Bioaccumulation of Pharmaceuticals and Other Anthropogenic Waste Indicators in Earthworms from Agricultural Soil Amended With Biosolid or Swine Manure

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Analysis of earthworms offers potential for assessing the transfer of organic anthropogenic waste indicators (AWIs) derived from land-applied biosolid or manure to biota. Earthworms and soil samples were collected from three Midwest agricultural fields to measure the presence and potential for transfer of 77 AWIs from land-applied biosolids and livestock manure to earthworms. The sites consisted of a soybean field with no amendments of human or livestock waste (Site 1), a soybean field amended with biosolids from a municipal wastewater treatment plant (Site 2), and a cornfield amended with swine manure (Site 3). The biosolid applied to Site 2 contained a diverse composition of 28 AWIs, reflecting the presence of human-use compounds. The swine manure contained 12 AWIs, and was dominated by biogenic sterols. Soil and earthworm samples were collected in the spring (about 30 days after soil amendment) and fall (140—155 days after soil amendment) at all field sites. Soils from Site 1 contained 21 AWIs and soil from Sites 2 and 3 contained 19 AWIs. The AWI profiles at Sites 2 and 3 generally reflected the relative composition of AWIs present in waste material applied. There were 20 AWIs detected in earthworms from Site 1 (three compounds exceeding concentrations of 1000 μg/kg), 25 AWIs in earthworms from Site 2 (seven compounds exceeding concentrations of 1000 μg/kg), and 21 AWIs in earthworms from Site 3 (five compounds exceeding concentrations of 1000 μg/kg). A number of compounds that were present in the earthworm tissue were at concentrations less than reporting levels in the corresponding soil samples. The AWIs detected in earthworm tissue from the three field sites included pharmaceuticals, synthetic fragrances, detergent metabolites, polycyclic aromatic hydrocarbons (PAHs), biogenic sterols, disinfectants, and pesticides, reflecting a wide range of physicochemical properties. For those contaminants detected in earthworm tissue and soil, bioaccumulation factors (BAF) ranged from 0.05 (galaxolide) to 27 (triclosan). This study documents that when AWIs are present in source materials that are land applied, such as biosolids and swine manure, AWIs can be transferred to earthworms.

Introduction

Municipal wastewater treatment produces solid byproducts, commonly referred to as sewage sludge. After additional treatment to meet regulatory standards for pathogen, nutrient, and metal content, this treated sewage sludge, now classified as biosolids, may be disposed of by land application. This organic carbon- and nutrient-rich material may be beneficially used as a fertilizer or in land-restoration projects (*1*). The daily per capita volume of wastewater produced in the United States is about 450 L, which contains about 240 mg/L suspended solids (*2, 3*). In 2006, the U.S. Environmental Protection Agency estimated that more than 8×10^6 dry tons of biosolids per year are produced in the United States (*4*); about 50% of which are land applied, with the remainder incinerated or disposed of in landfills (*1*). In Europe an estimated 37% of biosolids are land applied for a total 2.39 $\times$ 10^6 dry tons per year (*5*). Biosolids are predominantly applied on agricultural soil, but are also used on large-scale landscaping, home landscaping and gardens, remediation of abandoned mining sites, and revegetation projects (*6–8*).

Numerous organic contaminants, including pharmaceuticals, detergent metabolites, fragrances, antimicrobials, pesticides, and industrial products have been found in wastewater discharges (*9*) and are collectively referred to herein as anthropogenic waste indicators (AWIs). Some of these AWIs are unaltered or incompletely removed in wastewater-treatment plants (WWTPs), and subsequently have been identified in the environment, especially in surface waters receiving wastewater effluent (*9–12*). Various studies have raised concerns about the potential impacts of the environmental presence of AWIs on humans and wildlife, which includes reproductive impairment and antibiotic resistance among pathogenic bacteria (*13–19*).

During municipal wastewater treatment, the waste stream is separated into two components, solids and liquid effluent. A large fraction of the total AWIs entering WWTPs ultimately may reside in the biosolids (*21*). Many AWIs have moderate to large octanol–water partitioning coefficients and therefore can be predicted to undergo hydrophobic partitioning into the organic-rich solids phase during wastewater treatment. This is consistent with recent observations of AWIs in biosolids destined for land application, including detergent metabolites, synthetic fragrances, disinfectants, and pharmaceuticals (*21–27*).

