

Chemical Mixtures and Environmental Effects: A Pilot Study to Assess Ecological Exposure and Effects in Streams

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What chemicals occur together in our streams and at what concentrations? How do land-use and chemical-use patterns influence the complex chemical mixtures in streams across the Nation? How do these chemicals enter streams and do they persist? Can exposure to chemical mixtures result in unanticipated, adverse health effects in fish and other aquatic organisms? Could people be exposed to these chemical mixtures? This document presents some of the challenges associated with chemical mixtures in streams and describes an interagency (U.S. Geological Survey and U.S. Environmental Protection Agency), field-based investigation designed to help answer these and other questions and to provide information to guide future research.

Contaminant Mixtures in Surface Waters

Streams and lakes accumulate chemicals from many different human activities across the landscape (fig. 1). Watersheds collect water from precipitation that runs off the land surface, carrying with it chemicals from urban and residential areas (streets, sidewalks, parking lots, lawns, and gardens); crop lands treated with pesticides, manures, and

other organic and inorganic fertilizers; pastures and animal-production facilities; and lands where energy and mineral resources are extracted. Municipal, commercial and domestic wastewater-treatment systems, industries, and landfills can discharge their wastewaters directly to streams, and although the wastewater typically is treated, current treatment technologies are not designed to remove all chemicals. Groundwater, which discharges to streams and accounts for much of streamflow during dry seasons, also can carry contaminants from spills, leaks, septic systems, and land-applied chemicals that infiltrate

to the water table as well as naturally occurring chemicals that leach from aquifer material. Even chemicals from smokestacks, automobiles, and other emissions to the atmosphere can make their way into streams. As a result, surface waters can have complex mixtures of natural and anthropogenic chemicals that include pesticides, pharmaceuticals, household chemicals, and a wide range of chemicals produced as waste byproducts of commercial and industrial activities.

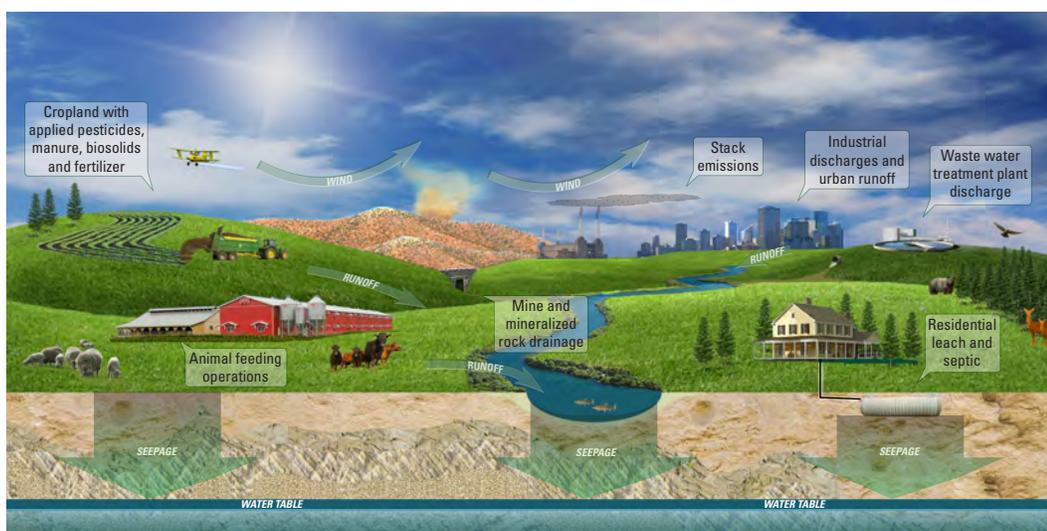


Figure 1. Contaminant sources and principal pathways to the environment. From Bright and others (2013).

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The Challenge of Chemical Mixtures

Assessment and management of the risks of exposure to complex chemical mixtures in streams are priorities for human and environmental health organizations around the world, including the U.S. Environmental Protection Agency (Doyle and others, 2014), the U.S. National Institute of Environmental Health Sciences (Carlin and others, 2013), the U.S. Geological Survey (Bright and others, 2013), the European Commission (European Commission, 2015), and the World Health Organization (World Health Organization, 2015). The current lack of information on the composition and variability of environmental mixtures and a limited understanding of the combined effects are fundamental obstacles to timely identification and prevention of adverse human and ecological effects of exposure to chemical mixtures.

