DEPARTMENT OF THE INTERIOR
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REGION 1

ENVIRONMENTAL CONTAMINANTS PROGRAM

ON-REFUGE INVESTIGATIONS SUB-ACTIVITY

NV - Polychlorinated Biphenyls at Ash Meadows National Wildlife Refuge

Project ID: 1261-1N35

Final Report

by

John F. Miesner

and

Cynthia T. Martinez

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Introduction

Ash Meadows National Wildlife Refuge (NWR) is located in the Mojave Desert, approximately 145 kilometers (kIn) northwest of Las Vegas, Nye County, Nevada (Figure 1). The refuge encompasses approximately 8,907 hectares (22,000 acres) of alkaline desert interspersed with several free-flowing springs and streams. The refuge was established by the U.S. Fish and Wildlife Service (FWS) in 1984 for the protection of threatened and endangered species of plants, invertebrates, and animals and their habitats, and is populated by 12 such species. The refuge also provides aquatic and riparian habitats essential for migratory and resident bird species. The refuge is surrounded by private lands and lands administered by the Bureau of Land Management. Other significant lands in the area include the Desert National Wildlife Range, the Department of Energy (DOE) Nevada Test Site, and the Spring Mountains National Recreation Area which includes a portion of the Toiyabe National Forest.

Five Springs is located within Ash Meadows NWR in Sections 22 and 23 of Township 17 South, Range 50 East. The spring system includes several small springs which converge in an outflow pool and stream. The outflow stream extends approximately 1 kIn before being lost through evaporation or infiltration (Figure 2). Several unique plants and animals occur in or near Five Springs and the outflow stream. This system is identified as critical habitat for the endangered Ash Meadows Amargosa pupfish *(Cyprinodon mionectes)* and the threatened spring-loving centaury plant *(Centaurium namophilum).* Three other threatened plants, Ash Meadows blazing star *(Mentzelia leucophylla),* Ash Meadows milkveteh *(Astragalus phoenix),* and Ash Meadows sunray *(Enceliopsis nudicaulis* var. *corrugata),* occur in the immediate vicinity of the spring and outflow stream. The spring also supports several unique invertebrates, including three endemic snails that are considered species of concern (Oasis Valley springsnail *Pyrgulopsis micrococcus,* distal-gland springsnail *Pyrgulopsis nasus,* and Amargosa tryonia *Tryonia variegata).* The threatened Ash Meadows naucorid *(Ambrysus amargosus),* an aquatic invertebrate, and the Ash Meadows montane vole *(Microtus montanus nevadensis),* a species of concern, may also occur in the vicinity (FWS 1990).

Spring orifices at Five Springs emerge from shallow rock outcroppings. Total flow of this system isO.01 cubic meters per second (cms) at a temperature of33 °C. Three primary aquatic habitat types are present in the Five Springs system. Outflow charmels are typically less than I meter (m) wide, and incised approximately 0.3 m. Terrestrial vegetation, primarily salt grass *(Distichlis spictata),* grows along the outflow charmels and completely covers the narrower charmels. Outflow charmels are generally non-depositional, and are only slightly erosional because of a hard carbonate layer which lines most stretches. This carbonate layer originates from the carbonate aquifer which supports Five Springs. Shallow cattail *(Typha* sp.) marshes are supported by outflow charmels in low gradient areas. The cattail marshes present at Five Springs are heavily overgrown and offer little open water habitat, except along the perimeters. Finally, an open pool which is supported by flows originating in the central portion of Five Springs is present. This pool appears to be manmade because of its regular shoreline, depth, and the

FIGURE 1: Map of Ash National Wildlife Refuge, Nye County, Nevada.