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In addition to municipal sources of human biosolids, an estimated 500 million tons of manure are generated annually from about 92 million swine, 109 million cattle, 292 million turkeys, and 7.5 billion chickens in the United States (*28*). Most of this manure is applied untreated to agricultural soil as a source of plant nutrients. Historically, environmental effects of animal manure were principally associated with nutrients (*29*). Recently, however, other constituents have garnered interest, such as veterinary medicines (*30–32*), naturally excreted hormones (*33–35*), and pathogens (*36, 37*). To fully consider the sources of AWIs to terrestrial environments we have included analysis of an agricultural field site amended with swine manure from a confined animal feeding operation, where use of select AWIs is expected.

Although some aspects of the environmental presence of AWIs have been addressed in recent reports (*16, 38–41*), the range of sources and loadings to terrestrial and aquatic environments, exposure of humans and other organisms, and the effects of exposure are only beginning to be identified and understood. Assessing the transfer of AWIs from biosolids and manure to biota is one means of determining the potential for ecosystem health effects associated with the land application of these materials. Research has documented uptake of various AWIs by plants and animals (*42–45*), including humans (*46, 47*). Most research investigating the effects or bioaccumulation of AWIs has focused on aquatic environments and organisms (*38, 48, 49*). Far less is known about the movement of these compounds into terrestrial organisms.

The study described in this paper addresses the potential transfer of a diverse array of AWIs from land-applied biosolids and livestock manure to earthworms in field settings. Earthworms are common primary consumers of organic matter in soil and are known to biomagnify inorganic and organic soil contaminants, including mercury, polycyclic aromatic hydrocarbons (PAHs), brominated fire retardants, and pesticides, through soil consumption or contact (*50–55*). Earthworms can comprise as much as 60–80% of total soil biomass in some locations (*56*), and thus may be ideal sentinel terrestrial organisms for identifying AWIs in the food web.

Materials and Methods

Field Sites. For this project, three agricultural fields separated by >160 km in the Midwestern United States were selected (Table S1 in the Supporting Information). Site 1 (minimally affected site) was a nonirrigated soybean field that had not been amended with either human or livestock waste for at least the previous seven years. Soil and earthworm samples were collected from this field on June 6 and September 29, 2005. Site 2 (biosolid amended site) was a no-till, nonirrigated soybean field receiving biosolid as a fertilizer for the first time. The biosolids were from a local WWTP that processed, on average, 10.52 million gallons per day of wastewater influent from residential, university, hospital and medical facility, industrial, and landfill leachate sources. Sludge from this WWTP is processed through three anaerobic digestion steps at 130, 95, and 95 °C, respectively. The sludge is pressed to decrease water content and stored on an outdoor pad for 3−6 months prior to land application. The biosolid applied at this field site was stored for about 6 months prior to application to Site 2 on April 18, 2005, at a rate of 1.8 Mg/1000 m^2. Soil and earthworm samples were collected from Site 2 on May 19 (31 days postapplication) and September 21 (156 days postapplication), 2005. Site 3 was a nonirrigated cornfield receiving liquid swine manure (from a 5000-animal facility) as an organic fertilizer (manure-amended site). Swine manure was applied to this field on May 1, 2005, at a rate of 3300 L/1000 m^2. The field was tilled the day after application, and then planted with corn. Soil and earthworm samples were collected from Site 3 on May 31 (30 days postapplication) and September 15 (139 days postapplication), 2005.

Field Sampling. At each field site, earthworms were removed from standard-sized holes in a manner similar to that described by Salogovic et al. (*57*). A 40-cm diameter circle of soil was removed to a depth of about 25 cm using a precleaned metal-blade spade and placed on a clean tarp (one tarp used at each field site). The soil was carefully sorted (while wearing nitrile gloves) to remove all earthworms observed. Undamaged worms were placed in a shipping container with airholes and loosely packed native soil. The samples were shipped to the laboratory in an ice-filled cooler within 24 h of collection. In the laboratory, the earthworms were cleaned using cool deionized (DI) water and allowed to depurate on wet filter paper for 24 h (*40, 58*) to ensure that AWIs detected in the worms originated from tissue and not ingested soil, and to avoid overestimating AWI content and BAFs. After depuration, the worms were cleaned with cool DI water and frozen for later extraction and analysis.

Once the earthworms were removed from the soil from a particular hole, the soil was homogenized by hand. Subsamples of soil from each hole were placed into a glass bowl, thoroughly mixed, split into baked-glass jars, and shipped to the laboratory with the earthworm samples for soil texture, soil organic carbon, and AWI content analyses.

Samples of the applied biosolids and swine manure were collected at the time of field application and frozen for later AWI analysis. This procedure included biosolid samples collected from the drying pad at the WWTP, and swine manure collected directly from a valve in the pipe used to transfer the manure from the earthen basin (which was aerated during this time to ensure complete mixing of the liquid waste) into a tank truck for application as a slurry to the agricultural soil.

