

Expanded Target-Chemical Analysis Reveals Extensive Mixed-Organic-Contaminant Exposure in U.S. Streams

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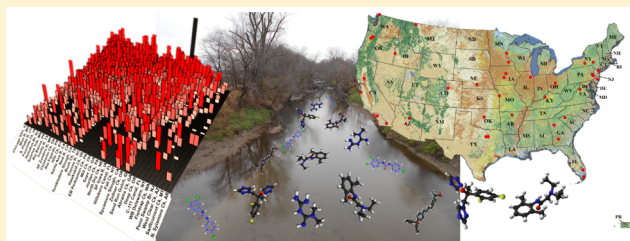
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S Supporting Information

ABSTRACT: Surface water from 38 streams nationwide was assessed using 14 target-organic methods (719 compounds). Designed-bioactive anthropogenic contaminants (biocides, pharmaceuticals) comprised 57% of 406 organics detected at least once. The 10 most-frequently detected anthropogenic-organics included eight pesticides (desulfinylflupronil, AMPA, chlorpyrifos, dieldrin, metolachlor, atrazine, CIAT, glyphosate) and two pharmaceuticals (caffeine, metformin) with detection frequencies ranging 66–84% of all sites. Detected contaminant concentrations varied from less than 1 ng L⁻¹ to greater than 10 μg L⁻¹, with 77 and 278 having median detected concentrations greater than 100 ng L⁻¹ and 10 ng L⁻¹, respectively. Cumulative detections and concentrations ranged 4–161 compounds (median 70) and 8.5–102 847 ng L⁻¹, respectively, and correlated significantly with wastewater discharge, watershed development, and toxic release inventory metrics. Log₁₀ concentrations of widely monitored HHCB, triclosan, and carbamazepine explained 71–82% of the variability in the total number of compounds detected (linear regression; *p*-values: < 0.001–0.012), providing a statistical inference tool for unmonitored contaminants. Due to multiple modes of action, high bioactivity, bioaccumulation, and direct environment application (pesticides), designed-bioactive organics (median 41 per site at μg L⁻¹ cumulative concentrations) in developed watersheds present aquatic health concerns, given their acknowledged potential for sublethal effects to sensitive species and lifecycle stages at low ng L⁻¹.



INTRODUCTION

Chemical-mixture exposures in streams are global concerns^{1,2} and the lack of information on the composition and associated effects of real-world-mixture exposures are fundamental obstacles to aquatic-ecosystem risk assessment.^{3–9} Comprehensive targeted-chemical surface water assessments are expensive and, consequently, uncommon^{10,11} despite the

acknowledged importance of improved understanding of surface water contaminant complexity^{2,8,12} and the direct

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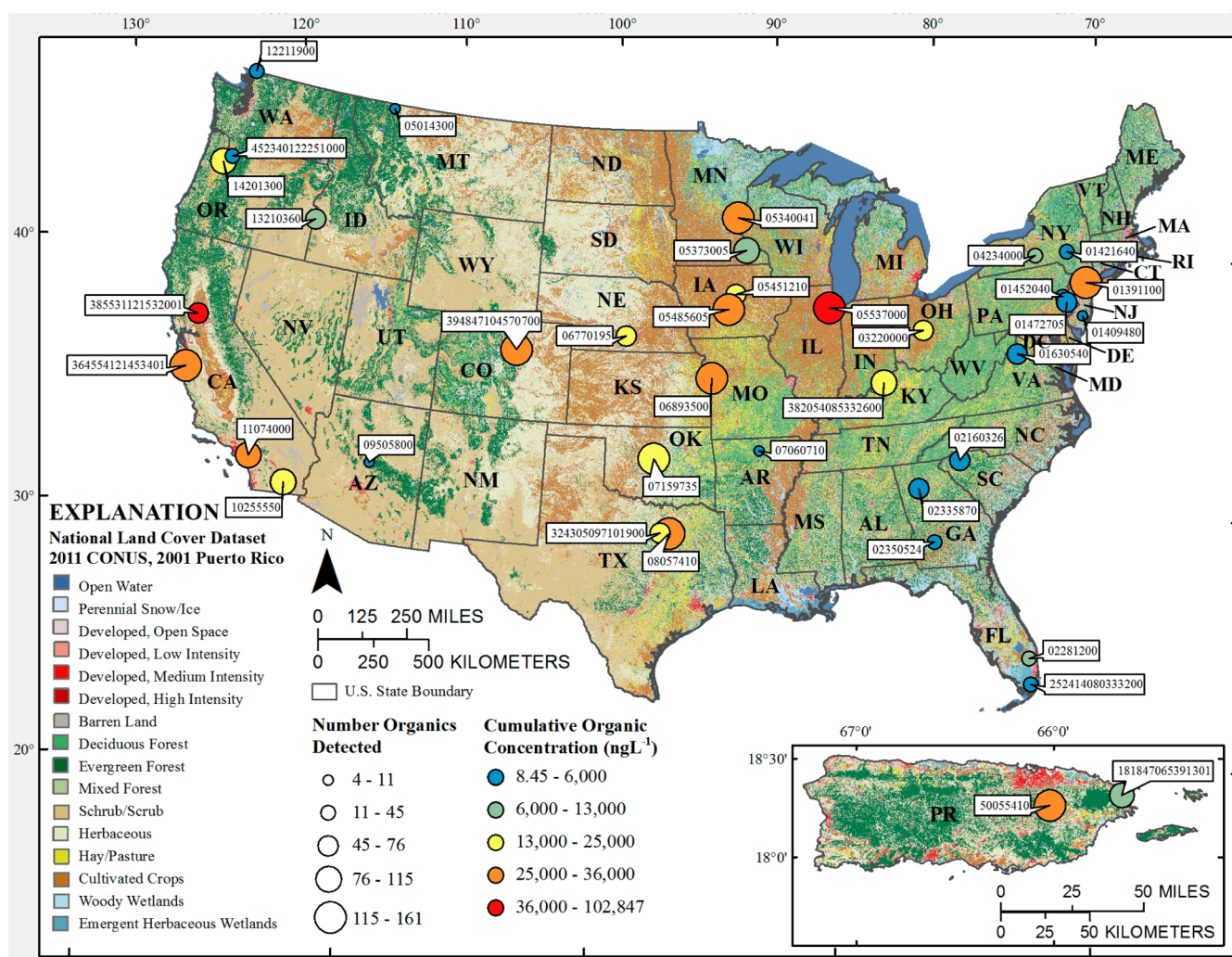


