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CONTAMINANT CONCENTRATIONS IN WATER AND SEDIMENTS FROM SHELTA CAVE

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CONTAMINANT CONCENTRATIONS IN WATER AND SEDIMENTS FROM SHELTA CAVE

by

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EXECUTIVE SUMMARY

Shelta Cave is a cavern system which lies under the northwestern portion of the City of Huntsville, Alabama. The National Speleological Society owns property which contains two pit entrances to the cave, and also maintains their headquarters office on a portion of this property.

Shelta Cave formerly housed a faunal community believed to be one of North America's richest in terms of species diversity and abundance. In recent years, there have been great declines in the number of aquatic troglobitic species present in Shelta Cave's subterranean lake. This study was designed to determine whether contaminants have been a factor contributing to the observed declines in aquatic cave fauna.

Ten sets of water samples were collected during an approximate 10-week period in the early summer of 1990. The samples were serially collected over an extended period of time to obtain representative samples of any contamination that might enter the cave via storm runoff conveyed through sinkholes. Sediment samples were also collected on the first and last trip into the cave.

Water samples were assayed for 28 chlorohydrocarbon compounds, 20 potentially toxic metals, 25 organophosphate and six carbamate pesticides, and six chlorophenoxy acid herbicides. Sediment samples were assayed for 27 chlorohydrocarbon compounds, 23 potentially toxic metals, 25 organophosphate and six carbamate pesticides, and six chlorophenoxy acid herbicides.

Although several of the water samples contained detectable traces of potentially harmful contaminants, only heptachlor epoxide was present at a concentration believed to be capable of adversely affecting aquatic cave life. Chlordane, dieldrin, and heptachlor epoxide concentrations detected in Shelta Cave sediments were believed capable of biologically impairing Shelta Cave's aquatic ecosystem. DDT, DDD, and DDE also appeared in the sediment samples, but at concentrations too low to clearly predict adverse biological effects.

Bioassays should be used to ascertain the acute and chronic toxicity (i.e., EC_{50} values) of Shelta Cave's sediments to determine if sediment-related toxicity may be a factor in the reported declines of the aquatic fauna.

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ABBREVIATIONS AND CONVERSION FACTORS

Abbreviations

liter	l
milliliter	ml
kilogram	kg
gram	g
parts per million	ppm
parts per billion	ppb
parts per trillion	ppt
milligram per kilogram	mg/kg
micrograms per gram	ug/g
micrograms per milliliter	ug/ml
micrograms per liter	ug/l
nanograms per liter	ng/l

Conversion Factors

micrograms per gram	ppm
micrograms per milliliter	ppm
milligrams per kilogram	ppm
micrograms per liter	ppb
nanograms per liter	ppt

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INTRODUCTION

Shelta Cave lies under the northwest portion of the City of Huntsville, Madison County, Alabama (Figure 1). Two pit entrances to the cave are located in a sinkhole on a wooded city lot immediately southeast of the National Speleological Society (NSS) Headquarters Office.

With regard to the biota of Shelta Cave, Hobbs and Bagley (unpublished) have stated the following:

> A number of biological investigations have indicated that Shelta Cave formerly housed one of the richest, most complex cave fauna communities known in North America. This was particularly true for the subterranean aquatic ecosystem. The cave's terrestrial fauna is represented by four phyla, ten classes, 22 orders and 32 minor identified taxa, with further taxonomic work yet to be completed. The aquatic fauna contains five phyla, ten classes, 16 orders and 30 minor identified taxa. Of this total, at least 22 species are troglobitic and Shelta Cave either is serving or will serve as the type locality for nine of these species (Cooper 1975). In addition to the organisms reported by Cooper, a basidiomycete (Leucoagaricus procerus) was reported in 1986.

Cooper (1975) focused primarily on three species of troglobitic crayfish, one of which is known to occur only in Shelta Cave. He also studied the troglobitic Alabama cave shrimp (Palaemonias alabamae), a federally listed endangered species, and the southern cave fish (Typhlichthys subterraneus). Cooper (1975) reported considerably fewer sightings of cave shrimp than he did for crayfish; however, he saw the greatest number of shrimp during November-December, 1968. More recent field work in both Shelta and Bobcat Caves, the only two locations where P. alabamae has been reported, confirmed the presence of a population in the latter cave. No individuals have been observed in Shelta Cave since those noted by Cooper. There is concern that the Shelta Cave population of P. alabamae may have been extirpated (Hobbs and Bagley, unpublished).

Shelta Cave also formerly contained a maternity colony of federally listed endangered gray bats (Myotis grisescens). It is thought that the bats may have abandoned the cave as a result of the installation of a gate and grating used to control access to Shelta Cave. The NSS acquired Shelta Cave from the Nature Conservancy in 1967 and installed the gate and grating prior to learning that gray bats are intolerant of gates. The actual size of the former gray bat colony associated with Shelta Cave is unknown, but probably included several hundred individuals.

Ironically, NSS's plan to protect the unique features and subterranean ecosystem of Shelta Cave through purchase and access control may have had virtually the opposite effect. The installation of the access gate and grate not only could have resulted in the loss of the

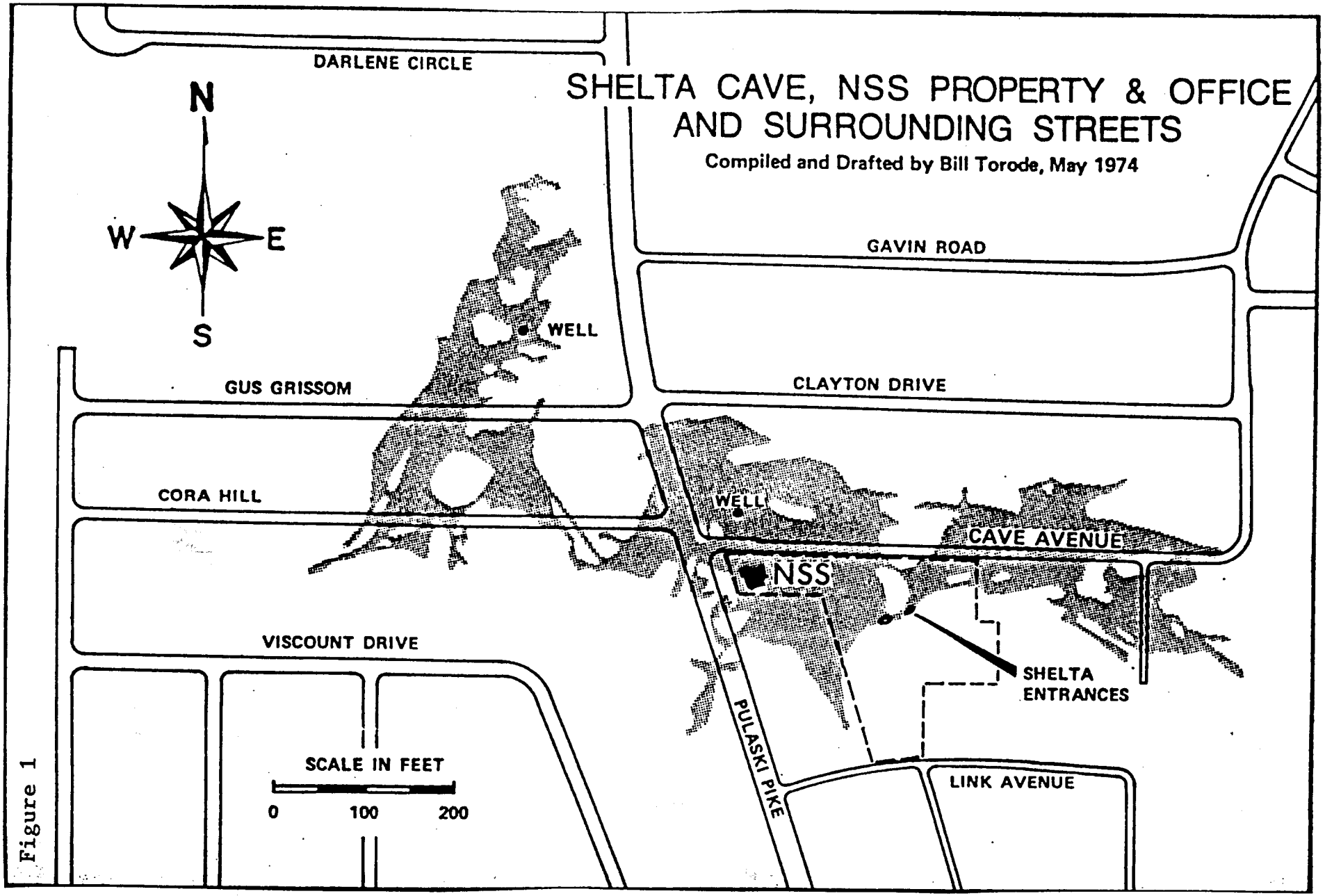
SHELTA CAVE, NSS PROPERTY & OFFICE
AND SURROUNDING STREETS
Compiled and Drafted by Bill Torode, May 1974
DARLENE CIRCLE
GAVIN ROAD
CLAYTON DRIVE
GUS GRISSOM
CORA HILL
VISCOUNT DRIVE
CAVE AVENUE
LINK AVENUE
PULASKI PIKE
WELL
WELL
NSS
SHELTA
ENTRANCES
N
S
E
W
SCALE IN FEET
0
100
200

Figure 1

endangered gray bat maternity colony, but also curtailed a primary source of allochthonous carbon for the grotto ecosystem. Bat guano probably provided a large source of carbon for terrestrial and aquatic organisms residing in Shelta Cave. Dead bats that fell onto the cave floor or into the subterranean lake also supplied a source of carbon. NSS officials believe that little drainage exists to import allochthonous carbon into Shelta Cave, and that once the bats abandoned the cave, a gross reduction in organic input to the cave probably occurred. The NSS has postulated this as a primary factor in the reduced numbers of cave fish and cave crayfish observed in recent years, and in the possible extirpation of the Alabama cave shrimp.