Earthworm, Soil, and Source Material Extraction and Instrumental Analysis. Earthworm, soil, and source samples were prepared in triplicate for AWI quantification. Two different extraction, cleanup, and quantification methods were required to encompass the range of compounds determined in this study. Both methods are based on previously published accelerated solvent extraction (ASE; Dionex-200, Dionex Corp., Sunnyvale, CA) methods developed for AWI determination in soil and sediment samples (*59, 60*).

The pharmaceuticals included in this study were quantified by externally calibrated, high-performance liquid chromatography (HPLC) coupled with electrospray ionization/quadrupole mass spectrometry in the positive ion mode (Hewlett-Packard/Agilent model series 1100 LC/MSD, Palo Alto, CA), similar to methods described by Cahill et al. (*61*). The remaining nonpolar AWIs were quantified using external calibration on an Agilent Technologies model 5973 gas chromatograph/mass spectrometer (*60*), by using electron-impact ionization (70 electron volts) in full-scan mode [from 45 to 550 mass/charge ratio (m/z)]. A detailed description of the extraction, quantification, and quality control methods employed is located in the Supporting Information.

Results and Discussion

Field-Applied Waste. For ease of comparison and discussion the AWIs included in this study have been grouped into 4 general categories: pharmaceuticals, personal care products, biogenic sterols, and others (e.g., PAHs and Alkyl-PAHs, wood preservative, skatol). Table 1 can be used to identify which compounds comprise each of these groups. Numerous AWIs were detected in the land-applied biosolid and swine manure. Twenty-eight of the 77 AWIs measured were detected in the biosolid (Table 1), including 3 pharmaceuticals, 10 personal-care products, 4 biogenic sterols, and 11 other AWIs

TABLE 1. Concentrations of AWIs Detected in Source, Soil, or Earthworm Samples[a]

