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Passive Sampling of Bioavailable Organic Chemicals in Perry County, Missouri Cave Streams

J. TYLER FOX,[†] GINNY ADAMS,[*,†]
MARTIN SHARUM,[‡] AND
KAREN L. STEELMAN[‡]

Departments of Biology and Chemistry, University of Central Arkansas, 201 Donaghey Ave., Conway, Arkansas 72035, United States

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Two types of passive samplers—semipermeable membrane devices (SPMDs) and polar organic chemical integrative samplers (POCIS)—were deployed in spring 2008 to assess bioavailable concentrations of aquatic contaminants in five cave streams and resurgences in Perry County, Missouri. Study sites represent areas of high cave biodiversity and the only known habitat for grotto sculpin (*Cottus carolinae*). Time-weighted average (TWA) water concentrations were calculated for 20 compounds ($n = 9$ SPMDs; $n = 11$ POCIS) originating primarily from agricultural sources, including two organochlorine insecticides, dieldrin and heptachlor epoxide, which were found at levels exceeding U.S. EPA criteria for the protection of aquatic life. GIS data were used to quantify and map sinkhole distribution and density within the study area. Infiltration of storm runoff and its influence on contaminant transport were also evaluated using land cover and hydrological data. This work provides evidence of cave stream contamination by a mix of organic chemicals and demonstrates the applicability of passive samplers for monitoring water quality in dynamic karst environments where rapid transmission of storm runoff makes instantaneous water sampling difficult.

Introduction

Clean groundwater is not only vital to human health, it is also fundamental to the survival of a diversity of aquatic organisms highly adapted to life in cave streams. There are 927 species and 46 subspecies of obligate cave fauna described in the United States. Of these, 50% are listed as vulnerable or threatened, while less than 4% benefit from federal protection status (*1*). Stygobites (aquatic cave organisms) comprise one-third of the cave species in the contiguous 48 states and 74% are known only from a single county or state (*1*). This study was prompted by an extirpation event that affected one such endemic species, known only from five cave systems in Perry County, Missouri—a cavefish allocated to *Cottus carolinae* (banded sculpin), and referred to as the grotto sculpin (*2*). Contamination of cave stream habitat from agricultural, industrial, and residential-sources has been implicated as a potential factor in grotto sculpin die-offs, as well in the declines of other sensitive cave species in Perry County (*2, 3*). The first die-off, which occurred in Running Bull Cave in 1999, was followed by a second in Mystery Cave in 2003, which prompted the U.S. Fish and Wildlife Service to list the grotto sculpin as a high priority candidate to the Endangered Species Act (ESA). The impacts of organic chemical contaminants on cave biota and groundwater resources are of particular concern in southern Missouri due to extensive agricultural land use in areas of well-developed karst. There are over 700 caves in Perry County, many of which support species-rich cave life communities (*4*). Furthermore, it is estimated that up to one-half of all sinkholes in Perry County, some with histories of use dating back more than a century, are used for disposal of anthropogenic wastes (*2*). In this work, we utilized two types of passive sampling devices (PSDs)—the semipermeable membrane device (SPMDs) and the polar organic chemical integrative sampler (POCIS), to assess bioavailable concentrations of organic chemicals in five cave streams representing the only known habitat for grotto sculpin.

Karst Vulnerability. In karst areas where agriculture is the dominant form of land use, pesticide contamination of groundwater is a major source of concern in terms of both environmental and human health. Soils in karst regions tend to be thin, which can allow chemical and bacterial contaminants to rapidly infiltrate the water table without undergoing significant remediation through adsorption, filtration, and microbial degradation (*5, 6*). In areas like Perry County where conduit flow is the dominant mode of groundwater movement, chemical-laden storm runoff can be rapidly transported from the surface by sinkholes and losing streams, to emerge at springs and resurgences far from its origins (*5*–*8*).

Contaminant Monitoring in Karst. Traditional methods of water quality monitoring involving collection of grab samples at regular intervals provide a snapshot of contaminant concentrations at specific moments in time. By extending the collection of samples over a period of weeks to months, time-weighted average (TWA) water concentrations can be calculated that indicate average exposure to a chemical contaminant over time. However, this method of calculating TWAs is unsuitable for detecting chemicals present in the environment at very low concentrations or only during episodic events, and requires a large investment of time, labor, and money (*9*). In karst regions where the interval between rain events and the arrival of storm runoff in caves and resurgence springs is brief, it can be especially difficult to collect representative samples of aquatic contaminant concentrations (*10*).

