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Human Pharmaceuticals, Hormones, and Personal Care Product Ingredients in Runoff from Agricultural Fields Irrigated with Treated Wastewater

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Irrigation of crops with treated wastewater has the potential to introduce effluent-derived organic microcontaminants into surface waters through agricultural runoff. To determine whether compounds indicative of the presence of treated effluent in irrigation water could be identified in agricultural runoff, surface runoff samples collected from effluent-irrigated and rain-fed cultivated fields were analyzed for a broad spectrum of organic compounds. A variety of compounds was identified that appeared to be associated with irrigation with treated wastewater. These compounds included human pharmaceuticals (e.g., carbamazepine, gemfibrozil, carisoprodol), personal care product ingredients (e.g., insect repellent, polycyclic musks), and alkyl phosphate flame retardant chemicals. Most of these compounds appear not to have been previously reported in agricultural runoff. These compounds were present at concentrations below the few published aquatic toxicology data available; however, their potential to elicit more subtle effects in aquatic organisms cannot be excluded. None of these compounds were detected by broad-spectrum analysis in samples from the same fields during runoff-producing rain events.

KEYWORDS: Pharmaceuticals; runoff; personal care product ingredients; hormones; flame retardant chemicals; irrigation; nonpoint source pollution

INTRODUCTION

Wastewater treatment plant (WWTP) effluent is being used increasingly to irrigate crops and public areas in arid regions of the United States and other countries. The obvious benefit of this practice is the reduced demand placed on water supplies. The potential exists, however, for contaminants present in treated wastewater to leach into groundwater supplies used as drinking water sources or to enter aquatic ecosystems through irrigation runoff. The few studies examining the long-term effects of effluent irrigation on groundwater have focused primarily on dissolved salts, nutrients, and heavy metals (*1*, *2*). However, concerns about effluent-derived organic microcontaminants entering aquifers used as drinking water sources are increasing (*2*). Indeed, Drewes et al. (*3*) and Heberer et al. (*4*) reported that soil-aquifer treatment and bank filtration did not completely remove the drugs carbamazepine and primidone. Less attention has been focused on the introduction into aquatic ecosystems of effluent-derived organic xenobiotics in runoff from fields irrigated with treated wastewater.

At present, the most stringent nonpotable water reuse regulations in the United States are the California Water Recycling Criteria (*5*). For irrigation of food crops, a minimum of secondary treatment is required with the intent of providing farm workers with some level of protection from exposure to wastewater-borne pathogens (*5*). For use on nonprocessed food crops, wastewater must be disinfected after secondary treatment. A higher level of treatment is required when reclaimed water comes in contact with the edible portion of food crops in order to minimize pathogen exposure to people consuming raw crops that were irrigated with effluent. The required treatment train for such use consists of secondary treatment followed by filtration and disinfection; coagulation and filter aids are used when required to meet product water standards (*5*). Effluent treated in this manner is referred to as "disinfected tertiary recycled water". However, effluent discharged to streams from which water is withdrawn for irrigation purposes needs to undergo only secondary treatment even if treated wastewater comprises the bulk of streamflow.

A large number of potentially toxicologically relevant organic microcontaminants have been identified in treated wastewater and in streams receiving treated effluent (e.g., refs *6* and *7*). Such contaminants may be present in runoff from effluent-irrigated fields. Currently, environmental monitoring programs analyze for targeted sets of organic chemicals (e.g., by U.S. EPA methods 624 and 625). This practice necessarily excludes a large number of potentially toxic "nontarget" chemicals. Classes of nontarget compounds identified or potentially present

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Table 1. Site Characteristics

site	nominal area (ha)	crop	irrigation water source	duration of runoff event(s) sampled (min)	approximate runoff volume (m^3)
A	9.9	strawberries	tertiary treated wastewater[a]	316	105
B	18.7	green onions	tertiary treated wastewater[a]	465, 480[b]	317, 273[b]
C	32.1	corn	effluent-dominated streamwater	350, 360[b]	103, 6528[b]
D	4.7	cilantro	effluent-dominated streamwater	457	88
E	2.4	bell peppers	tertiary treated wastewater[a]	355	571
F	7.4	celery	variable[c]	185	81

[a] Effluent piped directly to fields. [b] During rainfall event. [c] Irrigation source varied between groundwater and effluent-dominated streamwater.

in treated wastewater include human pharmaceuticals, personal care product ingredients (PCPIs), nutraceuticals/herbal remedies, flame retardant chemicals, plasticizers, and disinfection byproducts (*6*−*8*). The environmental fate and ecological effects of most of these compounds are poorly understood.

