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Standard Test Fish for India and the Neighboring Countries

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Among the battery of tests for environmental hazard evaluation of aquatic pollutants, the short-term or the 96-h LC 50 test with fish is one of the three base level aquatic toxicity tests that have to be conducted before registering a chemical for use in EEC (European Economic Community) countries. Especially in view of the need to evaluate the safety of new chemicals that are being added to the already existing 65,000 odd chemicals in common use (Lave and Omenn 1986), the need for adopting a standard test fish that aids in the standardization of the protocol has been well recognized. As early as 1951, Doudoroff et al stressed the need to develop uniform standard test procedures to maximize the comparability of data from a number of tests.

Sprague (1970) recommended the use of either the most important sensitive species or the most important local fish or a standard test fish to conduct aquatic toxicity tests. Cairns (1980) recommended the use of an aquatic'white rat' to enable comparison and standardization of toxicity test methods in a new place or a new laboratory, or by new personnel with well established standard. Buikema et al (1982) suggested that a standard test protocol with reference toxicants and a standard test species is essential to maximize comparability, replicability and reliability, and to answer questions on the relative toxicity of a compound or on the sensitivity of a test. Alongwith standard test fish, the use of reference toxicants has also been evaluated. The criteria for choosing reference toxicants were listed by Klaverkamp et al (1979) and elaborated upon by Fogels and Sprague (1977), and reviewed by Murty (1986).

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Several species of standard test fish have been evaluated in the industrialzed countries. Among them, Cyprinus carpio in Japan, Pimephales promelas and Lepomis macrochirus in USA, Salmo gairdneri and Jordanella floridae in Canada, USA and Europe, Rasbora heteromorpha and Cichlasoma nigrofasciatum in UK, Brachydanio rerio and Leusescus ides in Europe (OECD (Organisation for Economic Co-operation and Development) Guidelines, 1981), Lebestes reticulatus and Poecilia latipinna in the EEC countries are being used as standard test fish in the OECD and EEC countries.

Recently, following a remarkable increase in the use of agricultural and industrial chemicals in the developing countries and increasing awareness of the environmental problems, the need to ensure a clean environment is felt. Since aquatic toxicity testing is an integral part of the hazard evaluation process, the need to develop a standard aquatic toxicity test protocol in the developing countries, too, cannot be overemphasized. No standard test fish for routine hazard evaluation of chemicals have been identified in India and the neighboring countries. The purpose of the present study is to evaluate the suitability of some commonly occurring species of Indian fish for adoption as standard test fish for aquatic toxicity tests. We compared the data of two tests, viz., the acute toxicity (which is the most often performed toxicity test) and the avoidance behavior test, in considering the suitability of a fish species as a standard test fish.

MATERIALS AND METHODS

The test fish (Danio devario (Hamilton), Esomus danricus (Hamilton), Chela atpar (Hamilton), Ambassis gymnocephalus Cuvier, Gambusia affinis (Baird and Girard), Puntius sofore (Hamilton), Oryzias melanostigma (Mc Clelland), Aplocheilus panchax (Hamilton) and Puntius ticto (Hamilton) were collected from Guntur canal or natural waterbodies around Nagarjuna University campus, Nagarjuna Nagar, S. India. Fish were caught with a small dipnet without causing any damage to the fish. They were brought to the laboratory, acclimated for a period of 2 wk and exposed to the chosen concentrations of phenol, which was used as a reference toxicant. A gravity-aided continuous flow system described by Murty and Murthy (1988) was used to maintain proper loading density, adequate levels of dissolved oxygen, and a continuous replacement of the toxicant. The flow-through system consists of a water reservoir (WR), a main toxicant reservoir (MTR), a second step toxicant reservoir

(SSTR) and a mixing chamber (MC). The first two are placed at a higher level than the SSTR and the latter in turn is at a higher level than the MC to ensure gravitational flow. Constant levels in the WR and SSTR are maintained by a slightly higher inflow than outflow, the excess draining out through a side spout. Desired volumes of toxicant (from SSTR) and water (from WR) flow into the MC, the outflow from which is distributed equally between duplicate tanks. There are as many MTRs, SSTRs and MCs as there are desired number of concentrations. With this system, the fluctuations in the flow rates were less than 20% of the initially adjusted flow, in a 96-h test. The recommendations of the ASTM Commiittee for conducting aquatic toxicity tests (ASTM 1980) were followed. Groundwater pumped from a deep borewell was used, after sufficient aeration, for conducting the toxicity tests. Representative water quality data are; dissolved oxygen 6.5 to 8 mg/L, total alkalinity 550 to 584 mg/L, total carbon dioxide 405 to 426 mg/L, total hardness 96 to 102 mg/L and pH 7.5 to 7.9. The maximum size of all the species of fish used in the experiments was 5 cm. During the period of acclimation, the fish were fed daily with minced boiled egg albumin on an average at 3% of their body weight and were not fed for 2 d prior to and during the period of experimentation. With each test species, five concentrations of the toxicant, with ten fish exposed to each concentration, were employed. The 96-h LC 50 value was calculated by probit analysis (Roberts and Boyce 1972). Toxicity tests were conducted in duplicate.

The avoidance tests were conducted in a 1-m long and 2.5-cm wide (inner diam.) tube with inlets at both ends and an outlet in the center. The fish was let into the tube through an opening in the center which was later closed. The fish was acclimated in the tube for 1 hr by letting in water from both ends (at 20 mL/min). For the next 30 min, with water still entering from both ends, the distance travelled into and the time spent in each half of the tube by the fish were noted. During the next 1 hr, toxicant of chosen concentration from one end and water from the other (each at a flow rate of 20 mL/min) were let in and the fish was acclimated. The time spent by the fish in, and the distance of penetration into the toxicant portion or water were noted for the next 30 min. At least four to five concentrations were used with each test species and three different specimens were exposed to each concentration of the toxicant.