anthropogenic waste indicators	common use/source	source	source	Site 1 (minimally affected)				Site 2 (biosolid amended)				Site 3 (manure amended)			
		biosolid	swine manure	soil 6/6/05	worm 6/6/05	soil 9/29/05	worm 9/29/05	soil 5/19/05	worm 5/19/05	soil 9/21/05	worm 9/21/05	soil 5/31/05	worm 5/31/05	soil 9/15/05	worm 9/15/05
caffeine	stimulant (PH)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
carbamazepine	antiepileptic (PH)	390	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
thiabendazole	anthelmintic/pesticide (PH)	5 000	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
trimethoprim	antibiotic (PH)	ND	ND	0.64[b]	ND	ND	ND	ND	127	ND	ND	ND	61	ND	ND
diphenhydramine	antihistamine (PH)	7 000	ND	ND	ND	ND	ND	1.1[b]	ND	ND	ND	ND	ND	ND	ND
d-limonene	fragrance (PCP)	1 600	1 050	393	ND	ND	41	ND	ND	40	48	ND	ND	ND	63
galaxolide (HHCB)	fragrance (PCP)	427 000	ND	633	61	ND	ND	1 050	3 340	2 770	131	ND	ND	ND	49
tonalide (AHTN)	fragrance (PCP)	177 000	ND	113	19	ND	ND	287	279	773	75	ND	ND	ND	32
acetophenone	fragrance (PCP)	3 450	1 380	627	150	ND	137	ND	110	ND	101	147	ND	ND	222
indole	fragrance (PCP)	6 800	21 500	ND	2 320	673	1 480	285	1 950	540	1 300	410	3 130	673	1 750
isoborneol	fragrance (PCP)	ND	ND	267	ND	ND	NA	ND	ND	ND	NA	ND	ND	ND	NA
camphor	flavor and odorant (PCP)	ND	ND	ND	NA	ND	20[b]	ND	NA	ND	26[b]	ND	NA	ND	16[b]
isoquinoline	fragrance and flavor (PCP)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	15[b]	ND	ND	ND
menthol	cigarettes, cough drops, and mouthwash (PCP)	ND	ND	177	31[b]	ND	26[b]	ND	ND	ND	ND	ND	ND	ND	ND
4-tert-octylphenol	detergent metabolite (PCP)	ND	ND	ND	ND	ND	ND	ND	570	ND	186	ND	ND	ND	ND
para-nonylphenol-total	detergent metabolite (PCP)	483 000	ND	ND	ND	ND	ND	ND	5 200	3 570	7 690	ND	ND	ND	ND
nonylphenol monoethoxy-total	detergent metabolite (PCP)	25 300	ND	ND	ND	ND	ND	ND	ND	ND	1 520	700	ND	ND	ND
nonylphenol diethoxy-total	detergent metabolite (PCP)	760	120 000	ND	ND	ND	ND	ND	ND	ND	ND	1 230	ND	ND	ND
4-cumylphenol	detergent metabolite (PCP)	ND	ND	37	ND	ND	ND	ND	140	ND	ND	ND	ND	ND	ND
octylphenol, monoethoxy	detergent metabolite (PCP)	5 030	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
octylphenol, diethoxy	detergent metabolite (PCP)	ND	ND	ND	ND	42	ND	74	ND	46	ND	143	ND	42	ND
phenol	disinfectant (PCP)	6 270	552 000	5 970	NA	573	343	ND	NA	237	406	1 280	NA	573	751
triclosan	disinfectant (PCP)	10 500	ND	833	ND	ND	ND	160	1 740	96	2 610	69	ND	ND	ND
3-beta-coprostanol	biogenic sterol	467 000	1 570 000	ND	ND	ND	ND	1 910	ND	1 360	ND	1 570	1 830	ND	986
cholesterol	biogenic sterol	66 700	387 000	18 900	253 000	887	17 600	7 700	166 000	2 270	19 100	9 030	153 000	887	15 400
beta-sitosterol	biogenic sterol	177 000	659 000	24 000	11 600	3 730	4 770	4 570	7 030	3 530	4 360	4 200	8 430	3 730	5 110
stigmastanol	biogenic sterol	77 700	644 000	4 900	ND	1 090	483	1 300	ND	927	501	2 470	ND	1 090	519
naphthalene	PAH	610	ND	ND	ND	ND	15[b]	ND	ND	ND	22[b]	ND	ND	ND	ND
phenanthrene	PAH	1 730	ND	ND	ND	ND	ND	ND	ND	6[b]	ND	ND	ND	ND	ND
anthracene	PAH	320	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
fluoranthene	PAH	950	ND	ND	ND	23[b]	ND	ND	ND	22[b]	ND	9[b]	ND	23[b]	8[b]
pyrene	PAH	740	ND	ND	ND	ND	ND	ND	ND	ND	ND	9[b]	ND	ND	10[b]
benzo[*a*]pyrene	PAH	ND	ND	ND	ND	ND	ND	ND	ND	10[b]	ND	21[b]	ND	ND	ND
2-methylnapthalene	alkyl-PAH	ND	ND	ND	19[b]	ND	11[b]	ND	ND	ND	11[b]	ND	14[b]	ND	13[b]
1-methylnapthalene	alkyl-PAH	ND	ND	ND	19[b]	ND	7[b]	ND	ND	ND	8[b]	ND	12[b]	ND	9[b]
2,6-dimethyl-napthalene	alkyl-PAH	915	ND	ND	ND	ND	3[b]	ND	ND	ND	6[b]	ND	ND	ND	3[b]
bisphenol A	fireretardant and plasticizer	4 600	ND	147	ND	ND	ND	ND	ND	81	ND	ND	ND	ND	ND
tributylphosphate	antifoaming agent and flame retardant	ND	ND	2 130	200	1 100	169	523	250	3 570	196	393	213	1 100	182
diethylhexyl phthalate	plasticizer	3 330	727	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
para-cresol	wood preservative	4 970	30 000	2 200	125[b]	113[b]	ND	ND	270[b]	ND	70[b]	ND	1 290	113[b]	ND
diazinon	pesticide	ND	ND	ND	99	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
metolachlor	pesticide	ND	ND	320	ND	ND	ND	ND	720	ND	ND	ND	ND	ND	ND
skatol	fecal indicator	5 170	513 000	ND	260	113	154	143	230	74	156	150	106	113	240
benzophenone	fixative	ND	ND	ND	28[b]	ND	10[b]	ND	ND	ND	11[b]	ND	38	ND	8[b]
isophorone	industrial solvent	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	137	ND	ND	ND
	total AWI content (mg/kg)	1 971	4 501	61	268	8.3	28	18	189	20	43	22	170	8.4	25

[a] Average from 3 replicate composite samples, individual compound concentrations in μg/kg dry wt. AWI = anthropogenic waste indicator. ND = not detected. PAH = polycyclic aromatic hydrocarbon. PH = pharmaceutical. PCP = personal care product. [b] Value is below the statistically calculated method detection limit based on previously published work (Table S2; refs (*59*) and (*60*)).

