State College Field Office
Resource Contaminant Assessment Report No. 86-1

A Preliminary Survey of Tumors in Brown Bullheads in Presque Isle Bay, Lake Erie Erie, Pennsylvania

Prepared by: Cynthia L. Rice, David J. Putnam, Francis R. Plewa
Project Leader: Charles J. Kulp

EXECUTIVE SUMMARY

In the summer of 1984, the State College Field Office of the U.S. Fish and Wildlife Service (Service) conducted a two-day survey of fish in Presque Isle Bay, Lake Erie, to investigate a reported high incidence of skin tumors in brown bullheads. Less than 50 brown bullheads were examined, but many of these exhibited a wide array of external lesions. Skin and internal organ tissue samples were collected for gross pathological examination, and whole fish samples were saved for chemical analysis. The results verified the presence of benign skin tumors in a few of the sampled brown bullheads. Chemical analysis of the whole fish revealed the presence of a variety of organochlorine pesticides and an unexpectedly high level of PCB's.

While a number of researchers have discovered higher fish tumor rates in industrialized areas compared to relatively unpolluted waters, fish skin tumors cannot be considered absolute proof that chemical carcinogens are present. Fish liver tumors (not observed in the fish we collected) would more strongly indicate the presence of a carcinogenic agent. However, detailed histopathological examinations of the bullhead livers were not conducted during this survey, raising the possibility that liver tumors may have been present and were simply missed during gross examination of the tissues.

The Service conducted a more detailed study of Presque Isle Bay brown bullheads in the spring of 1985. Over 100 brown bullheads were collected and detailed histopathological work is currently underway on skin and internal organ tissues obtained during this effort. When the results of this second survey are available, we should be better able to address questions concerning contaminants and fish health in Presque Isle Bay.

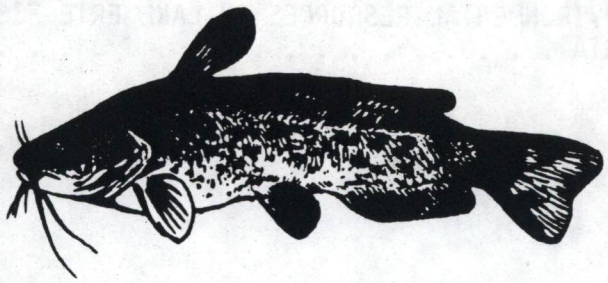


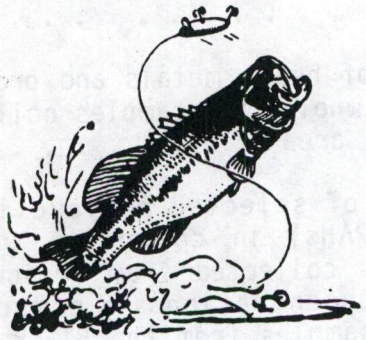
TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGMENTS

We are grateful to the following individuals for their assistance during various stages of this survey: Robert J. Wellington, Aquatic Biologist, Erie County Department of Health, Erie, Pennsylvania; Dr. Paul C. Baumann, Leader, Columbia National Fisheries Research Laboratory Field Research Station, U.S. Fish and Wildlife Service, Columbus, Ohio; Dr. John C. Harshbarger, Director, Registry of Tumors in Lower Animals, Smithsonian Institution, Washington, D.C.; Jim L. Zajicek, Chemist, and Christopher Schmitt, Fisheries Biologist, Columbia National Fisheries Research Laboratory, U.S. Fish and Wildlife Service, Columbia, Missouri; and Arnold Julin, Regional RCA Specialist, U.S. Fish and Wildlife Service, Newton Corner, Massachusetts.



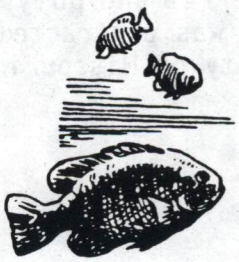
INTRODUCTION

The U.S. Fish and Wildlife Service (Service) has been concerned with environmental contaminants since the late 1940's when researchers began investigating the impacts of synthetic organic pesticides, such as DDT, on fish and wildlife resources. Publication of Rachel Carson's Silent Spring focused nationwide attention on the biological and ecological effects of massive use of persistent chemical compounds.

Over the past several years, the Service has been working to improve its field operations capabilities to address and enhance the quality of fish and wildlife resources impacted by environmental contaminants. Service field offices nationwide have established Resource Contaminant Assessment programs with responsibility for identifying contaminant threats to fish and wildlife and recommending actions to eliminate those threats. The results of the Service's monitoring, field assessment and research initiatives are indicating that a broad spectrum of contaminants are affecting fish and wildlife throughout the United States. Aberrations such as backbone deformation, liver tumors, alteration of enzyme activity and function, and reduced nesting success, are being detected with increasing regularity in research and field studies of natural populations.

The general public in the United States has become increasingly well-educated about chemical contaminant issues and private citizens frequently alert the Service to pollution problems. Such was the case in 1984, when the Service's State College, Pennsylvania, Field Office (SCFO) began receiving periodic reports from citizens in the vicinity of Erie, Pennsylvania, that brown bullheads (Ictalurus nebulosus) caught by fishermen in Presque Isle Bay exhibited skin and lip "tumors." In response to these reports, SCFO staff collected brown bullheads from the Presque Isle Bay area in the summer of 1984. The collection effort was designed as a preliminary survey to verify the persistent rumors and determine whether a more elaborate study of the problem was warranted.

The following report details our methods and the results of the survey.



METHODS

On July 31 and August 1, 1984, SCFO biologists David Putnam, Cindy Rice, and Frank Plewa, assisted by Erie County Department of Health Aquatic Biologist Robert Wellington, collected fish from Presque Isle Bay. An electrofishing boat and trap nets (standard 3-foot hoop frames and D-frame hoop nets; see Figure 1) were used. Collections were made in three areas we designated "Upper Bay," "Inner Harbor" and "Outer Harbor" (see Figure 2). Specific net locations are designated with "X" marks in Figure 2 and electrofishing areas are marked with a black line and the letters "EF". Nets were set on the afternoon of July 31 and checked on the morning of August 1. The electrofishing effort was conducted for three hours during the night of July 31.

Although over 20 species of fish were collected during the 2-day effort, brown bullheads were the primary focus of our study and most of the other fish were returned to the water in good condition. All 46 of the brown bullheads captured were kept, as well as five bluegills (Lepomis macrochirus) and four largemouth bass (Micropterus salmoides). The selected fish specimens were either processed immediately or placed on ice and processed within six hours. Lengths and weights of the fish were recorded. All fish were examined externally and internally for abnormalities (such as lumps or discolored areas on the skin, lumps on the liver, etc.), which were removed and preserved in 10% buffered formalin in whirl-pack bags. Sections of liver and kidneys were collected from most fish whether or not they appeared abnormal. The tissue samples were later mailed to Dr. Paul Baumann, the Service's expert in "fish tumor" research, located in Columbus, Ohio.

A composite sample of five whole brown bullheads from each area was saved for chemical analysis, as were a composite sample of four whole largemouth bass from the Outer Harbor and five whole bluegills from the Upper Bay. Sample numbers, collection locations and individual fish lengths and weights are provided in Table 1. Fish saved for chemical analysis were individually wrapped in aluminum foil, then placed in plastic bags and frozen. The samples were later shipped on dry ice to the Service's Columbia National Fisheries Research Laboratory (CNFRL) in Columbia, Missouri. We requested that all five composite samples be analyzed for organochlorine pesticides and PCBs, and for the following heavy metals: aluminum, arsenic, cadmium, copper, lead, mercury, selenium, thallium, vanadium, and zinc. In addition, two of the brown bullhead composite samples were analyzed for polycyclic aromatic hydrocarbons. The CNFRL conducted the organochlorine/PCB and polycyclic aromatic hydrocarbon analyses; the heavy metals analysis was contracted to the Environmental Trace Substance Laboratory at the University of Missouri.

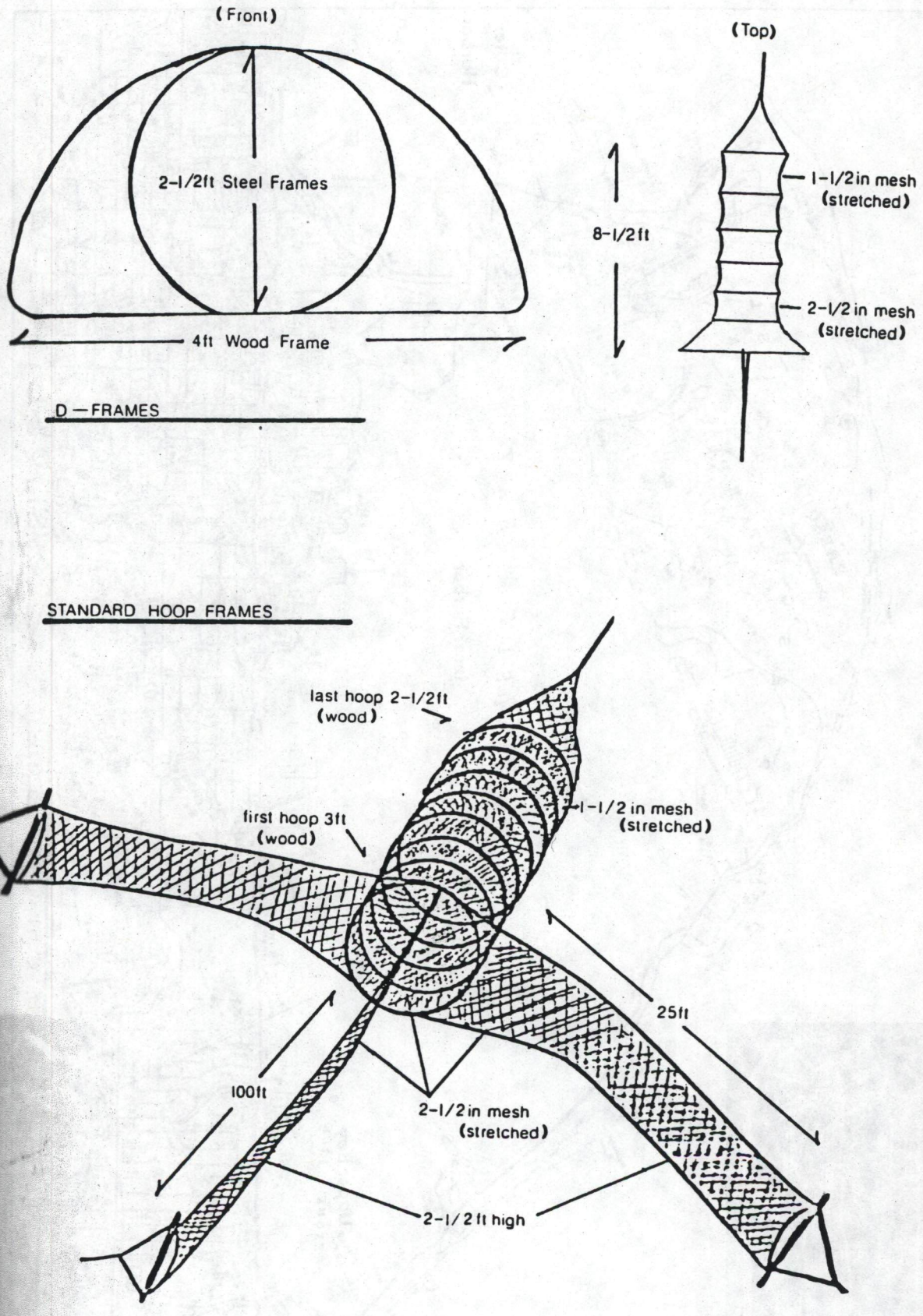


Figure 1. Standard hoop frame and D-frame nets used in the Presque Isle Bay fish collection effort.

