Decline in Liver Neoplasms in Wild Brown Bullhead Catfish after Coking Plant Closes and Environmental PAHs Plummets

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Polycyclic aromatic hydrocarbons (PAHs) in both sediment and brown bullhead catfish tissues from the Black River in Lorain County, Ohio, declined by 65% and 93%, respectively, between 1980 and 1982. Sediment PAHs declined an additional 99% by 1987, coincident with the closure of a coking facility in 1983. Contemporaneously, liver cancer in 3- to 4-year-old brown bullheads declined to about one-quarter the 1982 frequency (10% versus 39%) by 1987, while the percentage of livers without any proliferative lesions doubled (42% versus 20%). These changes were significant within age group. Our data affirm a cause-and-effect relationship between PAH exposure and liver cancer in wild fish. The data also support the efficacy of natural, assisted remediation once the source of the pollution is eliminated. Key words: brown bullhead catfish, cancer, catfish, neoplasms, polycyclic aromatic hydrocarbons, remediation, sediment. Environ Health Perspect 103:168–170 (1995)

Liver neoplasms are prevalent in populations of benthic fish inhabiting dozens of chemically contaminated waterways (1), and research data strongly link these tumors with high sediment levels of polycyclic aromatic hydrocarbons (PAHs) (2–4). We have studied liver tumor frequencies in a population of freshwater fish, the brown bullhead catfish (Ameiurus nebulosus), from a PAH-contaminated location, the Black River, Ohio, periodically since 1980 (2). After a sharp decline in the steel and associated coking industry in 1982, the USX coking facility on the Black River was closed in 1983. This provided an opportunity to test time-order and consistency in an epizootic study by comparing liver tumor frequencies of fish ages 3 and 4 hatched after 1982 with those of fish the same ages sampled in 1982 (5).

Methods

The Black River empties into Lake Erie at Lorain, Ohio. The USX steel facility and inoperative coking plant are co-located approximately 5.5 km upstream. Surficial sediments (approximately the top 5 cm) were collected and analyzed in a series of studies between 1980 and 1987 (2,6–8). All of these studies except the one in 1980 (2) collected sediment by dredge from at least five depositional sites within 1 km from the coking plant outfall downstream (7). In 1980, all sediments were collected within 100 m of the coke plant outfall (2). After extraction, sediment PAHs were analyzed by capillary column gas chromatography and gas chromatography–mass spectrometry (7,8). A suite of metals and organochlorines were also analyzed in the Black River sediment sample taken in 1984 (9).

We collected brown bullhead of 250 mm or longer, corresponding to age 3 or older, within 1 km of the coke plant discharge pipe by overnight sets of Fryke nets (5) during 1980, 1981, 1982, and 1987. Brown bullhead were sampled for chemical analysis in May 1980 (two composites of five fish each), September 1981 (two composites of three fish each), and September 1982 (eight individual fish). We analyzed whole fish for PAHs using a gas chromatography–mass spectrometry procedure (10).

Brown bullhead >250 mm were randomly selected in the spring of 1982 (N = 124) and 1987 (N = 80) for necropsy and histopathology (5). Livers were excised and preserved in 10% neutral-buffered formalin. Tissue blocks were cut at approximately 1-cm intervals from four to seven levels with increasing liver size and to include grossly visible lesions. Blocks were sectioned at 5 µm, stained with Mayer’s progressive hematoxylin and counter-stained by alcoholic eosin phloxine. Lesions were diagnosed and slides archived at the Registry of Tumors in Lower Animals, Smithsonian Institution.

We determined the age of the fish by examining pectoral spines. Spines were removed in the field and processed by a modification (5) of the methods of Marzolf (11) and Sholl (12). Spines were decalcified and sectioned with a microtome cryostat to varying (25–150 µm) thicknesses. We counted rings denoting annual growth cycles using both a dissecting scope with refracted light and projection scope.

Liver lesions of hepatocellular origin were diagnosed at three levels of severity by criteria already described (5). Hepatocytic neoplasms ranged from a pre- or incipient stage (hepatocellular alteration) through a benign stage (hepatocellular adenoma) to cancer (hepatocellular carcinoma). Cholangiocytic (biliary) neoplasms ranged from a benign stage (cholangioma) to cancer (cholangiocarcinoma) (5). Individual fish with multiple lesions, which was usually the case, were categorized only according to the most advanced liver lesion of each type diagnosed histologically. Thus, a specimen with cancer as well as less advanced lesions would be included only with the segment of the population having cancer. However, specimens were enumerated separately for both biliary and hepatic neoplasms. We used a chi-square test to statistically compare categories of age and tumor frequency.