The objective of this study was to determine whether irrigation of row and vegetable crops with disinfected tertiary recycled water or effluent-dominated streamwater resulted in the introduction of human pharmaceuticals, hormones, and PCPIs to receiving waters. In this paper, we use the term effluent irrigation to refer to the direct application of treated wastewater to agricultural fields, as well as to the practice of irrigating with effluent-dominated streamwater, which can be regarded as indirect effluent irrigation. Effluent-dominated streams have flows composed primarily of treated wastewater. Surface runoff samples collected from effluent-irrigated fields in southern California were screened for human pharmaceuticals, hormones, and PCPIs using a broad-spectrum gas chromatography−mass spectrometry (GC-MS) approach (*8*). When compounds of interest were detected, a quantitative target group analysis (*9*) for these compounds was conducted to quantify specific analytes.

MATERIALS AND METHODS

Site Description. The fields investigated in this study were located in the Calleguas Creek watershed in coastal Ventura County in southern California. Crops grown in the watershed included avocados, beans, celery, cilantro, cole crops, cucurbits, lemons, lettuce, onions, peppers, spinach, strawberries, sweet corn, and tomatoes. Six irrigated fields bordering the stream system and planted in a variety of row and vegetable crops were sampled during both dry and wet weather over the course of two growing seasons (July 1999−April 2000) (**Table 1**). Sampled sections of fields ranged in size from 1 to 19 ha and drained to discrete outlets. Row and vegetable crops were irrigated by sprinkler and/or furrow techniques throughout the dry season, as well as between storms in the rainy season. Irrigation water was drawn from shallow aquifers, pumped from the stream system or piped directly from wastewater treatment plants. Historically, streams in the watershed were intermittent, fed by springs and surface runoff; at present, 25−89% of dry weather flow is composed of secondary and tertiary treated effluent in most of the stream system (*10*). A growing trend exists in Ventura County to use treated wastewater for irrigation rather than discharging effluent into the stream systems (Henry Graumlich, Camrosa Water District, personal communication, 2001). The effluent conveyed directly to row and vegetable crops is disinfected tertiary recycled water. The treatment train employed included activated sludge treatment with extended aeration, sedimentation, nitrification/denitrification, sand filtration, and chlorination. The purpose of the extended aeration is to enhance the reduction of biological oxygen demand (BOD_5). Effluent discharged to the streams and subsequently used for irrigation received primary and secondary treatment (including nitrification/denitrification) followed by multimedia filtration (anthracite, sand, and gravel), chlorination, and dechlorination. The WWTP providing the bulk of the flow for the stream used to irrigate sites C and D received discharge from one hospital. Neither domestic animal manure fertilizers nor sewage sludge-derived soil amendments were used on the fields sampled. Seabird and bat guano find some use in the watershed and so may have been applied to the fields sampled.

Sample Collection. Two-liter water samples were collected at approximately half-hour intervals over the course of selected runoff-producing irrigation and storm events using an Isco 3700 portable sampler or by taking grab samples. Samples (Teledyne Isco, Inc., Lincoln, NE) were stored on ice until transported to the laboratory, where they were refrigerated at 4 °C until processed.

Sample Preparation. Runoff samples were filtered through 0.7-μm glass fiber filters (Whatman, Maidstone, U.K.) in a Teflon-lined hazardous waste filtration unit (Millipore Corp., Bedford, MA). The filters had been precleaned by the manufacturer; we further treated the filters by heating at 150−175 °C overnight to remove volatiles and dry before weighing (*11*). Dissolved and particle-associated compounds were thus operationally defined as those passing through or retained by the 0.7-μm filter. Filtrates (pH 7) were liquid−liquid extracted with dichloromethane (DCM) following U.S. EPA method 3510C using acenaphthene-d_{10} and tributyl phosphate as surrogate standards (*12*). Liquid−liquid extraction (LLE) was performed at pH 7 because samples were also analyzed for organophosphorus insecticides (*13*). LLE recoveries were evaluated for the targeted compounds. Briefly, 1 mL of a standard mixture was spiked into 1 L of 18 MΩ·cm resistivity water (DI water; Milli-Q Plus water system, Millipore Corp., Bedford, MA) to obtain final target analyte concentrations of 0.5−5 μg L^{-1}. The solution was mixed thoroughly, and the pH was adjusted to 7 using H_3PO_4 or NaOH. Spiked DI water was serially extracted with three 60-mL aliquots of DCM in a 2-L separatory funnel with a PFTE stopcock. The DCM extract was dried by passage through a hydrophobic membrane and concentrated to 1 mL using a Pyrex accelerated one-step extractor/concentrator (Corning Corp., Acton, MA). Recovery samples were extracted in triplicate.