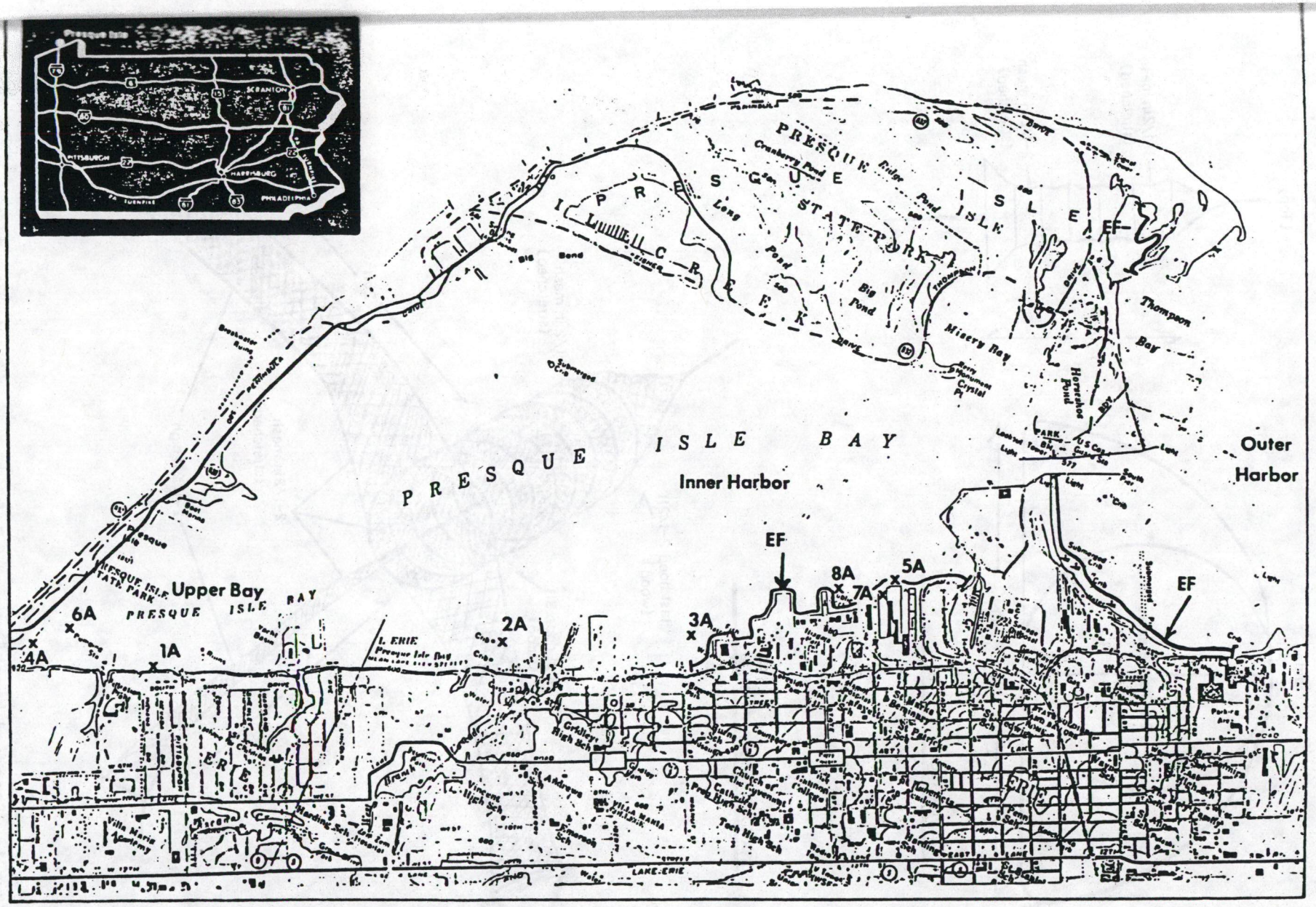


Figure 2. Fish sampling locations, Presque Isle Bay and Lake Erie, July 31 and August 1, 1984.

Table 1. Species, collection location, length and weight of Presque Isle Bay fish submitted for chemical analysis.

Sample No.	Species	Collection Location	Length (mm)	Weight (g)
84-5-SCFO-40-1	Largemouth bass	Outer harbor	384	992
			288	488
			287	388
			272	287
84-5-SCFO-40-2	Brown bullhead	Outer harbor	220	140
			304	388
			326	390
			326	510
			341	685
84-5-SCFO-41-3	Bluegill	Upper bay	200	180
			188	168
			194	160
			202	190
			180	135
84-5-SCFO-42-1	Brown bullhead	Inner harbor	300	381
			302	375
			328	480
			311	405
			---	---
84-5-SCFO-43-1	Brown bullhead	Upper bay	---	---

RESULTS

Chemical Analysis

Tables 2 and 3 detail the results of the laboratory analysis of heavy metals, pesticides, PCBs, and polycylic aromatic hydrocarbons (known as "PAHs", "PNAs", or "PAC's") in the whole brown bullhead, largemouth bass, and bluegill samples. Readers should note that whole fish, not fillets were analyzed because the legal mandate of the Fish and Wildlife Service is to evaluate the effects of contaminants on fish and wildlife, not humans. Whole fish analysis includes the entire animal -- skin, bones, internal organs, etc. -- and provides a measure of the amount of contaminants that would be available to wildlife or another fish that preyed upon the sample fish. Fat immediately below the skin in fish and organs such as the liver tend to accumulate more contaminants than muscle tissue. Therefore, whole fish residues are expected to be higher than fillet ("edible portion") residues. Therefore, our results should not be compared to U.S. Food and Drug Administration (FDA) "Action Levels," which are based only on edible portion residues.

Pathological

The brown bullheads collected for this study were afflicted with a wide array of external lesions that made them rather appalling to look at (see Figures 3 and 4), and we could easily understand why Presque Isle Bay fishermen have become alarmed. In recent years a number of popular magazines have publicized studies revealing a high incidence of tumors in fish from polluted waters, and it is natural that public awareness of the possible significance of abnormal, unhealthy-looking fish has increased.

Almost all of the brown bullheads collected exhibited fin erosion to various degrees; on some fish one or more fins were completely missing or only a stub remained. Barbels were also frequently eroded or "burned" in appearance. Less frequently, red areas on the skin, or dark skin patches were observed. The most striking abnormality we noted was a high incidence of mouth sores and/or lumps around the outside of the mouth. The sores were so extensive in one fish that the roof of the mouth appeared as if it had been severely burned. There was no noticeable difference in overall appearance between bullheads collected in the Upper Bay compared to those collected in the Inner Harbor area; Outer Harbor fish seemed to have fewer abnormalities.

As previously stated in the METHODS section, all of these abnormal skin lesions, as well as samples from the livers and other internal organs, were sent to the Service's Dr. Paul Baumann in Columbus, Ohio. Dr. Baumann examined all of the tissues and selected specimens from six bullheads that appeared to be "tumors". Dr. Baumann based his selection only on gross examination of the tissues, and does not rule out the possibility that histopathological examination might have discovered additional tumors.

The suspected tumors identified by Dr. Baumann were forwarded for verification to Dr. John C. Harshbarger, Director, Registry of Tumors in Lower Animals (RTLA) at the Smithsonian Institution in Washington, D.C. Table 4 presents Dr. Harshbarger's diagnosis of the tissues, and the lengths and weights of the bullheads affected. "Epidermal papillomas" (benign skin tumors) were

Table 2. Concentrations of elemental and organochlorine contaminants in whole fish samples collected from Presque Isle Bay area. Results in ppm wet weight. See Appendix A for complete laboratory report.