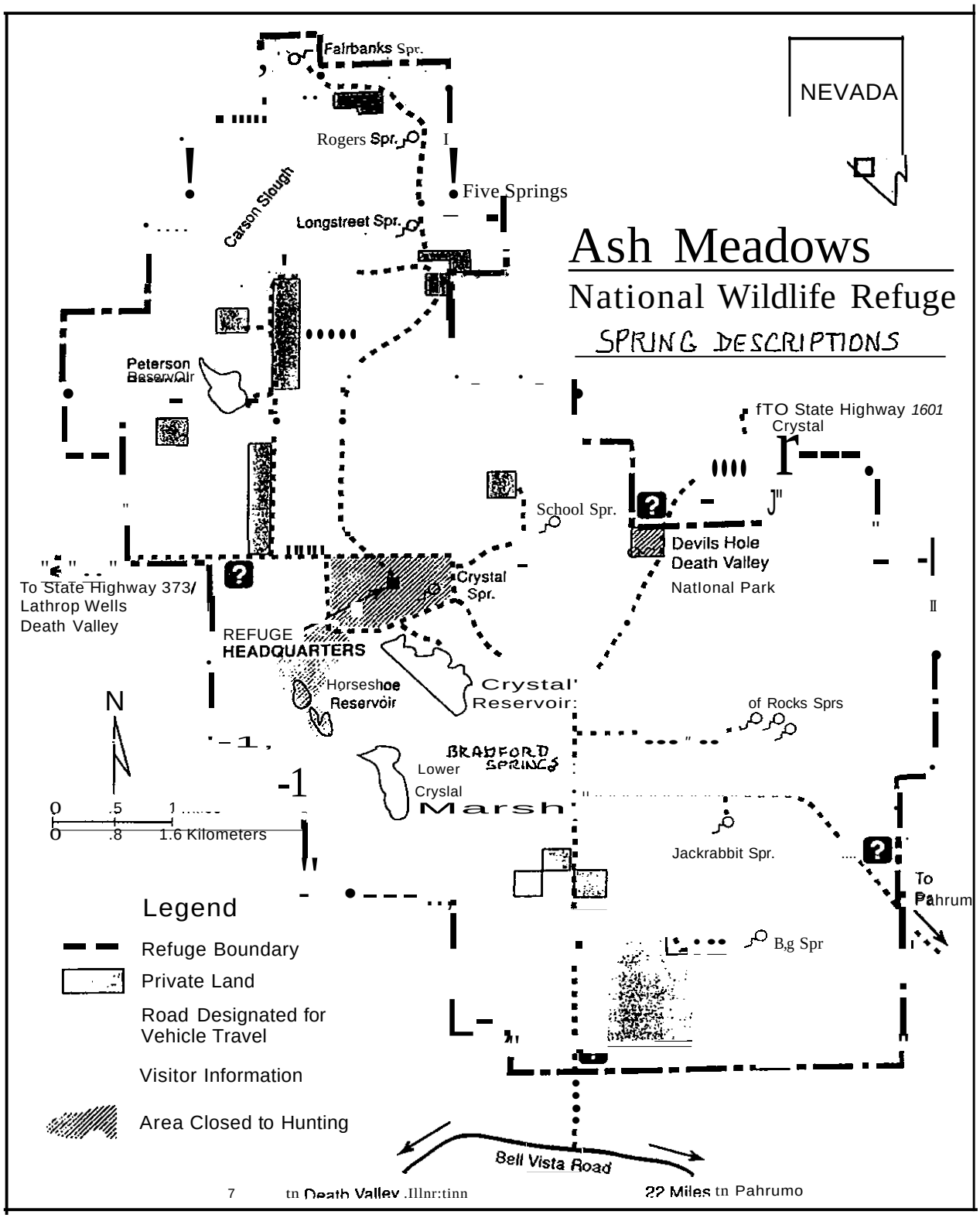
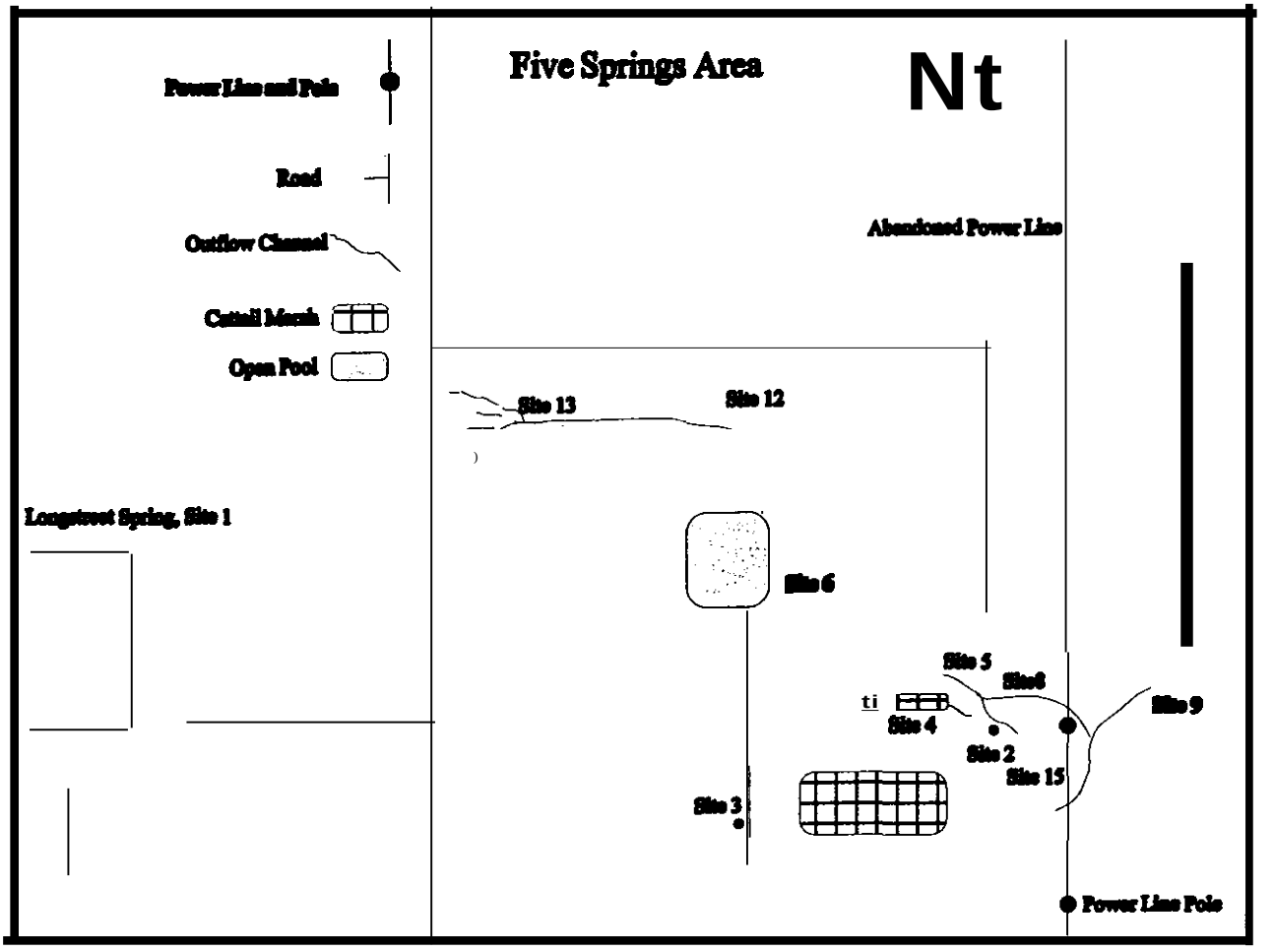


Figure 2: Generalized site map of Five Springs, Ash Meadows National Wildlife Refuge, Nye County Nevada. Note, map is not to scale.



presence of conspicuous piles of soil near the pool. Water in the pool is very clear with bottom features easily visible. Algae, the primary food source for species present in the pool, grow in large masses in the center of the pool. A complete description of sample sites is presented in Appendix A.

To identify possible effects to groundwater and spring discharges resulting from activities at the Nevada Test Site, approximately 32 Ian (20 miles) north of Ash Meadows NWR, the U.S. Geological Survey (USGS), in cooperation with DOE, routinely monitored spring discharge rate and water quality throughout the region. Activities at the Nevada Test Site which could have resulted in contamination of the region include both atmospheric and subterranean tests of nuclear devices and other tests involving radioactive materials, controlled atmospheric releases of numerous gaseous materials, and disposal and destruction of various types of solid and liquid wastes. In August 1990, monitoring of springs at Ash Meadows NWR detected polychlorinated

biphenyls (PCBs) at a concentration of 0.2 micrograms/liter in water collected from Five Springs Well Ash Meadows NWR. The concentration had increased to 1.1 $\mu g/L$ in a water sample collected at this site in April 1992. The concentration then returned to 0.2 $\mu g/L$ in December 1994, although samples collected in August 1992, and October 1994 did not contain PCBs (La Camera and Westenburg 1994). USGS had not published additional water quality data on Ash Meadows NWR at the time of this study (April 1996). Monitoring of six other springs on Ash Meadows NWR by USGS has not detected PCB contamination.