Suspended particles were dried, and 0.03−8.3 g was extracted with supercritical CO_2 at 36.0 MPa and 120 °C with 250 μL each of water, methanol, and DCM added as polarity modifiers following a method originally developed for polycyclic aromatic hydrocarbons (*14*) and applied for extraction of organophosphorus insecticides as part of a larger study (*13*). The mass of suspended particles analyzed represented 1.4−1.8 L of runoff. Suspended particle concentrations in the runoff samples analyzed ranged from 15 to 4500 mg L^{-1}. Because the extraction scheme was not selected specifically for the analysis of human pharmaceuticals, hormones, and PCPIs, we assessed supercritical fluid extraction (SFE) efficiency using field soils spiked with the target analytes. Briefly, autoclaved soil was mixed with sodium phosphate-buffered water (pH 7.0) spiked with the target analytes to obtain initial dissolved concentrations of 10−100 μg L^{-1}. After 24 h of agitation, soil particles were separated from the water phase by filtration through a 0.7-μm glass fiber filter. The spiked solution and filtrates were extracted by LLE, dried soil solids were extracted by SFE, and extracts were analyzed by GC-MS. Theoretical sorbed concentrations were calculated from the difference between those in the spiked solution and the filtrate after correction for LLE recovery. Recovery samples were processed in triplicate. We note that SFE efficiency using freshly spiked soils may not reflect the extractability of aged residues of pharmaceuticals and PCPIs.

Instrumental Analysis. Sample extracts were analyzed by a broad-spectrum GC-MS approach (*8*; system 1) and a recently developed rapid

Table 2. Method Detection Limits and Recoveries for Water and Suspended Particle Extracts for GC-MS Analyses Using System 2[a]

	water extracts[b]			suspended particle extracts[c]		
analyte	MDL[d] (ng L^{-1})	LLE recovery (%, mean ± SD)	RSD	MDL[e] (μg kg^{-1})	SFE recovery (%, mean ± SD)	RSD
		Human Pharmaceuticals				
caffeine	294	61 ± 4	7	120	2.0 ± 0.6	31
carbamazepine	225	95 ± 6	6	80	108 ± 14	13
carisoprodol	302	75 ± 10	14	80	74 ± 7	9
diazepam	488	82 ± 11	13	200	44 ± 17	39
fenofibrate	660	73 ± 12	17	160	69 ± 6	8
gemfibrozil	131	86 ± 14	16	80	17 ± 6	37
ibuprofen	1207	27 ± 3	10	160	2.4 ± 0.3	11
		Pharmaceutical Metabolites				
p-toluenesulfonamide	592	53 ± 8	15	160	25 ± 2	7
		Steroidal Hormones				
17β-estradiol	438	67 ± 13	19	160	55 ± 14	26
estriol	780	33 ± 7	22	680	18 ± 4	24
estrone	186	87 ± 3	3	160	61 ± 22	35
17α-ethinyl estradiol	577	83 ± 5	6	160	47 ± 13	28
progesterone	203	88 ± 10	11	80	27 ± 12	44
stanolone	252	93 ± 17	18	80	45 ± 23	51
testosterone	282	71 ± 8	12	80	17 ± 4	25
		Antioxidants				
butylated hydroxyanisole	268	63 ± 9	14	80	10 ± 3	32
butylated hydroxytoluene	2380	47 ± 2	4	64	5.9 ± 0.8	13
		Plasticizers				
N-butylbenzenesulfonamide	283	80 ± 12	15	80	111 ± 3	2

[a] Abbreviations: LLE, liquid–liquid extraction; MDL, method detection limit; RSD, relative standard deviation; SFE, supercritical fluid extraction. [b] Reference *9*. [c] Reference *15*. [d] Detection limits derived from those determined for continuous LLE of 40-L matrix-free water samples (*9*) adjusted for 2-L sample volume and LLE recovery at pH 7. Matrix effects could either increase or decrease recoveries. [e] Based on a 5-g sample of spiked field sediment ($n = 3$).

GC-MS method (*9*; system 2). Detailed descriptions of the methods employed are presented in the cited papers. System 1 was used for broad-spectrum analyses to tentatively identify compounds of potential interest. System 2 was employed in both full scan and selected ion monitoring (SIM) modes for targeted analysis of seven human pharmaceuticals, one drug metabolite, seven natural and synthetic steroid hormones, two antioxidants, and a plasticizer (cf. **Table 2**). With the exception of carbamazepine and caffeine, the pharmaceuticals and two of the hormones (17β-estradiol and 17α-ethiynl estradiol) rank among the most commonly prescribed drugs in the United States (*15*). For the broad-spectrum analyses, concentrations of tentatively identified compounds were not estimated; we report only their presence. Nontarget mass spectrometry identification criteria were based on those of ref *8*. Nontarget compounds reported had library search purity (SI) values of ≥900. Library and nontarget compound mass spectra were visually compared to assess the reasonableness of structural assignments.

