Validation of Mega Composite Sampling and Nationwide Mass Inventories for 26 Previously Unmonitored Contaminants in Archived Biosolids from the U.S National Biosolids Repository

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14 pages

Supplementary information:

Materials and methods:

Sampling procedure

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.

Sample analysis

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. Hydrocodone and codeine were reported to suffer from analytical cross interference arising from their similar molecular weights and formulae. To address this challenge, the contract laboratory routinely utilizes an algebraic correction that is based on the peaks for the two compounds. The interference is taken into account and use of the algebraic correction was shown to lower the rate of false-positive occurrence.

Modeling of annual loading to agricultural soil

The release rates of individual PPCPs were calculated as the products of the total amount of biosolids yearly (7.2 million dry tons), the concentration of compound detected in the samples and the % of biosolids used beneficially (55%) as reported in NEBRA (2007). Although representing a crude estimate, the computed rates serve to inform on the approximate magnitude of chemical releases to soils from biosolids-borne compounds.

Table S1. 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-Epichlortetracycline (ECTC) 4-Epioxytetracycline (EOTC) 4-Epitetracycline (ETC) Isochlortetracycline (ICTC) Minocycline	

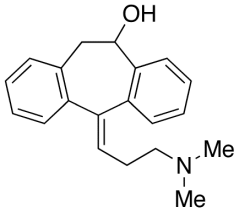
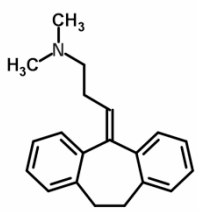
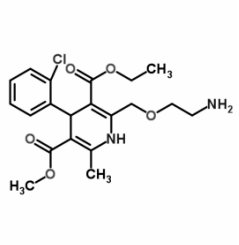
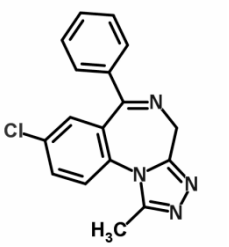
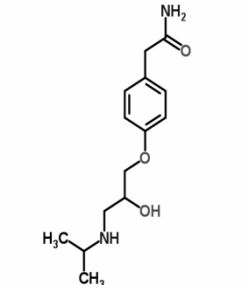
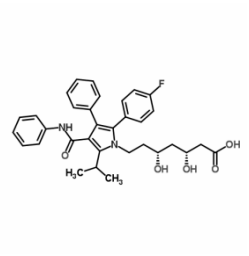
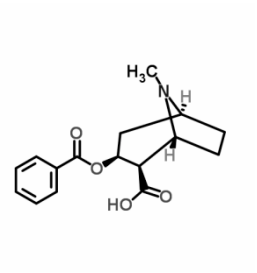
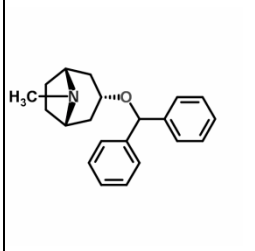
	4-Epianhydrochlortetracycline (EACTC) 4-Epianhydrotetracycline (EATC)	Oxytetracycline (OTC) Tetracycline (TC)
List 3 (Acid extraction, negative ESI)	**Bisphenol A** **Furosemide** Gemfibrozil **Glipizide** **Glyburide** **Hydrochlorothiazide** **2-hydroxy-ibuprofen**	Ibuprofen Naproxen Triclocarban Triclosan 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**

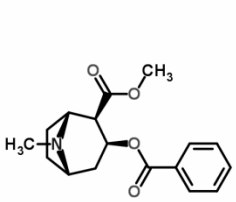
[1] Due to instability accuracy of Ampicillin data is unknown.

Table S2. Twenty-six PPCPs that were newly detected as a part of this analytical study. The number of positive occurrences in the five samples analyzed are marked beside the mean concentrations and for compounds that were inconsistently detected the maximum concentration has been 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. Structure and chemical properties of each compound was taken from the Royal Society of Chemistry database.

Compound

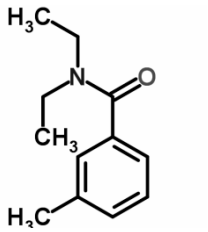
10-hydroxy-amitriptyline  CAS: 1159-82-6 OPR Recovery %: 70-130 $logK_{ow}$: 2.66 Half-life: N.A Soil loading: 32-80 kg yr^{-1} EC_{50}: N.A	Amitriptyline  CAS: 50-48-6 OPR Recovery %: 70-130 $logK_{ow}$: 4.92 Half-life: 120 d^{-1} Soil loading: 570-1402 kg yr^{-1} EC_{50}: 500 µg L^{-1} Rank: 143	Amlodipine *  CAS: 88150-42-9 OPR Recovery %: 45-130 $logK_{ow}$: 3.0 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 OPR Recovery %: 70-130 $logK_{ow}$: 2.12 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 OPR Recovery %: 70-130 $logK_{ow}$: 0.16 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 OPR Recovery %: 20-130 $logK_{ow}$: 3.85 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 OPR Recovery %: 70-130 $logK_{ow}$: -1.32 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 OPR Recovery %: 70-130 $logK_{ow}$: 4.28 Half-life: 75 d^{-1} Soil loading: 11-12 kg yr^{-1} EC_{50}: 48 µg L^{-1} Rank: -