The source of the reported PCB contamination at Five Springs is unknown. Spring discharge on Ash Meadows NWR emerges from a deep carbonaceous aquifer. Contamination of this aquifer is highly unlikely. Five Springs was inhabited by humans prior to establishment of Ash Meadows NWR. The period of human habitation is uncertain. Structures, utility poles, and debris remain at the site. Electrical transformers on Ash Meadows have been identified as containing PCBs, and are a suspected source of contamination. Transformers containing PCBs were removed from the refuge in the late 1980s and early 1990s. Unconfirmed reports indicate the electrical transformers were found on the ground. near Five Springs (Greg Fasano, Scientific Applications Investigation Consultants, 1994, pers. comm.). No transformers were found at the site in August 1993 (Suzanne Baird, Ash Meadows NWR, 1993, pers. comm.). Additionally, an attempt by the authors in 1996 to locate a source of PCBs by sampling stained soil near the site of an old power pole did not detect PCBs. The location of the source and extent of contamination on the refuge remains unanswered. PCB concentrations reported in August 1990, April 1992, and December 1994 from Five Springs Well exceeded State and Federal water quality criteria and several published fish and wildlife effect levels (Eisler 1986). However, the reported concentrations did not exceed State action levels for water, and the State of Nevada declined to initiate a removal action. Because of the high lipid solubility of PCBs, contamination in biological systems is of greater concern than the water quality violations.

Adverse effects to aquatic organisms from exposure to PCBs have been documented for concentrations in the reported range (0.2 - 1.1 $\mu g/L$ in water; Eisler 1986). LC50 values for various mixtures of Aroclor compounds can vary from 0.1 to 10.0 $\mu g/L$ to sensitive freshwater and marine species. Toxicity tends to increase with increasing exposure durations. PCBs can also cause various sub-lethal impacts, including skin lesions, immunotoxicity, reproductive toxicity, genotoxicity, and various hepatic effects.

The primary objective of this study was to determine the nature (i.e., type and magnitude of effects on biota) and extent of PCB contamination in soils, water, sediment, and biota at Five Springs, Ash Meadows NWR, Nye County, Nevada.

Methods

We selected sample locations during a preliminary field survey of the site immediately prior to sampling. Selection criteria included: 1) Sampling the outflows of the major springs in the area; 2) sampling downstream of the confluence of major and minor outflows; 3) sampling

depositional areas; 4) sampling pools or larger flows which might contain all species of biota; and 5) sampling downstream in the single major stream leaving the Five Springs area Longstreet Spring was selected as a reference site as it is believed to originate from the same aquifer and is located in the general vicinity of the Five Springs system. We collected water, sediment, red-rim melania snails *(Melanoides tuberculata),* crayfish *(Procambarus* and Ash Meadows Amargosa pupfish samples on March 31 through April 2, 1997, from the sites (Figure 2 and Table 1) using the techniques described below.

Table I: Sample site matrix for Ash Meadows NWR, Nye County, Nevada, 1997. Refer to Figure I for sample locations and Appendix A for site descriptions.

Site Number	Water	Sediment	Snail	Crayfish	Pupfish	Other Tissue
I	X	X	X	X	X (+histology)	
2	X	X	X			
3	X	X		X		
4	X	X	X	X	X	
5	X	X	X	X	X (+histology)	
6	X	X	X	X	X	
7						X (bait)
8		X				
9		X				
10		X				
11		X				
12		X				
13		X				
15		X (soil)				

Note: Samples from Site 14 were not submitted for analysis because of budget constraints. However, the original site numeration has been retained.

We wore latex gloves, which were changed between each sample collected. We placed all samples in glass sample containers which were certified PCB-clean by the manufacturer and were sealed with teflon-lined, screw top lids. We cleaned field sampling equipment, when possible, with Alconox® (use of trade names does not imply endorsement by the Federal government), followed by sequential rinses with deionized water, reagent grade hexane, and

deionized water. We then wrapped the equipment in hexane-rinsed aluminum foil. We recleaned all equipment between the collection of each sample. Some equipment, such as the minnow traps and dip-nets, could not be cleaned using procedure. We thoroughly rinsed this equipment with site water before use at the sample locations. We weighed all sediment and tissue samples and placed the sample jars in plastic "zip-lock" bags to eliminate the possibility of cross-contamination. We immediately stored on water ice or blue ice in the field, and placed them in a freezer at -20°C upon return to the laboratory. We shipped all samples, except water, to the analytical laboratory on dry ice. We shipped water samples to the analytical laboratory on wet ice.

We submitted all samples for chemical analysis to the Mississippi State Chemical Laboratory for organochlorine scan residue analysis. Analytical methods followed procedures and quality controVquality assurance guidelines described in Patuxent Analytical Control Facility (1995). Samples were analyzed for the following constituents: Total PCBs; alpha-, beta-, delta-, and gamma-benzene hexachloride; alpha- and gamma-chlordane; cis- and trans-nonachlor; oxychlordane; dieldrin; endrin; heptachlor epoxide; mirex; *o,p'-DDD,* DDE, and DDT; *p,p'-* DDD, DDE, and DDT; and toxaphene. The lower detection limit on a wet weight basis for sediment and tissue samples was O.Olmilligramslkilogram (mg/kg), with the exception of total PCBs and toxaphene which were 0.05 mg/kg. The lower detection limit for all constituents in water was 0.1 μg/L.

We collected water samples by hand in two 500 milliliter (ml; 1000 ml total sample size from each location) amber glass jars. We filled each jar approximately half ful1 and rinsed it by vigorous shaking. We discarded this water, re-submersed the jar in the water, filled it to the brim, capped it, and stored it on wet ice for shipment. We shipped al1 water samples to the analytical laboratory on the day of collection because of the short holding time for PCBs in water samples.

We collected sediment and soil samples in 200 ml glass jars. We varied the method of col1ection according to the depth and grain size of the sediments present at each site. The preferred method of collection was with a sediment core col1ector. This device uses a 5 centimeter (em) diameter tube which is pushed into the sediment to the desired depth. A vacuum is then created by blocking the top end with one hand and extracting the col1ector from the sediment, removing a grab sample of the sediment. This method works well with fine-grained sediments in shal10w water and deeper depositional areas. This type of substrate was not present at al1 sites; therefore, we col1ected composite sediment samples with a stainless steel spoon at sites where the sediment was too coarse-grained or too shallow to sample with the coring device. In addition to the six sites where we collected all possible samples, we col1ected sediment samples from the main outflow of the Five Springs system. Sample sites extended to the point where flows had almost completely evaporated or infiltrated. Additional1y, in 1996, we col1ected two samples of stained soil from a point near an abandoned power line and the center outflow of Five Springs (Site 2) and submitted them for organochlorine scans.

