007a) for the analysis of PPCPs in various matrices afforded the opportunity to analyze biosolids samples using a standardized protocol.

The U.S. EPA has performed national sewage sludge surveys (NSSS) in 1989, 2001, and 2007. The survey conducted in 2001 served to evaluate the potential need for regulations of trace levels of dioxins and polychlorinated biphenyls (USEPA, 2007b). After the 2001 survey was completed, unused samples were released to a nationwide repository of biosolids samples now maintained at the Biodesign Institute at Arizona State University.

This investigation evaluated occurrences and concentrations of PPCPs in biosolids from the year 2001 to enable risk assessments and to establish a national baseline for evaluating temporal trends of PPCPs in U.S. biosolids. The analysis of composite samples by EPA Method 1694 was employed to determine average concentrations of 72 PPCPs in archived biosolids collected by the EPA, as a representative sample of the more than 16,000 treatment plants located in the contiguous United States.

2. Materials and methods

2.1. Sampling procedure

Biosolids samples with solid contents between 1% and 30% were obtained from 94 wastewater treatment plants in 32 states and the District of Columbia for the 2001 National Sewage Sludge Survey (USEPA, 2007b). They were selected by the U.S. EPA to obtain a representative estimate of the occurrence of chemical contaminants in sewage sludge that is disposed of primarily by land application. Information on the exact sampling locations is available in Table S1 of the supplementary material (SM). This survey aimed to estimate levels of dioxins, dibenzofurans, and coplanar polychlorinated biphenyls in biosolids. The sampling was conducted by the U.S. EPA between February and March 2001, and samples were collected according to sampling procedures developed by the U.S. EPA (USEPA, 2001). Samples were only taken from fully processed sewage sludges intended for disposal. Eighty-nine of the 94 WWTPs had one single system for sludge treatment, therefore one sample was collected. Five facilities had two systems for treating their sludges, therefore two samples were taken from each of these plants. In addition, duplicate samples were collected from 15% of facilities (14 samples) for precision analysis. This amounted to 113 samples overall. After completion of the 2001 NSSS, the samples were acquired by the Halden laboratory for further studies. For the 7-year period between acquisition and analysis in the spring of 2008, samples were stored at -20°C .

2.2. Composite sample preparation

From the 113 biosolids samples acquired from the EPA, three were excluded from analysis because the sample containers were broken or compromised; the remaining 110 samples were randomly grouped into five groups. Composite samples were prepared by weighing out approximately 1 g of dry weight from each sample and pooling it to obtain 5 composites each containing solids from between 21 and 24 individual samples. A duplicate of composite sample #3 was prepared to serve as a blind duplicate.

2.3. Sample analysis

The samples were analyzed by AXYS Analytical Services (2045 Mills Road West, Sydney, British Columbia, Canada V8L 3S8) according to EPA method 1694 (USEPA, 2007a). For the purpose of compound detection, the 72 analytes were divided into four groups. All analytes were separated by liquid chromatography and detected by tandem mass spectrometry. For compounds with a respective labeled analog, the concentration was determined using the isotope dilution technique. The corresponding concentrations are deemed to be of high quality. For compounds where a labeled analog was not available, the concentration was determined using an external calibration. Quantitative data for analytes not determined by the isotope dilution method are judged to be less robust. More detailed information on the analysis method and accuracy and precision criteria for acceptance of analytical data is available in

supplementary material (SM). Further information on study limitations can be found in the discussion section.

2.4. Quality assurance

To ensure system and laboratory performance, several tests were performed before sample analysis. Calibration accuracy was verified using a calibration standard solution with labeled and native analytes. Retention times of native and labeled compounds had to be within ± 15 seconds of the respective retention time established during the previous calibration. In addition, ongoing precision and recovery were ensured. Lab blanks were analyzed before each sample analysis. A duplicate sample analysis was performed by the lab for each batch between 7 and 20 samples. In addition to these standard procedures, a blind duplicate was included in the sample set to evaluate analysis precision. Precision is expressed as relative percent difference (RPD) between each pair of measured concentrations. It was calculated using the following equation,

$$\text{RPD}[\%] = \frac{|C_{\text{sample}} - C_{\text{duplicate}}| * 100}{\frac{C_{\text{sample}} + C_{\text{duplicate}}}{2}} \quad (1)$$

where C_{sample} and $C_{\text{duplicate}}$ are, respectively, the concentration detected in the original sample and in its duplicate sample, and RPD is the relative percent difference.

