Title: Occurrence of Pharmaceuticals, Hormones, and other Organic Wastewater Contaminants in Cave Water within the Lower Neosho and Illinois River Basins, Oklahoma

Start Date: 3/1/2006

End Date: 2/28/2007

Congressional District: District 3 (University), District 2 (Field Sites)

Focus Category: GW, NPP, WQL

Descriptors: Organic wastewater contaminants, cave streams, passive sampler

Principal Investigators: Joseph R. Bidwell, Department of Zoology, Oklahoma State University

Publications: A manuscript that will be submitted to a peer-reviewed publication is currently in preparation.

Problem and Research Objectives: The headwaters and upper basins of the Illinois River and the eastern part of the Lower Neosho River are located in northwestern Arkansas and northeastern Oklahoma. In Arkansas, this area is experiencing significant urban development and is one of the most productive poultry producing regions in the United States (Galloway et al. 2004). While there has been widespread concern about the health and continued aesthetic quality of these systems due to nutrient input and associated water quality effects, effluent and/or runoff from livestock production facilities and municipal wastewater treatment plants may contain a number of organic wastewater contaminants (OWCs) such as antibiotics, hormone residues, various pharmaceutical compounds, and other trace organics that are ultimately transferred to aquatic habitats. The frequency with which these contaminants occur in U.S. surface waters was clearly indicated by a U.S. Geological Survey (USGS) study that found 80% of the 139 streams sampled contained detectable levels of OWCs (Kolpin et al. 2002). Another recent USGS study (Galloway et al. 2004) sampled surface water in Benton and Washington counties, Arkansas, and similarly reported the presence of selected OWCs in some streams receiving input from municipal wastewater treatment plants.

In addition to the land use characteristics stated above, the basins of the Illinois and Lower Neosho Rivers are situated within the Ozark Plateau, an uplifted region with a karst topography that is characterized by sinkholes, disappearing springs, and caves. As a result of these features, surface water contaminants may enter the groundwater and associated cave ecosystems. The presence of contaminants in water flowing through caves is a concern since these habitats often support a highly specialized assemblage of organisms which may be particularly susceptible to water-quality impacts due to their generally low population densities and unique life history requirements (Graening and Brown, 2003). Several aquatic species that are federally-listed or considered species of special concern are known to use caves in the Ozark Plateau of Oklahoma. These species include the Ozark cavefish, *Amblyopsis rosae*, federally- and

state-listed as threatened, and the Oklahoma cave crayfish, *Cambarus tartraus*, state-listed as endangered. An unnamed cave crayfish, *C. subterraneus*, and the Ozark cave amphipod, *Stygobromus ozarkensis,* both considered critically imperiled by the Oklahoma Natural Heritage Inventory, also occur in caves of this region (NatureServe, 2005). Environmental contamination has been identified as one of the most significant threats to cave-dwelling fishes (Proudlove, 2001), and chemical and septic system pollution has been implicated in the loss of both invertebrate and vertebrate taxa from cave ecosystems (Aley, 1976; Crunkilton, 1984; Simon and Buikema, 1997).

The prevalence and potential impacts of pharmaceuticals, hormones, and other OWCs in the Ozark cave habitats of Oklahoma are currently unknown. However, given the agricultural activities, wastewater discharges, and urban development that are occurring in the Lower Neosho and Illinois River basins, the karstic nature of these basins, and the recent detection of OWCs in streams within them (Galloway et al. 2004), contamination of ground water and associated cave habitats may be occurring. The presence of OWCs and related compounds could have significant implications for the long-term management of cave habitats since the potential for effects due to exposure to these contaminants may result in the need to relocate populations of some organisms and make it necessary to revise recovery plans for those groups listed as threatened or endangered. By determining the extent to which OWC residues are present in caves, this project will provide an important first step toward understanding the risk these chemicals pose to these sensitive habitats.

The objectives of this study were to 1) determine the presence of selected lipid soluble and water soluble pharmaceuticals, hormones, and other organic wastewater contaminants at two surface-water sites and in ground water in six caves in northeastern Oklahoma and western Arkansas using Semi Permeable Membrane Devices (SPMD) and Polar Organic Chemical Integrated Samplers (POCIS), and 2) evaluate the potential for sub-lethal effects associated with exposure to water from the sampling sites through 7-day bioassays with the fathead minnow, *Pimephales promelas*, a standard US EPA test organism.

Methodology: *Site selection*: Caves were selected in the Illinois and Lower Neosho River basins with the guidance of Mr. Steve Hensley and Mr. Richard Stark, US Fish and Wildlife Service, Tulsa Field Office, and were restricted to sites in which populations of Ozark cavefish, *A. rosae*, were known to have occurred. The caves evaluated included four systems (Twin, Starr, Mgee and Long, and January-Stansbury) in Delaware County, OK, and two systems (Logan and Cave Springs) in Benton County, AK. In addition, sampling devices were deployed at one surface water site in Oklahoma and one in Arkansas. The Oklahoma site (designated OK-Surface) was located in an unnamed creek near the town of Jay, OK, with the sampler deployed approximately 250 m downstream from the outfall of a municipal wastewater treatment plant. A previous study indicated a link between water in this stream and that occurring in Star Cave (Aley, 2005). The Arkansas surface water site was located in Little Osage Creek (designated AK-Surface) near Osage Mills, AK.

Passive sampler deployment: The cave and surface-water sites were sampled using two types of passive, *in situ*, samplers; the polar organic chemical integrative sampler or POCIS and the semi-permeable membrane device or SPMD. The POCIS is designed to sample transient water-soluble (polar or hydrophilic) organic chemicals from aquatic environments (USGS, 2004), while SPMDs passively accumulate transient hydrophobic organic compounds, such as PCBs, PAHs, and organochlorine pesticides (Huff, 2005). Both of these samplers have been successfully used to monitor OWCs in surface and groundwater (Jones-Lepp et al. 2004; Vrana et al. 2005). The POCIS and SPMDs were purchased from Environmental Sampling Technologies (EST), St. Joseph, MO, and were sent to Oklahoma State University under argon gas in sealed metal cans. The sampling membranes were held on stainless steel racks and deployed in stainless steel canisters (also obtained from EST, Figure 1 & 2). Each canister included three POCIS and three SPMD samplers, with a single canister placed at each site. During deployment, the samplers were removed from their original metal cans and placed in the deployment canisters as quickly as possible to minimize air exposure. In the caves, the cages were tied off to some object on the shoreline with a length of nylon rope (Figure 2). At the surface water sites, the racks were affixed to a concrete block with lengths of stainless steel cable and submerged. The time taken to transfer the sampler to the cage and then to submerge the cage in water was recorded during each deployment. A set of six trip blanks consisting of metal cans that contained either one of three POSIS or SPMD membranes were opened and exposed to air during the time the water samplers were being deployed as well.

