TWIN CAVE WATER QUALITY AND POLLUTION SOURCE ASSESSMENT

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1.0 INTRODUCTION

1.1 HISTORY

Twin Cave is part of an underground cave network situated in a crinoidal limestone formation. Over time, the cave and its hydrological complex have been inhabited by a variety of subterranean organisms. This geologic rarity has evolved into a fragile ecosystem dependent on high water quality and allochthonous energy inputs. Currently, the cave is habitat for the federally endangered Ozark cavefish (*Amblyopsis rosae*), blind cave crayfish (*Cambarus subterraneus*) and gray bats (*Myotis grisescens*), as well as several rare and potentially endangered species. The fragile ecosystem is vulnerable to human developmental pressures and surface and groundwater pollution. In an effort to protect the cave fauna, The Nature Conservancy (TNC) has developed a Twin Cave Conservation plan to prevent and protect the site from degradation and has attempted to acquire the lands above the cave system and critical areas in the cave watershed. During the preliminary investigation of the site, several potential sources of pollution were identified.

Limited water quality investigations using semi permeable membrane devices (SPMDs) showed the presence of 48 organic compounds, including two halogenated hydrocarbons, which potentially threatened the cave fauna. These results can be found in Appendix A. The Nature Conservancy worked with the Oklahoma Conservation Commission (OCC) and the EPA Region VI Laboratory (Houston, Texas) to quantify and monitor the toxicity of water and sediment in the cave system.

Twin Cave and the entire cave complex are particularly susceptible to water quality degradation due to NPS pollution. In 1993, Nature Conservancy research using SPMDs identified 48 synthetic organic compounds in the cave water that could harm the aquatic life. The presence of synthetic organic compounds, regardless of their toxicity, illustrates the vulnerable nature of the cave system to non-point source (NPS) pollution. Various land-uses and housing developments in the watershed are potential sources of NPS pollution. Identified sources of concern include:

1. illegal dumps in sink holes and in losing streams,
2. the spreading of chicken litter and the expanding chicken industry of NE Oklahoma,
3. land clearing for cattle production,
4. cattle in the surface streams and riparian areas,
5. home wells,
6. septic systems, and
7. fuel storage tanks (both surface and underground).

Development and pressure from human activities places the cave complex at risk. As part of the goal of Oklahoma's NPS Management Plan, high quality areas need to be protected from degradation. In order to protect this area, a comprehensive investigation of the watershed, including the surface and groundwater aspects, must be undertaken.

Although these are identified sources of concern, the extent to which these influence the cave system is generally unknown. Dye studies within the surface watershed conducted in 1990 and 1991 failed to completely delineate the cave watershed, indicating a need for an expanded dye study outside of the surface watershed to further delineate the watershed. Subsequently, the purpose for this project was to identify the current and potential NPS pollutant causes and sources that can influence the water quality of the Twin Cave subterranean complex.

Delineating the cave watershed, overlaying land use and potential sources will in turn allow the OCC in partnership with TNC, Natural Resources Conservation Service (NRCS) and US Fish and Wildlife Service (USFW) to draft a 319(h) workplan for education in the area and demonstration of best management practices (BMP) to protect the cave water quality.

1.2 PROJECT BACKGROUND

1.2.1 Project Site

The project site is located at the western edge of the Ozark highlands (Figure 1). It is surrounded by areas of forests, shrubs, pasture/hay fields and wetlands. Twin Cave is approximately one mile south of the Drowning Creek arm of Grand Lake of the Cherokees in Delaware County, Oklahoma, and is approximately six miles west of Jay, Oklahoma. A legal description of the site is as follows: S/4 Sec. 13, Sec. 24 (less portion of SW/4), E/2, NE/4 SE/4 Sec. 23, NE/4 Sec. 25, all in T23N-R22E; SW/4 Sec. 18, W/2 NW/4 and SW/4 Sec. 19, W/2, W/2 SE/4 Sec. 30, N/2 NW/4 Sec. 31 all in T23N-R23E. Locations of the cave entrance have been keep confidential to prevent vandalism and other human disturbances. The cave is located within the boundaries of a retirement and second home community, Lakemont Shores and Bay Club. This community encompasses approximately 2,000 acres and has been subdivided into 5,000 lots.

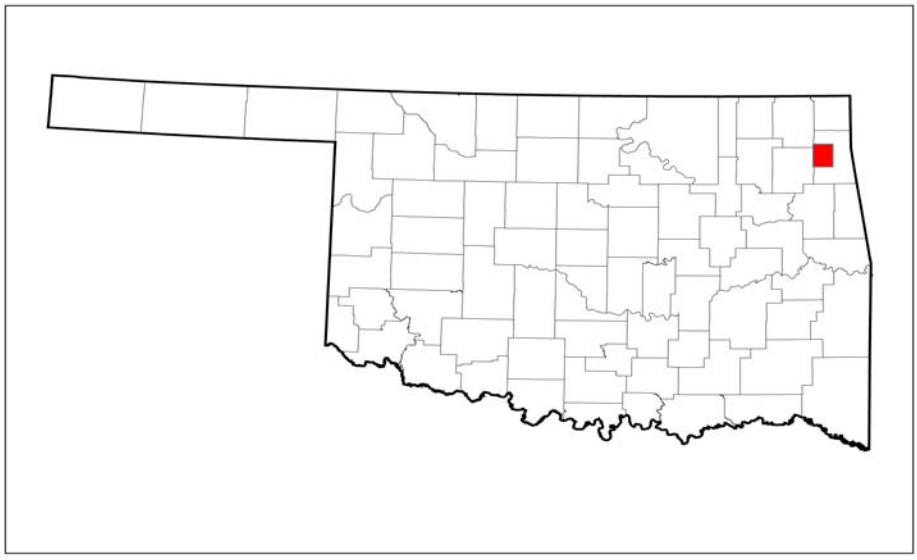

Figure 1: Twin Cave recharge area location.

1.2.2 Project Overview

The objectives of this project were to begin a process to protect the cave system, along with the endangered and threatened fauna, from water quality threats. The specific objectives for which the project tasks were written included:

1. Cave Water Quality Assessment - identification of suspected pollutants entering the cave stream;
2. Cave Watershed Delineation – determine watershed recharge area
3. Cave Watershed Hydrological Investigation – investigate and assess the watershed hydrology;
4. Cave Watershed Land Use Assessment and NPS Assessment - assess the watershed land-use and pollutants; and
5. Cave Watershed Protection Project - drafting an implementation plan and a 319(h)-workplan for education and demonstration of best management practices to protect the cave system.

The OCC has joined forces with TNC, Natural Resources Conservation Service (NRCS), Oklahoma Water Resources Board (OWRB), Delaware County Conservation District, Tulsa Regional Oklahoma Grotto (TROG), and the US Fish and Wildlife Service (USFW) to undertake this effort.

The questions being addressed by the data collected under the project's approved QAPP were: 1.) What are the current conditions in the Twin Cave complex concerning water quality? 2.) What is the recharge area for the cave complex? 3.) How does the groundwater hydrology affect the cave system? 4.) What is the land-use watershed area and what are potential NPS pollution sources?

This project was established as a preliminary study to determine potential threats to the Twin Cave ecosystem. This project consisted of five activities. The first characterized the Twin Cave system through a water quality assessment of chemical and biological parameters. The data resulting from this portion of the study will be used to determine baseline conditions which will be used to establish existing conditions, potentially indicating areas of concern. In addition, the toxic nature of any synthetic organic compounds present will be identified along with other water quality parameters of concern. Depending on the findings of the water quality investigation, these results may serve as an impetus for restoring the ecosystem. Based on the results of this study, a remediation workplan can be completed as needed.

The second objective delineated the Twin Cave watershed, showing where the water flows. This information can be used to identify regions around the cave complex that require particular protective activities. Future activities can then be established that will limit detrimental impacts.

The Twin Cave watershed hydrological investigation was the third objective. This identified water wells that could be a conduit for groundwater contamination and refined

the watershed delineation through groundwater flow maps. Like the delineation of the watershed, the data from this portion of the study will allow the targeting of areas near the cave that should be protected to limit further damage to the cave ecosystem.

A land use assessment and NPS assessment comprised the fourth objective of this study. The results of these evaluations will generate a list of activities in the watershed that potentially influence the cave. Current and future land-use and other sources of NPS pollution were identified, which will aid in the protection of the cave system.
The last objective was the development of a workplan to facilitate preservation of the Twin Cave ecosystem. This was to be accomplished through education and BMP demonstration.