2.5. Modeling of soil and porewater concentration

For assessment of their potential environmental impact, the concentrations of PPCPs after mixing with agricultural soil were calculated. The mixing ratio with soil was assumed based on the EPA-recommended rate of biosolids application of up to 4.5 dry kg per m^2 and by further assuming incorporation into soil to a depth of 10 cm (USEPA, 1994). The soil bulk density was assumed to equal 1.3 g cm^{-3} and the biosolids bulk density 1.6 g cm^{-3} . Though soil moisture content is known to vary widely depending on soil properties, for the purpose of this calculation, a soil moisture content of 22% (v/v) was used, as reported by others for agricultural soil (De Lannoy et al., 2006). A typical soil organic carbon fraction was assumed (Causarano et al., 2008), as was a biosolids organic carbon fraction of 0.4 (USEPA, 2007b). The concentration in soil and porewater at equilibrium was calculated based on the organic carbon fraction of the soil-biosolids mixture and the compound specific organic-carbon distribution coefficient (K_{OC}) of each analyte (Chalew and Halden, 2008). Calculations took into consideration the soil moisture content, as well as the volume of biosolids added to the soil, and were conducted for an environmentally relevant pH range of 7–9. The concentrations of PPCPs in porewater of soil/biosolid mixtures were calculated using the equations below,

$$C_{\text{porewater}} = \frac{\frac{m_{\text{biosolids}}}{m_{\text{soil+biosolids}}} C_{\text{biosolids}}}{\frac{f_{\text{porewater}} * \rho_{\text{porewater}}}{m_{\text{soil+biosolids}}} + K_{\text{OC}} * f_{\text{OC}}} \quad (2)$$

$$C_{\text{soil}} = \frac{m_{\text{biosolids}}}{m_{\text{soil+biosolids}}} C_{\text{biosolids}} - \frac{f_{\text{porewater}} * \rho_{\text{porewater}}}{m_{\text{soil+biosolids}}} C_{\text{porewater}} \quad (3)$$

where m is the dry mass in kg m^{-3} of the solid matrix, C the concentration in $\mu\text{g kg}^{-1}$, ρ the density in kg m^{-3} , and $f_{\text{porewater}}$

and f_{OC} the dimensionless fractions of, respectively, porewater and organic carbon in the soil/biosolids mixture.

2.6. Drug usage and ecotoxicity data

Information of prescription drug consumption was obtained from Internet sources (www.drugtopics.com). Exotoxicity data were taken from EPA's Ecotox database (www.epa.gov/ecotox).

2.7. Modeling of annual loading to agricultural soil

The annual loading of PPCPs contained in biosolids was based on a production of 5.6–7 million dry tons of sewage sludge in the U.S., of which 50–60% is applied to land (National Research Council, 2002; Jones-Lepp and Stevens, 2007; NEBRA, 2007).

3. Results

3.1. Data quality assurance

Lab blanks showed no detections above the detection limit for any of the analytes except for ciprofloxacin [$61 \mu\text{g kg}^{-1}$ dry weight (dw); detection limit (DL) $32 \mu\text{g kg}^{-1}$ dw] and erythromycin- H_2O ($2.6 \mu\text{g kg}^{-1}$ dw; DL $1.5 \mu\text{g kg}^{-1}$ dw). However, concentrations detected in biosolids were 100- and 40-times greater than the background level detected in lab blanks. Therefore, measured concentrations for both analytes were accepted.

Recovery for all analytes typically was good with an average of 112% but some notable outliers were observed leading to a range of 12–493% (See Table 1 and Table S2, SM). A total of 10 analytes exceeded the methods' lower and upper control limits. For 5 analytes (anhydrotetracycline, azithromycin, cimetidine, 4-epianhydrochlortetracycline, and 4-epianhydrotetracycline) recovery rates were below the method's lower control limit of 40%. The concentrations reported for these analytes in biosolids may represent underestimates. The recovery rates of 5 analytes (clinafloxacin, enrofloxacin, lomefloxacin, ofloxacin, and sarafloxacin) were above the method's upper control limits. The respective concentrations reported for these analytes may represent overestimations. All of the above mentioned analytes were quantified using labeled surrogate standards. In the case of clinafloxacin, enrofloxacin, lomefloxacin, ofloxacin, and sarafloxacin, $^{13}\text{C}_3\text{-}^{15}\text{N}$ -ciprofloxacin was used as an isotope-labeled surrogate standard. The recoveries were determined from spiked quality control samples, where recovery of $^{13}\text{C}_3\text{-}^{15}\text{N}$ -ciprofloxacin was below the method's lower control limit. This led to extremely high recoveries of the above mentioned analytes. In the biosolids samples, recovery of $^{13}\text{C}_3\text{-}^{15}\text{N}$ -ciprofloxacin was always within the method's control limits. However, the lack of adequate recovery of $^{13}\text{C}_3\text{-}^{15}\text{N}$ -ciprofloxacin in quality control samples suggests that concentrations reported in biosolids for clinafloxacin, enrofloxacin, lomefloxacin, ofloxacin, and sarafloxacin should be interpreted with caution.