The samplers were left on site for approximately 30 days (exact duration is presented in results). During retrieval, the stainless steel canisters were first moved from the water to the shoreline where they were opened. The racks holding the sampling membranes were removed and placed in the original metal cans. As for deployment, trip blanks for both membrane types were exposed to the air from the time the samplers were first removed from the stainless steel canisters until they were placed in the holding cans and the cans sealed by gently tapping their metal lids into place. The sealed cans were placed on ice as soon as possible and transported back to Oklahoma State University where they were held at -20°C until being shipped overnight to Environmental Sampling Technologies (also on ice) for extraction.

The membrane extraction procedures followed standard protocols used by Environmental Sampling Technologies for the POCIS and SPMD. The final extract from each sampling cage was a composite of either the three POCIS or SPMD membranes. Similarly, the replicate trip blanks were composited into one extract. The final combined extracts were transferred to 2 ml amber ampules and the ampules sealed in preparation for analyses. In total, the samples were analyzed for 159 different compounds, including 68 common wastewater organics (USGS Schedule 1433), 33 antibiotic and pharmaceutical compounds and 58 additional organics including a number of halogenated forms (Tables 1-3). The analyses were conducted using liquid chromatography-mass spectroscopy (LC-MS) and gas chromatography-mass spectroscopy (GC-MS) at the USGS National Water Quality Laboratory, Denver, Colorado, under the direction of Dr. Steven Zaugg (analytes listed in Table 1 & 3) and at

the Organic Geochemistry Laboratory, USGS Kansas Water Science Center, Lawrence, Kansas, under the direction of Dr. Michael Meyer (antibiotics listed in Table 2). As indicated in Tables 1-3, some of these analyses were conducted on extracts from both samplers, while others were restricted to a particular membrane type. The methods used for analyses of the OWCs and antibiotics listed in Tables 1 &2 followed established protocols (Zaugg et al. 2001; Alvarez et al. 2005) that allowed detection at the microgram (1×10^{-6}g) level. The 58 organic compounds listed in Table 3 were quantified at the nanogram (1×10^{-9}g) level by a technique that is currently under development and is not yet published (S. Zaugg, USGS, personal communication). The results of chemical analyses are qualitative (presence/absence) or semi-quantitative (relative concentrations) since calculations of water concentrations based on levels sequestered in the sampling devices requires an *in situ* sampling rate for each chemical and this value is not known for all analytes (Alvarez et al. 2005).

Field water chemistry and fathead minnow bioassays: Basic water chemistry parameters including specific conductance, pH, temperature and dissolved oxygen were measured at cave and surface-water sites when samplers were deployed and collected using a YSI XL-600 multimeter. These same parameters and alkalinity and hardness were determined for each of the water samples collected for the fathead minnow bioassays. Water for these bioassays was collected in acid-washed, 3-liter plastic containers at each cave and surface water site and placed on ice as soon as possible for transport back to the University. Fathead minnow bioassays were conducted at the Ecotoxicology and Water Quality Research Laboratory, Oklahoma State University. General test protocols (test chamber size, loading rate, water renewal, feeding, etc.) followed methods described in US EPA (2002). Briefly, larval fish (<24 h) were exposed to sample water for 7 days and their survival and growth (as dry weight) were compared to that of fish maintained in laboratory water formulated to have similar hardness as the site water (US EPA 2002). Statistical analyses of growth and survival data were conducted using the Comprehensive Environmental Toxicity Information System (CETIS ver 1.1.1, Tidepool Scientific Software, McKinleyville, CA) and followed the standard US EPA decision tree for chronic toxicity data (US EPA 2002). In cases where a significant difference in growth and/or survival was observed between laboratory reference and field samples, the site was re-sampled and a dilution series of the site water was prepared (using laboratory water as the diluent) to determine if a dose response could be generated.

Figure 1. POCIS (left) and SPMD (right) sampling membranes deployed at the cave and surface water sites.

Figure 2. Deployment of a stainless steel canister in a cave (left) and at one of the surface sites (right).

Table 1. Organic wastewater compounds that were targeted in analyses of the extracts from both the POCIS and SPMD passive sampling devices. Descriptions of chemicals and associated laboratory reporting levels were taken from Galloway et al. (2004) and Alvarez et al. (2005). *-Compound analyzed in extracts from SPMD only

Compound	Description	Laboratory Reporting Level (µg/L)
1,4-Dichlorobenzene	Moth repellant, fumigant, deodorant	0.5
1-Methylnapthalene	Component of gasoline/diesel/crude oil	0.5
2,6-Dimethylnapthalene	Component of diesel and kerosene	0.5
2-Methylnapthalene	Component of gasoline/diesel/crude oil	0.5
3,4-Dichlorophenylisocyanate	Herbicide intermediate	
3-beta-Coprostanol	Carnivore fecal indicator	2.0
4-Cumylphenol	Nonionic detergent metabolite	1.0
17-beta-Estradiol*	Estrogen replacement therapy and metabolite	5.0
4-n-Octylphenol	Nonionic detergent metabolite	1.0
4-tert-Octylphenol	Nonionic detergent metabolite	1.0
5-methyl-1H-Benzotriazle	Antifreeze component, deicer	2.0
2,2',4,4'-tetrabromodiphenylether*	Flame retardant	
Acetophenone*	Fragrance in soap, detergent, tobacco; flavor in beverages	0.5
Anthracene	Wood preservative, component of tar/diesel/crude	0.5
Anthraquinone	Used in Manufacture of dye/textiles	0.5
Atrazine	Herbicide	
Benzo(a)pyrene	Polyaromatic hydrocarbon, by-product of combustion	0.5
Benzophenone	Fixative for perfumes and soaps	0.5
Beta-sitosterol	Generally a plant sterol	2.0
BisphenolA	Manufacture of resins; antioxidant	1.0
Bromacil	Herbicide- non-crop grass/brush control	0.5
Bromoform	By-product of wastewater ozination	0.5
Caffeine	Stimulant	0.5
Camphor	Flavor, odorant, in ointments	0.5