NSS and U.S. Fish and Wildlife Service (FWS) officials have also been concerned about the role which contaminants may have played in the reduced numbers of cave organisms observed in Shelta Cave. Based upon observed numbers and diversity of the subterranean fauna (Hobbs and Bagley, unpublished), water quality in the cave was believed to have remained good until 1975. However, the Tennessee Valley Authority (TVA) found that water samples from the cave contained trace amounts of heptachlor epoxide and dieldrin (French and Strunk 1990). Samples collected in March and May, 1987, had heptachlor epoxide concentrations of 0.5 and 0.04 micrograms per liter (ug/l), respectively. A trace of dieldrin (0.1 ug/l) was detected in the water sample which TVA collected in May, 1987.

Heptachlor epoxide is a degradation product of heptachlor, a long-lasting cyclodiene soil insecticide previously used as a termiticide. In addition, heptachlor comprises approximately 10 percent of technical grade chlordane, another former termite pesticide. The sale and distribution of all products containing chlordane or heptachlor were halted by order of the U.S. Environmental Protection Agency on April 15, 1988.

Dieldrin is a highly stable cyclodiene compound similar to heptachlor epoxide. Dieldrin can also originate as a degradation product of aldrin, another cyclodiene compound formerly used as a pesticide. Because of dieldrin's persistence in the environment and its high toxicity to fish and other aquatic organisms (at levels as low as 2-4 micrograms per liter), all uses of this compound were cancelled by the early 1980's.

These levels of heptachlor epoxide and dieldrin pose an acute toxicity risk to aquatic troglobites, and a chronic risk due to their tendency to accumulate in tissues. Bioaccumulated chlorohydrocarbon pesticides tend to become more concentrated through the food chain, potentially exposing predators and scavengers to levels much higher than those present in the ambient environment. These compounds may also produce adverse secondary effects such as aberrant behavior or reproductive inhibition in some species.

A very high level (48 ug/l) of cadmium (Cd) was also detected in the May, 1987, water sample analyzed by TVA. This level greatly exceeds EPA's water quality criteria for the protection of freshwater aquatic life (3.9 ug/l), and the drinking water maximum contaminant level of 10 ug/l. Cadmium levels of this magnitude do not occur naturally, and presumably resulted from an effluent discharge of some sort (e.g., industrial metal plating).

The discovery of these contaminants in water samples collected from the cave in 1987 caused FWS biologists to question the role of water quality in the reduced numbers of troglobitic organisms residing in the cave. Shelta Cave underlies urban/suburban Huntsville in a karst area where storm event surface runoff can rapidly convey both point and nonpoint source pollutants into the groundwater via sinkholes. Urban runoff potentially poses an insidious threat to subterranean organisms because the contaminants may be present in the aquatic environment for only short periods of time following a storm event. If water samples are not collected shortly following the storm-related inflow, most contamination entering the cave will not be detected. However, periodic pulses of contamination associated with urban runoff are just as capable of causing adverse impacts to sensitive aquatic organisms as is chronic exposure. This is particularly the case with some of the cyclodiene pesticides like heptachlor epoxide and dieldrin, which even at low aqueous concentrations are capable of bioaccumulating within the tissues of living organisms, until some threshold level of impairment occurs.

The three main purposes of this study were to: 1) determine whether the cadmium and cyclodiene pesticides previously reported by TVA were still present in the cave's subterranean lake; 2) determine if contaminant levels in the subterranean lake fluctuate significantly, possibly in association with inflows of urban storm runoff; and 3) analyze sediment samples to help provide a historical record for some of the more persistent contaminants.

METHODS

The FWS contracted with Mr. Lee Tucker of the NSS to make a serial collection of ten triplicate water samples from the subterranean lake in Shelta Cave during late spring and summer 1990. Mr. Tucker collected samples on:

May 22 and 30
June 5, 13, 20 and 27
July 10, 16, 24 and 31

During each trip into the cave, Mr. Tucker collected: 1) a 1000 ml water sample for metals analysis; 2) a 500 ml water sample for herbicide and chlorohydrocarbon analysis; and 3) a 500 ml water sample for carbamate and organophosphate pesticide analysis. Triplicate 250 ml composite sediment samples also were collected on the first (May 22) and last trip (July 31) into the cave.

Each 1000 ml water sample was collected (unfiltered) in a high density polyethylene bottle which had been chemically precleaned to EPA specifications by the vendor. Prior to providing sample containers to the contract sample collector, the FWS pipetted eight ml of lab grade (70%) nitric acid into each 1000 ml bottle and subsequently recapped them with teflon-lined lids. The 1000 ml sample bottles containing the concentrated nitric acid

remained closed until filled with the water. Water samples were collected by submersing one of the 500 ml bottles in the subterranean lake until full and pouring the contents into the 1000 ml bottle containing the acid. When filled, each 1000 ml bottle contained a water sample acidified to a pH of approximately 2.0.

The 500 ml water samples were collected in acid-cleaned glass bottles. Samples were collected by submersing the bottles in the lake. Filled bottles were capped with teflon-lined lids. The samples were refrigerated awaiting shipment to a FWS contract laboratory.

Duplicate composite sediment samples were collected during the first and last sample collection trips into Shelta Cave. Sediments were collected by dredging each 250 ml wide-mouth jar through sediments at several separate locations in Shelta Cave's subterranean lake. Excess water was decanted and the jars were sealed with teflon-lined caps and transported to the surface. Upon exiting the cave, the jars were wiped clean, labeled and placed in a freezer until they were packaged for shipping.

Most samples were shipped the day following collection via an overnight delivery company. Water and sediment samples were wrapped in bubble wrap and packed with "Blue Ice" for shipment.

A set of ten 500 ml water samples and two sediment samples were shipped to the Mississippi State Chemical Laboratory (MSCL) for analysis. Using methods described in Appendices A and B, water and sediment samples were analyzed for: 28 chlorohydrocarbon compounds and six chlorophenoxy acid herbicides. Percent moisture for the sediment samples was also determined and results were reported as dry weight values in microgram/gram (ug/g), or parts-per-billion (ppb).

A set of ten 500 ml water samples also was sent to the U.S. Fish and Wildlife Service's Patuxent Wildlife Research Center (PWRC) in Laurel, Maryland, for analysis of organophosphate and carbamate pesticide content. After methylene chloride extraction, analyses were performed using the techniques described in Appendix C.

Ten 1000 ml water samples and two sediment samples were shipped to Research Triangle Institute (RTI) for analysis of 20 metals. After preconcentration, samples were analyzed by inductively coupled plasma (ICP) emission spectrometry (Appendix D). ICP analysis without preconcentration was used for sediments. Sediment samples were also analyzed for arsenic (As), mercury (Hg) and selenium (Se) by atomic absorption spectrophotometry (Appendix D).

RESULTS

Chlorohydrocarbon Constituents

In the water samples which were collected, only four of the 28 chlorohydrocarbon compounds analyzed (14%) were present above analytical detection limits. Alpha-chlordane, gamma-chlordane, heptachlor epoxide and dieldrin were present at concentrations ranging from 0.01 to 0.16 ppb (Table 1). The other 24 chlorohydrocarbon compounds (Table 2) were not present at the 0.005 ppb detection limit.

For the sediment samples which were collected, eight of the 28 chlorohydrocarbon compounds analyzed (28%) were present above analytical detection limits. In addition to the four compounds present in the water samples, three DDT related compounds (p,p'-DDT, -DDE, and -DDD) and dieldrin were present in at concentrations ranging from 0.01 to 0.321 ppm (Table 3). The remaining chlorohydrocarbon compounds were not present at the 0.01 ppm detection limit. Toxaphene and PCBs were not present at the 0.05 ppm detection limit.

Chlorophenoxy Acid Herbicides

Both water and sediment samples were analyzed for six chlorophenoxy acid herbicides. These included: Dicamba; Dichlorprop; 2,4-D; Silvex; 2,4,5-T; and 2,4-DB. No concentrations of these compounds were measured above the 0.005 ppm or 0.01 ppm detection limits in water or sediment samples, respectively.

Organophosphate and Carbamate Pesticides

None of the 25 organophosphate or six carbamate pesticides were detected in the water samples. These analyses were not performed on the sediment samples.

Metals Analyses: Water

Of the 20 metals which were analyzed in the water samples (Table 4), only barium (Ba), magnesium (Mg) and strontium (Sr) were present in all ten samples. Zinc (Zn) was present in nine of the samples, and ranged from 0.006 to 0.034 ppm. Aluminum (Al), antimony (Sb), chromium (Cr), cobalt (Co) and lead (Pb) were each measured in only one sample. None of the water samples contained beryllium (Be), cadmium (Cd), molybdenum (Mo), silver (Ag), tin (Sn) or vanadium (Vn) at concentrations above their respective detection limits (Table 4). Detection limits for all metals are given in Table 5.

Table 1. Shelta Cave Water Sample Results Above Detection Limits (ppb) - Chlorohydrocarbon Scan

	Sample Number				
Compound	SCWAT-1A	SCWAT-2A	SCWAT-3A	SCWAT-4A	SCWAT-5A
Alpha-chlordane	0.03	0.03	0.02	0.02	0.03
Gamma-chlordane	0.01	0.02	0.01	0.01	0.02
Hept. epoxide**	0.08	0.08	0.08	0.08	0.04
Dieldrin	0.10	0.16	0.14	0.15	0.15

Compound	SCWAT-6A	SCWAT-7A	SCWAT-8A	SCWAT-9A	SCWAT-10A
Alpha-chlordane	0.02	0.02	0.02	0.02	0.02
Gamma-chlordane	0.02	0.01	0.02	0.02	0.01
Hept. epoxide**	0.07	0.07	0.09	0.07	0.07
Dieldrin	0.15	0.12	0.15	0.15	0.13

* The detection limit for chlorohydrocarbon compounds in water was 0.005 ppb.
** Heptachlor epoxide.