We collected red-rim melania snails by hand and placed them in 200 ml glass jars. We attempted to select the larger snails at each site to ensure adequate sample size. We also attempted to shuck the collected snails as the weight of the shells would have biased the analytical results. However, the shells of *M. tuberculata* were thicker than anticipated and attempts to shuck some snails resulted in complete destruction of the inner tissue; therefore these individuals were discarded. We sent the remaining snail samples to the analytical laboratory with shells intact.

We collected crayfish using baited minnow traps. We placed a small amount of canned cat food (Smith's Brand Fisherman's Platter) in a small Whirl-Pak bag, sealed the bag, punched several small holes into it with a knife, and placed the bag in the minnow trap. We placed the minnow trap in the stream or pool to be sampled for between 4 and 12 hours, or until a sufficient sample was collected. We removed the crayfish from the traps, and placed them in 200 ml glass jars. We submitted all crayfish samples to the analytical laboratory with exoskeletons intact.

We obtained a scientific collection permit from Region 1 of the FWS to ensure that the collection of Ash Meadows Amargosa pupfish would not represent a significant impact to this endangered species. As a condition of this collection permit, the population of pupfish in the proposed sampling areas was estimated and the study was limited to collection of no more than 10 percent of the estimated population. The population was estimated by visually counting the fish and was conducted by FWS personnel from Ash Meadows NWR. Based on the results of this population estimate, the requested sample sizes could easily be collected without posing an undue impact to the survival of the species (Cynthia T. Martinez, FWS, pers. comm.).

For Ash Meadows Amargosa pupfish, the requested sample weight required the collection of larger adult individuals, including pre-spawn females ripe with eggs. We collected pupfish with baited minnow traps using methods similar to those used to collect crayfish. At the sites located in pools, individual fish were captured with a dip net, as it proved to be a faster method, until the fish were driven to hiding cover. We collected at least 10 individuals comprising a minimum of 25 grams (g) for each sample. We placed the samples in 250 ml glass jars. Ash Meadows Amargosa pupfish were not present at three sites (Sites 2, 3 and 4). We collected mosquitofish *(Gambusia affinis)* as an alternate species at Site 4. No fish were present at Sites 2 and 3 and no sample was collected. In addition, we collected five individual pupfish each from Sites 1 and 5 , stored them in Davidson's fixative, and submitted them to the California-Nevada Fish Health Laboratory for histological analysis. Histology is used to determine health of the animal and effects related to exposure to environmental contaminants (McCarthy and Shugart 1990).

In order to ensure that the bait used in the minnow traps used for crayfish and pupfish capture did not affect the results of the residue analysis, we collected one bait sample at random from the four cans of catfood used during this study and submitted it for organochlorine scan residue analysis.

We documented sample collection activities on 35 millimeter slide film. We logged each individual sample onto a data sheet which recorded information on sample time and date,

environmental conditions, sample matrix, and individual sample numbers. We kept a notebook which documented miscellaneous information about the site, including a hand-drawn map of the Five Springs area.

Results

PCBs or other organochlorine compounds were not detected in water from any site sampled during this study. Detection limits reported by the analytical laboratory (0.1 μg/L) were less than the lowest PCB concentration reported by USGS (0.2 μg/L; 1990). One water sample container from Site 4 broke during shipment resulting in only 500 ml being analyzed. This caused the reported detection limit to double to 0.2 μg/L for this sample, which was still sufficient to detect the minimum concentration previously reported.

PCBs or other organochlorine compounds were not detected in sediment from any site sampled during this study. Reported dry weight detection limits ranged from 0.068 to 0.147 mglkg. All soil and sediment samples were subjected to particle size analysis, which determines the percentage composition of sands, silts, and clays. Grain size analysis for all soil and sediment samples is presented in Table AI.

Total PCBs (0.37 mglkg dry weight) were detected in the sample of red-rim melania snails collected from Site 2, near where USGS collected water samples in the earlier monitoring program. PCBs were not detected in any other samples collected at or downgradient of this site (Table 2).

PCBs or other organochlorine compounds were not detected in any crayfish samples. Reported dry weight detection limits ranged from 0.222 to 0.303 mg/kg.

Table 2. Detection of total PCBs in *Melania* snails from Ash Meadows NWR, 1997.

Site	ppm Dry Weight		ppm Wet Weight	
Number	Result	Detection Limit	Result	Detection Limit
	< 0.11	0.11	<0.05	0.05
2	0.37	0.08	0.24	0.05
4	<0.08	0.08	<0.05	0.05
5	<0.09	0.09	<0.05	0.05
6	<0.08	0.08	<0.05	0.05

• Note: < means Below Detection Limit.

PCBs or other organochlorines were not detected in pupfish (or mosquitofish; Site 4) from any site sampled. Reported dry weight detection limits ranged from 0.250 to 0.278 mglkg.

A sample of the bait used to capture pupfish and crayfish contained *p,p'*-DDE at 0.65 mglkg on a dry weight basis; no other organochlorines were detected. DDE was not detected in any sample captured using the baited minnow traps even though the detection limits for all other samples were generally an order of magnitude less than the reported concentration in the bait sample. The placement of the bait in plastic bags in such a manner that the fish and crayfish could only smell it and not consume it may have resulted in the lack of DDE in any other sample. Also, we set traps for only enough time to collect a sample and removed organisms immediately, which prevented organisms from consuming the bait.

Table 3: Detection of *p,p'*-DDE in a sample of the bait used to capture fish and crayfish at Ash Meadows NWR, 1997.