PSDs, such as Chemcatchers, Empore disks, PISCES, SPMDs, and POCIS simplify determination of TWA exposure concentrations by concentrating trace levels of organic chemicals over extended deployment periods without significant loss of sampled analytes (*11*). In this work, we selected SPMDs and POCIS to assess cave stream water quality because of their demonstrated use and effectiveness in sampling dissolved organic chemicals in widely varying environments and field conditions (*9*–*15*). SPMDs generally accumulate more nonpolar, hydrophobic organic compounds, while POCIS generally concentrate polar, hydrophilic compounds (*14*). The use of SPMDs and POCIS allows for a comprehensive assessment of legacy and current-use pesticides, as well as many residential and industrial contaminants.

This study is among the first to utilize SPMDs and POCIS together for assessment of chemical contaminants in sensitive

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[*] Corresponding author phone: 501.450.5917; fax: 501 0.450.5914; e-mail: gadams@uca.edu.

[†] University of Central Arkansas, United States Department of Biology.

[‡] University of Central Arkansas, United States Department of Chemistry.

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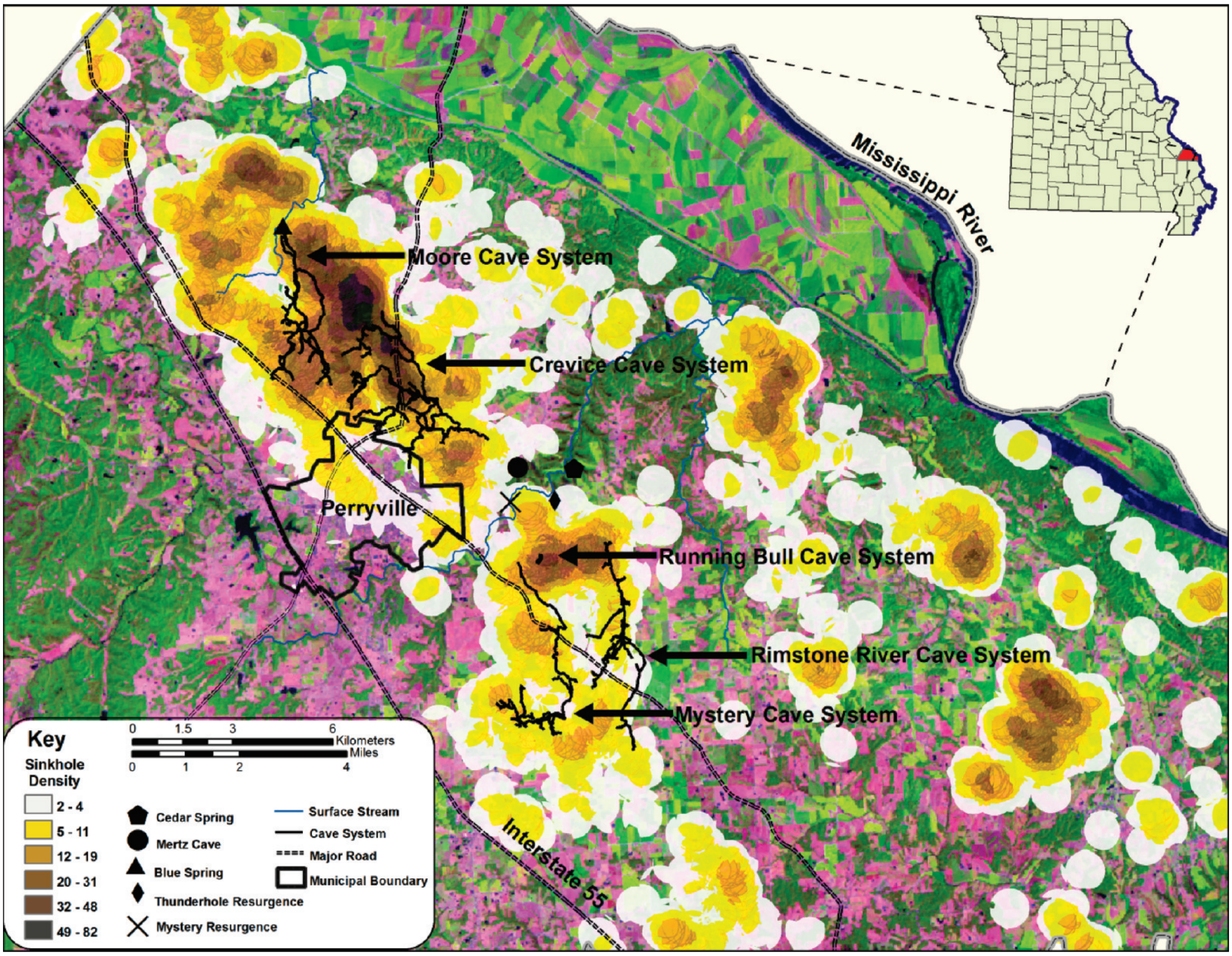

FIGURE 1. False color LandSat 7 +ETM map of the Perryville sinkhole plain showing sinkhole density, deployment sites and associated cave systems. Exposed soils and disturbed areas appear as shades of purple with young vegetation and agricultural fields appearing light green.