A subset of samples was reanalyzed by liquid chromatography−tandem mass spectrometry (LC-MS/MS) at the Southern Nevada Water Authority to confirm the presence of some target analytes (*16*). The LC-MS/MS analysis did not employ the internal and surrogate standards listed in the published method. Therefore, the concentrations reported from the LC-MS/MS analyses shouuld be considered estimates only.

RESULTS AND DISCUSSION

Table 2 summarizes analyte recoveries from matrix-free water by LLE at pH 7 and from laboratory-spiked field sediments by SFE. LLE recoveries ranged from 27 to 95% with relative standard deviations (RSD) between 3 and 22%. As expected, mean recoveries from water of acidic analytes (e.g., ibuprofen) were low at pH 7. Mean LLE recovery was highest for carbamazepine (95%) and lowest for ibuprofen (27%). Caffeine, 17β-estradiol, estriol, the antioxidants, and *p*-toluenesulfonamide displayed intermediate extraction efficiency into DCM (33−67%).

Pharmaceuticals and plasticizers with intermediate to high K_{ow} values (log $K_{ow} = 2.3-5.2$) that were neutral at pH 7 were most efficiently extracted by SFE. These compounds included *N*-butylbenzenesulfonamide (111%), carbamazepine (108%), carisoprodol (74%), and fenofibrate (69%). Recoveries of the steroidal hormones and antioxidants were less than those of neutral pharmaceuticals with comparable K_{ow} values. As was the case with LLE, acidic pharmaceuticals were not efficiently extracted (generally <20%). In general, steroidal hormones exhibited low to intermediate SFE efficiency (17−61%). For most target analytes, recovery variability as measured by RSD was higher for SFE than for LLE. The authors know of no previous attempt to extract these compounds from suspended particles with supercritical CO_2.

A number of target and nontarget human pharmaceuticals, pharmaceutical metabolites, steroidal hormones, and PCPIs were identified by GC-MS in surface runoff from fields irrigated with treated effluent and effluent-dominated streamwater (**Tables 3** and **4**). Only two prior papers have appeared indicating the presence of human pharmaceuticals and PCPIs in agricultural runoff (*8*, *17*). Concentrations reported in **Table 3** were not corrected for recovery and so represent conservative estimates, especially for the more poorly recovered compounds.

Broad-spectrum analysis proved to be useful in identifying compounds of potential relevance and in directing subsequent target analysis. In our previous work (*8*), broad-spectrum analysis (system 1) allowed the identification of several PCPIs (viz., AHTN, benzophenone, *N*,*N*-diethyltoluamide, HHCB), human pharmaceuticals and their metabolites (e.g., carisoprodol, *p*-toluenesulfonamide), and flame retardant chemicals in agricultural runoff. The PCPIs may have escaped notice had we employed the "targeted" method alone. The targeted approach employed here allowed the identification and quantitation of several additional pharmaceutically active compounds (PhACs). In this study, only 3 of the 19 target analytes were present in runoff from effluent-irrigated at concentrations exceeding their GC-MS method detection limits (MDLs). Analysis by LC-MS/MS confirmed the presence of 6 additional target analytes in

Table 3. Target Human Pharmaceuticals, Steroidal Hormones, and Antioxidants Present in Runoff from Fields Irrigated with Treated Effluent[a]

compound	use	site[c]	concentration[b] (ng L^{-1}) runoff	WWTP effluent	stream[d]
		Human Pharmaceutical Compounds			
caffeine	stimulant	E*[v]	14*	<15–9500[e–j]	<310
		[C–F]	<330		(3–6000)[e–j]
carbamazepine	antiepileptic	C, E, F	320–440	<10–6300[e,i,k–n]	0.14–0.24
		[D]			(<30–1100)[g,l,m,o]
carisoprodol	muscle relaxant	E	680	<10–63[j]	0.30
		[A, B, D, F]			(–)
fenofibrate	hypolipidemic	[all]	<730	20–160[e,l,n,o]	<1400
					(<10)[l]
gemfibrozil	hypolipidemic	C, E	190–790	<10–4760[j,l,n,o]	160–360
		[D, F]			(<10–790)[j,l]
ibuprofen	antiinflamatory	E*	11*	~2–7110[i,m–p]	<1300
		[C–F]	<1300		(<0.2–1000)[k,n]
p-toluenesulfonamide	hyperglycemic metabolite	[all]	<650	115–184[j]	<630
					(–)
		Steroidal Hormones			
17β-estradiol	natural estrogen	B*	3*	0.1–64[h,j,p–s]	<470
					(0.05–200)[h,p,q]
estrone	estradiol metabolite	E*	52*	<0.4–70[h,j,p,q]	<200
					(<0.5–1600)[j,k,q]
progesterone	natural progestin	E*	3*	<10[j]	ND
					(<0.5–199)[j,k]
testosterone	natural androgen	E*	16*	<10[j]	<300
					(<5–214)[j,k]
		Antioxidants			
butylated hydroxyanisole	antioxidant	[all]	<300	16–1000[j,v]	<290
					(<10–24)[j]
butylated hydroxytoluene	antioxidant	[A–C, E]	<2600	<8–45[f]	<2600
					(<8–100)[j,k]
		Plasticizers			
N-butylbenzenesulfonamide	plasticizer	A,C,E	350–590	351–6000[f,j,t]	<300
		[B, D, F]			(690–890)[j,t,u]