Table 1. Continued

Compound	Description	Laboratory Reporting Level (µg/L)
Carbazole	Manufacture of dyes, explosives, and lubricants	0.5
Chlorpyrifos*	Organophosphorous insecticide	0.5
Cholesterol	Fecal indicator, also a plant sterol	2.0
Cotinine	Primary nicotine metabolite	1.0
Cumene	Manuf phenol/acetone; component of fuels/paint thinner	0.5
Diazinon	Organophosphorous insecticide	0.5
Dichlorvos	Organophosphorous insecticide	1.0
Diethylhexylphthalate	Plasticizer	0.5
Diethylphthalate	Plasticizer	0.5
d-Limonene	Antimicrobial antiviral; fragrance in aerosols	0.5
Estrone*	Hormone	5.0
Ethanol,2-butoxy-,phosphate	Flame retardant	0.5
Ethylcitrate	Cosmetic component	0.5
Fluoranthene	Component of coal tar/asphalt	0.5
Galaxolide (HHCB)*	Musk fragrance	0.5
Indole	Fragrance	0.5
Isoborneol	Fragrance	0.5
Isophorone	Solvent for lacquers, plastics, oils, silicon, resins	0.5
Isoquinoline	Manuf phenol/acetone; component of fuels/paint thinner	0.5
Menthol	Cigarettes, cough drops, liniment, mouthwash	0.5
Metalaxyl	Fungicide	0.5
Methylsalicylate	Liniment, food, beverage, UV-absorbing lotions	0.5
Metolachlor	Herbicide	0.5
N,N-diethyltoluamide (DEET)	Insect repellant	0.5
Naphthalene	Fumigant	0.5
Nonylphenol di-ethoxylates (total)	Nonionic detergent metabolite	5.0
Octylphenol di-ethoxylates (total)	Nonionic detergent metabolite	1.0
Octylphenol monoethoxylates (total)	Nonionic detergent metabolite termite control	1.0
Para-cresol*	Wood preservative	1.0

Table 1. Continued

Compound	Description	Laboratory Reporting Level (µg/L)
Para-nonylphenol (total)	Nonionic detergent metabolite	5.0
Pentachlorophenol	Insecticide	2.0
Phenanthrene	Component of tar/diesel/crude	0.5
Phenol*	Disinfectant	0.5
Prometon	Herbicide	0.5
Pyrene	common in coal tar/asphalt	0.5
s3-Methyl-1(H)-indole (skatol)	Odor in feces and coal tar	1.0
Stigmastanol	Generally a plant sterol	2.0
Tetrachloroethylene	Solvent, degreaser; Veterinary: anthelminic	0.5
Tonalide (AHTN)*	Musk fragrance	0.5
Triclosan*	Antimicrobial in soaps	1.0
tri(2-Chloroethyl)phosphate	Plasticizer and flame retardant	0.5
tri(Dichlorisopropyl)phosphate	Flame retardant	0.5
Tributylphosphate	Antifoaming agent and flame retardant	0.5
Triphenylphosphate	Plasticizer, resins, waxes, finishes, roofing paper, Flame retardant	0.5

Table 2. Antibiotics and other pharmaceutical compounds that were additionally targeted in analyses of the extracts from the POCIS samplers.

Compound	Description	Laboratory Reporting Level (µg/L)
Azithromycin	Antibiotic	0.005
Carbamazapine	Anticonvulsant	0.005
Chloramphenicol	Antibiotic	0.020
Chlorotetracycline	Antibiotic	0.01
Ciproflaxacin	Antibiotic	0.005
Doxycycline	Antibiotic	0.01
Enrofloxacin	Antibiotic	0.005
Epi-chlorotetracycline	Antibiotic	0.01
Epi-iso-chlorotetracycline	Antibiotic	0.010
Epi-oxytetracycline	Antibiotic	0.010
Epi-tetracycline	Antibiotic	0.010
Erythromycin	Antibiotic	0.005
Erythromycin-H2O	Antibiotic	0.005
Ibuprofen	Analgesic	0.020

Table 2. Continued

Compound	Description	Laboratory Reporting Level (µg/L)
Iso-chlorotetracycline	Antibiotic	0.010
Lincomycin	Antibiotic	0.005
Lomefloxacin	Antibiotic	0.005
Norfloxacin	Antibiotic	0.005
Ofloxacin	Antibiotic	0.005
Ormetoprim	Antibiotic	0.005
Oxytetracycline	Antibiotic	0.010
Roxithromycin	Antibiotic	0.005
Sarafloxacin	Antibiotic	0.005
Sulfachloropyridazine	Antibiotic	0.005
Sulfadiazine	Antibiotic	0.005
Sulfadimethoxine	Antibiotic	0.005
Sulfamethazine	Antibiotic	0.005
Sulfamethoxazole	Antibiotic	0.005
Sulfathiazole	Antibiotic	0.005
Tetracycline	Antibiotic	0.010
Trimethoprim	Antibiotic	0.005
Tylosin	Antibiotic	0.005
Virginiamycin	Antibiotic	0.005

Table 3. Additional organic compounds that were targeted in analyses of the extracts from the SPMD samplers only. Analyses were conducted with a method that allowed lower detection limits.

Compound	Description	Laboratory Reporting Level (ng/L)
BDE100 (Brominated di-phenyl ether)	Flame Retardant	0.2
BDE138	Flame Retardant	0.5
BDE153	Flame Retardant	0.5
BDE154	Flame Retardant	0.5
BDE183	Flame Retardant	0.5
BDE47	Flame Retardant	0.5
BDE66	Flame Retardant	0.5
BDE71	Flame Retardant	0.5
BDE85	Flame Retardant	0.2
BDE99	Flame Retardant	0.2
Chlorpyrifos	Organophosphorous insecticide	0.5

Table 3. Continued

Compound	Description	Laboratory Reporting Level (ng/L)
Chlorthalonil	Organochlirine, Fungicide	10
cis-Chlordane	Organochlorine, Insecticide	0.2
cis-Nonachlor	Organochlorine, Insecticide	0.2
Cyfluthrin	Pyrethroid insecticide	0.5
Cyhalothrin	Pyrethroid insecticide	0.5
DCPA	Phthalate/herbicide	0.2
desulfnylFipronil	Organochlorine, Insecticide	0.2
Dieldrin	Organochlorine, Insecticide	0.2
Endosulfan I	Organochlorine, Insecticide	0.2
Fipronil	Organochlorine, Insecticide	0.2
FipronilSulfide	Organochlorine, Insecticide	0.2
Firemaster	Flame Retardant	0.5
HCB	Organochlorine, Fungicide	0.2
Octachlorostyrene	Organochlorine biproduct	1
Oxychlordane	Organochlorine breakdown product	1
Oxyfluorfen	Herbicide	10
p,p-DDT	Organochlorine, Insecticide	5
p,p'-DDE	DDT metabolite	1
p,p'-DDD	DDT metabolite	2
PCA (p-chloroaniline)	Dye intermediates, agricultural chemicals and pharmaceuticals	0.2
PCB101 (Polychlorinated biphenyl)	Organochlorine, formerly used in hydraulic oils and some other industrial applications	2
PCB110		1
PCB118		0.5
PCB138		0.5
PCB146		0.5
PCB149		1
PCB151		1
PCB170		0.5
PCB174		0.5
PCB177		0.5
PCB180		0.5
PCB183		0.5