The duplicate analysis revealed 18% relative percent difference (RPD) for all analytes. The blind duplicate analysis of a subset of 10 analytes revealed 28% RPD. Both RPD values

Table 1 – Analytical results and summary statistics for pharmaceuticals and personal care products detected in U.S. biosolids collected in 2001 and reported on a dry weight basis.

Substance name	CAS RN	Detection limit [$\mu\text{g kg}^{-1}$]	Standard deviation of detection limit [$\mu\text{g kg}^{-1}$] n = 5	Frequency measured [%]	Maximum concentration [$\mu\text{g kg}^{-1}$]	Mean detected concentration [$\mu\text{g kg}^{-1}$]	Standard deviation of mean concentration [$\mu\text{g kg}^{-1}$] n = 5	Recovery [%]	Isotope dilution quantification	Use	Lowest effect concentration for aquatic biota [$\mu\text{g L}^{-1}$]	Projected annual land application [kg yr^{-1}] ^f
Anhydrotetracycline ^g	4496-85-9	74.1	7.4	100	880	392	249	51		Antibiotic		1300–1600
Azithromycin ^g	83905-01-5	5.6	1.6	100	1220	838	224	12		Antibiotic		2800–3500
Caffeine	58-08-2	59.0	16.4	100	643	248	200	78	✓	Stimulant	0.05 ^a (Bantle et al., 1994)	830–1000
Carbamazepine	298-46-4	5.6	1.6	100	238	163	56.4	139		Anticonvulsant	100 ^b (Jos et al., 2003)	550–680
Chlortetracycline	57-62-5	22.9	6.2	60	43.5	23.4	16.9	159		Antibiotic	36 ^c (Brain et al., 2004)	80–100
Cimetidine ^g	51481-61-9	6.4	2.0	100	893	504	208	41		Antacid		1700–2100
Ciprofloxacin	85721-33-1	20.9	6.4	100	10800	6858	2348	98	✓	Antibiotic	5 ^d (Halling-Sorensen, 2001)	23,000–28,000
Clarithromycin	81103-11-9	5.6	1.6	100	94.6	66.2	25.5	114		Antibiotic		220–270
Codeine	76-57-3	11.2	3.2	20	29.7	n.d.		114		Analgesic		
Cotinine	486-56-6	9.9	3.3	100	38.6	28.1	8.3	103	✓	Nicotine metabolite		100–120
Diltiazem	42399-41-7	1.3	0.1	100	109	45.2	34.2	110		Antianginal		150–190
Diphenhydramine	58-73-1	2.3	0.6	100	1740	1166	516	101		Antihistamine		3900–4800
Doxycycline	564-25-0	23.9	6.3	100	1780	966	436	85		Antibiotic		3200–4000
Enrofloxacin ^h	93106-60-6	19.5	6.5	60	28.6	n.d.		190		Antibiotic	49 ^d (Robinson et al., 2005)	
4-Epianhydro-tetracycline ^g	4465-65-0	77.1	20.2	100	399	261	71.7	39		Antibiotic		900–1100
4-Epichlor-tetracycline	14297-93-9	56.3	16.0	40	93.0	n.d.		161		Antibiotic		
4-Epitetracycline	23313-80-6	74.4	18.8	100	3040	2376	517	82		Antibiotic		8000–9800
Erythromycin-H ₂ O	114-07-8	1.6	0.2	100	183	81.5	52.3	97	✓	Antibiotic	22 700 ^e (Williams et al., 1992)	270–340
Fluoxetine	54910-89-3	8.2	1.0	100	258	171	46.6	89	✓	Antidepressant	36 ^h . ^c (Flaherty and Dodson, 2005)	580–710
Gemfibrozil	25812-30-0	12.2	12.3	100	159	152	13.2	107	✓	Antihyperlipidemic	30,040 ^c (Zurita et al., 2007)	510–630
Ibuprofen	15687-27-1	122	123	80	359	246	121	109	✓	Anti-inflammatory		830–1000
Isochlortetracycline	514-53-4	22.5	6.4	60	36.0	n.d.		70		Antibiotic		
Lomefloxacin ⁱ	98079-51-7	13.2	2.1	40	16.1	n.d.		388		Antibiotic	106 ^e (Robinson et al., 2005)	
Metformin	657-24-9	119	32.5	80	456	305	152	116	✓	Antidiabetic		1000–1300
Miconazole	22916-47-8	5.8	1.3	100	1100	777	266	86		Antifungal		2600–3200
Minocycline	10118-90-8	1880	524	80	2630	1884	939	54		Antibiotic		6300–7800

(continued on next page)

Table 1 (continued)