aquatic cave ecosystems (*16*). The primary goals of this study were to: (1) evaluate groundwater contamination in shallow karst during a period of intensive agricultural pest management and (2) provide a quantitative assessment of organic chemical contaminants in cave stream habitats utilized by grotto sculpin in light of its designation as a priority candidate species to the ESA. Despite extremely heavy rainfall in spring 2008 that delayed planting of corn and other row crops in many areas, SPMDs and POCIS from all five study sites accumulated multiple organic chemical contaminants at levels above method detection limits (MDLs). TWA water concentrations were calculated for a total of 20 compounds, including two organochlorine insecticides present at levels exceeding US EPA ambient water quality Criterion Continuous Concentrations (CCC) for protection of aquatic life.

Materials and Methods

Study Area. Perry County is located $\sim$100 km southeast of St. Louis, Missouri on the eastern edge of the Salem Plateau subprovince of the Ozark Mountains. Much of eastern Perry County lies within a 41 km^2 sinkhole plain formed within Ordovician dolostones and limestones of the Perryville Karst. Sinkholes number in the thousands, range in size from a meter to several kilometers in diameter, and reach depths of up to 30 m. Unique to this area, are the numerous large river caves, concentrated primarily in the central portion of the county in the vicinity of the city of Perryville (population 8192 in 2009). Most of these cave systems are at shallow depths and are entered through sinkholes located in the upper cave stream reaches (*17*).

Deployment sites included Mertz Cave (Crevice Cave System) and four cave stream resurgence springs: Blue Spring (Moore Cave); Thunderhole Resurgence (Running Bull Cave); Cedar Spring (Rimstone River Cave); and Mystery Resurgence (Mystery Cave) (Figure 1). Resurgence sites are the most downstream location for each cave system and were selected in an effort to capture contamination emanating from diffuse upstream sources. Cave streams involved in this work all support populations of grotto sculpin, in addition to a high diversity of other stygobitic and troglobitic species. Mystery Cave has the highest species diversity of any cave in Perry County with 59 species, including eight listed as Missouri Species of Concern because of extreme rarity or small population size, and five that are rare or threatened on a global scale (4).

Equipment and Chemicals. Commercially available SPMDs and POCIS were provided by Environmental Sampling Technologies (EST, St. Joseph, Missouri). SPMDs were constructed following a design developed by Huckins et al. (*12*) and consisted of thin (75−90 μm), low density polyethylene tubes (91.4 × 2.5 cm) containing 1 mL ultra high purity (>99%), synthetic triglyceride triolein (1,2,3-tri[cis-9-octadecenoyl] glycerol) purified using methods developed by Lebo et al. (*18*), which greatly reduced the incidence of analytically interfering compounds. POCIS were the pharmaceutical variety (*14*), with each disk constructed of two microporous polyethersulfone membranes (130-μm thickness) enclosing 0.228 g Oasis hydrophilic−lipophilic copolymer as the sorbent, and an effective surface area of 41 cm^2. Automated multiprobe datasondes (Hydrolab; Hach

Environmental, Loveland, CO) were provided by the Missouri Department of Conservation (MDC) to record abiotic conditions (turbidity, specific conductivity, temperature, etc.) of the cave streams during PSD deployment. Ultra high-purity analytical chemical standards were purchased from Ultra Scientific and AccuStandard, Inc. and all organic solvents (Optima or Pesticide Analysis grade) were purchased from Fisher Scientific.

Deployment and Collection. SPMDs and POCIS were deployed from mid-May until mid-July 2008 for two consecutive periods lasting 31and 32 days, respectively. Upon collection, the condition and extent of biofouling of samplers were recorded. Samples were then placed into solvent-rinsed metal cans and transferred to a cooler where they were maintained at 0°C during transport. SPMDs and POCIS were stored at 20°C in the laboratory prior to cleanup, extraction, and analysis. Additional details of deployment methods are given in S6−S7 of the Supporting Information (SI).

QA/QC. SPMDs were spiked during construction with 10 μg of four analytically noninterfering performance reference compounds (PRCs) of moderate to high fugacity: anthracene-d$_{10}$; phenanthrene-d$_{10}$; pyrene-d$_{10}$; and chrysene-d$_{12}$. During aquatic deployment PRCs slowly dissipate from SPMDs, with the rate of PRC loss increasing as logarithmic water−octanol partition coefficient (log K_{ow}) value decreases (*19*). The rate of PRC loss (PRC uncertainty factors are listed in SI Table S1) was derived from measuring concentrations at the beginning ("day 0") and end of the exposure periods, enabling correction of laboratory sampling rates to account for the effects of specific environmental variables such as turbulence, biofouling, and temperature (*19*−*22*). SPMD fabrication blanks were used to calculate initial PRC concentrations and to account for any existing laboratory contamination. Additional quality control measures included the use of replicate field blanks (SPMD and POCIS) and laboratory reagent blanks.

