SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 34, No. 11, pp. 2475–2488, 2015
© 2015 SETAC
Printed in the USA

PHARMACEUTICALS AND OTHER ANTHROPOGENIC TRACERS IN SURFACE WATER: A RANDOMIZED SURVEY OF 50 MINNESOTA LAKES

Mark L. Ferrey,*† Steven Heiskary,† Richard Grace,‡ M. Coreen Hamilton,‡ and April Lueck†
†Minnesota Pollution Control Agency, St. Paul, Minnesota, USA
‡AXYS Analytical Services, Sidney, British Columbia, Canada

(Submitted 24 March 2015; Returned for Revision 16 April 2015; Accepted 17 June 2015)

Abstract: Water from 50 randomly selected lakes across Minnesota, USA, was analyzed for pharmaceuticals, personal care products, hormones, and other commercial or industrial chemicals in conjunction with the US Environmental Protection Agency's 2012 National Lakes Assessment. Thirty-eight of the 125 chemicals analyzed were detected at least once, all at parts per trillion concentrations. The most widely detected was N,N-diethyl-m-toluamide, present in 48% of the lakes sampled. Amitriptyline, a widely used antidepressant, was found in 28% of the lakes. The endocrine active chemicals bisphenol A, androstenedione, and nonylphenol were found in 42%, 30%, and 10% of the lakes, respectively. Cocaine was found in 32% of the lakes, and its degradation product, benzoylecgonine, was detected at 28% of the locations. Carbadox, an antibiotic used solely in the production of swine, was also present in 28% of the lakes sampled. The means by which these and other chemicals were transported to several of the remote lakes is unclear but may involve atmospheric transport. *Environ Toxicol Chem* 2015;34:2475–2488. © 2015 SETAC

Keywords: Pharmaceuticals Endocrine active chemicals Surface water Lake

INTRODUCTION

A growing body of literature demonstrates that human and veterinary pharmaceuticals, personal care products, hormones, and other commercial and industrial chemicals are present in the surface waters of the United States, particularly in those impacted by wastewater treatment plant (WWTP) effluent [1–3]. This is coupled with mounting evidence that many of these chemicals are exerting adverse effects on aquatic organisms and ecosystems. Endocrine active chemicals are known to affect fish behavior [4], physiology, reproductive biology [5–8], and genetic expression [9,10] at parts per trillion concentrations. In aquatic environments, endocrine active chemicals may be affecting not only individual organisms but perhaps entire populations and ecosystems [11].

Much less is known about the presence of pharmaceuticals and endocrine active chemicals in freshwater lakes [12]. In Minnesota, USA, concern over these contaminants in surface water and their potentially harmful effects resulted in a 2008 study of 11 urban and rural lakes [7]. Lakes selected for that study represented different trophic conditions across the state, and most were impacted by some form of shoreline development, although 2 were selected as reference sites because of their remote locations. The results showed the presence of pharmaceuticals and personal care products in all of the lakes, as well as evidence of endocrine disruption in caged fathead minnows and in wild fish, including the reference lake locations.

In the present investigation, we expanded the scope of that work with an analysis of water samples from 50 randomly selected lakes from across Minnesota, many without shoreline development. The present study relied on the framework established by the US Environmental Protection Agency (USEPA) National Lakes Assessment and benefited from the extensive data collection of that effort. The results reveal a chemical profile of contaminants in lake water that is similar to that of surface water impacted by treated wastewater effluent. This widespread occurrence of pharmaceuticals, personal care products, detergent metabolites, and illicit drugs in lakes lacking obvious, proximate sources of contamination indicates that their presence in surface water is not always attributable to the direct discharges of domestic or municipal wastewater.

MATERIALS AND METHODS

Study area

Minnesota has among the highest number of lakes in the lower 48 states, with more than 12 000 lakes greater than 4 ha in size (Supplemental Data, Figure S1). The distribution, size, and depth of the lakes are largely a function of various glacial advances and retreats across the state, resulting in distinct regional patterns in landform, soils, natural vegetation, and ultimately land use (Figure 1). These patterns provided the basis for the USEPA mapping of Minnesota's ecoregions. Ecoregions and their influence on lake condition have been addressed elsewhere [13]. The 3 aggregated ecoregions that are referred to in the present study (Figure 2) are the northern forested region, where more than 79% is characterized by forest or wetland land use; the highly agricultural great plains region, where 78% is cultivated cropland; and the transitional eastern temperate forests region, which is characterized by a mixture of land uses (35% cropland and 20% forest). Developed land use comprises a small proportion of each ecoregion at 2%, 9%, and 6%, respectively, for the northern forested region, the eastern temperate forests region, and the great plains region.

National Lakes Assessment selection process

The USEPA conducts national randomized surveys of lakes, rivers, wetlands, and estuaries to measure the health of US waters. The 2012 National Lakes Assessment was the 11th in a

All Supplemental Data may be found in the online version of this article.
* Address correspondence to Mark.ferrey@state.mn.us.
Published online 15 October 2015 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/etc.3125

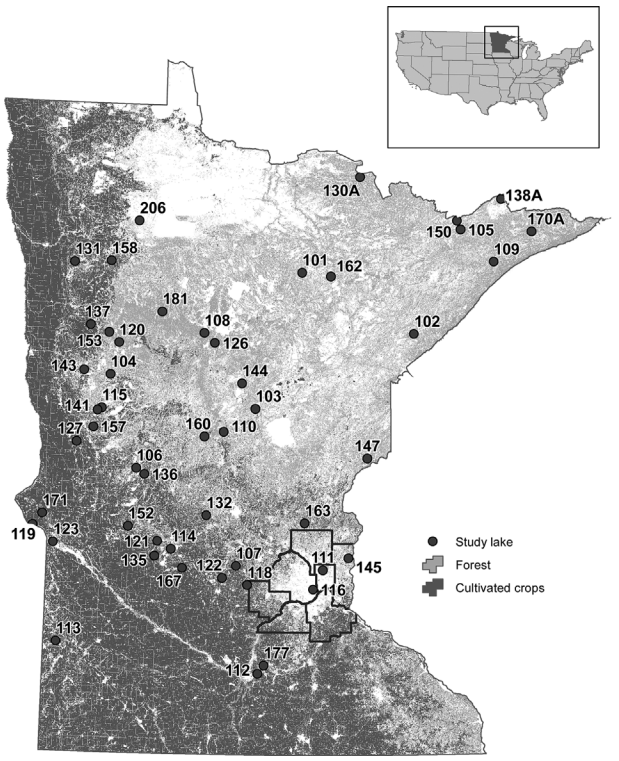

Figure 1. Minnesota's 2012 National Lake Study sampling locations, with the location in reference to the lower 48 states of the United States included for perspective. Forest and cropland land use and location of the 7-county Twin Cities metropolitan area are included to provide a geographic context for Minnesota and the survey lakes.

series of those surveys. In 2012, the survey included 904 natural lakes, ponds, and reservoirs across the lower 48 states [14]. Lakes with a minimum of 11 ha in size and a depth of at least 11 m were eligible for inclusion. Lakes were selected by the USEPA using a generalized random tessellation stratified design [15]. This design allows for probabilistic surveys that can be stratified by multiple lake size classes while maintaining spatial balance throughout the state. Five lake size classes (1–4 ha, 4–10 ha, 10–20 ha, 20–50 ha, and >50 ha) were used in the 2012 survey. The relative abundance of lakes in each of the size classes allows for weighting of results from the survey lakes when applied to the overall population of lakes in that size class (e.g., population of Minnesota lakes 1–4 ha in size).

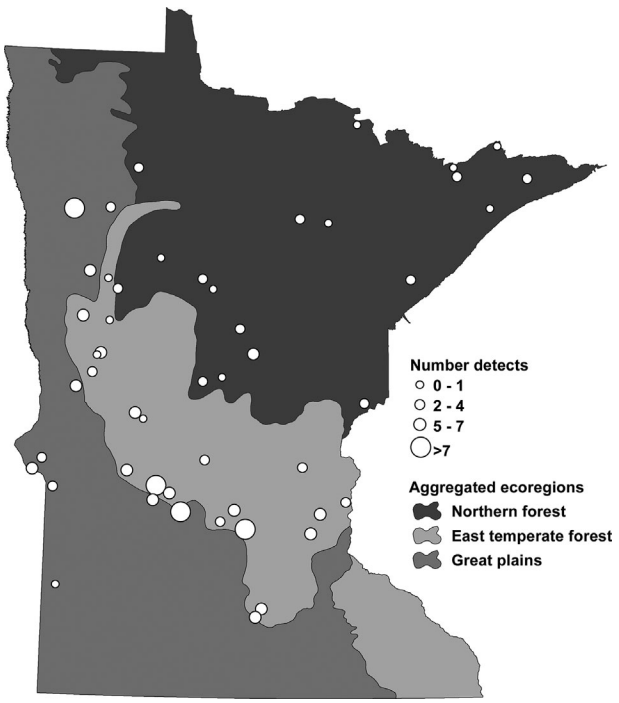

Figure 2. Number of contaminant detections by location and by US Environmental Protection Agency aggregated ecoregions.

Fifty Minnesota lakes were included in the national survey. Minnesota's lakes ranged in size from 11 ha to 4822 ha, with a median of 41 ha (Table 1). The total population of Minnesota lakes >1 ha was 15 046. A sample size of 50 allowed for condition estimates at the state level with 90% confidence in the estimates, as determined by the USEPA using a binomial distribution (T. Olson, USEPA, Corvallis, OR, personal communication). The weights of the individual sample lakes allow extrapolation of the data to generate a statistically valid estimate of the condition of lakes statewide [16]. A more complete explanation of the USEPA National Lakes Assessment lake selection and study procedures is provided in USEPA publications [14].

