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Acute Toxicity of Phenol and Long-Term Effects on Food Consumption and Growth of Juvenile Rohu *Labeo rohita* (Ham.) under Tropical Conditions

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Abstract

The study was designed to arrive at an experimentally-determined application factor for fixing tentative water quality criteria for phenol in tropical freshwaters. Larval rohu *Labeo rohita* (14-18 mm total length, 118-139 mg wet weight) were subjected to static lethal bioassay. The 24-h LC_{50} value was found to be 32 $mg \cdot l^{-1}$ at 29±1°C. Juvenile rohu (26-33 mm total length, 226-273 mg wet weight) were exposed to sublethal phenol concentrations (0.1, 0.5, 2, 5 and 10 $mg \cdot l^{-1}$) for 28 d at 29±1°C without aeration. Treatment media were replaced every 48 h. Fish were fed a pelleted diet at 6% wet body weight per day. Rohu exposed to 5 and 10 $mg \cdot l^{-1}$ phenol showed significantly lower mean wet weight gain, specific growth rate, food conversion efficiency, and dry matter and protein digestibility. These fish also had higher moisture content, and lower protein, lipid and ash contents, as body nutrients were depleted. Juveniles exposed to the lower phenol concentrations of 0.1, 0.5 and 2 $mg \cdot l^{-1}$ were not significantly different from the control. The maximum allowable toxicant concentration for juvenile rohu was 3.16 $mg \cdot l^{-1}$, indicating an application factor of 0.10.

Introduction

A review of phenol toxicity to fish by Alabaster and Lloyd (1982) discussed the tentative water quality criteria for temperate waters supporting commercial fisheries. Sublethal bioassay studies on the effect of phenol on tropical freshwater fishes are limited (Razani et al. 1986a). A road tanker accident in June 1993 and the resultant phenol spillage into the Peechi Reservoir (Kerala State, South India) that affected the drinking water supply in central Kerala triggered the present investigation. The present study experimentally determines an application factor (according to Stephan and Mount 1973) for fixing a tentative water quality criterion for phenol in tropical waters. The Indian major carp *Labeo rohita* (Ham.) was used as the test animal because it is present in

almost all freshwater reservoirs in India and is suitable for toxicity monitoring (Ashraf et al. 1992). The effects of phenol on food consumption and growth in rohu were monitored as suggested by Webb (1978) for environmental toxicants in general.

Materials and Methods

Test Animals

The larvae and juveniles of rohu (*L. rohita*, Cyprinidae) were supplied by the carp hatchery of the College of Fisheries, Cochin, India. The lethal toxicity test used larval fish (14-18 mm total length, 118-139 mg wet weight) and the sublethal toxicity test used juvenile fish (26-33 mm total length, 226-273 mg wet weight). The fish were acclimated in well water (28-30°C water temperature; 7.2-8.0 $mg \cdot l^{-1}$ dissolved oxygen; 6.8-7.7 pH) for 10 d prior to the experiment. The fish were fed *ad libitum* once a day on natural plankton or pelleted carp feed.

Phenol

Analar monohydric phenol (C_6H_5OH, MW 94.1) produced by New India Chemical Enterprises, Cochin, was used throughout the study. The nominal concentrations were prepared from fresh stock solutions every time.

Lethal Toxicity

After exploratory tests, seven phenol concentrations from 29 $mg \cdot l^{-1}$ (no mortality) to 35 $mg \cdot l^{-1}$ (100% mortality) were chosen for the final 24-h test to determine the 50% lethal concentration (LC_{50}). Static bioassay was carried out in triplicate in glass containers with 4 l well water and 12 fish each. Fish were taken at random from the stock and starved for 24 h prior to the experiment. As a result of the addition of phenol, the dissolved oxygen level was reduced to 0.4 $mg \cdot l^{-1}$ at all test concentrations (control = 8 $mg \cdot l^{-1}$). Mortality during the 24-h exposure period was recorded for each treatment. The 24-h LC_{50} and its 95% confidence limits were calculated by linear regression analysis after probit transformation of mean mortality and $\log_{10}$ transformation of the test concentrations as described by Finney (1971).