SPMD Processing. The patented procedures used by EST for processing and hexane dialysis of SPMDs are similar to those described by Huckins et al. (*22*). Following dialytic recovery of accumulated compounds, SPMD extracts containing PRCs were purified using gel permeation chromatography (GPC) with methylene chloride as the mobile phase, a process which removes nearly all polyethylene waxes, and 95−99% of oleic acid and methyl oleate (*18*). This, in addition to the use of ion trap mass spectrometry in selective ion storage (SIS) mode for quantitation of contaminant concentrations, obviated the need for additional chemical cleanup. In order to increase likelihood of detection, extracts from two SPMD replicates from each site were combined to create a composite sample for each deployment period. Composite samples were evaporated to dryness and then reconstituted to a final volume of 1 mL with the addition of pentachloronitrobenzene (PCNB) at a concentration of 2 μg/mL in hexane as the internal standard prior to GC/MS analysis.

POCIS Processing. Chemical residues were recovered from POCIS following procedures described by Alvarez et al. (*9*). Compounds sequestered in the sorbent material were extracted from POCIS replicates with 40 mL of high purity methanol. Methanol extracts from POCIS replicates were combined and evaporated to dryness before being reconstituted to a final volume of 1 mL in methanol with PCNB as the internal standard.

Chemical Analysis. Samples were analyzed using a Varian CP 3800 gas chromatograph coupled to a Saturn 2000 ion trap mass spectrometer. Separation was achieved through injection of 1 μL of sample onto a Varian HP-5 ms column (30 m × 0.25 mm × 0.32 μm) following temperature programs shown in Table 1. Analyte peaks were identified by comparing GC/MS retention times and the three most abundant ions with those of commercial standards. Quantitation of all compounds was performed by means of selective ion storage (SIS) using six-point calibration curves. To account for loss of analyte during evaporation, 1 mL of each standard solution was also evaporated to dryness, before being reconstituted to a final volume of 1 mL in hexane or methanol (SPMD and POCIS, respectively) and spiked with the internal standard PCNB. No recovery experiments were performed, as extracts and standard solutions were processed in parallel to compensate for any loss of analyte. Method detection limits (MDLs) were calculated as the mean signal-to-noise ratio at an analyte's retention time plus three standard deviations from replicate blanks. Method quantitation limits (MQLs) were calculated at 10 standard deviations (Tables S2,S3 of the SI).

TABLE 1. GC/MS Temperature Programs Used for Chemical Analysis of SPMD and POCIS Extracts

	T (°C)	hold (min)	ramp (°C/min)
SPMD	70		15
	165		2
	250		10
	320		
POCIS	90	1	10
	180	5	5
	230	5	10
	320	2	

Water Concentration Estimates—SPMDs. Researchers have previously used two different equations in order to estimate TWA water concentrations (C_w) of selected contaminants depending upon whether uptake was linear or equilibrium-based (*21*). Recently, a concentration equation was introduced by Huckins et al. (*22*), which, unlike previous methods, can be used to calculate C_w regardless of the mode of analyte uptake. Values derived for C_w using the updated method are comparable to those derived by previous methods, though the newer equation tends to result in slightly lower values (*23*). For this work, an empirically-based SPMD water concentration calculator developed by the USGS (v.5) was used to estimate TWA aqueous concentrations from the amounts of chemicals absorbed by SPMDs (*24*). Log K_{ow} values used for calculating TWA water concentrations are given in Table S2, SI.

Water Concentration Estimates—POCIS. Estimation of TWA ambient water concentrations from the amount of chemicals accumulated by POCIS is dependent on the availability of R_s values generated in laboratory calibration studies. Chemical uptake by POCIS generally follows first-order kinetics with water sampling rates (R_s; L or mL/day) controlled primarily by resistance to mass transfer imparted by the water boundary layer (WBL) present at the surface of the POCIS membrane, as well as by the water-filled pores of the polyethersulfone membrane itself (*14*). Observed increases in the rates of chemical uptake by POCIS associated with higher water flow or turbulence appears to be largely due to a reduction in the effective thickness of the WBL, especially for chemicals with log K_{ow} values < 3 (*14*). A suitable PRC method for POCIS has not yet been developed, although several compounds showing potential for use as PRCs have recently been identified (*25*).