Sample collection

Minnesota Pollution Control Agency staff collected all samples. Mid-lake grab samples were collected in amber glass or high-density polyethylene bottles, depending on the specific analysis performed by the laboratory. Field staff did not apply fragrances, insect repellant, or sunscreen and wore disposable powder-free, nitrile gloves while sampling. Sample bottles were transported to the site in resealable plastic bags. For all samples, bottles were rinsed with the surface water 3 times by filling and emptying the bottle completely. Bottles were immersed below the surface to prevent contact of the exposed skin above the gloved hand with the surface water. The final sample was collected without headspace in the bottle. Once filled, bottles were recapped, resealed in the plastic bags, and chilled on ice.

Field blanks were collected by transporting duplicate sample bottles to a site where samples were collected. Field blank water, also supplied by the laboratory in identical glass or high-density polyethylene bottles, was transported to the site. Field blank water bottles were transported in identical resealable plastic bags and in coolers to replicate the procedure used for actual samples. Using gloved hands and facing upwind, we poured the field blank water from the lab-supplied bottles into empty sample bottles without rinsing. The field blank samples were then recapped, resealed in plastic bags, and treated identically to the surface water samples.

After returning from the field, samples were refrigerated at 4 °C. All samples were kept in the original resealable plastic bags and shipped overnight to the laboratory (AXYS Analytical Services) for chemical analysis. The maximum holding time for samples, from the date of collection to their extraction in the laboratory, was 7 d.

All chemicals listed in Table 2 were analyzed in samples from each of the 50 lakes except for bisphenol A and triclosan, which were analyzed for 21 of the 50 lakes because of sampling logistics and budgetary restrictions.

Analyses

Five separate analyses were performed to target the 125 compounds (Table 2) analyzed in the present study as described below with further details provided in the Supplemental Data.

Table 1. Characteristics of the lakes and their watersheds

Lake ID	Area (ha)	Total watershed (ha)	Watershed: lake ratio	Developed (%)	Crop (%)	Rangeland (%)	Forest (%)	Open water (%)	Wetland (%)	No. feedlots	Public access	% Disturbed (crop % + developed %)	% Undisturbed (forested % + wetland %)
MN-101	146	643	4.4	5.5	0.0	0.02	53.4	23.3	17.6	0	Y	5.5	71.1
MN-102	40	1238	31.0	2.2	0.0	0.7	52.6	9.6	35.0	0	Y	2.2	87.6
MN-103	92	1243	13.6	1.8	0.7	12.7	42.8	11.8	30.2	0		2.5	73.0
MN-104	54	276	5.1	7.0	17.0	23.6	28.3	21.3	0.6	1		24.0	28.9
MN-105	24	253	10.5	0.0	0.0	0.8	65.5	10.7	23.1	0		0.0	88.5
MN-106	468	46 489	99.4	6.1	30.1	23.0	16.3	21.1	3.3	155	Y	36.2	19.7
MN-107	220	11 864	54.0	6.4	72.5	10.8	3.8	4.8	1.7	24	Y	78.9	5.5
MN-108	105	1777	16.9	2.9	0.1	5.1	80.6	9.5	1.7	0	Y	3.0	82.3
MN-109	42	189	4.5	2.4	0.0	0.0	61.6	20.8	15.1	0		2.4	76.7
MN-110	144	4397	30.5	3.7	8.8	15.7	41.2	3.7	26.9	2	Y	12.5	68.1
MN-111	64	396	6.2	67.1	0.0	0.5	10.8	14.9	6.7	0	Y	67.1	17.5
MN-112	187	2146	11.5	4.0	57.0	5.9	4.5	14.5	14.1	4	Y	61.0	18.7
MN-113	34	1060	31.1	4.9	46.0	33.9	1.1	9.8	4.3	3		51.0	5.4
MN-114	73	235	3.2	3.5	19.0	12.6	24.3	28.1	12.5	1		22.4	36.8
MN-115	35	331	9.6	4.0	25.7	15.5	38.3	13.7	2.9	0	Y	29.7	41.1
MN-116	81	1194	14.8	82.9	0.0	0.8	1.8	8.0	6.3	0	Y	82.9	8.1
MN-118	70	277	3.9	3.8	42.4	16.5	6.3	26.5	4.5	1		46.2	10.8
MN-119	4822	197 188	40.9	4.6	51.5	28.6	3.0	5.8	6.5	54	Y	56.0	9.5
MN-120	743	8780	11.8	1.0	0.1	2.6	71.6	15.8	8.8	0	Y	1.1	80.5
MN-121	907	9650	10.6	3.5	53.1	17.1	9.4	13.7	3.2	39	Y	56.7	12.6
MN-122	428	5007	11.7	4.5	57.3	9.8	7.6	17.2	3.6	11	Y	61.7	11.3
MN-123	2	186	82.0	11.2	66.3	16.0	0.2	1.3	5.0	0		77.5	5.2
MN-126	2	231	153.8	0.4	0.0	0.4	92.3	3.3	3.7	0		0.4	96.0
MN-127	6	111	19.0	1.6	53.4	0.0	1.1	15.1	28.8	0		55.1	29.9
MN-130A	44	442	10.1	0.0	0.0	0.0	72.5	10.8	16.6	0		0.0	89.2
MN-131	8	68	9.0	8.4	49.2	2.4	4.6	8.8	26.6	0		57.6	31.2
MN-132	46	1204	26.2	2.9	31.5	23.0	23.2	12.4	6.9	8	Y	34.4	30.1
MN-135	23	493	21.8	3.9	57.2	15.0	3.9	13.9	6.0	1		61.1	10.0
MN-136	3	171	50.2	0.6	41.7	34.3	16.0	2.5	4.9	0		42.2	20.9
MN-137	15	155	10.1	7.9	44.8	16.9	0.0	23.1	7.3	1		52.7	7.3
MN-138A	8	190	23.5	0.0	0.0	0.0	74.1	5.7	20.2	0		0.0	94.3
MN-141	2	1596	685.9	3.7	16.6	21.9	21.5	20.8	15.4	0		20.3	36.9
MN-143	4	24	6.1	0.0	17.4	9.6	47.0	17.0	8.9	1		17.4	55.9
MN-144	6	49	8.8	1.7	1.9	22.8	2.2	5.9	65.6	0	Y	3.5	67.8
MN-145	45	852	19.0	3.3	6.8	26.9	46.2	12.1	4.7	0	Y	10.1	51.0
MN-147	17	93	5.3	1.8	18.8	20.5	23.9	19.8	15.1	0	Y	20.6	39.1
MN-150	12	204	17.0	0.0	0.0	0.0	45.5	6.0	48.5	0		0.0	94.0
MN-152	3	440	136.6	3.4	66.6	19.0	1.8	8.4	0.9	0		70.0	2.6
MN-153	13	110	8.8	0.0	0.0	0.0	60.8	25.9	13.3	0		0.0	74.1
MN-157	35	152	4.3	2.9	30.1	11.1	23.1	31.7	1.1	0		33.0	24.2
MN-158	16	416	26.6	3.9	51.3	19.3	5.0	10.5	10.0	2		55.3	15.0
MN-160	3	62	23.0	0.0	0.0	0.0	85.6	10.0	4.4	0		0.0	90.0
MN-162	11	61	5.6	3.7	0.0	2.4	66.3	17.2	10.5	0		3.7	76.8
MN-163	141	3006	21.3	5.3	35.6	11.1	25.7	10.8	11.6	1	Y	40.9	37.3
MN-167	57	625	10.9	2.9	57.7	13.7	7.9	12.3	5.4	2	Y	60.6	13.3
MN-170A	79	341	4.3	0.7	0.0	0.0	69.5	22.8	7.0	0	Y	0.7	76.5
MN-171	1	20	18.1	9.8	63.6	0.0	0.0	26.7	0.0	0		73.3	0.0
MN-177	10	106	10.3	1.4	50.3	22.0	15.6	8.9	1.8	2		51.7	17.4
MN-181	42	333	7.9	3.0	0.7	8.9	67.8	14.9	4.6	1		3.7	72.4
MN-206	49	634	13.0	0.0	0.0	0.0	0.5	9.3	90.2	0		0.0	90.7

Table 2. List of analytes

PPCP list 1	PPCP list 2	PPCP list 3	Hormones ESI^+	Hormones ESI^-	PPCP list 5	Alkylphenols
Acetaminophen	Anhydrochlortetracycline	Bisphenol A	Allyl trenbolone	17α-Dihydroequilin	Alprazolam	4-Nonylphenol monoethoxylates
Azithromycin	Anhydrotetracycline	Furosemide	Androstenedione	Equilenin	Amitriptyline	4-Nonylphenol diethoxylates
Caffeine	Chlortetracycline	Gemfibrozil	Androsterone	Equilin	Amlodipine	4-Nonylphenols
Carbadox	Demeclocycline	Glipizide	Desogestrel	17β-Estradiol	Benzoylecgonine	Octylphenol
Carbamazepine	Doxycycline	Glyburide	Estriol	17α-Estradiol	Benztropine	
Cefotaxime	4-Epianhydrochlortetracycline	Hydrochlorothiazide	Mestranol	Estrone	Betamethasone	Bisphenol A
Ciprofloxacin	4-Epianhydrotetracycline	2-Hydroxy-ibuprofen	Norethindrone	17α-Ethinyl estradiol	Cocaine	
Clarithromycin	4-Epichlortetracycline	Ibuprofen	Norgestrel		DEET	Triclosan
Clinafloxacin	4-Epioxytetracycline	Naproxen	Progesterone		Desmethyldiltiazem	
Cloxacillin	4-Epitetracycline	Triclocarban	Testosterone		Diazepam	
Dehydronifedipine	Isochlortetracycline	Triclosan			Fluocinonide	
Diphenhydramine	Minocycline	Warfarin			Fluticasone propionate	
Diltiazem	Oxytetracycline				Hydrocortisone	
Digoxin	Tetracycline				10-Hydroxy-amitriptyline	
Digoxigenin					Meprobamate	
Enrofloxacin					Methylprednisolone	
Erythromycin-H_2O					Metoprolol	
Flumequine					Norfluoxetine	
Fluoxetine					Norverapamil	
Lincomycin					Paroxetine	
Lomefloxacin					Prednisolone	
Miconazole					Prednisone	
Norfloxacin					Promethazine	
Norgestimate					Propoxyphene	
Ofloxacin					Propranolol	
Ormetoprim					Sertraline	
Oxacillin					Simvastatin	
Oxolinic acid					Theophylline	
Penicillin G					Trenbolone	
Penicillin V					Trenbolone acetate	
Roxithromycin					Valsartan	
Sarafloxacin					Verapamil	
Sulfachloropyridazine						
Sulfadiazine						
Sulfadimethoxine						
Sulfamerazine						
Sulfamethazine						
Sulfamethizole						
Sulfamethoxazole						
Sulfanilamide						
Sulfathiazole						
Thiabendazole						
Trimethoprim						
Tylosin						
Virginiamycin						
1,7-Dimethylxanthine						

PPCP = pharmaceuticals and personal care products; ESI = electron spray ionization; DEET = *N,N*-diethyl-m-toluamide.