Sublethal Toxicity

Five nominal concentrations (0.1, 0.5, 2, 5 and 10 $mg \cdot l^{-1}$) of phenol and a control were tested for 28 d in a static system with 48-h treatment water replacement. One-third of the 24-h LC_{50} was taken as the maximum sublethal concentration (Konar 1969) and the lower concentrations were chosen according to Sprague (1973). Each treatment had three replicates (6 x 3 = 18 sets). Twelve rohu juveniles chosen at random from the main stock were maintained in 12 l well water in seasoned cement cisterns. The ratio of animal wet weight to water volume was 0.22-0.50 $g \cdot l^{-1}$ during the 28-d exposure period. The dissolved oxygen and pH during the different treatments are presented in

Table 1. The ranges are for measurements taken immediately before and after phenol dosing every 48 h. Water temperature was maintained at 29±1°C throughout the study.

The fish were fed once a day on a pelleted carp feed (40% clam meat, 25% rice bran, 25% groundnut oil cake and 10% tapioca flour) at 6% wet body weight adjusted against weekly weight measurements. The feed had a proximate composition of 8.23% moisture, 40.0% crude protein, 4.94% crude fat, 1.84% crude fiber and 5.02% ash. Feed remnants and fecal matter were collected by siphoning every 24 h. On days 6, 13, 20 and 27, the fish were starved for 24 h, and all weighed to the nearest milligram on a monopan electronic balance after blotting off the excess moisture using filter paper. The specific growth rate (SGR, % d^{-1}) during the exposure period was calculated using the equation:

$$SGR = \frac{\ln W_f - \ln W_i}{28} \cdot 100$$

where W_i and W_f are mean initial and final body weights, respectively.

The feed remnants were dried to a constant weight and used for determining the total and average daily food consumption (ADFC, mg·d^{-1}). From the total weight gain and food consumption, the food conversion ratio (FCR) was calculated. Digestibility coefficients for dry matter and crude protein were determined by direct quantitative feces collection. The feces collected daily from each tank were dried to a constant weight and pooled over 28 d. The feces were analyzed for crude protein content (AOAC 1984). Apparent dry matter digestibility (ADMD) and apparent crude protein digestibility (ACPD) were computed following Maynard and Loosli (1969).

Six randomly selected fish from each set were sacrificed after the 28-d exposure and analyzed for body proximate composition. Moisture, crude protein and ash contents were determined by AOAC (1984) methods. Fat was extracted using Folch's procedure (Folch et al. 1957) and was gravimetrically quantified.

Mortality observed during the exposure period was used to calculate percentage survival at the end of the experiment. Treatment means of the various biological measurements were compared by one-way ANOVA following Snedecor and Cochran (1973).

Table 1. The ranges of dissolved oxygen and pH in six different phenol treatments during the 28-d sublethal bioassay.

Nominal phenol concentations (mg·l^{-1})	Dissolved oxygen (mg·l^{-1})	pH
0.0	7.2 - 8.0	6.83 - 7.75
0.1	6.6 - 7.8	6.83 - 7.50
0.5	5.8 - 7.8	6.82 - 7.40
2.0	5.4 - 7.4	6.75 - 7.40
5.0	4.2 - 7.2	6.70 - 7.20
10.0	3.8 - 7.0	6.60 - 7.10

Results

Lethal Toxicity

The calculated 24-h LC_{50} of phenol to larval rohu at 29±1°C is 31.97 $mg \cdot l^{-1}$ in static water without aeration. The 95% confidence limits are 31.50-32.44 $mg \cdot l^{-1}$.

Sublethal Toxicity

The summarized results of growth parameters in rohu juveniles due to a 28-d exposure to various sublethal concentrations of phenol are presented in Table 2. The weekly progression of growth in wet weight is represented in Fig. 1. Compared to control fish, fish exposed to the lower concentration of 0.1 $mg \cdot l^{-1}$ phenol showed a significantly higher wet weight gain. The wet weight gain and SGR were significantly lower in fish exposed to 5 and 10 $mg \cdot l^{-1}$ phenol than in the control and the lower concentrations. FCR, ADMD and ACPD values showed the same trend as SGR. Control fish converted 45.45% of the ration to wet weight, while fish exposed to 5 and 10 $mg \cdot l^{-1}$ phenol converted only 24.40% and 21.30%, respectively. Digestibility of dry matter and crude protein were also significantly reduced in these higher concentrations. Thus, these variables can be considered to be of long-term biological significance and to be consistent end points for dose-related effects of phenol on fish.