Substance name	CAS RN	Detection limit [$\mu\text{g kg}^{-1}$]	Standard deviation of detection limit [$\mu\text{g kg}^{-1}$] n = 5	Frequency measured [%]	Maximum concentration [$\mu\text{g kg}^{-1}$]	Mean detected concentration [$\mu\text{g kg}^{-1}$]	Standard deviation of mean concentration [$\mu\text{g kg}^{-1}$] n = 5	Recovery [%]	Isotope dilution quantification	Use	Lowest effect concentration for aquatic biota [$\mu\text{g L}^{-1}$]	Projected annual land application [kg yr^{-1}] ^f
Naproxen	22204-53-1	24.3	24.6	100	273	119	79	106	✓	Anti-inflammatory		400–500
Norfloxacin	70458-96-7	63.5	6.3	100	418	289	74.0	136		Antibiotic	18 000 ^b (Yang et al., 2008)	970–1200
Ofloxacin ^h	82419-36-1	7.7	4.2	100	8140	5446	1941	493		Antibiotic	21 ^d (Robinson et al., 2005)	18000–23,000
Oxytetracyclin	79-57-2	22.8	5.8	100	114	87.5	22.2	90		Antibiotic	50 ^d (Hanson et al., 2006)	300–360
Ranitidine	66357-35-5	6.5	1.7	100	30.1	21.0	8.0	51		Antacid		70–90
Sulfamethoxazole	723-46-6	2.9	0.7	20	3.3	n.d.		98	✓	Antibiotic	9 ^e (Brain et al., 2008)	
Sulfanilamide	63-74-1	56.2	16.0	40	87.3	n.d.		101		Antibiotic		
Tetracycline	60-54-8	57.5	15.5	100	2790	1914	691	124		Antibiotic	47 ^e (Brain et al., 2004)	6400–7900
Thiabendazole	148-79-8	5.9	1.2	100	370	110	131	103	✓	Fungicide	310 ^c (EPA, 2000)	370–460
Triclocarban	101-20-2	183	67.0	100	48100	36060	8049	96	✓	Disinfectant	0.101 ^c (EPA/OTS, 1992)	120,000–150,000
Triclosan	3380-34-5	487	493	100	19700	12640	3816	105	✓	Disinfectant	0.12 ^b (Wilson et al., 2003)	42,000–52,000
Trimethoprim	738-70-5	10.6	3.8	60	60.5	26.0	21.5	97	✓	Antibiotic	16 000 ^b (Luetzhof et al., 1999)	90–110

a Amphibium.

b Green algae.

c Crustacean.

d Cyanobacteria.

e Macrophyte.

f Projected deposition rates of PPCPs on land based on 5.6–6.9 million dry tons of annual sewage sludge production and a land application rate of 60% (National Research Council, 2002; USEPA, 2003; USGS, 2006; Jones-Lepp and Stevens, 2007).

g Concentration may be underestimated due to low recovery in quality control samples.

h Concentration may be overestimated due to high recovery in quality control samples.

were in control with respect to the target RPD of 30% or less. The RPD value improved to 11% for 9 analytes, when excluding results for metformin, whose lack of detection in the blind duplicate unfavorably increased the summary statistic for measurement precision. A non-blinded duplicate analysis, performed by the contract laboratory for these 10 analytes, showed an RPD value of 11%.

3.2. Study representativeness and sample integrity

The prolonged storage of samples between sampling event and analysis may have allowed for the chemical degradation of labile analytes to occur. Therefore, any results of this study are conservative with respect to the detection frequency and concentration of compounds found. In other words, due to pooling of a large number of samples, analytes occurring infrequently and at low concentrations may have been diluted out to below the detection limit. A comparison of the presented data with the EPA data (USEPA, 2009), reveals that the mean concentrations of all analytes show no statistically significant difference within the 95th percentile confidence interval. Therefore, the prolonged storage did not impair the detection of multiple analytes at elevated concentrations in archived samples.

3.3. Occurrence of PPCPs in biosolids

All composites tested positive for at least 26 analytes. Of the 72 PPCPs targeted, 38 (54%) were detected at concentrations ranging from the low parts-per-billion (ppb) to the parts-per-million (ppm) range. These 38 PPCPs include 8 that have not previously been reported in biosolids in the peer-reviewed literature but that also were observed in the U.S. EPA's TNSSS published online (USEPA, 2009). The remaining 34 PPCPs were

not detected in any of the composite biosolids samples. The mean total concentration of all targeted PPCPs combined in the five composite samples was $74.4 \text{ mg kg}^{-1} \text{ dw}$ of sewage sludge $\pm 21.4 \text{ mg kg}^{-1}$ standard deviation ($n = 5$).

The two most abundant contaminants were the disinfectants triclocarban (48% of total detected PPCP mass) and triclosan (17%) (Fig. 1). Their mean concentrations were 36 ± 8 and $12.6 \pm 3.8 \text{ mg kg}^{-1} \text{ dw}$ ($n = 5$), respectively (Table 1). The second most abundant class of PPCPs found was antibiotics. In order of decreasing concentration, ciprofloxacin, ofloxacin, 4-epitetracycline, tetracycline, minocycline, doxycycline and azithromycin were found at concentrations between 6.8 ± 2.3 and $0.8 \pm 0.2 \text{ mg kg}^{-1} \text{ dw}$ ($n = 5$) (Table 1). The combined mass of all antibiotics constituted about 29% of the total mass of PPCPs per sample.