	Sample Number (84-5-SCFO-___) and Species				
	43-1 Brown Bullhead	42-1 Brown Bullhead	40-2 Brown Bullhead	40-1 Largemouth Bass	41-3 Bluegill
Collection Location	Upper Bay	Inner Harbor	Outer Harbor	Outer Harbor	Upper Bay
% Lipid	6.5	9.1	5.6	9.9	5.6
Elements (ppm)					
aluminum	100.	36.	93.	3.2	4.5
arsenic	0.26	0.1	0.09	0.15	0.1
cadmium	0.03	0.061	0.039	0.034	0.03
copper	0.88	0.005	0.98	0.59	0.46
mercury	0.03	0.03	0.04	0.19	0.04
lead	0.80	0.77	0.46	0.09	0.24
selenium	0.21	0.28	0.26	0.44	0.53
thallium	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
vanadium	0.64	0.49	0.60	0.39	0.35
zinc	16.	18.	13.	16.	22.
Organochlorines (ppm)					
pentachloroanisole (PCA)	ND	ND	ND	ND	ND
α - BHC	ND	0.01	ND	ND	ND
γ - BHC	ND	0.01	ND	ND	ND
β - BHC	ND	ND	ND	ND	ND
Δ - BHC	ND	ND	ND	ND	ND
oxy-chlordane	0.02	0.01	ND	0.03	0.12
heptachlor[1]	ND	0.01	ND	0.01	0.03
trans-chlordane	0.04	0.07	0.04	0.03	ND
trans-nonachlor	0.11	0.13	0.11	0.14	0.26
cis-chlordane	0.12	0.16	0.13	0.09	0.08
o,p'-DDE	ND	ND	ND	ND	ND
p,p-DDE	0.04	0.04	0.04	0.03	0.07
o,p-DDD	ND	ND	ND	0.02	ND
cis-nonachlor	0.03	0.05	0.04	0.05	0.07
o,p-DDT	ND	ND	ND	0.02	ND
p,p'-DDD	0.16	0.21	0.23	0.25	0.06
p,p'-DDT	ND	ND	ND	0.1	ND
methoxychlor	ND	ND	ND	ND	ND
PCBs[2]	6.8	7.1	7.5	7.8	4.8
toxaphene	0.6	0.08	0.8	0.9	0.08
dacthal	ND	ND	ND	ND	ND
dieldrin	0.2	ND	ND	ND	0.12
endrin	ND	ND	ND	ND	ND
HCB	ND	ND	ND	ND	ND
mirex	ND	ND	ND	ND	ND

[1]Includes heptachlor epoxide.
[2]Total PCB residue levels expressed as the sum of 105 congeners/isomers.
ND = not detected.

Table 3. Concentrations of selected polycyclic aromatic hydrocarbons (PAH's) in composite whole brown bullhead samples collected from Presque Isle Bay area, vs. PAH concentrations in composite brown bullhead samples from the Black River, Ohio. Results in ppm wet weight. (Source: U.S. Fish and Wildlife Service, Columbia National Fisheries Research Laboratory, September 1985). The values reported below are uncorrected for percent recovery; see Appendix A for complete laboratory report.

	Lake Erie			Black River
	84-5-SCFO -43-1	84-5-SCFO -40-2		Mean concentration of five determinations
	Rep 1	Rep 1	Rep 2	
naphthalene[c]	.036	.092	.034	.129
1-methylnaphthalene	.020	.036	<.019[a]	
2,3-dimethylnaphthalene	<.015[a]	<.002[a]	<.002[a]	
acenaphthylene[c]	<.015[a]	ND	ND	.157
acenaphthene[c]	ND	.046	ND	.127
fluorene[c]	ND	<.003[a]	ND	.488
dibenzothiophene[d]	ND	ND	ND	.173
phenanthrene[c]	ND	<.013[a]	<.015[a]	1.219
anthracene[c]	ND	ND	ND	.248
methyl-phenanthrene	ND	<.015[b]	ND	
fluoranthene[c]	ND	<.048[b]	ND	.775
pyrene[c]	ND	ND	ND	.423
benzo(b)naphtho(2,1-d)-thiophene[d]	ND	ND	ND	<.010
benzo(a)anthracene[c]	ND	ND	ND	<.010
chrysene[c]	ND	ND	ND	.047
benzo(b)fluoranthene[c]	ND	ND	ND	<.017
benzo(k)fluoranthene[c]	ND	ND	ND	<.016
benzo(a)pyrene[c]	ND	ND	ND	<.019
perylene	ND	ND	ND	<.014
indeno(1,2,3-c,d)pyrene[c]	ND	ND	ND	
dibenz(a,h)anthracene[c]	ND	ND	ND	
benzo(g,h,i)perylene[c]	ND	ND	ND	<.054

< - less than - Indicates that a peak was detected for this residue and the calculated concentration is less than the estimated method determination limit.

[a] - method determination limit is 0.02 ppm.

[b] - method determination limit is 0.05 ppm.

ND - not detected - Indicates that no peak was detected for this residue.

[c] - EPA priority pollutant PNA.

[d] - sulfur heterocyclic PNA.

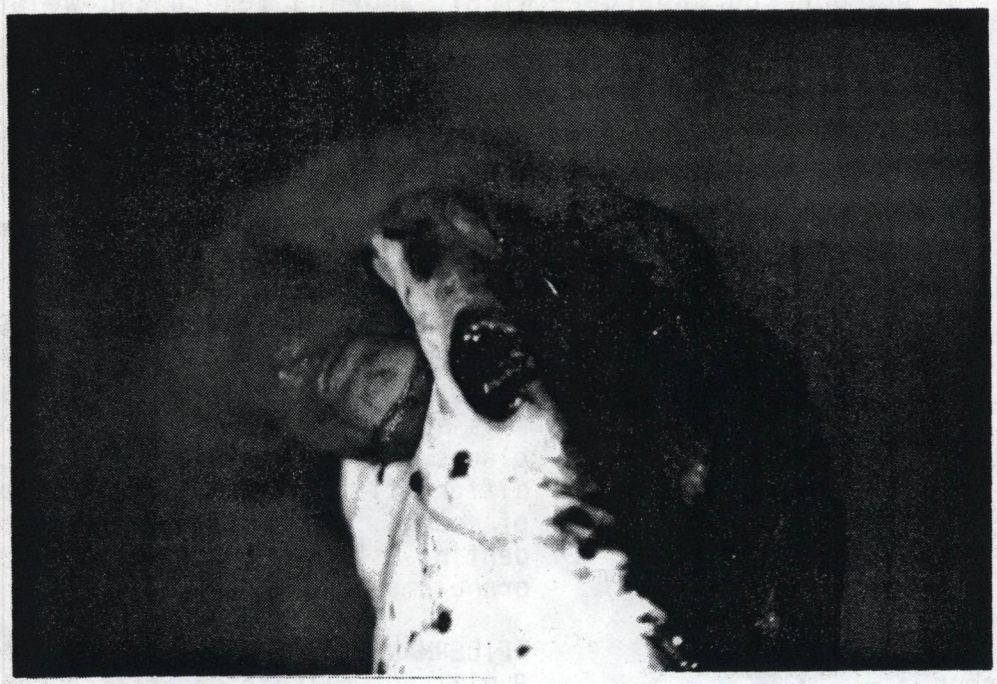


Figure 3. Photo of skin sore on Presque Isle Bay brown bullhead.

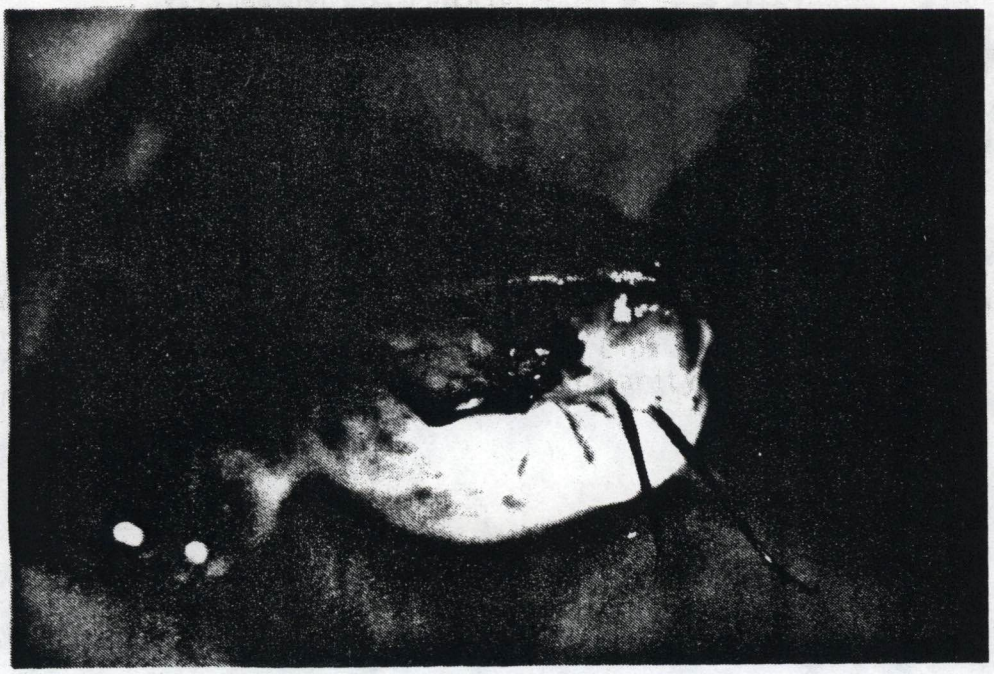
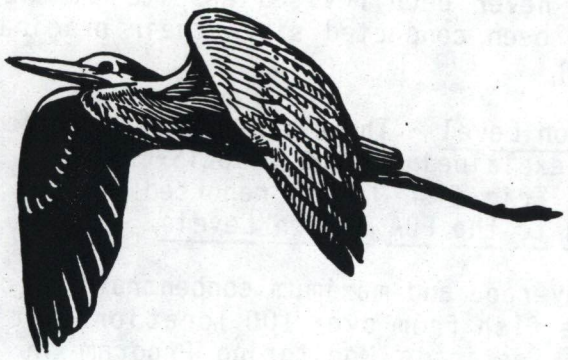


Figure 4. Photo of lip lesion on Presque Isle Bay brown bullhead.

Table 4. Diagnosis of selected tissues from Presque Isle Bay brown bullheads by Dr. John Harshbarger (RTLA), showing length and weight of the affected fish. (Diagnosis by Dr. Harshbarger dated August 19, 1985.)

Length (mm), weight (g), collection location	Tissues sent to RTLA from Dr. Baumann	Dr. Harshbarger's diagnosis
345, 430 Innner Harbor	Upper lip, pectoral fin, skin from right side of head	EPIDERMAL PAPILLOMA; ODONTOMA This is an oral neoplasm with a range of pictures. Principally the lip has a large mass of papillary epidermis which in places is undergoing ameloblastic differentiation with occasional production of enamel. Deeper areas may be producing dentin, osteodentin and bone. Helminthic granulomas are also present.
337, 456 Inner Harbor	Upper jaw	EPIDERMAL PAPILLOMA Papillary well-differentiated epidermis of lip.
324, 350 Inner Harbor	Lower jaw; skin to left of lower jaw	INJURY Tissue shows congested vessels, hemorrhage and inflammation
285, 372 Upper Bay	Left jaw	EPIDERMAL HYPERPLASIA Thickened epidermis.
314, 430 Upper Bay	Upper lip; kidney	EPIDERMAL PAPILLOMA White area of kidney is normal corpuscle of Stannius. Skin and lip tissues exhibit epidermal papillarity.
306, 355 Upper Bay	Lower jaw	EPIDERMAL PAPILLOMA Skin is ulcerated with inflammation in the underlying tissues, probably due to injury. Lips have epidermal thickening and papillarity but no invasion.

identified in tissues from four of the six bullheads. Tissues from one of the bullheads exhibited an "epidermal hyperplasia," or non-neoplastic thickening of the skin. The suspicious tissues from the sixth fish proved to be the result of an injury.



