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Changes in Bluegill Activity as a Monitor of Acute Benzene Exposure

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Monitoring of waterways with biological organisms is an approach that is gaining acceptance (Cairns and van Der Shalie 1980). Various techniques have been used. The most commonly used automated, non-contact methods involve transducers which are sensitive to ventilation and activity of aquatic organisms. Behavioral bioassays are valuable tools to assess water quality since the information can be more rapidly derived than from other sensitive toxicity testing procedures (e.g. growth and reproduction).

The purpose of this study was to determine the acute sensitivity of bluegill (Lepomis macrochirus) to benzene using a newly developed automated activity monitor (Fisher et al. 1982; 1983). Benzene has a relatively high degree of water solubility, and information concerning its toxicity is available (Pickering and Henderson 1966; Moles et al. 1979; Degraeve et al. 1982; Brockson and Bailey 1973; and Maynard and Weber, 1981). Benzene is a major constituent of the water soluble fraction of most crude and refined fuels.

METHODS AND MATERIALS

Bluegills were obtained from Kurtz Fish Farm, Elverson, Pennsylvania and held in the laboratory at least two weeks prior to experimentation. The mean length and weight of these fish were 11.9 cm (SD ± 0.08) and 27.3 g (SD ± 2.6), respectively. Water quality characteristics were a temperature of 16-19°C, pH 6.7-6.9, 400-500 μmhos conductivity, and 5.5-7.5 ppm dissolved oxygen. Individual fish were acclimated to a test chamber for two days before data collection was initiated. Usually five to seven chambers were available for use. All experiments were conducted from 1200-1500 hours. Control activity was monitored for three days, exposure activity on the fourth day, and, in one case, postexposure activity on the fifth day.

Individual fish were contained within each testing chamber in an area slightly larger than the fish itself. Bluegills were isolated from each other by placement of dark paper on the aquaria sides. An adjustable stainless steel screen with mounting bracket was used to facilitate positioning of a

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stainless steel paddle and to contain the fish (Fig. 1). The stainless steel paddle was beveled on each side of its stem and bonded strain-gages were attached. The paddle was placed adjacent to the fish and responded to water disturbances created by fin and body movement. These water disturbances were used to judge fish activity. The use of this technique has been described by Fisher et al. (1982 and 1983).

The testing chambers were 13.3 cm X 56.5 cm X 14.7 cm and filled with 5 liters of water to a depth of 6.3 cm. The chambers were placed on a 1540 kg table top supported by compressed air to dampen low frequency vibration (Micro-G table, Technical Manufacturing Corporation). The table was isolated from the laboratory with a plywood frame and surgical sheet enclosure. A 40 watt light bulb was used to maintain a 12-hour light-dark cycle. Water was metered into each chamber at 0.3 liter per minute. A multichannel Masterflex pump was used to introduce the toxicant. Well water was initially preconditioned via iron and charcoal filtration, then a blend of iron and charcoal filtered and reverse osmosis treated water was used to acquire proper water quality.

The μ volt analog signals from the strain-gage paddles were conditioned (Bay model 7100, 5 volts excitation) and amplified (Bay model 5503) about 2900X. A low pass filter was set at 10 Hz. The conditioned and amplified analog signals were then fed into a multichannel Brush recorder and a Data Translation model 4025 microcomputer (Digital Equipment Corporation, LSI 11/2 processor). The analog signals were sampled at 50 Hz, and maximum peak to peak voltages were retained in 10 second intervals. Graphic displays of activity time courses on a model 4025 Tektronix graphic terminal were retained with a model 4631 hardcopy unit. A model 5000 Data Royal line printer was available if hard copies of the raw values were needed. The collected data were transferred directly from the floppy diskette to an IBM 370 mainframe for data reduction and statistical analyses. Four computer programs were written to perform the functions described.

All test statistics were computed using the Statistical Analysis System (SAS) package (Goodnight 1979). The raw 10 second interval data were transformed into 10 minute and hourly values by summing the 10 second intervals.The values of the 10 second intervals ranged from 0.05 to 2.0 volts. A range of 0.05 to 0.2 volts represented low fish activity, while 0.2 to 1.0 volts indicated fin movement (pectoral and caudal) and whole body movement; above 1.0 volt, the fish displayed intensified fin and body movement. Total activity for each day was calculated by summing the three hourly values. A paired t-test (Goodnight 1979) was used to compare control total daily pre-exposure activity to total daily exposure activity. This test was chosen because it is non-assumptive towards homogeneity of variances. Variability in relative activity was observed in fish experiencing similar environmental conditions. Control fish activity was examined for hourly fluctuations, both during