In addition to the 3 tetracyclines reported above, three tetracycline antibiotics were found for which peer-reviewed occurrence data in sewage sludge thus far were lacking. Anhydrotetracycline, 4-epianhydrotetracycline, and chlortetracycline (in order of decreasing concentration) together accounted for $6.9 \pm 2.5 \text{ mg kg}^{-1} \text{ dw}$ ($n = 5$) (Table 1). Also not previously reported in biosolids were two prescription drugs, metformin and ranitidine. They together accounted for 0.4% of the total mass of PPCPs found in sewage sludge composites.

4. Discussion

4.1. Study limitations

For this study, a relatively large number of individual samples were combined to form 5 composite pools or mega composites. This approach served to reduce the number of samples to be analyzed in order to obtain a defensible estimate of mean

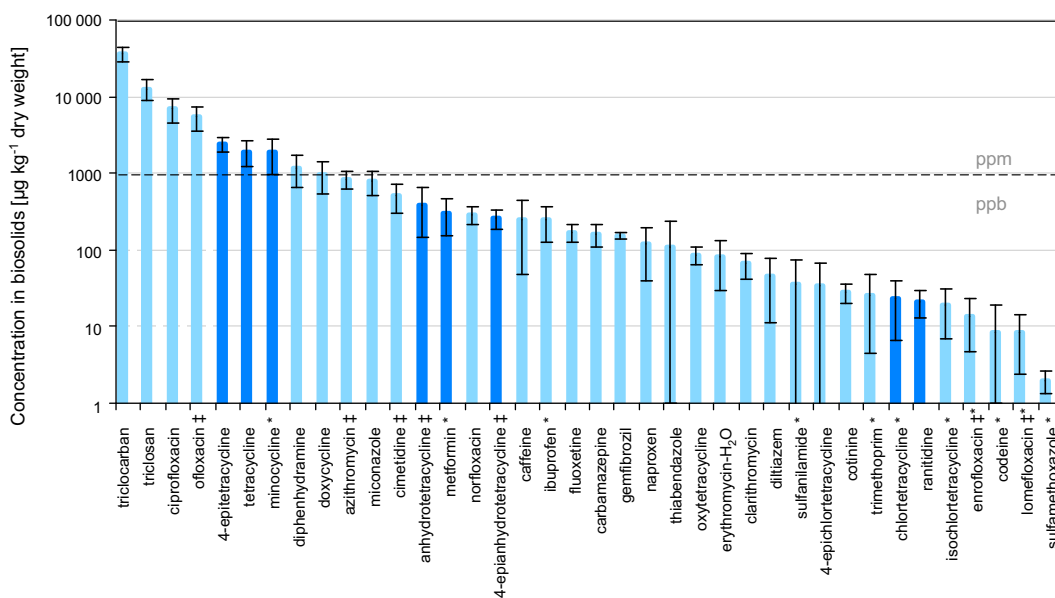


Fig. 1 – Rank order of mean concentrations for 38 PPCPs detected in composites of a total of 110 U S biosolids samples from 94 treatment plants in 32 states and the District of Columbia. Newly detected compounds are shown in darker hue. Error bars depict \pm one standard deviation ($n = 5$). Some concentrations represent estimates only (†) and some analytes were detected inconsistently (*).

analyte concentrations across the various treatment facilities represented. While being efficient and economical for the intended purpose, this approach was not well suited to capture the full spectrum of concentrations of individual PPCPs as a function of plant type, treatment processes employed, populations served, as well as of geographical locations and climate zones represented. As a comparison with the EPA TNSSS data reveals (USEPA, 2009), the detection frequency of less abundant analytes was significantly reduced in composite samples compared to individual sample analysis. Therefore, analytes that were not detected will represent a conservative estimate, and may still occur at detectable concentrations in individual samples from specific plants. While the mega composite approach cannot serve to determine variability between the large numbers of WWTPs studied, it was found to be suitable for identifying major contaminants of concern as well as their average concentration in a large sample set.