Site	ppm Dry Weight		ppm Wet Weight	
Number	Result	Detection Limit	Result	Detection Limit
7	0.65	0.125	0.052	0.0\

Histologic examination revealed a few minor abnormalities in the six pupfish examined, but did not reveal any obvious health problems. Lipofuscin pigment, which is a breakdown pigment produced by oxidative cellular damage, was observed in the heart, liver, pancreatic and kidney tissue of all fish examined. It was unclear whether this is a sign of an environmental stressor or a normal aging characteristic. Other abnormalities, such as shrinkage of the kidneys and epithelial separation of secondary gi1llamellae, could be attributed to problems with the fixation method used. These particular tissues may require a different fixation technique. One fish had pronounced vacuoles in the optic lobe (mesencephalon) of the brain. No indications of inflammation or parasites were observed in any of the fish (J. Scott Foott, FWS, written communication). All slides, tissue blocks, and remaining tissues were returned to the FWS-Reno Field Office for educational purposes.

Discussion

We were unable to definitively the presence of PCBs in the Five Springs system of Ash Meadows NWR, including a source or sink, through the analysis of various environmental matrices collected in 1996 and 1997. The only detection of total PCBs was from a red-rim melania snail sample at Site 2 (Table 2). Although this result appears to be valid, as it is approximately five times the reported detection limit for that sample, the lack of confirmation by any other sample collected at or downstream of this site makes interpretation of this result difficult. For example, Eisler (1986) reported bioconcentration factors for a specific type of

PCB, Aroclor 1254, which is a commercial mixture of PCBs, in crayfish of 5,IOO after a 21 day exposure to a water concentration of 1.2 $\mu g/L$, and in pinfish *(Lagodon rhomboides)* of 2I,800 after a 35 day exposure to a water concentration of 5.0 $\mu g/L$. The crayfish and pupfish at Site 5 are exposed to the same water as the snails at Site 2 as the two sites are separated by approximately 10 m of stream channel. If there were a continuous presence of PCBs at the concentration reported by USGS in 1992 (1.1 $\mu g/L$) in this area, the expected PCB concentration in crayfish at Site 5 would be approximately 5.6 mglkg on a wet weight basis. The expected PCB concentration in pupfish would be approximately 23.9 mglkg on a wet weight basis. No PCBs were detected in crayfish or pupfish at Site 5, with wet weight detection limits of 0.05 mglkg for both matrices. Therefore, if PCBs were still present in the system as in earlier sampling efforts, they should have been detected by our study.

Most biological samples were present at the six sites intensively sampled. The lack of pupfish at Sites 2 and 3 can be explained by physical barriers precluding fish access to those sites. The lack of red-rim melania snails at Site 3 is not quite as clear, although it, too, may be related to the same physical barrier which precludes pupfish. The lack of pupfish at Site 4 is probably related to poor habitat characteristics at this site.

Summary and Recommendations

PCBs and other organochlorine compounds were detected in only two logically unrelated samples collected from the Five Springs area. PCBs were detected in a red-rim melania snail sample from Site 2 (near the point of the original PCB detections), and *p,p'*-DOE was detected in a sample of the bait used in the minnow traps to capture fish and crayfish. The number, frequency, and magnitude of the detections do not confirm the presence of PCBs, as reported by USGS, or other organochlorine compound contamination. No obvious health problems were observed in Ash Meadows Amargosa fish samples submitted for histologic examination.

The results of this study do not suggest the need for remedial actions to address PCB or other organochlorine contamination. It is possible that PCBs may have been present at this site, and others, possibly originating from the release of insulating oils from deteriorating submersible pumps or other electrical equipment placed in well casings (see the description for Site 2 in Appendix A.). We recommend that the possible presence of electrical equipment be considered should any effort be made to remove abandoned well casings such as those in the Five Springs area. Drilling or well-maintenance records may indicate if any sort of electrical equipment was placed within any well. Disturbance of such equipment with a deteriorated housing could result in the release of insulating oils which may contain PCBs. Additionally, care should be exercised when removing other abandoned electrical equipment that may contain PCBs.

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APPENDIX A: Site Descriptions

Site I: Longstreet Spring.
This was sampled as the reference site. The spring emerges in the center of a large (approximately 30 x 40 m) pool and the outflow stream flows south Peterson Reservoir. Spring discharge is 0.08 cms (1500 gallons per minute) and 28°C year round. The site provides habitat for 400 to 600 Ash Meadows Amargosa pupfish along with introduced species (mosquitofish, sailfin mollies *[Poecilia latipinna]*, and crayfish). Sediments collected from the pool contained the highest percentage of sands of all sites sampled, with a low total organic carbon content. See Table **Al** for a description of sediment composition.

Five Springs.
Five Springs is located approximately 1.2 km (3/4 mile) east of Longstreet Spring. Five primary outflow points, some comprised of numerous smaller outflows, converge and flow west for a total distance of approximately I km. Wells have been installed at some outflows to enhance flow rates. Some well casings remain in place. Overhead power lines crossed Five Springs, but were removed in the 1970s and 1980s. The site was formerly inhabited by humans, but no dwelling remains today. Trash piles are present along the access road and the major outflow stream.

Site 2: Central outflow, Five Springs.
We believe that this site was sampled by USGS in their monitoring program which reported PCB detections in 1990, 1992, and 1994. A USGS surveyor's monument is located approximately 20 m north of this spring, near the entrance road. This spring emerges from a rock outcropping and has a well casing still in place. This site is surrounded by very little vegetation, mostly small alkaline grasses. We oberved two electrical wires inside the well casing which lead to an unknown point below the water surface. We suspect these wires are attached to a submersible pump or other sort of electrical equipment which may have been installed in the well, and may still be in place. The outflow of this spring collects in a small pool (approximately I m^2) and flows through a small pipe which spills approximately 0.5 m into the outflow channel. The outflow charmel is approximately I m wide, and flows towards the confluence upstream of Site 5. Very little loose sediment is present at this site. The bed of the outflow charmel consists of a relatively solid carbonate rock layer. We found one small sediment deposition area near where the outflow pipe spills into the outflow stream and collected the sediment sample here with a stainless steel spoon. The sediment sample was moderately coarse with a low total organic carbon content. Snails collected here were relatively small. Crayfish and pupfish were not present at this site. See Table **Al** for a description of sediment composition.

Site 3: Southern Outflow, Five Springs.
This site consists of a relatively low flow charmel fed by a spring and existing well casing. The outflow charmel is thickly covered over with terrestrial grasses which must be pulled away to actually see the stream. The well casing has several small holes which allow water to spill into the outflow charmel. Some flow originates up-gradient of the well casing, but we did not

determine its exact location. The channel at Site 3 is approximately 1 m wide. Most of the water flowing in this channel originates from the well casing. Flows from this spring combine with flows from Site 4 upstream of the pool sampled as Site 6. Sediments at Site 3 were relatively coarse with a low total organic carbon content. A sufficient number of live snails to comprise an adequate sample size was not present at this site, although we observed a large number of snail shells in the flow channel. Pupfish were not present at this site. See Table A1 for a description of sediment composition.