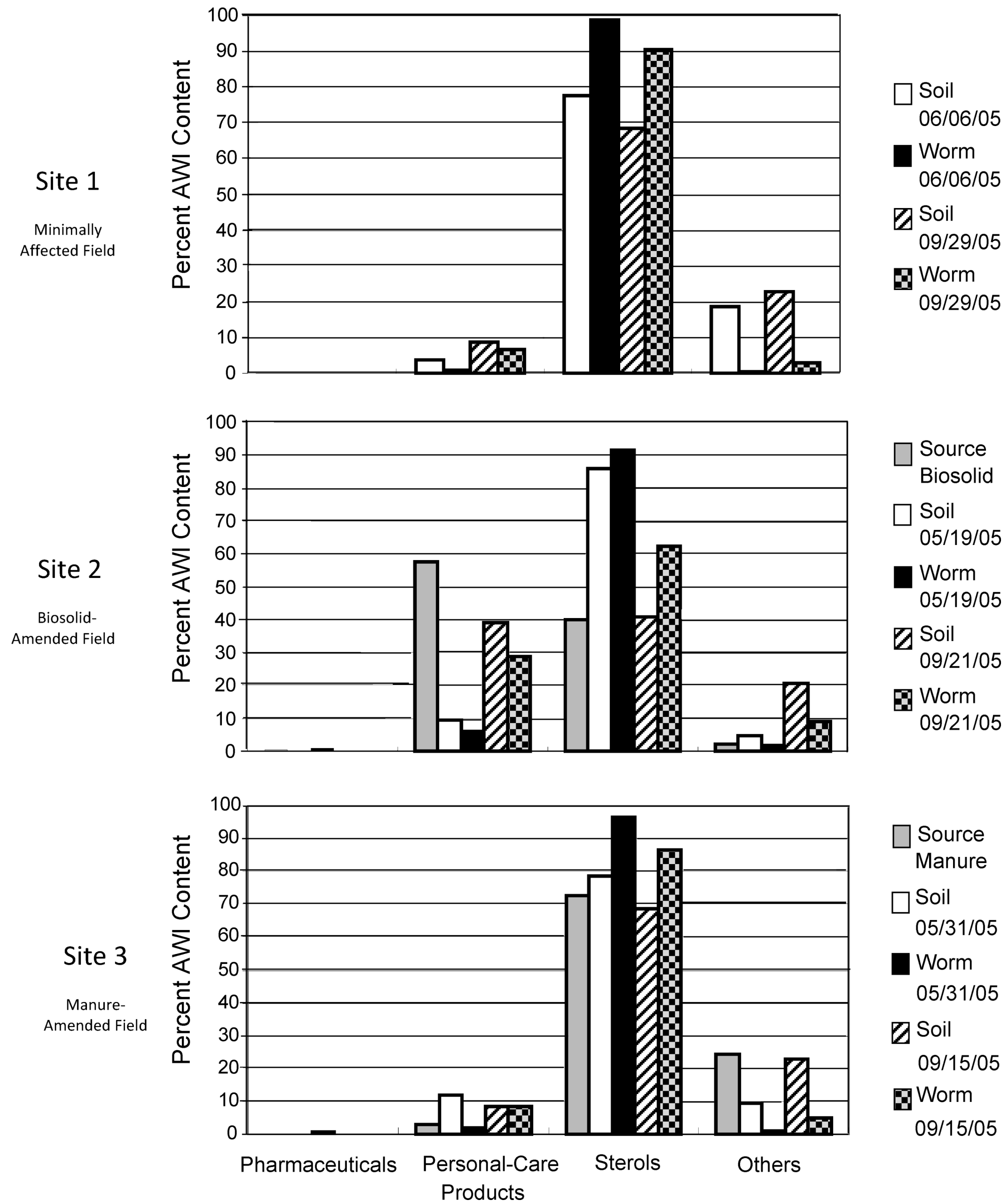

FIGURE 1. Relative contribution of pharmaceuticals, fragrances, biogenic sterols, and other anthropogentic waste indicators (AWIs) to the overall AWI composition of source, soil, and earthworm samples.

(Figure 1); the concentrations of 21 compounds exceeded 1000 μg/kg and 9 compounds exceeded 10 000 μg/kg. The highest concentrations were observed for biogenic sterols, fragrances (galaxolide and tonalide), detergent metabolites (mono- and diethoxy nonylphenol), and triclosan (Table 1). The concentrations of many of the AWIs detected in the biosolid applied at Site 2 were greater than the mean concentrations of the same AWIs reported in a recent survey of biosolids (*27*).