The number of chemicals registered for use in the United States is immense and increasing rapidly. In comparison, assessment of health risks and subsequent development of environmental regulations have progressed slowly, been conducted largely on a chemical-by-chemical basis, and focused primarily on mortality, acute toxicity, and carcinogenicity. As a result, the vast majority of these chemicals remain untested for their biological mode(s) of action and toxicity, and very few mixtures have been assessed for potential chemical interactions and their combined effect. To address this knowledge gap, high throughput testing and other automated methods are being developed for more efficient evaluation of the biological activity and toxicity of chemicals (National Research Council, 2007; Collins and others, 2008). However, even robust chemical characterization of environmental chemical mixtures has been found to explain only a small fraction of the observed biological response to exposure (Tang and others, 2014).

Environmental Health Effects of Chemical Mixtures

Predicting the Combined Effects of Chemical Mixtures

Testing for the health effects of all potential chemical mixtures is not feasible. Therefore, multiple approaches have been developed for using the results of chemical-specific testing to assess the combined effects of chemical mixtures. Chemicals often are assumed to act additively in terms of their concentration, dose, potency, or biological response to exposure. These approaches are challenged by changes in response with exposure time, the biological interaction of chemicals within an organism, and complex dose-response relations including the effects thresholds below which individual chemicals do not demonstrate effects. Adverse health effects have been demonstrated from exposure to multiple chemicals at low concentrations, which individually would not cause harm (Brian and others, 2007; Smith and others, 2013; Kortenkamp,

2014). Chemical interactions can result in impacts that are greater than (synergistic) or less than (antagonistic) those predicted by additive approaches. An everyday example of an unanticipated chemical interaction is the need to avoid consuming grapefruit when taking specific medications due to its potential to amplify pharmacological effects in some patients (Kane and Lipsky, 2000). Examples of environmental contaminants known to interact synergistically or antagonistically include polycyclic aromatic hydrocarbons (Schneider and others, 2002), pesticides (Belden and Lydy, 2006; Laetz and others, 2009; Christen and others, 2014; Chen and others, 2015), metals (Norwood and others, 2003), pesticides and metals (Chen and others, 2015), antibiotics (Gonzalez-Pleiter and others, 2013), pharmaceuticals with similar (Cleuvers, 2004) and different modes of action (Pomati and others, 2008), pharmaceuticals and metals (Aslop and Wood, 2013), pharmaceuticals and synthetic musks (Schnell and others, 2009), polycyclic aromatic hydrocarbons and metals (Wang and others, 2013), and more diverse chemical mixtures (Boltes and others, 2012; Orton and others, 2014).

Health Outcomes

Adverse human and environmental health outcomes other than acute toxicity and mortality may occur in streams even at low contaminant concentrations due to the complexity of chemical mixtures and associated chemical modes of action. Modes of action may include carcinogenicity, genotoxicity, mutagenicity, toxicity modulation, endocrine disruption, and selection of microbial antibiotic resistance.

As an example, many of the chemicals that enter our streams can disrupt the endocrine systems of aquatic organisms. Hormones, secreted by an organism's endocrine system, play a major role in biological function and metabolic stability. Endocrine disrupting chemicals can interfere with natural hormone signaling in exposed organisms, resulting in altered growth, sexual development and function, neurological development, metabolism, and stress response (Diamanti-Kandarakis and others, 2009). Estrogenically active contaminants can interfere with natural estrogen binding, affecting sexual differentiation and development, among other things. Estrogenic contaminants in streams may include estrogens produced naturally in animals and subsequently excreted (biogenic estrogens), estrogenic chemicals produced in plants (phytoestrogens), synthetic pharmaceutical estrogens (ovulation inhibitors or estrogen supplements), and estrogen mimics (a wide range of chemicals with industrial and commercial uses including some pesticides, flame retardants, surfactants, and products in plastics). In one study, severe population declines were documented in fish in a Canadian lake dosed with a single compound, 17- α ethinyl estradiol, an active ingredient in human birth control pills (Kidd and others, 2007). Fish and other aquatic organisms that inhabit contaminated streams are often exposed to many endocrine-active chemicals, not just one. Because endocrine disruption effects

often do not conform to established, dose-response relations, these chemicals pose a serious challenge to traditional toxicological risk assessment practices (Fuhrman and others, 2015).