It is considered possible to arrive at a reliable maximum allowable toxicant concentration (MATC) based on these end points.

$$MATC = (NOEC \times LOEC)^{1/2}$$

NOEC is the no-observable-effect concentration, and LOEC is the least-observable-effect concentration. The MATC of phenol to rohu juveniles is 3.16 $mg \cdot l^{-1}$ under a 28-d static system (with 48-h replacement) at 29±1°C. This gives an application factor (MATC/24-h LC_{50}) of 0.10.

The data on SGR, FCR, ADMD and ACDP for the different treatments were further analyzed to find the threshold level or median effective concentration (EC_{50}) of phenol. A good estimate of the threshold level was taken (from Webb and Brett 1973) as the intersection point between the regression lines for effective concentrations (2, 5 and 10 $mg \cdot l^{-1}$) and no-effect concentrations (0.1, 0.5 and 2 $mg \cdot l^{-1}$). The phenol concentration of 2 $mg \cdot l^{-1}$, being the experimental threshold level, is taken as a common concentration factor. The EC_{50} of phenol is 1.8 $mg \cdot l^{-1}$ from this analysis.

The carcass proximate composition at the end of the 28-d exposure period between control fish and those exposed to 5 and 10 $mg \cdot l^{-1}$ was significantly different. The moisture levels were significantly high; and the crude protein, crude fat and ash contents were significantly low at 5-10 $mg \cdot l^{-1}$ phenol. Fish exposed to lower phenol concentrations of 0.1, 0.5 and 2 $mg \cdot l^{-1}$ showed a similar body composition as the control fish.

After 23 d of exposure, mortality occurred at 5 and 10 $mg \cdot l^{-1}$ phenol test concentrations. Percentage survival after 28 d was 92±7% and 72±24%, respectively. No mortality was observed in the lower test concentrations and control during the entire period of exposure. Juveniles of *L. rohita* are

Table 2. Growth parameters (mean±SD) for rohu *Labeo rohita* juveniles during 28-d exposure to various sublethal concentrations of phenol. Numbers in parenthesis are percentages of control values.

Parameters	Nominal phenol concentrations ($mg \cdot l^{-1}$)					
	0.0	0.1	0.5	2.0	5.0	10.0
Initial weight (mg)	$230.0^{a} \pm 12.0$	$250.0^{a} \pm 17.0$	$226.0^{a} \pm 7.0$	$238.0^{a} \pm 10.0$	$240.0^{a} \pm 6.0$	$240.0^{a} \pm 11.0$
Final weight (mg)	450.0 ± 19.0	488.0 ± 16.0	440.0 ± 5.0	460.0 ± 14.0	325.0 ± 5.0	312.0 ± 9.0
Mean wet weight gain (mg)	$220.0^{a} \pm 2.8$	$238.0^{b} \pm 2.8$	$214.0^{a} \pm 3.3$	$222.0^{a} \pm 4.2$	$85.0^{c} \pm 2.2$	$67.0^{d} \pm 2.9$
Specific growth rate (% d^{-1})	$2.4^{a} \pm 0.08$	$2.39^{a} \pm 0.1$	$2.38^{a} \pm 0.08$	$2.36^{a} \pm 0.06$	$1.08^{b} \pm 0.05$	$0.86^{b} \pm 0.07$
	(100.0)	(99.6)	(99.2)	(98.3)	(45.0)	(35.8)
Average daily food consumption ($mg \cdot d^{-1}$)	$16.9^{ab} \pm 2.5$	$20.4^{a} \pm 3.7$	$18.3^{a} \pm 3.1$	$18.5^{a} \pm 2.0$	$12.3^{b} \pm 1.9$	$11.3^{b} \pm 2.0$
Food conversion ratio	$2.2^{a} \pm 0.3$	$2.4^{a} \pm 0.5$	$2.4^{a} \pm 0.4$	$2.3^{a} \pm 0.2$	$4.1^{b} \pm 0.6$	$4.7^{b} \pm 0.9$
	(100.0)	(109.1)	(109.1)	(104.5)	(186.4)	(213.6)
Food conversion efficiency (%)	45.4^{a}	41.6^{a}	41.6^{a}	43.4^{a}	24.4^{b}	21.3^{b}
Apparent dry matter digestibility (%)	$66.5^{a} \pm 2.9$	$65.5^{a} \pm 3.4$	$64.9^{a} \pm 3.0$	$63.9^{a} \pm 3.1$	$51.9^{b} \pm 2.2$	$48.1^{b} \pm 2.1$
	(100.0)	(98.5)	(97.6)	(96.1)	(78.0)	(72.3)
Apparent crude protein digestibility (%)	$82.7^{a} \pm 0.4$	$82.4^{a} \pm 0.5$	$82.2^{a} \pm 0.5$	$81.9^{a} \pm 0.5$	$76.7^{b} \pm 1.0$	$75.5^{b} \pm 1.4$
	(100.0)	(99.6)	(99.4)	(99.0)	(92.7)	(91.3)
Percentage survival	100.0	100.0	100.0	100.0	91.6 ± 6.8	72.2 ± 23.9