DISCUSSION

In general, elemental contaminant levels in all five composite fish samples appeared to be within expected values. However, aluminum levels were high in the three bullhead composite samples (Zajicek, pers. comm., 12/20/85). It is possible that aluminum-containing sediments were present in the gastrointestinal tract of the bullheads, thus biasing the results. Brumbaugh and Kane (1985) have recommended that the gastrointestinal tract and its contents be removed before chemical analysis to provide a more accurate assessment of "true" aluminum concentrations in whole fish tissues.

Table 5 shows the geometric mean and maximum concentrations of organochlorine contaminants and PCBs found in our samples, compared with several guidelines:

1) NAS/NAE Criteria - Maximum residue levels in whole fish established by the National Academy of Sciences/National Academy of Engineering (1972) for the protection of fish and piscivorous (fish-eating) wildlife. These criteria represent the only published reference for evaluating the possible biological significance of organochlorine residues in fish. Unfortunately, the criteria are now 15 years old and have never been revised despite the wealth of relevant research that has been conducted since their original publication (Schmitt et al. 1983)

2) FDA Action Level - These values are provided for reference only. For reasons explained in the RESULTS-Chemical Analysis section (page 6), the Lake Erie fish levels reported in this survey cannot be directly compared to the FDA Action Levels.

3) NPMP - Average and maximum concentrations of these compounds detected in whole fish from over 100 locations nationwide for the Service's National Pesticide Monitoring Program in 1980 and 1981 (Schmitt et al., 1985).

All five whole fish samples exceeded the NAS/NAE criteria for combined organochlorines (excluding DDT) and chlordane alone. Two of the bullhead samples (40-2 and 43-1) exceeded the NAS/NAE criteria for toxaphene alone, as did the bass and bluegill samples. The bluegill sample and bullhead 43-1 also exceeded the NAS/NAE criteria for dieldrin.

PCBs far exceeded the NAS/NAE criteria of 0.5 ppm in all five fish samples, ranging from 4.8 to 7.8 ppm. The Pennsylvania Department of Environmental Resources (Appendix B) and the Erie County Department of Health (ECDH), in commenting on our results, noted that these PCB levels are disturbingly high, even for whole fish samples. The results seem especially high in view of the fact that bullhead skinless fillets collected by the ECDH in October 1984 and March, May and September 1985 have consistently contained less than 0.5 ppm PCBs.

We requested that our laboratory analyze two bullhead samples for PAHs because these compounds have typically been found at high levels in sediments and fish from areas where brown bullheads exhibit skin or liver tumors. As noted in the laboratory report (Appendix A), however, the PAH levels in the Lake Erie bullheads "do not appear to be consistent with high PNA contamination, such as

Table 5. Geometric mean and maximum concentrations of organochlorine contaminants and PCBs in Lake Erie whole fish samples compared with NAS/NAE criteria and NPMP geometric mean and maximum levels for 1980-1981. The FDA action level is also provided as a reference only. FDA levels apply to edible portions of fish, not whole fish which include contaminant-accumulating internal organs. All values reported as ppm wet weight.

NAS/NAE CRITERIA (Whole Fish)	COMPOUND	FDA ACTION LEVEL (Edible Portion)	NPMP 1980-1981 (Whole Fish) Geometric Mean	NPMP 1980-1981 (Whole Fish) Max.	LAKE ERIE[1] (Whole Fish) Geometric[2] Mean	LAKE ERIE[1] (Whole Fish) Max.
0.1 ppm, singly or in combination with other organochlorine insecticides, excluding DDT	α-BHC		<0.01	0.04	<0.01	0.01
	γ-BHC		<0.01	0.03	<0.01	0.01
	heptachlor[3]	0.3 ppm	0.01	0.27	0.01	0.03
	trans-chlordane		0.02	0.22	0.03	0.07
	trans-nonachlor		0.04	0.77	0.17	0.26
	oxychlordane	0.3 ppm	0.01	0.33	0.05	0.12
	cis-nonachlor		0.02	0.27	0.05	0.07
	cis-chlordane		0.03	0.36	0.10	0.16
	toxaphene	5.0 ppm	0.27	21.0	0.44	0.9
	dieldrin	0.3 ppm	0.04	0.72	0.06	0.2
1.0 ppm	p,p'-DDE	5.0 ppm total, excluding any levels below 0.2 ppm	0.20	2.57	0.05	0.07
	o,p'-DDD				<0.01	0.02
	o,p'-DDT				<0.01	0.02
	p,p'-DDD		0.07	3.43	0.17	0.25
	p,p'-DDT		0.05	2.69	0.03	0.1
0.5 ppm	PCB's	2.0 ppm	0.53	11.30	6.5	7.8

[1]Collected by the U.S. Fish and Wildlife Service for this preliminary survey on July 31 and August 1, 1984.

[2]Wet weight concentrations were normalized by applying the ($\log_{10}$[residue concentration + 1.0]) transformation before computation of the mean in the following manner: 1/3[(three bullhead residues)/3 + largemouth bass residue + bluegill residue] (per Schmitt, 1/3/86).

[3]Includes heptachlor epoxide.

that found in brown bullheads from the Black River in Ohio." However, the laboratory analysis did not include the metabolites of the PAH compounds, believed to be the actual inducers of PAH-related fish tumors. Some fish tumor researchers theorize that PAH levels in fish tissues may undergo seasonal changes, perhaps being high in the spring following the winter period of low metabolic activity, and low in late summer; during periods of low PAH tissue levels, PAH metabolites might be present in higher concentrations. Unfortunately, this is just a theory and laboratory techniques for analyzing PAH metabolites are not fully developed at this time (pers. comm., Zajicek (CNFRL), 11/25/85).

The ECDH has conducted sediment sampling at various locations in Presque Isle Bay on several occasions. Results of PAH analysis of the sediments have shown that, while present, the PAH levels are much lower than those reported from the Niagara River area, where external or internal neoplasms have been found in at least seven species of fish (Black 1983), or the Black River, Ohio, where liver tumors occur in 33% of brown bullheads 3 years old and older (Baumann et al., 1982). ECDH's most recent sediment collection, however, identified a few PAH "hot spots" in the Bay with PAH levels within the range of the Niagara River area values. An ECDH report summarizing the sediment analysis results was not yet available for inclusion in this report.

The open sores and epidermal papillomas observed in some of the Presque Isle Bay brown bullheads in this survey would appear to be a cause for concern, but it is not possible at this time to conclusively pinpoint a cause. Viruses have been found to cause tumors in some species of fish, but electron microscopy has failed to detect any viral agent associated with bullhead tumors (Harshbarger, pers. comm., 11/22/85).

There is increasing interest among researchers in the possibility that tumors in fish populations could offer an early-warning signal of environmental contamination. Such was the case in the early 1960's, when an international epidemic of liver cancer in hatchery-reared rainbow trout led to the discovery that a mold growing on peanuts used in trout food produced aflatoxins (Morell 1984). Alfatoxins have been identified as one of the most potent carcinogens known and are now regulated by the FDA.

Sonstegard (1977) necropsied over 50,000 fish from the Great Lakes and found epizootics (epidemic incidence) of tumors in carp (Cyprinus carpio), goldfish (Carassius auratus), carp x goldfish hybrids (Cyprinus carpio x Carassius auratus) and white suckers (Catostomus commersoni). In many cases, the high tumor incidences were clustered around polluted areas. Brown et al. (1973) compared fish from the polluted Fox River in Illinois to unpolluted Canadian waters (Lake of the Woods, Ontario) and found a much higher tumor incidence in Fox River fish vs. fish from the same species at Lake of the Woods. Black (1983) has established a direct cause-effect link between contaminated sediments and skin tumors in brown bullheads; he painted a laboratory population of brown bullheads with an extract of PAH-laden Buffalo River sediments, and observed the development of epidermal hyperplasia and neoplasms within 12 months. Black used the same technique on mice and skin cancer developed almost immediately (Morell 1984).

Thus, while the presence of epidermal hyperplasia and neoplasms would seem to indicate the presence of chemical carcinogens in Presque Isle Bay, both Drs. Baumann and Harshbarger (pers. comm., 11/22/85) have advised us that skin

are not absolute proof of carcinogens. Much stronger indication would be the presence of liver tumors, but none were observed bullheads we collected. However, some types of liver cancer, especially hepatocarcinomas, are seldom grossly visible and might easily have missed without extensive histopathology on normal appearing livers (Baumann, pers. comm., 12/9/85). Black (1983) has pointed out that the liver is the major organ for metabolizing toxic chemicals into forms that can be eliminated from an organism. Thus, it is logical that liver tumors would occur more frequently in contaminant-stressed populations.

Skin tumor rates have been shown to be higher in industrialized areas compared to relatively clean areas. Even if these tumors were virally induced, it would seem that the polluted nature of the fishes' habitat somehow makes them more susceptible to the viral agents, possibly through increased stress (Baumann, pers. comm., 11/22/85). If chemical stress is a factor in tumor development in Presque Isle Bay bullheads, it may also be a factor in the development of the mouth sores noted in these fish (i.e., perhaps the bullheads are more susceptible to a disease agent that causes the sores, and/or perhaps chemical stress has affected the bullheads' immune system and, consequently, their ability to heal).

If there are carcinogens present in Presque Isle Bay in sufficient concentrations to affect the bullhead population, identifying them may be extremely difficult. While ECDH work has identified a few areas of relatively high levels in sediments, PAH's are far from the only known or suspected carcinogen.

CONCLUSIONS

As stated earlier, the purpose of this survey was to verify the reported occurrence of tumors in Presque Isle Bay bullheads and determine whether a more extensive study was warranted. In this brief survey, we have verified the presence of skin tumors (benign) in several bullheads, but no liver tumors and relatively low levels of PAH in the fish tissues were found.