Table 3. Continued

Compound	Description	Laboratory Reporting Level (ng/L)
PCB187		0.5
PCB194		0.5
PCB206		0.5
PCB44		5
PCB49		5
PCB52		5
PCB70		2
Pendimethalin	Herbicide	5
Pentabromotoluene	Flame Retardant	1
Pentachloronitrobenzene	Water treatment/fungicide	0.2
Tefluthrin	Pyrethroid insecticide	0.2
Tetradifon	Acaricide	0.5
trans-Chlordane	Organochlorine, Insecticide	0.2
trans-Nonachlor	Organochlorine, Insecticide	0.2
Triclosan	Antimicrobial in soaps	10
Methoxytriclosan	Antiseptic, metabolite of Triclosan	2
Trifluralin	Herbicide	0.2

Principal Findings and Significance: The deployment time for the samplers ranged from 28 to 35 days (Table 4). The first deployment at Star Cave in May – June 2006 failed because the cave stream went dry before the end of the exposure time. A second successful deployment was conducted in June –August 2006 in which the sampler was placed in more permanent water farther into the cave. Due to the possible connection between the OK surface water site and this cave, a second sampler was deployed at the OK surface site in conjunction with the second deployment at Star Cave.

Table 4. Deployment and retrieval dates and durations of exposure for the POCIS and SPMD samplers at each cave and surface water site.

Site	Deploy date	Retrieve date	Exposure time (Days)
AK-Surface	8 May 2006	5 June 2006	28
OK-Surface-1st Deployment*	8 May 2006	7 June 2006	30
OK-Surface-2nd Deployment*	27 June 2006	1 August 2006	35
Cave Springs (AK)	2 May 2006	5 June 2006	34
January-Stansbury (OK)	1 May 2006	1 June 2006	31
Logan (AK)	2 May 2006	5 June 2006	34
Mgee and Long (OK)	1 May 2006	1 June 2006	31
Star (OK)- 1st Deployment*	8 May 2006	7 June 2006	30
Star – 2nd Deployment*	27 June 2006	1 August 2006	35
Twin (OK)	6 May 2006	6 June 2006	31

*= The stream in Star Cave went dry during the first deployment and a new sampler had to be redeployed. A second sampler was also placed at the OK-Surface because of the potential link between it and Star Cave.

On-site water chemistry: The water chemistry values measured at each of the sites are presented in Table 5. Due to instrument malfunctions, values were not available for all sites on all visits. The measured temperature at the cave sites ranged from 13-15 °C, pH was near neutral and dissolved oxygen was near saturation. Conductivity levels for the Oklahoma caves were higher than those for the Arkansas caves. The temperature values at the Oklahoma surface water site were approximately 10°C higher than that in the caves, and dissolved oxygen was below saturation.

Table 5. Water chemistry values at surface water and cave sites during deployment/retrieval of the samplers. Due to instrument malfunctions, data for some of the sites were not available (NA).

	AK-Surface	OK-Surface	Cave Springs	January-Stansbury	Logan	Mgee and Long	Star	Twin
Temperature (°C)	NA	21.6[1]/23.5[2]	14.9/NA	13.5/14.2	14.3/NA	13.8/15.0	15.8[3]/NA	15.7[4]
Dissolved Oxygen (mg/L)	NA	7.2[1]/5.6[2]	8.8/NA	10.2/10.1	9.3/NA	9.2/7.6	6.2[3]/NA	8.5[4]
pH	NA	6.9[1]/6.5[2]	6.9/NA	7.0/7.0	7.1/NA	6.8/6.8	6.4[3]/NA	7.6[4]
Conductivity (uS/cm)	NA	494[1]/602[2]	269/NA	207/293	293/NA	NA/258	456[3]/NA	398[4]

1-Value from 7 June 2006 retrieval of sampler

2-Value from 27 June 2006 deployment of sampler

3-Value from 27 June 2006 deployment of sampler

4-Value from 3 December 2005 reconnaissance of site

Detection of target compounds in passive sampler extracts: Lists of the compounds detected in the extracts from the POCIS and SPMD samplers are presented in Tables 6-9. These tables are differentiated based on analytical technique used and compounds analyzed. The data presented in Tables 6 & 7 were derived from the analyses for standard wastewater compounds (e.g. Zaugg et al. 2001), the data in Table 8 summarizes the antibiotic residues detected, while that in Table 9 summarizes the results of analyses for chlorinated and other organics using the experimental, unpublished analytical technique which provides lower detection limits. An additional summary of all detections is presented in Figure 3. Regardless of the analytical technique used, more compounds were detected in the surface water sites than in caves and more were detected in the OK-Surface site than in the AK-Surface site. This is not a surprising result given that the OK samplers were placed directly downstream from the outfall of a municipal wastewater treatment plant.

A total of 27 different organic wastewater compounds were detected in the POCIS and SPMD extracts from the surface water and cave sites, with the majority of these found in the extracts from the POCIS samplers (Tables 6 & 7). Of these 27 compounds, 11 OWCs were detected in the caves, and Star Cave had the greatest number of detects, followed by Cave Springs Cave. Cholesterol and diethylexylphthalate were the most commonly detected compounds in the POCIS extracts, while no consistent trend in compound detection was apparent for the SPMD extracts.

Measurable levels of antibiotics/pharmaceuticals were only found in the extracts from samplers at the OK surface water site and in Star Cave, with 8 compounds detected in the surface water and 2, carbamazapine and sulfamethoxazole, detected in the cave (Table 8). In most cases, the level of antibiotic measured in these extracts was 5 times the detection limit or higher.

As would be expected, the majority of compound detections were observed in the SPMD extracts that were analyzed with the experimental method allowing for lower detection limits (Table 9). A total of 44 compounds were measured using this method, with this number including those with estimated levels below the laboratory reporting limit (LRL). Since this analytical technique is still being developed, a more conservative approach was taken when interpreting the data. Specifically, a “detect” was considered to have occurred only if the level of compound in the extract was at least 2X the LRL or level measured in the blanks. With this approach, 32 detections were observed, with 23 of these occurring in extracts from the cave samplers. In the OK surface site, the most commonly encountered residues were selected BDE and PCB congeners, organochlorine pesticides, and the common wastewater contaminants triclosan and methoxytriclosan. For the caves, the most common residues were BDEs and other selected flame retardants, organochlorine pesticides and triclosan and methoxytriclosan. Most of these residues were observed in the samples from January-Stansbury and Logan caves, although Star cave also had a number of detects at lower (<2X LRL) levels. The compounds triclosan and chlorpyrifos were also targeted as part of the OWC analyses (Table 1), although chlorpyrifos was only measured using the method with nanogram detection and triclosan was detected at more sites using this more sensitive method (Table 9).

Table 6. Compounds detected in extracts from the POCIS samplers. *(D)*=Detection at less than the laboratory reporting limit (LRL); D=Detection above, but less than 2X, LRL or average blank concentration; D-2X=Detection at or above 2X, but less than 5X, LRL or average blank concentration, D-5X=Detection at or above 5X LRL or average blank concentration.