Cocaine



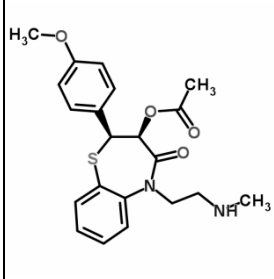
CAS: 50-36-2
OPR Recovery %: 70-130
$logK_{ow}$: 2.30
Half-life: 75 d^{-1}
Soil loading: 7-36 kg yr^{-1}
EC_{50}: 740 µg L^{-1}

DEET



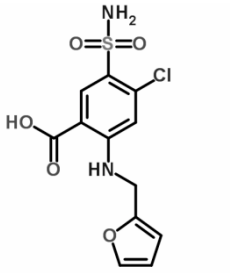
CAS: 134-62-3
OPR Recovery %: 70-130
$logK_{ow}$: 2.18
Half-life: 75 d^{-1}
Soil loading: 19-45 kg yr^{-1}
EC_{50}: 91 µg L^{-1}

Desmethyl-Diltiazem



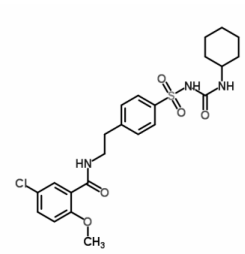
CAS: 86408-45-9
OPR Recovery %: 3-357
$logK_{ow}$: 2.58
Half-life: N.A
Soil loading: 8-69 kg yr^{-1}
EC_{50}: N.A

Furosemide*



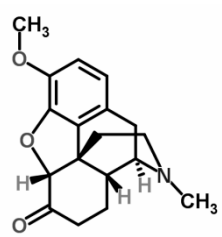
CAS: 54-31-9
OPR Recovery %: 65-130
$logK_{ow}$: 2.03
Half-life:120 d^{-1}
Soil loading: 356-483 kg yr^{-1}
EC_{50}: 1400 µg L^{-1}
Rank: 98

Glyburide*



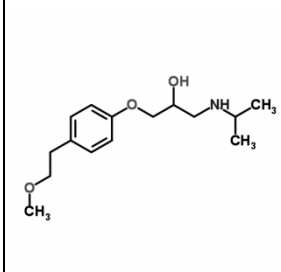
CAS: 10238-21-8
OPR Recovery %: 50-180
$logK_{ow}$: 4.79
Half-life: 360 d^{-1}
Soil loading: 23-82 kg yr^{-1}
EC_{50}: 2 µg L^{-1}
Rank: 149

Hydrocodone*



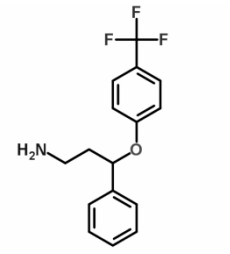
CAS: 126-29-1
OPR Recovery %: 70-130
$logK_{ow}$: 2.16
Half-life: N.A
Soil loading: 24-86 kg yr^{-1}
EC_{50}: 730 µg L^{-1}
Rank: 1

Metoprolol



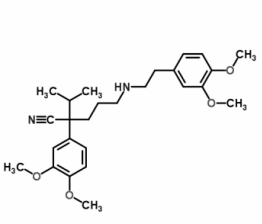
CAS: 51384-51-1
OPR Recovery %: 70-130
$logK_{ow}$: 1.88
Half-life: 75 d^{-1}
Soil loading: 46-150 kg yr^{-1}
EC_{50}: 1400 µg L^{-1}
Rank: 170

Norfluoxetine



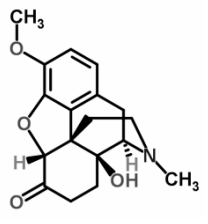
CAS: 83891-03-6
OPR Recovery %: 70-130
$logK_{ow}$: 4.18
Half-life: N.A
Soil loading: 8-329 kg yr^{-1}
EC_{50}: 300 µg L^{-1}