Table 2. Chlorohydrocarbon Compounds Below Detection Limit* in All Water Samples.

Compound
HCB
Alpha-BHC
Beta-BHC
Delta-BHC
Gamma-BHC
Oxychlordane
Trans-nonachlor
Cis-nonachlor
Toxaphene
PCB's (total)
O, P'- DDT
P, P'- DDT
O, P'- DDE
P, P'- DDE
O, P'- DDD
P, P'- DDD
Endrin
Mirex
8-monohydromirex
10-monohydromirex
2,8-dihydromirex
(cis)5,10-dihyromirex
(trans)5,10-dihydromirex
Octachlorostyrene

* 0.005 ppb detection limit.

Table 3. Shelta Cave Sediment Sample Results (ppm) - Chlorohydrocarbon Scan*

Compound	Sample Number			
	SCSED-1A		SCSED-2A	
	Wet Weight	Dry Weight	Wet Weight	Dry Weight
Alpha-chlordane	0.03	0.107	0.02	0.081
Gamma-chlordane	0.03	0.107	0.02	0.081
Trans-nonachlor	0.01	0.036	0.01	0.040
Hept. epoxide**	0.04	0.143	0.02	0.081
P, P'- DDT	0.02	0.071	ND	ND
P, P'- DDE	0.01	0.036	0.01	0.040
P, P'- DDD	0.01	0.036	0.01	0.040
Dieldrin	0.09	0.321	0.06	0.242
Sample weight	245 grams		400 grams	
% Moisture	28.0		24.8	

* The detection limit for chlorohydrocarbon compounds in sediment was 0.01 ppm, and was 0.05 ppm for toxaphene and PCB's.
** Heptachlor epoxide.

Table 4. Analytical Results for Shelta Cave Water Samples (ppm) - ICP Metals Scan

	Sample Number				
Element	SCWAT-1C	SCWAT-2C	SCWAT-3C	SCWAT-4C	SCWAT-5C
Aluminum	<0.050	<0.050	<0.050	<0.050	<0.050
Antimony	<0.030	<0.030	<0.030	<0.030	<0.030
Barium	0.011	0.013	0.011	0.010	0.010
Beryllium	<0.001	<0.001	<0.001	<0.001	<0.001
Boron	<0.003	<0.003	<0.003	<0.003	<0.003
Cadmium	<0.002	<0.002	<0.002	<0.002	<0.002
Cobalt	0.019	<0.008	<0.008	<0.008	<0.008
Chromium	0.006	<0.005	<0.005	<0.005	<0.005
Copper	<0.008	<0.008	<0.008	<0.008	0.016
Iron	0.044	<0.030	<0.030	<0.030	<0.030
Lead	<0.010	<0.010	<0.010	<0.010	<0.010
Magnesium	4.63	5.14	4.43	4.47	3.87
Manganese	0.003	<0.002	0.004	0.002	<0.002
Molybdenum	<0.010	<0.010	<0.010	<0.010	<0.010
Nickel	0.023	<0.010	<0.010	<0.010	<0.010
Silver	<0.010	<0.010	<0.010	<0.010	<0.010
Strontium	0.060	0.066	0.059	0.059	0.055
Tin	<0.020	<0.020	<0.020	<0.020	<0.020
Vanadium	<0.005	<0.005	<0.005	<0.005	<0.005
Zinc	0.011	0.013	<0.005	0.008	0.034

Table 4. (continued)

Element	Sample Number				
	SCWAT-1C	SCWAT-2C	SCWAT-3C	SCWAT-4C	SCWAT-5C
Aluminum	<0.050	0.156	<0.050	<0.050	<0.050
Antimony	0.035	<0.030	<0.030	<0.030	<0.030
Barium	0.012	0.013	0.011	0.020	0.011
Beryllium	<0.001	<0.001	<0.001	<0.001	<0.001
Boron	<0.003	0.005	0.004	0.004	<0.003
Cadmium	<0.002	<0.002	<0.002	<0.002	<0.002
Cobalt	<0.008	<0.008	<0.008	<0.008	<0.008
Chromium	<0.005	<0.005	<0.005	<0.005	<0.005
Copper	<0.008	<0.008	<0.008	<0.008	<0.008
Iron	0.061	<0.215	<0.030	<0.350	<0.030
Lead	<0.010	<0.010	<0.010	<0.010	0.014
Magnesium	4.59	4.42	4.17	4.42	4.47
Manganese	0.011	<0.030	0.003	0.028	<0.003
Molybdenum	<0.010	<0.010	<0.010	<0.010	<0.010
Nickel	0.010	<0.010	<0.010	<0.010	<0.010
Silver	<0.010	<0.010	<0.010	<0.010	<0.010
Strontium	0.062	0.060	0.057	0.057	0.056
Tin	<0.020	<0.020	<0.020	<0.020	<0.020
Vanadium	<0.005	<0.005	<0.005	<0.005	<0.005
Zinc	0.007	0.020	0.010	0.022	0.006

Table 5. Detection Limits For The ICP and AA Methods (ppm)

ICP DETECTION LIMITS			AA METHOD DETECTION LIMITS	
Element	Water* ug/ml	Sediment ug/g (dry weight)	Element	Sediment ug/g (dry wt.)
Al	0.05	100	As	0.3
Sb	0.03	35	Hg (CVAA)	0.02
Ba	0.003	2.0	Se	0.3
Be	0.001	0.3		
B	0.003	3.0		
Cd	0.002	0.7		
Co	0.008	10		
Cr	0.005	3.0		
Cu	0.008	3.0		
Fe	0.03	100		
Pb	0.010	7.0		
Mg	0.02	100		
Mn	0.002	2.0		
Mo	0.010	5.0		
Ni	0.010	5.0		
Ag	0.010	10		
Sr	0.005	5.0		
Sn	O.020	25		
V	0.005	2.0		
Zn	0.005	5.0		

* Preconcentration Microwave

Metals Analyses: Sediment

Sediment samples were tested for the same 20 metals analyzed in the water samples. Of these metals, Ag, Mo, Sb and Sn were not measured above their respective detection limits (Tables 5, 6) in either sediment sample. The 16 other metals were present at concentrations ranging from 1.34 ppm (Be) to 2.23% (Fe) on a dry weight basis (Table 6). While these 16 metals were present in both sediment samples (May 21 and July 31), concentrations generally tended to be greater in the sample collected on July 31 (Table 6).

Arsenic (As), mercury (Hg) and selenium (Se) were also analyzed in the sediment samples (Table 7). Arsenic ranged from 8.44 to 19.7 ppm, and was higher in the latter sample (July 31). Mercury varied from 0.055 to <0.02 ppm, and was not detected in the sample collected on July 31 (SCSED-2C). Selenium, total volatile solids and percent moisture values were similar in both sediment samples (Table 7).

Table 6. Shelta Cave Sediment Sample Results (ppm) - ICP Metals Scan

Element	Sample Number			
	SCSED-1C		SCSED-2C	
	Wet Weight	Dry Weight	Wet Weight	Dry Weight
Aluminum	3869	5520	4421	5810
Antimony	<35	<35	<35	<35
Barium	100.2	143	214	281
Beryllium	0.94	1.34	1.98	2.60
Boron	20.26	28.9	43.2	56.8
Cadmium	1.09	1.56	3.27	4.30
Cobalt	14.6	20.8	32.4	42.6
Chromium	34.8	49.6	44.7	58.8
Copper	3.0	4.26	20.8	27.4
Iron	12,408	17,700	16,970	22,300
Lead	31.6	45.1	48.7	64
Magnesium	228	325	390	513
Manganese	1,416	2,020	2,686	3,530
Molybdenum	<5.0	<5.0	<5.0	<5.0
Nickel	40.0	57.0	147.6	194
Silver	<10	<10	<10	<10
Strontium	4.6	6.53	8.1	10.7
Tin	<25	<25	<25	<25
Vanadium	44.2	63.0	71.2	93.6
Zinc	145	207	303	398

Sediment Analysis - Arsenic, Mercury, Selenium

Table 7. Shelta Cave Sediment Sample Results (ppm) - AA Analysis

	Sample Number			
Element	SCSED-1C		SCSED-2C	
	Wet Weight	Dry Weight	Wet Weight	Dry Weight
Arsenic	5.92	8.44	15.0	19.7
Mercury (CVAA)*	0.0383	0.0547	<0.02	<0.02
Selenium	0.378	0.539	0.307	0.404
Total volatile Solids	3.96%		4.46%	
% Moisture	29.9		23.9	

* Cold Vapor Atomic Absorption

DISCUSSION

Chlorohydrocarbon Constituents

The water samples which TVA collected in Shelta Cave in March and May of 1987 contained 0.5 and 0.04 ug/l of heptachlor epoxide, respectively (French and Strunk 1990). Low levels of the pesticide dieldrin were also detected in TVA's water samples. Our chlorohydrocarbon scan of Shelta Cave during May 22-July 31, 1990 (Table 1), corroborated TVA's previous findings of low levels of the pesticides heptachlor epoxide and dieldrin. In addition, our water samples also contained 0.03 to 0.05 ug/l total chlordane.[1]

The sediment samples collected on May 22 and July 31, 1990, showed traces of the same chlorohydrocarbon compounds detected in the water samples. These included alpha and gamma-chlordane, heptachlor epoxide and dieldrin (Table 3). In addition, both sediment samples contained traces of four chlorohydrocarbon compounds not detected in the water samples: trans-nonachlor; p,p'-DDT; p'-DDD and p,p'-DDE.

