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Acute Toxicity of PAH Contaminated Sediments to the Estuarine Fish, *Leiostomus xanthurus*

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High concentrations of polycyclic aromatic hydrocarbons (PAH) have been found in the Elizabeth River, Virginia, especially in the Southern Branch near a former creosote wood preservation plant (Bieri et al. 1981, 1986; Lu 1982). Locally high concentrations may also be found near shipping, transfer and storage facilities. At one heavily contaminated site (Station 217, Fig. 1), sediment concentrations as high as 390 ug/g dry weight have been reported (Bieri et al. 1981; Hargis et al. 1984). In a core collected near Station 217 the concentration was 10 ug/g in the top 2 cm but over 200 ug/g at 30 cm depth (Lu 1982).

Although acute mortality of fish directly attributable to high PAH concentrations in sediment and water is unreported in the Elizabeth River, fish of several species often exhibit fin erosion and other external lesions (Huggett et al. 1987; Hargis and Colvocoresses 1986). Fish with lesions are more prevalent near Station 217 than elsewhere. During previous laboratory experiments with spot (Leiostomus xanthurus) exposed to naturally contaminated sediments from Elizabeth River Station 217 we observed acute mortalities within 8 days as well as fin erosion, ulceration of the lateral body surface, and several types of lesions of internal organs (Hargis et al. 1984). Exposure to effluent from primary exposure tanks resulted in ulcerations and cataracts, but no mortalities.

The present study was conducted to determine 1) the concentration of contaminated sediment causing an acute lethal effect on L. xanthurus exposed either to sediment or to water which had been in contact with sediment.

MATERIALS AND METHODS

Juvenile spot were seined from an uncontaminated site in the Ware River, Virginia (salinity = $20^{o}/_{oo}$). Spot were acclimated for 2 wk in outdoor fiberglass aquaria while being fed Ziegler Chow, Salmon Starter No. 3. No mortalities occurred during acclimation.

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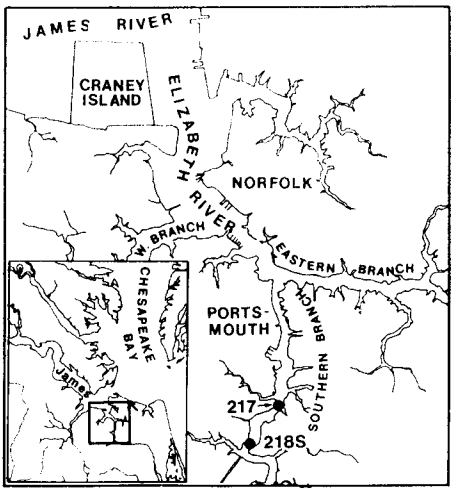

Figure 1. Map of Elizabeth River, Virginia showing Station ER217.

PAH-contaminated sediments were collected from Elizabeth River Station 217 (Fig. 1). The sediment samples, designated 100% ER217 sediments, were mixed manually. Control sediments were collected at a site about 365 m offshore from the Institute's laboratory on the York River. Sediments from this site have a particle size distribution similar to those from the Elizabeth River site (RJ Diaz, VIMS, personal communication).

Contaminated sediments were used as collected or in mixtures containing 1, 3.2, 10, or 32% of ER217 sediment diluted with York River sediment (v/v). Mixtures were stirred manually and placed in 37-L glass aquaria to a depth of 5 cm. Aquaria were filled with 10 um filtered estuarine water; a continuous flow of filtered water was then initiated to each aquarium (ca 400 mL/min). Overflow from each aquarium, including entrained resuspended solids, was passed to a second aquarium with no sediment on the bottom (effluent aquarium). Sediment samples were collected by coring each sediment-containing aquarium before and after the experiment. Samples were frozen in clean glass bottles until analyzed for PAH.

Spot were measured and weighed prior to the test. Fish were fed Ziegler chow at a rate of 3% of body wet weight per day. To evaluate whether observed effects resulted from starvation rather than toxicants directly, a second pair of control aquaria (sediment and effluent) were established in which the fish were unfed. Spot were measured and weighed upon death or when sacrificed after 28 d.