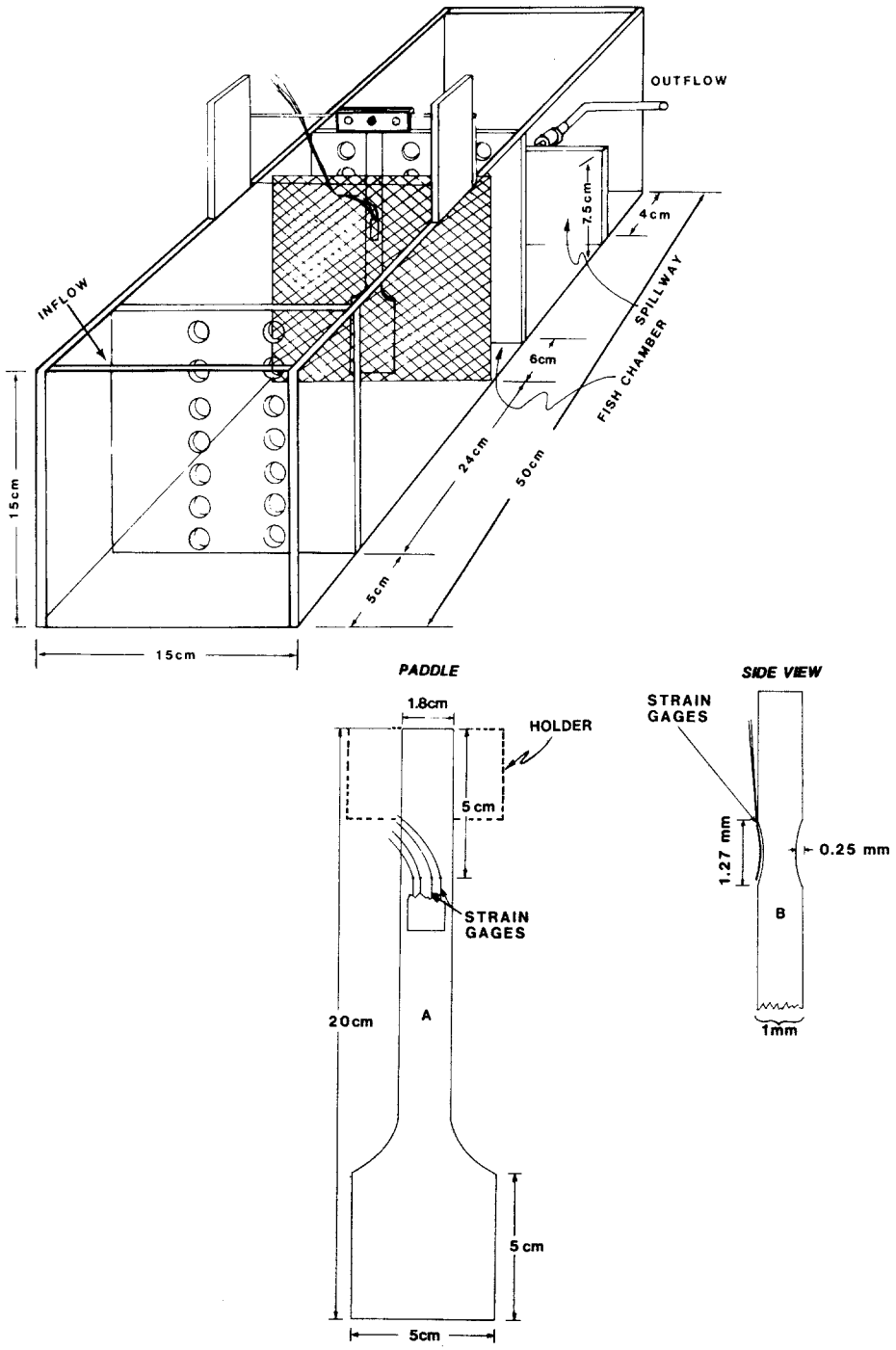

Figure 1. Fish testing chamber with strain-gage paddle attached and front and side view of strain-gage attached to paddle.

the three hour testing period of each day and throughout the three day period. Fish activity was examined for hourly fluctuations during the exposure period.

Benzene (99%) was purchased from Fisher Scientific Products. Stock solutions were prepared by mixing 5 to 9 ml of benzene with 9 liters of water in a stoppered glass container for 24 hours and allowing to stand for 24 hours before use. A Tekmar model ALS (Automatic Laboratory Sampler) multichannel purge and trap and a Perkin Elmer 900 gas chromatograph interfaced with a Varian Vista 900 data system were used to measure benzene concentrations in water.