Site 4: Cattail pool, Five Springs.
This site consists of a relatively low flow spring which is immediately collected into a pool (15 m wide by 40 m long) overgrown with cattails. The preliminary site investigation indicated that this pool was connected to the outflow of Site 2; however, after closer observation we found that the Site 2 outflow only passed close to the Site 4 outflow, but was not physically connected. The outflow from Site 4 combines with the outflow from Site 3 to feed the large pool sampled as Site 6. Pupfish were not present at this site and mosquitofish were collected instead. All other sample types were present at Site 4. Sediments were much finer than those at other sites, with a relatively higher total organic carbon concentration, typical of a cattail marsh in a low flow, Mojave Desert enviromnent. See Table A1 for a description of sediment composition.

Site 5: Confluence of Site 2 outflow with outflow from Northern spring.
This site was located immediately downstream of the confluence of the Site 2 outflow with the flow originating from the northern spring, and represents all waters originating in the northern half of the study area. This spring is located approximately 20 m east of the Site 2 spring, and divides to flow both to the large cattail marsh northeast of the sampling area and to the west towards the sampling area. The channel at Site 5 is similar to the channel at Site 3, being somewhat overgrown by terrestrial vegetation; however, the channel here is visible without the need to comb away the overgrown grasses. All sample types were present at Site 5, however the channel was not deep enough to allow sampling of pupfish by minnow trap. The pupfish sample for Site 5 was collected by chasing the fish into the small pool at Site 8 and capturing them using a dip net. Sediment samples were similar to other flow channel sediments with low total organic carbon content. Snails sampled here were relatively larger than at other sites and a large number of snail shells were present in the stream channel, being the dominant substrate in places. Pupfish were collected from a small pool near the outflow of the northern spring. See Table A1 for a description of sediment composition.

Site 6: Large, open pool, Five Springs.
This site is a large (15 x 20 m) open pool located downstream of the confluence of the outflow channel of Site 3 and the outflow channels of Sites 2 and The confluence of the inflow channel and the pool has a significant drop of approximately m, which would make an effective barrier to pupfish movement upstream, and may explain the absence of pupfish at Site 3. This pool appears to be manmade, as it has a very regular shoreline with nearly right-angle comers and almost vertical sides with a uniform, I m depth. Dense terrestrial grasses surround the pool and overgrow the edges to form deep "undercut" bank habitats. All sample types were

present at this site. This site is most similar to the reference site at Longstreet Spring, except for differences in size and total flow. Sediments at this site, however, more closely resembled those from Site 4, both in grain size ratios and in total organic carbon content. Snails were larger here than at other sites and easily collected by hand. Crayfish and pupfish were numerous and quickly collected with baited minnow traps. The outflow of Site 6 combines with the flows from a small spring head in the southwest portion of the study area (Site 10) to form the main outflow channel. See Table A1 for a description of sediment composition.

Site 7: Bait sample.
The sample representing Site 7 is a small sample of the bait used in the minnow traps for this study. This sample was selected at random from one of four cans used to collect pupfish and crayfish at all sites.

Site 8: Outflow from northern spring, Five Springs.
The sediment sample from Site 8 was collected in the small pool where the pupfish from Site 5 were collected. Sediments here resembled channel sediments rather than pool sediments. See Table A1 for a description of sediment composition.

Sites 9: Outflow channel flowing towards northern, large cattail marsh, Five Springs.
The sediment sample collected at Site 9 was from an outflow channel with a similar source as Site 8. The multiple spring orifices at this site are divided by local topography to flow in almost opposite directions. Sediments at this site are intermediate between typical channel and pool sediments. See Table A1 for a description of sediment composition.

Site 10: Southwestern spring head, Five Springs.
This site is located in the southwestern extent of the sampling area and comprises approximately four spring orifices. The channel is "S"-shaped, first flowing east, then west, then north to the confluence with the outflow from Site 6. The upstream end of this channel is near a small (3 m) rock outcropping and could be considered deeply incised. The small spring head and associated channel are heavily overgrown with cattails and it was difficult to determine exactly where the channel was located, especially towards its downstream end. The sediment sample was collected in an area heavily doininated by cattails upstream of the confluence with the Site 6 outflow, and was similar to sediment samples collected from other areas dominated by cattail growth. See Table A1 for a description of sediment composition.

Site 11: Below confluence of sites 6 and 10, Five Springs.
This site represents water originating from the southern half of the study area. Terrestrial vegetation at this site is dominated by heavy brush. Cattails dominate the aquatic vegetation. Sediments collected here resembled other sediments collected in cattail dominated habitats. See Table A1 for a description of sediment composition.

Site 12: Below confluence of Site 11 (Sites 3, 4, 6 and 10) and Site 5 (Sites 2 and 8).
site is immediately downstream of the primary confluence of all outflows of the Five Springs system. Brush and trees dominate the terrestrial vegetation, and cattails dominate the aquatic vegetation. Sediments collected here resembled other sediments collected in cattail dominated habitats. See Table A1 for a description of sediment composition.

Site 13: Main outflow system, Five Springs.
This site is towards the downstream extent of flowing water originating from Five Springs. Terrestrial and aquatic vegetation are similar to those at Site 12, but the stream channel is much less stable and flows have begun to fan out and resemble a depositional zone. Evaporation and infiltration, along with the widening stream channel, determine habitats, biological communities, and flow patterns. Sediments here resemble those collected at Sites 11 and 12. See Table A1 for a description of sediment composition.

Site 15: Location of power pole near Site 2, Five Springs.
Soils collected at Site 15 were from the general vicinity of those soil samples collected in 1996. This sample was collected near the base of an abandoned utility pole. Soils in this area are extremely alkaline, with an encrustation of alkaline salts approximately 2 cm thick. We collected the soil sample here by scraping away the overlying alkaline encrustation, and sampling the underlying soil. See Table A1 for a description of soil composition.

Table AI. Composition of sediment and soil samples collected at Ash Meadows NWR, 1997.