4.2. Sanitizing agents

Triclocarban, which in previous U.S. studies had been found in concentrations ranging from 5.97 to 51 mg kg⁻¹ dry weight (dw) (Heidler et al., 2006; Chu and Metcalfe, 2007; Sapkota et al., 2007), was detected in every composite sample assayed. Also found in significant amounts was triclosan, which has been observed in a number of U.S. studies of sewage sludge with reported concentrations ranging from 0.53 to 30 mg kg⁻¹ dw (Chu and Metcalfe, 2007; Heidler and Halden, 2007; Kinney et al., 2008; McAvoy et al., 2002; USEPA, 2003). The EPA TNSSS (USEPA, 2009) found triclocarban and triclosan at concentrations of up to 441 and 133 mg kg⁻¹ dw, respectively, with mean concentrations at 38.7 ± 59.7 and 12 ± 18 mg kg⁻¹ dw (n = 74), respectively. The relatively high mean concentrations of triclocarban and triclosan reported here and by the U.S. EPA (USEPA, 2009) in U.S. biosolids are in line with the intense usage of these antimicrobials and their high octanol/water partitioning coefficient (log K_{OW}) of 4.9 and 4.8, respectively (both at neutral pH), which indicates significant potential of both compounds for sorption to biosolids (Halden and Paull, 2005). In addition, triclosan was found to be persistent in biosolids after aeration (Ying et al., 2007). Both triclosan and triclocarban concentrations found in the present study fall within the range of concentrations previously reported. Information on previously reported concentrations of PPCPs in biosolids is available in Table S2, SM.

4.3. Antibiotics

The most abundant antibiotic was ciprofloxacin (6.8 ± 2.3 mg kg⁻¹ dw; n = 5), which is among the 30 most prescribed drugs in the United States, according to Internet sources. Ciprofloxacin, a metabolite of enrofloxacin, is polar and therefore prone to electrostatic interactions with the negatively charged surfaces of microbes that are found in high concentrations especially in secondary sludge (Ternes et al., 2004b). Ciprofloxacin has been detected in sewage sludge by several studies conducted in Sweden and Switzerland. Detected concentrations ranged from 6 × 10⁻⁵–11 mg kg⁻¹ dw (Golet et al., 2002; Lindberg et al., 2005, 2006, 2007). Therefore, the mean concentration found in the present study (6.8 ± 2.3 mg kg⁻¹

dw; n = 5) falls in the mid range of concentrations reported from outside of the U.S. The EPA TNSSS also identified ciprofloxacin as an abundant microcontaminant in sewage sludge, which was detected in every sample analyzed, yielding a mean concentration of 8.7 ± 8.5 mg kg⁻¹ dw.

At a mean concentration of 5.4 ± 1.9 mg kg⁻¹ dw, ofloxacin was the fourth most abundant contaminant found in biosolids. It is fairly hydrophilic (log K_{OW} of -0.2) and does not appear on the list of the top 200 prescription drugs. Its detection at elevated levels in every composite sample came as a surprise. However, ofloxacin had been found in concentrations of up to 2 mg kg⁻¹ dw in biosolids from Sweden (Lindberg et al., 2005). It can be speculated that the carboxyl group contained in ofloxacin may form an ion complex with exchangeable cations associated with negatively charged surfaces.

Among the 10 most abundant PPCPs of the present study were three tetracycline antibiotics, 4-epitetracycline, tetracycline and minocycline, which had not been reported in biosolids before in the peer-reviewed literature. In addition, doxycycline was found as the ninth most abundant PPCP at a mean concentration of 1 ± 0.4 mg kg⁻¹ dw. It had not been detected in biosolids from the U.S. before and only once in a Swedish study that found concentrations similar to those reported here (Lindberg et al., 2005). All tetracycline antibiotics are fairly hydrophilic (log K_{OW} of -1.33 for tetracycline/4-epitetracycline, -0.42 for minocycline, and -1.36 for doxycycline). Despite their hydrophilic character, the mean concentrations reported here are around 1–2 mg kg⁻¹ dw. Tetracycline antibiotics are known to precipitate with ions of magnesium, calcium and ferric iron, and therefore accumulate in the solid fraction during wastewater treatment. Tetracycline, doxycycline and minocycline also rank among the 200 prescription drugs most widely used in the U.S. Other tetracycline antibiotics analyzed in this study were only found at mean concentrations below 0.4 mg kg⁻¹ dw, but the concentrations of anhydrotetracycline, 4-epianhydrotetracycline and 4-epianhydrochlortetracycline were possibly underestimated due to low recoveries. The sum of tetracycline antibiotics found in sewage sludge constitute about 8 ± 1.3 mg kg⁻¹ dw, which is similar to the findings of the EPA TNSSS that found about 5 mg kg⁻¹ dw (USEPA, 2009).

Azithromycin was found at 0.8 ± 0.2 mg kg⁻¹ dw. It ranked as the 6th most frequently prescribed drug in 2007 and is also fairly hydrophobic. Due to both these properties and the fact that biodegradation of azithromycin was found to be insignificant (Ericson, 2007), one would expect high concentrations in biosolids. Yet, there are 7 drugs (not counting triclocarban and triclosan) that were found at higher concentrations. However, a low recovery of azithromycin of only 12% may indicate that actual azithromycin concentrations in biosolids are much higher than the detected concentration. Azithromycin has been detected in previous studies at concentrations of up to 6.5 mg kg⁻¹ dw in the U.S. (Jones-Lepp and Stevens, 2007) and at up to 0.16 mg kg⁻¹ dw in sludge from Germany and Switzerland (Gobel et al., 2005a, 2005b).