Values with different superscripts in the same row differ significantly ($P < 0.05$).

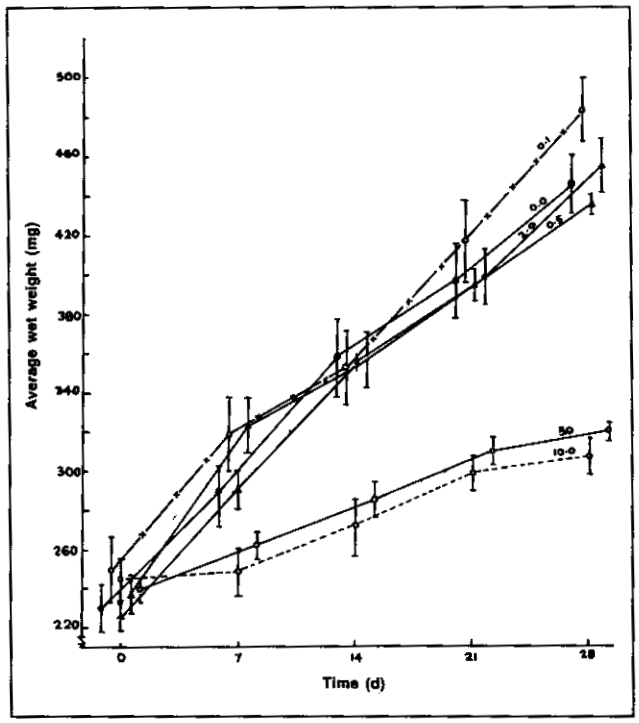

Fig. 1. Growth of rohu juveniles during the 28-d bioassay in sublethal concentrations of phenol (the nominal phenol concentrations in $mg.l^{-1}$ given beside growth curves.

good test animals as they yield relatively consistent results in toxicological assays and a steady control survival in freshwater at 29±1°C.

Discussion

Phenol vs. Oxygen Toxicity, a Perspective

Phenol adversely affects freshwater fishes by direct toxicity (including irreversible changes in the proteins) and by its oxygen demand and the resulting oxygen depletion in the water (Alabaster and Lloyd 1982). The bioassay tests were carried out in water with reduced oxygen content due to the toxicant and, in fact, this is the condition that often prevails in natural waters (as suggested by Katz 1971). In attempting to solve the problem of testing an oxygen-consuming waste, it would sometimes be most realistic to regard the increased toxicity of the waste, caused by lowered oxygen, as a legitimate component of its toxic action (Sprague 1973). A reduction in dissolved oxygen from 100 to 50% of air saturation value reduces the threshold LC_{50} of phenol by about 20% (Lloyd 1961). Reduction in tolerance to progressive hypoxia when exposed to sublethal levels of phenol (0-12 ppm) has been reported in stoneroller minnow (Hlohowskj and Chagnon 1991).