Spot were held 28 d in cages immersed in each aquarium with the bottom mesh slightly submerged in the sediment. This allowed fish direct contact with contaminated sediment but prevented them from burying themselves in sediment and thereby asphyxiating themselves. Cages were constructed of plastic-coated wire mesh (0.64 cm) and wood coated with clear epoxy. Cage bottoms in sediment-free effluent aquaria were covered with plastic to retain food. Because

sediment was frequently resuspended by fish foraging and swimming, there was considerable sediment oxygen demand thus necessitating aeration.

Water temperature in each aquarium was measured daily with a stem thermometer. Salinity was measured with a YSI (Yellowsprings, Ohio) Environmental Monitor and dissolved oxygen with a YSI Model 54 oxygen meter. Ambient pH was monitored daily with a Fisher Acumet pH meter (Pittsburg, Pennsylvania).

For PAH analysis, sediment samples were thawed, freeze-dried, and homogenized. Dried sediment was extracted with methylene chloride in a Soxhlet apparatus. Extracts were fractionated by gel permeation chromatography and high-performance liquid chromatography to eliminate biogenic organic compounds. The non-biogenic, non-polar fraction was spiked with 2,2'-binaphthyl and decachloro-biphenyl (internal standards) and analyzed by glass capillary gas chromatography with a Flame Ionization Detector (Varian 3700, Sunnyvale, California). Peaks were identified and quantified by computer analysis (Hewlett Packard, HP3350A Computer, Palo Alto, California) of retention indices and peak heights (Bieri et al. 1981; 1986).

RESULTS AND DISCUSSION

Sediment collected from Station 217 for this experiment contained 21,200 to 33,000 ug total PAH/g dry weight of sediment. Control sediments collected from the York River contained only 2 to 4 ug/g. PAH concentrations in sediments prepared by dilution of Elizabeth River sediment with sediments from the York River were always considerably less than the nominal concentration (Table 1).

Table 1. Total PAH concentration in sediments (ug/g dry weight) from the Elizabeth River and York River.

Treatment (% ER217)	Day 0	Day 15
Control	2	4
1.0	81	44
3.2	322	139
10	443	1720
32	2580	3820
100	21200	33100

While there were obvious quantitative differences in total PAH concentrations between treatments, proportions of each constituent PAH did not differ markedly among various sediment dilutions, or controls. Naphthalenes were an abundant low molecular weight fraction, representing 2 to 7% of the total resolved PAHs. Of the high molecular weight aromatic compounds, fluorene and pyrene were abundant. Phenanthrene was exceptionally abundant (20% of total resolved PAHs) in undiluted sediment (Table 2) when compared to concentrations from other samples (Bieri et al. 1981, 1986).

Water temperature was $25.8 \pm 1.2^{o}C$, and salinity was $15.1 \pm 1.0^{o}/_{oo}$

during the experiment with no significant differences among aquaria in either temperature or salinity (ANOVA, $p>0.05$). The mean oxygen concentration in sediment tanks for all treatments combined was 7.0 ± 0.8 mg/L whereas in effluent tanks, it was 5.7 ± 1.5 mg/L for all treatments combined. As a result of sediment resuspended by feeding activity, oxygen concentrations were depressed briefly in some aquaria to as low as 1.0 mg/L. The lowest mean oxygen concentrations, observed in the 3.2% effluent and 10% effluent tanks, were 4.66 ± 1.0 and 4.70 ± 0.8 mg/L respectively. In the corresponding sediment tanks, oxygen concentrations were much higher at 7.0 ± 0.7 and 6.7 ± 0.6 mg/L respectively.

Spot averaged 74 mm in standard length (90 mm total length) and 9.0 g wet weight at the beginning of the experiment. Fish size did not differ among treatments. Fed control fish did not increase in length or weight during the experiment indicating that the food ration closely approximated a previously determined maintenance level (Fisher 1985).

Table 2. Initial concentrations of selected PAH constituents (in ng/kg) in sediments from the Elizabeth and York Rivers.

