Polycyclic Aromatic Hydrocarbon Investigation for the San Juan River, San Juan County, New Mexico 1993

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Table 1. Abbreviations and Conversions

Abbreviations

liter									-																-		I
milliliter					-				-																		ml
kilogram						-	-		-																		kg
gram.									-																		g
parts per	r n	nill	lic	n																							ppm
parts per	' b	illi	io	n						•																-	ppb
parts per	r ti	rill	io	n																							ppt
milligran	n p	ber	r k	il	20	gr	а	m	۱																		mg/kg
microgra	arr	าร	pe	ər	g	ra	an	n																			mcg/g
microgra	am	าร	pe	ər	ñ	۱il	li	li	te	۶r	•												-	-		-	mcg/ml
microgra	am	າຮ່	pe	ər	li	te	er																		,		mcg/l
nanogra	m	s p	je	r I	it	eı	r				-	-										-				-	ng/l

Conversion Factors

micrograms per gram	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	-	•	•	•	•	•	•	•	ppm
micrograms per milliliter		•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	ppm
micrograms per liter	:	:	:	:	:		:	:	:			:	:	:	:	:	:	:	:	:	:	2	:	:	:	:	daa
nanograms per liter	•			•	•	•	•	•	•	•	-	•	•	•	•	•	•	•	•	•		•	•	•	•	•	ppt

Conversions

Wet weight = (dry weight)(1- (percent moisture/100))

Dry weight = (wet weight)/(1 - (percent moisture/I 00))

Executive Summary

In October of 1992, Environmental Contaminants Program personnel from the U.S. Fish and Wildlife Service's New Mexico Ecological Services State Office assayed bile collected from San Juan River flannelmouth suckers for polycyclic aromatic hydrocarbon (PAH) metabolites. The objective of this study was to determine if San Juan fish, including endangered fish (i.e., Colorado squawfish), are being exposed to PAH contamination. Thirty-two bile samples from twenty-four flannelmouth suckers were collected along five separate reaches of the San Juan River between river mile markers 80 and 156.

This report presents data showing the presence of three PAH compounds, naphthalene, phenanthrene and benzo[a]pyrene(BaP), and the findings of a literature search concerning the possible deleterious effects of these compounds on fish in the San Juan River.

All of the fish samples analyzed contained levels of naphthalene and phenanthrene and eleven of the twenty-four samples contained levels of BaP. Variations in fish species, life histories, habitat preferences, feeding habits, and physiology limit the conclusions that can be drawn from noting the observed levels of PAHs. Also, the PAH aquatic biotoxicity database is probably too limited at this point, especially for flannelmouth suckers, to use the data presented in this report to forecast ecological risks to San Juan River fish communities. However, due to the high carcinogenicity of this group of compounds, the fact that there is evidence of widespread contamination in one fish species warrants concern for the health of the entire San Juan River aquatic ecosystem.

Introduction

Polycyclic aromatic hydrocarbons (PAH) are composed of hydrogen and carbon atoms arranged as two or more benzene rings (Eisler 1987). Basic structures of naphthalene, phenanthrene and benzo[a]pyrene are shown below in Figure 1. As the molecular weight of these compounds increases, solubility in lipids increases and resistance to oxidation and reduction decreases. Therefore, PAHs will vary in their behavior in the environment and in their biological effects. Low molecular weight PAHs such as naphthalene and phenanthrene are highly mobile in the aquatic environment and present significant acute toxicity to many organisms. Also, many higher molecular weight PAHs such as benzo[a]pyrene have been shown to be highly carcinogenic (Eisler 1987).





Naphthalene $C_{10}H_8$; mol. wt. 128.16

Phenanthrene $C_{14}H_{10}$; mol. wt. 178.22



Benzo[a]pyrene C₂₀H₁₂; mol. wt. 252.3

Figure 1. Chemical structures and molecular weights of naphthalene, phenanthrene and benzo[a]pyrene (adapted from Eisler 1987).