2.0 MATERIALS AND METHODS

2.1 CAVE WATER QUALITY ASSESSMENT

Water quality assessment in the cave system began on 9 May 2000 and concluded on 12 March 2002. One run-off event in May 2000 yielded surface samples from five sites. Grab samples of water from the cave were coupled with SPMD analysis. The first part of the sampling protocol involved cave stream water grab samples. These were usually made on a bi-monthly or quarterly basis as established by the workplan; however, the intermittent nature of the watershed precluded collection on occasions when no water flow was present upstream. On-site parameters measured included dissolved oxygen (DO), pH, alkalinity, temperature, conductivity, and turbidity. Collections were made for hardness, nutrients (total phosphorous (TP), ortho phosphate (PO_4), ammonia, total Kjeldahl nitrogen (TKN), nitrate (NO_3) and nitrite (NO_2)), five day biochemical oxygen demand (BOD_5), sulfate (SO_4), chloride (Cl^-), total suspended solids (TSS), bacteria (fecal coliform, *Escherichia coli* (*E. coli*), enterococcus), semi-volatile and priority pollutant analysis. After collection, samples were stored on ice and transported to a certified laboratory within 24 hours. Sampling procedures followed those outlined in the OCC Water Quality Division's standard operating procedures (nos. 1, 8, 9, 10, 12, 14, 15, 18, 24, and 32). The samples were analyzed by the Oklahoma City County Health Department (OCCHD) Lab, the Department of Environmental Quality (DEQ) Lab, or the Department of Agriculture Lab, depending on which laboratory was under contract at the time. EPA Region VI Laboratory conducted all semi-volatile and priority pollutant analyses.

Toxicity testing makes up the second part of the protocol. Water and sediment collections were made quarterly for a year, beginning 25 February 1998, and were analyzed by EPA Region VI Laboratory (Houston, Texas). Tests were performed on the water and sediments to identify any toxic effects. For both sediment and water, a seven day survival and reproduction analysis was performed using ten individuals of the test organism *Ceriodaphnia dubia*. This test organism is quite sensitive, offering a good indication of the toxicity of the sample. The percent mortality was calculated for both the control and the Twin Cave sample. Additionally, the mean young per female produced after seven days of exposure was tabulated. A seven day embryo/larval analysis was also

performed on both sediment and water samples using the test organism *Pimphales promelas*. Thirty organisms were exposed to both the water and eluate samples and the percentage of organisms affected was noted. The effects included the combined number of dead organisms, as well as organisms exhibiting terata and abnormal swimming behavior.

The third integral part of the sampling protocol involved the SPMDs. These samplers passively monitor for the presence of a variety of contaminants over an extended period of time (≥28 days). They are designed to mimic the bioaccumulation of organic compounds in fatty tissues. These units are advantageous because they are extremely sensitive and reproducible (Huckins et al., 1997). Previous tests have shown organic compounds in the cave waters; therefore, SPMDs were deployed in conjunction with the collection of grab samples for toxicity testing. The devices were strategically placed to further determine the source of the contamination. The two categories of deployment used were hypogean (below ground) and epigean (above ground). The hypogean deployment evaluated the cave water along with wells in the watershed. The epigean deployment investigated the surface drainages in the watershed, such as losing streams.

2.2 CAVE WATERSHED DELINEATION

Recharge areas for the cave could not be identified solely from the topographical features because of the karst environment. Instead, Ozark Underground Laboratory (OUL) of Protem, Missouri, conducted a more intensive evaluation using tracers to delineate the cave watershed. A previous tracer study, using rhodamine WT dye, identified a portion of the watershed, but a more extensive survey was needed to refine the delineated recharge area. Dye tracing activities focused on a series of groundwater traces. Points of injection included suspected recharge areas, and areas in proximity to poor quality waters that were potential sources of groundwater pollution. The specific methods used by OUL can be found in Aley and Aley (1999). The work effort from OUL was supplemented by the OCC field staff and volunteer labor from TNC and the Tulsa Regional Oklahoma Grotto (TROG) a local chapter of the National Speleological Society.

2.3 CAVE WATERSHED HYDROLOGICAL INVESTIGATION

Determination of groundwater elevations is a crucial factor in understanding cave hydrology. The direction of groundwater flow and the location of groundwater divides was determined in order to delineate the watershed. One of the most direct ways to obtain groundwater elevations is to measure water levels in existing wells.

Elevations of springs in the area and stage measurements of the cave stream and Grand Lake were associated with the well water level elevations to create groundwater elevation coverage. The coverage was then used to create groundwater flow maps. Because groundwater flow in a karst system can vary under different water levels, measurements were taken during different water level conditions. A comparison of groundwater flow maps representing high and low water level conditions determined if groundwater flow patterns change under different conditions.

A cursory examination of the OWRB well completion records revealed about 28 domestic water wells in the study area. (The area of study encompasses the initial recharge area plus a two-mile radius.) Total depths of the wells range from 100 to 600 feet. Only shallower wells, representative of the hydrologic system of the cave, were appropriate for this study. OWRB evaluated well construction records to determine the appropriate wells for water level measurements.

Abandoned water wells and water wells without a surface seal provide potential pathways for contamination to enter the groundwater. Therefore, in order to control sources of pollution, such wells should be identified and corrected. The OWRB well completion record database is not a complete record of water wells. A field investigation was therefore required to inventory all wells. All wells in the study area were identified, inventoried, and inspected to identify pathways that NPS contaminants may follow to reach the cave system. The locations of the wells were marked using a Trimble Global Positioning Survey (GPS) Pathfinder Pro XR receiver. All wells were inspected to determine if they met current, minimum, well construction standards. Only wells that met the construction standards were used for water level measurement. (Corrective action was taken on wells not meeting the basic construction standards, independent of this project.) The GPS unit provided latitude, longitude and elevation coordinates. The wells were evaluated to determine appropriate ones for water level measurement. Measurements were taken once during high water table conditions, typically occurring during the spring months when precipitation is greatest, and once during low water table conditions, typically occurring during the summer months. Depth-to-water (DTW) measurements were obtained in these wells using steel tapes with 0.01-foot increments. Based on this information, OWRB produced a groundwater hydraulic report that provides information on the groundwater elevations, spring elevations, and the stages of the cave stream and Grand Lake. Groundwater water flow maps generated from this information were used to evaluate the cave recharge area.

2.4 CAVE WATERSHED LAND USE AND NPS ASSESSMENTS

A detailed land use assessment following OCC SOP 46 (OCC, 1997a) was conducted by OCC field staff on 21 November 2002 throughout the cave watershed. Prior to this, Ace Aerial Photography, Inc. flew over the project area on 17 October 2002 taking aerial photos. Results were digitized for GIS applications to track land use, identify non-point sources of pollution, and facilitate preparation of an implementation plan. GIS coverage incorporated the OWRB well locations and other results.

2.5 CAVE WATERSHED PROTECTION PROJECT

Based upon land-use and NPS pollution inventories in the cave recharge area, the OCC in concert with the Delaware County Conservation District, NRCS, USFWS, OWRB and TNC may draft a 319(h)-workplan of eligible activities for future funding to protect the cave ecosystem. That workplan would include both an educational component and demonstration of BMPs to protect the cave system. The OCC will coordinate with various Oklahoma regulatory agencies, DEQ and USFWS, to work with 319 ineligible

problems (i.e. point sources, storage tanks and landfills), as necessary. This document would be prepared under support of the OCC staff support workplan.

3.0 RESULTS AND DISCUSSION

3.1 CAVE WATER QUALITY ASSESSMENT

The results of the grab samples, toxicity samples, and SPMDs all indicate that the Twin Cave system is healthy. No contaminants were present at levels high enough to cause toxicity. At this time, the cave water quality is acceptable for cave fauna.

3.11 Grab Samples

Eight grab samples were obtained. The results of the grab samples indicate that the water in the Twin Cave system is supportive of the cave's aquatic organisms (Tables 1 and 2). Dissolved oxygen contents ranged from 5.02 to 9.48. The pH range was fairly narrow from 6.57 to 7.45. The water in the cave was moderately hard, clear and relatively free of suspended solids. The results of the BOD_5 and $cBOD_5$ tests (Table 2) indicated that there was little biodegradable waste, suggesting that the water is mostly clean. Nutrients do not appear to be negatively impacting the system. Phosphorus levels were highest in May and are most likely affected by the contribution of bat guano to the cave system. Levels of chloride and sulfate do not indicate cause for concern; chloride levels were always below 27.0 mg/l and sulfate stayed below 14.0 mg/l.