Twelve AWIs were detected in the swine manure (Table 1), including 4 personal-care products, 4 biogenic sterols, and 4 other AWIs (Figure 1). Concentrations of 11 compounds exceeded 1000 μg/kg and nine exceeded 10 000 μg/kg; the highest AWI concentrations were of biogenic sterols (Figure 1). Although fewer AWIs were detected in the swine manure, the total AWI concentration (about 4500 mg/kg) in the manure was greater than that in the biosolid (about 1970 mg/kg). This result is primarily due to the high concentrations of biogenic sterols (Table 1) that comprised over 70% of the total AWI concentration in the swine manure (Figure 1) and may reflect the untreated nature of the source swine manure compared to the biosolid. The biosolid contained a broader range of compounds, particularly those indicative of human use (Figure 1). Some AWIs were present at much higher concentrations in the source swine manure compared to the source biosolid, including nonylphenol-diethoxy detergent metabolites, para-cresol, and phenol (Table 2). This suggests that land-applied manures should be considered as potential sources of some AWIs to terrestrial environments.

Amended Soils. Unexpectedly, 21 AWIs were detected in the soil samples from Site 1 (Table 1). Biogenic sterols comprised about 70–80% of the total AWI concentrations in these soil samples (Figure 1). Biogenic sterols are natural components of fecal materials and may have originated from indigenous terrestrial wildlife and/or soil fauna. Other AWIs detected at Site 1 (e.g., galaxolide, tonalide, and triclosan) are commonly found in personal-care products and detected in the environment (*9, 25*). Human-use AWIs detected at Site 1 may have originated from a septic system near the boundary of the field, or from fields up gradient from Site 1, but this could not be confirmed. The detections of AWIs at Site 1 documents the difficulty in identifying a true control site given the ubiquitous nature of many AWIs; thus

TABLE 2. Chemical Properties and Bioaccumulation Factors (BAF)[a] for AWIs Detected in Soils or Earthworms

anthropogenic waste indicators	log K_{ow}[b]	water solubility (mg/L)[b]	vapor pressure (mm Hg)[b]	Site 1 minimally affected BAF 6/6/05[c]	Site 1 minimally affected BAF 9/29/05[c]	Site 2 biosolid amended BAF 5/19/05[c]	Site 2 biosolid amended BAF 9/21/05[c]	Site 3 manure amended BAF 5/31/05[c]	Site 3 manure amended BAF 9/15/05[c]
caffeine	−0.07	2.16×10^{4}	7.33×10^{-9}	–	–	NC	–	NC	–
trimethoprim	0.91	400	9.88×10^{-9}	0	–	NC	–	NC	–
d-limonene	4.57	13.8	1.98	0	NC	–	1.2	–	NC
galaxolide (HHCB)	5.70	1.25	5.12×10^{-4}	0.10	–	3.1	0.05	–	NC
tonalide (AHTN)	5.90	1.75	5.45×10^{-4}	0.17	–	1.0	0.1	–	NC
acetophenone	1.59	6.13×10^{3}	0.397	0.24	NC	NC	NC	0	NC
indole	2.14	3.560×10^{3}	0.0122	NC	2.19	6.8	2.4	7.6	2.6
camphor	2.38	1.600×10^{3}	0.65	–	NC	–	NC	–	NC
menthol	3.4	456	0.11	0.17	NC	–	–	–	–
4-tert-octylphenol	5.28	5	4.78×10^{-4}	–	–	NC	NC	–	–
para-nonylphenol-total	5.92	5×10^{3}	9.42×10^{-5}	–	–	NC	2.1	–	–
nonylphenol monoethoxy-total	4.17[d]	NA	NA	–	–	–	NC	0	–
4-cumylphenol	4.12	43.3	5.94×10^{-5}	0	–	NC	–	–	–
tributylphosphate	4	280	1.20×10^{-4}	0.09	0.15	0.5	0.06	0.54	0.16
3-beta-coprostanol	8.82	2.03×10^{-4}	5.47×10^{-10}	–	–	0	0	1.2	NC
cholesterol	8.74	0.095	7.79×10^{-10}	13	20	21.4	8.3	17	17
beta-sitosterol	9.65	1.3×10^{-5}	3.77×10^{-10}	0.48	1.3	1.5	1.2	2.0	1.4
stigmastanol	NA	NA	NA	0	0.44	0	0.5	0	0.47
naphthalene	3.30	31	0.85	–	NC	–	NC	–	–
2-methylnapthalene	3.86	24.6	0.055	NC	NC	–	NC	NC	NC
1-methylnapthalene	3.87	25.8	0.067	NC	NC	–	NC	NC	NC
2,6-dimethylnapthalene	4.31	2	4.3×10^{-3}	–	NC	–	NC	–	NC
phenol	1.50	8.28×10^{4}	0.35	–	0.59	–	1.7	–	1.3
triclosan	4.53	10	6.45×10^{-7}	0	–	10.8	27	0	–
diethyl phthalate	7.88	0.27	1.42×10^{-7}	NC	NC	1.4	NC	NC	NC
para-cresol	1.97	2.15×10^{4}	0.11	0.06	0	NC	NC	NC	–
diazinon	3.81	40	9.01×10^{-5}	NC	–	–	–	–	–
metolachlor	3.13	530	3.14×10^{-5}	0	–	NC	–	–	–
skatol	2.60	498	5.55×10^{-3}	NC	1.3	1.6	2.1	0.70	2.1
benzophenone	3.18	137	1.93×10^{-3}	NC	NC	–	NC	NC	NC