PAHs are ubiquitous in the environment, produced in small quantities through terrestrial vegetation and microbial synthesis and volcanic activity and on a larger scale by forest and prairie fires as well as man's anthropogenic processes (Eisler 1987). Anthropogenic activities which distribute PAH contaminants throughout the San Juan River basin include petroleum production and refining, coal mining, fossil fuel power generation, disposal of municipal and domestic sewage, and agricultural irrigation projects. Throughout the basin, there are in excess of 20,000 oil and gas wells and several petroleum processing plants, including oil refineries, gas processing plants, and conveyance pipelines. These energy-development facilities can all contribute heavily to the release of fossil fuel related contamination, including PAHs, into the surrounding environment. (Petty et al. 1992) Some PAH compounds have received much attention because of their suspected or confirmed carcinogenic and mutagenic nature (Eisler 1987). Exposure of biota to PAHs can cause deleterious effects at the site of contact and via the circulatory system, throughout the various organs and tissues (Lee and Grant 1981). Laboratory animals exposed to PAHs have exhibited carcinogenic responses and bottom dwelling fish from PAH polluted waterways have produced oral, dermal, and hepatic neoplasms (Pucknat 1981; Couch and Harshbarger 1985). The presence of pollutants such as BaP in fish taken from contaminated environments generally reflects sedimentary PAH concentrations (Johnston and Baumann 1989). Because of the variation among these compounds and individual species response, it is difficult to make generalizations regarding the class of PAH metabolites (Lee and Grant 1981). However, biliary concentrations of specific PAHs can be compared between fish from polluted waterways with those from reference sites (Krahn et al 1984).

The San Juan River basin is important habitat for a wide array of fish and wildlife resources. Major habitat types include aquatic, riparian, grassland, desert shrub, pinon/juniper woodland, ponderosa pine, mixed conifer, and alpine tundra. One hundred

ninety-eight of the three hundred-eleven-bird species in the basin depend in some way on the riparian habitat. Ninety-three of these are directly dependent on this zone for reproduction and survival. There are also ninety-nine species of mammals, thirty-four species of reptiles, fourteen species of amphibians and a variety of aquatic invertebrates permanently resident in the San Juan River basin. Some parts of the river also provide habitat for migrating raptors and other birds including bald eagles, peregrine falcons and whooping cranes. Approximately 500,000 waterfowl winter along the San Juan River and its riparian habitat. (Petty et al. 1992)

There are fifty species of fish found in the San Juan basin, sixteen of which are native. Endangered fish such as the Colorado squawfish and razorback sucker, and roundtail chub, a category 2 candidate species, require backwater habitat and low flow channel areas for spawning and nursery areas.

Fish and wildlife habitat requirements in the Basin are in competition with the more than 50,000 people that use scarce water supplies for agricultural, industrial and domestic purposes. Contamination of this waterway is not only a major concern for the fish and wildlife resources in the basin but also for the human population. All public water utilities that provide water to 2,500 people or more utilize surface waters. Those smaller communities which use groundwater from the shallow alluvial deposit in the basin can have their water supplies contaminated by anthropogenic activity in the area. (Petty et al. 1992)

Eisler (1987) states several factors which influence uptake, retention, and translocation of PAHs by aquatic organisms. Most species of aquatic organisms will rapidly accumulate PAHs from their surrounding environment, but will also metabolize quite rapidly according to species specific metabolizing factors. This suggests that for any given water system, each species will accumulate different quantities of PAHs and any resultant deleterious effects will vary. A study of American lobsters by Sirota and Uthe (1981), showed higher PAH concentration in large lobsters than in small lobsters, implying a correlation in age or body size to PAH accumulation dynamics. Johnston and Baumann (1989) suggest that certain species of fish may serve as better indicators of PAH contamination than others. They also state that species with relatively low biliary concentrations may exhibit a higher frequency of tumors with more metabolites retained as adducts than a species with high biliary concentrations and low tumor frequency. Johnston and Baumann suggest combining the HPLC/florescence method with histopathological and sediment analyses to determine source and extent of exposure to freshwater fish populations.

The ability of many organisms to convert PAH to various metabolites such as phenols and dihydrodiols, leads to problems in assessing exposure of these animals to PAH compounds using analysis of tissue samples. However, using a HPLC/ florescence method to determine concentration of biliary PAH will give an indication of current levels of exposure (McDonald et al. 1990; Johnston and Baumann 1989; Krahn et al. 1987, 1986a, 1984).