Norverapamil



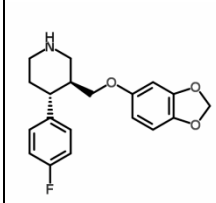
CAS: 67018-85-3

Oxycodone*



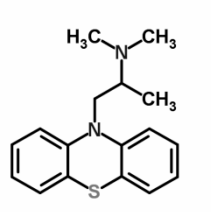
CAS: 76-42-6
OPR Recovery %: 65-130

Paroxetine



CAS: 61869-08-7
OPR Recovery %: 70-130

Promethazine



CAS: 60-87-7
OPR Recovery %: 70-130

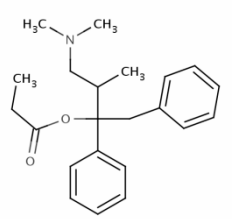
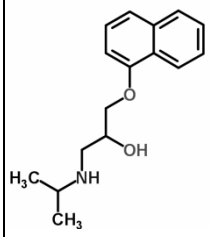
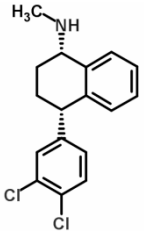
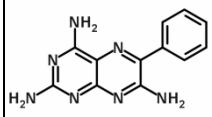
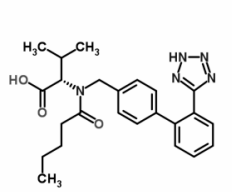
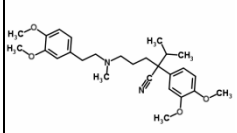
OPR Recovery %: 55-130 $logK_{ow}$: 4.59 Half-life: N.A Soil loading: 499-2336 kg yr^{-1} EC_{50}: N.A	$logK_{ow}$: 0.66 MDL: 1.2 µg kg^{-1} Mean ± Std. dev: nd (n=3) Recovery: 108 % Half-life: 360 d^{-1} Soil loading: 43-622 kg yr^{-1} EC_{50}: 5400 µg L^{-1} Rank: 33	$logK_{ow}$: 2.57 Half-life: N.A Soil loading: 122-356 kg yr^{-1} EC_{50}: 140 µg L^{-1} Rank: 127	$logK_{ow}$: 4.81 Half-life: 120 d^{-1} Soil loading: 60-117 kg yr^{-1} EC_{50}: 34 µg L^{-1} Rank: 113
Propoxyphene  CAS: 469-62-5 OPR Recovery %: 70-130 $logK_{ow}$: 4.18 Half-life: 120 d^{-1} Soil loading: 64-304 kg yr^{-1} EC_{50}: 15 µg L^{-1} Rank: 79	Propranolol  CAS: 525-66-6 OPR Recovery %: 70-150 $logK_{ow}$: 3.48 Half-life: 30 d^{-1} Soil loading: 231-578 kg yr^{-1} EC_{50}: 360 µg L^{-1} Rank: -	Sertraline  CAS: 79617-96-2 OPR Recovery %: 50-130 $logK_{ow}$: 5.29 Half-life: N.A Soil loading: 883-2519 kg yr^{-1} EC_{50}: 4.3 x 10^4 µg L^{-1} Rank: 28	Triamterene  CAS: 396-01-0 OPR Recovery %: 70-140 $logK_{ow}$: 0.98 Half-life: 75 d^{-1} Soil loading: 1030-2309 kg yr^{-1} EC_{50}: 4600 µg L^{-1} Rank: 106
Valsartan*  CAS: 137862-53-4 OPR Recovery %: 70-130 $logK_{ow}$: 3.65 Half-life: N.A Soil loading: 95-254 kg yr^{-1} EC_{50}: N.A Rank: 30	Verapamil  CAS: 52-53-9 OPR Recovery %: 70-145 $logK_{ow}$: 3.79 Half-life: 360 d^{-1} Soil loading: 586-2665 kg yr^{-1} EC_{50}: 1.3 µg L^{-1} Rank: 198		

Table S3. World-wide occurrences of the 26 PPCPs not previously detected in biosolids and reported 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.

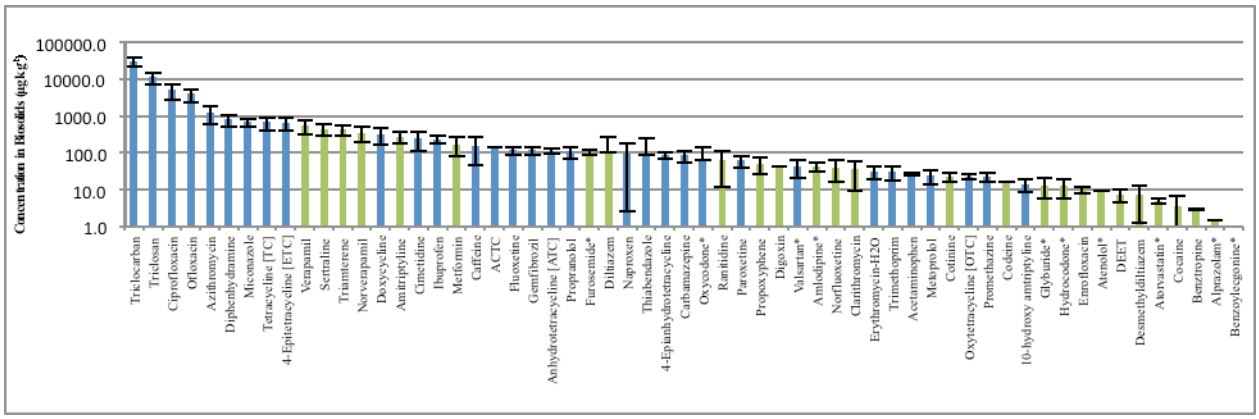


Figure S1. Rank order of mean concentrations of 59 PPCPs that were detected in composites of 110 U.S biosolids samples archived from the 2001 NSSS. Error bars depict ± one standard deviation (n=5), and compounds marked with (*) indicate inconsistent detection.

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