4.4. Bioavailability and soil/porewater equilibria

To explore the importance of bioavailability of PPCPs sequestered in biosolids, the concentrations of individual PPCPs that are anticipated to occur in the solid and liquid

phases upon mixing of land applied biosolids into soil were calculated (Fig. 2). In fully equilibrated soil-biosolids mixtures (assuming an EPA-recommended mixing ratio of approximate 25:1), concentrations of PPCPs on soil particles are expected to fall into the ppb range for most analytes, except for triclocarban, which was projected to be present at around 1 ppm_(w/w) dw. Since most of the PPCPs found in biosolids are fairly hydrophobic, their calculated concentrations in porewater at equilibrium typically were quite low (Fig. 2 B) (Kinney et al., 2008). A comparison of these estimated dissolved PPCP levels in porewater with the lowest effect concentrations for aquatic organisms (red circles in Fig. 2 B) suggests that the leaching of dissolved PPCPs into surface waters probably does not present an important mechanism for exposure of aquatic biota for the majority of analytes detected. Concentrations calculated for soil porewater typically were several orders of magnitude below the lowest effect concentration reported for aquatic organisms. Notable exceptions were 6 analytes (Fig. 2) that are expected to yield potentially problematic concentrations in porewater after land application and partitioning of biosolids-derived PPCPs. These include the antibiotics ciprofloxacin, ofloxacin and tetracycline, as well as the stimulant caffeine,

and the two sanitizing agents triclosan and triclocarban (Fig. 2).

4.5. Risk assessment data gaps

These results suggest that aside from the 7 notable exceptions discussed above, the majority of the PPCPs detected in biosolids in this study likely exert no acute effects on aquatic organisms, assuming that biosolids are applied as regulated by the EPA (USEPA, 1994) and that the migration of solids from agricultural land to surface water via soil erosion and runoff can be completely prevented. While the former assumption is plausible, the latter may not always apply, as soil erosion is a common phenomenon.

However, chronic toxicity as well as effects from mixtures of PPCPs on non-target organisms cannot be assessed due to lack of appropriate toxicity data. It has been speculated that the presence of sub-therapeutic concentrations of antibiotics may adversely affect soil microbial community structures as well as induce spreading of resistance among bacterial pathogens. In addition to influencing microbial populations, it has been shown that some