Date	DO (mg/l)	pH	Alk (mg/l)	Hard (mg/l)	Temp (C)	Cond (µS/cm)	SO_4 (mg/l)	Cl^- (mg/l)	TSS (mg/l)	Turb (NTU)
5/9/00	9.48	6.97	49	68.0	15.1	154.0	3.61	6.0		10.00
10/31/00	5.02	7.14	92	144.0	17.2	308.8	6.1	13.8	<1	1.07
2/3/01	8.38	6.57	120	80.2	12.8	200.0	7.0	8.8	5	1.26
3/13/01	9.09	6.98	82	100.0	11.9	228.9	8.1	9.0		5.93
5/9/01	9.3	6.97	90	122.0	12.7	283.6	8.4	14.8	6	1.73
9/26/01	9.45	7.43		164.4	15.3	345.0	10.3	23.1	<10	0.55
12/11/01	7.61	7.45	143		15.9	356.1	11.6	27.0	<10	2.05
3/12/02	7.71	6.96	112		13.7	223.4	13.74	18.3	<10	1.09

Table 1: Measurements of water quality parameters for Twin Cave.

Date	Total P (mg/l)	Ortho Phosphate (mg/l)	NH_3 (mg/l)	TKN (mg/l)	NO_3 (mg/l)	NO_2 (mg/l)	BOD_5 (mg/l)	$cBOD_5$ (mg/l)
5/9/00	0.069	0.032	<0.01	0.67	1.39	<0.005	<2.0	
10/31/00	0.015	0.009	<0.05	0.07	1.01	<0.05		
2/3/01	0.035	0.009	<0.05	0.23	2.85	<0.05	<2	
3/13/01	0.021	0.017	<0.05	0.17	<0.05	0.05		
5/9/01	0.041	0.014	<0.05		1.19	<0.05	3.71	
9/26/01	0.019	<0.005	0.026	0.174	1.39	<0.01		<2

12/11/01	<0.005	<0.005	<0.015		1.37	<0.01		6
3/12/02	0.015	0.006	<0.015	<0.11	1.53	<0.01		5

Table 2: Nutrient, BOD_5, and $cBOD_5$ measurements for Twin Cave.

Grab samples were also tested for the presence of metals. Table 3 contains the results of these tests. Based on the total hardness, the numerical criteria to protect the beneficial use of Fish and Wildlife Protection at the acute level for lead was 81.65 µg/l while the chronic level was 3.18 µg/l. Lead levels found in surface water grab samples are acceptable for Fish and Wildlife Propagation at the acute level; however, they fail Oklahoma's standards for chronic levels.

3.12 Toxicity Samples

Four toxicity sampling events were conducted. Usually, both sediment samples and water samples were collected each time. On 5 January 1999, a sediment sample was unavailable.

Neither the sediment nor the water from the Twin Cave system showed resulting toxicity to the test organisms. In all tests but one, no difference in toxicity existed between the Twin Cave sample and the control (Tables 4-17). One sediment analysis from the Lake Room resulted in a significant difference between the Twin Cave sample and that of the control (Table 4). The results from this test potentially indicated a slight toxicity to the sediment as there was a reduced reproductive capacity in the test organism. The ecological significance of this finding is questionable as all other tests did not support a determination of toxicity. Possibly, one organism was less fecund than the others for reasons other than toxicity.

3.13 SPMD Samples

Samples were collected on six occasions from six sites. Analyses were conducted to identify semi-volatile organic compounds and pesticides/PCBs. Additionally, some compounds were tentatively identified by the EPA lab using the best match with the NIST/Wiley mass spectral database and/or by manual interpretation; these are called TICs. SPMD analyses results of the pesticide/PCBs and TICs of semi-volatiles are located in Table 18. Semi-volatile compounds were not found above detection limits in routine analyses. Volatile analyses, including volatile TICs, were conducted on the 5/3/00-7/9/00 SPMDs collected. Volatile compounds in routine analyses and volatile TICs were not found above detection limits. These analyses were omitted from future SPMD tests.

Comparisons can be made to what compounds are found in the water column as grab samples were subjected to these same tests. No pesticides, PCBs, volatile or semi-volatile organic compounds were found in the water column at detection limits through routine analyses (Table 19). The only volatile TIC reported was ethyl acetate at 126 µg/l from the 10/31/00 sample. As the semi-volatile TICs found in the water column samples were

also found in the blanks, contamination of either the preservative or the bottles is the likely source of the compounds.

Technical chlordane, which includes both Alpha-Chlordane and Gamma-Chlordane, was the only insecticide present in each SPMD sample. There is no current commercial use of chlordane allowed (EPA, 2000). Both 4,4' DDE and 4,4' DDT were found in the 12 March 2002 sample at detectable levels. These pesticides have been excluded from current use in the United States (EPA, 2000).

Large volume injection analyses were initiated as they allow an increase in sample volume and are more sensitive to trace amounts of compounds in a sample. The results for the SPMD large volume injection analyses can be found in Appendix B and those for the water column in Appendix C. Concentrations and identifications of these compounds are tentative. For the compounds that could be identified, possible sources and the effects of the compounds are listed in Appendix D (National Library of Medicine, 2002; EPA, 2002). Of interest is the presence of caffeine and O-Benzyl-P-Chlorophenol. These are considered a very good indicators of contamination by human waste. Some septic tank contamination is most likely taking place. While it is not ecologically significant at this time, a potential exists for an increase in contamination with an increase in development in the area.

Based on the results of the analyses completed, the SPMD study indicates that there are no contaminants present at unusual levels in the cave at this time. Originally, volatile chemicals found in the cave system caused concern. The presence of these elements appears to have been of a transient nature. This could be accounted for by an episodic dumping of waste materials in a sinkhole in the recharge area. It should be noted that while the best available technology could render more sensitive detection limits for the compounds, the results of toxicity tests support the findings of the SPMD analyses that the levels of compounds are not threatening the cave fauna.

Site	Date	Al	Ba	Ca	Fe	Pb	Mg	Mn	K	Na	Zn
Site 01*	5/9-10/00	235	37	23100	258		931	9	2190	3000	
Robertson*	5/9-10/00		51	22400	114		1540		4640	5330	
Lakemont Shores*	5/9-10/00	380	48	19200	339		1200	12	2350	3250	
Stage Sampler 01A*	5/9-10/00	3140	60	9530	3350	3.5	1710	204	6790	1870	24
Stage Sampler 01B*	5/9-10/00	3490	59	4820	3500	3.6	1660	140	5840	2120	25
Upstream Lake Room	10/31/00		62.3	50200			1600		2310		
Upstream Lake Room	2/3/01		40.8	31500			1130	7.3	2160	7000	
Upstream Lake Room	5/9/01	118	54.7	47200			1510		1920	7840	
Upstream Lake Room	9/26/01		71.1	60000			1830		2510	10400	
Upstream Lake Room	3/12/02		51.1	44100			1450		1670	7450	

Table 3: Metals present in grab samples in µg/l.
*Surface grab samples.

Site	pH	Hardness (mg/l)	Alkalinity (mg/l)	Conductivity (mg/l)	Total Ammonia (mg/l)	Total Chlorine (mg/l)	Salinity (mg/l)	Mortality (%)	Young per Female
Control	7.7	188	184	462	<0.1	<0.1	0	0	17.9
Twin Cave	7.1	140	118	351	0.5	<0.1	0	0	15.7*

Table 4: Results from the seven day survival and reproduction analysis of Lake Room sediment on 25 February 1998.
*Significantly ($p \geq 0.95$) different from the control.

Site	pH	Hardness (mg/l)	Alkalinity (mg/l)	Conductivity (mg/l)	Total Ammonia (mg/l)	Total Chlorine (mg/l)	Salinity (mg/l)	Organisms Affected (%)
Control	8.1	196	182	463	<0.1	<0.1	0	3
Twin Cave	7.1	140	118	351	0.5	<0.1	0	0

Table 5: Results from the seven day embryo/larval analysis of Lake Room sediment on 25 February 1998.
After seven days, no significant effect was observed in organisms exposed to the Twin Cave eluate sample.

Site	pH	Hardness (mg/l)	Alkalinity (mg/l)	Conductivity (mg/l)	Total Ammonia (mg/l)	Total Chlorine (mg/l)	Salinity (mg/l)	Mortality (%)	Young per Female
Control	7.7	188	184	462	<0.1	<0.1	0	0	17.9
Twin Cave	7.8	124	104	260	0.4	<0.1	0	0	17.5

Table 6: Results from the seven day survival and reproduction analysis of Lake Room water on 25 February 1998. After seven days, no significant effect was observed in organisms exposed to the Twin Cave water sample.