Figure 1. Total numbers and cumulative concentrations (ng L⁻¹) of organic analytes detected in water during 2012–2014 sampling of 38 stream sites (SI Table S1) across the nation. Numeric labels indicate USGS NWIS⁹² stream station IDs.

influence of the scope of contaminant characterization on perceived risks to stream health.^{2,12} Combined approaches incorporating chemical and effects-based analyses simultaneously preserve targeted-chemical forensic capabilities, interrogate cumulative bioactivities at multiple molecular end points without prior knowledge of mixture composition, and promote technical improvement via cross-examination of respective results mismatches.^{3,8,9,11,13–16} To that end, the U.S. Geological Survey (USGS) and U.S. Environmental Protection Agency (EPA) conducted an extensive, field-based, split-sample assessment of targeted-chemical composition (14 methods, 916 total analytes, 893 organic analytes) and cumulative biochemical activity of water samples from urban/agricultural-impacted streams across the United States during 2012–2014.⁷ The results of the target-organic, contaminant-mixture exposure assessment are presented herein; split-sample biological-activity results are presented separately (e.g., ref 17). Candidate contaminant subsets for improved multiresidue chemical screening⁸ and potential land-use land-cover (LULC) predictors of surface water organic-contaminant-mixture complexity (composition and concentrations) are also assessed, herein. Results of this overall effort are expected to yield insights into chemical mixtures present in a wide range of surface waters

across the USA and their potential for eliciting adverse ecological health consequences.

MATERIAL AND METHODS

Site Selection. Thirty-four urban/agricultural-impacted sites and 4 undeveloped sites spanning 24 States and Puerto Rico (Figure 1, Supporting Information (SI) Table S1) were selected based on data from more than a thousand stream sites assessed in previous USGS studies of contaminants of emerging environmental concern.⁷ Upstream drainage areas and population densities ranged 12–16 000 km² and 0–1200 people per km², respectively.^{7,18} Site selection and sample collection timing focused on capturing a range of mixed-contaminant exposure profiles; no effort was made to address site-specific temporal variability or to target high-impact contamination events, such as seasonal pesticide application windows.

Sampling Methods. Water samples were collected once from each stream during November 2012 to June 2014. Sampling protocols, bottles, preservation supplies, preprinted laboratory request, and chain of custody forms, shipping labels, and cooler packing diagrams were provided to each sampling team, to ensure consistency in sampling methods. Grab samples were collected (approximately 1 h total sampling time) from

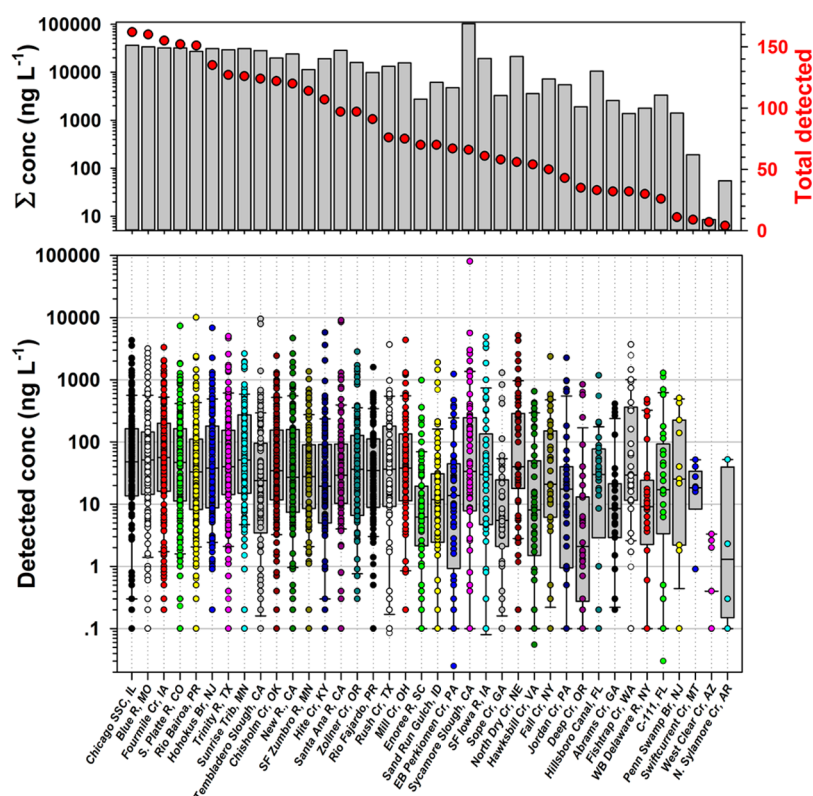


Figure 2. Top: Total numbers (red circles) and cumulative concentrations (ng L^{-1} ; bars) of organic analytes detected in water by site during 2012–2014 sampling of 38 streams across the nation. Bottom: Concentrations (ng L^{-1} , circles) of individual organics detected. Boxes, centerlines, and whiskers indicate interquartile range, median, and 5th and 95th percentiles, respectively.

the center of flow at each site by USGS staff using established trace-level protocols.^{19–23} Water samples were homogenized in Teflon churns, decanted into individual containers, and shipped overnight on ice to USGS and EPA laboratories for analysis. Complete sampling details are provided elsewhere.¹⁸