Species specific hepatic enzymatic variations determine that some fish species will show relatively high metabolites in bile versus other species. This makes it difficult to select one species as a surrogate for another (i.e. flannelmouth sucker for Colorado squawfish) for estimating PAH-related health threats. Dr. Susan J. McDonald of the Geochemical and Environmental Research Group at Texas A&M University (GERG) has stated that she has observed flannelmouth suckers to exhibit relatively low metabolite levels, while species of catfish seem to be more sensitive to PAH and will show higher metabolite levels (McDonald pers. comm. 1993). Therefore, a species such as black bullhead (*Ictalurus me/as*) or channel catfish (*Ictalurus punctatus*) could be used in further studies analyzing bile for PAH metabolites. However, these species are not found throughout the entire San Juan River basin. On the other hand, the flannelmouth sucker, which exhibits a much lower tendency to produce concentrations of PAH-related bile metabolites consistent with the concentrations present in water and/or sediment, is nearly ubiquitous throughout the Basin. Dr. McDonald has suggested that hepatic enzymatic assays might help normalize

PAH-related bile metabolite data observed in flannelmouth suckers to what is typically found in channel catfish, since these two fish species collected from the same stream reaches, typically exhibit widely variant concentrations of PAH-related bile metabolites (McDonald pers. comm. 1993)

Future studies of the San Juan River basin, in reference to PAH residues, will center on the use of a semipermeable polymeric membrane device (SPMD) containing a thin film of pure lipid (Triolein) for in situ passive concentration and separation of trace aquatic contaminants. The SPMDs make possible a cost-effective method to detect the presence of organic contaminants. In addition, the USFWS's National Fisheries Contaminant Research Center is conducting time trials with SPMDs in order to be able to predict average PAH concentrations in water based upon observed concentrations in SPMDs. Thus, in the future it may be possible to determine water and sediment PAH concentrations using SPMDs.

The analytical method used in the SPMD technology is much less complex than the current HPLC/fluorescence method used to detect PAHs in bile and tissue samples. The membrane bound lipid is easily extracted from the sample site and contaminant residues are dialyzed into an organic solvent. These devices will concentrate organic pollutants, including PAHs which are normally metabolized by aquatic organisms and provide an index of pollution severity including estimates of rates of uptake and exposure. SPMDs concentrate PAHs to theoretical maximum levels whereas species specific differences makes this impossible in live samples. SPMDs can be used in both lotic and lentic systems, groundwater, and processed waters. The data collected from tests using the SPMD technology will not only provide a measure of environmental concentrations of PAHs, but will provide necessary information on water quality in the San Juan River basin useful for estimating contaminant-related risks to endangered fish as well as other aspects of the aquatic ecosystem. (Huckins et al. 1990a, 1990b, 1990c) Research is currently being conducted to determine if the SPMD technology can also be used as a cleanup device (Meadows et al. 1993).

Procedures

Twenty-six fish were collected from five sites on the San Juan River between Farmington, New Mexico, and the Colorado-Utah border (see figure 1) using rafts equipped with gasoline generator-powered electroshocking equipment. The fish samples were dissected using stainless steel instruments, and the bile extracted directly from the gall bladder with sterile needles and evacuated blood specimen tubes. Individual fish were examined in the field for occurrences of external abnormalities. Bile samples were frozen and sent to the Geochemical and Environmental Research Group at Texas A & M University (GERG), an analytical laboratory under contract with the USFWS.

The samples were tested by GERG for the individual PAH metabolites, naphthalene, phenanthrene and benzo[a]pyrene, using high performance liquid chromatography (HPLC) with florescence detection. In this method, bile is injected directly onto an HPLC system and specific florescence is recorded at appropriate wavelengths for each of the three metabolites. Results were calculated in ng/g wet weight and can be found in Table 2 and in raw form in Appendix A.



Figure 1. Sites of October, 1992, flannel mouth sucker collection. San Juan River, San Juan County, New Mexico.

Sampling Area

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Results and Discussion

The initial results for all samples are listed in Table 2 below. Results for duplicate tests (every tenth sample) are given in Appendix A.

Sample Number	Naphthalene	Phenanthrene	Benzo[a]pyrene	Specimen Abnormalities
SJFB0192	35000	8300	<100	No
SJFB0292	29000	7000	<100	No
SJFB0392	38000	9500	100	No
SJFB0492	67000	14000	150	No
SJFB0592	53000	11000	130	Yes
SJFB0692	60000	12000	130	No
SJFB0792	56000	13000	140	No
SJFB0892	60000	12000	140	No
SJFB0992	56000	12000	120	No
SJFB1092	46000	12000	120	No
SJFB1192	72000	15000	200	No
SJFB1292	58000	12000	160	Yes
SJFB1492	35000	7200	<100	No
SJFB1592	27000	5600	<100	No
SJFB1692	50000	11000	160	No
SJFB1792	25000	5000	<100	No
SJFB1892	20000	4100	<100	Yes
SJFB1992	24000	5300	<100	No
SJFB2092	26000	5800	<100	No
SJFB2192	27000	5900	<100	No
SJFB2292	26000	5900	<100	Yes
SJFB2392	34000	7400	<100	No
SJFB2492	30000	5800	<100	No
SJFB2592	22000	4700	<100	No

Table 2. HPLC Analysis Results for Naphthalene, Phenanthrene and Benzo[a]pyrene (ng/g wet weight)¹ and species abnormalities.