Site	pH	Hardness (mg/l)	Alkalinity (mg/l)	Conductivity (mg/l)	Total Ammonia (mg/l)	Total Chlorine (mg/l)	Salinity (mg/l)	Organisms Affected (%)
Control	8.1	196	182	463	<0.1	<0.1	0	3
Twin Cave	7.8	124	104	260	0.4	<0.1	0	3

Table 7: Results from the seven day embryo/larval analysis of Lake Room water on 25 February 1998. After seven days, no significant effect was observed in organisms exposed to the Twin Cave water sample.

Site	pH	Hardness (mg/l)	Alkalinity (mg/l)	Conductivity (mg/l)	Total Ammonia (mg/l)	Total Chlorine (mg/l)	Salinity (mg/l)	Mortality (%)	Young per Female
Control	6.9	180	172	490	<0.1	<0.1	0	0	16.0
Twin Cave	7.3	116	102	404	0.4	<0.1	0	0	17.7

Table 8: Results from the seven day survival and reproduction on sediment from upstream of the natural opening on 2 June 1998. After seven days, no significant effect was observed in organisms exposed to the Twin Cave eluate sample.

Site	pH	Hardness (mg/l)	Alkalinity (mg/l)	Conductivity (mg/l)	Total Ammonia (mg/l)	Total Chlorine (mg/l)	Salinity (mg/l)	Organisms Affected (%)
Control	6.9	180	172	490	<0.1	<0.1	0	3
Twin Cave	7.3	116	102	404	0.4	<0.1	0	0

Table 9: Results from the seven day embryo/larval analysis on sediment from upstream of the natural opening on 2 June 1998. After seven days, no significant effect was observed in organisms exposed to the Twin Cave eluate sample.

Site	pH	Hardness (mg/l)	Alkalinity (mg/l)	Conductivity (mg/l)	Total Ammonia (mg/l)	Total Chlorine (mg/l)	Salinity (mg/l)	Mortality (%)	Young per Female
Control	6.9	180	172	490	<0.1	<0.1	0	0	16.0
Twin Cave	7.3	124	106	248	0.4	<0.1	0	0	16.5

Table 10: Results from the seven day survival/reproduction analysis of water from upstream of the natural opening on 2 June 1998. After seven days, no significant effect was observed in organisms exposed to the Twin Cave water sample.

Site	pH	Hardness (mg/l)	Alkalinity (mg/l)	Conductivity (mg/l)	Total Ammonia (mg/l)	Total Chlorine (mg/l)	Salinity (mg/l)	Organisms Affected (%)
Control	6.9	180	172	490	<0.1	<0.1	0	0
Twin Cave	7.3	124	106	248	0.4	<0.1	0	3

Table 11: Results from the seven day embryo/larval analysis of water from the natural opening on 2 June 1998. After seven days, no significant effect was observed in organisms exposed to the Twin Cave water sample.

Site	pH	Hardness (mg/l)	Alkalinity (mg/l)	Conductivity (mg/l)	Total Ammonia (mg/l)	Total Chlorine (mg/l)	Salinity (mg/l)	Mortality (%)	Young per Female
Control	8.4	112	92	214	<0.1	<0.1	0	0	17.9
Twin Cave	7.7	156	154	435	0.2	0.2	0	0	18.9

Table 12: Results from the seven day survival and reproduction analysis of sediment from the natural opening on 15 September 1998. After seven days, no significant effect was observed in organisms exposed to the Twin Cave eluate sample.

Site	pH	Hardness (mg/l)	Alkalinity (mg/l)	Conductivity (mg/l)	Total Ammonia (mg/l)	Total Chlorine (mg/l)	Salinity (mg/l)	Organisms Affected (%)
Control	8.6	139	154	480	<0.1	<0.1	0	3
Twin Cave	7.7	156	154	435	0.2	0.2	0	3

Table 13: Results from the seven day embryo/larval analysis of sediment from the natural opening on 15 September 1998. After seven days, no significant effect was observed in organisms exposed to the Twin Cave eluate sample.

Site	pH	Hardness (mg/l)	Alkalinity (mg/l)	Conductivity (mg/l)	Total Ammonia (mg/l)	Total Chlorine (mg/l)	Salinity (mg/l)	Mortality (%)	Young per Female
Control	8.4	112	92	214	<0.1	<0.1	0	0	17.9
Twin Cave	7.0	100	68	163	0.4	<0.1	0	0	19.2

Table 14: Results from the seven day survival and reproduction analysis on water from the natural opening on 15 September 1998. After seven days, no significant effect was observed in organisms exposed to the Twin Cave water sample.

Site	pH	Hardness (mg/l)	Alkalinity (mg/l)	Conductivity (mg/l)	Total Ammonia (mg/l)	Total Chlorine (mg/l)	Salinity (mg/l)	Organisms Affected (%)
Control	8.2	154	226	625	<0.1	<0.1	0	3
Twin Cave	7.0	100	68	163	0.4	<0.1	1000	0

Table 15: Results from the seven day embryo/larval analysis on water from the natural opening on 15 September 1998. After seven days, no significant effect was observed in organisms exposed to the Twin Cave water sample.

Site	pH	Hardness (mg/l)	Alkalinity (mg/l)	Conductivity (mg/l)	Total Ammonia (mg/l)	Total Chlorine (mg/l)	Salinity (mg/l)	Mortality (%)	Young per Female
Control	8.3	85	67	183	<0.1	<0.1	0	0	18.5
Twin Cave	7.7	111	101	219	0.2	<0.1	0	10	17.6

Table 16: Results from the seven day survival and reproduction analysis of Lake Room water on 5 January 1999. After seven days, no significant effect was observed in organisms exposed to the Twin Cave water sample.

Site	pH	Hardness (mg/l)	Alkalinity (mg/l)	Conductivity (mg/l)	Total Ammonia (mg/l)	Total Chlorine (mg/l)	Salinity (mg/l)	Organisms Affected (%)
Control	8.4	146	142	445	<0.1	<0.1	0	0
Twin Cave	7.7	111	101	219	0.2	<0.1	0	0

Table 17: Results from the seven day embryo/larval analysis of Lake Room water on 5 January 1999. After seven days, no significant effect was observed in organisms exposed to the Twin Cave water sample.

Date	TIC Semi-volatiles	Conc µg/L	Pesticides/PCBs	Conc µg/L
5/3/00- 7/9/00	•2 unknown hydrocarbons •Hexadonic acid •Methyl octadecenoate isomer •(Z)-9-Octadecenoic acid •Unknown ester •(Z)-9-Octadecenamide •Unknown	7 and 10 17 98 1180 98 7 196	•Technical chlordane	0.22
2/3/01	•Hexadecanoic acid •(Z)-9-Octadecenoic acid •Unknown ester	10 561 18	•Technical chlordane	0.071
5/9/01	•Unknown •Hexadecanoic acid •9-Octadecenoic acid, methyl ester •(Z)-9-Octadecenoic acid •Unknown ester	8 14 24 788 202	•Technical chlordane	0.10
9/26/01	•3 Unknowns •(Z)-9-Octadecenoic acid •(Z)-9-Octadecenamide •Unknown ester	10, 14, 32 508 13 15	•Technical chlordane	0.087
12/11/01	•2 unknowns •(Z)-9-Octadecenoic acid	11 and 22 131	•4,4' DDT •Technical chlordane	0.005 0.11
3/12/02	•(Z)-9-Octadecenoic acid	171	•4,4' DDE •4,4' DDT •Technical chlordane	0.009 0.007 0.17

Table 18: Results of the routine SPMD analyses of semi-volatile TICs, pesticides/PCBs and their estimated concentrations.
In routine water grab sample analyses, semi-volatile organic compounds were not found at detection levels.

Date	TIC Volatiles	Conc µg/L	TIC Semi-Volatiles	Conc µg/L
10/31/00	Ethyl acetate	126	•Phenothiazine* •[1,1'-Biphenyl]- 4,4'-diamine, 3,3',5,5'- tetramethyl*	13 14
2/3/01	ND		ND	
5/9/01	ND		[1,1'-Biphenyl]- 4,4'-diamine, 3,3',5,5'- tetramethyl*	10
9/26/01	ND		ND	
3/12/02	ND		ND	

Table 19: Water column volatile and semi-volatile TICs and estimated concentrations.
Volatile and semi-volatiles and pesticides/PCBs were not detected at detection limit (ND) in routine analyses. *This compound was found in the corresponding blank at levels comparable to or above those found in the sample, indicating contamination of either the preservative or bottles.

3.2 CAVE WATERSHED DELINEATION

Ozark Underground Laboratory conducted an investigation to delineate the watershed of the cave in 1999. Their results of the Twin Cave recharge area can be seen in Figure 3. This area is 2.43 square miles, contributes surface runoff from losing streams to Twin Cave, and includes the basin of the intermittent stream proximal to the cave (Aley and Aley, 1999). Aley and Aley found no source for the organic materials found through SPMD analyses (1999). High, moderate and low hazard areas were designated for this system (Aley and Aley, 1999) and can be seen in Figure 3. The results for this portion of the study can be found in the report completed for this task by Aley and Aley (1999).