EPA cancelled most agricultural uses of the cyclodiene insecticides aldrin, dieldrin, heptachlor and chlordane in the United States between 1975 and 1980. However, some of these chemicals, particularly chlordane, were heavily used to control termites and other soil-dwelling insects after 1980. Chlordane use was cancelled in 1988, and was the last of these cyclodiene compounds to be used as a termiticide. Aldrin decays to dieldrin and heptachlor epoxide. Heptachlor decays to heptachlor epoxide. Chlordane decays to a variety of compounds, including heptachlor epoxide, trans-nonachlor, and both alpha and gamma-chlordane.

The traces of dieldrin, chlordane and heptachlor epoxide detected in the samples of water and sediment collected from Shelta Cave may be the result of leaching from the soil around buildings that were treated for termites. The climate in northern Alabama is highly favorable for termites, and many buildings in the area have been treated to prevent infestations of these pests.

Chlordane

The insecticide chlordane is listed by the Environmental Protection Agency as one of 129 priority pollutants (Keith and Telliard 1979), is rapidly accumulated by aquatic organisms, has a tendency to biomagnify in the food chain, and has been shown to be an animal

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[1] Technical chlordane is a complex mixture of isomers of chlordane, closely related compounds and by-products (trans-chlordane 24%, cis-chlordane 19%, heptachlor 10%, Chlordene isomers 21.5%, cis-nonachlor and trans-nonachlor 7% and closely related hydrocarbon compounds 18.5%). Isomers of chlordane residue are generally summed to give a total value.

carcinogen in laboratory tests (U.S. EPA 1980). Mayer and Ellersieck (1986) performed extensive aquatic toxicity tests under standard laboratory conditions at the U.S. Fish and Wildlife Service's National Fisheries Contaminant Research Center (NFCRC). Their work determined that the 96-hour-LC_{50}[2] for largemouth bass averaging 4.2 grams was 56 ug/l, and was 57 ug/l for bluegill averaging 1.4 grams. In similar toxicity tests performed on invertebrates at the NFCRC, Johnson and Finley (1980) determined that the 96-hour LC_{50} for the amphipod Gammarus fasciatus averaged 40 ug/l. In flow-through tests, crayfish (Orconectes) were the least sensitive invertebrate; the 96-hour LC_{50} was 50 ug/l and the 35-day LC_{50} was 31.6 ug/l. Hunn, Multer and DeFelice (1989) indicated that fish and aquatic invertebrate LC_{50} in the range of 30-60 ug/l (i.e., less than 100 ug/l) placed chlordane in the extremely toxic category (Table 8).

Table 8. Relative Acute Toxicity Of Chemicals For Aquatic Species (Hunn et al. 1989)

Toxicity Rating	96-Hour LC50
Slightly Toxic	10,000 - 100,000 ppb
Moderately Toxic	1,000 - 10,000 ppb
Highly Toxic	100 - 1,000 ppb
Extremely Toxic	less than 100 ppb

Under the authority of the Clean Water Act, EPA developed water quality criteria (WQC) which are used by the states to develop water quality standards. To protect freshwater

[2]LC50 = The median lethal concentration - expressed as mg/l (ppm), mcg/l (ppb), etc. - of chemical that is estimated to produce 50 percent mortality in test organisms exposed for a designated time, e.g., 96 hours for fish.

aquatic life, EPA's recommended acute criterion for chlordane is 2.4 ug/l, and the chronic criterion is 0.0043 ug/l (EPA 1986). The State of Alabama has promulgated water quality standards to protect freshwater life and has adopted EPA's WQC for chlordane. However, these standards currently apply only to surface waters. Legally, therefore, the water in the subterranean lake within Shelta Cave would not be subject to the same protection as surface waters in the State of Alabama.

The chlordane concentrations (expressed as the sum of alpha and gamma-chlordane) observed in our Shelta Cave water samples ranged between 0.03 and 0.05 ug/l. These concentrations were an order of magnitude above the EPA chronic freshwater quality criterion (0.0043 ug/l), but were far below the acute criterion (2.4 ug/l). Our chlordane results were also substantially below those associated with toxicity to crayfish, amphipods and most species of fish by Johnson and Finley (1980).

The chlordane concentrations (expressed as alpha-chlordane, gamma-chlordane and trans-nonachlor) detected in the sediment samples (0.07 and 0.05 ug/g wet weight, respectively) were approximately a thousand times higher than in the water.

Long and Morgan (1990) reviewed data from the National Oceanic and Atmospheric Administration's (NOAA) National Status and Trends Program, and many literature references concerning different approaches to determining sediment criteria. They published some data indicating potential high and low range values for biological effects relative to sediment-sorbed contaminants. For estuarine sediments, Long and Morgan (1990) estimated that the potential for biological effects of chlordane sorbed to sediments was highest where its concentration exceeded 6 ppb dry weight, and was lowest in sediments where the concentration was <0.5 ppb dry weight (Table 9).

Results of two studies on biological effects associated with chlordane- contaminated freshwater sediments included in NOAA's analysis were: 1) significant Daphnia magna mortality associated with chlordane concentrations of 31.3 ± 29.4 ppb, and low mortality with concentrations of 1.7 ± 2.3 ppb in sediments of the Trinity River, Texas; and, 2) the least number of benthic macroinvertebrate taxa (6.7 ± 2.5/site) associated with chlordane concentrations of 25 ± 22.3 ppb, and the highest numbers (15.8 ± 2/site) with concentrations of 8.3 ± 4.3 ppb in sediments of the Dupage River in Illinois (Long and Morgan 1990).

The Shelta Cave sediment concentrations detected in this study (202 ppb and 250 ppb dry weight) greatly exceeded the values that NOAA used in determining a level of concern for chlordane in sediments. The sediment concentrations of chlordane associated with significant Daphnia magna mortality in the Trinity River was only 30-60 ppb dry weight. In the Dupage River, the least number of benthic macroinvertebrate taxa were associated with sediments containing concentrations of chlordane from 25 to approximately 50 ppb (Long and Morgan 1990). The concentrations of chlordane we detected in Shelta Cave sediments were an order of magnitude greater than those studied in either the Trinity River or the

Table 9. Levels of Sediment-Sorbed Chlordane Associated with High and Low Probabilities for Biological Effects (Long and Morgan 1990)[3].

	ER-M	ER-L
Compound	Sediment Concentration Associated with High Potential for Biological Effects (PPB Dry Weight)	Sediment Concentration Associated with Low Potential for Biological Effects (PPB Dry Weight)
Chlordane	6.0 ppb	0.5 ppb

Dupage River. They also are an order of magnitude greater than the USGS alert level (20 ppb) to flag 15-20% of samples analyzed, and are two orders of magnitude greater than NOAA's ER-M value of 6.0 ppb.

Results of our study are not a conclusive demonstration of a direct cause-and-effect relationship between chlordane detected in Shelta Cave sediment, and the apparent reductions in the abundance of cave fauna. Nevertheless, these data certainly suggest that chlordane concentration in Shelta Cave sediments may be acutely or chronicly toxic to some species of troglobitic fauna, and through bioconcentration/ magnification, could be capable of disrupting the ecological food web.

Dieldrin

Dieldrin is a man-made compound belonging to a subgroup of cyclodiene insecticides which also includes Aldrin, DDT, BHC and others. Both dieldrin and aldrin were manufactured in the United States by Shell Chemical Company until the EPA prohibited their manufacture

[3]The 6 ppb and 0.5 ppb concentrations correspond to NOAA's Effects Range Median (ER-M) and Effects Range Low (ER-L) values, respectively. The ER-M value is equivalent to the 50 percentile point in the screened available data. This is the concentration above which effects were frequently or always observed or predicted among most species. The ER-L value represents the lower end of the range in which effects had been observed. The ER-L is equivalent to the 10th percentile point in the screened available data.

in 1974. These pesticides were subsequently manufactured by Shell Chemical Company in Holland. Prior to 1974, both insecticides were available in the United States in various formulations for broad-spectrum insect control (Sittig 1981).

The EPA cancelled all uses of the insecticide dieldrin in the United States in 1974, and now lists it as one of 129 priority pollutants (Keith and Telliard 1979). Dieldrin is also among the 25 hazardous substances thought to pose the most significant potential threat to human health at priority superfund sites (U.S. Dept. of Health 1987). Waters sampled in the United States have shown aldrin or dieldrin contamination up to 0.05 ug/l (Sittig 1981).

Johnson and Finley (1980) summarize the effects of dieldrin upon aquatic invertebrates and fish as follows:

> Dieldrin rapidly accumulates in invertebrates from a few hundred to several thousand times the exposure level in concentrations as low as 50 ng/l. Plateau levels were reached within 3 days in Daphnia and the residue half-life was 2.5 days. Although concentrations of 560 ug/l were not acutely toxic, midge larvae were unable to survive beyond 14 days in concentrations of 180 ug/l, and less than half were able to complete metamorphosis at 5.6 ug/l. The toxicity of dieldrin approximately doubled for rainbow trout and bluegills when water temperatures were increased from 2° to 13° C and from 7° to 29° C, respectively. Water hardness did not appear to affect toxicity to fish or invertebrates. Dietary dieldrin significantly altered several physiological and biochemical factors, including serum amino acid composition, adrenal and thyroid function, ammonia detoxification and phenyl-keto acid metabolism. The ability to withstand stress was significantly reduced.

Dieldrin's persistence in the environment is due to its extremely low volatility (i.e., a vapor pressure of 1.78×10^{-7} mm mercury at 20°) and low solubility in water (186 ug/l at 25° to 29°). In addition, dieldrin is extremely apolar, resulting in a high affinity for lipids. This accounts for its retention in animal fats, plant waxes and other organic matter in the environment. The fat solubility of dieldrin results in the progressive accumulation in the food chain. This can result in the body burden concentration of dieldrin within an organism exceeding the lethal limit for a consumer (Sittig 1981).