[a] Abbreviations: MDL, method detection limit; ND, not detected; WWTP, wastewater treatment plant. [b] Water phase concentration. [c] Sites for which analyte was present but at concentrations indicated by [<MDL]. [d] Data from previous studies given in parentheses. [e] Reference *4*. [f] Reference *35*. [g] Reference *36*. [h] Reference *37*. [i] Reference *38*. [j] Reference *39*. [k] Reference *7*. [l] Reference *40*. [m] Reference *41*. [n] Reference *42*. [o] Reference *3*. [p] Reference *43*. [q] References *44* and *45*. [r] Reference *46*. [s] Reference *47*. [t] Reference *48*. [u] Reference *28*. [v] An asterisk (*) indicates the value was estimated from LC-MS/MS data.

some samples at levels below the MDLs of the GC-MS method and indicated the presence of 9 pharmaceuticals and PCPIs not targeted by the GC-MS method. An additional 12 nontarget pharmaceuticals, PCPIs, and flame retardant chemicals were detected by GC-MS. With the exception of estriol, all detected target analytes were present in the few irrigation water samples analyzed. None of the target compounds were detected by broad-spectrum GC-MS analysis of storm runoff from the agricultural fields sampled or field blanks.

Target analytes were not present in supercritical fluid extracts of suspended particles at levels allowing quantitation. Several target pharmaceuticals, one pharmaceutical metabolite, one plasticizer, and two antioxidants were detected in SFE extracts (viz., carbamazepine, carisoprodol, fenofibrate, *p*-toluenesulfonamide, *N*-butylbenzenesulfonamide, butylated hydroxyanisole, butylated hydroxytoluene), but their concentrations were below their MDLs. Analytes associated with suspended particles are not discussed further.

We note that in many samples, SIM indicated the presence of target analytes at concentrations below their MDLs. The MDLs were defined by the lower limit of the linear calibration range. Target compounds can therefore be detected below this level, but not reliably quantified. We indicate the presence of target analytes at concentrations <MDL in **Table 3**, but confine our discussion to the compounds we were able to quantify or that were nontarget analytes.

Concentrations of individual effluent-derived microcontaminants varied over an order of magnitude during runoff events. **Figure 1** displays exemplary data for a runoff event at site F. Estimated dissolved concentrations of the flame retardant chemical TBEP and the synthetic musks HHCB and AHTN varied between <0.1 and 3.5 μg L^{-1} during the event. The concentrations displayed in **Figure 1** were estimated relative to the response of an *n*-octacosane internal standard by GC with flame ionization detection (*8*). Below we summarize our results by compound class.

Human Pharmaceuticals. Three targeted human pharmaceuticals were present above their MDLs for the GC-MS method in runoff from fields irrigated with disinfected recycled water and effluent-dominated streamwater (**Table 3**). We tentatively identified several additional pharmaceuticals (viz., gabapentin, lidocaine, phenytoin) by matching against the mass spectral libraries and visual comparison of spectra following the criteria presented in ref *8* (**Table 4**). These compounds were not quantified. A further seven human pharmaceuticals were identified by LC-MS/MS (**Table 4**). Dissolved concentrations of detected target PhACs are summarized in **Table 3** along with information about previous reports of occurrence in WWTP effluent and streamwater. In general, concentrations were lower than the few published acute toxicity thresholds for aquatic organisms. However, the effects on aquatic organisms of chronic low-level exposure to these PhACs are currently unknown.

Table 4. Nontarget Human Pharmaceuticals, Personal Care Product Ingredients, and Flame Retardant Chemicals Present in Runoff from Fields Irrigated with Treated Effluent