Water Chemistry Assessment. Surface-water samples were analyzed using 14 methods for a total of 893 targeted organic analytes (719 nonredundant) at 3 USGS laboratories: National Water Quality Laboratory (NWQL), in Denver, Colorado (volatile organic compounds;²⁴ steroid hormones and related compounds;²⁵ wastewater indicator compounds;^{26,27} human-use pharmaceuticals, pharmaceutical metabolites, and polar organic compounds;²⁸ pesticides and pesticide metabolites;^{29–31} and halogenated organic compounds¹⁸); Organic Geochemistry Research Laboratory (OGRL), in Lawrence, Kansas (acetamide herbicides and degradation products;³² glyphosate, glufosinate, and aminomethylphosphonic acid;³³ steroid hormones and phytoestrogens;³⁴ triazine and phenyl-urea herbicides;³⁵ and antibiotics³³); and Organic Chemistry Research Laboratory (OCRL), in Sacramento, California (pesticide and pesticide degradates;³⁶ diuron, diuron degradates, and neonicotinoid insecticides;³⁷ and disinfection byproducts³⁸). This study included filtered and unfiltered sample methods, as noted in SI Table S3. Method details and complete results are provided at.^{18,39,40}

Quality Assurance Quality Control (QAQC). Field blanks and matrix spikes were completed at 15 and 13 sites, respectively, with 2–8 field blanks and 3–4 matrix spikes per method.¹⁸ Nine organics were detected (once each) in both blank and environmental samples. Because the median sample concentration exceeded the blank concentration, data for seven

of these are presented without censoring (SI Table S2); interpretation of environmental results below blank detection levels warrants caution. Because blank concentrations exceeded the median sample concentrations, hexachlorobenzene and pentachloroanisole data were censored to nondetect for this publication (SI Table S2). QAQC details are provided elsewhere.¹⁸

Statistical Analyses. Laboratory-reporting levels (LRL) were determined for each analyte based on the long-term method detection level (MDL).^{41,42} Detections between the LRL and MDL are confirmed identifications but considered semiquantitative (estimated).⁴¹ Results below the MDL were reported as censored ($<\text{LRL}$);⁴¹ censored data were set to half the LRL and estimated values were used as reported, for statistical analyses.

Site groupings with statistically distinct contaminant profiles were identified by unconstrained divisive cluster analysis (UncTree) of fourth-root-transformed data and statistical-significance testing (SimProf; $\alpha = 0.05$) routines of Primer 7 (PRIMER-E Ltd., Plymouth, UK^{43–45}). Statistical relations between Euclidean-distance-resemblance matrices for detected-contaminant and LULC were assessed by permutation-based, nonparametric, multivariate analysis (Primer 7).^{43–45} Resemblance matrices were calculated on log-transformed and normalized data to address method-related differences in reporting units and, thus, censoring levels.^{43–45} Nonmetric multidimensional scaling (NMDS), one-way analysis of similarity (ANOSIM), and permutation-based cophenetic correlation (RELATE, permutations = 999) routines (Primer 7)⁴⁵ were used to explore relationships between site-specific detected-contaminant and LULC resemblance matrices.

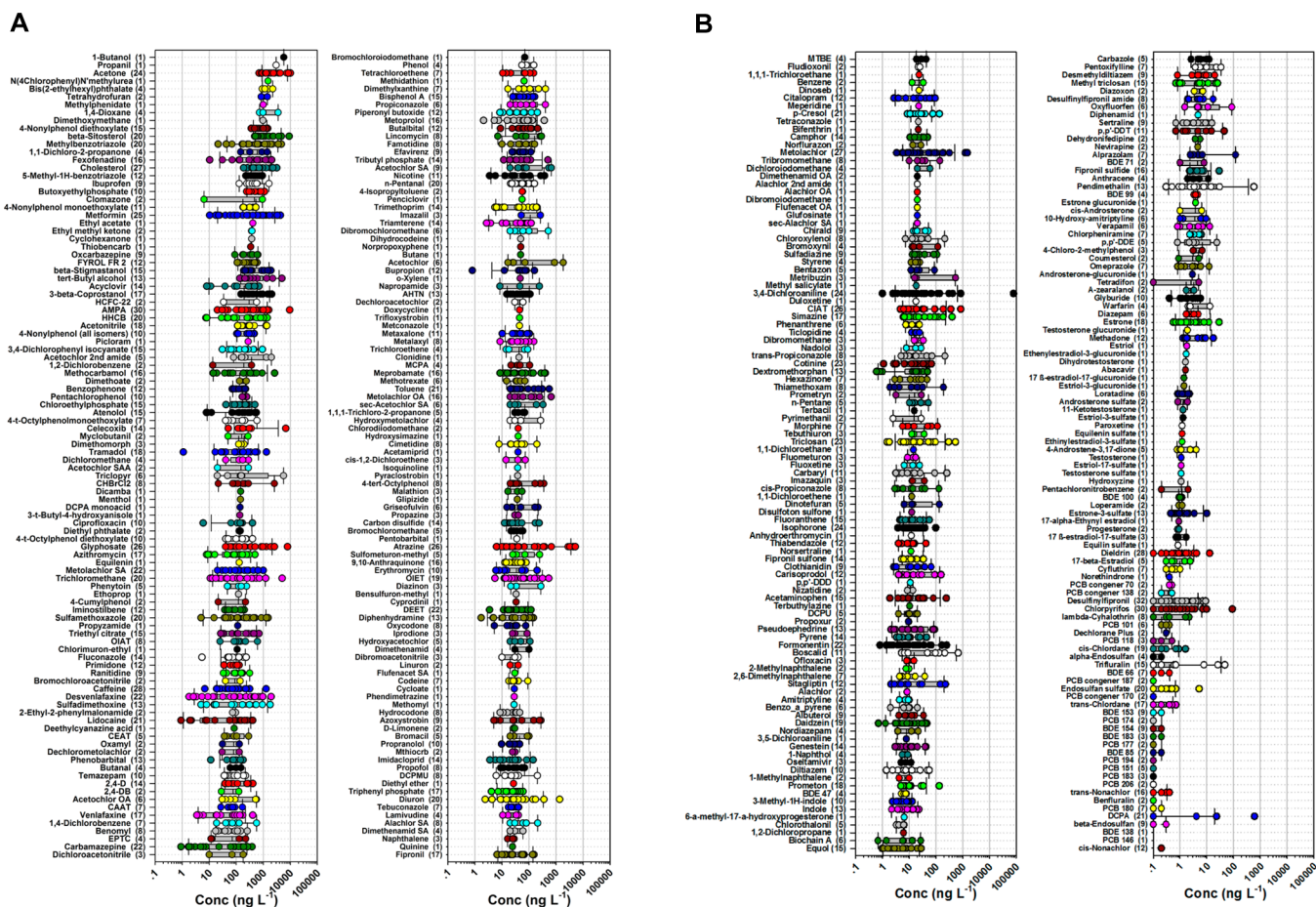


Figure 3. Detected concentrations (circles, ng L^{-1}) and number of sites (in parentheses) for 389 organic analytes (in order of decreasing median detected concentration, top to bottom left to right: 3a. 1–200; 3b. 201–389) in water samples during 2012–2014 sampling of 38 streams across the nation. Circles are data for individual samples. Boxes, centerlines, and whiskers indicate interquartile range, median, and 5th and 95th percentiles, respectively.