Pharmaceuticals and personal care products. The pharmaceuticals and personal care products selected for analysis were based on USEPA Method 1694 [17] with additional compounds incorporated into list 3 and an additional run for list 5 compounds (see Supplemental Data for AXYS Analytical Services lists 1, 3, 4, and 5). This expanded USEPA 1694 analyte list represents those pharmaceutical and personal care compounds identified by the USEPA, the Minnesota Pollution Control Agency, and other AXYS clients as priorities for assessment based on previous detection, annual consumption, expected toxicity, and persistence. For analysis of compounds from lists 1, 2, 3, and 5, a 1.0-L sample was filtered (1.6 μm), adjusted to pH 2 by addition of hydrochloric acid (HCl), spiked with a suite of isotopically labeled internal standards, and extracted by solid-phase extraction using an Oasis HLB cartridge (Waters). The extract was analyzed by liquid chromatography–tandem mass spectrometry (LC-MS/MS) operated in the electron spray ionization (ESI)–positive mode for list 1, 2, and 5 compounds and analyzed in the ESI-negative mode for list 3 compounds. Separate analysis runs and conditions were used for each of the lists of target analytes.

Hormones. A 1.0-L sample was filtered (1.6 μm), adjusted to pH 2 by addition of HCl, spiked with a suite of isotopically labeled internal standards, and extracted by solid-phase extraction using an Oasis HLB cartridge. The extract was analyzed by LC-MS/MS operated in the ESI-positive mode for the ESI^+ hormones and by LC-MS/MS operated in the ESI-negative mode for the ESI^- hormones (Table 2).

Alkylphenols. An unfiltered 1.0-L sample was spiked with isotopically labeled internal standards, adjusted to pH 11–12 by the addition of potassium carbonate solution, and acetylated with acetic anhydride. The extract was then acidified to pH 6 with HCl and extracted with hexane. Extracts were cleaned up by silica column chromatography and analyzed by gas chromatography–mass spectrometry operated in the multiple ion detection mode.

Bisphenol A. A 0.5-L sample was filtered (1.6 μm), adjusted to pH 2 by addition of HCl, spiked with deuterated bisphenol A internal standard, and extracted by solid-phase extraction using an Oasis HLB cartridge. The extract was analyzed by LC-MS/MS operated in the ESI-negative mode.

Triclosan. A 0.5-L sample was filtered (1.6 μm), adjusted to pH 2 by addition of HCl, spiked with [^{13}C]-labeled triclosan, and extracted by solid-phase extraction using an Oasis HLB cartridge. The extract was analyzed by LC-MS/MS operated in the ESI-negative mode.

Quality control and assurance

Methods were validated by USEPA Tier 1 procedures [18]. All analytes were quantified either by isotope dilution internal standard quantification or, when an isotopically labeled analog of the analyte was unavailable, by internal standard methods using a related labeled compound. This produces accurate results that are recovery-corrected for losses during the analysis procedure and compensated for LC-MS/MS suppression or enhancement because of the sample matrix. For each batch of samples analyzed, a lab blank was included to demonstrate that detected analytes were not the result of laboratory background or other external contamination. Results for laboratory blanks were compliant with AXYS's statistically determined blank control limits (mean plus 3 standard deviations of approximately 30 blanks), and any detections were used to censor field sample results. Each analysis batch also included a "known" or quality control sample to demonstrate the accuracy of the method for each analyte. Recoveries of all added labeled standards were monitored to ensure that analyses were in control and met regular method specifications. Detailed quality assurance and quality control information for all analyses can be found in the Supplemental Data.

Normally, a reporting limit is determined for each compound on a sample-specific basis as the concentration (corrected for surrogate recovery) corresponding to the lowest concentration calibration standard. Because of the detection of N,N-diethyl-m-toluamide (DEET) in some lab blanks and field blanks, a conservative reporting limit for DEET was determined as the mean (2.3 ppt) plus 3 standard deviations (3×3.3 ppt) of the results of 26 lab blanks (the 6 blanks associated with the samples in the present study plus the 10 blanks run immediately before and the 10 blanks run immediately after the present study). Any DEET result below 12.3 ppt was therefore treated as a nondetect. Any DEET result above the 12.3 ppt reporting limit was reported as a blank-corrected value (measured concentration minus the mean blank value of 2.3 ppt). None of the 3 field blanks analyzed for DEET returned a positive detection at the 12.3 ppt reporting limit.

Condition estimates

Data for each analyte were categorized as detect or nondetect. The categorical data were analyzed using a conditional estimation statistic in "R" statistical software [19] and "spsurvey" [20]. The conditional estimation calculates the percentage of lakes within the state likely to contain detectable concentrations of each analyte on a 95% confidence interval (CI).

All 50 lakes were mapped in ESRI Arc GIS 10.2. Watershed boundaries were delineated for all lakes using geographic information system–based tools, including the Minnesota Department of Natural Resources automated catchment tool. Digital elevations and stream flow lines were used to help establish the accuracy of the boundaries. Land use composition was determined for all lakes based on the 2006 National Land Cover Database. Aggregated categories were as follows: developed (all residential and urban classes), cropland (all cultivated cropland), rangeland (pasture and grassland), forest (deciduous, coniferous, mixed, and shrub/scrub), wetland, and open water (lakes). Land use was tabulated for each lake (Table 1) and expressed as a percentage of the total watershed draining to the lake. For some analyses, developed and cropland uses were combined to yield a "disturbed" land use category, and forest and wetland uses were combined to yield an "undisturbed" designation. In addition, the number, location, and type of animal feedlots were mapped.

Change-point analysis

Regression tree analysis was done in R statistical software (*rpart* in the *rpart* package). This method identifies thresholds by dividing samples into 2 groups based on differences in their means and variances [21]. Trees were constrained to a single split with a bucket size of 5 samples. Ninety percent confidence intervals were estimated with a bootstrap analysis in R statistical software that resampled 1000 times. A χ^2 test was used to assess whether the change point was significant at $\alpha = 0.05$. Nonsignificant change points were not used in further analyses.

RESULTS

Frequency of detections

Table 3 lists all chemicals that were found in these lakes and summarizes the ranges of detected concentrations. Analytical

Table 3. Summary statistics for chemicals detected

Chemicals	Min	Max (ppt)	Reporting limit range(ppt)[a]	Lakes with detects (%)
Pharmaceuticals				
Alprazolam	nd	0.369	0.28–0.34	2
Amitriptyline	nd	4.05	1.04–0.90	28
Benztropine	nd	0.72	0.66–0.80	4
Caffeine	nd	42.9	14.3–54.1	16
Carbadox	nd	121	1.43–31.6	28
Carbamazepine	nd	36.7	1.40–1.72	2
Ciprofloxacin	nd	19.4	5.71–96.1	2
Dehydronifedipine	nd	1.62	0.57–5.13	4
Diphenhydramine	nd	0.784	0.56–0.71	6
Fluoxetine	nd	4.52	1.43–1.72	6
Gemfibrozil	nd	13.2	1.40–3.44	4
Meprobamate	nd	29.4	0.38–0.51	2
Norverapamil	nd	0.24	0.14–0.47	2
Ofloxacin	nd	8.94	1.54–7.66	2
Sulfadimethoxine	nd	3.52	0.29–5.58	2
Sulfamerazine	nd	12.5	0.57–5.52	4
Sulfamethazine	nd	134	1.33–18.3	4
Sulfamethizole	nd	2.48	0.58–5.10	2
Sulfamethoxazole	nd	57.1	0.61–8.32	2
Sulfathiazole	nd	32.8	1.44–4.94	10
Theophylline	nd	90.6	58.1–122	2
Thiabendazole	nd	3.98	1.40–1.72	2
Trimethoprim	nd	6.22	1.49–7.17	4
Valsartan	nd	5.01	0.37–0.46	2
Hormones				
Androstenedione	nd	7.48	1.90–3.61	30
Androsterone	nd	406	19.5–158	8
Testosterone	nd	4.10	0.77–7.30	8
Mestranol	nd	71.7	19.4–82.3	2
Progesterone	nd	0.989	0.76–2.41	6
Estrone	nd	15.5	3.77–4.21	4
Allyl trenbolone	nd	1.14	0.77–1.94	4
Alkylphenols				
4-Nonylphenol	nd	20.1	3.53–12.5	10
Octylphenol	nd	3.76	0.69–4.58	4
Other				
Bisphenol A	nd	14.9	1.67–2.40	43
Triclosan	nd	11.8	4.01–8.87	14
DEET	nd	308.7	12.3[b]	48
Illicit drugs				
Cocaine	nd	5.26	0.14–0.31	32
Benzoylecgonine	nd	9.48	0.29–0.77	28

[a]A reporting limit was determined for each compound on a sample-specific basis as the concentration (corrected for surrogate recovery and actual volume of sample analyzed) corresponding to the lowest concentration calibration standard.
[b]The reporting limit for DEET was derived through a statistical evaluation of laboratory blank data as described in *Materials and Methods*.
nd = nondetect (below reporting limit); DEET = *N,N*-diethyl-m-toluamide.

results for the 11 most frequently detected contaminants for all of the 50 lakes sampled are found in Table 4.