compound	use	site
Pharmaceutically Active Compounds		
acetaminophen[a]	analgesic–antipyretic	E
cannabinol methyl derivative	illicit drug metabolite	stream
diclofenac[a]	antiinflammatory	E
erythromycin–H_2O[a]	antimicrobial degradate	E
gabapentin	anticonvulsant	A–C, E
hydrocodone[a]	opioid analgesic and antitussive	E
lidocaine	antiarrhythmic	E
meprobamate[a]	antianxeity agent	E
naproxen[a]	antiinflammatory	E
phenytoin	antiepileptic	E
sulfamethoxazole[a]	antimicrobial	E
trimethoprim[a]	antimicrobial	E
Personal Care Product Ingredients		
AHTN (Tonalide)	musk fragrance	A, F
benzophenone	fragrance fixative	A
N,*N*-diethyltoluamide (DEET)	insect repellant	F
HHCB (Galaxolide)	musk fragrance	A, F
oxybenzone[a]	UV screen	E
triclosan[a]	antibacterial agent	E
Flame Retardant Chemicals		
tris(2-chloroethyl) phosphate	flame retardant	F
tris(chloropropyl) phosphate	flame retardant	A, F
tris(2-butoxyethyl) phosphate	flame retardant	A, F
tris(1,3-dichloro-2-propyl) phosphate	flame retardant	A, F

[a] Determined by LC-MS/MS.

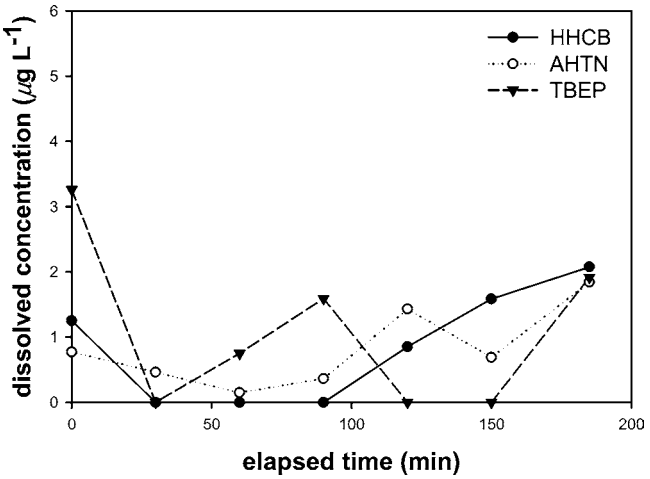

Figure 1. Variation in nontarget analyte concentrations over the course of a runoff event from an agricultural field irrigated with effluent-dominated streamwater. HHCB and AHTN are synthetic polycyclic musks, and TBEP is an alkyl phosphate flame retardant chemical. Concentrations were estimated as response relative to an *n*-octacosane internal standard by GC with flame ionization detection (*8*).

Below we discuss each class of PhACs that was present at levels exceeding their MDLs or that was not specifically targeted by the analytical method employed.

Antidyslipidemic Drugs. The fibric acid derivative gemfibrozil [5-(2,5-dimethylphenoxy)-2,2-dimethylpentanoic acid] is a hypolipidemic drug and was present at concentrations exceeding its MDL in surface runoff from two effluent-irrigated fields irrigated (viz., 190−790 ng L^{-1}). Dissolved gemfibrozil concentrations fell within the range of those previously reported in WWTP effluents (viz., <10−4760 ng L^{-1}).

Antiseizure Drugs. Three antiseizure drugs used to treat epilepsy were identified in runoff from fields irrigated with treated effluent: the iminostilbene derivative carbamazepine (5-carbamoyl-5*H*-dibenz[*b*,*f*]azepine), the hydantoin derivative phenytoin (Dilantin; 5,5-diphenyl-2,4-imidazolidinedione), and gabapentin [Neurotin; 1-(aminomethyl)cyclohexaneacetic acid]. Carbamazepine was quantifiable in runoff samples from three effluent-irrigated fields. Runoff concentrations of carbamazepine (320−440 ng L^{-1}) were similar to those reported by Drewes et al. (*3*) for tertiary treated effluent in the United States (viz., <MDL−445 ng L^{-1}). We also detected carbamazepine in irrigation water (140−240 ng L^{-1}). Carbamazepine is carcinogenic to rats and teratogenic in humans (*18*). Short-term (96-h) exposure of the microalgae *Ankistrodesmus braunii* and *Selenastrum capricornutum* to carbamazepine concentrations up to 20 mg L^{-1} resulted in negligible inhibition of growth (*19*).

Phenytoin was tentatively identified by GC-MS (and confirmed by LC-MS/MS) in site E runoff. We know of no previous report of this widely used antiseizure agent and antiarrhythmic drug in WWTP effluent. Because phenytoin experiences extensive first-pass hepatic metabolism (*18*), only minor amounts are expected to be excreted; however, the enzymes involved in phenytoin metabolism are saturable, so that if administered at higher doses or with other drugs metabolized by the same enzymes, excretion of larger amounts of the active compound is plausible. Gabapentin was tentatively identified in runoff from most sites. This compound has not been previously reported in WWTP effluent or in the environment.

Antiarrhythmic Drugs. The tentatively identified antiarrhythmic drug and local anesthetic lidocaine [2-(diethylamino)-*N*-2,6−dimethylphenylacetamide] was tentatively identified in runoff from site E. We know of no previous report of lidocaine in treated effluent or surface waters.