The adverse effects of antimicrobial resistance on human health and the potential significance of both clinical and natural environments in resistance selection are also widely acknowledged (Wellington and others, 2013; Martinez, 2014). The ability of microorganisms to resist antibiotic and antimicrobial agents can be affected by multiple chemical exposures and can be transferred between organisms (Kummerer, 2009). Chemicals with the potential to select resistance genes and resistant microbial populations include metals (Baker-Austin and others, 2006), quaternary ammonium compounds (Gaze and others, 2005), biocides (Sanchez and others, 2005), and herbicides (Kurenbach and others, 2015). Resistance to an individual antibiotic can be further affected by other antibiotics and metals acting on the same genetic target or on different targets on the same genetic element (Baker-Austin and others, 2006). Common antibiotic pathways to the environment include chemical use and disposal in animal agriculture, in healthcare facilities, and in the home.

The combined adverse health effects of exposure to multiple chemicals and of physical insults from other stressors that affect different organs or biological functions, as well as indirect effects on complex ecological relations such as host-pathogen interactions, are critical challenges (Sexton and Hattis, 2007; Lokke and others, 2013; Hadrup, 2014).

A Pilot Study

The U.S. Geological Survey (USGS) and the U.S. Environmental Protection Agency (EPA) are collaborating to provide new information on environmental chemical mixtures in United States streams. The objective of the study is to characterize chemical mixtures and biological activity in surface waters that are affected by diverse chemical sources. The study is intended to provide field-based information for the prioritization and design of future research on environmental chemical mixtures. The study represents the most comprehensive assessment of the chemical composition and biological activity of contaminant mixtures in surface waters ever performed in the United States.

Pilot Study Approach

Site Selection

Data from more than a thousand stream sites from previous USGS studies of contaminants of emerging environmental concern were used to select 38 stream sites spanning 24 States and Puerto Rico (fig. 2; table 1). Thirty-four stream sites were selected because they have highly developed watersheds with a wide range of contaminant sources; the remaining four stream sites were selected as undeveloped reference sites.

Sites vary widely in upstream drainage area, ranging from 4.6 square miles (mi²) for a small reference site on Penn Swamp Branch in the Pinelands of New Jersey to 6,265 mi² for the Trinity River, an arid southwestern stream in Texas (table 1). Most of the study watersheds cover areas less than 100 mi² (fig. 3A). Population density also varies widely (fig. 3B), ranging from zero (Swiftcurrent Creek, a reference site near Many Glacier, Montana) to almost 3,000 people per mi² (Rush Creek near Arlington, Texas).

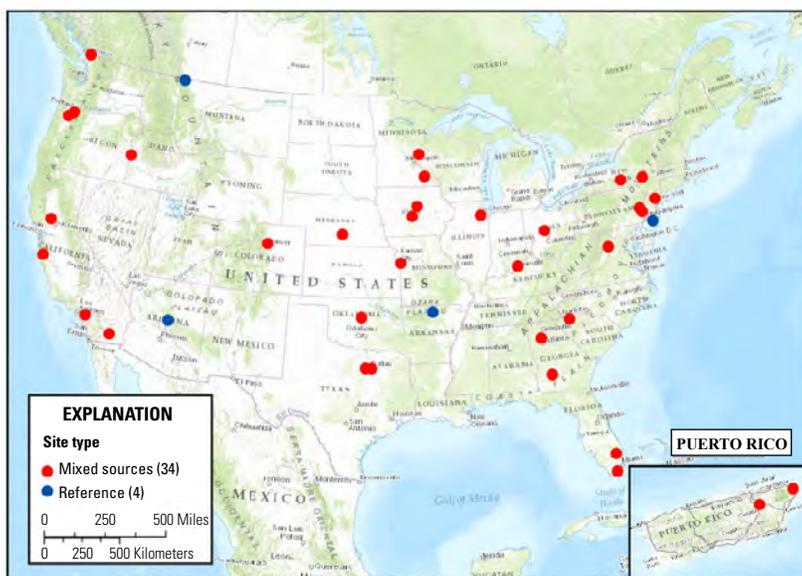


Figure 2. Stream sampling locations. Thirty-four of 38 stream sites (red markers) have a wide range of contaminant sources within their watersheds; the remaining four stream sites (blue markers) were selected as reference sites because their watersheds are minimally affected by contaminant sources and their biological communities are considered to be unaffected or minimally affected by development.