Although POCIS R_s values are available for a growing number of chemicals with bioaccumulative potential, flow conditions under which they are generated often vary among calibration studies, and may not be representative of faster water flows present under environmental exposure conditions (*15, 26, 27*). Alvarez et al. (*14*) reported a 5−6-fold difference in R_s values for nine chemicals measured under still and turbulent conditions. The exact flow rates for the cave streams were not measured during this study; however,

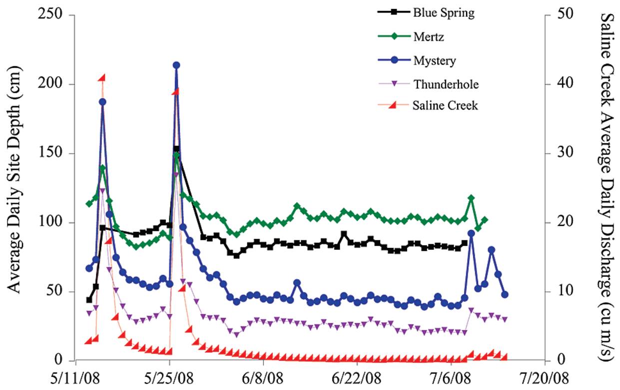

FIGURE 2. Hydrograph of cave stream and resurgence spring depth (cm) and discharge (m^3 s^{-1}) of South Saline Creek, a surface stream located near Perryville, Missouri.

rapid changes in site depth of up to 150 cm were observed following several significant storm events during May and June deployments indicating significant water flows and extremely turbulent conditions (Figure 2). For this reason, the highest available POCIS uptake rates were used when possible for calculating TWA water concentration estimates (SI, Tables S2−S3). Use of these higher R_s values may result in an underestimation of chemical concentrations; however, this difference is relatively minor and not orders of magnitude in size (David Alvarez, USGS, Pers. comm.).

Geospatial Data Analysis. Geographic information system (GIS) data were obtained from the Missouri Spatial Data Information Service (MSDIS) and the MDC (additional details given in section A, SI). These data were used with ArcGIS 9.3 software to map cave systems, monitoring sites, and sinkhole density within the study area (Figure 1). Data were log10-transformed to correct for nonnormality, and relationships between land cover, chemical concentrations, and Hydrolab data were analyzed for statistical significance ($\alpha = 0.05$) through multiple pairwise correlations using JMP 7 (SAS) statistical software.

Results and Discussion

Field Blanks. Phenanthrene, pyrene, and the mosquito repellent *N*,*N*-diethyltoluamide (DEET) were detected at measurable levels in field blanks. Reported values in Tables 2 and 3 for these compounds were corrected to reflect field blank concentrations. Fabrication and reagent blanks were not found to contain levels of chemicals above MDLs.

PRC Dissipation. Analysis of PRC dissipation during field exposures of SPMDs provided a means to correct laboratory-derived sampling rates and account for the effects of differing environmental conditions on chemical uptake among sampling locations. Pyrene-d_{10} and chrysene-d_{12} exhibited minimal losses with nearly 100% retention of both compounds. Dissipation of anthracene-d_{10} and phenanthrene-d_{10} was within the optimal range of 20 to 80% (*21*), which allowed the use of both PRCs in sampling rate corrections. PRC dissipation rates were substantially higher at all sampling locations during May deployment indicating that both water flow and the rate of chemical uptake were also higher than during June deployment.

Hydrological Monitoring. Data for cave steam depth (m) illustrate the speed at which stormwater runoff is funneled into the cave streams by sinkholes and conduits in the carbonate bedrock during significant rain events (Figure 2). The short lag time between rainfall and the arrival of stormwater can be seen through comparisons of cave stream depth to average daily discharge (m^3/sec) of South Fork Saline Creek, a surface steam located near the city of Perryville. Maximum average daily cave stream depth corresponded with peaks in Saline Creek discharge during large rain events in May, as well as several smaller rain events in July 2008. The extremely short transport time from recharge points to resurgence springs is reflective of the relatively thin soil and epikarst layers within the study area, which would act to both slow the rate of water flow and provide remediation of contaminants (*28*).

Contaminant Identification and Quantitation. GC/MS analyses of passive sampler extracts from the May and June deployments provide evidence of contamination of Perry County cave streams by a complex mixture of organic pollutants (Tables 2 and 3). Triazine and organochlorine pesticides and their degradation products were the most commonly detected classes of chemicals, followed by chloroacetanilide pesticides and polycyclic aromatic hydrocarbons (PAHs). A number of additional compounds of interest were tentatively identified by matching GC/MS retention times and mass signatures with those cataloged in the National Institute of Standards and Technology (NIST) chemical library (SI, Table S4). As the identities of these compounds were not confirmed, caution should be used when interpreting these results. However, they do provide a useful list of target chemicals for future water quality studies.

TWA water concentrations were calculated for a total of 20 chemicals (SPMD = 9; POCIS = 11) detected in samplers at levels above MDLs, the majority of which ($n = 16$) originated from agricultural pest management activities. At the 95% confidence level, no significant difference was found between the total number of chemicals detected during either May or June deployment periods. Four compounds were detected at all sites during both deployments: the cholora-cetanilide herbicides acetochlor and diethatyl-ethyl; the triazine herbicide atrazine; and its degradation product desethylatrazine (DEA). Four additional compounds were detected at all sites during either May or June deployment, including pyrene, metolachlor, DEET, and pentacholoroanisole, an organochlorine degradation product of pentachlorophenol. In addition, deisopropylatrazine (DIA) was also observed at every site except Thunderhole.