[a] NA = Not Available. NC = "Not calculated", analyte was detected in earthworm tissue; it was below detection in the soil. – = Not present in soil or earthworm tissue. [b] Physicochemical properties are from the following online database: http://www.syrres.com/esc/physdemo.htm (last viewed January 2008). [c] Sample collection date. [d] log K_{ow} value from Ahel and Giger (*72*).

"minimally affected" was chosen as the most appropriate descriptor for this site.

Nineteen of the AWIs studied were detected in the biosolid-amended soil at Site 2. Unlike the other two sites, human-use compounds, such as the synthetic fragrances and other personal-care products (Table 1, Figure 1), comprised a higher percentage of the total concentration of AWIs detected. Notably, many of the nonylphenol and octylphenol detergent metabolites and disinfectants detected at relatively high concentrations in the biosolid were not detected in the corresponding biosolid-amended soil samples (Table 1).

The soil at Site 3 contained detectable concentrations of 19 of the AWIs (Table 1). Biogenic sterols constituted about 86% of the total AWIs measured in the soil (Table 1, Figure 1), which is proportionally similar to the source material. With the exception of d-limonene, para-cresol, and diethylhexyl phthalate, AWIs detected in the source swine manure also were detected in the corresponding soil samples from Site 3 (Table 1, Site 3, 5/31/05).

There were differences in the detected AWIs measured in the temporally separated soil samples in this study, such as the presence of triclosan in the soil from Site 3 collected on 5/31/05 and absence of triclosan in the soil collected on 9/15/05 from this same site. These observations likely reflect degradation, volatilization, or leaching occurring between application and sampling dates, or interaction between these compounds and the surrounding soils (*39, 62*). This field study, however, was not designed to distinguish such factors. Attempts to collect the fall soil and earthworm samples immediately adjacent to collection points of the spring samples were not made. Thus, natural soil heterogeneity and the inherent heterogeneity of the waste products and their application likely contribute to the variations observed in chemical concentrations between the two sampling times.

Earthworms. Although unanticipated, 20 AWIs were detected in earthworms from Site 1, including seven personal-care products, three biogenic sterols, and 10 other AWIs (Table 1). Over 90% of the total AWI concentrations measured in the earthworms were biogenic sterols (Figure 1). Indole, cholesterol, and beta-sitosterol were the only AWIs measured in earthworm tissue at concentrations exceeding 1000 μg/kg at Site 1 (Table 1).

Earthworms collected from Site 2 had detectable concentrations of 25 AWIs including one pharmaceutical, 11 personal-care products, three biogenic steroids, and 10 other AWIs (Table 1). Although biogenic sterols were generally detected at the highest concentrations in the earthworm samples from Site 2, the relative contribution of other AWIs, especially personal-care products, is noticeably larger than at the other two field sites (Figure 1). Seven compounds in these tissue samples exceeded 1000 μg/kg (Table 1). Individual AWIs ranged in concentration from as little as 6 μg/kg of 2,6-dimethyl-napthalene to 2610 μg/kg of triclosan to 166 000 μg/kg of cholesterol.

Twenty-one AWIs were detected in earthworms collected from Site 3. The compounds detected included one pharmaceutical, six personal-care products, three sterols, and 10 other AWIs (Table 1). The AWI profile was dominated by the presence of biogenic sterols, comprising over 85% of the total measured concentration (Figure 1). Six compounds had tissue concentrations exceeding 1000 μg/kg in earthworm samples from Site 3. The biogenic sterols beta-sitosterol (153 000 μg/kg) and cholesterol (8430 μg/kg), and the para-cresol (1290 μg/kg) were among the AWIs detected at the highest concentrations in earthworms from Site 3.