	Control	100% *
Naphthalene	7	95000
Benzothiophene	72	2870
2-Methylnaphthalene	46	31800
1-Methylnaphthalene	18	nd
Biphenyl	18	85000
Fluorene	37	1250000
Dibenzothiophene	5	351000
Phenanthrene	97	4220000
Anthracene	18	264000
Fluoranthene	100	2370000
Pyrene	99	1350000
Benzo(a)fluorene	7	298000
Benzo(b)fluorene	28	282000
Benzo(a)anthracene	42	350000
Chrysene	58	317000
Benzofluoranthene	111	234000
Benzo(e)pyrene	42	78100
Benzo(a)pyrene	43	98500
Perylene	45	50700
Indeno(1,2,3-cd)pyrene	34	33800
Benzo(ghi)perylene	30	25500
Total resolvable PAH (in ug/g)	2	21200

* mean of 2 samples

The 24-h LC50 for sediment-exposed fish was 56% Elizabeth River sediment. The LC50 decreased to 51% after 7 days exposure, 16%

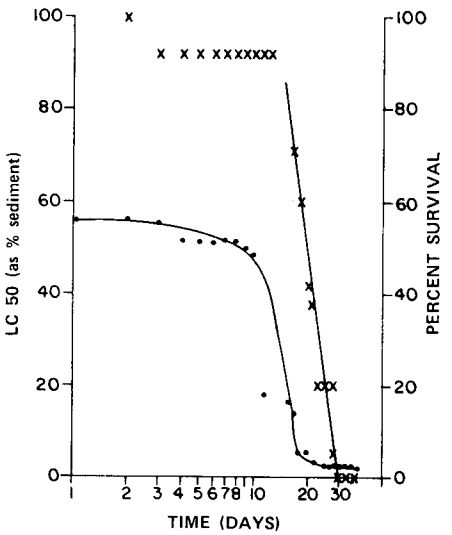

Figure 2. Comparison of temporal progression of LC50 (as percent sediment) with survivorship for starved control population. (^{x}---^{x} = starved control, ·----· = LC50 for Elizabeth River sediment).

after 12 days exposure, 2.9% after 21 days exposure, and 2.5% after 28 d exposure (Fig. 2). LC50s for the effluent exposed fish, expressed as percent sediment from which effluents were derived, were 80% after 24 hours exposure and 49% after 7 days exposure which is not different from the LC50 for fish exposed directly to sediment.

Without aeration, oxygen concentrations in several effluent aquaria were severely reduced on the eighth day causing partial or total mortality, a condition not observed in the sediment aquaria. After aeration was started, all spot were replaced in some effluent tanks on the eighth day. No further deaths attributable to oxygen deficiencies occurred after initiating aeration. The effects of effluent on replacement fish were much less severe on the day following exposure and after another 7 days than had been observed during the first 7 days, with a 24-h LC50 >100% ER217 and a 7-day LC50 equal to 77% ER217. Even after 20 days exposure, the effluent LC50 was 70% ER217.

All fish exposed to 100% contaminated sediment were dead within 2 h. The lethal time for 50% of the fish (LT50) was estimated graphically to be 57 m. Following these deaths, these highly contaminated sedi-ments were left undisturbed for 7 days during which the water flow was maintained. After that time another group of spot also exhi-bited rapid mortality, with none surviving longer than 5 hours; the LT50 was estimated to be 150 m even after a 7-d flushing period.

The LT50 for fish exposed to 3.2% sediment, a treatment with a sediment concentration slightly above the estimated 28-d LC50 concentration, was 19.5 d. Interestingly this group exhibited significant weight loss during the experiment despite receiving the same food ration as all other groups except the starved control

group. The starved control population of fish exhibited better than 90% survival for 11 days, followed by rapid mortality with no survivors after day 24 (Fig. 2). The estimated LT50 was 18 d.

LC50s calculated from the mortality data are expressed in terms of percentage contaminated sediment. Since fish respond to substances dissolved or suspended in the water column more than those in the sediment layer, sediment PAH concentration does not reflect actual exposure. Further, these experimental exposures do not permit one to unequivocally identify PAHs as the effective toxicant(s) since there are other potent toxic substances associated with Elizabeth River sediment (such as polar aromatic hydrocarbons, heterocyclic compounds, heavy metals, to mention a few) which were not measured. Many of these compounds are known toxicants which may contribute to the mortality observed in the present study; many PAHs identified in the sediments (e.g., naphthalenes and various pyrene derivatives, Table 2) or their decomposition and metabolic by-products are abundant and known to be toxic. Nevertheless, these LC50 values do serve to illustrate that sediments from ER217 are extremely toxic under the exposure conditions used and that a dose/time/response relationship exists.