Mertz Cave was the most severely impacted of all the sites based upon total number of chemical detections ($n =$ 17) and maximum chemical concentrations ($n = 9$), including those of atrazine (130 ng/L), DEA (27 ng/L), metribuzin (62 ng/L), and trans-chlordane (3 ng/L), as well as elevated concentrations of DIA (41 ng/L) and dieldrin (34 ng/L). Although the same number of contaminants were detected at Thunderhole Resurgence ($n = 17$) as at Mertz, fewer chemicals were present at their maximum study concentrations ($n = 4$). These included acetochlor (53 ng/L), dieldrin (34 ng/L), pendimethalin (88 ng/L), and the heptachlor degradation product heptachlor epoxide (7 ng/L). Mystery Resurgence had the fewest total number of detections ($n =$ 10), and was the only site at which no chemical was found at its maximum concentration during either May or June deployments.

Current-Use Agricultural Pesticides. The detection of acetochlor, atrazine, and the principal atrazine degradation product, DEA, at all sites during both deployments was not surprising considering the extensive agricultural land use within the study area. Atrazine is the most frequently detected herbicide in both ground and surface waters and has been shown to reduce egg production and cause gonadal abnormalities in fathead minnows (*Pimephales promelas*) at concentrations well below current EPA criteria for protection of aquatic life (*29*). TWA concentrations for atrazine ranged from 60 to 130 ng/L during May deployment and 23 to 120 ng/L during June deployment. These values were on the same order of magnitude with water quality grab sample data collected by the Missouri Department of Conservation (MDC) between April and December 2008 (twice a month on

TABLE 2. Estimated Time-Weighted Average (TWA) Concentrations (ng L^{-1}) of Chemicals Sequestered in SPMDs Deployed in Cave Stream and Resurgence Sites during May and June 2008

analyte	Blue Spring		Cedar Spring		Mertz		Mystery		Thunderhole	
	May	June	May	June	May	June	May	June	May	June
	ng L^{-1}		ng L^{-1}		ng L^{-1}		ng L^{-1}		ng L^{-1}	
chlorpyrifos	ND[a]	ND	5.2	9.3	ND	ND	ND	ND	ND	1.5
dieldrin	ND	ND	ND	ND	34	ND	ND	ND	34	17
heptachlor epoxide	ND	ND	ND	ND	ND	ND	ND	ND	ND	7.1
pendimethalin	ND	ND	7.4	14	9.7	4.1	ND	ND	ND	88
pentachloroanisole	0.71	0.96	ND	0.47	0.44	1.3	0.46	0.44	0.15	0.29
phenanthrene	<MQL	ND	<MQL	ND	ND	ND	ND	ND	<MQL	ND
pyrene	0.57	1.2	<MQL	ND	2.4	2.6	<MQL	1.1	<MQL	<MQL
trans-chlordane	0.68	1.8	ND	ND	1.3	3.1	ND	ND	0.67	1.0
trans-nonachlor	0.51	1.4	ND	0.64	ND	0.56	ND	ND	0.40	1.2

[a] Nondetection (ND); values below method detection limits (MDL; mean +3 × SD). [b] (<MQL); values below method quantitation limits (MQL; mean +10 × SD).

TABLE 3. Estimated Time-Weighted Average (TWA) Water Concentrations (ng L^{-1}) of Chemicals Sequestered in POCIS Deployed in Cave Stream and Resurgence Sites during May and June 2008

analyte	Blue Spring		Cedar Spring		Mertz		Mystery		Thunderhole	
	May	June	May	June	May	June	May	June	May	June
	ng L^{-1}		ng L^{-1}		ng L^{-1}		ng L^{-1}		ng L^{-1}	
acetochlor	43	9.9	10	2.9	18	10	21	4.8	40	53
atrazine	60	23	61	120	130	74	70	31	85	88
DEET	*<MQL*[a]	5.7	2.4	<MQL	2.5	3.4	<MQL	*ND*[b]	3.0	3.5
desethylatrazine	11	8.9	10	23	27	9.4	11	8.9	11	9.9
deisopropylatrazine	28	16	28	55	41	19	25	15	ND	ND
diethatyl ethyl	4.0	8.7	2.1	3.9	1.7	2.7	3.4	1.8	4.2	7.8
dimethenamid	ND	ND	5.9	40	5.9	ND	ND	3.6	ND	8.9
metolachlor	ND	6.2	10	9.3	4.1	13	2.3	5.3	2.3	2.4
metribuzin	43	7.7	ND	ND	62	9.1	4.9	3.8	6.9	ND
prometon	ND	ND	ND	ND	7.1	9.5	ND	ND	ND	ND
terbuthylazine	ND	ND	ND	<MQL	16	ND	ND	ND	ND	ND