3.3 CAVE WATERSHED HYDROLOGICAL INVESTIGATION

OWRB conducted a study of the cave's groundwater watershed in 2000. This study confirmed the recharge area as that established by Aley and Aley (1999) and that the watershed of the groundwater reflects that of the surface (Osborn and Penderson, 2000). The report for this portion of the study was completed by the OWRB and should be consulted for further details on this task (Osborn and Penderson, 2000).

3.4 CAVE WATERSHED LAND USE AND NPS ASSESSMENTS

The land in the Twin Cave recharge area is predominantly moderately used by cattle forest (1-10% of surface area is bare soil), good condition grassland (≤1% bare soil) and fair condition grassland (1-5% bare soil) (Table 20, Figure 2). Potential areas of concern for the land use in the recharge area were noted by Aley and Aley (1999). These areas were scrutinized through both the aerial photographs and followed up by the detailed land use survey performed on ground. Chicken houses, dumps, and fuel tanks comprised the bulk of these potential sources for pollution (Table 21). The locations of these sites can be seen in Figure 3. As nothing was found in the SPMDs, the toxicity tests, or the water quality, the land uses in this area are not currently deemed to be negatively impacting the cave ecosystem.

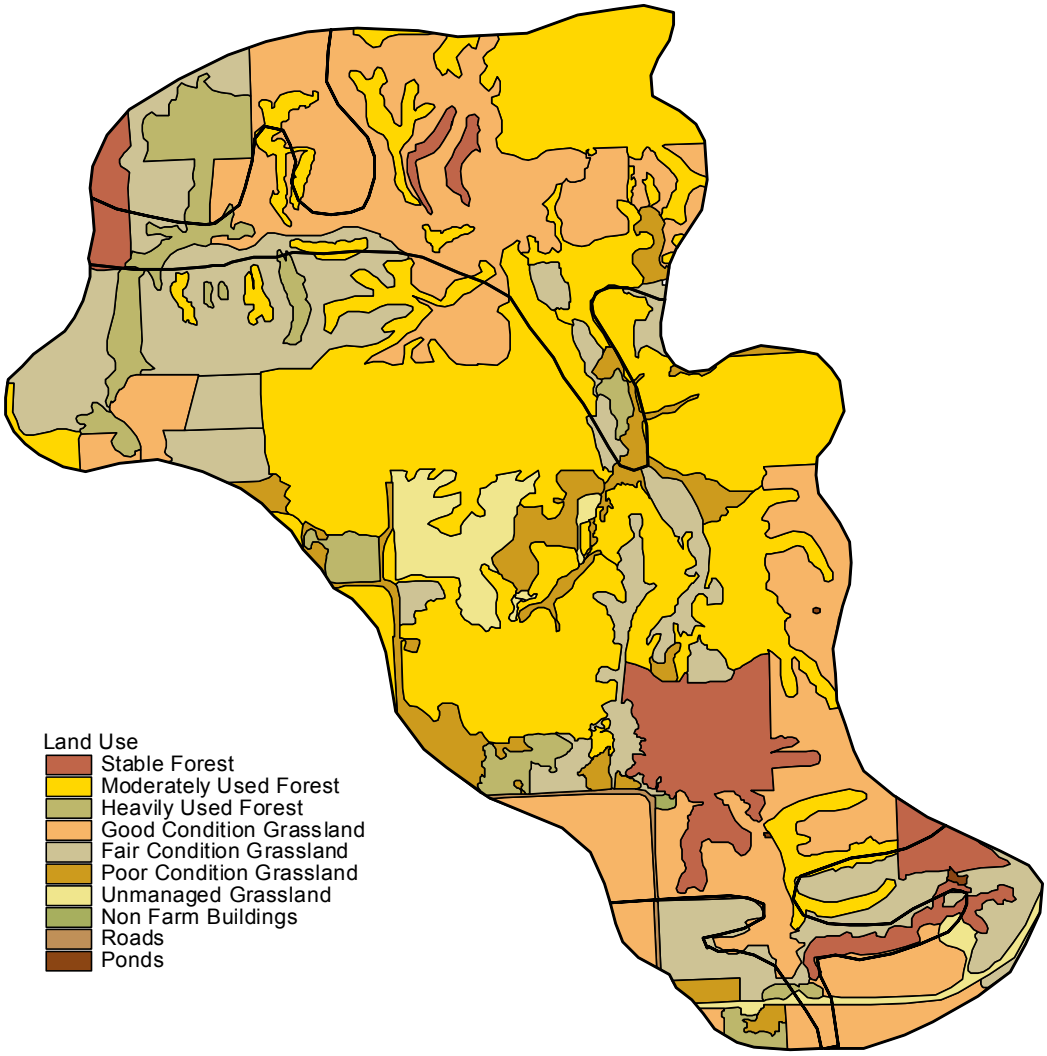

Figure 2: Land use of Twin Cave recharge area based on aerial photographs and ground survey.

Land Use	Acres	Percentage
Stable Forest	1223.52	7.27
Moderately Used Forest	6050.17	35.97
Heavily Used Forest	867.22	5.16
Good Condition Grasslands	4238.00	25.19
Fair Condition Grasslands	3015.50	17.93
Poor Condition Grasslands	823.42	4.90
Unmanaged Grasslands	547.47	3.25
Non-farm Buildings	8.98	0.05
Roads	39.71	0.24
Ponds	8.19	0.05

Table 20: Land use acres and percent coverage of the Twin Cave recharge area.

Site Letter	Description (Aley and Aley, 1999)	Current Status
A	Small dump and auto repair shop	No dump found
B	One confined animal house	Large active chicken house (approx. 20,000 bird capacity)
C	Medium-sized cattle feeding site in dry stream bed	Small holding pen/feeding area in dry stream bed
D	Above-ground gasoline storage tank	No fuel tank found; one large 500-1000 gal. reserve water tank from well at storage barn
E	Two large storage tanks located in maintenance yard	Two large fuel storage tanks in parking lot of maintenance yard
F	Fire station; not a significant ground water hazard	Fire station—no obvious threat observed
G	One large confined animal house	Large active chicken house (approx. 20,000 bird capacity)
H	Two large confined animal houses	Two large active chicken houses (each approx. 20,000 bird capacity resulting in 36,000 to 40,000 birds)
I	Small dump and muffler shop	No dump or shop found
J	Small salvage yard	Small (1 to 2 acres) salvage/junk yard—tractors, large trucks, cars—not commercially active
K	Medium-sized dump	Small active household dump
L	House with 1500 gal. storage tank	Barn with 1500 gal. reserve water tank 100-150 meters from two small (50 gal.) fuel tanks on stands in yard

Table 21: Potential sources of pollution to the Twin Cave recharge area updated and adapted from Aley and Aley (1999).