Table 1. Stream sampling sites, location, watershed drainage area, and date sampled.

[Latitude and longitude in decimal units, referenced to the North American Datum of 1983 (NAD 83); drainage area in square miles]

Stream name	Location	Station number	Latitude	Longitude	Drainage area	Sample date
Developed watersheds						
Abrams Creek	near Oakfield, Georgia	2350524	31.7187847	-83.98851469	79.58	July 23, 2013
Blue River	at Kansas City, Missouri	06893500	38.957	-94.5588889	184.19	February 11, 2014
C-111 Canal	near Homestead, Florida	252414080333200	25.40428063	-80.5586705	51.04	April 9, 2013
Chicago Sanitary and Ship Canal	at Lockport, Illinois	05537000	41.57027778	-88.0794444	749.34	February 11, 2014
Chisholm Creek	at Edmond, Oklahoma	07159735	35.72560669	-97.5272639	38.78	November 28, 2012
Deep Creek	at Highway 224, Oregon	452340122251000	45.39429	-122.42064	48.98	January 13, 2014
East Branch Perkiomen Creek	near Derstines, Pennsylvania	01472705	40.35402778	-75.31327778	29.35	January 14, 2014
Enoree River	at Pelham, South Carolina	02160326	34.85650727	-82.22622468	84.80	May 19, 2014
Fall Creek	near Ithaca, New York	423400	42.4533333	-76.47277778	126.35	June 9, 2014
Fishtrap Creek	near Lynden, Washington	12211900	49.00261838	-122.4073816	16.45	December 10, 2013
Fourmile Creek	downstream of the Ankeny WWTP outfall, Iowa	05485605	41.7173771	-93.5701332	60.94	November 19, 2012
Hawksbill Creek	at Route 648, Springfield, Virginia	01630540	38.70817319	-78.4563966	68.52	April 15, 2014
Hillsboro Canal	at S-6, near Shawano, Florida	02281200	26.4720184	-80.4458857	311.34	March 12, 2013
Hite Creek	at Sleepy Hollow Road near Prospect, Kentucky	382054085332600	38.34833	-85.55722	5.54	May 20, 2014
Hohokus Brook	at Ho-Ho-Kus, New Jersey	01391100	40.95527778	-74.1005556	20.47	November 28, 2012
Jordan Creek	at the mouth, at Allentown, Pennsylvania	01452040	40.6018	-75.46208889	82.32	June 17, 2013
Mill Creek	near Bellepoint, Ohio	03220000	40.24839457	-83.1738026	177.93	June 4, 2013
New River	near Westmorland, California	10255550	33.10476565	-115.6644382	1,471.12	March 5, 2013
North Dry Creek	South of Kearney, Nebraska	06770195	40.6411236	-99.1159223	78.74	June 10, 2013
Rio Bairoa	at the mouth, Puerto Rico	50055410	18.26106496	-66.0187762	7.68	March 19, 2013
Rio Fajardo	downstream of the Fajardo WWTP outfall, Puerto Rico	181932065383501	18.31297222	-65.6536111	20.38	March 25, 2013
Rush Ck	at Hwy 303 near Arlington, Texas	324305097101900	32.71794	-97.17201	28.89	May 13, 2013

Table 1. Stream sampling sites, location, watershed drainage area, and date sampled.—Continued

[Latitude and longitude in decimal units, referenced to the North American Datum of 1983 (NAD 83); drainage area in square miles]