[a] (<MQL); values below method quantitation limits (MQL; mean +10 × SD). [b] Nondetection (ND); values below method detection limits (MDL; mean +3 × SD).

average), with median atrazine concentrations ranging from 120 ng/L (Thunderhole) to 510 ng/L (Cedar Spring) (Brad Pobst, MDC, Pers. comm.). Our results for atrazine, DIA, DEA, metribuzin, acetochlor, and metolachlor were also consistent with median values reported by Lerch (*30*) for Boone County, MO from April 1999 to April 2002. Levels of corn production were similar in the two counties during both study periods, with 9100 ha of corn harvested in Perry County in 2008 and 8700 ha harvested in Boone County in 2002 (*31*).

Legacy-Use Chemicals. Levels of two chlorinated cyclodiene insecticides, dieldrin and heptachlor epoxide, exceeded established EPA criteria for the protection of aquatic life. Dieldrin and heptachlor have been previously linked to mortality of endangered gray bats (*Myotis grisescens*) and aquatic macroinvertebrates during the late 1970s and early 1980s at Hunter Cave and Devil's Icebox Cave, Boone County, Missouri (*32*).

Heptachlor epoxide is the primary degradation product of heptachlor, and its toxicity is considered to be similar to that of the parent compound (*33*). Heptachlor epoxide was detected at Thunderhole Resurgence during June deployment at a concentration of 7 ng/L, nearly double the EPA aquatic life criterion of 3.8 ng/L as a 24-h average (*33*). The origins of heptachlor epoxide detected at Thunderhole are difficult to determine. Heptachlor, itself, is a major constituent of technical chlordane along with trans-chlordane and trans-nonachlor, both of which were also detected at Thunderhole during May and June deployments. Chlordane and heptachlor are highly persistent, with biodegradation half-lives lasting decades (*33*), so it is possible that heptachlor epoxide, trans-chlordane, and trans-nonachlor originated from historical use. However, another potential source of these chemicals is the area's numerous trash-filled sinkholes into which pesticide and chemical containers are frequently dumped.

Dieldrin was used extensively in the past as a domestic pesticide, primarily for control of corn pests, and it is also a degradation product of the organochlorine insecticide aldrin (*34*). Dieldrin is strongly nonpolar, with a high affinity for bioaccumulation in animal fats and a half-life of up to 25 years in certain environments (*34*), factors that contributed to its permanent cancellation in 1987 on the basis of severe hazards to human health and aquatic life. The current US EPA Criterion Continuous Concentration (CCC) for chronic exposure to dieldrin (56 ng/L as a four day average) does not consider exposure though diet (*35*). Accumulation of dieldrin through food consumption is an important factor, especially for aquatic life occupying upper trophic levels, resulting in tissue concentrations many times those found in the ambient environment (*34*). Grotto sculpin are top predators in their typically nutrient-limited cave stream habitat, relying on several species of cave amphipods (*Gammarus* sp.) and isopods (*Cecidotea* sp.) as a primary source of prey (*36*). It is for this reason that we believe that the ambient water quality criterion previously established for dieldrin (1.9 ng/L as a 24-h average), which takes dietary exposure into account, is more suitable for assessing the potential for adverse effects of the chemical on grotto sculpin. TWA water concentrations of dieldrin exceeded the EPA criterion of 1.9 ng/L by more than 17× at Mertz and Thunderhole (34 ng/L) during May deployment and by more than 8× that amount at Thunderhole in June (17 ng/L).

Given its long half-life, high stability, and low volatility (*34*), it is possible that dieldrin detected during this work originated from registered uses prior to its final cancellation. However, the high concentrations of the insecticide suggest a more recent input into the cave streams. Dieldrin concentrations during June deployment were less than half those observed during May deployment when rainfall totals were substantially higher. We strongly suspect that chemical leaching of trash-filled sinkholes by storm runoff is a major factor contributing to the critically high TWA concentrations of dieldrin at Mertz and Thunderhole. Identification and recovery of sinkholes found to contain nonvegetative agricultural and industrial debris, as well as implementation of buy-back programs aimed at preventing improper disposal of pesticides in the process of being phased-out, should be considered in order to minimize the threat of future groundwater contamination.