Aquatic toxicity tests performed under standard conditions at the NFCRC indicated dieldrin was extremely toxic (96-hour $LC_{50} < 10$ ug/l) to rainbow trout (Mayer and Ellersieck 1986). Tests by other researchers also indicated that dieldrin was highly toxic to other species of fish (96-hour LC_{50} 100 to 1000 ug/l) and crayfish (Morgan and Brunson 1989). In other aquatic toxicity tests with dieldrin, Johnson and Finley (1980) reported that the 96-hour LC_{50} averaged 3.5 ug/l for largemouth bass and 3.1 ug/l for bluegill.

The levels of dieldrin (0.10 to 0.16 ug/l) detected in the Shelta Cave water samples were significantly below those which NFCRC's studies determined were acutely toxic to largemouth

bass, bluegills and invertebrates. The rainbow trout was the most sensitive to dieldrin of any fish species that the NFCRC tested. Even so, the concentrations of dieldrin detected in the Shelta Cave water samples were an order of magnitude less than the 96-hour LC_{50} (1.2 ug/l) for rainbow trout, and further, are approximately three orders of magnitude less than the levels NFCRC's tests indicated were acutely toxic to midge larvae. However, the early life stages of the southern cave fish or Alabama cave shrimp could be more sensitive to dieldrin than the rainbow trout or invertebrates tested at the NFCRC.

As previously mentioned, the NFCRC indicated that invertebrates can rapidly accumulate and bioconcentrate (up to several thousand times the environmental concentration) dieldrin from concentrations in water as low as 50 ng/l. The concentrations of dieldrin in the Shelta Cave water samples were about three times greater than the 50 ng/l level determined by NFCRC as the approximate minimum threshold for daphnids to accumulate and bioconcentrate this chemical. Water concentrations of dieldrin detected in this study were about one order of magnitude less than those which the NFCRC determined to cause metamorphic impairment in midge larvae.

With regard to sediment, there is very little useful data available for interpreting and/or predicting the biological significance of dieldrin detected in the Shelta Cave sediments. As with chlordane, possibly the best information regarding the potential toxicity of sediment-sorbed dieldrin comes from the NOAA study done by Long and Morgan (1990). They have suggested that the potential for biological effects resulting from dieldrin sorbed to sediments is highest where its concentration exceeded 8 ppb dry weight, and lowest where its concentration is <0.02 ppb dry weight.

The dry weight concentrations of dieldrin detected in the two sediment samples from Shelta Cave were 0.321 and 0.242 ug/g (ppm). These are 30-40 times greater than the 8 ppb ER-M value (50 percentile point in the screened, available data) which NOAA suggests is the sediment concentration above which effects were frequently or always observed.

The concentrations of dieldrin detected in Shelta Cave sediments not only exceed NOAA's ER-M value[4], but were also an order of magnitude higher than the U.S. Geological Survey's (USGS) alert level (20 ppb), which flags 15-20 percent of samples analyzed (Long and Morgan 1990). Furthermore, concentrations of dieldrin in Shelta Cave sediments surpass EPA's (USEPA 1988) interim mean freshwater sediment quality criteria (199 ppb) at 1% total organic carbon (TOC).

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[4]In their report, NOAA cautions that the degree of confidence in these values is low (Long and Morgan 1990). Also, many of the dieldrin sediment concentration values included in NOAA's analysis were from marine environments, which may exhibit potentials for sorption and desorption of this compound which differ from freshwater.

No direct relationship can be established between the concentrations of dieldrin in Shelta Cave sediment samples and apparent reductions in the abundance of cave fauna. However, comparative data from other studies strongly imply that dieldrin levels in the cave sediments could be acutely or subacutely toxic to some species of troglobitic fauna. Dieldrin's capacity for bioconcentration/ magnification also poses an indirect threat to cave fauna through potential disruption of the ecological food chain.

Heptachlor Epoxide

The insecticide heptachlor is rapidly metabolized to heptachlor epoxide by many organisms. The EPA lists both heptachlor and heptachlor epoxide among 129 priority pollutants (Keith and Telliard 1979). Heptachlor was formerly widely used to control soil insects, crop pests, fire ants and termites. Heptachlor has been demonstrated to: be highly toxic to aquatic life; be persistent in the environment; bioconcentrate in organisms at various trophic levels; and exhibit carcinogenicity in mice. The principle metabolite of heptachlor, heptachlor epoxide, is more acutely toxic than heptachlor (Sittig 1981). Exposure symptoms in animals include tremors, convulsions and liver damage. Also, the carcinogenic potency of heptachlor is very high (Brown and Donnelly 1988).

Heptachlor is a minor, but relatively toxic, component of technical chlordane. Although some authors have concluded that most environmental residues of heptachlor epoxide originated from the use of heptachlor, other have concluded that lethal residues in birds originated from technical chlordane (Schmitt et al. 1985).

The EPA chronic freshwater aquatic life criterion for heptachlor is 0.0038 ug/l, while the acture criterion is 0.52 ug/l (USEPA 1986). The State of Alabama's water quality standards with regard to heptachlor parallel the EPA freshwater aquatic life criterion. Neither EPA or Alabama have water quality regulations specific to the compound heptachlor epoxide. Furthermore, Alabama's water quality regulations with regard to heptachlor currently apply only to surface waters and would not be legally applicable to the subterranean lake in Shelta Cave.

Heptachlor epoxide levels detected in Shelta Cave water samples (0.04 to 0.09 ug/l) exceeded the EPA (heptachlor) chronic freshwater aquatic life criterion (0.0038 ug/l) by more than an order of magnitude. This, and the fact that heptachlor epoxide can be more toxic than heptachlor, indicate that the levels of heptachlor epoxide detected in Shelta Cave water may pose a chronic threat to troglobitic fauna. The concentrations of heptachlor epoxide detected in water from Shelta Cave may not be acutely toxic to aquatic cave fauna, but may cause stress, or intensify the effects of other environmental stress. It is unclear whether heptachlor epoxide is more toxic to early life stages than to adult forms of the various types of troglobitic fauna.

The dry weight values of heptachlor epoxide detected in the two Shelta Cave sediment samples were 81 and 143 ppb. Although NOAA did not have enough data available to calculate ER-L and ER-M values for concentrations of heptachlor (or heptaclor epoxide) in sediments, the

observed concentrations greatly exceed the USGS alert level (20 ppb) to flag 15-20% of samples analyzed (Long and Morgan 1990).

DDT, DDD, DDE

Dichloro-diphenyl-trichloroethane (DDT) was formerly an inexpensive broad spectrum insecticide. However, an extensive review of health and environmental hazards of the use of DDT resulted in EPA banning further use of this compound in December, 1972. This decision was based upon several properties of DDT that had been well evidenced: 1) DDT and its degradation products (DDD and DDE) are toxicants with long-term persistence in soil and water; 2) it is widely dispersed by erosion, runoff and volatilization; and 3) the low water solubility (0.0012 ppm) and high lipophilicity (100,000 ppm) of DDT result in concentrated accumulation of DDT in the fat of wildlife and humans which may be hazardous (Sittig 1981).

DDT is of moderate acute toxicity to man and most other vertebrate organisms. Its principle breakdown product, DDE, has very similar properties. Because DDT and DDE compounds are highly persistent in living organisms, the major concern with DDT toxicity is related to its potential chronic effect (Sittig 1981).

The EPA chronic freshwater aquatic life criterion for DDT is 0.0010 ug/l, and the acute criterion is 1.1 ug/l (USEPA 1986). In aquatic toxicity tests with DDT done by the NFCRC, the 96-hour LC_{50} averaged: 1.5 ug/l for largemouth bass; 8.6 ug/l for bluegill; 12.2 ug/l for fathead minnows; and averaged 0.18 ug/l for crayfish (Orconectes sp.) (Johnson & Finley 1980). A summary of NFCRC's findings with regard to DDT toxicity tests on the previously mentioned species (and others) are presented below:

> The p,p'-isomer appears to be more toxic than the o,p-isomer (of DDT) to invertebrates. DDE is one of the primary metabolites of DDT in invertebrates and produces biological effects similar to those of the parent compound. DDT rapidly accumulates in invertebrates to several thousand times the exposure level in concentrations as low as 80 ng/l. The residue half-life was 7 days in Daphnia. A 60% reproductive impairment was observed in Daphnia at 100 ug/l. The 96-hour LC50 for 19 species of fish ranged from 1.8 to 22 ug/l. Toxicity to bluegills increased slightly when temperatures were increased from 7° to 29° C. No difference in toxicity was noted between hard and soft water. Although isomers tested were toxic to rainbow trout sac fry, the more polar compounds appeared more toxic than the less polar ones. DDT detrimentally altered several physiological characteristics, including normal ratios of serum amino acids, thyroid activity, and the ability to withstand stress. Food seems to be more important than water as a source of body residues. Although DDT was not observed to affect gonad maturation, the mortality of fry produced by treated parents was high, especially during the terminal stages of yolk absorption.

No DDT or related degradation products were detected in the Shelta Cave water samples. This is not surprising because these compounds (DDT, DDD and DDE) have a very low solubility in water, and the uses of DDT have been cancelled for nearly twenty years. Very low concentrations (i.e., below standard detection capabilities) of DDT or its breakdown products may be dissolved in the subterranean lake within Shelta Cave. However, we believe that direct contact with the very low levels of dissolved DDT in the aqueous environment probably represents an insignificant threat to troglobitic fauna. A greater threat is likely posed by the levels of DDT and its breakdown components detected in the cave sediments, and by the corresponding level of accumulation/biomagnification that occurs as a result of contaminated sediment.