Stream name	Location	Station number	Latitude	Longitude	Drainage area	Sample date
Developed watersheds						
Sand Run Gulch	at Highway 95 crossing near Parma, Idaho	13210360	43.7640501	-116.9120956	79.49	June 18, 2013
Santa Ana River	downstream of Prado Dam, California	11074000	33.88334875	-117.6453296	2,261.44	March 25, 2014
Sope Creek	near Marietta, Georgia	02335870	33.95388889	-84.4433333	30.81	April 16, 2013
South Fork Iowa River	near New Providence, Iowa	05451210	42.31498375	-93.1524252	266.39	May 20, 2013
South Fork Zumbro River	downstream of WWTP near Rochester, Minnesota	05373005	44.0738534	-92.46767819	312.00	December 5, 2012
South Platte River	near Commerce, Colorado	39484710457700	39.8130418	-104.9524794	4,082.21	December 11, 2012
Sunrise River Tributary	near Lindstrom, Minnesota	05340041	45.4076111	-92.8805889	6.59	December 4, 2012
Sycamore Slough	at County Line Road near Dunnigan, California	385531121532001	38.92527778	-121.8888889	64.30	June 18, 2013
Tembladero Slough	at Castroville, California	364554121453401	36.765	-121.7594444	153.97	March 3, 2014
Trinity River	downstream of Dallas, Texas	08057410	32.70763139	-96.7358319	6,264.63	December 4, 2012
West Branch Delaware River	at Hobart, New York	01421640	42.3436111	-74.72	48.22	November 19, 2012
Zollner Creek	near Mount Angel, Oregon	14201300	45.10039816	-122.8217596	15.90	May 19, 2014
Reference Sites						
North Sylamore Creek	near Fifty Six, Arkansas	07060710	35.99166667	-92.2138889	58.7	January 7, 2014
Penn Swamp Branch	near Batsto, New Jersey	01409480	39.68416667	-74.65027778	4.6	December 10, 2012
Swiftcurrent Creek	upstream of Swiftcurrent Lake at Many Glacier, Montana	05014300	48.7954444	-113.6805833	15.4	August 28, 2013
West Clear Creek	near Camp Verde, Arizona	09505800	34.53863606	-111.6940356	241	January 15, 2014

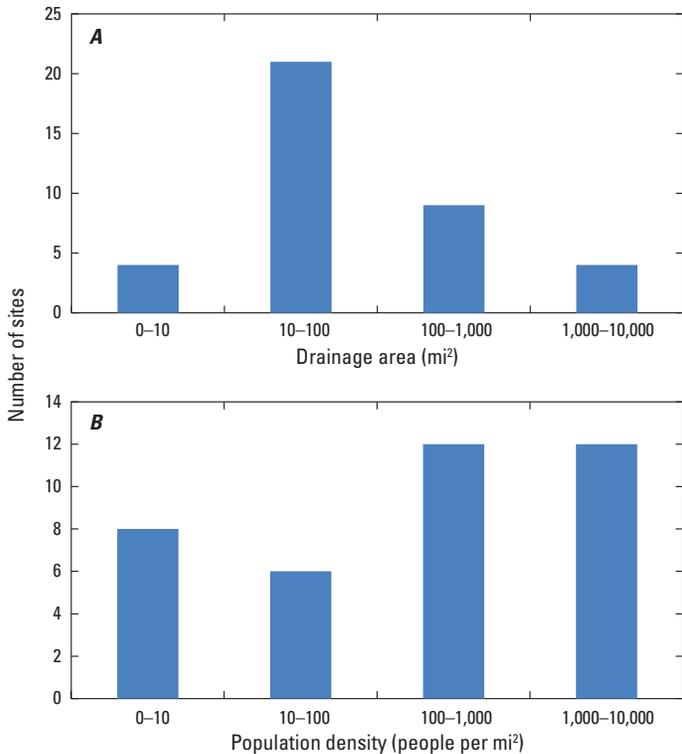


Figure 3. Watershed characteristics of sampled stream sites. *A*, Distribution of drainage areas in square miles. *B*, Distribution of watershed population densities in people per square mile.

Some watersheds are characterized almost entirely by agricultural land (fig. 4A). Mixed urban/agricultural land use is common, with urban land (fig. 4B) generally covering less area than agricultural land. The Hite Creek site in Kentucky (fig. 5) is an example of a developed watershed affected by highly varied contaminant sources. The 5.5 mi² watershed is approximately 52 percent urban land and 10 percent agricultural land. It has a population density of about 2,323 people per mi² and includes a National Pollutant Discharge Elimination System (NPDES) major discharger.