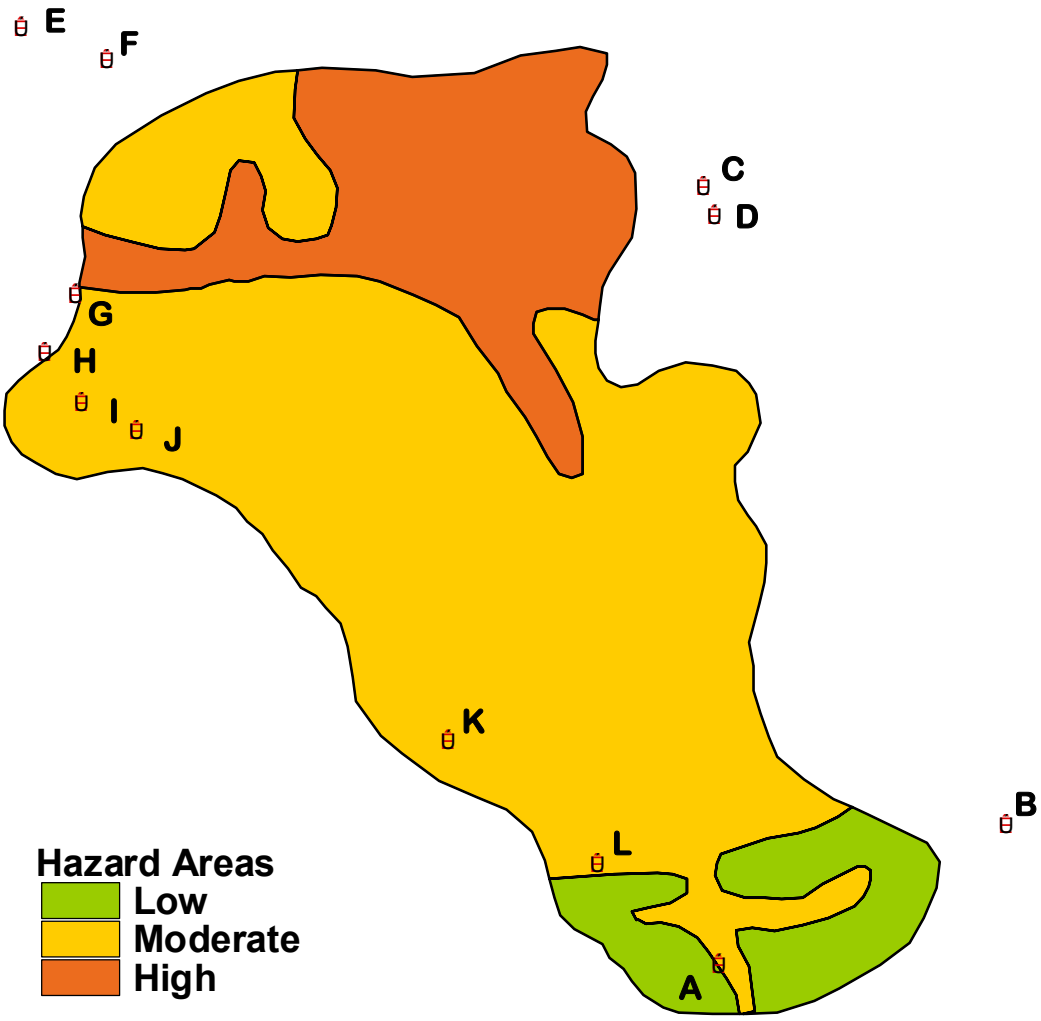

Figure 3: Twin Cave recharge hazard areas and potential pollution sources in area.
*Potential sources with letters corresponding to Table 21.

4.0 CONCLUSIONS AND SUGGESTIONS FOR PRESERVATION

The Twin Cave ecosystem currently appears to be healthy. In order to maintain this status, the cave should be monitored periodically for both water and habitat quality. This should include assessing the presence and levels of metals such as lead and chemicals such as chlordane. Additionally, land use should be periodically monitored. Should any negative changes be observed in the results, community education programs and best management practices could then be implemented to preserve the environment. The land use currently in practice in this area should be maintained as is.

5.0 REFERENCES

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Appendix A: TNC Report of Organic Compounds Identified at Houston Lab

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
REGION 6
HOUSTON BRANCH
10625 FALLSTONE RD.
HOUSTON, TEXAS 77099

January 15, 1997

MEMORANDUM

SUBJECT: Laboratory Analysis of Nature Conservancy Samples

FROM: Douglas A. Lipka, Chief
Environmental Services Branch
Management Division

TO: Charlie Howell
Water Quality Protection Division

The following information concerns the mass spectral identification of previously unknown peaks from GC-ECD analysis of Nature Conservancy samples.

It would be extremely difficult to determine which peaks in the ECD chromatogram correspond to peaks in the total ion chromatogram from the mass spectral analysis. Based on the ECD analysis, it was believed that some of these peaks could be halogenated because they responded to the detector. Only 2 peaks were found that contained halogens (1-Bromo-heptane and 1-bromo-octane). No chlorination patterns were observed. That does not preclude their presence due to the difference in sensitivity between the two instruments.

In order to increase sensitivity for mass spectral analyses, the four "real" samples were combined and concentrated to 1ml. (4-5 ml each). This generated a 16-20 times increase in sensitivity. Using Hewlett-Packard's PTV on 6890 GC, 10 ul was injected to further increase sensitivity (routine injections are 1ul). The SPMD blank and the solvent blank were also concentrated and 10 ul injections performed. The results from the 4 combined samples were compared with the blanks in order to remove any blank related compounds. A compound was considered blank related when it was present in either blank and in the sample. However, if it was present in the sample at more than 5 times the blank concentration, it was reported.

The following results are the compounds found in the combined sample listed in order of retention time. All blank related compounds present at greater than 5 times the blank concentration are denoted with an "*".

RT	Compound	Molecular Formula (if known)
7.25	unknown	
7.57	Heptanal	C7H14O
8.17	Benzaldehyde	C7H6O
8.41	3-Octanone	C8H16O
8.49	Octamethylcyclotetrasiloxane	C8H24O4Si4
8.77	branched hydrocarbon	CxHy
8.84	branched hydrocarbon	CxHy
8.89	hydrocarbon	CxHy
8.98	1-Bromo-heptane	C7H15Br
9.07	branched hydrocarbon	CxHy
9.13	possible alcohol	CxHyOH?
9.19	unknown (possibly contains N)	
9.26	branched hydrocarbon	CxHy
9.34	branched hydrocarbon	CxHy
9.44	possible alcohol or branched hydrocarbon	CxHyOH or CxHy?
9.62	branched hydrocarbon	CxHy
9.65	branched hydrocarbon	CxHy
9.77	branched hydrocarbon	CxHy
9.84	unknown siloxane	
9.85	1-Bromo-octane	C8H17Br
9.90	branched hydrocarbon	CxHy
10.01	branched hydrocarbon	CxHy
10.12	branched hydrocarbon	CxHy
10.31	Decanal	C10H20O
10.60	unknown	
10.74*	2-Decenal	C10H18O
10.98	unknown aldehyde	CxHyO
11.12	unknown siloxane	
11.34*	unknown	
11.45*	Undecenal	C11H20O
12.14	.delta.-deca-2,4-Dienolactone	C10H14O2
13.33	possible alkene or alcohol	CxHy or CxHyOH?
13.37	8-Heptadecene	C17H34
13.99	possible ethyl ester	R-CO2-C2H5?
14.02	unknown aldehyde	CxHyO
14.56	2-Heptadecanone	C17H34O
14.89	Ethyl 9-hexadecanoate	C18H34O2
15.00*	Hexadecanoic acid ethyl ester	C18H36O2

RT	Compound	Molecular Formula (if known)
15.04	straight chain hydrocarbon	CxHy
15.06	9-Octadecenal (Z)	C18H34O
15.42	unknown	
15.63	Hexadecanoic acid	C16H32O2
15.90	9-Octadecenoic acid	C18H34O2
15.97*	9-Octadecenoic acid ethyl ester	C20H38O2
16.14	Elaidinicacid isopropyl ester	C21H40O2
16.57	Citroflex A or Acetyl-tri-n-butyl citrate	C20H34O8 or C20H36O8
17.33	possible ester	R-CO2-R'
17.39	possible ester	R-CO2-R'

The mass spectra for each of the above retention times are attached. The pages are from a Library Search Compound report (or LSC). If you look at one of these pages, there will be 4 spectra shown. The top spectrum is from the actual sample and has been subtracted for background. The bottom 3 spectra correspond to reference spectra from the library matches listed below "Hit#". Additional subtractions of spectra were performed when necessary, and they are attached. Also, additional reference spectra are attached when the library search did not identify the compound correctly.

It is very possible that the siloxanes were a contamination from this lab. Please use information with caution.

Because no comparisons are made for retention times and spectra using verified standards, all identifications are tentative.

If you have any questions concerning this report, please contact Richard McMillin at (281) 983-2107.