When possible, bioaccumulation factors (BAFs, ratio of the contaminant concentration in earthworm tissue to the contaminant concentration in soil) were calculated for the AWIs in earthworm tissue (Table 2). The BAFs ranged from 0.05 (galaxolide from Site 2) to 27 (triclosan from Site 2). The log K_{ow} and water solubility of AWIs detected in earthworm tissue ranged from −0.07 to 9.65 and 1.3×10^{-3} to 8.28×10^{4} mg/L, respectively, reflecting the diverse physicochemical nature of the AWIs accumulating in the earthworms. Physicochemical properties, such as log K_{ow} and water solubility, are not significant indicators of BAFs based on Spearman's Rank Correlation, $P > 0.35$ and $P > 0.54$, respectively.

A majority of the 28 AWIs detected in the earthworm samples were below detectable concentrations in the corresponding soil samples (Table 1). This phenomenon suggests these AWIs may be more persistent in earthworm tissue compared to soil, and that by bioaccumulating these organic contaminants, earthworms act as integrating samplers, effectively improving the overall detection of AWIs in soil environments. In such instances, a BAF value could not be calculated, which is represented by "NC" in Table 2.

The general profile of AWI content in the earthworms mirrored that of the soil from which they were collected (Figure 1). There was little consistency in the BAFs for compounds detected in earthworms in both the first and second samples from each site (Table 2). The mean difference between the BAFs is almost ±75%. We hypothesize that these observations reflect natural soil heterogeneity, spatial variability in the sample locations, changing bioavailability of AWIs, or detoxification by elimination in the earthworms, all of which may be influenced by differences in soil texture, organic carbon content, or earthworm species (*40, 63–65*). In addition, some of the reported values for individual AWIs in earthworm and corresponding soil samples were at, or slightly below, the statistically calculated method detection limits (Tables 1 and S2; refs (*59*) and (*60*)), which could contribute to the uncertainty inherent in the calculation of BAFs.

One unanswered question regarding the practice of land application of biosolids is whether such practices affect human or ecological health. Earthworms occupy a low trophic position in the food web and can facilitate the movement of organic soil contaminants into higher trophic levels by consuming soil particles (*40, 53, 66*). Earthworms are known to be consumed by many bird species, representing up to 90% by weight of the diet of some species (*67*). Species of mammals, reptiles, amphibians, fish, and other invertebrates feed upon earthworms (*68*). The presence of earthworms in the biosolid- and manure-amended soil suggests these materials and AWIs they contain are not immediately toxic to earthworms. However, it might be appropriate for future research to consider chronic effects of these substances on earthworm behavior, growth, and reproduction that might indirectly affect soil fertility or modify the quality of earthworms as a food source (*40, 68*).

The results presented here demonstrate that organic contaminants, many of which are distinctly anthropogenic, can be transferred from source materials, such as biosolids, to soil-dwelling earthworms. While many researchers have reported the bioaccumulation of specific organic contaminants in a variety of earthworm species, particularly in laboratory controlled experiments (*40, 50, 54, 63, 69*), these results demonstrate that earthworms in common agricultural soil environments can accumulate a wide range of chemically diverse organic contaminants originating from biosolids or manure applied to terrestrial ecosystems. Given that application of municipal biosolids and manure is common practice worldwide (*1, 5*), the transfer of AWIs into the food web via earthworms is globally relevant. The most abundant AWIs in the samples analyzed were the biogenic sterols, which have no known ecological or human health threat. Although this study was not designed to consider potential impacts on human health or directly evaluate the effects that exposure and bioaccumulation of AWIs might have on earthworm health, some of the AWIs detected in earthworm tissue are known or suspected endocrine-disrupting compounds, including the nonylphenol detergent metabolites and benzophenone (*70*). In addition, the synthetic fragrances galaxolide and tonalide, which have been observed to accumulate in human tissue, are suspected to result in liver disorders (*71*). Based on these findings, future consideration of AWI bioaccumulation and exposure on earthworms is warranted. Further field and laboratory experiments are necessary to clarify the exact mechanisms of bioaccumulation of AWIs; however, it is evident that AWIs in biosolids and livestock manure are actively mobilized from the solid phase to earthworms. This finding suggests that through predation of earthworms, these compounds could be further dispersed beyond the point of application in terrestrial ecosystems.

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Supporting Information Available

Details pertaining to the methods utilized and Tables S1 and S2. This material is available free of charge via the Internet at http://pubs.acs.org.

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