Toxicity of Chemical Mixtures. Predicting aquatic toxicity of pesticides in cave environments is difficult, as environmental contaminants represent just one class of stressors to which cave organisms are exposed (*37*). Additionally, individual contaminants may act synergistically in mixtures with additive or greater-than-additive effects. Toxicity of the organophosphate chlorpyrifos to midge larvae (*Chironomus tentans*), for example, has been shown to increase in the presence of atrazine (*38*), and in combination with DEA in the amphipod, *Hyalella azteca* (*39*). Fish exposed to mixtures containing organophosphate and carbamate pesticides exhibit overt symptoms of sublethal anticholinesterase toxicity not present during exposure to individual chemicals, including loss of equilibrium, rapid gilling, increased mucous production, and altered startle response (*40*). Model organisms for toxicity studies are typically surface dwelling invertebrates, and data are still lacking on the toxicity of observed contaminants on cave-adapted aquatic organisms including crustaceans and fishes. Therefore, direct toxicity studies for grotto sculpin and other stygobites with these detected compounds, both alone and in combination, are much needed in order to better inform management practices in karst areas with extensive agriculture.

Statistical and Geospatial Analysis. Hydrological recharge areas of the cave systems range in size from 567 ha (Running Bull Cave) to 3035 ha (Crevice Cave), with cropland and grassland comprising between 74% (Crevice Cave) and 87% (Mystery Cave) of land cover within the recharge zones (*41*) (SI, Table S5). Few significant statistical relationships were detected between land cover and chemical concentration variables, likely due to the similarly high proportion of agricultural land cover among all the recharge areas. However, average daily turbidity of cave streams was positively correlated with total (additive) chemical concentrations in POCIS ($n = 4$; $R^2 = 0.983$; $p = 0.017$) and with DEA concentration ($n = 4$; $R^2 = 0.999$; $p = 0.0004$) during May deployment. Maximum turbidity was strongly correlated with total chemical concentrations in POCIS ($n = 4$; $R^2 = 0.957$; $p = 0.043$) during May deployment, and with total number of chemical detections during June deployment ($n = 4$; $R^2 = 0.987$; $p = 0.014$).

The fact that Crevice Cave had the most chemical detections and lowest amount of cropland and grassland within its recharge area, with the opposite trend observed for Mystery Cave, indicates that land use alone is not the best predictor of contamination risk in these systems. Cave length and recharge area size also do not appear to be the greatest factors influencing chemical concentrations as evidenced by the similarly high levels of contaminants observed at Mertz and Thunderhole Resurgence. This suggests that intensity of agricultural land management practices and their location within the cave recharge areas, as well as proximity of point sources of pollution, such as trash-filled sinkholes, are more important predictors of cave stream vulnerability.

Point density analysis showed the highest concentrations of sinkholes centered just north of Perryville in the region of Crevice Cave and southeast of the city close to Running Bull Cave (Figure 1). These two cave systems also had both the greatest total number of chemical detections, and detections of chemicals at their maximum study concentrations. The upstream reaches of Crevice Cave within the region of highest sinkhole density are adjacent to an industrial zone housing several manufactures of foam, plastics, and other petroleum products, in addition to over 50 ha of vacant land available for future development. Particular care should be taken to ensure that development within this area of high sinkhole density is undertaken in a manner that minimizes the risk of contaminant infiltration.

Further Considerations. SPMDs and POCIS were deployed following extreme rainfall (~83 cm) during the period March through May, which caused widespread flooding of many low-lying agricultural fields. As a result, planting and pest management activities were delayed in a large number of fields, leading to a 23% (2954 ha) reduction in corn acreage planted in Perry County during 2008 compared to 2007, and a 25% (3076 ha) reduction in corn harvested. Along with an overall reduction in acreage treated with pesticides, heavy rainfall and flooding likely mobilized residual chemicals in soils and diluted concentrations of chemicals in cave streams during sampler deployment. This, along with our use of higher R_s values for calculating TWA water concentrations for POCIS, suggests that our results represent a conservative assessment of contaminant concentrations in Perry County cave streams during a typical growing season and are a baseline to which future contaminant data may be compared.

Assessment of the bioavailable fractions of organic chemical contaminants in Perry County, Missouri cave streams is a primary step in evaluating the threat of pollution to grotto sculpin and other aquatic cave organisms. The results of this study demonstrate the susceptibility of shallow groundwater in a karst watershed to contamination by a variety of synthetic organic chemicals. In addition, PSDs are shown to be useful for monitoring organic chemicals in highly dynamic karst environments where rapid infiltration and transmission of surface runoff strongly influence contaminant concentrations.

Effective management of cave streamwater quality must be based upon sound knowledge of karst watershed hydrology and modes of chemical transport. Increasing public and municipal awareness of surface connectivity to the subterranean is also crucial to protection of karst groundwater resources. Future research in Perry County may focus more closely on how specific land use practices, local climate patterns, and proximity to point sources of pollution contribute to the contaminant burden of the region's cave streams and resurgence springs.

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Supporting Information Available

Further details on the data and methods, tentatively indentified compounds, and cave recharge areas. This information is available free of charge via the Internet at http://pubs.acs.org/.

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