Appendix B: Large Volume Injection Results of Tentatively Identified Compounds Found in SPMDs

Date	TIC	Estimated Concentration (µg/ml)
2/3/2001	Undecane	0.37
	Nonanal	1.8
	Decahydro-dimethylnaphthalene isomer	0.66
	Nonanoic acid	1.1
	Undecenal	0.58
	Cyclic hydrocarbon	0.48
	C15H24 isomer	0.28
	Dimethyl decalol isomer	0.36
	Dodecanoic acid	0.44
	Unsaturated hydrocarbon	0.56
	Tetradecanoic acid	1.5
	Pentadecanoic acid	0.69
	Hexadecanoic acid-methyl ester	0.42
	Hexadecenoic acid*	14*
	Cyanopentadecene isomer?	1.4
	Octadecenoic acid-methyl ester*	6.7*
	Unsaturated hydrocarbon*	6.8*
	Octadecenamide*	14*
	Tetracosahexaene	1.8
	Unsaturated hydrocarbon*	22*
	Unknown ester	1.1
5/9/2001	Unknown cyclic hydrocarbon	0.46
	Unknown hydrocarbon	0.29
	Unknown cyclic hydrocarbon	1.1
	Unknown hydrocarbon	0.75
	Unknown hydrocarbon	0.29
	Unknown hydrocarbon	2.2
	Unknown	0.46
	Decane	0.43
	Undecane	1.4
	Nonanal	1.1
	Unknown cyclic hydrocarbon	0.26
	Dodecanoic acid	0.57
	Unknown	2.7
	Tetradecanoic acid	1.8
	C18H30O Phenol	0.42
	Unknown	0.47
	Hexadecanoic acid-methyl ester	2.4
	Unknown (possible Hexadecene compound)	Reported
	Hexadecanoic acid	Reported

Date	TIC	Estimated Concentration (µg/ml)
5/9/2001	2-Methylhexadecanoic acid-methyl ester	0.85
	Unknown	0.89
	Unknown	0.46
	Unknown	0.86
	Octadecenoic acid-methyl ester isomer	Reported
	Octadecenoic acid-ethyl ester isomer	Unk
	Unknown amide	Unk
	11-Eicosenoic acid-methyl ester	Unk
	9-Octadecenoic acid	Reported
	Unknown	0.64
	Unknown ester	0.31
	9-Octadecenamide	5.1
	C22H42Oe4 adipate	2.1
	Unknown ester	1.1
	Unknown	6.3
	Unknown	0.55
	Unknown paraffin	0.45
	Unknown	1.1
	Unknown	0.65
	Unknown ester	1.2
	Unknown isoprenoid compound	1.1
	Unknown paraffin	0.46
	Unknown ester	Reported
	Unknown	0.73
	Unknown	0.68
	Unknown ester	1.0
	Unknown	2.6
	Unknown ester	3.0
	Unknown	0.99
	Unknown	0.85
	Unknown ester	1.7
9/26/2001	3,4-Dihydro-6-methyl-2H-pyran	7.48
	1-Methylcyclopentanol	89.1
	3-Methylcyclopentanol	10.7
	3-Methylcyclopentanone	2.50
	Unknown	2.66
	Unknown	20.7
	Unknown	33.9
	Unknown	67.6
	Unknown	7.60

Date	TIC	Estimated Concentration (µg/ml)
9/26/2001	Unknown	6.46
	Undecane	3.38
	1,2,3,4-Tetrahydronaphthalene-d12	2.32
	Tetradecanoic acid	5.76
	Pentadecanoic acid	2.70
	Hexadecanoic acid, methyl ester	0.66
	9-Hexadecenoic acid	12.1
	Hexadecanoic acid	32.8
	Octadecenoic acid, methylester isomer	9.72
	(Z)-9-Octadecenoic acid	393
	Unknown carboxylic acid	18.0
	(Z)-9-Octadecenamide	11.9
	Unknown ester	8.08
	Unknown	0.42
12/11/2001	3,4-Dihydro-6-methyl-2H-pyran	5.01
	1-Methylcyclopentanol	43.9
	2-Hexanol	6.15
	3-Methylcyclopentanone	9.11
	Unknown	5.38
	Unknown	7.67
	Unknown	1.24
	Unknown	14.5
	Unknown	1.89
	Unknown substituted cyclohexane	2.64
	Unknown	1.17
	Decane	2.98
	Unknown paraffin	<1
	Unknown paraffin	<1
	C11H22 isomer	1.75
	Unknown branched paraffin	<1
	Decahydronaphthalene isomer	2.48
	Unknown	1.65
	C11H22 isomer	1.17
	Undecane	2.37
	Dodecane	<1
	Tetradecanoic acid	1.26
	Unknown	2.72
	Hexadecanoic acid	7.04
	Octadecenoic acid, methyl ester isomer	<1
	(Z)-9-Octadecenoic acid	147

Date	TIC	Estimated Concentration (µg/ml)
12/11/2001	Octadecanoic acid	8.75
	Di(2-ethylhexyl)adipate	1.36
	Squalene	<1
3/12/2002	Unknown	1.12
	Unknown**	2.46**
	Unknown ketone	<1
	Unknown cyclic compound	<1
	Decane	<1
	Methylhydroxypropyl propenoic acid ester isomer	2.16
	Unknown substituted cyclohexane	<1
	Unknown substituted cyclohexane	1.26
	Unknown paraffin	<1
	C11H22 isomer	2.16
	Unknown substituted cyclohexane	1.34
	Unknown substituted cyclohexane	1.24
	Undecane	1.82
	Nonanal	3.70
	Unknown phenol	1.98
	Z-11-Hexadecenoic acid	1.86

*Estimated value reported is above concentration normally reported in ABN TIC report; therefore, concentration and/or presence should be suspect.
**<5 times instrument blank concentration.

Appendix C: Large Volume Injection Results of Tentatively Identified Compounds Found in Water Column

Date	TIC	Estimated Concentration (µg/ml)
2/3/2001	4-Ethoxy benzoic acid, ethyl ester	0.25
	Straight chain paraffin	0.24
5/9/2001	4-Methyl-3-penten-2-one	0.29
	4-Hydroxy-4-methyl-2-pentanone	0.29
	2-Butoxyethanol	0.33
	C9H10O2 isomer	0.19
	Unknown ester	0.56
	Unknown	0.46
	Ethyl-4-ethoxybenzoate	1.9
	Unknown phthalate	0.28
	Hexadecanoic acid	0.19
	Phenothiazine	8.0
	3,3',5,5'-Tetramethylbenzidine	13.0
	Unknown	0.31
9/26/2001	Unknown	1.09
	4-Hydroxy-4-methyl-2-pentanone	4.99
	Unknown ether	0.64
	Unknown	0.61
	Nonanal	1.14
	(2-Butoxyethoxy)ethanol isomer	0.95
	2-Phenoxyethanol	0.62
	Unknown	0.19
	Unknown	0.21
	Unknown paraffin	0.19
	Tetradecanoic acid	0.28
	Caffeine	0.26
	Unknown alcohol or alkene	0.61
	4-Chloro-2-(phenylmethyl) phenol	1.88
	Hexadecanoic acid	1.24
	Octadecanoic acid	0.28
	Unknown amide	0.34
3/12/2002	N,N-Dimethylformamide	0.11
	2-Butoxyethanol	0.24
	Unknown	0.46
	N-Hexadecanoic acid	0.32
	Unknown branched paraffin	0.16

Appendix D: Effects and Possible Sources of PCBs, Pesticides, and Tentatively Identified Compounds Found in the Water Column and SPMDs

Water Column TICs		
Compound	**Source**	**Effect**
2-Phenoxyethanol	•Solvent for ink, resin, and cellulose acetate •Perfume fixative •Used in photographic and manufacturing industries	•No significant bioconcentration in aquatic organisms •Soluble in water •Rainbow trout, *Salmo gairdneri*, exposed to concentrations ranging from 0.25 to 0.75 ml/l stopped schooling, became hypoactive, and lost equilibrium before dying
Mesityl oxide/ 4 methyl 3 penten 2 one	•Intermediate solvent for resins •Production of : Medication Insecticides Pesticides Stain removers Carburator cleaners •Leaches into soil	•No significant bioconcentration in aquatic organisms •Does not hydrolyze in water •No significant adsorption to sediment •4.403 mM decreases population of the protozoan *Tetrahymena pyriformis*
4-hydroxy-4-methyl-2-pentanone	•Solvent for variety of substances including pesticides •Additive/intermediate for variety of substances including fuel and antifreeze additives and insecticides •Leaches in soil •High water solubility	•No significant bioconcentration in aquatic organisms •High solubility in water •No significant adsorption to sediments •8930 mg/L causes mortality in golden ide, *Leuciscus idus melanotus*
Ethylene glycol/ Mono-N-butyl ether/2-butoxyethanol	•Production of hydraulic fluids and plasticizers •Coupling agent for cleaners and cutting oils •Solvent for resins, lacquers, and a variety of cleaners	•Low potential for bioconcentration in aquatic organisms •1490 mg/L causes mortality in golden ide, *Leuciscus idus melanotus*
Palmitic acid/ hexadecanoic acid	•Natural occurrence in fats and oils •Manufacture of soap, cosmetics and surface coating oils	•Very high potential for bioconcentration in aquatic organisms •12000 µg/L causes mortality in Coho salmon, *Oncorhynchus kisutch*
Phenothiazine	•Insecticide •Pharmaceuticals •Dyes	•High potential for bioconcentration in aquatic organisms 3000 µg/L decreases growth and can cause mortality in the fathead minnow, *Pimephales promelas*