One of the two sediment samples from Shelta Cave (SCSED-1A) contained a combined total of 0.143 ug/g (dry weight) of DDT_R (the combined levels of DDT, DDE and DDD). The other sample (SCSED-2A) contained a 0.08 ug/g (dry weight) combination of DDE and DDD. One half (0.71 ug/g) of the 0.143 ug/g (dry weight) level of DDT_R detected in sediment sample SCSED-1A consisted of DDT and the other half consisted of equal portions of DDD (0.36 ug/g) and DDE (0.36 ug/g). The DDT_R in sediment sample SCSED-2A consisted of equal portions of DDD (0.04 ug/g) and DDE (0.04 ug/g). All of the DDT, DDE and DDD detected was of the more toxic p,p'-isomer form.

There are few published concern levels for DDT and its degradation products in sediment. NOAA has published the best data suggesting potential high and low range values (Table 10) for biological effects relative to sediment-sorbed DDT, DDD and DDE (Long and Morgan 1990).

Table 10. Levels of Sediment-Sorbed DDT, DDD and DDE Associated With High and Low Probabilities for Biological Effects (Long and Morgan 1990)

	ER-M	ER-L
Compound	Sediment Concentration Associated With High Probability for Biological Effects (ppb dry weight)	Sediment Concentration Associated With Low Probability for Biological Effects (ppb dry weight)
DDT	350	3
DDD	20	2
DDE	15	2

The dry weight levels of DDD and DDE detected in the Shelta Cave sediment samples were each 36 ppb in the first sample collected, and 40 ppb in the second sample. These concentrations exceeded NOAA's ER-M value[5] (Table 10) for these compounds, suggesting a high probability for some level of biological effects. The 71 ppb concentration of DDT detected in the first sediment sample exceeded the 20 ppb (dry weight) alert level used by USGS to flag 15-20% of samples analyzed.

The level of DDT_R in Shelta Cave sediments is high enough to be cause for concern. Further, synergistic biological effects could result from combined exposures to DDT, DDD and DDE since the concentrations of each suggest potential chronic toxicity in freshwater aquatic life.

Chlorophenoxy Acid Herbicides

None of the six herbicides were detected in water or sediment samples from Shelta Cave. Analyses were conducted because Shelta Cave receives runoff from a golf course, domestic residences, and businesses where herbicides may be used.

Organophosphate and Carbamate Pesticides

Of the 25 organophosphate and six carbamate pesticide analytes tested, none were detected in water samples from Shelta Cave. Because these compounds rapidly degrade in the environment, water was the only matrix tested. They do not typically accumulate in sediments or tissue. In fact, many of these compounds degrade so rapidly in the environment that it can be difficult to detect their present in water samples. Thus, the absence of organophosphate or carbamate pesticide compounds in the Shelta Cave water samples does eliminate the possibility that these compounds could have been present in low concentration when the water samples were collected. It also does not dismiss the possibility of periodic, storm event-related influxes of these compounds into the waters of Shelta Cave. Because no organophosphate or carbamate pesticides were detected in any water samples, adverse influences of these compounds on the cave ecosystem are likely less significant than other contaminant-related influences (i.e., chlorohydrocarbon compounds in sediment).

Metals

With regard to metallic constituents, the water quality of Shelta Cave appeared quite good. Metal concentrations generally were below or slightly above, the level of detection. None of

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[6]In their report, NOAA cautions that the degree of confidence in these values is low because of the limited data available (Long and Morgan 1990).

the metals detected were present at concentrations shown to cause acute or chronic toxicity to biota.

Most of the metals detected in the sediments seemed to be close to normal background levels. Lead, nickel and zinc appeared somewhat elevated with respect to the ER-L and ER-M values (Table 11) developed by Long and Morgan (1990). Releases of wastes from metal plating industries, other local industries, and domestic sources of these metals may have increased the concentrations of nickel, lead and zinc above historical background levels. However, with the possible exceptions of nickel and zinc in sample SCSED-2, the concentrations of these three metals in Shelta Cave sediments were very similar to the sediment concentration ranges of nickel (13-59 ug/g), lead (36-70 ug/g) and zinc (64-260 ug/g) that TVA (Meinert 1991) noted in the sediments of Wheeler Reservoir, near Huntsville, Alabama. Although nickel, lead and zinc concentrations observed in Shelta Cave sediments may be somewhat high, they are probably within what could be considered the current normal background range for the Huntsville area.

Table 11. Comparison of the Concentrations of Toxic Trace Metals Detected in Shelta Cave Sediments With Sediment Values Associated With Low and Moderate Effects Upon Biota (Long and Morgan 1990)

	Observed Concentration in Shelta Cave Sediments (ug/g Dry Weight)			
Metal	SCSED-1	SCSED-2	ER-L Value	ER-M Value
Arsenic	8.44	19.7	33.0	85.0
Cadmium	1.56	4.30	5.0	9.0
Chromium	49.6	58.8	80	145
Copper	4.26	27.4	70	390
Lead	45.1	64.0	35	110
Mercury	.0547	<0.02	0.15	1.3
Nickel	57	194	30	50
Zinc	207	398	120	270

Lead and zinc concentrations were far below levels observed in sediments from ecosystems known to be heavily impacted by lead and zinc mining. For example, Allen and Wilson (1992) found that zinc concentrations in sediments of the Spring River in Cherokee and Crawford Counties, Kansas, averaged slightly under 4,000 ug/g. Concentrations of lead from the same area averaged slightly above 300 ug/g. Although fish and benthic macroinvertebrates collected within the more highly contaminated reaches of Spring River did show elevated levels of zinc and lead in their tissues, there was no obvious evidence that these levels of sedimentary zinc and lead had biologically impaired these populations.

Lead (Pb)

Lead is a biologically unessential element; long recognized as a cumulative poison (Allen and Wilson 1992). Lead becomes available to aquatic environments via atmospheric deposition, urban runoff, industrial discharges and other sources (USFWS 1983). The aquatic chemistry of lead is complex. Most lead compounds are relatively insoluble in water except where the pH is low. Lead is most soluble and bioavailable under conditions of low pH, low organic content, low concentrations of suspended sediments, and low concentrations of the salts of calcium, iron, manganese, zinc and cadmium (Eisler 1988). Lead was more acutely and chronically toxic to all aquatic species tested when bioassays were performed in soft water (USFWS 1983).

The U.S. EPA (1986) summarizes the aquatic toxicity characteristics of lead as follows:

> The acute toxicity of lead to several species of freshwater animals has been shown to decrease as the hardness of water increases. At a hardness of 50 mg/l, the acute sensitivities of 10 species range from 142.5 ug/l for an amphipod to 235,900 ug/l for a midge. Data on the chronic effects of lead on freshwater animals are available for two fish and two invertebrate species. The chronic toxicity of lead also decreases as hardness increases and the lowest and highest available chronic values (12.26 and 128.1 ug/l) are both for a cladoceran, but in soft and hard water, respectively. Acute-chronic ratios are available for three species and range from 18 to 62. Freshwater algae are affected by concentrations of lead above 500 ug/l, based upon data for four species. Bioconcentration factors are available for four invertebrate and two fish species and range from 42 to 1,700.

The EPA (1986) chronic water quality criterion for protecting freshwater aquatic life effects of lead is 3.2 ug/l (24-hr average), and the acute criterion is 82 ug/l (at 100 mg/l hardness). Soluble lead concentrations in Shelta Cave waters appeared to be 3 to 5 times below the levels which have been associated with chronic or acute toxicity to freshwater aquatic life.

Knowlton et al. (1983) determined that crayfish exposed to contaminated sediment accumulated lead principally through adsorption to the exoskeleton and lost lead through molting, although

internal uptake and elimination without molting was measurable. Further, exposure to lead leached from sediment, surface to weight ratios, and frequency of molting seemed to influence lead uptake by crayfish.

In view of the available information, the moderate sedimentary concentrations of lead observed in Shelta Cave would appear to present a low order of risk to troglobitic fauna, particularly when compared to the risks posed by sedimentary levels of chlorohydrocarbon pesticide compounds.

Nickel (Ni)

Nearly 0.02 percent of the earth's crust is nickel; however, elemental nickel is rarely found in nature. Because nickel salts are highly soluble in water, nickel usually occurs as a divalent cation strongly sorbed to other metal oxides (USFWS, 1983). The geometric mean for U.S. soils was 13 ug/g, although concentrations as high as 700 ug/g have been observed (Shacklette and Boerngen 1984).

In freshwater systems, the toxicity of nickel is influenced by water hardness. At comparable water hardness, the LC_{50} values vary among species from 0.5 ppm for Daphnia spp. to 33.5 ppm for stonefly and 46.5 ppm for killifish (USFWS 1983). Phillips and Russo (1978) indicated that nickel has a low tendency to bioaccumulate. The American Fisheries Society (AFS 1979) has stated that an EPA-suggested criterion of 0.1 mg/l is not adequate for protecting freshwater organisms. The current EPA freshwater chronic aquatic life criterion for nickel is 160 ug/l (24-hr average), and the acute criterion is 1400 ug/l at 100 mg/l hardness (USEPA 1986). The greatest observed concentration of nickel in Shelta Cave water was 23 ug/l, which was within the levels recommended by the American Fisheries Society or EPA for the protection of freshwater biota.

Nickel has been considered less problematic than many other heavy metals (Allen and Wilson 1992), and little useful information is available to determine the biological risks associated with concentrations of sedimentary nickel observed in Shelta Cave. In view of the available information, the observed concentrations of sedimentary nickel in Shelta Cave probably represent only a moderate risk to troglobitic fauna, particularly when compared to the risks posed by sedimentary levels of chlorohydrocarbon pesticide compounds.

Zinc (Zn)

Zinc is an elemental nutrient required for metabolic processes of most organisms. In most instances, its biological transport is homeostatically controlled. It is never found free in nature, but occurs as a sulfide, oxide or carbonate. In aqueous solution, zinc is a divalent cation and is soluble in both neutral and acidic solutions, making it one of the most mobile of the heavy metals.