	AK-Surface	OK-Surface	Cave Springs Cave	January-Stansbury Cave	Logan Cave	Mgee and Long Cave	Star Cave	Twin Cave
4-tert-octylphenol	ND	***(D)***	***(D)***	ND	ND	ND	ND	ND
Anthracene	ND	***(D)***	ND	ND	ND	ND	ND	ND
Atrazine	***(D)***	ND	ND	ND	ND	ND	ND	ND
Benzophenone	ND	***(D)***	ND	ND	ND	ND	**D**	ND
beta-Sitosterol	***(D)***	**D-2X**	ND	ND	ND	ND	ND	ND
Bromacil	ND	**D-5X**	ND	ND	ND	ND	ND	ND
Caffeine	***(D)***	**D**	ND	ND	ND	ND	ND	ND
Cholesterol	**D**	**D-5X**	**D-2X**	**D-2X**	**D**	**D-2X**	**D**	**D-5X**
Diethylhexylphthalate	**D**	**D-5X**	***(D)***	ND	***(D)***	***(D)***	**D-5X**	**D-2X**
Diethylphthalate	ND	**D**	ND	ND	ND	ND	**D-5X**	ND
d-Limonene	ND	ND	**D-5X**	ND	ND	ND	ND	ND
Ethanol,2-butoxy-,phosphate	ND	**D-5X**	ND	ND	ND	ND	ND	ND
Indole	ND	**D-5X**	ND	ND	ND	ND	ND	ND
Methylsalicylate	ND	***(D)***	ND	ND	ND	**D-5X**	ND	ND
N,N-diethyltoluamide(DEET)	***(D)***	**D-2X**	***(D)***	ND	ND	ND	***D***	ND
Naphthalene	ND	***(D)***	ND	ND	ND	ND	ND	ND
Octylphenol monoethoxylates (total)	ND	***(D)***	ND	ND	ND	ND	***D***	ND
Prometon	***(D)***	ND	ND	ND	ND	ND	***D***	ND
Skatol	ND	***(D)***	ND	ND	ND	ND	ND	ND
tri(2-Chloroethyl)phosphate	ND	**D**	ND	ND	ND	ND	ND	ND
tri(Dichlorisopropyl)phosphate	ND	***(D)***	ND	ND	ND	ND	ND	ND
Number of Detections	**7**	**18**	**5**	**1**	**2**	**3**	**7**	**2**

Table 7. Compounds detected in extracts from the SPMD samplers. *(D)*=Detection at less than the laboratory reporting limit (LRL); D=Detection above, but less than 2X, LRL or average blank concentration; D-2X=Detection at or above 2X, but less than 5X, LRL or average blank concentration, D-5X=Detection at or above 5X LRL or average blank concentration.

Compound	AK-Surface	OK-Surface	Cave Springs Cave	January-Stansbury Cave	Logan Cave	Mgee and Long Cave	Star Cave	Twin Cave
2,2',4,4'-Tetrabromodiphenylether	ND	D	ND	ND	ND	ND	ND	ND
Diethylhexylphthalate	ND	ND	ND	ND	ND	ND	ND	ND
Diethylphthalate	**D-5X**	D	ND	ND	ND	ND	ND	ND
Fluoranthene	ND	D	ND	ND	ND	ND	ND	ND
Pyrene	ND	D	ND	ND	ND	ND	ND	ND
Tetrachloroethylene	ND	ND	ND	(***D)***	ND	ND	ND	ND
Tonalide (AHTN)	ND	**D-5X**	ND	ND	ND	ND	ND	ND
Triclosan	ND	**D-5X**	ND	ND	ND	ND	ND	ND
Number of Detections	**1**	**2**	**0**	**1**	**0**	**0**	**0**	**0**

Table 8. Antibiotic and other pharmaceutical compounds detected in extracts from the POCIS samplers. *(D)*=Detection at less than the laboratory reporting limit (LRL); D=Detection above, but less than 2X, LRL or average blank concentration; D-2X=Detection at or above 2X, but less than 5X, LRL or average blank concentration, D-5X=Detection at or above 5X LRL or average blank concentration.

Compound	AK-Surface	OK-Surface	Cave Springs Cave	January-Stansbury Cave	Logan Cave	Mgee and Long Cave	Star Cave	Twin Cave
Azithromycin	ND	D-5X	ND	ND	ND	ND	ND	ND
Carbamazapine	ND	D-5X	ND	ND	ND	ND	D-5X	ND
Erythromycin-H2O	ND	D-2X	ND	ND	ND	ND	ND	ND
Ibuprofen	ND	D-5X	ND	ND	ND	ND	ND	ND
Lincomycin	ND	D-5X	ND	ND	ND	ND	ND	ND
Sulfamethoxazole	ND	D-5X	ND	ND	ND	ND	D-5X	ND
Trimethoprim	ND	D-5X	ND	ND	ND	ND	ND	ND
Tylosin	ND	D-5X	ND	ND	ND	ND	ND	ND
Number of Detections	**0**	**8**	**0**	**0**	**0**	**0**	**2**	**0**

Table 9. Chlorinated and other compounds detected in extracts from the SPMD samplers using the experimental analytical method with lower detection limits. *(D)*=Detection at less than the laboratory reporting limit (LRL); D=Detection above, but less than 2X, LRL or average blank concentration; D-2X=Detection at or above 2X, but less than 5X, LRL or average blank concentration, D-5X=Detection at or above 5X LRL or average blank concentration.

