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Environmental Contamination and Toxicology

Evidence for Polynuclear Aromatic Hydrocarbons in the Diet of Bottom-Feeding Fish

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Aquatic environments in industrialized areas receive inputs of pollutants which may be toxic, mutagenic, or carcinogenic. Several studies have suggested that increased frequencies of neoplasms in fish from impacted areas may be due to environmental pollution (Dawe et al. 1964; Brown et al. 1973; Sonstegard 1977; Pierce et al. 1978; Black et al. 1981; Baumann et al. 1982; Black 1983). It has also been suggested that bottom-dwelling fish are exposed to carcinogens during feeding and thus could be useful monitors for environmental hazards (Dawe et al. 1964; Sonstegard 1977).

Among environmental pollutants, polynuclear aromatic hydrocarbons (PAH) are of interest because of their widespread occurrence (Andelman and Suess 1970) and the human carcinogenicity of their metabolic products (Gelboin and Ts'o 1978). Most PAH are relatively water insoluble and ultimately are deposited in sediments. In localized areas of eastern Lake Erie, sediments have been shown to have high concentrations of PAH (Black et al. 1981; Black 1983). In addition, several species of bottom-dwelling fish from these areas were found to have a variety of neoplasms and pre-neoplastic lesions (Black 1983). As a part of our ongoing research on environmental carcinogenesis, we have been examining possible routes of exposure to carcinogens in feral fish. We report here data indicating that the diet of bottom-feeding fish can contain substantial concentrations of PAH. Some of these PAH can be metabolically activated to carcinogens and may be involved in neoplasia in these fish.

MATERIALS AND METHODS

Fish were collected by gill netting from three sites in eastern Lake Erie (Figure 1). Fish were identified, weighed, measured, examined for gross pathology and selected tissues were fixed in 10% buffered formalin. Stomach contents from white suckers (Catastomus commersoni) were removed and frozen for subsequent analysis (Table 1). In general, the nature of the material in the stomachs was impossible to determine, although on occasion, small snails and clams were observed.

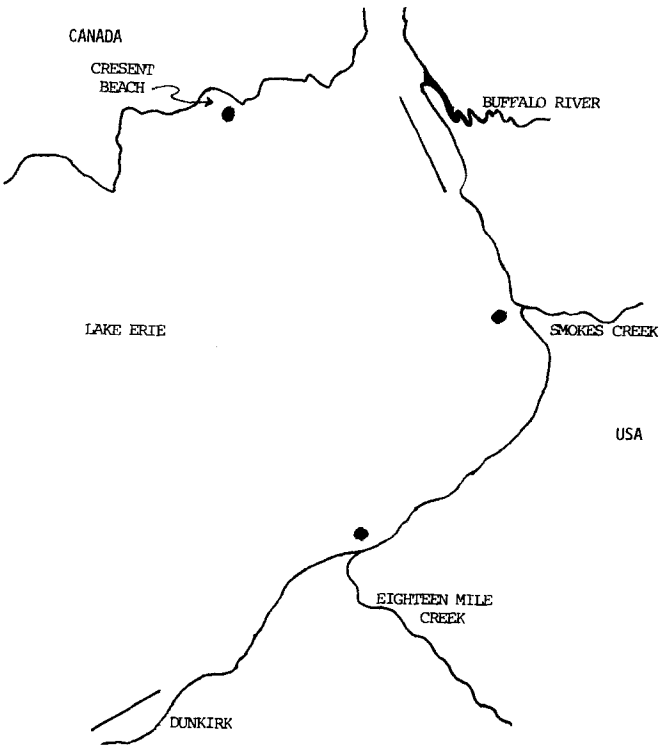

Figure 1. Diagramatic map of eastern Lake Erie showing location of sampling sites (●).

Table 1. Summary of fish samples taken for pathology and analysis of stomach contents.

SITE	NUMBER OF FISH	LENGTH (cm)[a]	WEIGHT (g)[a]	STOMACH CONTENTS(g)[b]
SMOKES	11	45.5±3.2	964.8±141.8	48.2
18 MILE	6	46.1±2.4	964.0±133.8	26.2
CRESCENT	5	42.6±2.2	910.8±175.1	20.8

a values are mean ± standard deviation
b total wet weight of pooled stomach contents extracted

Stomach contents were thawed at room temperature and samples from the same site were pooled. The total wet weight of each pooled sample was determined and the samples were transferred to 500 mL round bottom flasks for extraction with 150 mL of ethanol and 7 g of potassium hydroxide. After three hours of refluxing, the sample mixtures were liquid/liquid partitioned into cyclohexane and then concentrated on a flash evaporator to approximately 15 mL. The concentrated samples were then cleaned up on a column of 2.5% deactivated Florisil (Black et al. 1979). Columns were eluted with 80 mL of hexane followed by 150 mL methylene chloride: hexane (1:1 volume) and the PAH containing fraction was collected. The methylene chloride:hexane fraction was solvent exchanged into dimethyl sulfoxide (DMSO) and back extracted into cyclohexane (Dunn and Stich 1976). Finally, the samples were solvent exchanged into fresh DMSO with the volume adjusted so that the concentration of original wet weight extracted was the same in each sample.

