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Avoidance Behavior Test as an Alternative to Acute Toxicity Test

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Short-term or 96-h LC50 test with fish forms one of the three base level mandatory aquatic toxicity tests that have to be conducted for the environmental hazard evaluation of a chemical before registering it for use in the EEC (European Economic Community) countries. Though it is time-, and cost-efficient, considering the large number of tests needed to evaluate the hazard of the vast array of chemicals that are already in use and are being added (Lave and Omenn 1986), even a low cost test like the acute toxicity test strains the resources of a standard laboratory. Hence other tests which take much less time than the traditional acute toxicity test (and at the same time yield equally scientifically and legally defensible and reproducible data), are being evaluated for the hazard evaluation of chemicals. Among the battery of aquatic toxicity tests being attempted, behavioral studies could be effectively used for assessing the safety of chemicals (Sprague and Drury 1969; Hansen et al. 1972; Rand 1985).

Present study deals with the avoidance or preference of a reference chemical (phenol) by nine species of Indian test fish. The results of avoidance test are compared with those of the 96-h LC50 test and the acceptability of the former as an effective alternative to the latter is evaluated.

MATERIALS AND METHODS

The test fish Danio devario (Hamilton), Esomus danricus (Hamilton), Chela atpar (Hamilton), Ambassis gymnocephalus Cuvier, Gambusia affinis (Bard and Gerard), Puntius sofore (Hamilton), Oryzias melanostigma (McClelland), Aplocheilus panchax (Hamilton) and Puntius ticto (Hamilton) were collected from Guntur canal or natural water bodies around Nagarjuna University campus, Nagarjunanagar, S. India.

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The reasons for choosing these species, their distribution, maintenance of the fish and test conditions have already been described (Murty and Kondaiah 1991). Phenol (99.5% pure) was used as a reference toxicant in 96-h LC50 tests. The 96-h LC50 value was calculated using the probit method. The avoidance or preference of chosen concentrations of phenol by different species of experimental fish were studied in a 1-m long and 2.5-cm inner diameter tube with inlets at both ends and an outlet at the center. The fish was let into the tube through another opening in the center which was later closed tightly with a bark-cork. The fish was acclimated by letting water in, from both ends (at 40 mL/min from each end) for 1 hr. For the next 30 min, with water still entering from both the ends, the distance traversed and the time spent in each half of the tube by the fish were noted. These were taken as the control observations in water. During the next 1 hr, toxicant of chosen concentration from one end and water from the other end (each at a flow-rate of 40 mL/min) were let in and the fish was acclimated to the toxicant medium. The time spent by the fish in, and the distance of penetration into the toxicant portion of the tube were noted for the next 30 min. The concentrations of phenol used with each test species were 0.1, 1,10 and 50 mg/L and three different fish were exposed to each concentration to determine the avoidance or preference of a particular concentration of the toxicant.

To ascertain whether the avoidance or preference is influenced by the flow of the toxicant either from the left or from the right side of the tube, the toxicant (in the different replicates of the same concentration) was let in randomly from either side of the tube. The time spent in the toxicant or water was expressed as percent of the total period of observations. Subsequently, following arc-sine transformation of the percentages, the data were subjected to statistical analysis. To test whether the fish evinces any preference either for the left or the right half of the tube, the time spent by the fish in each half of the tube while in water (control values) were averaged and the mean time spent in one half of the tube was tested for any significant difference from the expected 50% (i.e., the time supposed to be spent in each half of the tube) using the Student's t-test. The control values as well as the time spent in the toxicant half (with different concentrations of the toxicant) were subjected to analysis of variance. Further, the replicate data of each concentration were compared with those of the controls using least significant difference test, to determine whether the time spent by the fish in a particular concentration of the toxicant

was significantly different from the time spent by it in the identical half of the tube while in water.

RESULTS AND DISCUSSION

Though 108 tests were performed (i.e., with nine species, four concentrations, each with three replicates and controls) as a representative of the rest, only the movements of the fish in the lowest concentration detected by G. affinis are presented in Fig. 1. The 96-h LC 50 values and the lowest concentration detected in the behavioral test by the nine test fish are shown in Table 1. Actually four species, i.e., P. ticto, O.melanostigma, E. danricus, and G. affinis, are best suited for behavioral tests as they satisfy the criteria suggested by Adelman and Smith (1976) such as availability throughout the year, small size, the ease with which they can be handled and transported to the laboratory, short life cycle, and broad sensitivity to a wide range of test chemicals.