Subsequently, correlations between site-specific summary contaminant metrics (cumulative, maximum, and median concentrations/detections) and individual LULC metrics, as well as individual contaminant metrics, were assessed by Spearman Rank Correlation (SigmaPlot 13, Systat Software, San Jose, CA).

RESULTS AND DISCUSSION

Building on prior studies largely in Europe (see for overview⁴⁶), in 1999–2000 the USGS Toxics program surveyed 139 stream sites across the U.S. using five target-organic-chemical methods (95 total organic waste analytes; geospatial-chemical space of 13k cells);¹⁰ this study established the ubiquity and corresponding geospatial importance of organic contaminants of emerging concern as potential drivers of aquatic-ecosystem and downstream human health in the U.S.⁴⁷ The Kolpin et al. study¹⁰ also provided one of the earliest glimpses into the complexity of surface water organic-contaminant mixtures, despite the fact that comprehensive chemical characterization was not a study objective and methods for some well-established environmental-contaminant classes (e.g., pesticides and VOC) were not included.

In 2012–2014 the USGS and EPA conducted a follow-up survey of surface water samples from 38 streams across the nation employing a substantially expanded target-organic-analysis toolbox (14 chemical methods covering 719 unique

compounds; a 2-fold increase in geospatial-chemical space to 27k cells), to more realistically characterize surface water bioactive-contaminant space (contaminant-mixture complexity). Complementary biological-effects assays were employed to link chemical exposures with the potential for associated stream-ecosystem health effects. The current results represent one of the most comprehensive quantitative target-organic-contaminant assessments conducted to date, particularly with respect to polar organics (see⁴⁸ for regional semivolatile organic compound analysis approach), and, to our knowledge, the most comprehensive national/regional-scale investigation involving split-sample chemical/effects-based analyses (see for comparison: 42 sites in 37 drainage basins and 33 target organics;⁴⁹ 18 Great Lakes Basin sites and 132 target organics;^{11,13,50} 9 sites, 3 streams and 405 target organics⁵¹). The national-scale urban/agricultural-development gradient provides a unique opportunity to assess the scope and occurrence frequency of in-stream contaminants, the range and site-to-site variability of in-stream-contaminant complexity, and the associated relation to readily available, watershed-scale LULC metrics.

HUNDREDS OF BIOACTIVE CONTAMINANTS DETECTED IN U.S. STREAMS

Accounting for method overlap, the 893 organic-chemical analytes targeted in this study corresponded to 719 unique organic compounds (SI Table S3). This exceeds the number of