Samples (5µL) were injected onto a high pressure liquid chromatograph equipped with a Vydac reversed phase PAH selective column (201TP 54.6 C_{18}, The Separations Group). A linear gradient from 40% acetonitrile in water to 100% acetonitrile was run over 40 minutes with a final hold at 100% for 15 minutes. Compounds were detected sequentially by absorbance (254 nm) and fluorescence (excitation 295 nm, emmission 410 nm). Compounds were identified by retention relative to phenanthrene and chrysene (absorbance chromatograms) or to benzanthracene and benzo(k)fluoranthene (fluorescence chromatograms). Individual PAH response factors were used for quantitation by peak area.

RESULTS AND DISCUSSION

HPLC analysis of stomach contents demonstrated varying amounts of PAH depending on sampling site (Figure 2). Samples from Crescent Beach and 18 Mile Creek had similar chromatographic profiles, although the concentrations of certain PAH were different. The chromatographic profile of Smokes Creek was more complex, having more compounds. Moreover, higher concentrations of individual PAH were observed in samples from Smokes Creek with the exception of a very high concentration of fluoranthene found in Crescent Beach samples (Table 2).

In general, levels of PAH in stomach contents of fish from Crescent Beach and Smokes Creek paralleled values reported for sediments from these sites (Black 1983). These sites are considered to be clean (Crescent Beach) and polluted (Smokes Creek) with respect to PAH input. In addition, the 18 Mile Creek site is also considered to be relatively clean as it is not directly impacted by any industrialized discharges (Black unpublished). Sediment from Smokes Creek had a chromatographic PAH profile similar to that observed for the stomach contents of fish from Smokes Creek (Figure 3). Each peak that was resolved in the stomach contents chromatogram was also seen in sediment chromatograms, albeit at higher concentrations. Black et al.

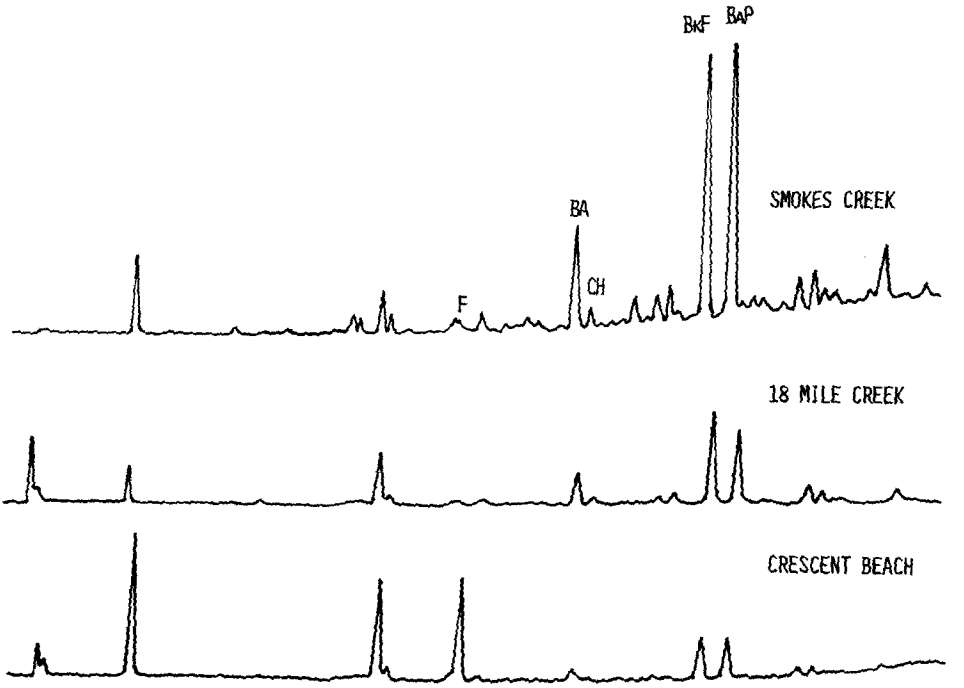

Figure 2. HPLC chromatograms (fluorescence detector) comparing levels of PAH in stomach contents of fish from three sites. F-fluoranthene, BA-benzanthracene, CH-chrysene, BkF-benzo(k)-fluoranthene, BaP-benzo(a)pyrene.