Table 1 - Comparison of LC50 and the lowest concentration of phenol avoided (indicated in the last column) by nine species of fish

Species	96-hr LC50 values (mg/L)		Ac[1] mg/L
	Replicates		
	1	2	
D. devario	29.2 (25.3-33.7)	28.1 (24.2-32.7)	50
E. danricus	30.6 (26.6-35.2)	30.4 (26.1-35.4)	50
C. atpar	13.2 (10.8-16.3)	12.1 (7.2-20.2)	10
A. gymno-cephalus	8.1 (3.2-20.1)	5.6 (2.4-13.7)	*50
G. affinis	15.1 (13.7-16.6)	15.4 (14.1-17.1)	10
P. sofore	14.5 (10.8-19.4)	13.7 (12.6-15.0)	*50
O. melano-stigma	9.9 (8.7-11.1)	9.3 (8.3-10.4)	10
A.panchax	9.6 (8.1-11.3)	9.4 (8.6-10.4)	*50
P. ticto	15.6 (14.2-17.1)	15.4 (14.0-17.1)	*50

[1]AC= Avoidance concentration
*Values significantly different from LC50 at p = 0.05

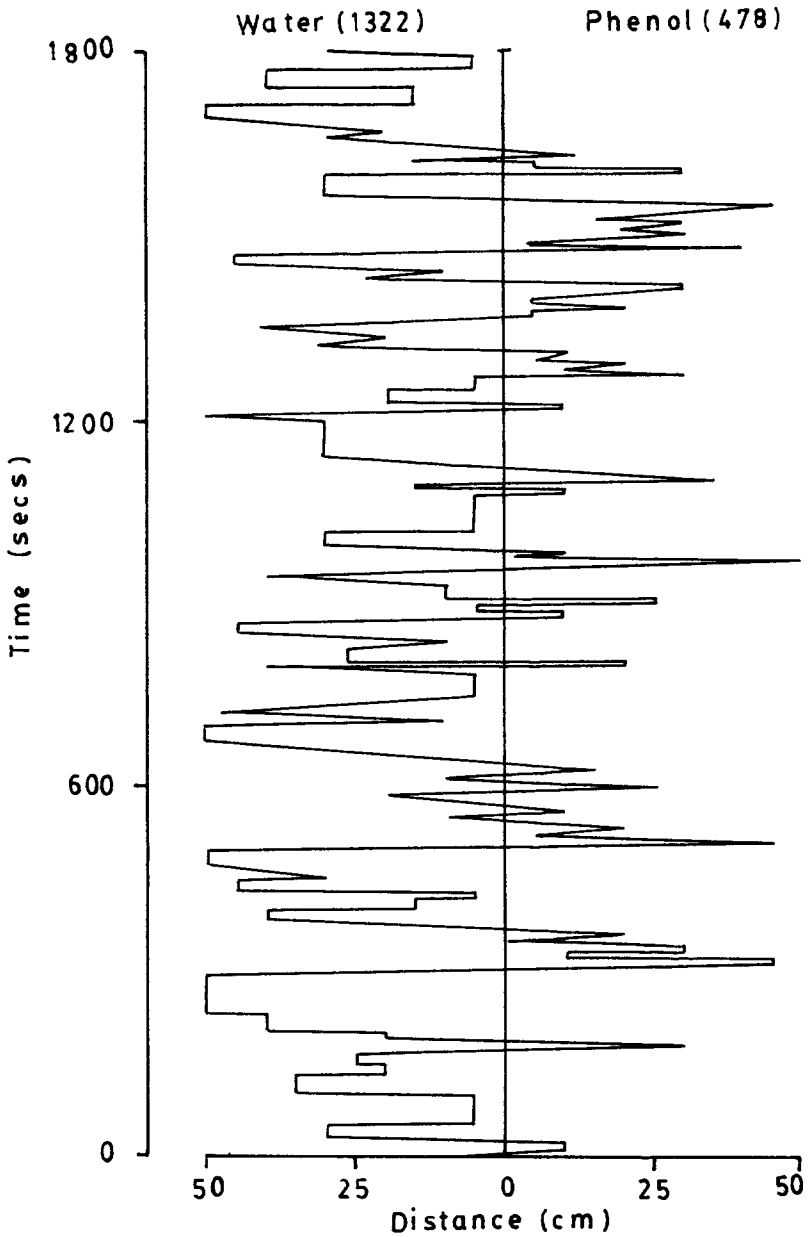

Figure1. Time spent and distance traversed by Gambusia affinis in the toxicant or aqueous half (each 50 cm) of the test apparatus. The time (in sec) spent in each half is shown at the top (Conc. of Phenol = 10mg/L).