RESULTS AND DISCUSSION

Two days of acclimation in the testing chambers were followed by three days of control activity data collection from 1200 to 1500 hours. Differences in hourly activity levels for individual fish during the three hour control test period were least on the third day. On day 1, one-half of the fish displayed from 30 to 50 percent variation in hourly activity while the remainder of the fish were less varied in their activity. On day 2, seventy percent of the fish displayed from 10 to 30 percent variation in hourly activity. The day 3 hourly fish activity varied from less than 10 percent to 20 percent for all fish. Therefore, day 3 control bluegill activity was used to assess benzene induced activity changes on the fourth day from 1200 to 1500 hours. Comparisons of activity on control (day 3) and exposure (day 4) days revealed a dose related response (Table I) with the 5.0, 7.1, and 9.0 mg/L groups showing increased activity as benzene concentrations increased ($p \leq 0.05$). No observable changes in activity occurred at the 1.3 mg/L concentration ($p < 0.05$). During exposure, fish activity for the 7.1 and 9.0 mg/L groups was characterized by an initial marked increase in activity the first hour followed by a gradual decline in activity for the remainder of the exposure period. Activity values, however, were still elevated well above control levels. The 7.1 mg/L group was monitored 21 hours after exposure for three hours, and fish activity had returned to pre-exposure levels (paired t-test, $p \leq 0.05$).

Benzene reached maximum concentrations in the testing chambers within 60 to 75 minutes and after exposure decreased to well under 1.0 mg/L $\leq$ within two hours. No benzene was detected 24 hours postexposure.

Toxicity of benzene appears to be similar among various species of teleosts. Six salmonid species, Oncorhynchus tshawytscha, O. kisutch, O. gorbuscha, O. nerka, Salvelinus alpinus, and S. malma, had 96 hr median tolerance limits (TLM) ranging from 11.7 to 14.7 mg/L. Gasterosteus aculeatus and Cottus cognatus had 96 hr TLM values of 24.8 and 15.4 mg/L, respectively (Moles et al. 1979). Benzene 96 hr TLM values for Lepomis macrochirus, Pimephales promelas, Carassium auratus, and Poecilia reiculata ranged from 22.5 to 36.6 mg/L (Pickering and Henderson, 1966).

Table I. Mean hourly fish activity levels collected by the strain-gage paddle system. Both control and benzene exposed mean hourly activity levels are presented.

Fish Activity

Sample Size	Control (Day 3) Hour 1	Control (Day 3) Hour 2	Control (Day 3) Hour 3	Exposure (Day 4) Hour 1	Exposure (Day 4) Hour 2	Exposure (Day 4) Hour 3	Mean Benzene Conc. mg/L
5	50.5 (33.5)[1]	45.9 (21.3)	[2]	50.5 (29.7)	58.5 (33.9)	52.4 (28.0)	1.3 (0.7)
10	44.5 (18.0)	54.5 (26.1)	48.9 (14.1)	92.4 (49.0)	91.1 (39.7)	75.4 (34.3)	5.0 (0.7)[3]
5	46.1 (25.7)	35.7 (17.6)	36.4 (15.9)	149.2 (53.5)	116.9 (49.3)	101.9 (86.2)	7.1 (0.8)[3]
8	33.8 (18.5)	28.1 (16.9)	28.6 (13.6)	238.8 (136.9)	218.1 (124.5)	107.7 (66.6)	9.0 (1.3)[3]

1 Number in parentheses is standard deviation of the mean.

2 Data were not collected.

3 These water concentrations of benzene produced a significant ($p \leq 0.05$) increase in activity.

Degraeve et al. (1982) reported acute benzene toxicity values of 5.3 mg/L and 15.1 mg/L for Salmo gairdneri and Pimephales promelas, respectively. Fish in developmental stages exhibited various toxic responses to benzene. Reproduction in Pacific herring (Clupea harengus pallas) exposed to parts per billion of benzene for 48 hours prior to spawning displayed a reduction in egg survival. For Coho salmon eggs (O. kisutch), the TLM value was 339-542 mg/L and for the emergent fry, 12.4-17.1 mg/L (Struhsaker 1977).

The use of sublethal biological endpoints has demonstrated threshold responses to benzene concentrations below acute toxicity values. Coho salmon avoided benzene at concentrations nearly one seventh of the 96 hr TLM during one hour exposures (Maynard and Weber 1981). Juvenile striped bass and chinook salmon increased their respiration after 24 and 48 hour exposures to 5 ppm benzene (Brocksen and Bailey 1973). In our study, a benzene concentration of 1.3 mg/L did not affect bluegill activity, but 5.0 mg/L tended to increase fish activity within a 3 hour exposure. This is about one fifth of the 96 hr benzene TLM for bluegills (Pickering and Henderson 1966).

The use of fish behavior as an indirect means to assess water quality appears to be sensitive and responsive. Clues regarding ecological death may be derived from observing sublethal effects on behavior. This information is not available in traditional toxicity testing (Henry and Atchison 1979). The strain gage paddle system is fairly sensitive to slight movements of fish and may prove useful to other investigators.

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