Sorption is the dominant process that results in zinc enrichment of suspended and bed sediments when pH exceeds 7. Zinc is desorbed from sediments as salinity increases, and zinc forms are potentially toxic if they can be sorbed or bound by biological tissue (USFWS 1983). The availability of zinc is determined principally by pH. However, zinc toxicity to aquatic animals is significantly affected by several environmental factors, notably hardness, dissolved oxygen and temperature (Flora et al. 1984). Furthermore, salts of alkaline earth metals are antagonistic to the action of zinc salts, and salts of certain heavy metals are synergistically toxic in soft water. An increase in temperature or a reduction in dissolved oxygen will increase the toxicity of zinc. In toxic concentrations, zinc compounds can have a variety of deleterious effects on both the morphology and physiology of fish (Flora et al. 1984).

Allen and Wilson (1991) concluded that zinc concentrations are generally well controlled metabolically by most organisms, and that the bioaccumulation or bioconcentration of zinc is highly dependent upon location, feeding habits and the life stage of the organism being studied.

The EPA (1986) chronic freshwater aquatic life criterion for zinc is 110 ug/l as a 24-hour average, and the acute criterion is 120 ug/l (at 100 mg/l hardness). The American Fisheries Society Task Force (AFS 1979) recommended the following freshwater zinc criteria for the hardness ranges shown:

Hardness (mg/l as $CaCO_3$)	Zinc (mg/l total Zn)
1-150	0.05
150-300	0.10
300-400	0.30
>400	0.60

Hardness was not measured when water samples were collected at Shelta Cave, and recent water chemistry studies for caves in the Huntsville area could not be located. A reasonable estimate of hardness was obtained by contacting the Huntsville Water Department. The City of Huntsville's water supply is obtained from wells and the average hardness is approximately 140 mg/l. Assuming that the hardness of Shelta Cave lake water is 140 mg/l, then, according to the American Fisheries Society Task Force's formula, total dissolved zinc concentrations should be no higher than 0.05 mg/l. The greatest concentration of total recoverable zinc recorded in this study was 0.022 mg/l, or about one-half of the Task Force's recommended criterion. Thus the concentrations of zinc associated with the waters of Shelta Cave are unlikely to pose a serious threat to aquatic life.

The concentrations of zinc in both of the sediment samples from Shelta Cave exceed NOAA's ER-L values and that the second sample collected (SCSED-2) substantially exceeded the ER-M value (Table 11). However, Allen and Wilson (1992) determined that fish, crayfish and benthic

invertebrates seemed to be relatively unaffected in areas with higher levels (i.e., 4,000 ug/g) of sedimentary zinc. Thus, it apprears that the levels of zinc detected in Shelta Cave sediments are likely to pose only a moderate threat to aquatic life in Shelta Cave. Furthermore, it is probable that any threat of zinc-related toxicity is overshadowed by that posed by chlorohydrocarbon compounds which were present in the cave sediments.

RECOMMENDATIONS

This study has shown that toxicants, particularly residues from chlorohydrocarbon pesticides, are present in the aquatic environment of Shelta Cave, and that these contaminants occur at concentrations which are likely to pose a significant level of risk to cave fauna. Therefore, we recommend that a series of bioassays be conducted to determine the actual level of sediment-related toxicity which may be caused by the presence of these contaminants.

Bioassays using several different organisms representative of the various trophic levels within the Shelta Cave aquatic ecosystem should be conducted using both water and sediments from Shelta Cave. Potentially suitable indicator organisms include, but should not be limited to: Photobacterium phosphoreum, a luminescent bacteria available from Microbics, Inc.; a microinvertebrate such as the cladoceran, Ceriodaphnia dubia; a sediment dwelling invertebrate such as the amphipod, Hyallela azteca; and larval fathead minnows (Pimephales promelas).

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APPENDIX A

SAMPLE PREPARATION AND ANALYTICAL METHODOLOGY FOR CHLOROHYDROCARBON PESTICIDES, POLYNUCLEAR AROMATIC HYDROCARBONS AND CHLOROPHENOXY ACID HERBICIDES IN WATER

WATER SAMPLE PREPARATION

This procedure was developed to allow sample extraction to be performed in the same container in which the sample is collected, thus eliminating the need to transfer the sample to other glassware and eliminating the possibility of incomplete extraction of any analyte that may adhere to glass surfaces. Five- pint acid bottles, calibrated at two liters, are convenient for sample collection/extraction.

This procedure may be used with any volume of sample with the appropriate volume reduction of solvents, reagents, and glassware. However, if transfer from the collection container is necessary, this container should be rinsed with the extraction solvent to insure complete recovery of all analytes.

1. Reduce sample volume to 2 liters, or record volume if < 2 liters.

2. Add PRQ 6N KOH to sample, seal and shake vigorously 30 times. Test pH with glass rod (ph = 8). Adjust to proper pH by dropwise addition of 6N KOH. Allow to stand one hour at pH 8.

3. Add 100 ml CH_2CL_2 and shake 2 minutes with periodic venting. Remove CH_2CL_2 into 500 ml French Square bottle using glass suction device attached to aspirator system. Reverse suction device to return water in CH_2CL_2 layer to the bottle. Repeat 2 X 100 ml CH_2CL_2. After final step, assure return of all water to bottle using Pasteur pipet. This combined extract holds chlorohydrocarbon, nitrogen and phosphorus-containing pesticides, aliphatic hydrocarbons and polynuclear aromatic hydrocarbons.

4. Acidify water with PRQ 12N H_2SO_4. Shake 30 times and test pH with glass rod. Adjust pH to 2 with dropwise addition of acid if necessary.

5. Extract water with 200 ml ethyl ether (EtoEt) by shaking two minutes. Remove ether layer with suction device to 1000 ml French Square. Return excess water to bottle. Repeat 2 X 100 ml EtoEt. Extract with a final aliquot of 100 ml petroleum ether. Remove all water from French Square with Pasteur pipet. This combined extract contains chlorophenoxy acid herbicides.

6. Concentrate acid and neutral extracts with Kuderna-Danish evaporators and reduce volume to adequate size for column clean-up.

<u>Column Clean-Up:</u>
*NEUTRAL FRACTION (N/P and chlorohydrocarbon pesticides, aliphatic and polynuclear aromatic hydrocarbons) - adjust sample extract to exact volume and remove an appropriate aliquot for column clean-up techniques specific to analyte, for pesticides use Mini-florisil (described in Method 2), for hydrocarbons use 1% deactivated silica gel (described in Method 4).

*ACID FRACTION (Chlorophenoxy acid herbicides) -

Derivitization: Reduce sample volume to approximately 0.5 ml and ethylate using diazoethane (15 min.). Exchange to hexane (N-EVAP) and reduce volume to 0.2 ml.

Column Clean-Up: Place 2.0 grams of 1% deactivated silica gel in a 7 mm i.d. chromatography column (#22 Kontes). Top with 1 cm Na_2SO_4 and pre-wet column with 10 ml hexane. Collect sample effluents in three fractions as follows:

FRACTION A: Add sample and rinse container with two 0.5 ml washes of 20 % benzene in hexane. Elute with 9 ml of the same solution (contains PCP).

FRACTION B: Add 10 ml 40% benzene in hexane. Add 10 ml 60% benzene in hexane (contains Dalapon, PNP, Silvex, Dinoseb, portion of Dicamba).

FRACTION C: Add 10 ml 80% benzene in hexane. Add 10 ml 100% benzene (contains remaining Dicamba, Dichlorprop, 2,4-D, 2,4,5-T, 2,4-DB, Bentazon and Blazer).

APPENDIX B

SAMPLE PREPARATION AND ANALYTICAL METHODOLOGY FOR CHLOROHYDROCARBON PESTICIDES, POLYNUCLEAR AROMATIC HYDROCARBONS AND CHLOROPHENOXY ACID HERBICIDES IN SEDIMENT

SEDIMENT SAMPLE PREPARATION

1. Weigh 20 grams soil into a PRQ centrifuge bottle. Add 10 ml PRQ H_2O to dry samples. Adjust pH to $\leq$ 2 with PRQ 12N sulfuric acid (about 1 ml). Add 50 ml acetone and shake 6 times over a one and one-half hour period (about every 15 min.). Add 50 ml of a 1:1 petroleum ether/ethyl ether mixture and repeat shaking. Centrifuge and decant liquid into a 500 ml separatory funnel containing 200 ml PRQ water. Re-extract soil by shaking one minute with 50 ml 1:1 PE:EtoEt (may need to add 10 ml H_2O & adjust to pH $\leq$ 2), then centrifuge and decant liquid into a separatory funnel.

2. Using PRQ 6N KOH (5 ml), adjust contents of separatory funnel to pH $\geq$ 12. Shake vigorously 2 minutes, then allow to stand 30 minutes with intermittent shaking. Drain water layer and reserve ether layer. Re-extract H_20 layer with 100 ml 1:1 PE:EtoEt. Cap and reserve combined ether extracts (this contains chlorohydrocarbon pesticides, aliphatic and polynuclear aromatic hydrocarbons).

3. Adjust aqueous layer to ph $\leq$ 2 using 3 ml of PRQ 12N sulfuric acid and extract with 100 ml 1:1 PE:EtoEt. Reserve this extract and re-extract H_20 with 100 ml 1:1 PE:EtoEt. Combine extracts (these extracts contain chlorophenoxy acid herbicides).

4. Concentrate acid and basic extracts with Kuderna-Danish evaporators and reduce volume to adequate size for column clean-up.

Column Clean-Up:
*NEUTRAL FRACTION (N/P and chlorohydrocarbon pesticides, aliphatic and polynuclear aromatic hydrocarbons) - adjust sample extract to exact volume and remove an appropriate aliquot for column clean-up techniques specific to analyte, for pesticides use Mini-florisil (described in Method 2), for hydrocarbons use 1% deactivated silica gel (described in Method 4).