The pathological and chemical results of this study were not yet available when the spring 1985 field season arrived, but we decided that, based on our own gross observations of the bullheads during the 1984 survey, further study of the area was warranted. In May 1985, we assembled a team of biologists and pathologists to undertake a more comprehensive examination of the fish. Over 100 bullheads were collected, internal and external examinations were conducted similar to those performed in 1984, and samples of the liver and other internal organs were preserved for histopathological examination. We are still waiting for the pathological and chemical analysis results of the May study.

Thus, at this time, many questions remain about the cause of skin sores and tumors in Presque Isle Bay bullheads. The results of this limited survey simply do not provide enough evidence to conclude that chemical carcinogens are causing the observed fish skin abnormalities. The results of the 1985 survey, encompassing a larger number of fish and focusing more closely on the condition of the fish livers and contaminant levels in Bay sediments (collected by ECDH), should provide enough information to better assess the situation.

LITERATURE CITED

Baumann, Paul C., W. D. Smith, and M. Ribick. 1982. Hepatic tumor rates and polynuclear aromatic hydrocarbon levels in two populations of brown bullhead (Ictalurus nebulosus). Pages 93-102 in Polynuclear Aromatic Hydrocarbons: Sixth International Symposium on Physical and Biological Chemistry, ed. by M. W. Cooke et al. Battelle Press, Columbus, Ohio.

Black, J. J. 1983. Field and laboratory studies of environmental carcinogenesis in Niagara River fish. J. Great Lakes Res. 9(2):326-334.

Brown, E. R. et al. 1973. Frequency of fish tumors found in a polluted watershed as compared to nonpolluted Canadian waters. Cancer Res. 33:189-198.

Brumbaugh, W. G., and D. A. Kane. 1985. Variability of aluminum concentrations in organs and whole bodies of smallmouth bass (Micropterus dolomieui). Environ. Sci. Technol. 19(9):828-831.

Morell, V. 1984. Fishing for trouble. International Wildlife 14(4):40-43.

National Academy of Sciences/National Academy of Engineering, Committee on Water Quality Criteria. 1972. Water Quality Criteria, 1972. Washington, D.C. 594 pp.

Schmitt, C. J., M. A. Ribick, J. L. Ludke, and T. W. May. 1983. National Pesticide Monitoring Program: Organochlorine Residues in Freshwater Fish, 1976-79. U.S. Fish and Wildlife Service, Washington, D.C. Resource Publ. 152. 62 pp.

Schmitt, C. J., J. L. Zajicek, and M. A. Ribick. 1985. National Pesticide Monitoring Program: Residues of organochlorine chemicals in freswater fish, 1980-81. Arch. Environ. Contam. Toxicol. 14:225-260.

Sonstegard, R. A. 1977. Environmental carcinogenesis studies in fishes of the Great Lakes of North America. Ann. New York Acad. Sci. 298: 261-269.

U.S. Fish and Wildlife Service. 1983. National Pesticide Monitoring Program (NPMP) 1980-81 freshwater fish residue data. Columbia National Fisheries Research Laboratory, Columbia, Missouri. (Unpublished)

APPENDIX A

RESULTS OF CHEMICAL ANALYSIS OF LAKE ERIE FISH - LABORATORY REPORTS

Organochlorine Pesticides and PCB's

UNITED STATES GOVERNMENT

memorandum

DATE: May 28, 1985

FROM: Assistant to the Director, CNFRL

SUBJECT: Sample Analyse FY84

TO: RCA Coordinator, Region 5

Attached are the results of analyses of fish from the Blosenski Landfill and from Presque Isle Bay. The two brook trout (#s 228 and 229) are from the Blosenski Landfill.

Larry Ludke has left CNFRL to take a position with the Coastal Ecosystem Team in Slidell, LA. According to the notes he left with me, we still have to analyze a composite of the brook trout and the white sucker from the Bolenski Landfill for PNA's. These will be done when we complete the PNAs on the remaining samples from the Elizabeth River, which are in the process of being analyzed.

Richard J. Graham

RJG:lw

UNITED STATES GOVERNMENT

memorandum

21 May 1985

Ted R. Schwartz

Residue Analysis Job 30, 31

Dr. Richard Graham

Samples of homogenized fish tissue were submitted for organochlorine residue analysis on 26 November 1984. Capillary gas chromatography were used to measure residues of 23 organochlorine contaminants (Table 1). The limits of determination for individual compounds were 0.01 ug/g. For toxaphene and PCBs, the limits of determination were 0.1 ug/g (wet weight). On the basis of systematic recovery studies in which ^{14}C-radiolabeled and non-radiolabeled spiked fish tissue blanks were carried through the extraction and fractionation steps, it was determined that recovery efficiency exceeded 85% for all compounds measured except PCA (80%), α-BHC (68%), HCB (48%) and heptachlor (74%). Results were not adjusted for percent recovery. The following references may be consulted for details of the analytical procedures used:

Schwartz, T.R. et al. Anal. Chem. 1984, 56, 1303.
Schmitt, C.J. et al. Arch. Environ. Contam. Toxicol. 1985, 14, 225.

Ted R Schwartz

T.R. Schwartz

TRS/rcb

OPTIONAL FORM NO. 10

Lab Code	Investigator Designation	
F513-141C-1	84-5-SCFO-2-2-A	Brooktrout - BLF #229
F514-141C-2	84-5-SCFO-2-2	Brooktrout - BLF #228
F515-142C-1	84-5-SCFO-43-1	Brown Bullhead #240
F516-142C-2	84-5-SCFO-42-1	Brown Bullhead #239
F517-142C-3	84-5-SCFO-40-2	Brown Bullhead #237
F518-142C-4	84-5-SCFO-41-3	Bluegill #238
F519-142C-5	84-5-SCFO-40-1	Largemouth Bass #236

Table 1. Residues measured.

Residue	Chemical Abstracts No.	Chemical names	Principal uses and occurrence
DDE	72-55-9	2,2-bis(p-chlorophenyl)-1,1-dichloroethylene	Insecticide; DDT-metabolite
DDD (TDE)	72-54-8	2,2-bis(p-chlorophenyl)-1,1-dichloroethane	Insecticide; DDT-metabolite
DDT	50-29-3	2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane	Insecticide
PCBs			
Aroclor 1242	53469-21-9	Polychlorinated biphenyl averaging 42% chlorine by weight	Dielectric fluid in capacitors; transformer fluid; vacuum pump lubricants; gas turbine lubricants; hydraulic fluids; plasticizers; heat transfer fluids; wax, pesticide, ink, lubricant, and cutting oil extenders; dedusting agents; carbonless reproducing paper
Aroclor 1248	12672-29-6	Polychlorinated biphenyl averaging 48% chlorine by weight	
Aroclor 1254	11097-69-1	Polychlorinated biphenyl averaging 54% chlorine by weight	
Aroclor 1260	11096-82-5	Polychlorinated biphenyl averaging 60% chlorine by weight	
Dieldrin	60-57-1	1,2,3,4,10,10-hexachloro-exo-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-exo-5,8-dimethanonaphthalene	Insecticide; aldrin metabolite
Endrin	72-20-8	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo,endo-5,8-dimethanonaphthalene	Insecticide
Heptachlor	76-44-8	1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7 methanoindene	Insecticide; minor constituent of technical chlordane
Heptachlor epoxide	1024-57-3	1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindane	Heptachlor metabolite
cis-Chlordane	5103-71-9	1-exo-2-exo-4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane	Insecticide; constituent of technical chlordane

Residue	Chemical Abstracts No.	Chemical names	Principal uses and occurrence
trans-Chlordane	5103-74-2	1-exo-2-endo-4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane	Insecticide; chlordane constituent
cis-Nonachlor	29555-47-3	1-exo-2-exo-3-exo-4,5,6,7,8,8-nonachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane	Insecticide; chlordane constituent
trans-Nonachlor	3734-49-4	1-exo-2-endo-3-exo-4,5,6,7,8,8-nonachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane	Insecticide; chlordane constituent
Oxychlordane	26880-48-8	1-exo-2-endo-4,5,6,7,8,8-octachloro-exo-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindane	Chlordane metabolite
Toxaphene	8001-35-2	Chlorinated camphene mixture averaging 62% chlorine by weight	Insecticide; herbicide
α-Benzene hexachloride (BHC)	319-84-6	α-isomer of 1,2,3,4,5,6-hexachlorocyclohexane	Constituent of insecticide mixture containing various BHC isomers
γ-Benzene hexachloride (BHC; lindane)	58-89-9	γ-isomer of 1,2,3,4,5,6-hexachlorocyclohexane	Insecticide; BHC constituent
Hexachlorobenzene (HCB)	118-74-1	perchlorobenzene	Fungicide; industrial intermediate
Methoxychlor	72-43-5	2,2-bis(p-methoxyphenyl)-1,1,1-trichloroethane	Insecticide
Mirex	2385-85-5	dodecachlorooctahydro-1,3,4-methano-2H-cyclobuta (cd) pentalene	Insecticide; fire retardant
Dacthal	1861-32-1	dimethyl tetrachloroterephthalate	Herbicide
Pentachloroanisole (PCA)	1825-21-4	2,3,4,5,6-pentachloroanisole	Pentachlorophenol metabolite

Table II. Organochlorine residues (ug/g, wet weight).