The watersheds of the four stream reference sites are largely undeveloped, minimally affected by contaminant sources, and considered to have minimally disturbed fish and aquatic macro-invertebrate communities. For example, the 15.4 mi² Swiftcurrent Creek watershed in Montana (fig. 6) has no urban land, 0.1 percent agricultural land, and no major NPDES dischargers. The population density of the watershed is less than 1 person per mi².

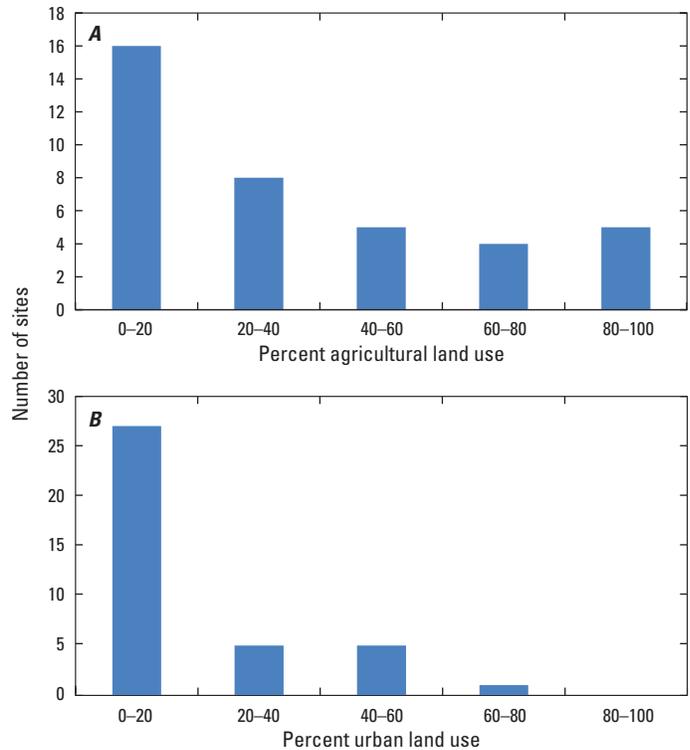


Figure 4. Distribution of *A*, Agricultural and *B*, Urban land use as a percentage of total drainage area for stream-site watersheds. (2011 National Land Cover Database [conterminous United States, <http://www.mrlc.gov/nlcd2011.php>]; Puerto Rico 2001 National Land Cover Database [Puerto Rico, http://www.mrlc.gov/nlcd01_data.php])



Figure 5. Hite Creek Kentucky, looking upstream at the sampling location. Photograph by Angela Crain, USGS.



Figure 6. Swiftcurrent Creek, Montana, looking upstream at the sampling location. Photograph by Hannah Nilges, USGS.

Sample Collection and Analysis

Water samples were collected once from each stream from December 2012 to June 2014. Samples taken from the center of flow at each stream were collected by USGS staff using established trace-level protocols (Shelton, 1994; Wilde, 2004a; Wilde and others, 2004b; Hladik and others, 2009). Composite water samples were homogenized and decanted into the specified container or collected within the specified container as appropriate. Water samples were kept at 3 degrees Celsius (°C) while in the field and more than 60 sample bottles were shipped overnight to USGS and EPA national laboratories for analysis (fig. 7).



Figure 7. U.S. Geological Survey scientist collecting water samples and measuring water field properties at North Sylamore Creek, Arkansas, on January 7, 2014, a study reference site. Photograph by J. Tyler Mays, USGS.

Stream water samples were tested using (1) sensitive and specific direct analysis for more than 700 organic and inorganic chemicals and physical properties (table 2), (2) environmental diagnostics for identification of unknown chemical contaminants using Gas Chromatography-Mass Spectrometry and Liquid Chromatography-Time of Flight Mass Spectrometry, and (3) a variety of bioassays to evaluate biological activity and toxicity, including hormonal activity, carcinogenicity, and mutagenicity (table 3). In addition, a subset of 8 ambient water extracts from sites selected from a range of environmental settings were screened for approximately 80 different biological activities using the Attagene, Inc., subset of ToxCast™ assays (U.S. Environmental Protection Agency, 2015). The chemical analytes measured include anthropogenic and naturally occurring chemicals as well as associated metabolites and environmental transformation byproducts.