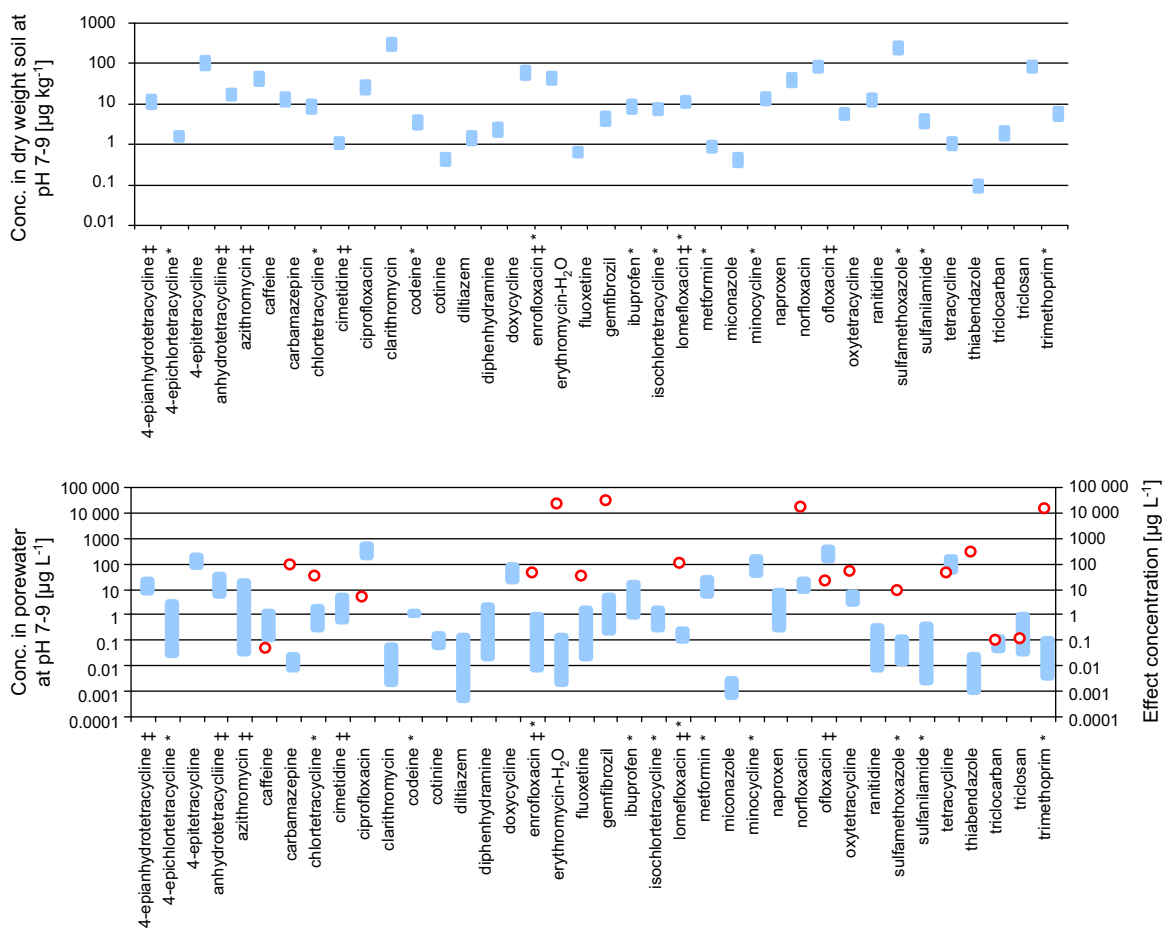


Fig. 2 – Predicted equilibrium concentrations of PPCPs associated with particulates of soil-biosolids mixtures (top) and dissolved in porewater (bottom) after land application of biosolids on agricultural soil. Data depict the environmentally relevant range between pH 7 and 9. Circles represent the lowest ecological effect concentrations contained in the EPA Ecotox database. Some concentrations were calculated based on estimates only (†) and some analytes were detected inconsistently (*).

antibiotics, specifically drugs belonging to the tetracyclines, fluoroquinolones and sulfonamides, may be taken up by crop plants (Migliore et al., 2003; Kumar et al., 2005; Dolliver et al., 2007). This presents a potential exposure pathway to humans through ingestion of contaminated food and may result in the promotion of resistant bacteria in humans (Shoemaker et al., 2001).

Furthermore, information is lacking to determine risks to the health of agricultural soils and soil-dwelling organisms. Few studies have examined the half-lives in soils of PPCPs sequestered in biosolids. The effect of ppm levels of sanitizing agents on soil microbial communities has rarely been investigated to date (Liu et al., 2009). Similarly, the half-life of PPCPs in biosolids-amended soils will require additional research to inform risk assessment analyses. Passage through municipal digesters and chemical aging may reduce the bioavailability of PPCPs and with it the risk of chemical uptake of and exposure to soil-dwelling organisms. However, a reduced bioavailability also may imply a prolonged half-life of these compounds in the environment, with possible delayed release in sensitive compartments. Furthermore, studies are lacking on the potential of biosolids-derived antimicrobials and antibiotics to exert selective pressure for the enrichment of drug-resistant microorganisms, a scenario demonstrated in vitro (Braoudaki and Hilton, 2004). Also unavailable are threshold concentrations for toxic effects in terrestrial organisms of many PPCPs, including some that have been detected in biosolids at ppm levels. The EPA's Ecotox database (www.epa.gov/ecotox) provides only some toxicity values in aquatic organisms for the PPCPs investigated here. Bioaccumulation and biomagnification are other aspects that will need to be considered for risk assessment purposes. Although PPCPs are not typically thought of as representing persistent hydrophobic pollutants, some may be subject to bioaccumulation and possibly biomagnification thereafter in both terrestrial and aquatic environments. Bioaccumulation was demonstrated for triclosan and triclocarban in lab and field studies that examined uptake of these compounds from soil, sediment and water (Coogan and La Point, 2008; Higgins et al., 2009; Kinney et al., 2008).

5. Conclusions

Overall, this study reemphasizes the significance of biosolids recycling as a mechanism for the release of PPCPs into the environment. Based on the mean concentrations of all analytes detected, it is estimated that the total loading to U.S. soils from nationwide biosolids recycling is on the order of 210–250 metric tons per year for the 72 PPCPs investigated here.

It is concluded that, despite large variations found by the U.S. EPA between different treatment plants, mean concentrations of PPCPs in U.S. biosolids on a nationwide basis have remained fairly constant between 2001 and 2007 and possibly longer. Good agreement between this and the U.S. EPA study further suggests that the here demonstrated use of mega composite samples represents a cost-effective approach for collecting regional and nationwide information on average concentrations of contaminants in biosolids.

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Appendix. Supplementary material

Additional details regarding experimental procedures and results can be found in the Supplementary Material accompanying this article. This information is available free of charge via the Internet at www.iwaponline.com.

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.watres.2009.12.032](https://doi.org/10.1016/j.watres.2009.12.032).

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