	lipid %	PCA	α-BHC	γ-BHC	β-BHC	Δ-BHC	Oxy-chlordane	Heptachlor(a)	trans-Chlordane	trans-Nonachlor	cis-Chlordane
F513; 84-5-SCFO-2-1	4.6	ND	ND	ND	ND	ND	ND	ND	ND	0.006	ND
F514; 84-5-SCFO-2-2	6.5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
F515; 84-5-SCFO-43-1	6.5	ND	ND	ND	ND	ND	0.02	ND	0.04	0.11	0.12
F516; 84-5-SCFO-42-1	9.1	ND	0.01	0.01	ND	ND	0.01	0.01	0.07	0.13	0.16
F517; 84-5-SCFO-40-2	5.6	ND	ND	ND	ND	ND	ND	ND	0.04	0.11	0.13
F518; 84-5-SCFO-41-3	5.6	ND	ND	ND	ND	ND	0.12	0.03	ND	0.26	0.08
F519; 84-5-SCFO-40-1	9.9	ND	ND	ND	ND	ND	0.03	0.01	0.03	0.14	0.09
Procedure Blank		ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

	o,p'-DDE	p,p-DDE	o,p-DDD	cis-nonachlor	o,p-DDT	p,p'-DDD	p,p'-DDT	Meth-oxychlor	PCBs(b)	Toxaphene
F513; 84-5-SCFO-2-1	ND	ND	ND	ND	ND	ND	ND	ND	0.44	0.07
F514; 84-5-SCFO-2-2	ND	ND	ND	ND	ND	ND	ND	ND	1.3	0.07
F515; 84-5-SCFO-43-1	ND	0.04	ND	0.03	ND	0.16	ND	ND	6.8	0.6
F516; 84-5-SCFO-42-1	ND	0.04	ND	0.05	ND	0.21	ND	ND	7.1	0.08
F517; 84-5-SCFO-40-2	ND	0.04	ND	0.04	ND	0.23	ND	ND	7.5	0.8
F518; 84-5-SCFO-41-3	ND	0.07	ND	0.07	ND	0.06	ND	ND	4.8	0.8
F519; 84-5-SCFO-40-1	ND	0.03	0.02	0.05	0.02	0.25	0.1	ND	7.8	0.9
F511-^{14}C spike recovery							95		94	
F512-^{14}C spike recovery							104		100	
Procedure Blank	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

	Dacthal	Dieldrin	Endrin	HCB	Mirex
F513; 84-5-SCFO-2-1	ND	ND	ND	ND	ND
F514; 84-5-SCFO-2-2	ND	ND	ND	ND	ND
F515; 84-5-SCFO-43-1	ND	0.02	ND	ND	ND
F516; 84-5-SCFO-42-1	ND	ND	ND	ND	ND
F517; 84-5-SCFO-40-2	ND	ND	ND	ND	ND
F518; 84-5-SCFO-41-3	ND	0.12	ND	ND	ND
F519; 84-5-SCFO-40-1	ND	ND	ND	ND	ND
F475-QC spike recovery	NA	NA	NA	NA	NA
Procedure Blank	ND	ND	ND	ND	ND

NA, not analyzed
ND, not detected
(a), Includes Heptachlor epoxide
(b), Total PCB residue levels expressed as the sum of 105 PCB congeners/isomers

Elements

Environmental Trace Substances Research Center
U.S.D.I. - Graham
Batch #R5-31
(Units are µg/g wet weight)

Customer ID	84090236 Largemouth Bass 84-5-SCFO-40-1	84090237 Brown Bullhead 84-5-SCFO-40-2	84090238 Bluegill 84-5-SCFO-41-3
Al	3.2	93.	4.5
As	0.15	0.09	0.1
Cd	0.034	0.039	0.03
Cu	0.59	0.98	0.46
Hg	0.19	0.04	0.04
Pb	0.09	0.46	0.24
Se	0.44	0.26	0.53
Tl	<0.1	<0.1	<0.1
V	0.39	0.60	0.35
Zn	16.	13.	22.
% Moisture	75.2	77.5	73.5

Customer ID	84090239 Brown Bullhead 84-5-SCFO-42-1	84090240 Brown Bullhead 84-5-SCFO-43-1
Al	36.	100.
As	0.1	0.26
Cd	0.061	0.03
Cu	0.005	0.88
Hg	0.03	0.03
Pb	0.77	0.80
Se	0.28	0.21
Tl	<0.1	<0.1
V	0.49	0.64
Zn	18.	16.
% Moisture	74.5	75.7

Customer: U.S.D.I. - Graham

ETSRC ID: 84090236

The attached pages have been checked and verified.

J. S McAllister 4-23-85
Date

Edward J. Hunkeberger 4-24-85
Date

Lynn A. Hartman 4-30-85
Date

U.S.D.I. - GRAHAM
Batch #R5-31
Quality Control
(Units are µg/g wet weight)

Duplicates

U.S.D.I. #84-5-SCFO-40-1

ETSRC ID	Element	Sample	Duplicate	% Deviation
84090236	Al	3.3	2.9	13.
	As	0.15	0.14	6.9
	Cd	0.035	0.033	5.9
	Cu	0.58	0.60	3.4
	Hg	0.19	0.18	5.4
	Pb	0.06	0.08	29.
	Se	0.42	0.45	6.9
	Tl	<0.1	<0.1	0.0
	V	0.39	0.39	0.0
	Zn	16.	16.	0.0
			Average % Deviation	7.1

Spikes

U.S.D.I. #84-5-SCFO-42-1

ETSRC ID	Element	Sample	Duplicate	% Recovery
84090239	Al	36.	41.	*
	As	0.1	1.9	92.
	Cd	0.06	0.25	109.
	Cu	0.05	0.24	104.
	Hg	0.03	0.11	91.
	Pb	0.77	2.9	108.
	Se	0.28	6.6	88.
	Tl	<0.1	4.2	114.
	V	0.49	1.8	73.
	Zn	18.	58.	108.
			Average % Recovery	98.

*Spiked at <1/2 sample conc.

U.S.D.I.-Graham
Batch #R5-31
Blind Quality Control
(Units are µg/g)

NBS ID	ETSRC ID	Element	Sample	NBS Certified Value
Oyster Tissue	84090241	As	11.	13.4 ± 1.9
1566		Cd	4.3	3.5 ± 0.4
		Cu	68.	63.0 ± 3.5
		Pb	0.49	0.48 ± 0.04
		Hg	0.047	0.057 ± 0.015
		Se	2.2	2.1 ± 0.5
		V	2.2	(2.8)

NBS ID	ETSRC ID	Element	Sample	NBS Certified Value
Spinach	84090242	Al	-	870. ± 50.
1570		As	0.1	0.15 ± 0.05
		Cd	1.6	(1.5)
		Cu	12.	12.2 ± 2.
		Pb	1.3	1.2 ± 0.2
		Hg	0.02	0.030 ± 0.005
		Tl	<0.1	(0.03)
		Zn	45.	50. ± 2

Polycyclic Aromatic Hydrocarbons

UNITED STATES GOVERNMENT

memorandum

DATE: September 25, 1985

REPLY TO ATTN OF: Assistant to the Director, CNFRL

SUBJECT: Analytical Report

TO: RCA Coordinator, Region 5

Attached are the PNA analyses for 2 bullheads from Presque Isle Bay, Lake Erie. Jim Petty said that the remaining samples to be analyzed for PNAs will be completed in about a week.

RJG

Richard J. Graham

RJG:lw

SEP 0

OPTIONAL FORM NO. 10
(REV. 1-80)
GSA FPMR (41 CFR) 101-11.6
5010-114

*** MEMO ***

Jab. 31

Date : Sept 19, 1985

Reply to
Attn of : J. L. Zajicek

Subject : Report for PNA Analyses on Samples Submitted from USFWS Region 5 Collected from Lake Erie. Work unit 641.01.

To : L. Smith, J. Petty, T. Schwartz and R. Graham

Attached are the PNA residue results (Table 1) for two brown bullhead individuals (BRB-84-5-SCFO-43-1/#240 and BRB-84-5-SCFO-40-2/84090237/#237) collected from Lake Erie and submitted to CNFRL by A. Julien. These results are not corrected for recovery losses.

Residues for most of the PNAs analyzed in these two brown bullhead samples were in general below or slightly greater than the determination limit(s) of the gas chromatographic method used. Only naphthalene and 1-methylnaphthalene were detected in both samples at concentrations above the estimated method determination limit. Overall, these residue concentrations do not appear to be consistent with high level PNA contamination, such as that found in brown bullheads collected from the Black River in Ohio (Table 2).

Also attached is a detailed description of the materials and methods used in these analyses, and a brief discussion of the recovery data for spikes and Black River brown bullhead PNA QC environmental controls analyzed concurrently with these environmental samples.

MATERIALS AND METHODS:

All biological samples were ground as described by C. J. Schmitt et. al., 1984 (1). Briefly, whole body fish were cut into two to three inch cubes, then ground in a meat grinder, the ground tissue was manually mixed until it appeared homogeneous, and again passed through the grinder.

For analysis of PNAs, a 5.0 g aliquant of each sample and Q C material (including: 1. method reagent blank, 2. fish tissue blank(s), 3. fish tissue blanks spiked with a mixture of PNAs at nominally 500 ng/g per individual PNA, and 4. PNA environmental controls) was mixed with 15.0 mL of 3.5 M KOH using a Polytron tissue homogenizer (Brinkman Instruments) until a very fine suspension was obtained. This suspension was then transferred to a 50 mL centrifuge tube with teflon lined screw cap and for selected samples was mixed with 26,500 dpm of C-14 labeled dibenzanthracene (C-14DBA). Saponification was then carried out at 90 C in a heated water bath for 90 min. After 45 min. each sample was vortexed and then returned to the water bath. The samples were cooled with tap water and then 8.0 mL of a hydrochloric acid-phosphate (HCl-P04) buffer was added to each sample and Q C material and the pH was adjusted with 1M HCl or 1M NaOH to between 5 and 6. This minimized emulsion formation in the subsequent extraction step. The HCl-P04 buffer was prepared by dissolving 110 g NaH2P04 H20 in 300 mL HPLC grade water then adding 363 ml of concentrated HCl and finally diluting to a total volume of 880 mL with HPLC grade water.

Following pH adjustment, each saponified sample was extracted three suscessive times with separate 15.0 mL portions of a 20:80 (v/v %) methylene chloride:cyclopentane mixture. The extractions were carried out in the original 50 mL centrifuge tubes using manual shaking for five minutes. Each extract was then centrifuged for a minimum of 15 min. at 3000 rpm using a Dynac centrifuge (Clay Adams). After the upper organic layer had separated from the lower aqueous layer, all but approximately 1 mL of the organic layer was transferred into a 100 mL double reservoir evaporation flask (DB-flask, (2)). This process was repeated with a second and a third portion of the extraction solvent. The combined extracts were then reduced in volume to 5 mL by rotoevaporation at room temperature.