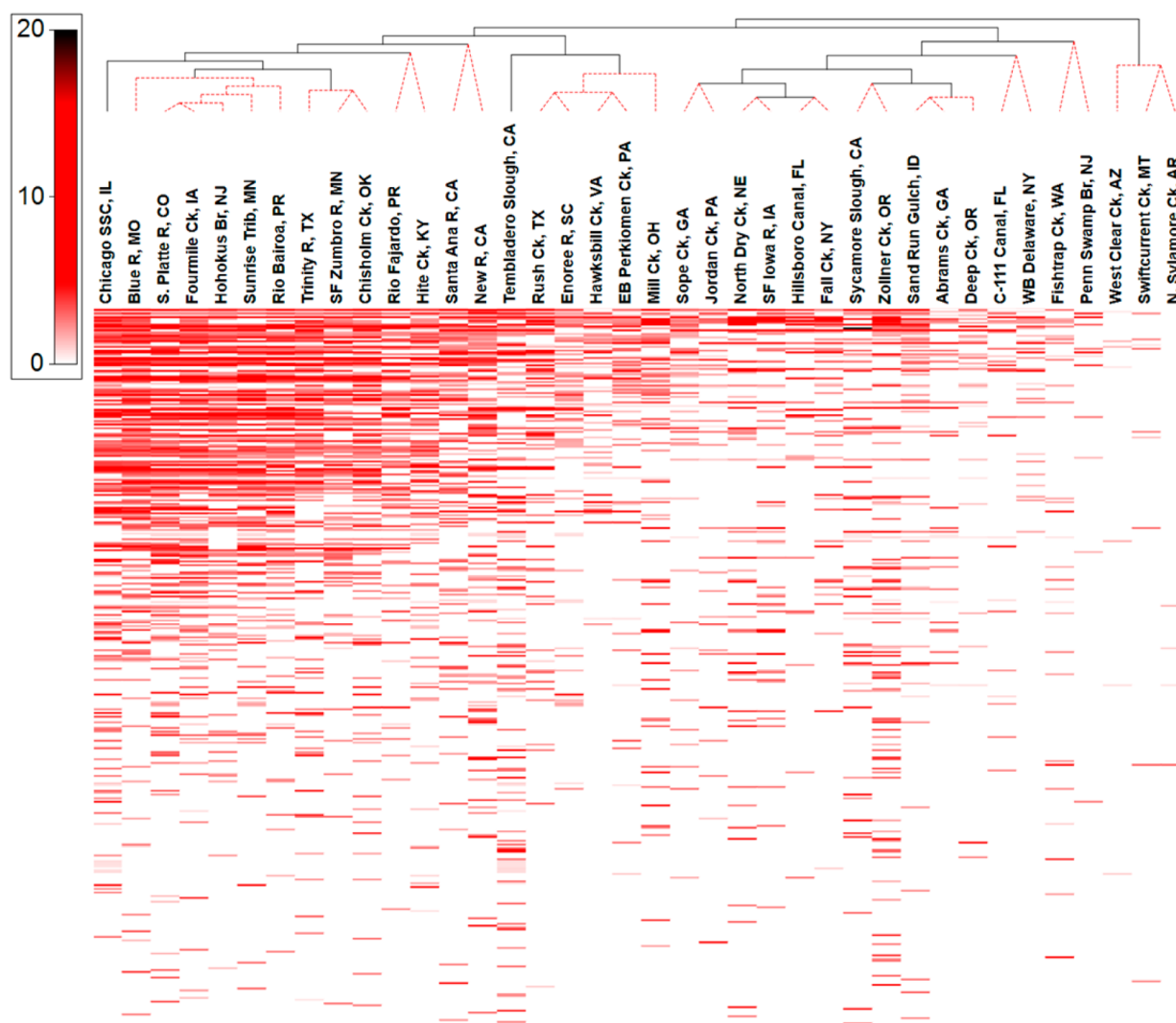


Figure 4. Shadeplot of concentrations (scale in $\mu\text{g L}^{-1}$) of 406 detected organic analytes (unlabeled, top to bottom in order of decreasing median detected concentration) in water from 38 stream sites, clustered (UncTree) by mixture pattern. White indicates less than MDL (minimum detection limit). Dashed-red dendrogram lines indicate sites not statistically different (SimProf; $\alpha = 0.05$).

contaminant classes (e.g., 433 pesticide and degradates,¹² 107 pharmaceuticals⁵²) or total analytes (e.g., 95–405 mixed, polar-organic contaminants in^{1,2,10,13,50,51,53–55}) assessed quantitatively in previous target-chemical-based, regional/national investigations. Approximately 57% of these unique organics (406/719) were detected at least once across all sites (Figures 1, 2, and 3, SI Table S3). Bioactive-anthropogenic-organic compounds (biocides [pesticides and antimicrobials] and pharmaceuticals) comprised 55% of the targeted organic analytes and 57% of those detected at least once in this study. The following discussion of frequently detected anthropogenic-organic contaminants excludes naturally occurring sterols (cholesterol, coprostanol, B-sitosterol, etc.), which are not uniquely associated with contamination.

The 10 most frequently detected anthropogenic-organic contaminants (not including cholesterol) were all designed-bioactive chemicals (Figure 3; Table 3). Eight were pesticides or pesticide degradates (desulfinylfipronil, AMPA [α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid], chlorpyrifos, diel-drin, metolachlor, atrazine, CIAT [deethyl atrazine], glyphosate), with detection frequencies ranging from 26 to 32 sites. Desulfinylfipronil (fipronil-insecticide degradate) was pervasive,

occurring at 32 sites (84%). The broad-spectrum herbicide, glyphosate, or its common environmental degradate, AMPA (also potential organophosphonate degradate^{56,57}), was detected at 30 sites (79%). Likewise, the broad-spectrum herbicide, atrazine, or its environmental degradate, CIAT, was detected at 27 sites (71%). The remaining two were caffeine (28 sites, 74%) and the antidiabetic medication, metformin (25 sites, 66%). High detection frequencies for caffeine and metformin in this study are consistent with but notably lower than those (92% and 97% of sites, respectively) reported in a recent survey of pharmaceutical contaminants in 59 wadeable Piedmont streams of the southeastern U.S.⁵² Caffeine was the second most frequently detected compound (77.5% of samples) in a recent survey of 33 trace organic contaminants in 37 stream basins across the U.S. by Bernot et al.,⁴⁹ the most frequently detected contaminant, sucralose (87.5% of samples), in Bernot et al.⁴⁹ was not assessed in the present study. Thirty compounds (not including cholesterol and β -sitosterol) were detected at more than 50% of the sites in the current study; of these, 21 (70%) had designed-bioactivities, including biocidal (13 pesticides or degradates), bacteriostatic (triclosan), and pharmacological (7 pharmaceuticals or metabolites/degradates)

activities. Designed-bioactivity is emphasized here as a conservative indicator of ecosystem concern, because established predictive-ecotoxicology models (e.g., Structure Activity Relationship, Quantitative Structure Activity Relationship) assume a nonspecific toxicity mode of action which can substantially underestimate molecularly targeted toxicity and a range of ecologically relevant, sublethal effects.^{58–61}

Concentrations of detected organic contaminants varied by more than 5 orders of magnitude from less than 1 ng L⁻¹ up to greater than 10 µg L⁻¹ (Figure 3, SI Table S3). The highest detected concentration by far was the greater than 80 µg L⁻¹ of 3,4-dichloroaniline in the sample from Sycamore Slough CA. Although 3,4-dichloroaniline has number of potential agricultural⁶² and industrial⁶³ sources, in this setting it is most readily attributed to environmental degradation of propanil, a contact herbicide widely employed in rice agriculture in California's Sacramento Valley and specifically in the Sycamore Slough watershed.^{64,65} Acetone and the glyphosate degradate, AMPA, were detected at maximum concentrations of approximately 10 µg L⁻¹. Across all sites, 37 organic contaminants (not including cholesterol, 3β-coprostanol, β-sitosterol, β-stigmastanol) had maximum detected concentrations greater than 1 µg L⁻¹, among which 20 were designed-bioactive contaminants including 10 pesticides or pesticide degradates (all herbicides or herbicide degradates) and 10 pharmaceuticals and metabolites.

In this study, six organic contaminants had greater than 1 µg L⁻¹ median detected concentrations, while 77 and 278 (not including cholesterol, 3β-coprostanol, β-sitosterol, β-stigmastanol) had median detected concentrations greater than 100 ng L⁻¹ and 10 ng L⁻¹, respectively. Designed-bioactive contaminants included 14 pesticides and 12 pharmaceuticals with median detected concentrations above 100 ng L⁻¹ and 86 pesticides/pesticide degradates and 58 pharmaceuticals with median detected concentrations above 10 ng L⁻¹. Detection of ng L⁻¹ to µg L⁻¹ concentrations of individual bioactive contaminants and frequent co-occurrence of multiple bioactive contaminants represent aquatic health concerns. Several of these contaminants are expected to elicit sublethal effects at low ng L⁻¹ concentrations^{66–68} and many others remain largely uncharacterized regarding pathway-specific toxicities for sensitive species and lifecycle stages in biodiverse stream environments.