Compound	AK-Surface	OK-Surface	Cave Springs Cave	January-Stansbury Cave	Logan Cave	Mgee and Long Cave	Star Cave	Twin Cave
BDE100	**D-2X**	**D-5X**	**D-2X**	**D-2X**	**D-2X**	**D**	**D**	**D**
BDE153	ND	**D-5X**	**D-2X**	**D-2X**	**D-2X**	**D-2X**	ND	**D**
BDE154	**D**	**D-5X**	***(D)***	**D-2X**	**D-2X**	**D-2X**	**D**	**D**
BDE183	ND	**D-2X**	**D-2X**	ND	**D-5X**	ND	ND	**D-2X**
BDE47	**D**	**D-5X**	**D-2X**	**D-2X**	**D-2X**	**D-2X**	**D**	**D**
BDE66	ND	**D-5X**	ND	ND	ND	ND	ND	ND
BDE71	**D-5X**	ND	ND	ND	ND	ND	ND	ND
BDE85	ND	**D-5X**	ND	**D-2X**	**D-2X**	**D**	**D**	ND
BDE99	**D**	**D-5X**	**D**	**D-2X**	**D-2X**	**D-2X**	**D**	**D**
Chlorpyrifos	**D-2X**	**D-5X**	ND	ND	ND	ND	**D**	ND
cis-Chlordane	**D-2X**	**D-5X**	**D-5X**	**D-2X**	**D-2X**	**D**	**D**	**D-2X**
cis-Nonachlor	**D-5X**	**D-5X**	**D-5X**	**D-2X**	**D-2X**	ND	**D-2X**	**D**
desulfnylFipronil	ND	ND	ND	**D-2X**	ND	ND	ND	ND
DCPA	ND	***(D)***	ND	ND	ND	ND	ND	ND
Dieldrin	**D-2X**	**D-5X**	**D-2X**	ND	ND	ND	**D-2X**	ND
Fipronil	ND	ND	ND	**D-2X**	ND	ND	ND	ND
FipronilSulfide	ND	ND	ND	**D-2X**	ND	ND	ND	ND
Firemaster	ND	**D-5X**	ND	**D-2X**	**D-2X**	ND	ND	ND
HCB	**D-5X**	**D-5X**	ND	**D-2X**	**D-2X**	ND	**D**	ND
Oxychlordane	**D**	**D-5X**	**D**	ND	ND	ND	ND	ND
p,p'-DDD	ND	**D-5X**	ND	ND	ND	ND	ND	ND
p,p'-DDE	**D-2X**	**D-5X**	**D-2X**	ND	**D-2X**	ND	**D**	**D-2X**
p,p-DDT	ND	**D-5X**	ND	ND	ND	ND	ND	ND
PCA	**D-5X**	**D-5X**	**D-2X**	ND	ND	ND	**D**	ND
PCB110	ND	**D-2X**	ND	ND	ND	ND	ND	ND
PCB118	ND	**D-5X**	**D**	**D-2X**	ND	ND	**D**	**D**
PCB146	ND	**D-2X**	**D**	**D**	**D**	**D**	ND	ND

Table 9. Continued

Compound	AK-Surface	OK-Surface	Cave Springs Cave	January-Stansbury Cave	Logan Cave	Mgee and Long Cave	Star Cave	Twin Cave
PCB149	ND	**D**	ND	ND	ND	ND	ND	ND
PCB151	ND	**D**	ND	ND	ND	ND	ND	ND
PCB170	ND	ND	ND	ND	(***D)***	ND	ND	ND
PCB174	ND	**D**	ND	**D**	(***D)***	ND	ND	ND
PCB180	ND	**D-2X**	**D**	**D-2X**	**D**	ND	ND	ND
PCB183	ND	ND	ND	**D**	ND	ND	ND	(***D)***
PCB187	ND	**D-2X**	ND	**D**	(***D)***	ND	ND	**D**
PCB194	ND	ND	ND	**D**	ND	ND	ND	ND
PCB206	ND	ND	ND	**D**	ND	ND	ND	ND
PCB44	ND	**D**	ND	ND	ND	ND	ND	ND
PCB52	ND	ND	ND	ND	ND	ND	**D**	ND
Pendimethalin	**D-2X**	ND	ND	ND	ND	ND	ND	ND
Pentachloronitrobenzene	ND	**D-2X**	ND	**D-2X**	ND	**D-2X**	**D-2X**	**D-2X**
trans-Chlordane	**D-2X**	**D-5X**	**D-5X**	**D-2X**	**D-2X**	**D-2X**	**D-2X**	**D-2X**
trans-Nonachlor	**D-5X**	**D-5X**	**D-5X**	**D-2X**	**D-5X**	**D-2X**	**D-2X**	**D-2X**
Triclosan	***(D)***	**D-5X**	ND	**D-5X**	**D-2X**	**D**	**D-2X**	**D-2X**
Methoxytriclosan	**D-5X**	**D-5X**	ND	**D-5X**	**D-5X**	**D-5X**	**D-5X**	**D-5X**
Number of Detections								
(D)	**1**	**1**	**1**	**0**	**3**	**0**	**0**	**1**
D	**4**	**4**	**5**	**6**	**2**	**5**	**12**	**8**
D-2X	**7**	**6**	**7**	**18**	**13**	**7**	**6**	**7**
D-5X	**6**	**23**	**4**	**2**	**3**	**1**	**1**	**1**

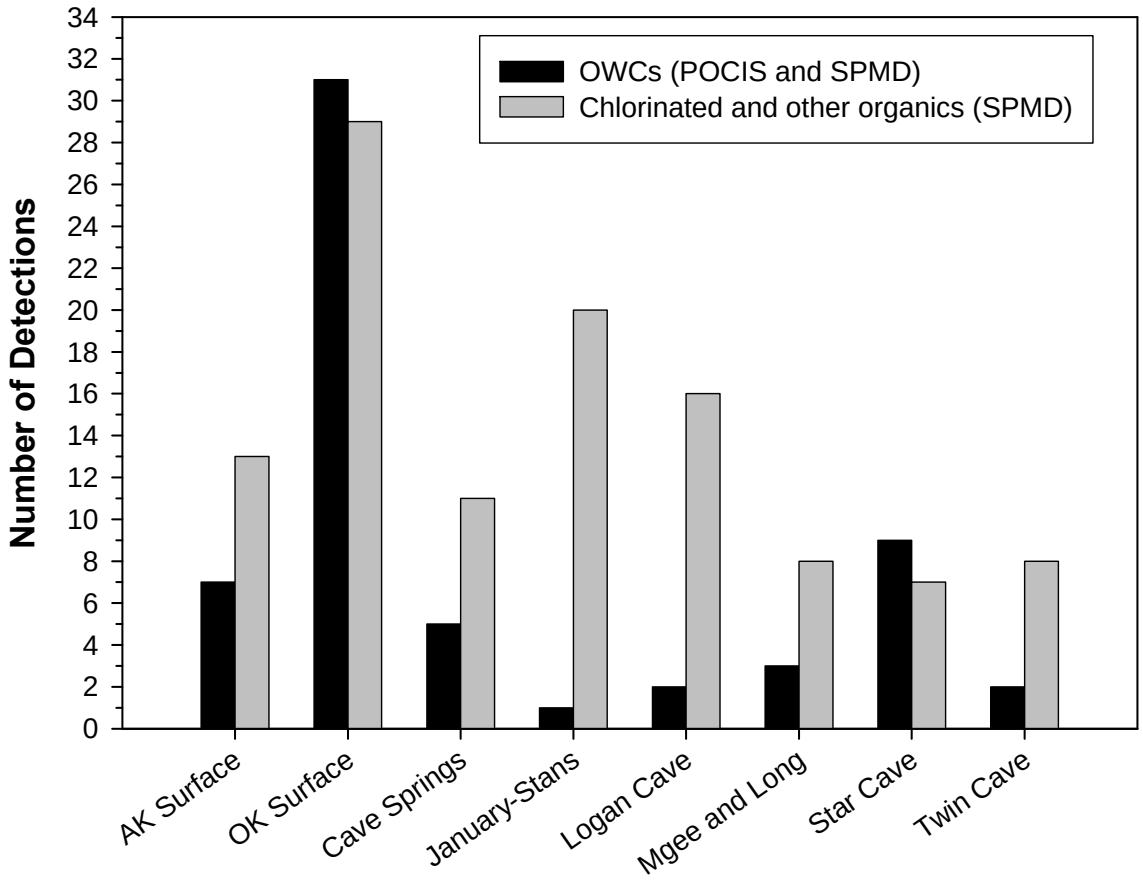

Figure 3. Summary of compound detections in extracts from the POCIS and SPMD samplers. OWCs (including antibiotics) were analyzed in the µg range while the chlorinated and other organics were analyzed in the ηg range using an experimental method that is under development.