The concentrated extracts (not more than 500 mg of lipid) were then quantitatively applied to a potassium silicate-silica gel adsorption column. Approximately 99.5 % of the extracted biological lipid was removed by this single adsorption step. The column was prepared as follows:

A 1.1 cm ID. X 30.0 cm column with a 4.8 cm OD. X 8.0 cm long reservoir at the top and a Teflon stopcock with a glass tip at the bottom was used. A small amount of glass wool was conformed to the bottom of the column and topped with ca. 0.5 cm layer of sintered sodium sulfate. Next 8.0 g of EM 60 silica gel (activated in 190 C oven for at least 48 hours) was added followed by 20 g 130 C activated potassium silicate, 7.0 g 50/50 (w/w %) potassium silicate (130 C activated)/sintered sodium sulfate and topped with an ca. 1 cm layer of sintered sodium sulfate. The potassium silicate was prepared as follows:

250 mL of methanol was placed into a 1000 mL round bottom flask sitting in an ice water bath, and 84 g of KOH pellets were allowed to slowly disolve. When the KOH had dissolved, 150 g of unwashed EM 60 silica gel was added to the flask along with an additional 125 mL of methanol. The flask was then attached to a rotoevaporator (caution! no vacuum applied) and allowed to rotate for 90 minutes in a 55 C water bath. This material was then poured into a large glass column (ca. 5 cm X 100 cm) with a plug of glass wool at the bottom. A second batch was

prepared and also added to the large column. The methanolic KOH was allowed to drain away, after which the column was washed with 500 ml of methanol, then with 500 mL of methylene chloride. The column was dried with nitrogen gas or heluim and then activated in a shallow pyrex glass pan for heat activation at 130 C. After activation for a minimum of 48 hours, the potassium silicate was stored in a 130 C oven until used. Note! All adsorbants were washed extensively with methanol followed by pesticide grade methylene chloride prior to heat activation. The sintered sodium sulfate was baked at 475 C for 10 hrs.

After applying a sample to its potassium silicate column, the DB-flask was rinsed with two ca. 1 mL portions of the elution solvent, and these rinses were also applied to the column. The column was then eluted with the remaining portion of the 75 mL of 20:80 (v/v %) methylene chloride:cyclopentane elution solvent. The eluate from each column was then collected in 100 mL DB-flasks and the volume was reduced to approximately 5 mL using rotoevaporation at room temperature. These 5 mL samples were filtered using 2 mL Pasteur transfer-pipets that had a small plug of glass fibers at the bottom. Each pipet was rinsed with approximately two mL of methylene chloride to effect quantative transfer of the samples into 15 mL graduated, conical tipped centrifuge tubes. Each sample extract was then evaporated to approximately 0.5 mL under a gentle stream of nitrogen at room temperature. The filtration step was required to remove small (100-500 uM diameter) particles that would plug up the GPC column in the subsequent step.

These 0.5 mL samples were then quantitatively transferred into one mL sample loops of a modified GPC Autoprep 1001 (ABC Labs). A Waters Associates Model 6000 HPLC pump was substituted for the original pump, and two 1 cm X 22 cm long columns each packed with 3.8 g of SX-3 Biobeads, 270-320 mesh and connected in series were used as the separatory column. In addition the GPC effluent was connected in series with an LDC Model UVIII monitor with the wavelength set at 254 nm. From the resulting GPC/UV chromatogram of a standard mixture of two through five ring PNAs, the GPC dump and collect volumes were quickly determined prior to running each new group of samples. A 50:50 (v/v %) methylene chloride: cyclopentane mixture was used as the GPC mobile phase at a flow rate of approximately 1.3 mL/ min. This resulted in a dump volume of 26 mL and a collect volume of 14 mL. The Samples were collected in 15 mL graduated, conical tipped, centrifuge tubes and the solvent was evaporated to approximately 2ml using a gentle stream of helium.

At this point, 1.0 mL of internal standard spiking solution (containing 23.8 ug of 1-chloronaphthalene (1ClN), 19.8 ug of 9-chloroanthracene (9ClA) and 20.6 ug of 1-chloro-9,10,-diphenylanthracene (1Cl9,10DPA) in ethylene dichloride was added to each sample. The solvent volume was then further reduced to 1.0 mL using a gentle stream of helium, and a 100 uL aliquot of each spiked sample was removed for determination of C-14 dibenzanthracene recovery efficiency. Approximately 250 uL of each sample was transferred to 300 ul SCI-VI micro-crimp-cap autosampler vials (Chemical Research Supplies, Schaumberg, Il.) from which 3 uL of each sample was automatically injected into a PerKin-Elmer Model Sigma 2B gas-chromatograph (GC) using a Perkin-Elmer Model AS-100 autosampler.

The GC system consisted of a packed column injector (modified to allow for a variable septum purge, and containing an in-house designed quartz direct injection insert) operated in a capillary direct injection mode, connected to a 1.0 M X 0.32 mm ID. piece of uncoated fused silica capillary tubing via a 1/4 inch graphite-Vespel reducing ferrule. The outlet of the uncoated capillary was connected to a 15 M X 0.32 mm ID. analytical capillary column coated with a 1.0 uM Film of DB-5 (J&W Sci) using a low-dead-volume butt connector (Supelco, Bellefonte, PA). The outlet end of the analytical column was then connected via a second butt connector to a terminal 30 cm X 0.32 mm piece of uncoated fused

silica capillary tubing which extended up to within 1 mm of the detection chamber of a Perkin Elmer Photoionization detector. Installation of this terminal piece of uncoated fused silica facilitated operation of the temperatur restrictive PID at 270 C, without any detectable cold trapping of the less volitle five ring PNAs inside the relatively cool detector.

The following points should be kept in mind when connecting a capillary analytical column as described above to a PID.

1) Five ring PNAs are not eluted from the stationary phase of the analytical column until the GC-oven temperature reaches 280 C to 295 C. Thus, with the analytical column installed directly up to the PID detection chamber, a detecto temperature of 300 C is required to prevent cold trapping of these five ring PNAs inside the section of analytical column extenting into the detector.
2) When the outlet of the analytical column is connected to the PID detector insert at the outside of the detector, the chromatography is unacceptable due t the relatively large volume and/or activity of this insert. With this type of column to detector geometry, even at a detector temperature of 300 C the chromatography is characterized by poor sensitivity and excessive peak tailing.
3) It has been shown previously by the PID lamp manufacturer, that the PID lamp life-time, stability and signal-to-noise ratio are dramatically decreased as th detector temperature exceeds 275 C and approaches 300 C.

Hydrogen at a linear velocity of ca. 100 cm/sec (measured at 50 C oven temperature under constant pressure control @ 7.5 psig) was used as carrier and GC temperatures were: injector, 260 C; detector, 270 C; oven temperature programmed from 50 C with 2 min hold, then 4 C/min. up to 296 C with a final hold of 5 min.

Data was collected using a model 763SB interface (Nelson Analytical) and Nelsor Analytical Model 2600 chromatography software running on an IBM PC. Individual PNA components were tenatively identified using retention indices (RIs,(3)) based on three internal reference compounds (1C1N:RI=235.86, 9C1A:RI=336.38, ar C19,10DPA:RI=503.48). The RIs for all component peaks in each sample and standard chromatogram were calculated using software developed in-house and written in Digital MUMPS. In addition, these programs compared the calculated sample RIs with a reference template (library) of RIs we generated from chromatography of standard mixtures of PNAs under identical GC conditions. Whe sample component RIs matched those found in the template, the programs also calculated the component concentration by internal standard methods using 1C1N, 9C1A, and 1C19,10DPA as quantitative internal standards (QISs) and relative response factors calculated from external standards (at five concentration levels) of the 22 measured PNAs vs. these QISs.

DISCUSSION of RECOVERY DATA:

As described in the materials and methods section, selected QC samples were spiked with C-14DBA prior to beginning the saponification step. These C-14DBA recoveries therefore reflect the recovery of spiked C-14DBA through the entire saponification, extraction and clean-up procedure prior to GC/PID analysis. Table 3a contains the calculated recovery efficiencies for those QC materials analyzed concurrently with the Lake Erie samples. The average C-14DBA percent recovery was 80 percent, which is comparable to recoveries obtained by us in previous PNA analyses (mean recoveries from previous analyses ranged from 76% 85%).

Table 3b contains the percent recovery data for non-radiolabeled PNAs extracte from fish tissue spikes analyzed concurrently with the Lake Erie samples. The: recovery efficiencies are generally lower than than those obtained for C-14DBA

and include additional losses due to compound specific differences in volatility, solubility, and capillary GC/PID Detection. For the most part, the recovery efficiencies for non-radiolabeled PNAs were compound dependent and showed the largest differences for the more volatile two and three PNAs. Although these recovery efficiencies are lower than we are used to seeing for organochlorine pesticide analyses, they generally are similar to the 72 and 87 percent recoveries reported for anthracene and phenanthro(2,3-b)thiophene respectively in PNA analyses performed for CNFRL in the past by Dr. D. Vassilaros of Dr. M. L. Lee's laboratory at Brigham Young University.

Table 4 contains the residue results for the three PNA environmental control (PNA QC) samples analyzed concurrently with these Lake Erie samples. The EPA recommends that the control limits be equal to the mean plus or minus three times the standard deviation (99 % confidence level). And that plus or minus two times the standard deviation (95 % confidence level) be used for the method warning limits. As indicated by the superscripts in Table 4, all residue values with the exception of those for naphthalene and acenaphthene and one observation each for acenaphthylene and anthracene fall within plus or minus two times the standard deviations from their respective means listed in Table 2. This is rather good agreement considering that the control limits are based on only five observations each. Since, it is generally accepted that at least 20 observations are required to accurately determine the control mean and standard deviation values.

REFERENCES:

(1) Schmitt, C.J., Zajicek, J.L., and Ribick, M.A., 1985. National pesticide monitoring program: Residues of organochlorine chemicals in freshwater fish, 1980-81. Archives of Contamination and Toxicology (In Press).

(2) May, T.W. and Stalling, D.L., 1979. Double-resevoir rotoevaporation vessel for residue analysis. Anal. Chem. 51:169-170.

(3) Vassilaros, D.L., Kong, R.C., Later, D.W., and Lee, M.L., 1982. Linear retention index system for polycyclic aromatic compounds: Critical evaluation and additional indices. J. Chromatogr., 252:1-20.