*ACID FRACTION (Chlorophenoxy acid herbicides) -

Derivitization: Reduce sample volume to approximately 0.5 ml and ethylate using diazoethane (15 min.). Exchange to hexane (N-EVAP) and reduce volume to 0.2 ml.

Column Clean-Up: Place 2.0 grams of 1% deactivated silica gel in a 7 mm i.d. chromatography column (#22 Kontes). Top with 1 cm Na_2SO_4 and pre-wet column with 10 ml hexane. collect sample effluents in three fractions as follows:

FRACTION A: Add sample and rinse container with two 0.5 ml washes of 20 % benzene in hexane. Elute with 9 ml of the same solution (contains PCP).

FRACTION B: Add 10 ml 40% benzene in hexane. Add 10 ml 60% benzene in hexane (contains Dalapon, PNP, Silvex, Dinoseb, portion of Dicamba).

FRACTION C: Add 10 ml 80% benzene in hexane. Add 10 ml 100% benzene (contains remaining Dicamba, Dichlorprop, 2,4-D, 2,4,5-T, 2,4-DB, Bentazon and Blazer).

Reference for column clean-up for chlorophenoxy acid herbicides:

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Elution Profiles for Florisil, Silica Gel and
Silicic Acid Column Separations

A. Florisil Column:

1. **Fraction I** (6% ethyl ether containing 2% ethanol, 94% petroleum ether)

   HCB, alpha-BHC, beta-BHC, gama-BHC, delta-BHC, oxychlordane, heptachlor epoxide, gamma-chlordane, trans-nonachlor, toxaphene, PCBs, o,p'-DDE, alpha-chlordane, p,p'-DDE, p,p'-DDT, cis-nonachlor, o,p'-DDT, p,p'-DDD, mirex, dicofol, endosulfan I (split with F-II).

2. **Fraction II** (15% ethyl ether containing 2% ethanol, 85% petroleum ether)

   dieldrin, endrin, dacthal, endosulfan I (split with F-I), endosulfan II (split with F-III), endosulfan sulfate (split with F-III).

3. **Fraction III** (50% ethyl ether containing 2% ethanol, 50% petroleum ether)

   endosulfan II (split with F-II), endosulfan sulfate (split with F-II), malathion.

B. Florisil Mini-Column:

1. **Fraction I** (12 ml hexane followed by 12 ml 1% methanol in hexane)

   HCB, gamma-BHC (25%), alpha-BHC (splits with F-II), trans-nonachlor, o,p'-DDE, p,p'-DDE, o,p'-DDD, p,p'-DDD (splits with F-II), o,p'-DDT, p,p'-DDT, mirex, cis-nonachlor, cis-chlordane, trans-chlordane, PCBs, photomirex and derivatives.

2. **Fraction II** (24 ml 1% methanol in hexane)

   gamma-BHC (75%), beta-BHC, alpha-BHC (splits with F-I), delta-BHC, oxychlordane, heptachlor epoxide, toxaphene, dicofol, dacthal, endosulfan I, endosulfan II, endosulfan sulfate, octachlorostyrene, kepone (with additional 12 ml 1% methanol in hexane).

C. Silica Gel:

1. **SG Fraction I** (100 ml petroleum ether)

   n-dodecane, n-tridecane, n-tetradecane, ocylcyclohexane, n-pentadecane, nonycyclohexane, n-hexadecane, n-heptadecane, pristane, n-octadecane, phytane, n-nonadecane, n-eicosane.

2. **SG Fraction II** (100 ml 40% methylene chloride in petroleum ether followed by 50 ml methylene chloride)

   napthalene, fluorene, phenanthrene, anthracene, fluoranthrene, pyrene, 1,2-benzanthracene, chrysene, benzo [b] fluoranthrene, benzo [k] fluoranthrene, benzo [e] pyrene, benzo [a] pyrene, 1,2:5,6-dibenzanthracene, benzo [g,h,i] perylene.

D. Silicic Acid:

1. **SA Fraction I** (20 ml petroleum ether)

   HCB, mirex

2. **SA Fraction II** (100 ml petroleum ether)

   PCBs, p,p'-DDE (splits with SA-III)

3. **SA Fraction III** (20 ml mixed solvent: 1% acetonitrile, 80% methylene chloride, 19% hexane)

alpha-BHC, beta-BHC, gamma-BHC, delta-BHC, oxychlordane, heptachlor expoxide, gamma-chlordane, trans-chlordane, toxaphene, o,p'-DDE, alpha-chlordane, p,p'-DDE (splits with SA-II), cis-nonachlor, o,p'-DDT, p,p'-DDD, p,p'-DDT, dicofol.

APPENDIX C

SAMPLE PREPARATION AND ANALYTICAL METHODOLOGY FOR ORGANOPHOSPHATE AND CARBAMATE PESTICIDES IN WATER SAMPLES

ANALYTICAL METHODOLOGY FOR ORGANOPHOSPHATE/CARBAMATE SCANNING

MATRIX - WATER

SAMPLE PREPARATION DATE - 6/6/90

COMPLETION DATE - 8/17/90

SUMMARY - The extraction procedure was a modified version of the one described in the EPA test Method #608, Section 10[1]. The water samples were extracted three times with methylene chloride (all containers were rinsed three times as well). The methylene chloride extracts were combined, dried with sodium sulfate and concentrated on a rotary evaporator. Sample extracts were refrigerated prior to analysis. Residues were quantified by gas chromatography using either an instrument selective for organophosphate pesticides (Flame Photometric Detector) or one selective for carbamate pesticides (Nitrogen Phosphorus Detector) similar to Belisle et al (1988)[2]. Megabore capillary columns were used for the GC separation.

REFERENCES

[1]. EPA test method #608, Selection 10 (sample extraction), 1982.

[2]. Belisle, A.A. and D.M. Swineford. 1988. Simple, Specific Analysis of Organophosphorus and Carbamate Pesticides in Sediments Using Column Extraction and Gas Chromatography. Environ. Toxicol. and Chem. 7(9):749-752.

*Note: Due to low recovery of oxamyl (38%), detection limits were raised accordingly.

APPENDIX D

SAMPLE PREPARATION AND ANALYTICAL METHODS FOR INDUCTIVELY COUPLED PLASMA EMISSION (ICP) MEASUREMENT, GRAPHITE FURNACE ATOMIC ABSORPTION (GFAA) MEASUREMENT, AND MERCURY MEASUREMENT BY COLD VAPOR ATOMIC ABSORPTION

METHODOLOGY

SEDIMENT PREPARATION

1. **Homogenization** - Following freeze drying, samples were ground to approximately 100 mesh using a glass mortar and pestle.

2. **Digestion for Inductively Coupled Plasma Emission ICP) measurement** - Some 0.25 to 0.5 grams of sediment were placed in a 120 ml teflon microwave vessel. One ml each of HCL, HF and $HClO_4$, and 10 ml of HNO_3 were added to the vessel. The vessel was then capped according to the manufacturer's instructions and was heated in a CEM microwave oven for two minutes at 120 watts, three minutes at 180 watts, and ten minutes at 600 watts. The resulting residue was diluted to 100 ml with 5% HCl. This solution was then filtered through Whatman 41 filter paper prior to ICP measurement. An HF resistance torch tip was used for these digests during the ICP measurement.

3. **Digestion for Graphite Furnace Atomic Absorption (GFAA) measurement** - Using a CEM microwave oven, 0.25 to 0.5 g of freeze dried sediment was heated in a capped 120 ml teflon vessel in the presence of 5 ml of Baker Instra-Analyzed nitric acid for three minutes at 120 watts, three minutes at 300 watts, and fifteen minutes at 450 watts. The residue was then diluted to 50 ml with laboratory pure water.

4. **Digestion for mercury measurement by Cold Vapor Atomic Absorption (CVAA)** Some 0.25 to 0.5 g of sample were refluxed for two hours in 10 ml HNO_3 (Baker Instr-Analyzed) and diluted to 50 ml with 1% HCl.

WATER SAMPLE PREPARATION

1. **Preconcentration Digestion for Inductively Coupled Plasma Emission ICP) measurement** - Using a CEM microwave oven, 50 ml of water is heated in a capped 120 ml teflon vessel in the presence of 5 ml of Baker Instra-Analyzed nitric acid for three minutes at 120 watts, three minutes at 300 watts, and 35 minutes at 450 watts. The vessel contents are then allowed to cool and the cap is removed and rinsed carefully with 3 ml of HNO_3 adding the rinsings with the vessel contents. The uncapped vessel is then returned to the microwave oven and heated until the vessel contents are less than 1 ml in volume. The contents are carefully rinsed with laboratory pure water into a 10 ml glass volumetric vessel and made to volume with additional laboratory pure water. The sample is now ready for ICP analysis.

2. **Digestion for Graphite Furnace Atomic Absorption (GFAA) measurement** - Using a CEM microwave oven, 50 ml of water sample was heated in a capped 120 ml teflon

vessel in the presence of 5 ml of Baker Instra-Analyzed nitric acid for 15 minutes at 300 watts. The residue was then diluted to 100 ml with laboratory pure water.

3. **Digestion for mercury measurement by Cold Vapor Atomic Absorption (CVAA)** Ten ml of water sample was refluxed for two hours in 10 ml HNO_3 (Baker Instr-Analyzed) and diluted to 50 ml with 1% HCl.

MEASUREMENT

1. **ICP** - ICP measurements were made using a Leeman Labs Plasma Spec I sequential spectrometer.

2. **GFAA** - GFAA measurements were made using a Perkin Elmer Zeeman 3030 atomic absorption spectrophotometer with an HGA-600 graphite furnace and an AS-60 autosampler.

3. **CVAA** - Mercury measurements were conducted using $SNCL_4$ as the reducing agent. An Instrumentation Laboratories Model 251 AA spectrophotometer was employed.