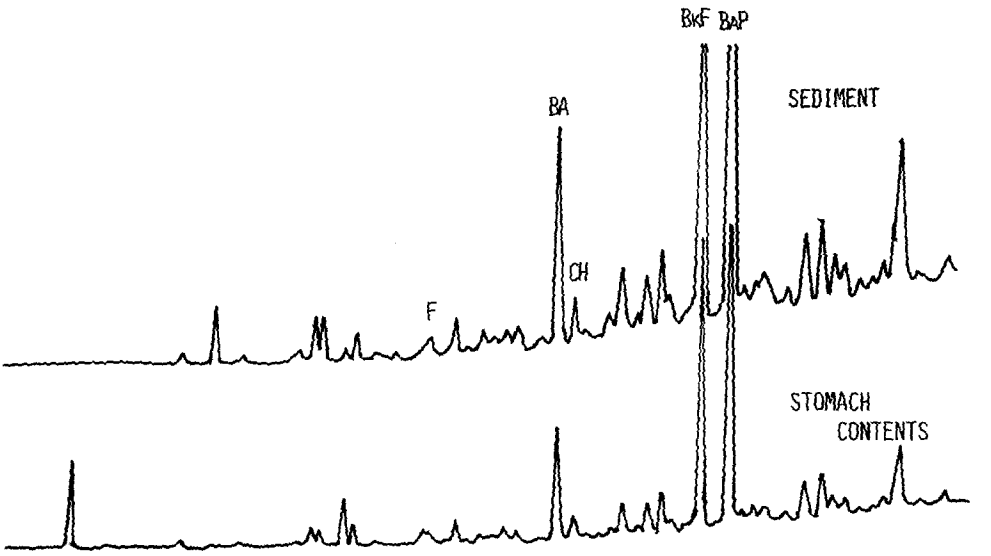

Figure 3. HPLC chromatograms (fluorescence detector) comparing levels of PAH in sediment (1X conc.) and stomach contents (20X conc.) samples from Smokes Creek. F-fluoranthene, BA-benzanthracene, CH-chrysene, BkF-benzo(k)fluoranthene, BaP-benzo(a)pyrene.

(1981) reported similar results for worms and sediment taken from the Buffalo River, Buffalo, New York. It is reasonable to assume that the fish in this study are receiving at least part of their PAH load via ingestion of PAH contaminated food or sediment. Moreover, stomach contents may reflect an integrated sampling of PAH in sediment because white suckers would be expected to browse a fairly large bottom area. This fact, coupled with the correlation of relative concentration of PAH in stomach contents reflecting the relative concentration of PAH in sediments, suggests that analysis of stomach contents of bottom-feeding fish might be used to determine PAH levels in areas where sediment samples are difficult to obtain.

With respect to individual PAH, the carcinogens benzo(a)pyrene and benzanthracene are of particular interest. In this study, the stomach contents of white suckers from Smokes Creek had the highest concentration of these two PAH (Table 2).

Table 2. Concentrations[a] of selected PAH in stomach contents of white suckers as determined by HPLC.

PAH	CRESCENT	18 MILE	SMOKES
Fluorene[b]	ND[c]	ND[c]	15.40
Phenanthrene[b]	23.37	25.65	43.07
Anthracene[b]	1.95	2.17	2.11
Fluoranthene	731.84	ND	109.99
Pyrene	8.17	2.42	73.63
Benzanthracene	4.00	9.63	25.88
Chrysene	3.35	9.70	26.11
Benzo(b)fluoranthene	3.24	6.20	17.00
Benzo(k)fluoranthene	2.08	4.37	11.08
Benzo(a)pyrene	1.94	3.93	15.48
Dibenz(a,h)anthracene	1.17	1.63	4.11
Benzo(g,h,i)perylene	2.68	2.04	14.07
Indeno(1,2,3-c,d)-pyrene[b]	ND	ND	14.95
TOTAL	783.79	67.74	372.88

a concentration in ng/g wet weight stomach contents
b determined by U.V. absorbance, all others determined by fluorescence
c ND = not detected

Observations indicate that among the three sites compared in this study, fish from Smokes Creek have a high incidence of neoplasms and pre-neoplastic lesions (Black 1983). Although further studies are required, the correlation of PAH levels and tumor frequency suggests that the two may be related (even though PAH compounds

per se may not be the actual carcinogens). This suggestion is further supported by the demonstration of carcinogenic effects of river sediment extracts in both liver and skin (using dietary and skin painting exposures, respectively) of brown bullheads (Black 1983).

It has been demonstrated that fish can metabolize PAH from contaminated food and sediments to carcinogenic compounds (Varanasi and Gmur 1981a,b; Gmur and Varanasi 1982). Moreover, tumor formation in fish fed carcinogen-contaminated diets has been observed (Halver 1967; Sinnhuber et al. 1968; Wales et al. 1978; Hendricks 1982). Although it is possible that the fish in our study may be taking PAH from their diet or the sediment and metabolizing them to carcinogens, a direct link between PAH found in the stomach contents and neoplasia in the white suckers has not been demonstrated. As additional observations and data are reported from more sites, it is becoming increasingly apparent that feral fish are good sentinel animals for environmental pollution and could be extremely valuable as early indicators of potential health hazards to man.

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