Results and Discussion

Surficial sediment concentrations of PAHs declined rapidly from 1980 through 1987 (Table 1). PAHs in sediment had decreased to 34.8%, 16.9%, and 0.39% of the 1980 total by 1982, 1984, and 1987, respectively. Residues of PAHs in whole bullheads declined even more rapidly between 1980 and 1982 (Table 2). The 1982 concentrations of 11 PAHs identified ranged from about one-quarter (chrysene) to one-thirtieth (phenanthrene) of the corresponding average residue levels for 1980 and 1981. Analysis of sediment samples taken from five Great Lakes tributaries in 1984 demonstrated that the Black River sediment contained lower levels of arsenic, cadmium, chromium, nickel, polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, and non-ortho-polychlorinated biphenyls than did sediment from rivers where no epizootic of neoplasia existed in bullheads (9). Although the 1980 sediment sample collection had been concentrated closer to the coke plant outfall than that in 1982, the more than 90% decline in PAH residues in fish between 1980–1981 and 1982 argues for a real decrease in PAH exposure. Observational data on freighter traffic indicated a severe decline in the steel industry in 1982, before the final closure of the coke plant in 1983.

Neoplasms of all types in brown bullheads aged 3 and older declined by 46% between 1982 and 1987, whereas frank
Table 1. Concentrations of selected polycyclic aromatic hydrocarbons (PAH) in sediment taken from the Black River, Ohio, in 1980 (2), 1982 (6), 1984 (7), and 1987 (8)

| PAH                  | 1980   | 1982   | 1984   | 1987   |
|---------------------|--------|--------|--------|--------|
| Acenaphthylene      | 40.0   | 8.00   | 17.00  | 0.08   |
| Acenaphthene        | 36.0   | 7.20   | 2.50   | 0.14   |
| Phenanthrene        | 390.0  | 100.0  | 57.00  | 0.79   |
| Fluoranthene        | 220.0  | 76.00  | 33.00  | 0.79   |
| Pyrene              | 140.0  | 56.00  | 24.00  | 0.93   |
| Benzo[a]anthracene  | 51.00  | 23.00  | 11.00  | 0.74*  |
| Chrysene/triphenylene | 51.00   | 25.00  | 10.00  |        |
| Benzo[ghi]perylene  | 43.00  | 21.00  | 6.80   | 0.24   |
| Benzo[a]pyrene      | 75.00  | 37.00  | 15.00  | 0.59   |
| Benzo[ghi]pyrene    | 24.00  | 13.00  | 5.40   | 0.03   |
| Indeno[1,2,3-cd]pyrene | 26.00   | 15.00  | 6.40   | 0.01   |
| Total               | 1096   | 381.2  | 185.1  | 4.27   |

*Combined peaks for benzo[a]anthracene, chrysene, and triphenylene.

Table 2. Whole-body polycyclic aromatic hydrocarbon (PAH) residues in 3-year-old brown bullhead catfish from the Black River, Ohio, in 1980 (2), 1981, and 1982

| PAH                  | 1980   | 1981   | 1982   |
|---------------------|--------|--------|--------|
| Dibenzothiophene    | 509    | 832    | 91.9   |
| Phenanthrene        | 3930   | 7570   | 161    |
| Fluoranthene        | 1260   | 4040   | 129    |
| Pyrene              | 756    | 1570   | 83.9   |
| Chrysene            | 60.5   | 42.0   | 13.2   |
| Total               | 6515.5 | 14,054 | 478.1  |

carcinomas dropped by 74% (Table 3). Both of these changes were significant (p ≤ 0.01). Conversely, the percentage of fish with livers free of neoplasms and foci of hepatocellular alteration doubled between 1982 and 1987. Frequencies of hepatocellular alteration did not show a decline.

When compared within age group, the decline in cancers and increase in normal livers remained significant (Table 4). For each age group, the percentage of bullheads with normal livers approximately doubled between 1982 and 1987, while the cancer rate decreased by three-fourths. Frequencies of hepatocellular alteration and benign neoplasms did not show any significant change within age classes.

Biliary cancers declined significantly for both age 3-year-old and 4-year-old fish between 1982 and 1987 (Table 4). Frequencies in 1987 were about one-fourth those in 1982. Benign biliary lesions declined for both age groups, but the decline was not significant. Hepatocellular cancer also decreased significantly for both age groups (Table 4). Three-year-old fish had only one-seventh the cancer frequency in 1987 as in 1982, and no hepatocarcinomas were found in age 4 fish in 1987, even though this age group had a 16% frequency of those lesions in 1982. In contrast, the frequency of hepatocellular alterations (in 3 year olds) and hepatocellular adenomas (in 4 year olds) increased significantly between 1982 and 1987.