3,3',5,5'-Tetramethylbenzidine	•Research chemical	•Suspected carcinogen, some mutagenic effects but toxicology is not thoroughly studied
Myristic acid/ tetradecanoic acid	•Naturally present in animal and vegetable fats •Manufacturing soaps, lubricants, cosmetics, and perfumes •By-product of tanneries and municipal waste	•Very high potential for bioconcentration in aquatic organisms •Susceptible to biodegradation •5000 µg/L causes observable stress in bluegill, *Lepomis macrochirus*, and rainbow trout, *Oncorhynchus mykiss*
Caffeine	•Naturally occurring in tea and cocoa nuts •Production of soft drinks and pharmaceuticals	•No bioconcentration in aquatic organisms •20000 µg/L slows growth of fathead minnow, *Pimephales promelas*, 40000-110000 µg/L causes abnormal growth, and 720000 µg/L causes mortality
o-Benzyl-p-chlorophenol/ 4 chloro 2 phenylmethyl phenol	•Manufacturing hospital disinfectant and fungicide	•Low potential for bioconcentration in aquatic organisms •Bluegill sunfish, *Lepomis machrochirus*, metabolized and eliminated the compound rapidly
Stearic acid/ octadecanoic acid	•Naturally present in cotton, corn, rapeseed, soybean and sunflower oils •Manufacturing stearates, stearate driers, medications, lubricants, and varnishes	•Very high potential of bioconcentration in aquatic organisms •12000 µg/L causes mortality in Coho salmon, *Oncorhynchus kisutch*
n,n-Dimethylformamide	•Chemical solvent •Intermediate •Additive	•Low toxicity for aquatic organisms •Rapid degradation in water
Ethyl-4-ethoxybenzoate	•Suspension medium	•Caused acute toxicity in *Salmo gairdneri*, *Daphnia magna* and *Selenastrum capricornutum*
SPMD TICs		
n-Undecane	•Production of paper processing, rubber, crude oil •Solvent •Oil spills	•Very high potential for bioconcentration in aquatic organisms; structurally similar compound (n-pentadecane) has low potential for bioconcentration in aquatic organisms (tested on carp) and may reflect more accurately a low potential for bioconcentration in n-undecane as n-undecane has low water solubility resulting in an inaccurate regression

		equation •Toxin targets specific organs, usually lungs
Decane	•Used in petroleum and gasoline industries •Production of lacquers, solvents, and pastes •Production of rubber materials	•Potential for bioconcentration in aquatic organisms •No effect on growth rate of mussel larvae, *Mytilus edulis* •Affects feeding behavior of rainbow trout, *Oncorhynchus mykiss*, at 0.84-74 ng/g
Dodecanoic acid	•Naturally occurs in essential oils of some plants and in animal waste	•Moderate potential for bioconcentration in aquatic organisms •25-50 ppm causes mortality in bluegill, *Lepomis macrochirus*
Erythromycin/ Pentadecanoic acid	•Antibiotic •Mycotoxin •Various amines and amides	•Genotoxic effects
Methyl palmitate/ hexadecanoic acid methyl ester	•Naturally present as a flavor component in some foods •Used as a synthetic intermediate	•High potential for bioconcentration in aquatic organisms •No tests available assessing acute toxicity, chronic toxicity, developmental/reproductive toxicity, mutagenicity, ecotoxicity and environmental fate
(Z)-9-Octadecenamide	•Lubricating oil additive. •Slip agent for polyethylene extrusion •Additive in wax, ink, and cosmetics	•No tests available assessing acute toxicity, carcinogenicity, chronic toxicity, developmental or reproductive toxicity, ecotoxicity, environmental fate and neurotoxicity
Phenol	•Naturally present in decomposition of leaf litter and animal wastes •Used as a chemical intermediate and disinfectant/antiseptic •Leaches from tires	•No bioconcentration in aquatic organisms •Very toxic to fish •Impacts on grey mullet: Elevated blood sugar Increased activities of aspartate aminotransferase and lactate dehydrogenase in blood plasma Lowered concentrations of blood hemoglobin, hematocrit values,

		cholesterol, proteins, and glycerides Increased activity of aspartate aminotransferase and GPT Damaged gills, liver, gallbladder, and kidneys Death •Causes inhibition of cell multiplication in the algae *Microcystis aeruginosa*
Oleic acid/9 octadecenoic acid	•Naturally present in essential plant oils and foods •Released in wastewater effluents from pulp/paper mills, waste treatment plants and urban runoff	•Potential for bioconcentration in aquatic organisms •In 24 hours, 285000 µg/L causes mortality in fathead minnow, *Pimephales promelas*
Tetralin/1 2 3 4 tetrahydronaphthalene	•Painting parts in automobile assembly plants •Runoff from land •Effluent from paper mills, petroleum refineries, and advance waste treatments	•Bioconcentrates in aquatic organisms incapable of metabolizing it •106-192 mg/kg causes mortality in common carp, *Cyprinus carpio* •2412 µg/L immobilizes the water flea, *Daphnia pulex*
2-Hexanol	•Used as base fluid for drilling fluids, as organic solvent, in lacquers, and in compounds for cleaning metals	•Neurotoxic
Dodecane	•Manufacturing rubber, paper processing, solvents, and gasoline	•Low potential for bioconcentration in aquatic organisms indicated by tests on golden ide and golden orfes •Affects feeding behavior, relation of organ weight in relation to body weight, and general histology of rainbow trout, *Oncorhynchus mykiss*
Bis(2-ethylhexyl) adipate/ di (2-ethylhexyl) adipate/Bis(2-ethylhexyl)ester hexanedioic acid	•Does not occur naturally •Production of PVC materials •Effluents from wastewater, POTW and chemical manufacturing plants	•Low potential for bioconcentration in aquatic organisms •480-850 µg/L causes mortality in fathead minnow, *Pimephales promelas*, and bluegill, *Lepomis macrochirus*
Sodium o-isopropyl xanthate/ z 11 Sodium isopropyl xanthogenate/z 11 Hexadecenoic acid	•Chemical weed killer •Fortifies certain oils •Reagent for metal ores	•Rainbow trout continuous flow bioassay exposure resulted in 200-2000 times more toxicity than static bioassays with larger fish more affected than smaller fish •10 mg/L causes mortality in channel

		catfish, *Ictalurus punctatus*, and bluegill, *Lepomis macrochirus*
Ethyl acetate (4-ethoxybenzoid acid, ethyl ester)	•Naturally occurs in animal waste, fermentation, and microbes •Used in medications, artificial leather, and solvents	•Low potential for bioconcentration in aquatic organisms •Common indian catfish, *Heteropneustes fossilus*, reactions: Changes in metabolism of carbohydrates A decline in hepatic glycogen levels Little change in muscle glycogen content Elevated levels of blood pyruvate Hyperglycemia Loss of equilibrium before death •Toxic effects most likely resulted from the impaired carbohydrate metabolism
PCBs/Pesticides		
Chlordane	•Previously used as an insecticide •No current uses approved in US •Does not occur naturally	•Significant potential for bioconcentration. •Very persistent in aquatic habitats •Freshwater fish, *Saccobranchus fossilus*, exposed to chlordane experienced hyperactivity, storage of dark bodies in the fat body, structural disruption of caecal lining and malpighian tubules, and cellular damage from chlordane rapidly penetrating the proventricular plates' cuticle lining of internal digestive tract •Causes mortality in tubificid worm, *Branchiura sowerbyi* •Affects growth in the eastern oyster, *Crassostrea virginica* •Stimulates growth of chlamydomonas; at higher concentrations, inhibits cell division •Most toxic to dunegrass crab, *Cancer magister*, at the zoel life stage
DDT/4 4 ddt/2,2'-Bis(p-chlorophenyl)-1,1,1-trichloroethane	•Previously used as a broad spectrum pesticide now banned in US •Can be used in emergencies as approved by the EPA •Does not occur naturally	•Very high potential for bioconcentration in aquatic organisms •Very persistent in environment •Highly toxic to fish and aquatic invertebrates

		•Causes hepatomas in trout •Smaller and younger fish are more susceptible to the effects of DDT than larger and older fish •Bioaccumulates more in female fish and in predators like pike perch and asp •Significantly affects net construction in hydropsyche larvae •Changes enzyme production, hormonal balance, and calcium metabolism, ultimately affecting reproduction and behavior •Fish less susceptible than invertebrates
DDE/4 4 dde/2,2-Bis(p-chlorophenyl)-1,1-dichloroethene	•Impurity in and degradation product of DDT •Released as result of DDT use	•Very high potential for bioconcentration in aquatic organisms •Uptake in fish is suspected to occur through water as well as food •In conjunction with PCBs, causes twice the amount of death in trout fry as opposed to control fry •Inhibits ATPase activities in fish •Induces temperature shift in brook trout fingerlings •Inhibits brain mitochondrial ATPase in bluegill sunfish, *Lepomis machrochirus*