Fathead minnow bioassay results: The water chemistry results derived from the fathead minnow bioassays were consistent with the data that were available from the field sampling (Table 5 & Table 10). The hardness of the water from the sites ranged between 76-190 mg/L as $CaCO_3$, and a moderately hard laboratory water (80 -100 mg/L as $CaCO_3$, USEPA 2002) was used as the reference water for the bioassays. For the majority of tests conducted, there were no significant differences between survival and growth of fish in laboratory versus cave water (Table 11). In Test 1 (3 May 2006), survival of fish exposed to water from Cave Springs Cave was reduced, although the difference was not statistically significant from that in the laboratory reference water. A follow-up bioassay with diluted Cave Springs water was conducted (Test 4, 7 June), with no effects observed. Similarly in Test 5 (9 June), a significant reduction in survival of fish exposed to Logan Cave water was observed, but a follow-up bioassay (Test 6, 20 June) indicated no effects. These data may suggest the presence of transitory stressors in the cave water that may be associated with run off events (Cave Springs Cave water was turbid with high flow on the day the sample was collected for Test 1), but consistent chronic effects were not indicated by the limited number of bioassays that were conducted for this study.

Table 10. Water chemistry ranges for all fathead minnow bioassays conducted with the surface and cave water samples.

	AK-Surface	OK-Surface	Cave Springs	January-Stansbury	Logan	Mgee and Long	Star	Twin
pH	6.8-7.1	6.8-7.5	6.4-7.3	6.4-7.3	6.5-7.0	6.7-7.2	6.3-7.0	6.7-7.2
Conductivity (uS/cm)	375-379	472-549	274-357	209-280	272-393	227-303	335-436	343-363
Dissolved Oxygen (mg/L)	8.3-9.0	7.1-7.9	8.0-8.7	8.5-10.0	8.4-9.7	8.0-8.9	8.2-8.3	8.5-8.6
Alkalinity (mg/L $CaCO_3$)	110-124	64-92	110-140	72-120	114-140	102-118	74-106	78-96
Hardness (mg/L $CaCO_3$)	120-146	104-114	120-190	76-116	132-154	106-132	90-156	96-122

Table 11. Results from bioassays with fathead minnows (*Pimephales promelas*) exposed to water from the caves and surface water sites evaluated in the study.

Test 1, Date: 3 May 2006-10 May 2006

Site	% Survival	Av. Dry Biomass (mg)	Range (mg)
Laboratory water	90	0.292	0.232-0.430
Cave Springs Cave	66	0.230	0.192-0.275
January-Stansbury Cave	92	0.277	0.226-0.324
Logan Cave	86	0.297	0.211-0.392
Mgee and Long Cave	90	0.320	0.225-0.476

Test 2, Date: 9 May -16 May 2006.

Site	% Survival	Av. Dry Biomass (mg)	Range (mg)
Laboratory water	100	0.463	0.398-0.559
AK-Surface	98	0.436	0.402-0.483
OK-Surface	94	0.439	0.392-0.490
Star Cave	96	0.460	0.395-0.494
Twin Cave	98	0.466	0.446-0.492

Test 3, Date: 2 June 2006-9 June 2006.

Site	% Survival	Av. Dry Biomass (mg)	Range (mg)
Laboratory water	100	0.291	0.222-0.370
January-Stansbury Cave	100	0.328	0.306-0.351
Mgee and Long Cave	96	0.290	0.249-0.333

Test 4, Date: 7 June 2006-14 June 2006. Results of bioassays with fathead minnows exposed to water from Cave Spring Cave.

Site	% Survival	Av. Dry Biomass (mg)	Range (mg)
Laboratory water	100	0.185	0.163-0.207
32% Cave water	94	0.213	0.198-0.219
42% Cave water	88	0.400	0.187-1.172
56% Cave water	92	0.231	0.176-0.285
75% Cave water	90	0.248	0.186-0.309
100% Cave water	92	0.249	0.226-0.265

Test 5, Date: 9 June 2006-16 June 2006. *- Survival significantly different from that in laboratory water at α=0.05.

Site	% Survival	Av. Dry Biomass (mg)	Range (mg)
Laboratory water	100	0.364	0.337-0.409
Logan Cave	72*	0.337	0.229-0.420
AK Surface	82	0.421	0.315-0.484
OK Surface	88	0.413	0.351-0.470
Twin Cave	86	0.375	0.339-0.410

Table 11. Continued.

Test 6, Date: 20 June 2006-27 June 2006. Results of bioassays with fathead minnows exposed to water from Logan Cave.

Site	% Survival	Av. Dry Biomass (mg)	Range (mg)
Laboratory water	100	0.502	0.388-0.698
12.5% Cave water	100	0.495	0.380-0.643
25% Cave water	100	0.563	0.444-0.720
50% Cave water	100	0.422	0.304-0.523
100% Cave water	100	0.461	0.347-0.559

Test 7, Date: 29 June 2006-6 July 2006.

Site	% Survival	Av. Dry Biomass (mg)	Range (mg)
Laboratory water	96	0.366	0.331-0.391
Star Cave	88	0.425	0.361-0.478
OK Surface	96	0.373	0.139-0.649

Test 8, Date: 2 August 2006-9 August 2006.

Site	% Survival	Av. Dry Biomass (mg)	Range (mg)
Laboratory water	92	0.405	0.274-0.558
Star Cave	96	0.445	0.348-0.523
OK Surface	98	0.474	0.442-0.537

Test 9, Date: 27 October 2006-3 November 2006.