¹ Raw data results including duplicate sample results are listed in Appendix A.

Several abnormalities in fish were observed in the field examinations mentioned in the procedures section above. Sample SJFB0592 had a possible healed over lesion. The fish measuring 442 mm in length and weighing 660 g of the composite sample SJFB1292 had some petechial hemorrhaging (leaching of blood to outer dermal area). Sample SJFB1892 had lordosis and a missing eye. Sample SJFB2292 had lernia (ectoparasite infection) near the dorsal fin.

Most species of fish will readily assimilate PAHs from contaminated food, and metabolism and excretion is relatively rapid (Maccubbin et al. 1985; Malins et al. 1985a, 1985b; Neff 1982). Nevertheless, laboratory aquatic ecosystem studies have shown that BaP can be accumulated to potentially hazardous levels in fish and invertebrates (Lu et al. 1977). In a study by Varanasi and Gmur (1981), the formation of reactive intermediates such as diol and phenol epoxides of BaP (implicated in mutagenesis and carcinogenesis of mammals) are the direct result of considerable metabolism of BaP. Bottom-dwelling fish with liver in rainbow trout exposed via diet. BaP can be readily accumulated in the ovary, wolffian ducts, oocytes, gonads and semen of fish and consequently transferred to developing gametes (Hose et al. 1981; Reichert and Varanasi 1982). BaP has also been shown to bind to both liver and gonadal proteins and DNA (Varanasi et al. 1982).

The role of sediments in retention of PAHs in the environment are of some importance to bottom dwelling fish such as the flannelmouth suckers. Johnston and Baumann (1989) reported BaP concentrations in brown bullhead which generally reflected sediment concentrations in the area where fish were collected. Baumann et al. (1982) reported the same PAH metabolites in fish that were in sediments from that site. The amount of harmful PAH metabolites and their distribution in the environment will depend on the chemical properties of specific compounds. However, the presence of numerous facilities for the utilization or processing of petroleum hydrocarbon compounds (i.e coal-fired power production, petroleum refining, automobiles, etc.) can contribute heavily to local or widespread contamination of a riverine environment (AI-Saad and AI-Timari 1989; Johnston and Baumann 1989; Krahn et al. 1986b).

Eisler (1987) states that many PAHs such as naphthalene and phenanthrene are acutely toxic at whole body concentrations above 50,000 ppm and deleterious sublethal responses occur at concentrations in the range of 100 to 5000 ppm. The six-ring aromatic hydrocarbon, BaP, is among the more toxic PAH compounds, causing deleterious effects at whole body concentrations above 100 ppm. Eleven of the twenty-four flannelmouth sucker samples collected for this study had biliary concentrations of BaP in excess of Eisler's recommended lower limit for deleterious effects to aquatic life. Naphthalene and phenanthrene are also found in the aquatic environment, although these PAHs are not known carcinogens or mutagens. The levels of naphthalene, phenanthrene, and BaP in flannelmouth suckers collected for this study are much lower than levels reported in fish from a similar study of Lake Powell in Utah (the eventual destination of San Juan River drainage) which were clearly hazardous from a carcinogenic risk level (Waddell and Weins, 1993). The concentrations of BaP metabolites that Waddell and Weins observed in the San Juan arm of Lake Powell ranged from 110 to 1,500 ng/g wet weight; naphthalene metabolites ranged from 16,000 to 810,000 ng/g and phenanthrene metabolites ranged from 3,900 to 330,000 ng/g. Bruce Waddell, Environmental Contaminant Specialist with the Service's Ecological Services State Office in Salt Lake City, indicated that, according to Texas A&M, the higher of these ranges are clearly hazardous to fish species from a carcinogenic risk level (Waddell, pers. comm., 1994).

Although the elevated PAH levels in flannelmouth suckers cannot be translated into quantifiable health-related risks to fish and other biota within the San Juan River ecosystem, the presence of these potentially toxic substances provides a legitimate basis for initial concern and a more detailed assessment of PAH-related risks to important elements of the San Juan River aquatic ecosystem.