The extremely high tumor frequency noted in 1982 was not a single-year phenomenon. Surveys of brown bullheads in the Black River began in 1980 and 1981, with only grossly visible lesions being counted and preserved for histopathology (2). In 1980 and 1981, 3-year-old fish had a tumor frequency of 18% and 12%, respectively, and 4-year-old fish had a tumor frequency of 44% and 29%, respectively (13). All of the tumors preserved were diagnosed as cholangiocarcinomas. Hepatocellular lesions were not greatly apparent in the livers. These gross tumor frequencies from 1980 and 1981 are similar to the cholangiocarcinoma frequencies in the 1982 study (Table 4).

Three-year-old and 4-year-old fish captured in 1987 would have hatched in years 1984 and 1983, respectively. The significant decline in liver cancer in these fish compared to mature fish captured in 1982 (and exposed during prior years) corresponds to the significant decline in PAHs found in surfiacial sediment between 1980 and 1987 and the decline in PAHs residues from bullheads captured in 1982. Because bullheads primarily eat on or near the surface of the sediment (13), a decrease in exposure would be expected if PAH levels in sediment had been reduced. Reduced PAH input, siltation processes, and perhaps degradation have contributed to the observed decline in PAH levels in the surficial sediment.

Liver neoplasms in fish have been strongly associated with environmental carcinogens. At least 16 species have been documented with epizootic liver tumors in association with contaminated sediment in several dozen North American waterways. One extensive series of studies focused on liver tumor frequency in English sole (Pleuronectes vetulus) from different areas within Puget Sound, Washington (3). Only sediment concentrations of PAHs were significantly correlated with liver tumors in English sole; concentrations of metals and chlorinated hydrocarbons were not (14).

Similarly, mummichog (Fundulus heteroclitus) from a PAH-polluted site on the Elizabeth River, Virginia, had a 93% incidence of liver lesions and a 33% frequency of liver cancer, whereas a population of the same species directly across the river (600 m away) had no detectable lesions (4).

A cause-and-effect relationship between PAHs and liver neoplasia in populations of wild fish has been further strengthened by the experimental induction of liver tumors in fish by PAHs (15,16). Metcalfe et al. (17) induced liver tumors in rainbow trout (Oncorhynchus mykiss) by injecting sac fuel with extracts of PAH-contaminated sediment from Hamilton Harbour, Ontario. Similarly, Black et al. (18) induced both liver tissue and bile-duct tumors in brown bullheads fed commercial trout food spiked with extracts from the PAH-contaminated sediment from the Buffalo River, New York. Sediment extracts from the Black River, Ohio, induced an 80% tumor frequency when painted on Swiss mice (18). This was a higher tumor frequency than that induced in mice painted with 200 μg/ml benzo[a]pyrene as a positive control (64%).

This study is the first to record a reduc-

Table 3. Frequency by age of various liver pathologies in brown bullhead catfish collected from the Black River

| Age and year | N     | Normal | Cellular alteration | Noncancer neoplasms | Cancer |
|-------------|-------|--------|--------------------|---------------------|--------|
| Ages 3–6    |       |        |                    |                     |        |
| 1982        | 124   | 23.4   | 16.1               | 60.5**              | 38.7   |
| 1987        | 80    | 45.5   | 25.0               | 32.5**              | 10.0** |
| Age 3       |       |        |                    |                     |        |
| 1982        | 48    | 27.1   | 16.7               | 25.0                | 31.2   |
| 1987        | 42    | 45.2*  | 33.3               | 14.3                | 2.1**  |
| Age 4       |       |        |                    |                     |        |
| 1982        | 73    | 21.9   | 16.4               | 20.5                | 41.1   |
| 1987        | 29    | 41.4*  | 17.2               | 34.5                | 6.9**  |

*Significant difference between years, p<0.05.

**Significant difference between years, p<0.01.
Table 4. Frequency by age of specific neoplasms in brown bullhead catfish collected from the Black River

| Age and year | N  | HCA | HA | HC | C | CC |
|--------------|----|-----|----|----|---|----|
| Age 3        | 1982 | 48  | 16.7 | 18.8 | 16.7 | 8.3 | 18.8 |
| Age 4        | 1982 | 73  | 16.4 | 13.7 | 16.4 | 11.0 | 32.9 |
|              | 1987 | 29  | 17.2 | 34.5 | 0* | 3.4 | 6.9**|

Abbreviations: HCA, hepatocellular alteration; HA, hepatoadenoma; HC, hepatocarcinoma; C, cholangioma; CC, cholangiocarcinoma.
*Significant difference between years, p<0.05.
**Significant difference between years, p<0.01.

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