From three well documented studies by previous authors on largemouth bass, common carp and coho salmon, Brett (1979) concluded that an oxygen concentration of close to 5 ppm is critical for growth, below which a drop of 1 ppm dissolved oxygen causes a 30% reduction in growth rate. In the present study, the treatments with 5 and 10 $mg \cdot l^{-1}$ phenol had dissolved oxygen levels

of 4.2 and 3.8 ppm. The SGRs in these concentrations were reduced by 55 and 64% of the control, suggesting only a complimentary role for dissolved oxygen as a toxicant.

Significant reduction in ADFC was shown by fish in the 5 and 10 $mg \cdot l^{-1}$ phenol treatments when compared to fish in the 0.1, 0.5 and 2 $mg \cdot l^{-1}$ phenol treatments. According to Brett (1979), a restricted (reduced in the present case) ration is accompanied by a lowering of the daily metabolic rate and consequently a reduced oxygen demand. Thus it might be expected that the critical oxygen level would be lowered in 5 and 10 $mg \cdot l^{-1}$ phenol, and thus suggests a reduced role for oxygen as a toxicant. The deleterious effects to the two highest phenol concentrations may then be more due to the increased uptake of phenol from the medium due to reduced oxygen content, as suggested by Jones (1964). Within the range of pH 6.5-8.5, there was little or no difference in toxicity of monohydric phenols to rainbow trout (Herbert 1962).

Lethal Toxicity

Earlier studies have shown lethal phenol concentrations (24-h LC_{50}) of 25 ppm at 18°C in crucian carp (Lukanenko and Flerov 1963), 25 ppm at 18°C roach and 15 ppm at 18°C in perch (Anon. 1969), and 28 ppm at 24±1°C in zebrafish (Razani et al. 1986b). Pickering and Henderson (1966) reported a 48-h LC_{50} at 44.5 ppm at 25°C in goldfish.The lower 24-h lethal toxicity (32 $mg \cdot l^{-1}$) shown by rohu larvae may be due to the high temperature of 29±1°C in the present study. Brown et al. (1969) observed a decrease in specific duration toxicity due to phenol with increase in temperature.

Sublethal Toxicity

The effect of phenol at 5 and 10 $mg \cdot l^{-1}$ may be similar to that of detergents which damage taste and smell receptors and thereby limit food consumption (Bardach et al. 1965). Impairment of taste and smell in carp due to phenolic substances and oils released from aqueous flora has been reported by Hara (1971).

In the lower concentrations of 0.5 and 2 $mg \cdot l^{-1}$ phenol, the values of SGR, FCR, ADMD and ACPD do not vary significantly from those of control fish. The apparent increase in ADFC without a corresponding increase in SGR and mean wet weight gain may indicate an elevated maintenance metabolism due to toxicant stress. Studying the toxicity of whole bleached kraft mill effluent on sockeye salmon, Webb and Brett (1972) suggested that elevated maintenance metabolism could be compensated for by increased food intake to maintain growth. In the higher concentrations of 5 and 10 $mg \cdot l^{-1}$ phenol, digestibility of dry matter and crude protein were significantly reduced as was food conversion efficiency. Coupled with this was the marked decrease in food consumption leading to a significantly reduced SGR in these higher phenol concentrations. Weight loss in common carp exposed for 2 months to 12.5 $mg \cdot l^{-1}$ phenol, and 20% growth reduction in rainbow trout exposed for 18 weeks to 1-5 $mg \cdot l^{-1}$ phenol are reported by Alabaster and Lloyd (1982).

Significant differences in the carcass composition of rohu exposed to 5 and 10 $mg \cdot l^{-1}$ phenol were the direct result of reduced food consumption and food conversion efficiency as was observed in pinfish (*Lagodon rhomboides*) exposed to sublethal concentrations of bleached kraft mill effluent (Stoner and

Livingston 1978). At lower levels (0.1, 0.5 and 2 $mg \cdot l^{-1}$) of phenol, rohu can withstand the toxicant effect without depleting its body nutrients. At higher phenol levels, rohu deplete body protein and fat to meet energy demand.