There is evidence of a diminution of toxicity with time. This effect is most clearly seen in the longer LT50 for fish exposed to 100% sediment after a 7-d flushing versus newly-prepared sediment. This effect also appears in the higher 7-d LC50 in effluent tanks following fish replacement, although one could argue that the initial low LC50 values were due to low dissolved oxygen.

The lack of data documenting actual aqueous concentrations of PAH in both sediment and effluent tanks limits the interpretation of these data. Analysis of water in tanks was deemed impractical because of the large volumes of water to be extracted and the number of treat-ments to be analyzed. However, the similarity in LC50s for sedi-ment- and effluent-exposed fish after 7 days suggests that the immediately critical factor was not a toxicant in bottom sediment (since the effluent-exposed fish never contacted bottom sediment directly) but rather a toxicant in the water column, whether dis-solved or associated with suspended particles. If this were not the case, one would expect a lower LC50 for fish permitted direct con-tact with sediment than for fish with no contact.

Several studies (Neff and Anderson 1977) tested the effects of PAH-contaminated sediments. In these experiments, PAH sources such as crude oil or single compounds, were mixed with clean sediments, usually fine sands. Fish or other organisms were then exposed to these sediments. Based upon these studies, it is generally held that low molecular weight PAH (e.g. naphthalenes) are more toxic than high molecular weight compounds, partly because the former, being more water soluble, are more bioavailable than the latter. The present study neither confirms nor refutes this notion, though low molecular weight compounds were abundant in the test sediments. No direct comparisons of these earlier data and our study are possible because of different bases for evaluation of toxic responses.

Some evidence in the present study indicates that inability of fish to feed effectively may have contributed to the mortality. If one plots the progression of LC50s expressed as percent sediment against time, the LC50 remained relatively constant at 50-55% until day 8 and thereafter plummeted rapidly (Fig. 2). There was a second plateau in response from day 11 to 17 with a second rapid decrease in LC50 until day 21 when it stabilized at 2.5-2.9%. In contrast, starved control fish exhibited excellent survival until about day 15-16, nearly equivalent to that for fed control fish. Between day 15 and day 25, all starved control fish died, with a median lethal time of 18 days coincident with the second rapid change in LC50 for fish exposed to contaminated sediments or effluents. Further, spot exposed to low sediment concentrations exhibited significant weight loss, suggesting reduced ingestion or assimilation of food while fed control fish survived well and did not lose weight. While these manifestations may be mere coincidence, it is possible that PAH concentrations much below an acutely toxic concentration may so inhibit the total feeding process that fish may die of starvation. If such inhibition occurs in the Elizabeth River, there are potentially far-reaching consequences for fish populations in this river.

The initial objective of this experiment was to define sediment con-centrations which would allow prolonged exposure of spot to PAH con-taminated sediment for studies of chronic effects. This purpose has been partially accomplished even though various limitations of the data preclude a definitive interpretation of possible ecosystem effects in terms of sediment concentration. It is clear that concentrations of <10% ER217 sediment will be necessary for the long-term exposures originally contemplated, which translates to a PAH-sediment concentration of <500 ug/g. Sediment concentrations in the Elizabeth River may approach this level.

The 21,200 ug/g concentration of PAH in the 100% sediment is much higher than reported previously from sediments in the Elizabeth River, Virginia (Bieri et al. 1981, 1986; Lu 1982), though samples for all these studies were analyzed in the same laboratory. Sediment samples used in this study included material not only from the sediment surface, but also from layers perhaps as deep as 20-30 cm. Deep sediment layers contain higher concentrations of PAH than surface layers (Lu 1982). No other samples have been collected with as high a concentration as sediment used in this study.

The 500 ug/g corresponding to 10% ER217 sediment is well above the maximum concentration of PAH reported in estuarine sediments from around the world. The maximum concentration in sediment from a single site in Massachusetts Bay was 120 ug/g (Mix 1984; data of Windsor and Hites 1979). Concentrations similar to those in Massachusetts have been reported from Casco Bay, Maine (Larsen et al. 1983). The similarity between the long-term LC50 (expressed as ug PAH/g sediment) and measured PAH concentrations in sediments is consistent with histopathological effects observed in fishes from the Elizabeth River (Hargis and Colvocoresses 1986).

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