The number of detections per lake ranged from 0 to 13, and the average number of chemicals detected per lake was 3.7. The lake with the greatest number of detections (National Lakes Assessment 12 MN 118, with 13 detections) was the only survey lake that receives treated domestic wastewater. Thirty-eight of the 125 analytes were found in at least 1 lake. Ordered by frequency of detection (Supplemental Data, Figure S2), DEET was the most common, found in 48% of the lakes that were sampled for the present study. It was also measured at the highest concentration of any of the detected contaminants at 308 ppt in a rural lake with less than 2% developed land use (Tables 1 and 4). This was followed by bisphenol A, detected in 43% of the lakes at a maximum concentration of 14.9 ppt in a rural lake in an agricultural setting and little developed watershed land use. Cocaine and its breakdown product, benzoylecgonine, were detected in 32% and 28% of the lakes, respectively, with the greatest concentrations of both these analytes—5.26 ppt for cocaine and 9.48 ppt for benzoylecgonine—occurring in the same lake. The highest concentration of the steroidal hormone androstenedione, found in 30% of the lakes, was 7.48 ppt in the only lake that receives treated wastewater. This lake also had the highest concentration of caffeine, considered a reliable tracer for wastewater, at 42.9 ppt. Caffeine was detected in 16% of the lakes. The antidepressant amitriptyline was detected in 28% of the study lakes, up to 4.05 ppt in a lake influenced by shoreline residential development. The swine antibiotic carbadox also was found in 28% of the lakes, with the highest concentration of 121 ppt occurring in a rural lake with a nearby swine feedlot. Triclosan and nonylphenol were detected in 14% and 10% of the lakes, respectively. The highest concentrations of these contaminants, at 11.8 ppt and 20.1 ppt, respectively, were found in lakes impacted by shoreline residential development. Several antibiotics in addition to carbadox were found at lower frequencies, primarily sulfonamides including sulfathiazole, sulfamerazine, sulfamethazine, sulfadimethoxine, sulfamethizole, sulfamethoxazole, and trimethoprim. Two fluoroquinolone antibiotics, ciprofloxacin and ofloxacin, were detected in 2% of the lakes.

Other pharmaceuticals that were detected at lower frequencies included the antidepressant fluoxetine and diphenhydramine, both found in water from 6% of the locations at concentrations up to 4.52 ppt and 0.784 ppt, respectively. Dehydronifedipine (a metabolite of nifedipine, a blood pressure regulator), the lipid regulator gemfibrozil, and the anticholinergic benztropine were each found in 4% of the lake samples. Carbamazepine (an anticonvulsive drug) the fungicide thiabendazole, the sedatives alprazolam and meprobamate, the blood pressure regulators norverapamil and valsartan, and the asthma medication theophylline were each found in 2% of the lakes.

In addition to the hormone androstenedione, the hormones androsterone and testosterone were each detected in 8% of the lakes at concentrations up to 406 ppt and 4.10 ppt, respectively, and progesterone, a female steroidal hormone, was detected in 6% of the samples at concentrations up to 0.989 ppt. Allyl trenbolone, a hormone used to increase muscle mass in livestock, and estrone, an estrogen, were each found in 4% of the lakes at 1.14 ppt and 15.5 ppt, respectively. Mestranol, an estrogen used in oral contraceptives, was found in 1 lake at 71.7 ppt.

Spatial occurrence

In general, the number of detections was low in the lakes of the northern forests ecoregion, with most lakes having 2 or fewer detections (Figure 2). The number of detections per lake increased significantly in the east temporal forests region, characterized by increased developed and agricultural land uses, and in the agriculturally dominated great plains region.

The geographic distribution of 4 of the most widely detected contaminants is shown in Figure 3. Figure 3A shows that DEET was detected widely across all 3 ecoregions of the state, including remote areas in northeast Minnesota that are heavily forested and have little developed land use. By contrast, amitriptyline (Figure 3B), cocaine and its degradation product benzoylecgonine (Figure 3C), and carbadox (Figure 3D) were not found in the relatively undeveloped, sparsely populated northeast area of the state. However, these contaminants were dispersed widely among lakes in the other 2 ecoregions of the state with a more developed or agricultural land use. Detections

Table 4. Concentration data for the 11 most frequently detected contaminants[a]

Lake ID	DEET (ng/L)	Bisphenol A (ng/L)	Cocaine (ng/L)	Androstenedione (ng/L)	Carbadox (ng/L)	Amitriptyline (ng/L)	Benzoylecgonine (ng/L)	Caffeine (ng/L)	Triclosan (ng/L)	4-Nonylphenols (ng/L)	Sulfathiazole (ng/L)
MN-101	51.0	14.9[b]	<0.145	<1.93	<6.66	<0.291	<0.294	<14.5	4.75	<3.74	<2.25
MN-102	11.8	B 4.86	B 1.38	2.29	<9.64	<0.482	<0.300	<15.0	<6.21	B 15.4	<1.50
MN-103	42.9		1.58	<1.99	<15.6	0.41	<0.320	17.5		10.5	<2.65
MN-104	B 8.92		B 0.175	2.2	B 7.11	B 1.04	<0.297	<14.8		<3.53	<2.06
MN-105	B 7		<0.196	<2.02	<8.83	<0.929	<0.316	<15.8		B 25.7	<1.58
MN-106	48.2	4.72	2.59	<2.11	<22.6	<0.319	0.702	23.7	<4.75	18.5[b]	<2.24
MN-107	10.7	B 4.31	B 0.632	2.2	<6.13	<1.06	<0.297	<14.9	<4.83	B 24.0	<1.49
MN-108	12.6		B 0.359	2.62	<2.57	<0.298	<0.298	<14.9		B 20.5	<1.49
MN-109	27.5	<2.60	<0.155	<2.57	<4.68	K 0.918	<0.310	<15.5	<5.03	<7.02	<1.55
MN-110	95.4	<1.99	<0.153	<2.28	<1.53	<0.307	<0.307	<15.3	<5.05	<6.25	<1.53
MN-111	57.3	B 3.54	<0.153	3.41	8.9	0.528	<0.658	<15.3	<4.77	B 12.3	<1.53
MN-112	11.6	<2.07	0.66	<2.55	62.2	<1.57	2.64	<15.7	11.8[b]	<5.41	<4.21
MN-113	B 6.58		0.219	<2.03	<13.6	K 1.76	<0.449	<15.1		B 432	<2.92
MN-114	37.6		<0.152	4.64	35.1	4.05[b]	<0.558	<15.2		<7.61	18.1
MN-115	30.0	<1.67	0.785	<2.04	<7.34	<0.300	0.621	20.3	<5.43	<4.49	<1.50
MN-116	132.7	2.24	<0.149	<1.95	6.51	K 0.756	0.622	17.4	6.23	<4.20	<1.52
MN-118	55.4		<0.158	7.48[b]	75.9	K 10.2	<0.543	42.9[b]		B 12.0	<2.87
MN-119	14.6	3.37	1.12	<2.02	43.3	0.794	1.18	<19.7	<4.68	4.59	<2.02
MN-120	B 2.51	B 2.18	B 0.410	4.47	<6.85	0.751	<0.446	<15.0	<8.87	B 26.1	<1.50
MN-121	41.7	2.92	5.26[b]	4.18	31.9	1.36	9.48[b]	24.8	<4.87	<5.31	<2.45
MN-122	10.1	B 5.36	B 0.347	2.39	<9.66	<0.398	2.33	<15.9	<4.27	B 14.2	<1.59
MN-123	B 4.48		0.364	<1.93	<7.59	<0.293	<0.293	<14.7		B 346	<1.64
MN-126	B 6.30		B 0.724	<2.48	<1.49	K 0.630	<0.297	<14.9		<4.71	<1.49
MN-127	B 6.41		<0.148	<2.12	<18.2	3.86	<0.316	<14.8		12.2	18.9
MN-130A	B 2.17		<0.172	<1.99	<5.26	K 0.500	<0.344	<17.2		B 23.5	<1.72
MN-131	B 6.39	4.23	0.499	3.24	24.4	1.04	0.73	<21.5	<4.82	<5.36	<3.73
MN-132	B 8.19		<0.150	<2.00	16.3	<0.898	<0.300	<15.0		B 14.6	<1.50
MN-135	B 10.2		1.32	<2.68	113	D 1.73	1.56	<30.9		B 224	18.1
MN-136	B 0.62		<0.154	<2.07	<1.54	<0.308	<0.308	<15.4		B 24.3	<1.54
MN-137	15.4		<0.309	7.31	58.1	K 16.0	<0.770	<15.5		B 36.5	<1.55
MN-138A	B 5.1		B 0.377	<2.01	<5.97	<0.792	<0.297	<14.9		B 16.1	<1.49
MN-141	27.1		B 0.219	<1.96	<9.50	<0.519	<0.307	<15.4		<4.18	<1.54
MN-143	B 9.55	2.58	1.18	<2.69	<18.0	1.62	0.466	<18.2	<5.08	<8.18	<2.56
MN-144	122.7	B 7.85	<0.146	2.7	<10.8	<0.865	<0.291	<54.1	<5.15	B 32.0	<2.18
MN-145	B 8.94	B 2.27	<0.157	<1.99	<6.56	0.789	<0.314	<15.7	<4.01	B 22.8	<1.57
MN-147	308.7[b]	2.82	<0.143	<1.89	<1.43	K 0.515	<0.286	<14.3	<5.00	B 2.45	<1.80
MN-150	11.9		B 0.280	<1.97	<5.93	K 0.972	<0.472	<14.8		B 16.2	<1.48
MN-152	B 6.35		4.11	<2.26	<11.4	<0.309	6.92	21.6		B 188	32.8[b]
MN-153	B 6.83		0.237	<1.90	<7.47	K 0.574	<0.321	<14.6		B 344	<2.27
MN-157	B 7.51		<0.143	K 3.45	<7.89	3.87	1.04	<14.3		<4.78	<2.58
MN-158	B 4.82		<0.190	<2.98	34.3	2.82	<0.542	<15.4		<11.7	<4.32
MN-160	10.1		0.466	<2.57	<13.5	0.636	<0.298	<15.1		B 231	<3.78
MN-162	B 7.52	<2.40	<0.148	<2.63	<22.0	<1.94	<0.296	<25.8	<5.11	<12.5	<1.48
MN-163	54.7	B 2.10	B 0.623	<1.98	<7.79	<0.348	<0.290	<14.5	<6.12	B 22.6	<1.45
MN-167	B 9.8	1.67	1.52	<1.97	25	<0.298	1.34	23.2	<4.70	<11.8	15.5
MN-170A	B 10.1		<0.144	<2.51	<1.44	<0.533	<0.287	<14.4		B 32.5	<1.44
MN-171	B 5.84		<0.170	4.53	<31.6	<3.08	<0.434	<14.0		B 20.6	<4.23
MN-177	B 9.11		2.11	<3.61	121[b]	1.89	2.17	<18.4		B 188	<4.94
MN-181	B 2.46		<0.150	<2.41	<10.1	K 1.00	<0.299	<17.0		<3.86	<1.50
MN-206	B 3.49		<0.145	3.24	<4.64	K 1.03	<0.458	<14.5		B 25.3	<1.45