Site	% Survival	Av. Dry Biomass (mg)	Range (mg)
Laboratory water	100	0.518	0.234-0.648
January-Stansbury Cave	92	0.535	0.502-0.580
Logan Cave	90	0.525	0.313-0.646
Mgee and Long Cave	96	0.622	0.497-0.680

Signiicance of Results: Links between land use activities and residues in surface water and cave sites: A key objective in the management of both surface and cave water habitats is to understand the linkage between land use activities and water quality. In this study, a greater number of analytes were measured in the surface waters than in caves which is expected given the potential for aerial deposition and the larger drainage area available to influence surface water quality. As previously mentioned, the higher number of detections at the Oklahoma surface water site was expected given the proximity to a wastewater treatment outfall, and the number of detections is consistent with previous studies that analyzed either water samples or used passive sampling devices to evaluate the presence of OWCs downstream from WWTP outfalls (Galloway et al. 2004; Alvarez et al. 2005). Given the preliminary nature of this study, it is not possible to make any definitive conclusions regarding land use activities and the compounds that were detected in the caves, although some of the results are compelling. For example, antibiotic residues were only found at the OK surface site and Star Cave, the two sites known to have a hydrologic link (Aley 2005). Star Cave also

ranked higher than others in the number of organic wastewater contaminants that were detected in the sampler extracts from that site. The presence of the antibacterial compounds triclosan and methoxytriclosan at most of the cave sites may suggest a wastewater influence at these sites as well.

The higher number of chlorinated organic residues that were detected at January-Stansbury, Logan, and to some extent Cave Springs caves is also intriguing. Both Logan and Cave Springs caves occur in drainage areas where there is increasing urban development, but January-Stansbury Cave is in a relatively undeveloped area. One common attribute of these sites is that the sampling canisters were placed relatively close to the cave entrance (versus Star and Twin caves in which a significant penetration of the system was required to reach permanent water). The water at these sites may therefore be more influenced by aerial deposition of dusts that contain these persistent organochlorine residues.

The significance of this study is that it did indicate the presence of a range of organic wastewater contaminants and other organic compounds in water of the caves examined. While the levels of these compounds are quite low (mostly in the 1×10^{-9}g range), their presence in these systems is a concern since so little is known about how contaminants may influence cave stream fauna. Additional studies that further quantify OWC levels in these habitats and investigate links between land use activities and cave water quality are critical to understand the risk these chemicals pose to cave habitats.

Acknowledgement
This study would not have been possible if not for the efforts of a number of individuals who gave their time and input toward project design and deploying the sampling devices. Carol Becker of the USGS Oklahoma Water Science Center was instrumental in the inception of the project and assisting with sampling. Steve Hensley and Richard Stark of the US Fish and Wildlife Service, Tulsa Field Office, arranged access to the caves and also helped with sample collection. Dr. Steven Zaugg of the USGS National Water Quality Laboratory, Denver, Colorado, and Dr. Michael Meyer of the Organic Geochemistry Laboratory, USGS Kansas Water Science Center analyzed the extracts from the passive samplers for the target compounds.

References Cited

Aley, T.J. 1976. Hydrology and surface management. In: The National Cave Management Symposium Proceedings. Speleo-books, Albuquerque, NM, pp. 44-45.

Aley, T.J. 2005. Groundwater recharge area delineation and vulnerability mapping of Star cave and nearby hydrologically associated springs, Delaware County, Oklahoma: Report submitted to the U.S. Fish and Wildlife Service, October, 2005.

Alvarez, D.A., P.E. Stackelberg, J.D. Petty, J.N. Huckins, E.T. Furlong, S.D. Zaugg and M.T. Meyer. 2005. Comparison of a novel passive sampler to standard water-

column sampling for organic contaminants associated with wastewater effluents enetering a New Jersey stream. Chemsophere, 61:610-622.

Crunkilton, R. 1984. Subterranean contamination of Meramec Spring by ammonium nitrate and urea fertilizer and its implication on rare cave biota. Proceedings of the 1984 National Cave Management Symposium. Journal of the Missouri Speological Society, 25:151-158.

Galloway, J.M., B.E. Haggard, M.T. Meyers and W.R. Green. 2004. Occurrence of pharmaceuticals and other organic wastewater constituents in selected streams in Northern Arkansas. U.S. Geological Survey, Scientific Investigations Report 2005-5140, 25p. Online at http://pubs.usgs.gov/sir/2005/5140/SIR2005-5140.pdf, accessed October 15, 2005.

Graening, G. and A. Brown. 2003. Ecosystem dynamics of an Ozark cave stream. Journal of the American Water Resources Association, 39:1497-1507.

Huff, T. 2005. Overview of semipermeable membrane devices. Online at http://wwwaux.cerc.cr.usgs.gov/spmd/spmd_overview.htm, accessed 21 October 2005.

Jones-Lepp, T.L., D.A. Alvarez, J.D. Petty and J.N. Huckins. 2004. Ploar organic chemical integrative sampling and liquid chromatography-electroscopy/ion-trap mass spectroscopy for assessing selected prescription and illicit drugs in treated sewage effluents. Archive of Environmental Contamination and Toxicology, 47:427-439.

Kolpin, D.W., E.T. Furlong, M.T. Meyer, E.M. Thurman, S.D. Zaugg, L.B. Barber and H.T. Buxton. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000: A national reconnaissance. Environmental Science and Technology, 36:202-1211.

NatureServe. 2005. NatureServe Explorer: An online encyclopedia of life [web application]. Version 4.5. NatureServe, Arlington, Virginia. Online at http://www.natureserve.org/explorer, accessed 28 September 2005.

Proudlove, G. S. 2001. The conservation status of hypogean fishes. Environmental Biology of Fishes, 62:201–213.

Simon , K. S. and A. L. Buikema, Jr. 1997. Organic Pollution of an Appalachian cave: changes in macroinvertebtrates Populations and food. American Midland Naturalist, 138:387-401.

U.S. Environmental Protection Agency. 2002. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, Fourth Edition, EPA-821-R-02-013

U.S. Geological Survey, 2004, Polar organic chemical integrative sampler (POCIS), Online at http://www.cerc.usgs.gov/pubs/center/pdfDocs/POCIS.pdf, accessed 24 September 2005.

Vrana, B., H. Paschke, A. Paschke, P. Popp and G. Schuurmann. 2005. Performance of semipermeable memberane devices for sampling of organic contaminants in groundwater. Journal of Environmental Monitoring, 7:500-508.

Zaugg, S.D., S.G. Smith, M.P. Schroeder, L.B. Barber, and M.R. Burkhardt,2001. Methods of analysis by the U.S. Geological Survey National Water Quality Laboratory – Determination of wastewater compounds by polystyrene-divinylbenzene solid-phase extraction and capillary-column gas chromatography/mass spectrometry: U.S. Geological Survey Water Resources Investigations Report 01-4186.

Notable Achievements and Awards: The notable achievement is the detection of these compounds in the cave water. This establishes the basis/need for additional study of these cave systems.

Student Support: A summary of the number of students, their degree level and discipline supported by the project in the following table:

Student Status	Number	Disciplines
Undergraduate	1	Zoology
M.S.	1	Zoology
Ph.D.	1	Zoology
Post Doc		
Total	3	