Miscellaneous PhACs. Carisoprodol (Soma; *N*-isopropyl-2-methyl-2-propyl-1,3-propanediol dicarbamate) is a centrally acting, orally administered skeletal muscle relaxant and analgesic that is sometimes abused (*20*). Carisoprodol was present above its MDL (302 ng L^{-1} for a 2-L sample) in runoff from one field irrigated with disinfected tertiary recycled water (680 ng L^{-1}) and in effluent-dominated streamwater used for irrigation (300 ng L^{-1}; 3.8-L sample). We are unaware of any previous report in the literature on the occurrence of carisoprodol in treated effluent. The environmental fate of carisoprodol and its potential effects on nontarget organisms are unknown.

Caffeine (1,3,7-trimethylxanthine) is an ingredient in some over-the-counter analgesics and diuretics and is sometimes prescribed for the treatment of prolonged apnea in preterm infants; however, the most important source in the United States is caffeine-containing beverages (*18*). Caffeine was not present above its MDL of 290 ng L^{-1} in runoff from the fields sampled. A dissolved concentration of 31 ng L^{-1} was estimated from the LC-MS/MS analysis of runoff from site E. This concentration is significantly below those reported to be acutely toxic to aquatic organisms [e.g., *Pimephales promelas* 96-h LC_{50} value of 40 mg L^{-1} (*21*)].

A methyl derivative of cannabinol, an active component of marijuana, was detected in effluent-dominated streamwater but not in runoff. Marijuana is the most widely used illicit drug in the United States (*18*).

Analysis of runoff samples by LC-MS/MS indicated the presence of low levels of additional PhACs including acetaminophen, diclofenac, erythromycin−H_2O, hydrocodone, meprobamate, naproxen, sulfamethoxazole, and trimethoprim.

Natural and Synthetic Hormones. None of the targeted natural and synthetic steroidal hormones were present in runoff samples at levels quantifiable by GC-MS (**Table 3**). Analysis

by LC-MS/MS indicated that low levels of estrone (~50 ng L^{-1}), progesterone (~3 ng L^{-1}), and testosterone (~20 ng L^{-1}) were present in runoff from site E and that 17β-estradiol (~3 ng L^{-1}) was present in runoff from site B (**Table 3**). These levels are similar to those reported previously for treated wastewater and streamwater.

Personal Care Product Ingredients. In addition to PhACs and steroidal hormones, several PCPIs were tentatively identified in runoff from effluent-irrigated fields (**Table 4**). The synthetic polycyclic musk fragrances HHCB (Galaxolide; 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[*g*]-2-benzopyran) and AHTN (Tonalide; 7-acetyl-1,1,3,4,4,6-hexamethyltetrahydronaphthalene) were tentatively identified in the water phase of runoff from two fields. Synthetic musks find widespread use in scented consumer products such as perfumes, cosmetics, soaps, shampoos, laundry detergent, and fabric softeners and have been reported in municipal WWTP effluent in Europe and North America (*22*−*24*). The aquatic toxicology of polycyclic musks has not been well studied, but the "No Observed Effects Concentrations" for the few aquatic invertebrate and freshwater fish tested [viz., 35−374 μg L^{-1} for AHTN and 68−201 μg L^{-1} for HHCB (*25*)] are at least 1−2 orders of magnitude higher than levels previously reported in effluent from U.S. WWTPs (0.024−2.21 μg L^{-1}) (*23*, *24*). The polycyclic musks AHTN and HHCB exert antiandrogenic effects both in vitro and in vivo in zebrafish (*Danio rerio*) (*26*).

Other PCPIs tentatively identified by GC-MS in water phase extracts of runoff from effluent-irrigated fields included *N*,*N*-diethyltoluamide (DEET) and benzophenone. DEET is the active ingredient in many topical insect repellants and was among the most frequently detected organic wastewater contaminants in U.S. streams affected by intense urbanization or livestock production (*7*). Benzophenone is an anthropogenic fragrance fixative for strong perfumes (e.g., in soaps, shampoos) and is occasionally used as a flavor ingredient. LC-MS/MS indicated the presence of two additional PCPIs in some runoff samples: oxybenzone and triclosan (**Table 4**).

Plasticizers and Flame Retardant Chemicals. One targeted plasticizer was detected, and several nontarget flame retardant chemicals were tentatively identified in surface runoff samples (**Tables 3** and **4**). *N*-Butylbenzenesulfonamide was present in surface runoff from two effluent-irrigated fields and one field irrigated with effluent-dominated streamwater. This compound is used as a plasticizer for polyamide materials and as a starting material for the synthesis of sulfonyl carbamate herbicides (*27*). *N*-Butylbenzenesulfonamide exhibits neurotoxicity to laboratory mammals, apparently affecting choline acetyltransferase (*27*). The occurrence of this compound in the environment has been reported several times in the literature (*3*, *8*, *28*).