Site Number	Matrix	% TOC'	% Sand	% Silt	% Clay
1	Sediment	'0.44	75.0	10.7	14.3
2	Sediment	0.92	52.1	42.6	5.3
3	Sediment	0.55	74.4	16.2	9.4
4	Sediment	1.1	25.0	55.0	20.0
5	Sediment	0.27	75.3	15.0	9.7
6	Sediment	1.0	39.8	46.2	14.0
8	Sediment	0.68	86.0	9.8	4.2
9	Sediment	0.82	62.7	30.9	6.4
10	Sediment	2.3	42.1	50.8	7.1
11	Sediment	0.83	56.5	35.2	8.3
12	Sediment	0.78	58.8	28.9	12.3
13	Sediment	1.1	55.1	37.8	7.1
15	Soil	0.91	69.7	22.1	8.2

"TOC = Total Organic Carbon.

APPENDIX B: Response to Peer Review Comments:

B1: Response to Comments from Jeremy Buck, USFWS, Oregon State Office, Portland, Oregon

Note, all comments were written by hand in the margins of the draft document. I have attempted to accurately interpret them here.

Comment 1: Page 2, paragraph 2.
Question regrading the common name of *Centaurium namophilum.*

Response:
Confirm that it is the "spring-loving centaury plant".

Comment 2: Page 4, paragraph 1.
Asks for clarification of activities at the Nevada Test Site that could lead to contamination of groundwater.

Response:
The text was expanded and clarified to include a partial list of activities at the Nevada Test Site which could have resulted in groundwater contamination. It should be noted that there were, and continue to be, activities at the Test Site that remain classified, and this report will not attempt to divulge any such information. Activities at the Nevada Test Site include both atmospheric and subterranean tests of nuclear devices and other tests involving radioactive materials, controlled atmospheric releases of numerous gaseous materials, and disposal of various types of solid and liquid wastes.

Comment 3: Page 4, paragraph 2.
Question for clarification of text regarding period of habitation of the site.

Response:
The reviewers question is correct, it was intended for the text to indicate the period of HUMAN habitation was unknown. The text was clarified as requested.

Comment 4: Page 6, paragraph 2.
A question of the methods used to sample water. Why were two 500 ml jars used for each sample rather than one 1000 ml jar.

Response:
It is agreed that by using multiple sample containers, we increased the possibility of contaminating our samples. However, 1000 ml jars would not fit into our sample shipping container, and the 1000 ml containers were much more than incrementally more expensive than the 500 ml jars. Although it is not a great justification, in the end, sample contamination was not an issue.

Comment 5: Page 7, paragraph 2.
Question concerns whether the sediment samples were composites or simple grabs.

Response:
We attempted to collect simple grab samples using the sediment coring device. However, this device was not useable at all sample sites, in which case, collection of composite samples resulted. The text was clarified.

Comment 6: Page 7, paragraph 3.
Question regarding what type of snail samples were shipped to the lab.

Response:
The text was clarified to indicate that only un-shucked snails were analyzed. We attempted to shuck the snails, but it quickly became obvious that this was not going to work, so only two or three snails were shucked. These were removed from the sample over concerns of sample contamination. The remainder of the unshucked snails were delivered to the laboratory.

Comment 7: Page 8, paragraph 5.
Question regarding evaluation of sediment grain size, and an assumption that fines were sampled.

Response:
The text was clarified to indicate that sediment grain size was analyzed, and reported in Table A1. In addition, the text was clarified to more accurately describe the sites, and indicate that "fines" were not always present. of the outflow channels had calcium carbonate accumulations so thick that they resembled concrete-lined stream channelization projects; at these sites fines are not present.

Comment 8: Page 8, paragraph 6.
Question analytical results for melania snails from sites below Site 2.

Response:
The text was clarified to indicate that PCBs were detected in melania snails only at Site 2. FUrther, the text was clarified to indicate that melania snails were only sampled at Sites 1, 2, **4,** 5, and 6.

Coniment 9: Page 9, paragraph 1.
Suggested edits to text.

Response:
Editorial suggestions were incorporated.

Comment 10: Page 10, paragraph 2.
Suggested edits to text.

Response:
Editorial suggestions were incorporated.

Comment 11: Page 10, paragraph 4.
Question to clarify relation of samples.

Response:
The text was clarified to indicate that the two samples that had detections were logically unrelated. One detection was of PCBs in snails, and the other was of p,p'-DDE in bait (sample collected from can). It would be improper to link or relate these detections with each other in any way to indicate a source-sink, fate-transport, or other type of pathway. If, however, we had detected PCBs in water at one site, and in sediments at a site immediately downstream of the first site, one could draw a logical connection between the two sites. The purpose of the statement in question in the CONCLUSIONS section of this document was to indicate that no logical conclusions could be made based on measured contaminant concentrations.

Comment 12: Page 11, References
Suggested edit for La Camera reference.

Response:
The reference was corrected.

Comment 13: Page 14, paragraph 2.
Question: Why does the bait represent Site 7? It was just analyzed at Site 7 as a QA/QC sample - correct?

Response:
To evaluate the results of this study, we assembled a database that could logically relate the results of each analysis conducted on the various sample matrices from the various sites. That is, we could query the database and view the results for Total PCBs from all sample types at Site 5, or the PCB results from sediments at all etc. To do this, we had to give the bait sample a unique sample site number to indicate that this was a separate sample which was not logically related to anyone site. The results for this sample could be included in the database queries described above if desired. There is no physical Site 7, it exists as nomenclature We analyzed this sample to ensure that any detections of analytes in samples collected with the bait did not originate from the bait, but originated from the environment. In this way, it is a Quality Assurance sample.

Appendix B2: Response to Comments Provided by Tom O'Brien and John Wolfe, USFWS, Region I, Portland, Oregon

Comment 1: Page 3, Figure 1.
Locate Five Springs on the map of AMNWR.

Response:
The Figure was changed.

Comment 2: Page 4, Figure 2.
Orient map so that North arrow is at top of page and change all corresponding Legend information to reflect the new orientation.

Response:
The Figure was changed.

Comment 3: Page 4, paragraph 2.
To simplify the text use a Table which summarizes the previous 1990, 1992, and 1994 PCB reports by US Geological Survey's study for US Department of Energy. We recommend using a foot note to indicate State and Federal water quality criteria for PCBs. The action level or threshold criteria for the State of Nevada to implement a remedial action could also be added.

Response:
The authors believe that replacing the existing text with a simple table summarizing the USGS data would not benefit the final report. The reviewer should note that State and Federal water quality standards are bits of information in this report as PCBs were not detected in water, and therefore, no comparisons to water quality standards can be made.