[a]Analytical qualifiers: < = analyte concentration less than the reporting limit; B = analyte detected in the sample and the associated laboratory blank; K = peak detected but did not meet quantification criteria (the result reported represents the estimated maximum possible concentration).
[b]Value denotes the highest concentration detected for that analyte.
DEET = *N,N*-diethyl-m-toluamide.

Figure 3. Maps of detections for N,N-diethyl-m-toluamide (**A**), amitriptyline (**B**), cocaine and benzoylecgonine (**C**), and carbadox (**D**), with US Environmental Protection Agency ecoregions noted.

of these contaminants do not appear clustered, nor are they situated near the state's more densely populated areas (Supplemental Data, Figure S3).

Because the present study was designed to allow an estimate of the condition of the overall population of the state's lakes, analytical results were extrapolated to give reasonably accurate estimates of the proportion of the total population of Minnesota lakes likely to contain the most frequently detected chemicals. The results of this statistical condition estimate, which incorporate a relative weighting of the sample lakes, are shown in Table 5. Based on a detection frequency for DEET in the sampled lakes of 48%, it was likely present in 59% of the total population of the state's lakes, or 8968 lakes. Similarly, the hormone androstenedione was likely present in 27% of the overall population of Minnesota's lakes, or 4114 lakes. Cocaine and benzoylecgonine were likely present in nearly 26% and 15% of the state's lakes, respectively, and amitriptyline in 24%.

Chemical detections were further analyzed spatially with respect to watershed land use for each lake (using the previously described land use categories) and the number and proximity to feedlots. No discernible relationships emerged from comparisons of chemical detection to either cropped or developed land use categories, when considered separately. However, when cropped and developed land use were combined to yield "disturbed" land use (Table 1), the number of contaminant detections increased with the proportion of disturbed land (Figure 4A). Change-point analysis revealed a significant ($p = 0.0005$) change point at 44% (90% CI, 31–72%) for the disturbed land use and 62% (90% CI, 49–115%) for the undisturbed land use (forest and wetland) categories (Figure 4B). There is a statistically significant increase in the number of detections when disturbed watershed land use increased above 44% or undisturbed land use decreased below 62%.

Table 5. Condition estimates of Minnesota, USA lakes for select contaminants based on study detection frequency

Contaminant	Estimate of total lakes affected (%)	Standard error	Estimate of no. of lakes affected
DEET	59.60	13.20	8968
Androstenedione	27.35	9.59	4114
Cocaine	25.96	7.85	3906
Amitriptyline	24.17	8.84	3637
Benzoylecgonine	14.90	4.60	2241
Carbadox	7.98	2.28	1201
Caffeine	7.60	3.32	1144
Sulfathiazole	2.06	1.07	310
Nonylphenol	1.46	1.05	219

DEET = *N,N*-diethyl-m-toluamide.

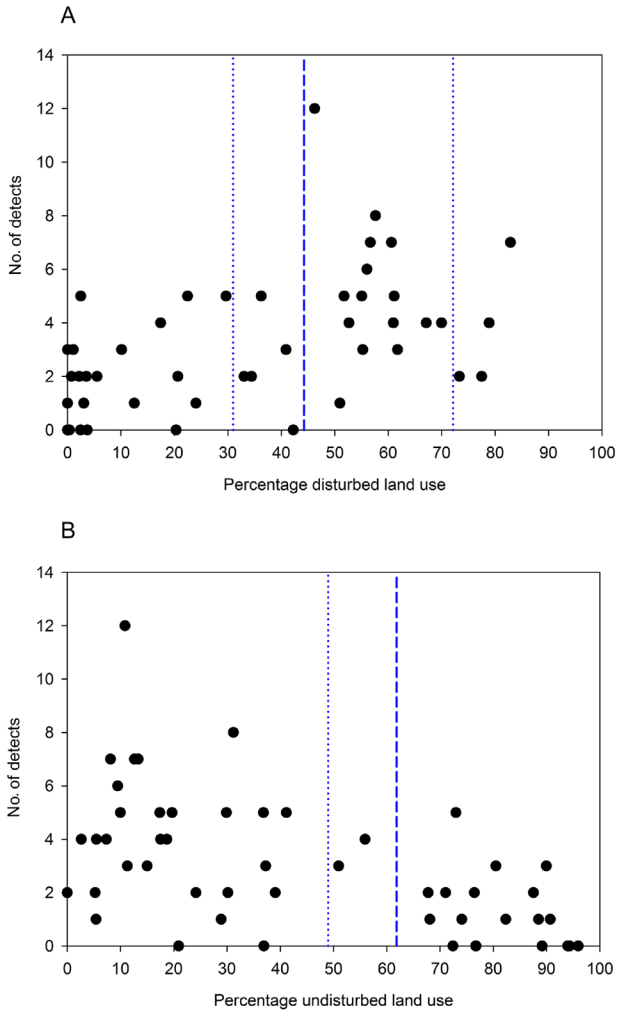

Figure 4. Change-point analysis of contaminants as a function of land use categorized as disturbed (**A**) or undisturbed (**B**). Dashed line indicates change point; dotted line indicates 90% confidence interval of the change point. There is no upper confidence limit in panel (**B**) because it was greater than 100%.

Several of the contaminants that were frequently detected in lakes were analyzed with respect to land use that could increase the likelihood of a contaminant being detected. Example hypotheses for 3 commonly detected analytes follow.

Cocaine—Hypothesis: Cocaine detections are greater in more developed watersheds with higher population density. There was no difference in the percentage of developed land use among lakes where cocaine was detected and where it was not detected (Figure 5A). Further, it was detected in lakes with no developed land use in the watershed or on the shoreline. Also, there was no apparent association with major cities throughout the state, based on a qualitative comparison of mapped detections (Figure 3C) and major cities (population of 20 000 or more) in Minnesota (Supplemental Data, Figure S3). Thus, this hypothesis is rejected.

Carbadox—Hypothesis: The frequency of detection for carbadox is greater in lakes with swine feedlots in their watersheds. Twelve of 14 lakes where carbadox was detected had 1 or more swine feedlots in the corresponding watershed or within 5 miles of the lake (Figure 6). Two lakes in the Twin Cities metropolitan area were within 7 miles of the University of Minnesota, St. Paul campus, where swine are raised. However, conducting a similar exercise for lakes without carbadox detections indicated that an equal or greater number of lakes had swine feedlots in the watershed or within 5 miles. The presence of a swine feedlot in the watershed did not increase the probability of carbadox detection (Figure 5B). The hypothesis is rejected.

Amitriptyline—Hypothesis: Amitriptyline detections occur only in watersheds with developed land use or lake shoreline. The frequency of amitriptyline detection increases with developed land use in the watershed. There was no significant difference in the percentage of developed land use for lakes with amitriptyline detections compared with lakes without amitriptyline detections. Although it was not detected in more sparsely populated northeastern Minnesota (Figure 3B), it was detected in lakes with no developed land use (Figure 5C). Thus, this hypothesis is rejected.