Though it is a commonly occurring species, and its behavioral response was good (it could detect a concentration less than the 96-hr LC50), C. atpar should not be considered for behavioral test as its mortality during transportation and acclimatization was unusually high despite any amount of care taken. Four other species, viz., D. devario, P. sofore, A. gymnocephalus and A. panchax, were also found unsuitable for conducting behavioral tests and sometimes short-term toxicity tests, too, as they were highly sensitive. Besides, A. gymnocephalus was very sluggish in both tests, P. sofore manifested high mortality in the holding tanks, and A. panchax showed erratic movements in the tests.

The lowest concentration that could be detected by C. atpar, G. affinis and O. melanostigma was 10 mg/L, while all others could detect only a phenol concentration of 50 mg/L. The LC50 value and the lowest concentration detected in the behavioral test agreed with each other in the case of O. melanostigma and E. danricus, while the difference was considerable in the case of A. gymnocephalus, P. sofore, A. panchax and P. ticto.

Statistical evaluation of the results not only indicated the concentration or concentrations that were significantly avoided by each species of fish studied, but also helped in the identification of those concentrations to which different species were attracted (Table 2). The latter is evident from the significantly more time spent by a particular fish in the toxicant half than in water as in the case of A. gymnocephalus and P. sofore which were attracted to a phenol concentration of 10 mg/L.

Phenols and phenolic compounds are commonly encountered in the wastes coming from coal distilleries and also in road surfacing materials, sheep dips, and effluent released in the manufacture of plastics, dyes and disinfectants. Experiments conducted by Jones (1951), Skrapek (1963), Ishio (1965), Summerfelt and Lewis (1967), and Sprague and Drury(1969) showed that fishes could detect and avoid concentrations that were 1.1 to 2.5 times the median lethal concentration. In the present study, too, different species of fish could detect and avoid concentration in the same range.

That fish are attracted by lower concentrations of certain toxicants is very well known (Jones 1947; Lindahl and Marcstrom 1958). Also, avoidance of lower concentrations seems to be common with some substances like copper (Maciorowski et al. 1977; Black and Birge, 1980; Giattina et al. 1982). In the present study A.

Table 2.Statistical evaluation of the time spent in the toxicant half in the avoidance test[a,b,c]

Fish species	t-Value[d]	F-Value[e]	Least significant difference test[f,g]			
			T1-T2	T1-T3	T1-T4	T1-T5
D. devario	0.36	6.16*	0.52	0.71	1.9	4.82#
E. danricus	0.61	6.65#	1.04	0.08	-1.03	4.72#
C. atpar	1.56	15.14#	0.05	-0.65	3.82#	6.78#
A. gymnocephalus	0.91	21.4#	-0.52	-2.06	-6.94*	4.37#
G. affinis	1.62	10.46#	-1.72	0.25	3.89#	4.65#
P. sofore	0.91	5.76#	-0.01	-0.12	-2.86*	3.19#
O. melanostigma	1.8	7.46#	-0.05	2.12	3.28#	4.57#
A. panchax	0.3	3.97*	-0.84	0.3	0.85	2.22#
P. ticto	1.27	3.22*	0.7	1.12	-1.16	2.98*

[a]The control data constitute T1 (=treatment 1).
[b]The exposure concentrations are 0.1, 1, 10 and 50 mg/L. (T2 to T5).
[c]Three replicates for each concentration.
[d]Comparison of the time spent by each fish in the right (or left) half of the tube during control observations, with the expected 50%, using Student's t-test; degrees of freedom 11.
[e]Degrees of freedom = 4,19;
[f]Degrees of freedom = 13.
[g]Negative sign indicates preference for the chemical.

*Significantly different at p = 0.05.
#Significantly different at p = 0.01.

gymnocephalus and P. sofore were attracted to concentrations that are in the range of the 96-hr LC50 value, but not higher concentrations.

The above results indicate that fish behavioral tests are very well suited to assess the environmental hazard of chemicals. The acceptability of behavioral tests for hazard evaluation has increased in the recent past, especially with the incorporation of objective means of recording and interpreting the observations.

The total time needed for conducting the behavioral test, as adopted in the present work is approximately 3 to 4 hrs. Further, with each species, only 12 to 15 specimens are needed to conduct the behavioral tests. In comparison, an acute toxicity test requires about 100 specimens and 96 hrs. Since the time, material costs, and the number of specimens used are less for behavior tests, such tests deserve to be tried on a much larger scale, especially in view of the fact that they yield as dependable and reproducible data as the standard acute toxicity test.

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