Extensive and Complex Surface Water Organic–Contaminant Mixtures. Detected anthropogenic-organic contaminants were numerous, diverse, and ubiquitous in the 38 streams sampled across the U.S. (Figures 1–4; SI Tables S1 and S3). Cumulative (sum of detected organic contaminants) concentrations ranged 8.5–102 847 ng L⁻¹ per site (Figures 1 and 2; SI Table S3). At least one unequivocally anthropogenic contaminant was detected at all 38 streams, including the four, targeted, undeveloped locations, with cumulative detections for all sites ranging 4–161 organic compounds/site and a median of 70 organic compounds/site (Figures 1 and 2).

Samples collected from the 4 nominally reference locations were characterized by the fewest detected analytes (Figures 1 and 2). Nevertheless, 27 organic compounds were detected at least once at these 4 sites, among which 19 were unequivocally anthropogenic (SI Table S3). Contaminant profiles for three sites (North Sylamore Creek, AR; Swiftcurrent Creek, MT; West Clear Creek, AZ) were statistically ($\alpha = 0.05$) different from all other sites including Penn Swamp Branch, the fourth targeted undeveloped site (Figure 4). The human population in

the four low-impact watersheds (SI Tables S5 and S6) ranged from 0 at Penn Swamp Branch, NJ and Swiftcurrent Creek, MT up to 80 at North Sylamore Creek, AR (SI Table S6). The cumulative detections and concentrations, respectively, of organic analytes ranged from 4 (North Sylamore Creek, AR) to 11 (Penn Swamp Branch, NJ) compounds/site and 55 (North Sylamore Creek, AR) to 1408 (Penn Swamp Branch, NJ) ng L⁻¹. Penn Swamp Branch drains a wetland area characterized by high natural organic content with the lowest pH (4.18) and fourth highest dissolved organic carbon concentration (15.9 mg L⁻¹) measured in this study; cholesterol and β-sitosterol comprised 66% of the cumulative concentration of detected organics at Penn Swamp Branch. Cumulative detections and concentrations, respectively, of anthropogenic-organic contaminants ranged from 4 to 6 compounds/site and 55–430 ng L⁻¹. Among the 19 anthropogenic-organic contaminants detected at targeted undeveloped sites, 58% were designed-bioactives (six pesticide/pesticide degradates and five pharmaceuticals), with cumulative detections and concentrations accounting for 27–50% of all compounds/site (median 38%) and 19–99% of the cumulative concentrations (median 76%), respectively. These results emphasize the environmental mobility, ubiquity, and, thus, potential concern of mixed, bioactive contaminants, in undeveloped and uninhabited watersheds.

The complexity and concentrations of organic contaminants detected in samples from the 34 urban/agricultural-impacted sites were up to 2 orders of magnitude greater than from undeveloped sites (Figures 1–4; SI Table S3). Cumulative detections and concentrations ranged 25–161 organic compounds/site and 1383–102 847 ng L⁻¹ per site. Median values for cumulative detections and concentrations of detected organics were 75 compounds/site and 15 800 ng L⁻¹, respectively, for the impacted sites, 1 and 2 orders of magnitude higher, respectively, than the 8 compounds/site and 123 ng L⁻¹ median values for targeted, undeveloped locations (Figures 1 and 2; SI Table S3). Cumulative detections and concentrations of designed-bioactive contaminants ranged 5–86 compounds/site (median 40.5) and 151–90 340 ng L⁻¹ (median 6223 ng L⁻¹), respectively, accounting for 20–69% of all compounds/site (median 50%) and 5–92% (median 35%) of the cumulative concentrations detected in samples from developed watersheds, respectively. Because antibacterial,⁶⁹ pharmaceutical,^{70,71} and pesticide^{70,71} compounds are formulated to target modes of action, generally have high bioactivity, are bioresistant, and have the potential to affect the entire foodweb (microorganisms, invertebrates, and vertebrates), multiple detections (median 41 per site) and µg L⁻¹ cumulative concentrations in developed watersheds suggest the likelihood of complex interactive effects and the critical need to apply integrating (net) effects tools to evaluate risks posed by actual environmental mixtures.

Chemical Predictors of Overall Contaminant–Mixture Complexity. The BEST BVSTEP model (termination criteria: $\rho > 0.95$, $\Delta\rho < 0.001$; Primer 7^{44,45}), with random start-variable selection, was used to identify combinations of the 55 most frequently detected contaminants that best explained the variability in the detected-contaminants resemblance matrix. Two potential screening subsets of 10–11 compounds each were identified: (1) azithromycin, fipronil, 2-methylnaphthalene, dimethenamid SA, linuron, lambda-cyhalothrin, ethyl methyl ketone, 1,1-dichloropropane, dichloromethane, loratadine ($\rho = 0.950$); and 2) dimethomorph, napropamide, atrazine, estrone,

AHTN, glyphosate, BDE153, PCB118, loratadine, methocarbamol, MTBE ($\rho = 0.951$).