DISCUSSION

The data presented here and in a smaller study of Minnesota lakes [7] reveal that lake surface water is prone to contamination

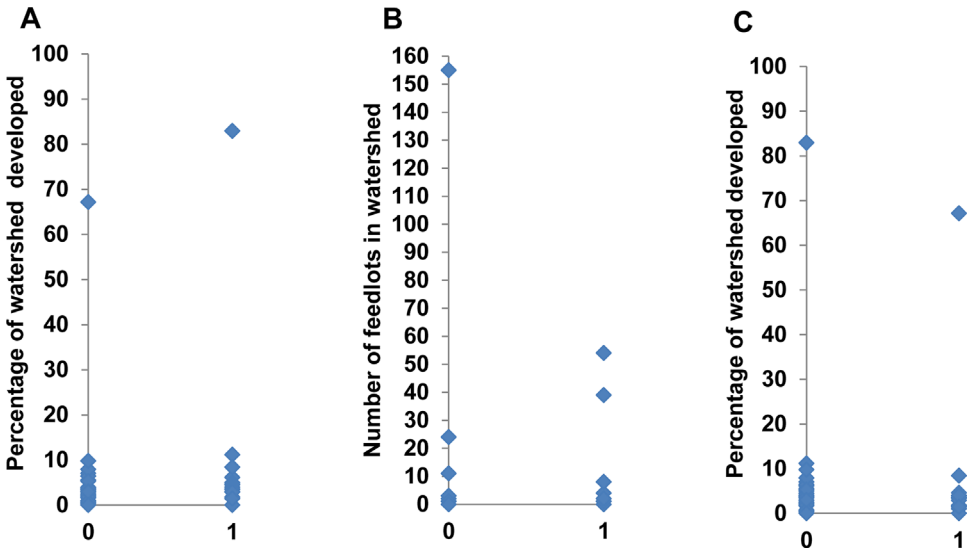

Figure 5. Proportion of watershed land use composition versus detections for cocaine (**A**), carbadox (**B**), and amitriptyline (**C**). Axis category labels: 0 = nondetect, 1 = detect.

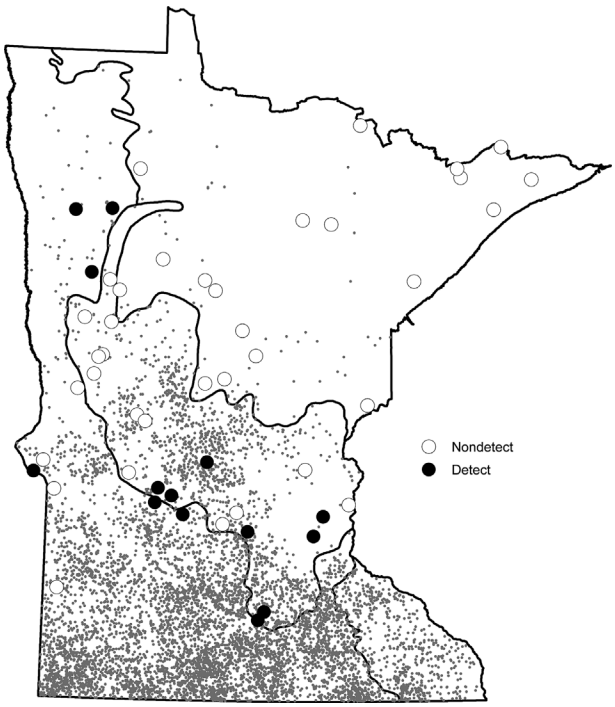

Figure 6. Carbadox detects versus nondetects compared with the statewide distribution of mapped swine feedlots (locations plotted in fine black dots).

by many of the same chemicals that are typically associated with wastewater, with 47 of the 50 lakes sampled in the present study containing at least 1 of the 125 pharmaceuticals and other contaminants analyzed for the present investigation. Although the maximum concentrations of some analytes corresponded to likely, proximate sources, we found no clear associations of land use categories (e.g., cropped or forested) with the overall frequency of detection of any of these contaminants. Moreover, some of the individual lakes where contaminants were found in the present study are characterized by an absence of shoreline development, making it difficult, in many cases, to comprehend how these chemicals reach them. Lake MN-160, for example, has no public access, no watershed development, and no nearby feedlot (Table 1) but contained DEET, cocaine, amitriptyline, and sulfamerazine (Table 4). The frequent detection of carbadox, a veterinary antibiotic approved only for use in the rearing of swine, further illustrates how the presence of a contaminant was not always associated with nearby sources. Some detections of carbadox were in areas with swine feedlots; however, we were generally unable to find convincing correlations between the detection of carbadox in lake water with nearby swine production facilities. These results lead us to suspect that the widespread occurrence of several of these chemicals is in part the result of atmospheric transport.

Atmospheric dispersion of chemicals throughout the environment via airborne particulate matter is established by other studies, including the detection of pesticides in remote mountain snowpack [22], the deposition of polychlorinated biphenyls in remote lakes [23], and the presence of atrazine in the Boundary Waters Canoe Area Wilderness (MN, USA) [24]. Cocaine is commonly present in air samples in some urban areas [25,26] and is associated with airborne particulate matter less than 2.5 μm in size [26], providing a means for the widespread atmospheric transport of this contaminant. Others have reported that a ratio of the concentration of cocaine to its metabolite benzoylecgonine of 1.2 to 2.0 indicates that excretion is the source of cocaine in water [27]. In the present study, the median ratio of cocaine to benzoylecgonine in samples where they co-occurred was 0.9, and 75% of the cocaine to benzoylecgonine ratios were less than 1.2. These data imply that cocaine found in several of the lakes was the result of some form of direct atmospheric deposition and not the transport of metabolized cocaine in wastewater. Although elucidating the mechanism of transport to lakes was beyond the scope of the present investigation, a plausible explanation for the widespread appearance of bisphenol A, amitriptyline, DEET, cocaine, carbadox, and other contaminants involves atmospheric transport via fine particulates and subsequent deposition to lakes.

Aside from mechanism of transport, the statistically significant increase in the number of detections when disturbed watershed land use increased above 42% suggests that the distribution of contaminants is not entirely random. This threshold is quite similar to the 40% disturbed land use threshold found by Cross and Jacobson [28] in their study of phosphorus concentrations in 1330 lakes in Minnesota.

Environmental effects of pharmaceuticals and endocrine active chemicals

The risk that these contaminants pose to aquatic organisms or to humans at environmentally relevant concentrations is not clear. Often, the therapeutic dose of a pharmaceutical is used as the "point of departure" for estimates of risk. The concentrations at which we have detected pharmaceuticals in surface water are orders of magnitude below a typical therapeutic dose; in the case of fluoxetine, 5000 L of water at 4.5 ppt are needed to reach a typical therapeutic dose of 20 mg.

Restricting an assessment of risk to intended therapeutic dose, however, fails to encompass the full range of possible hazards that a biologically active chemical might pose to either humans or wildlife for several reasons. For example, pharmaceuticals that are designed for transdermal delivery, such as hormones, may have unintended, adverse effects at far lower concentrations to another organism if ingested. Moreover, a drug's therapeutic dose, developed solely for the purpose of treating an adult human, may adversely affect fish and wildlife at much lower concentrations, exemplified by the lethal effect of very small amounts of diclofenac on *Gyps* vultures [29]. In the absence of extensive environmental effect data for pharmaceuticals in surface water, we grouped pharmaceuticals that were detected in these lakes according to their intended physiological endpoints (Supplemental Data, Table S1) as described by their Anatomical Therapeutic Classification [30], which have been referenced elsewhere as useful in understanding additive effects for the drugs in drinking water [31]. Pharmaceuticals designed to affect the nervous system were the most frequently detected in the present study, totaling 46 detections in the 50 lakes sampled, and included 3 psychoanaleptics, 2 psycholeptics, and 1 antiepileptic. Cocaine, a serotonin–norepinephrine–dopamine reuptake inhibitor (a triple uptake inhibitor), is included in this category. Eight other pharmaceuticals found in the present study are designated antiinfectives and antibacterials, including 2 fluoroquinolone antibiotics and 5 sulfonamides, with a total of 18 detections of pharmaceuticals in this category (Table 6). Three of the pharmaceuticals detected are classified as having effects on the cardiovascular system, and 4 are classified as dermatologicals, including the antiseptic triclosan. All listed designations for the pharmaceuticals detected in the present study are included in Table 6.

Table 6. Tally of the frequency at which pharmaceuticals appear in lakes based on the physiological system they are designed to affect

	Primary ATC designation						
Lake ID	C	N	J	S	R	D	Pharmaceutical
MN-101						1	Triclosan
MN-103	1	3		1			Caffeine, gemfibrozil, amitriptyline, cocaine
MN-106		2		1			Caffeine, cocaine
MN-107		1	1				Fluoxetine, sulfamethazine
MN-111		1					Amitriptyline
MN-112		1				1	Cocaine, triclosan
MN-113		1		1			Cocaine
MN-114		1	1				Sulfathiazole, amitriptyline
MN-115		2		1	1	1	Caffeine, diphenhydramine, cocaine
MN-116		2	2				Caffeine, thiabendazole, benztropine, triclosan
MN-118[a]	2	4	3				Caffeine, carbamazepine, diphenhydramine, ofloxacin, sulfamethazine, sulfamethoxazole, gemfibrozil, alprazolam, meprobamate, valsartan
MN-119		2		1			Amitriptyline, cocaine
MN-120		1	1				Sulfamethizole, amitriptyline
MN-121		3					Caffeine, amitriptyline, cocaine
MN-122		1					Fluoxetine
MN-123		1		1			Cocaine
MN-127	1	1	2	1			Ciprofloxacin, dehydronifedipine, sulfathiazole, amitriptyline
MN-131	1	2	2	1			Sulfamethazine, sulfadimethoxine, amitriptyline, cocaine, desmethyldiltiazem
MN-135		1	1	1			Sulfathiazole, cocaine
MN-137		1					Fluoxetine
MN-143		2		1			Amitriptyline, cocaine
MN-144			1				Trimethoprim
MN-145	1	1					Dehydronifedipine, amitriptyline
MN-147					1		Theophylline
MN-152		2	1	1			Caffeine, sulfathizole, cocaine
MN-153		1		1			Cocaine
MN-157		1					Amitriptyline
MN-158		1					Amitriptyline
MN-160		2	1	1		1	Sulfamerazine, amitriptyline, cocaine
MN-163	1						Norverapamil
MN-167		2	1	1	1	1	Caffeine, diphenhydramine, sulfathiazole, cocaine
MN-170A		1					Benztropine
MN-171			1				Trimethoprim
MN-177		2	1				Cocaine, amitriptyline

[a]Lake 118 currently receives domestic wastewater effluent.
ATC = Anatomical therapeutic classification; C = cardiovascular; N = nervous; J = anti-infective; S = sensory; R = respiratory; D = dermatological.