Comment 4: Page 7, paragraph 6.
The June 27, 1996 proposal specified that unbaited traps will be used to collect crayfish and fish and compute a catch per unit effort (CPUE) as part of the assessment of each sampling site. In the text, specify a for using baited minnow traps to collect native fish and crayfish and the rationale for not computing a CPUE.

Response:
Subsequent to the final proposal and prior to conducting field work, we were advised by biologists who were familiar with the study site to use baited traps to improve our sampling success, especially for crayfish. The baited minnow trap proved to be a successful method for capturing pupfish and mosquitofish, which were captured concurrently with the crayfish. We added a sample of bait (canned catfood) to be analyzed to ensure that the use of bait did not add to or create an indication of contamination.

Calculation of CPUE was included in the proposal as a requirement of our endangered species collecting permit. In order to ensure that conducted by this study did not severely impact this species, we were required to develop a pupfish population estimate at each site and were limited to collecting no more than 10% of the pupfish present at each site. This task was ultimately conducted by Refuge staff prior to our sampling efforts. As this requirement of our collecting permit had already been conducted, we did not collect the data needed to calculate CPUE, and only sampled long enough to collect a sufficient sample to avoid handling any more pupfish than necessary.

We do not believe that inclusion of this text will improve the final report.

Comment 5: Page 8, paragraph 2.
The report indicates that sample collecting was documented with 35 millimeter slides. It would be helpful to convert relevant slides into prints and use them as figures in the report.

Response:
Although the authors agree with the intent of this comment, there are two reasons that this comment will not be incorporated. First, the photographs taken of the sampling effort are not as "enlightening" or "telling" as the reviewers believe them to be. Second, the authors do not have the technical capabilities at hand to convert the slides to digital images so that they may be incorporated into the text.

Comment 6: Page 8, paragraph 5.
The proposal for this investigation specified that grain size and TOC would be analyzed for each sediment sample. A table in the appendix (Table AI) summarizes the results. It would be helpful to provide a few statements in'the text and refer the reader to Table AI.

Response:
A discussion of grain size and TOC was included in the text in an earlier version of the report. Because of the variation in grain size, especially the Sand and Silt categories, more than just "a few statements" are required to characterize the 15 sites from which sediment or soil was collected. Based on comments received to this early draft, Appendix A was added to the presentation of these results. A reference will be added at this point directing the reader to Appendix A.

Comment 7: Page 9, paragraph 2.
During a peer review of the original proposal for this investigation, Ted Schwartz commented the high likelihood of not detecting any histological effects with a very limited sample. The authors should include these comments before the summary of histological observations made by J. Scott Foott in order to evaluate probable health effects.

Response: .
The authors disagree that inclusion of such text would benefit the final report. Histological evaluation of tissues, along with chemical residue analysis, was included in this study to detennine not only if PCBs were present in the system, but if these contaminants were also impacting the health of a protected species. As there is very little information in the scientific literature on the implications of chemical contamination to pupfish health, the authors attempted to gather as much site-specific data as possible, including a basic health evaluation, to avoid a reliance on non-existent information. Results of histological examination alongside more standard chemical residue analysis, both of which indicated an apparent lack of PCB contamination and a population of apparently healthy fish at the site, should be viewed on their own merits, and do not need to be "minimized" or "qualified" with pre-project speculation.

Comment 8: Page 10, paragraph I (partl).
Since the analyses of tissues from AMNWR did not provide values for specific congeners, the authors need to be careful in citing Eisler. For example, pinfish is a marine and estuarine species of the Family Sparidae (porgies) with many taxonomic, evolutionary and physiological differences from pupfish and mosquitofish in the Family Cyprinodontidae (killifish) collected at AMNWR

Response (part I):
The text was clarified to clearly indicate where the authors are making a comparison between total PCBs reported by this study and individual congeners reported in the literature to evaluate the data. However, the reviewers should note that:

I) There were no reportable concentrations of PCBs, either total or congener-specific, in any of the fish analyzed. Regardless of whether the scientific literature is based on total PCBs or individual congeners, no detailed comparisons can be made. This information was presented to show the lower range of concentrations at which PCBs have been reported to elicit health impacts, and that our results were below that lower range, and:

2) to the authors knowledge, Ash Meadows Amargosa pupfish have previously never been used as a test subject in a published toxicological study. Given the fact that pupfish are the sole representatives of the family Cyprinodontidae (killifish are in Fundulidae, mosquito are in Poeciliidae), any comparison to previous studies in the scientific literature would necessarily result in a comparison of species that have" many taxonomic, evolutionary and physiological differences".

Comment 8 (continued):
In your results section, some clear language about the hazards associated with the presence of polychlorinated biphenyls (PCBs) should be included. With the extensive base of literature about PCBs the authors could make some comparisons to published papers.

Response (part 2):
A brief discussion of the of PCBs to aquatic biota has been added to the text, but it was added near the end of the Introduction section. The Results section is for presentation of the findings of this particular study, not a discussion of other efforts. However, regardless of the volume of scientific literature available, very little comparison can be made because PCBs were detected in 1 of 35 (2.8%) samples. See the first response to comment 8 above.

Comment 9: Page 10, paragraph 5.
The authors recommendation to determine if submersible pump and electrical equipment remain in any wells which might contain insulating oils, may prevent future releases of PCBs. The authors should highlight this proposed action item to ensure it is incorporated into the maintenance plan for AMNWR. If any removal actions are implemented, they should be coordinated with Enviromnental Contaminants staff. A recommendation could be added to periodically monitor AMNWR by retesting Site 2 and an additional downstream site, using water, sediment, and biota samples for analysis.

Response:
As the reviewer has noted, the authors have included text recommending caution should any efforts be made to remove well casings which may contain electrical eqnipment, or any other electrical equipment, from the refuge. The remaining portions of this comment are, in the author's view, beyond the scope of a contaminants study report. The authors do not believe that this report has the authority to force a management action into the refuge's Comprehensive Conservation Plan. The author's believe that a forum already exists to ensure coordination between the Refuge and Enviromnental Contaminants staff for any contaminant removal actions on a refuge. And, this report cannot guarantee the availability of funds to support a long-term contaminant monitoring program (Note: each iteration of the recommended sampling scheme would cost between $1,200 and $2,120, based on FY 1999 prices).