Recommendations

The results from this study should be used in conjunction with findings from other researchers to quantify PAH contamination in the San Juan River along with any resultant ecological risks posed by these hazardous compounds. The presence of PAH metabolites in the bile from these flannelmouth suckers indicates probable long-term exposure to PAH contamination, and this data should support the ongoing work with SPMD technology to assess total PAH contamination in the San Juan basin. Further studies of PAH-related metabolites in fish bile or tissue should also be accompanied by histopathological examinations and correlated with levels of waterborne and sediment loads of PAHs.

Acknowledgements

We would like to thank Dale Ryden of the Grand Junction Fish and Wildlife Colorado Fisheries Project office for providing ultra cold storage of bile samples prior to analysis. Dr. Susan J. McDonald of the Geochemical and Environmental Research Group at Texas A&M University provided valuable assistance regarding the interpretation of the PAH data that were collected. Carol Shanks, formerly with USFWS's Pinetop Fish Health Center (Pinetop, Az.), Mark Wilson and Matt Custer with the USFWS's Ecological Services New Mexico State Office (Albuquerque, NM), collected the bile samples and recorded observations of external abnormalities on the fish utilized for this study.

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Appendix A (page 1 of 2).

HPLC Analysis Results for Naphthalene, Phenanthrene and **Benzo[a]pyrene (ng/g** wet weight) for Bile Samples Collected on the San Juan River for the San Juan Fish Bile Study of October, 1992, San Juan County, New Mexico.

Samp. #	SJFB0192	SJFB0292	SJFB0392	SJFB0492	SJFB0592	SJFB0692
Matrix	Bile	Bile	Bile	Bile	Bile	Bile
Species	FM Sucker	FM Sucker	FM Sucker	FM Sucker	FM Sucker	FM Sucker
No/Comp ¹	1	1	1	1	1	1
Naphthalene Phenauthrene Benzo[a]Pyrene	35000 8300 • < 100	29000 7000 < 100	38000 9500 100	67000 14000 150	53000 11000 130	60000 12000 130
samp. #	SJFB0792	SJFB0892	SJFB0992	SJFB1092	SJFB1192	SJFB1292
Matrix	Bile	Bile	Bile	Bile	Bile	Bile
Species	FM Sucker	FM Sucker	FM Sucker	FM Sucker	FM Sucker	FM Sucker
No/Comp ¹	1	1	1	1	1	2
Naphthalene Phenanthrene Benzo[a]Pyrene	56000 13000 140	60000 12000 140	56000 12000 120	46000 12000 120	72000 15000 200	58000 12000 160

Samp. #	SJFB1492	SJFB1592	SJFB1692	SJFB1792	SJFB1892	SJFB1992
Matrix	Bile	Bile	Bile	Bile	Bile	Bile
Species	FM Sucker	FM Sucker	FM Sucker	FM Sucker	FM Sucker	FM Sucker
No/Comp ¹	2	1	1	1	1	1
Naphthalene Phenanthrene Benzo[a]Pyrene	35000 7200 c < 100	27000 5600 C100	50000 11000 160	25000 5000 C100	20000 4100 <100	24000 5300 C100

¹ No/Comp = number of samples in composite.

Appendix A (page 2 of 2).

HPLC Analysis Results for Naphthalene, Phenanthrene and **Benzo[a]pyrene (ng/g** wet weight) for Bile Samples Collected on the San Juan River for the San Juan Fish Bile Study of October, 1992, San Juan County, New Mexico.

Samp. #	SJFB2092	SJFB2192	SJFB2292	SJFB2392	SJFB2492	SJFB2592
Matrix	Bile	Bile	Bile	Bile	Bile	Bile
Species	FM Sucker					
No/Comp ¹	1	1	1	1	1	1
Naphthalene Phenauthrene Benzo[a]Purene	26000 5800	27000 5900	26000 5900	34000 7400	30000 5800	22000 4700

Samp. #2	SJFB0592D	SJFB1092D	SJFB1792D	SJFB2392D	SJFB0692D	SJFB0792D
Matrix	Bile	Bile	Bile	Bile	Bile	Bile
Species	FM Sucker	FM Sucker	FM Sucker	FM Sucker	PM Sucker	FM Sucker
No/Comp ¹	1	1	1	1	1	2
Naphthalene Phenanthrene	53000 11000	47000 12000	25000 4900	34000 7100		
Benzo[a]Pyrene	140				120	140

Samp. # ²	SJFB1492D	SJFB2492D	
Matrix	Bile	Bile	
Species	FM Sucker	FM Sucker	
No/Comp ¹	2	1	
Naphthalene Phenanthrene Benzo[a]Pyren	 e < 100	<100	

¹No/Comp = number of samples in composite.
² (Sample #)"D" = duplicate sample.