Research focused on the biological effect of mixtures and of particular contaminants that were found in the present investigation reveals that many of these chemicals may exert adverse effects at parts per trillion concentrations at the genetic, cellular, organism, and population levels. At the genetic level, Martinović-Weigelt et al. [9] applied unsupervised transcriptomic assays to assess the genetic effects in fathead minnows exposed to surface water that contained wastewater-derived chemicals. They reported that the expression of genes associated with teleost brain–pituitary–gonadal–hepatic axis function was altered when these fish were exposed to surface water that contained endocrine active chemicals at concentrations similar to those reported in the present investigation. Pomati et al. [32] showed that combinations of pharmaceuticals at parts per trillion concentrations repress cell division, alter the genetic expression of estrogen receptors on cells, and affect the transcription of DNA in human embryonic cells and in fish cells [10]. Parolini and Binelli [33] observed that diclofenac, gemfibrozil, and carbamazepine were toxic to zebra mussel hemocytes, gill cells, and digestive gland cells in the parts per billion range.

At the organism level, Painter et al. [4] found that antidepressants at environmentally relevant concentrations affect the escape responses of fathead minnow larvae, suggesting that even a short-term exposure to these chemicals at concentrations often found in the aquatic environment can disrupt a critical stage of physiological development in this species. Similarly, male fathead minnows that were exposed to nonylphenol at concentrations commonly found in the aquatic environment produced vitellogenin, the protein associated with the development of eggs in female fish. Exposure to nonylphenol also alters the ability of males to compete for nesting sites, implying that the reproductive competence of male minnows was compromised by this chemical [6]. Purdom [34] showed that male rainbow trout were feminized after exposure to wastewater effluent, demonstrating that chemicals in treated wastewater—and often in lakes—are estrogenic. Several pharmaceuticals, including antidepressants and carbamazepine, are detectable in fish tissues when fish are exposed to these pharmaceuticals in the environment [35].

Other research has demonstrated that pharmaceuticals have effects at the population level. Kidd et al. [11] demonstrated that the addition of the contraceptive ethinyl estradiol to a lake at 5 ppt caused the collapse of the fathead minnow population. Subsequent study of fathead minnows in mesocosms by Schwindt et al. [36] suggests that ethinyl estradiol has a permanent and transgenerational effect on reproduction and survival in these fish. Fluvoxamine and other selective serotonin

reuptake inhibitors have a dramatic effect on the spawning of freshwater mussels [37].

Although the biological effects of cocaine in the aquatic environment are not known, it has been shown to accumulate in eel tissue when these organisms are exposed to concentrations similar to those reported in the present study, raising concerns that long-term exposure to this neurologically active chemical might affect the reproductive behavior of aquatic organisms [38]. Benzoylecgonine caused sublethal but "notable adverse effects" in studies of freshwater mussels at 500 ppt [38,39]. Benzoylecgonine concentrations up to 740 ppt have been found in effluent-dominated surface water. In the present study, cocaine and benzoylecgonine were detected at up to 5.3 ppt and 9.5 ppt, respectively. Although cocaine has been reported in surface water down gradient of WWTPs [40–42], in sewage, and in biosolids collected from WWTPs [43], to our knowledge the present study is the first report of this drug in lakes that are unaffected by sources of wastewater.

A largely unexplored variable in determining the overall risk that these contaminants pose to our ecosystems is the degree to which they are recalcitrant to physical or biological degradation. Chemicals that resist anaerobic degradation, such as nonylphenol, can accumulate in sediment to levels that may adversely affect benthic organisms [3,44]; and a previous study suggests that concentrations of some contaminants are orders of magnitude higher in freshwater sediment than the surface water [7]. However, for most of the contaminants that were analyzed in the present investigation, information regarding their persistence in the surface water and sediment is not available.

In total, 276 pharmaceuticals, personal care products, and other commercially available chemicals have been analyzed (though not all detected) in Minnesota's surface water since 2008. These studies have shown that a large variety of hormones, surfactants, fragrances, pharmaceuticals, and personal care products are widespread in river and lake surface water [3,7]. Bisphenol A, DEET, carbamazepine, sulfonamide antibiotics, the alkylphenols octylphenol and nonylphenol, and alkylphenol ethoxylates have been found repeatedly in surface water and bed sediment. Bisphenol A, for example, was detected in 45% of the lakes in a previous lake study [7] and was again found in 42% of the lakes in the present study, suggesting that there is a constant flux of this chemical to our aquatic environment. The rates of detection for sulfonamide drugs and alkylphenols reported in the present study are also similar to previous analyses of surface water in the state.

However, the present study is is the first with a broad enough scope and randomized design to allow a statistical evaluation of the proportion of lakes in the state likely to contain a given contaminant based on the frequency of detection and a statistical weighting based on lake size (Table 5). The benefit of this condition estimate approach is that it provides a more accurate, and sometimes much different, perspective on the extent to which lakes are actually contaminated by these chemicals than what can be gained through simple detection frequency. Carbadox, for example, was detected in 28% of the sampled lakes; however, we estimate that a much lower proportion of the total number of lakes actually contained this antibiotic statewide. By contrast, the analysis of detection data for amitriptyline, which was found in 28% of the sampled lakes, indicated that it was likely present in 24% of the lakes across the state. These results demonstrate the benefit of, and the need for, a statistical treatment of detection data on which to base accurate conclusions about the true occurrence of these chemicals in the aquatic environment on a broader scale.

Table 7. Analytes detected in both lake water [7, present study] and water downstream of wastewater-treatment plants in Minnesota [3]

Analytes detected
17β-Estradiol
2,6-Di-*tert*-butyl-1,4-benzoquinone
4-Nonylphenol
4-Nonylphenol diethoxylate
4-Nonylphenol monoethoxylate
4-Nonylphenol triethoxylate
4-*tert*-Octylphenol
4-*tert*-Octylphenol diethoxylate
5-Methyl-1H-benzotriazole
Bisphenol A
Caffeine
Carbamazepine
Cholesterol
Coprostanol
Diphenhydramine
Estrone
Fluoxetine
Mestranol
DEET
Sulfamethoxazole
Triclosan
Trimethoprim

DEET = *N,N*-diethyl-m-toluamide.

From a more qualitative perspective, these results clearly demonstrate that pharmaceuticals and other chemicals most often associated with sewage are not restricted to surface water dominated by wastewater effluent. Inspection of Table 4 reveals that the highest concentrations for 9 of the 11 most frequently detected contaminants in the present study were not seen in the 1 lake that receives treated wastewater effluent (MN-118) but were distributed among lakes of varying land use, including some lakes that have no identifiable sources of the contaminants we detected. Twenty-two chemicals have been detected in surface water downstream of WWTPs [3] and in lake water in Minnesota, including pharmaceuticals, alkylphenols, alkylphenol ethoxylates, bisphenol A, the anticorrosive benzotriazole, and steroidal hormones (Table 7). Although WWTPs, septic systems, storm water runoff, agriculture, and areal transport are all sources of these contaminants to lakes, rivers, and streams, the degree to which any of these sources contributes particular chemicals to the environment is still not well understood. Further study on the presence of these contaminants in surface water should include investigations of how various sources, including airborne fine particulate matter, contribute contaminants to the aquatic environment.

SUPPLEMENTAL DATA

Tables S1–S5.
Lists S1, S3, S4, and S5. (1.3 MB DOC).
Figures S1–S3. (215 KB PDF).

Acknowledgment—The authors deeply appreciate the long hours, commitment, and hard work of our sampling crews that made this project possible: P. Anderson, J. Anderson, K. O'Hara, L. Engel, D. Richter, A. Garcia, C. Ahlers-Nelson, M. Kennedy, A. Swanson, and student workers W. Long and B. Larson. We also thank W. Bouchard for statistical analysis of data, K. Anderson for the attention to work plan and logistical details, and S. Nelson for the geographical information system assistance. This project was made possible by financial support from the Minnesota Clean Water Fund.

Data availability—Any of the data or methods used to generate conclusions or statistical derivations are available from the authors upon request. Inquiries should be directed to M. Ferrey, Minnesota Pollution Control Agency (mark.ferrey@state.mn.us).