Table 1. Concentrations of Selected PNA's in Lake Erie Brown Bullhead Submitted by Region 5. (CNFRL Sept. 1985)

Residue Name	Concentrations as ng/g, wet weight		
	BRB 84-5-SCFO-43-1/ (#240)	BRB 84-5-SCFO-40-2/ 84090237/ (#237)	
	Rep 1	Rep 1	Rep 2
naphthalene[c]	36	92	34
1-methylnaphthalene	20	36	<19[a]
2,3-dimethylnaphthalene	<15[a]	<2[a]	<2[a]
acenaphthylene[c]	<15[a]	ND	ND
acenaphthene[c]	ND	46	ND
fluorene[c]	ND	<3[a]	ND
dibenzothiophene[d]	ND	ND	ND
phenanthrene[c]	ND	<13[a]	<15[a]
anthracene[c]	ND	ND	ND
methyl-phenanthrene	ND	<15[b]	ND
fluoranthene[c]	ND	<48[b]	ND
pyrene[c]	ND	ND	ND
benzo(b)naphtho(2,1-d)-thiophene[d]	ND	ND	ND
benzo(a)anthracene[c]	ND	ND	ND
chrysene[c]	ND	ND	ND
benzo(b)fluoranthene[c]	ND	ND	ND
benzo(k)fluoranthene[c]	ND	ND	ND
benzo(a)pyrene[c]	ND	ND	ND
perylene	ND	ND	ND
indeno(1,2,3-c,d)pyrene[c]	ND	ND	ND
dibenz(a,h)anthracene[c]	ND	ND	ND
benzo(g,h,i)perylene[c]	ND	ND	ND

< - less than - Indicates that a peak was detected for this residue and the calculated concentration is less than the estimated method determination limit.

[a] - method determination limit is 20 ng/g.

[b] - method determination limit is 50 ng/g.

ND - not detected - Indicates that no peak was detected for this residue.

[c] - EPA priority pollutant PNA.

[d] - sulfur heterocyclic PNA.

Table 2. Stastical Summary of PNA Residue Concentrations in Black River Brown Bullhead PNA QC Environmental Control Sample Analyzed Five Times.

RESIDUE NAME	MEAN CONC. (ng/g, wet weight)	STANDARD DEV. (or RANGE) (ng/g)	% RS
APHTHALENE	129	13.8	10.7
CENAPHTHYLENE	157	22.5	14.4
CENAPHTHENE	127	28.3	22.2
LUORENE	488	78.1	16.0
HENANTHRENE	1219	423.6	34.7
NTHRACENE	248	26.4	10.6
LUORANTHENE	775	153.8	19.9
YRENE	423	73.9	17.5
ENZ(a)ANTHRACENE	<10	(ND - 31)	---
HRYSENE	47	26.8	57.5
ENZO(b)FLUORANTHENE	<17	(ND - 25)	---
ENZO(k)FLUORANTHENE	<16	(ND - 25)	---
ENZO(e)PYRENE	--- *	---	---
ENZO(a)PYRENE	<19	(ND - 30)	---
NDENO(1,2,3-cd)PYRENE	<13	(ND - 25)	---
,2,5,6-DIBENZANTHRACENE	--- *	---	---
ENZO(ghi)PERYLENE	<54	(ND - 110)	---
ERYLENE	<14	(ND - 26)	---
IBENZOTHIOPHENE	173	66.1	38.2
ENZO(b)NAPHTHO(2,1-d)-THIOPHENE	<10	(ND - 3)	---
	% OF WET WEIGHT N=3	STANDARD DEV.	% RSD
IPID	5.3	0.5	8.6
OISTURE	73.3	0.5	0.7

- Range - Indicates the lowest to highest values when the standard deviation was not calculated because the compound was not detected in one or more of the control samples.

< - less than - Indicates that the mean residue concentration is probably less than the concentration reported, since the residue was "not detected" in one or more of the samples analyzed. The "<" also indicates that the mean residue concentration was estimated by substituting a concentration of 10 ng/g (one half the estimated method detection limit) for all samples where a residue was "not detected".

ND - not detected- Indicates that the residue was was not detected. The method detection limit was estimated to be 20 ng/g, although for some samples we found residue levels below 20 ng/g (see RANGE above).

--- - no value determined.

* - Reference standard back-ordered.

Table 3a. ^{14}C Dibenzanthracene Recoveries from Spiked Fish Analyzed Concurrently with Region 5 Brown Bullheads.

Spiked Sample	% ^{14}C Dibenzanthracene Recovered
#1 Method Blank	82
#9 Tissue Blank	77
Mean % recovered	80

Table 3b. Recoveries for Selected Non-Radiolabeled PNA from Spiked Fish Tissue Analyzed Concurrently with Region 5 Brown Bullheads.

Residue Name	Spiking Level (ng/g)	% Recovery		Average % Recovery
		Spike 1	Spike 2	
nathphalene	498	19	68	44
acenaphthylene	459	64	82	73
fluorene	438	75	82	79
dibenzothiophene	618	74	82	78
phenanthrene	451	64	81	73
anthracene	269	41	70	56
fluoranthene	936	74	83	79
pyrene	695	59	66	63
benzo (b)naphtho (2,1-d)-thiophene	784	65	64	64
benzo (a)anthracene	582	74	68	71
chrysene	785	59	69	64
benzo (a)pyrene	178	106	61	84

Black River Brown
Five Times.

% RS

10.7
14.4
22.2
16.0

Table 4. PNA Residue Concentrations for Black River PNA QC Environmental Control Samples Analyzed Concurrently with Region 5 Brown Bullheads. (CNFRL Sept. 1985)

Residue Name	Concentration ng/g wet tissue		
	PNA QC-1	PNA QC-2	PNA QC-3
naphthalene	187[c]	81[c]	174[c]
acenaphthylene	229[c]	152[a]	162[a]
acenaphthene	211[b]	175[a]	186[b]
fluorene	589[a]	508[a]	493[a]
phenanthrene	1797[a]	1560[a]	1640[a]
anthracene	224[a]	249[a]	172[c]
fluoranthene	1070[a]	1080[a]	1070[a]
pyrene	563[a]	372[a]	523[a]
chrysene	65[a]	83[a]	43[a]
dibenzothiophene	248[a]	234[a]	219[a]

a - <± 2 SD
b - <± 3 SD
c - >± 3 SD

4 RS

10.7
14.4
22.2
16.0

APPENDIX B

COMMENTS FROM THE PENNSYLVANIA DEPARTMENT OF ENVIRONMENTAL RESOURCES ON LAKE ERIE FISH RESIDUE DATA

CR

COMMONWEALTH OF PENNSYLVANIA
DEPARTMENT OF ENVIRONMENTAL RESOURCES
Post Office Box 2063
Harrisburg, Pennsylvania 17120
August 12, 1985

717-787-2666

Mr. Edward W. Perry
Acting Field Supervisor
U.S. Fish and Wildlife Service
Suite 322
315 South Allen Street
State College, PA 16801

Dear Mr. Perry:

We have reviewed the raw data from fish tissue sampling conducted by your office in Presque Isle Bay on August 1, 1984 and contained in your June 24, 1985 transmittal. Our comments are presented below.

The type of tissue analyzed is not specified in the results we received. Were the samples whole fish or edible portion (fillets)? If the samples were fillets, were they skin-on or skin-off? The type of sample should be specified in order to allow proper comparison of this data to other samples and to FDA Action Levels. For your information, we are currently considering entering into an agreement with the EPA Great Lakes National Program Office which specifies the collection of skin-on fillets when sampling fish tissue from Lake Erie. This will allow the establishment of a common data base for all jurisdictions and will allow for comparison to FDA Action Levels. You may want to consider including skin-on fillets in any future work on Lake Erie so that your samples can be included in the data base.

My staff compared your data to FDA Action Levels, even though the type sample was unknown. The FDA Action Level for PCB was exceeded in the samples of brown bullhead (SCFO-40-2, 42-1, and 43-1), bluegill (SCFO-41-3), and largemouth bass (SCFO-40-1). The levels were all quite high (4.8 ppm or higher). The FDA Action Level for total chlordane was also exceeded in these samples. These levels indicate a cause for concern, even if they are in whole fish. If they were whole fish samples, we recommend resampling the edible portion to determine the levels to which sport fishermen are being exposed. Skin-on fillets should be collected for the reasons noted above.

The PCB and chlordane levels reported in your data are quite a bit higher than those found in our "CORE" fish tissue monitoring in Lake Erie and Presque Isle Bay over the last few years. In his comments to you, Robert Wellington of the Erie County Department of Health also noted that your results are considerably different from those found in their sampling and analysis. This discrepancy in results is another reason to resample and include the edible portion.

Please keep us in informed of your plans for any additional sampling in Presque Isle Bay and of any analysis results. If additional sampling reveals FDA Action Level exceedances in the edible portion, the public should be notified. Any required press release should be issued jointly by all agencies involved.

Sincerely,

Louis W. Bercheni

Louis W. Bercheni, Director
Bureau of Water Quality Management

CR

COMMONWEALTH OF PENNSYLVANIA
DEPARTMENT OF ENVIRONMENTAL RESOURCES
Post Office Box 2063
Harrisburg, Pennsylvania 17120

Bureau of Water Quality Management

717-787-9633

October 30, 1985

Mr. Edward W. Perry
Acting Field Supervisor
Fish and Wildlife Service
Suite 322
315 South Allen Street
State College, PA 16801

Re: DER File No. 18-12.2.1

Dear Mr. Perry:

We have reviewed the results of polynuclear aeromatic hydrocarbon (PNA) analysis on brown bullheads from Presque Isle Bay, Pennsylvania. We are a bit confused as to what was analyzed. Your cover letter indicates that the samples were composites of five whole fish. The correspondence from your laboratory indicates that two individuals were analyzed. The tissue type analyzed needs to be clarified.

We were pleased to note that the PNA levels in the Presque Isle samples were generally below the level of detection. As noted by your chemist, only naphthalene and 1-methylnaphthalene were found above detection limits. We concur with the statement by your chemist that these concentrations are not consistent with high levels of PNA's in the environment, as compared to the Black River, Ohio, samples attached to the report.

We look forward to receiving the histopathology results from Presque Isle Bay. Hopefully, they will also indicate few problems. If problems are indicated in the necropsies, we hope that definite cause/effect relationships can be established. This will allow the responsible agencies to begin developing strategies for any needed remedial actions. Our review of the histopathology results would be aided by comparisons to Black River, Ohio, fish because of the indications of contamination at that site.

Sincerely,

Richard H. Shertzer

Richard H. Shertzer, Chief
Quality Assessment Unit
Division of Water Quality