Table 2. Chemicals measured by direct analysis of stream water samples.

339	Pesticides and transformation byproducts
160	Pharmaceuticals and metabolites
32	Antibiotics and metabolites
38	Biogenic and synthetic hormones and conjugates
40	Volatile organic compounds
25	Disinfectants and byproducts
22	Metals
17	Flame retardants
17	Perfluorocarbons (PFCs)
11	Surfactants
10	Phytoestrogens and plant sterols
9	Fragrances
9	Nutrient species, and
	Other industrial chemicals

Table 3. Bioassays used to measure biological activity and toxicity of stream water samples.

Estrogen agonist and antagonist assays
Androgen assays
Glucocorticoid assays
Steroidogenesis (estrogen and testosterone production) assays
DNA binding transcription factor activity assays
Carcinogenicity and mutagenicity assays
DNA damage assays
Gene expression and metabolite profiling

Pilot Study Significance

The results of this pilot study will (1) document the mixtures and associated concentrations of chemicals found in stream waters, (2) help identify potential ecological and human exposures, (3) guide prioritization of toxicological studies of chemical mixtures, (4) provide insight into potential biological interaction of multiple contaminants, (5) help relate bioassay screening approaches to environmental chemical characterization in support of new monitoring strategies, and (6) provide a base of data for models to predict chemical mixtures in watersheds affected by diverse land-use and chemical-use patterns.

This pilot study also provides the basis for evaluating the feasibility of future field-based research on environmental chemical mixtures. The information about these sites can be used to design more detailed studies that address additional environmental media (sediment and tissue), multiple concurrent exposure pathways, temporal variations in chemical mixtures resulting from the timing of chemical-use practices and environmental transformation, acute and chronic effects of exposure to mixtures, and adverse health effects from varied chemical and physical insults at the organism, population, and community levels.

Integrated Approach to Chemical Mixtures Assessment, Source Identification, and Mitigation

Understanding and mitigating the human and ecological health risks associated with chemical- and land-use practices require knowledge of environmental chemical mixtures, the pathways different chemicals take to the environment, the environmental persistence of chemicals and associated transformation products, the way chemicals interact biologically, and the associated risk of human and ecological exposure. This knowledge must be sufficient to prioritize and inform ecological and human risk assessments; support regulation and motivate voluntary actions to reduce the use of specific chemicals; improve chemical and waste handling, use, and disposal practices that mitigate environmental releases; and effectively upgrade wastewater and drinking-water treatment technologies. An integrated approach (fig. 8) simultaneously employing field-based biological (Environmental Biology) and chemical (Environmental Chemistry) testing along with systematic laboratory assessment of the effects of environmentally relevant chemicals and chemical mixtures (Laboratory Testing) offers a promising means of addressing these multiple environmental health objectives.

Environmental bioassays allow for rapid assessment of environmental samples and can be used as cost-effective screening tools to identify susceptible environmental settings, the biological activity exhibited by environmental waters, and the associated adverse health outcomes on aquatic organisms. Field testing using test organisms (such as caged fish studies) can play an important role in documenting adverse health effects. Environmental chemical analyses permit spatial and temporal characterization of actual environmental chemical mixtures, allow for identification of unknowns (environmental diagnostics) for future chemical and biological methods development, and provide information on specific chemicals of concern that supports mitigation and management actions. Laboratory-based testing is well suited to identifying the range of biological activities and adverse health effects of specific chemicals and environmental chemical mixtures, defining the mechanisms of biological interaction across multiple modes of action, identifying exposure criteria for vulnerable species, and expanding the available bioassay toolbox. Use of field-based laboratories at long-term research sites provides unique laboratory control in a field-based setting. Continued use of high throughput testing of the biological activity of specific chemicals as well as more focused toxicological testing on a wide range of organisms are essential elements of the laboratory research component. Improved coordination among the three components of this integrated approach enables (1) feedback between field-based biological and chemical testing that results in detailed chemical characterization in environmental settings with documented biological activity and adverse health effects, (2) laboratory testing to identify the causes of toxicity in environmental chemical mixtures that are demonstrated to have biological consequences, and (3) improved, cost-efficient monitoring approaches that employ complementary use of bioassays and chemical analysis, and include high throughput testing of waters from demonstrated high-risk