REFERENCES

1. Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, Buxton HT. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999–2000: A national reconnaissance. *Environ Sci Technol* 36:1202–1211.
2. Focazio MJ, Kolpin DW, Barnes KK, Furlong ET, Meyer MT, Zaugg SD, Barber LB, Thurman EM. 2008. A national reconnaissance for pharmaceuticals and other organic wastewater contaminants in the United States—II) Untreated drinking water sources. *Sci Total Environ* 402:201–216.
3. Lee KE, Langer SK, Barber LB, Writer JH, Ferrey M, Schoenfuss HL, Gray JL, Revello RC, Martinovic D, Woodruff OP, Keefe SH, Brown GK, Taylor HE, Ferrer I, Thurman EM. 2011. Endocrine active chemicals, pharmaceuticals, and other chemicals of concern in surface water, wastewater effluent, and bottom sediment in Minnesota—Site description, methods, and data. US Geological Survey Data Series 575. Reston, VA.
4. Painter MM, Buerkley MA, Julius ML, Vajda AM, Norris DO, Barber LB, Furlong ET, Schultz MM, Schoenfuss HL. 2009. Antidepressants at environmentally relevant concentrations affect predator avoidance behavior of larval fathead minnows (*Pimephales promelas*). *Environ Toxicol Chem* 28:2677–2684.
5. Hinck JE, Blazer VS, Schmitt CJ, Papoulias DM, Tillitt DE. 2009. Widespread occurrence of intersex in black basses (*Micropterus* spp.) from U.S. rivers, 1995–2004. *Aquat Toxicol* 95:60–70.
6. Schoenfuss HL, Bartell SE, Bistodeau TB, Cediel RA, Grove KJ, Zintek L, Lee KE, Barber LB. 2008. Impairment of the reproductive potential of male fathead minnows by environmentally relevant exposures to 4-nonylphenol. *Aquat Toxicol* 86:91–98.
7. Writer JH, Barber LB, Brown GK, Taylor HE, Kiesling RL, Ferrey ML, Jahns ND, Bartell SE, Schoenfuss HL. 2010. Anthropogenic tracers, endocrine disrupting chemicals, and endocrine disruption in Minnesota lakes. *Sci Total Environ* 409:100–111.
8. Jobling S, Nolan M, Tyler CR, Brighty G, Sumpter JP. 1998. Widespread sexual disruption in wild fish. *Environ Sci Technol* 32:2498–2506.
9. Martinović-Weigelt D, Mehinto AC, Ankley GT, Denslow ND, Barber LB, Lee KE, King RJ, Schoenfuss HL, Schroeder AL, Villeneuve DL. 2014. Transcriptomic effects-based monitoring for endocrine active chemicals: Assessing relative contribution of treated wastewater to downstream pollution. *Environ Sci Technol* 48: 2385–2394.
10. Pomati F, Cotsapas CJ, Castiglioni S, Zuccato E, Calamari D. 2007. Gene expression profiles in zebrafish (*Danio rerio*) liver cells exposed to a mixture of pharmaceuticals at environmentally relevant concentrations. *Chemosphere* 70:65–73.
11. Kidd KA, Blanchfield PJ, Mills KH, Palace VP, Evans RE, Lazorchak JM, Flick RW. 2007. Collapse of a fish population after exposure to a synthetic estrogen. *Proc Natl Acad Sci U S A* 104:8897–8901.
12. Blair BD, Crago JP, Hedman CJ, Klaper RD. 2013. Pharmaceuticals and personal care products found in the Great Lakes above concentrations of environmental concern. *Chemosphere* 93:2116–2123.
13. Heiskary SA, Wilson CB. 2008. Minnesota's approach to lake nutrient criteria development. *Lake Reserv Manag* 24:282–297.
14. US Environmenal Protection Agency. 2011. National Lakes Assessment 2012: A fact sheet for communities. EPA 841/F-11/007. Washington, DC.
15. Stevens DL, Olsen AR. 2004. Spatially balanced sampling of natural resources. *J Am Stat Assoc* 99:262–278.
16. Peck DV, Olsen AR, Weber MH, Paulsen SG, Peterson C, Holdsworth SM. 2013. Survey design and extent estimates for the National Lakes Assessment. *Freshw Sci* 32:1231–1245.
17. US Environmenal Protection Agency. 2007. Method 1694: Pharmaceuticals and personal care products in water, soil, sediment, and biosolids by HPLC/MS/MS. EPA 821/R-08/002. Washington, DC.
18. US Environmenal Protection Agency. 1999. Protocol for EPA approval of new methods for organic and inorganic analytes in wastewater and drinking water. EPA 821/B-98/003. Washington, DC.
19. R Development Core Team. 2008. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.
20. Kincaid T. 2005. *User Guide for spsurvey.analysis_2.9 Probability Survey Data Analysis Functions*. US Environmental Protection Agency. [cited 2012 December 19] Available from: http://www.epa.gov/nheerl/arm/analysispages/user_guide.htm
21. Qian SS, King RS, Richardson CJ. 2003. Two statistical methods for the detection of environmental thresholds. *Ecol Model* 166:87–97.
22. Hageman KJ, Simonich SL, Campbell DH, Wilson GR, Landers DH. 2006. Atmospheric deposition of current-use and historic-use pesticides in snow at national parks in the western United States. *Environ Sci Technol* 40:3174–3180.
23. Swackhamer DL, Mcveety BD, Hites RA. 1988. Deposition and evaporation of polychlorobiphenyl congeners to and from Siskiwit Lake, Isle Royale, Lake Superior. *Environ Sci Technol* 22:664–672.
24. Minnesota Department of Agriculture. 2008. Minnesota National Lakes Assessment Project: Pesticides in Minnesota lakes. [cited 2015 April] Available from: http://www.mda.state.mn.us/news/publications/chemfert/pesticidesinmnlakes.pdf
25. Cecinato A, Balducci C, Nervegna G. 2009. Occurrence of cocaine in the air of the world's cities: An emerging problem? A new tool to investigate the social incidence of drugs? *Sci Total Environ* 407:1683–1690.
26. Viana M, Querol X, Alastuey A, Postigo C, De Alda MJL, Barceló D, Artíñano B. 2010. Drugs of abuse in airborne particulates in urban environments. *Environ Int* 36:527–534.
27. Postigo C, Lopez De Alda MJ, Barceló D. 2008. Analysis of drugs of abuse and their human metabolites in water by LC-MS2: A non-intrusive tool for drug abuse estimation at the community level. *Trends Anal Chem* 27:1053–1069.
28. Cross TK, Jacobson PC. 2013. Landscape factors influencing lake phosphorus concentrations across Minnesota. *Lake Reserv Manag* 29:1–12.
29. Oaks JL, Gilbert M, Virani MZ, Watson RT, Meteyer CU, Rideout BA, Shivaprasad HL, Ahmed S, Chaudhry MJ, Arshad M, Mahmood S, Ali A, Khan AA. 2004. Diclofenac residues as the cause of vulture population decline in Pakistan. *Nature* 427:630–633.
30. World Health Organization Collaborating Centre for Drug Statistics Methodology. 2015. *ATC/DDD Index 2015*. [cited 2014 December 29] Available from: http://www.whocc.no/atc_ddd_index/
31. Daughton CG. 2010. Pharmaceutical ingredients in drinking water: Overview of occurrence and significance of human exposure. In Halden RU, ed, *Contaminants of Emerging Concern in the Environment: Ecological and Human Health Considerations*. American Chemical Society, Washington, DC, pp 9–68.
32. Pomati F, Castiglioni S, Zuccato E, Fanelli R, Vigetti D, Rossetti C, Calamari D. 2006. Effects of a complex mixture of therapeutic drugs at environmental levels on human embryonic cells. *Environ Sci Technol* 40:2442–2447.
33. Parolini M, Binelli A. 2012. Sub-lethal effects induced by a mixture of three non-steroidal anti-inflammatory drugs (NSAIDs) on the freshwater bivalve *Dreissena polymorpha*. *Ecotoxicology* 21:379–392.
34. Purdom CE, Hardiman PA, Bye VJ, Eno CF, Tyler CR, Sumpter JP. 1994. Estrogenic effects of effluents from sewage treatment works. *Chem Ecol* 8:275–285.
35. Ramirez AJ, Brain RA, Usenko S, Mottaleb MA, O'Donnell JG, Stahl LL, Wathen JB, Snyder BD, Pitt JL, Perez-Hurtado P, Dobbins LL, Brooks BW, Chambliss CK. 2009. Occurrence of pharmaceuticals and personal care products in fish: Results of a national pilot study in the United States. *Environ Toxicol Chem* 28:2587–2597.
36. Schwindt AR, Winkelman DL, Keteles K, Murphy M, Vajda AM. 2014. An environmental oestrogen disrupts fish population dynamics through direct and transgenerational effects on survival and fecundity. *J Appl Ecol* 51:582–591.
37. Fong PP. 1998. Zebra mussel spawning is induced in low concentrations of putative serotonin reuptake inhibitors. *Biol Bull* 194: 143–149.
38. Capaldo A, Gay F, Maddaloni M, Valiante S, Falco M, Lenzi M, Laforgia V. 2011. Presence of cocaine in the tissues of the European eel, *Anguilla anguilla*, exposed to environmental cocaine concentrations. *Water Air Soil Pollut* 223:2137–2143.
39. Parolini M, Pedriali A, Riva C, Binelli A. 2013. Sub-lethal effects caused by the cocaine metabolite benzoylecgonine to the freshwater mussel *Dreissena polymorpha*. *Sci Total Environ* 444:43–50.
40. Postigo C, López De Alda MJ, Barceló D. 2010. Drugs of abuse and their metabolites in the Ebro River basin: Occurrence in sewage and surface water, sewage treatment plants removal efficiency, and collective drug usage estimation. *Environ Int* 36:75–84.

41. Zuccato E, Castiglioni S, Bagnati R, Chiabrando C, Grassi P, Fanelli R. 2008. Illicit drugs, a novel group of environmental contaminants. *Water Res* 42:961–968.
42. Bartelt-Hunt SL, Snow DD, Damon T, Shockley J, Hoagland K. 2009. The occurrence of illicit and therapeutic pharmaceuticals in wastewater effluent and surface waters in Nebraska. *Environ Pollut* 157:786–791.
43. Chari BP, Halden RU. 2012. Validation of mega composite sampling and nationwide mass inventories for 26 previously unmonitored contaminants in archived biosolids from the US National Biosolids Repository. *Water Res* 46:4814–4824.
44. Soares A, Guieysse B, Jefferson B, Cartmell E, Lester JN. 2008. Nonylphenol in the environment: A critical review on occurrence, fate, toxicity and treatment in wastewaters. *Environ Int* 34:1033–1049.