RESULTS AND DISCUSSION

The calculated 96-h LC 50 of phenol to the nine species of test fish are shown in Table 1. In the avoidance tests, Gambusia affinis and Chela atpar could detect and avoid concentrations much lower than the calculated 96-h LC 50, whereas Oryzias melanostigma avoided concentrations equal to the median lethal concentrations in the 96-h test.

Any fish to be chosen as a standard test fish must be small so that the recommended biomass loading (see the ASTM recommendations of 1980; Murty 1986), is not exceeded either in static or flow through tests. All the species chosen presently are small fish whose maximum size is about 15 cm (but usually specimens about 5 cm in length were used in the tests). According to Adelman and Smith (1976), any species to be accepted as test fish should be: 1) available all through the year; especially the desired size and age groups should be available in all seasons, 2) available in large quantities, 3) easily transportable to the laboratory and easy to handle and 4) neither too sensitive nor too resistant and should show a constant response to a wide range of toxicants under similar test conditions (for instance, because of its poor sensitivity, goldfish (Carassius sp.), which was often used in the early days of toxicity testing, is no longer accepted as a test species); furhter the test species should complete its life cycle in less than a year, so that whole life cycle tests can easily be conducted.

Although some of the species employed as standard test fish in the OECD countries have been reported from India, too, occasionally, owing to their sparse distribution and availability in small numbers, Indian species had to be evaluated for their suitability. Further, in a developing country like India, facilities for breeding the European species under controlled conditions are as yet non-existent, making it necessary to use abundantly available and easily maintainable indigenous species.

The range of the 96-h LC 50 values of phenol for the various species of Indian fish tested (5.6-30.6 mg/L) are comparable with those reported elsewhere (Fogels and Sprague 1977; Shelford 1917; Trama 1955 and Albersmayer and Erichsen 1959) which are in the range of 5.7 to 75 mg/L.

Among the nine species tested, we found that Danio devario, Ambassis gymnocephalus, Chela atpar, Aplocheilus panchax and Puntius sophore are not suitable as standard test fish. Danio devario is a very sensitive species resulting in heavy mortality during transportation and inexplicable mortality during acclimatization. Ambassis gymnocephalus was found unsuitable because of

Table 1. Calculated 96-h LC 50 of phenol to 9 spp. of Indian test fish

Fish species	96-h LC 50 values (mg/L)*	
	Replicate 1	Replicate 2
Danio devario	29.2 (25.3-33.7)	28.1 (24.2-32.7)
Esomus danricus	30.6 (26.6-35.2)	30.4 (26.1-35.4)
Chela atpar	13.2 (10.8-16.3)	12.1 (7.2-20.2)
Ambassis gymnocephalus	8.1 (3.2-20.1)	5.6 (2.4-13.7)
Gambusia affinis	15.1 (13.7-16.6)	15.4 (14.1-17.1)
Puntius sophore	14.5 (10.8-19.4)	13.7 (12.6-15)
Oryzias melanostigma	9.9 (8.7-11.1)	9.3 (8.3-10.4)
Aplocheilus panchax	9.6 (8.1-11.3)	9.4 (8.6-10.4)
Puntius ticto	15.6 (14.2-17.1)	15.4 (14-17.1)

*95% Confidence limits in parentheses

Table 2. Size range (in nature) and the geographical distribution of the test fish

Name of the fish	Length in cm	Distribution
Danio devario	4-10	India, Burma, Sri Lanka, Pakistan, Bangladesh, Nepal, Malaysia, China, Indonesia and Thailand
Esomus danricus	3-12	India, Burma, Sri Lanka, Nepal, Pakistan and Bangladesh
Chela atpar	4-10	India, Burma, Sri Lanka, Nepal, Pakistan and Bangladesh
Ambassis gymnocephalus	4-10	India, Burma, Indonesia, East coast of Africa, Nepal, Bngladesh, Pakistan and Malaysia
Gambusia affinis	4-12	India, Burma, Sri Lanka, Pakistan, Bangladesh, Malaysia, Taiwan, Hawaii, Thailand and other parts of the world
Puntius sofore	5-15	India, Pakistan, Nepal, Bangladesh, Burma and Yunnan
Oryzias melanostigma	2-5	India, Bangladesh, Burma, Pakistan and Thailand
Aplocheilus panchax	4-10	India, Bangladesh, Burma, Pakistan, Indonesia, Thailand and Malaysia
Puntius ticto	4-10	India, Bangladesh, Burma, Pakistan, Thailand and Sri Lanka

its sensitivity to any handling and its sluggishness in both the acute toxicity and avoidance tests. Chela atpar was very sensitive to handling. Despite any amount of care taken, mortality during transportation was very high. The mortality of Puntius sophore in the holding tanks was very high and it could not withstand any stress. Though Aplocheilus could easily be maintained ans was not excessively sensitive, it showed erratic movements in the tests and its avoidance reactions were not uniform.

The remaining four species, viz., E. danricus, G. affinis, P. ticto and O. melanostigma, in that order seem to be very well suited for being considered for recommendation as test fish as they could be easily transported to the laboratory, their mortality due to handling stress was negligible, no inexplicable control deaths were recorded and the required age and size groups were available all through the year. These four species also satisfy the criteria suggested by Adelman and Smith (1976). The maximum size attained by the nine species and their distribution are shown in Table 2. The reported optimum temperature for these species is 18-32^{o}C. Not only are the four species (that can be recommended as standard test species) distributed all over the Indian subcontinent, but their distribution extends into the neighboring Pakistan, Bangladesh, Nepal, Myanmar (Burma), Sri Lanka and Thailand also.

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