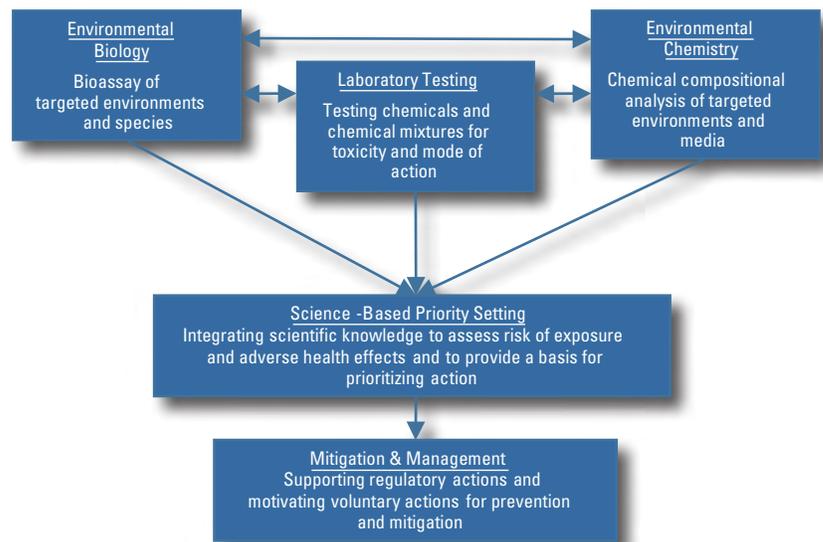


Figure 8. Diagram describing an approach to assessing environmental chemical mixtures that integrates field-based biological testing, field-based chemical testing, and systematic laboratory testing.

environmental settings across the Nation. Furthermore, such an integrated approach enables efficient development of a robust scientific basis for prioritization of preventive and mitigating actions, support of regulatory action, and motivation of voluntary action by industry and the public.

Federal Role

The pilot study described herein is a collaborative effort that draws on the unique capabilities of the USGS and the EPA to

- Address a wide range of environmental settings across the Nation,
- Employ uniform field and laboratory protocols with extensive quality-assurance programs, and
- Apply the unprecedented scope of chemical and biological analytical capabilities available at USGS and EPA laboratories.
- Implement an integrated approach to assessing environmental chemical mixtures as described above.

Federal agency collaboration and coordination is essential for national-scale assessment of the potential adverse health effects of chemical mixtures in the environment; examples of recent field-based efforts are Glassmeyer and others (2005), Glassmeyer and others (2009), Beaver and others (2014), Cavallin and others (2014), and Barber and others (2015). The study described herein and subsequent national-scale, field-based efforts will support development of more cost-effective, environmentally representative, and scientifically defensible assessment approaches for chemical mixtures in a range of surface-water settings.

Acknowledgments

This interagency project was made possible by the cooperation of numerous scientists and technicians within the USGS and the EPA, including USGS field crews who collected samples at each stream site, USGS and EPA staff at the laboratories who processed and analyzed the samples, and the principal investigators who played a major role in designing and implementing the study (table 4).

Table 4. U.S. Geological Survey and U.S. Environmental Protection Agency principal investigators on the interagency project team.

Principal Investigators	
U.S. Geological Survey	U.S. Environmental Protection Agency
<i>Project Design and Coordination</i>	<i>Project Coordination</i>
Herbert T. Buxton	Susan T. Glassmeyer
Dana W. Kolpin	Marc A. Mills (Chemistry)
Kathryn M. Kuivila	Daniel L. Villeneuve (Bioassays)
Timothy J. Reilly	
<i>Analytical Chemistry</i>	<i>Analytical Chemistry</i>
Larry B. Barber	Angela L. Batt
William T. Foreman	Mitchell Kostich
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Michele L. Hladik	Kathleen M. Schenck
Keith A. Loftin	
Michael T. Meyer	<i>Bioassays</i>
Kelly L. Smalling	Gerald T. Ankley
<i>Bioassays</i>	Adam D. Biales
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	David M. DeMarini
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Kristin Romanok	Phillip C. Hartig
James L. Orlando	David L. Lattier
Celeste Journey	James M. Lazorchak
	Jane Ellen Simmons
	Mary Jean See
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