Four alkylated phosphate ester flame retardant plasticizers were tentatively identified in water samples from effluent-irrigated sites: tris(2-chloroethyl) phosphate (TCEP), tris(chloropropyl) phosphate (TCPP), tris(2-butoxyethyl) phosphate (TBEP), and tris(1,3-dichloro-2−propyl) phosphate (TDCPP) (**Table 4**). None of these compounds are known to occur naturally. Alkylated phosphate esters are employed as flame retardants in polyurethane foams, resins, poly(vinyl chloride) compounds, floor polishes, lacquers, plastics, synthetic rubber, synthetic fibers, and cellulose ester compounds (*29*). The presence of alkylated phosphate ester flame retardants in streams and treated wastewater has been reported in Japan, Europe, and North America (e.g., refs *9* and *30*−*32*).

Ecotoxicological Implications. The ecotoxicological significance of the low concentrations of PhACs observed in agricultural runoff remains to be elucidated. At the levels observed in this study, acute effects such as lethality of nontarget organisms appear to be unlikely, but more subtle effects could potentially result in ecological consequences. Because PhACs are designed to elicit biological effects and are often resistant to degradation to avoid biological inactivation before exerting their therapeutic effect, the potential exists for these compounds to affect nontarget organisms. Although aquatic organisms share certain receptors with humans, the effects of PhACs on nontarget organisms are largely unknown. The modes of action of the PhACs and hormones detected in this study include interaction with peroxisome proliferator-activated receptors (fibric acid derivatives), inhibition of cyclic nucleotide phosphodiesterase (caffeine), inhibition of cyclooxygenase activity (acetaminophen, diclofenac, naproxen), prolongation of Na channel inactivation in neurons (carbamazepine, phenytoin), and binding to estrogen receptors. Fibric acid derivatives, carbamazepine and phenytoin induce various P450 enzymes. Several of the detected PhACs had unknown modes of action in humans (e.g., carisoprodol, gabapentin, hydrocodone).

The few data existing on the low-level toxicity of PhACs to aquatic organisms indicate that in some cases these compounds may induce unexpected effects. A few examples cited by Daughton and Ternes (*6*) serve to illustrate this potential. Although not identified in the present study, the selective serotonin-reuptake inhibitors employed as antidepressants elicit dramatic physiological responses in aquatic invertebrates at subnanomolar to micromolar levels including induction of spawning in male zebra mussels, enhanced production of ovarian proteins in crayfish, and accelerated sexual maturation in male fiddler crabs (see review in ref *6*). The effects of such changes on aquatic invertebrate populations are unknown.

Some PhACs are capable of sensitizing organisms to other toxicants (*6*). Many aquatic species, especially filter feeders and benthic organisms, possess multixenobiotic transporters that actively export potential toxicants from cells. Certain PhACs (e.g., verapamil, staurosporine) inhibit this transport system, leading to enhanced sensitivity to other toxic substances (chemosensitization) (*33*). Inhibition of multixenobiotic transporter proteins prevents elimination of toxic substances, dramatically lowering the concentration thresholds at which they exert their toxic effect (*33*).

In addition to potentially subtle effects elicited by PhACs, consideration must be given to additive and synergistic effects of these compounds. Additive effects of co-occurring compounds can result in toxicity even when the concentrations of individual compounds do not reach toxic levels. Flaherty et al. (*34*) recently showed that synergism between fluoxetine and clofibric acid resulted in significant mortality and deformities in *Daphnia magna*. Ecological risk assessment and the development of pollution control strategies should take the potential combined toxicity of such substances into account.

Management Implications. In arid areas, as more treated wastewater is used for irrigation rather than directly discharged into surface waters, runoff from agricultural fields will become an increasingly important source of contaminants traditionally associated with WWTP effluent. Whereas wastewater reuse may reduce overall impacts to surface waters, irrigation with treated effluent does not eliminate the introduction of organic microcontaminants into aquatic ecosystems. Although some reductions in contaminant load may be achieved by irrigation with treated effluent, this practice serves to shift the point of contaminant introduction from the treatment plant outfall to the edge of irrigated fields. The present study was not designed to assess

the degree of PhAC, hormone, and PCPI removal during crop irrigation; such research would improve our understanding of sewage-derived microcontaminant fate in agricultural systems. In addition to potential impacts to surface waters, effluent-derived xenobiotic contaminants may also leach into groundwater after they are applied to cultivated fields. Uptake of these compounds by crop plants may also warrant concern. In regions using treated wastewater for irrigation, application of advanced treatment technologies will be required to further reduce the introduction of PhACs and PCPIs into aquatic ecosystems.

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