Significant (operationally defined as $n > 19$; $\rho > 0.5$; p -value < 0.05) relations between site-specific summary metrics (cumulative detections, cumulative concentrations, maximum and median detected concentrations) and individual contaminant metrics also were explored using Spearman Rank Correlation (SI Table S4). A strong and highly significant ($\rho = 0.865$; p -value < 0.0001) correlation between cumulative detections and concentrations was observed; significant (p -value < 0.0001) but weaker ($\rho = 0.694$ – 0.716) correlations were observed for cumulative detects and maximum and median detected concentrations across all sites. Not including Sycamore Slough, a strong, highly significant linear relation (Pearson Correlation; adjusted- $r^2 = 0.821$, p -value < 0.001) between cumulative detections and concentrations was observed (Figure 5), providing a useful predictive relationship

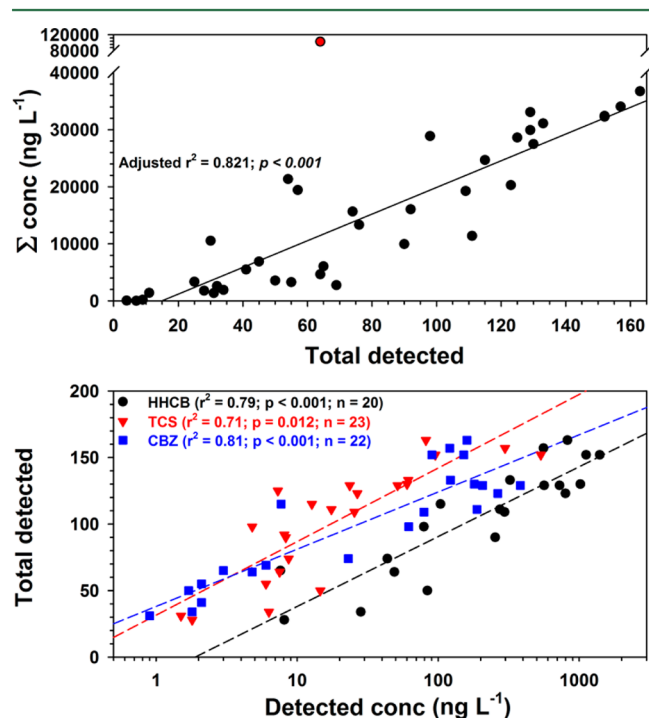


Figure 5. Top: Simple linear regression (line) of cumulative concentration and total number of detected organic analytes (black circles) in water from 37 streams across the nation (not including Sycamore Slough, red circle). Bottom: Simple linear regressions (lines) of total number of detected organic analytes and concentrations of HHCB (black circles), triclosan (TCS, red triangles), and carbamazepine (CBZ, blue squares).

and a means to identify outlying contaminant hot-spots/hot-events (e.g., 3,4-dichloroaniline in Sycamore Slough) that deviate substantially from the general trend.

Cumulative detections were significantly ($n > 19$; $\rho > 0.5$; p -value < 0.05) related to the concentrations of 14 individual contaminants (SI Table S4) including, most notably, HHCB ($n = 20$; $\rho = 0.882$), triclosan ($n = 23$; $\rho = 0.871$), carbamazepine ($n = 22$; $\rho = 0.815$), desvenlafaxine ($n = 22$; $\rho = 0.762$), and metformin ($n = 25$; $\rho = 0.735$), as well as ($n = 20$ – 32 ; $\rho = 0.503$ – 0.684) desulfinylfipronil, 3,4-dichloroaniline, acetone, cotinine, DEET, lidocaine, toluene, methyl-benzyltriazole, and sulfamethoxazole. Likewise, cumulative concentrations were significantly related to the concentrations of 16 individual

contaminants including, most notably, HHCB ($\rho = 0.780$), triclosan ($\rho = 0.772$), carbamazepine ($\rho = 0.757$), methyl-benzyltriazole ($\rho = 0.734$), and metformin ($\rho = 0.704$), as well as ($\rho = 0.538$ – 0.673) AMPA, atrazine, CIAT, 3,4-dichloroaniline, acetone, isophorone, cotinine, DEET, desvenlafaxine, sulfamethoxazole, and tribromomethane. Statistically significant (p -value: < 0.001 – 0.012) simple linear regressions indicated that individual concentrations of HHCB, triclosan, and carbamazepine in \log_{10} space explained 71%–81% of the variability in the total number of detected compounds (Figure 5). These statistical relations provide a tool (within the defined 0.1 – 100000 ng L^{-1} and 719 compound target-chemical space) to infer the presence of unmonitored contaminants based on concentrations of 3 widely monitored and generally well-documented contaminants.

Significant LULC Predictors of Overall Contaminant–Mixture Complexity. No significant relation between resemblance matrices for detected contaminants and all readily available LULC (SI Tables S5–S6) was detected (NMDS, ANOSIM, RELATE; Primer 7^{43–45}). However, significant (p -value = 0.001) but weak relations were identified between the detected-contaminant resemblance matrix and resemblance matrices for select subsets of LULC data including wastewater discharge (Global $R = 0.334$), EPA Toxic Release Inventory site (TRI; Global $R = 0.334$), and urban development classification (Global $R = 0.334$) metrics. Spearman Rank correlation (SI Table S7) identified significant ($|\rho| > 0.5$; p -value < 0.05) relations between detected-contaminant summary metrics and multiple LULC metrics related to wastewater discharge ($\rho = 0.528$ – 0.795 ; p -values < 0.001), population density/urban development ($\rho = 0.509$ – 0.668 ; p -values < 0.001), and TRI ($\rho = 0.583$; p -values < 0.0001). Among these metrics, the most strongly correlated to cumulative detections and concentrations were the number of major National Pollution Discharge Elimination System (NPDES) outfalls in the watershed (as defined in ref 72 $\rho = 0.795$, $p < 0.0001$ and $\rho = 0.656$, $p < 0.0001$, respectively) and the National Land Cover Database (NLCD) 2001⁷³ percent developed medium intensity in the watershed ($\rho = 0.668$, $p < 0.0001$, and $\rho = 0.579$, $p < 0.0001$, respectively), with TRI significantly correlated only to cumulative detected-organic contaminants. The results indicate LULC metrics are useful predictors of in-stream mixed-organic-contaminant complexity and, to a lesser extent, concentration.

Implications for Ecosystem Exposure Effects. Based only on these target-organic results, the complexity and composition of contaminant mixtures are substantial environmental health concerns in streams across the U.S. Although this study employed one of the most comprehensive targeted-analyte toolboxes currently available, particularly with respect to polar organics, 719 organic compounds is a fraction of the contaminant universe, estimated at more than 80 000 parent compounds in current use⁷⁴ and an incalculable chemical-space⁷⁵ of potential metabolites and degradates;⁷⁶ logically, actual surface water contaminant complexity and concentrations may substantially exceed the current observations. Nevertheless, the ng L^{-1} to $\mu\text{g L}^{-1}$ concentrations of individual contaminants and multiple detections per site (median = 70) at cumulative concentrations up to more than $102 \mu\text{g L}^{-1}$ are substantial concerns in their own right, because adverse environmental impacts have been documented for individual designed-bioactive contaminants at low ng L^{-1} concentrations⁶⁶ and interactive effects of contaminant mixtures are poorly understood and are global priorities.^{76–79} Because results of

direct assessments of exposure effects using multiple bioassay platforms are presented elsewhere,¹⁷ only select results are discussed here to emphasize the aquatic-health implications across the aquatic foodweb.

Detection of 17 pharmaceuticals (11 antibiotics, 4 antivirals, 2 antifungals), 9 fungicides, and triclosan (bacteriostat with recognized antibiotic selection potential⁶⁹) in this study with individual detection frequencies up to 53% and maximum concentrations up to $1.8 \mu\text{g L}^{-1}$ strongly suggests impacts at the microbial base of the aquatic foodweb in streams nationwide. Antibiotic-contaminant concentrations as low as $0.5 \mu\text{g L}^{-1}$ have been shown to affect the structure and composition of aquatic microbial communities.^{80,81} Individual detection frequencies (% of sites) and maximum and median detected concentrations for antibiotics alone ranged 3–53%, 12–1800 ng L^{-1} , and 12–135 ng L^{-1} , respectively, with multiple antibiotics (up to 8 per site) detected at 18 sites. Likewise, antibiotic-resistance selection is a global concern⁸² and sublethal selection of antibiotic-resistant bacteria has been documented at ciprofloxacin concentrations as low as 100 ng L^{-1} .⁸³ Ciprofloxacin was detected in this study at 26% of the sites, with maximum and median detected concentrations of 400 ng L^{-1} and 135 ng L^{-1} , respectively. Triclosan was detected at 61% of sites, with maximum and median detected concentrations of 543 ng L^{-1} and 14.6 ng L^{-1} , respectively.

Invertebrates comprise most animal biomass in aquatic ecosystems and the current results suggest substantial potential for adverse contaminant impacts. For example, the phenylpyrazole insecticide, fipronil, blocks GABA-gated chloride channels of insect central nervous systems leading to reduced reproduction and survival and at least two fipronil degradates (sulfide, sulfone) are reported to be more toxic to sensitive aquatic invertebrates than the parent compound.⁶⁸ In this national study, desulfinylfipronil, a fipronil degradate, was the most frequently detected contaminant (32 sites). Two or more fipronil-related compounds (fipronil, fipronyl sulfide, fipronil sulfone, desulfinylfipronil) were detected at 19 sites (50% of all sites; 56% of 34 impacted sites). The EPA Office of Pesticide Programs' fipronil 21-day-chronic and acute benchmarks for invertebrates are 11 ng L^{-1} and 110 ng L^{-1} , respectively,⁸⁴ but substantially lower acute end points (mean 96-h EC50) of 32.5 ng/L for fipronil and 7–10 ng/L for fipronil sulfide and fipronil sulfone have been reported for sensitive aquatic species.⁶⁸ Similarly, neonicotinoid insecticides can severely impair sensitive insect communities⁸⁵ and imidacloprid and clothianidin were detected at 37% and 24% of sites, respectively, with maximum and median concentrations ranging 175–475 ng L^{-1} and 66–143 ng L^{-1} , respectively. Acute and chronic ecological health thresholds below 200 ng L^{-1} and 35 ng L^{-1} , respectively, have been recommended to protect aquatic invertebrate communities.⁸⁵ Lastly, histamines are neurotransmitters for many aquatic insects⁸⁶ and exposure to approximately $2 \mu\text{g L}^{-1}$ fexofenadine has been shown to impair survival behavior (flight response) in damselfly (*Zygoptera*) species and result in bioconcentration up to 2000 times the dissolved concentration.⁸⁷ Multiple (fexofenadine, diphenhydramine, loratadine, hydroxyzine) antihistamines were detected in this study (16–42% of sites) at concentrations up to approximately $4 \mu\text{g L}^{-1}$. Fexofenadine was detected at 42% of sites, with maximum and median concentrations of 2047 ng L^{-1} and 576 ng L^{-1} , respectively; two or more antihistamines were detected at 13 sites (34%).

Pharmaceutical results best illustrate the concerns raised in this study with respect to aquatic-vertebrate impacts, because fish are widely used animal models for pharmaceutical efficacy testing.^{88,89} Eighty-four pharmaceuticals were detected across all sites, with detection frequencies for individual compounds ranging 3–74% of sites (median 24%). Frequent detection of metformin (66% of sites) at median concentrations greater than 400 ng L^{-1} , including seven sites with concentrations in the $\mu\text{g L}^{-1}$ range, is noteworthy because metformin is a designed endocrine-active compound and effluent-equivalent metformin exposures in the $\mu\text{g L}^{-1}$ range induce up-regulation of vitellogenin mRNA^{88,89} and male intersex⁹⁰ in fathead minnow (*Pimephales*). However, mixed pharmaceutical contamination was the norm in this study, with 0–61 pharmaceuticals detected per site (median 7.5 across all sites; median 12.5 for impacted sites) and cumulative concentrations ranging 0–9580 ng L^{-1} (median 327 ng L^{-1}).

Understanding and mitigating the human and ecological health risks associated with chemical- and land-use practices require knowledge of environmental chemical mixtures. The results presented herein provide the most extensive, target-polar-organic-chemical characterization of the composition and concentrations of contaminant-mixture exposures in streams available to date and support cross-examination of split-sample effects results (presented separately) as well as subsequent methods development, prioritization of future ecotoxicological studies of chemical mixtures, and in-silico modeling of potential biological effects of multiple contaminants. Nontarget screening of archived water samples, which has the potential to substantially expand the current contaminant profiles,⁹¹ is ongoing.

■ ASSOCIATED CONTENT

■ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.7b00012.

Data in this article are summarized in Tables S1–S8 and in ref 40. Complete primary data are downloadable from refs 39 and 92 (PDF) (XLSX)

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