Razani et al. (1986a, 1986b), studying the sensitive developmental stages of zebrafish (*Brachydanio rerio*), reported a 24-h LC_{50} of 28 ppm and NOEC, LOEC levels of 2.2 and 4.9 ppm phenol, respectively. The calculated MATC (3.28 ppm phenol) and application factor (0.11) for zebrafish support the results (3.16 $mg \cdot l^{-1}$ phenol and 0.10) for rohu juveniles.

Alabaster and Lloyd (1982) suggested tentative water quality criteria for phenol of 1 $mg \cdot l^{-1}$ for salmonids and 2 $mg \cdot l^{-1}$ for more resistant fishes at temperatures above 5°C in temperate waters. The recommended application factor is 0.05 and the suggested maximum phenol concentration is 0.1 $mg \cdot l^{-1}$ in North American freshwaters (Birge et al. 1979). We suggest an application factor of 0.10 of the 24-h LC_{50} (static bioassay without aeration) as the tentative water quality criterion for phenol for tropical freshwater coarse fishes.

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References

Alabaster, J.S. and R. Lloyd. 1982. Water quality criteria for freshwater fishes. 2nd edition. FAO, Butterworth Scientific Publications, London. 361 pp.

Anonymous. 1969. Water pollution research. U.K. Ministry of Technology, HMSO, London: 65-66.

Ashraf, M., M. Jafar and J. Tariq. 1992. Annual variation of selected trace metals in freshwater lake fish, *Labeo rohita*, as an index of environmental pollution. Toxicology and Environmental Chemistry 35: 1-7.

AOAC. 1984. Official methods of analysis. 14th edition (ed. S. William) Association of Official Analytical Chemists, Arlington, Virginia. 1141 pp.

Bardach, J.E., M. Fujiya and A. Hall. 1965. Detergents: Effects on the chemical senses of the fish *Ictalurus natalis* (Le Sueur). Science 148: 1605-1607.

Birge, W.J., J.A. Black, J.E. Hudson and D.M. Bruser. 1979. Embryo - larval toxicity tests with organic compounds. In: Aquatic toxicology (ed. L.L. Marking and R.A. Kimerle), pp. 131-147. ASTM STP 667, American Society for Testing and Materials, Philadelphia.

Brett, J.R. 1979. Environmental factors and growth. In: Fish physiology Vol. VIII (ed. W.S. Hoar, D.J. Randall and J.R. Brett), pp. 599-667. Academic Press, New York.

Brown, V.M., D.H.M. Jordan and B.A. Tiller. 1969. The acute toxicity to rainbow trout of fluctuating concentrations and mixtures of ammonia, phenol and zinc. Journal of Fish Biology 1: 1-9.

Finney, D.J. 1971. Probit analysis. Cambridge University Press, Cambridge. 333 pp.

Folch, J., M. Lees and G.H. Sloane-Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry 226: 497-509.

Hara, T.J. 1971. Chemoreception. In: Fish physiology Vol. V (ed. W.S. Hoar and D.J. Randall). pp. 79-120. Academic Press, New York.

Herbert, D.W.M. 1962. The toxicity to rainbow trout of spent still liquors from the

distillation of coal. Annals of Applied Biology 50: 755-777.
Hlohowskyj, I. and N. Chagnon. 1991. Reduction in tolerance to progressive hypoxia in the central stoneroller minnow following sublethal exposure to phenol. Water, Air and Soil Pollution 60: 189-196.
Jones, J.R.E. 1964. Fish and river pollution. Butterworth Scientific Publications, Washington D.C.
Katz, M. 1971. The effects of pollution upon aquatic life. In: Water and water pollution - Handbook (ed. L.L. Ciaccio) Marcel Dekker Inc., New York. 449 pp.
Konar, S.K. 1969. Two organophosphorus insecticides DDVP and phosphamidon as selective toxicants. Transactions of the American Fisheries Society 98: 430-437.
Lloyd, R. 1961. Effect of dissolved oxygen concentration on the toxicity of several poisons to rainbow trout (*Salmo gairdneri* Richardson). Journal of Experimental Biology 38: 447-455.
Lukanenko, V.I. and B.A. Flerov. 1963. Materialy so gidrobiologii biologii volzhih vodrohranilishch. Isd-vo Akademic Nauk. Moskva.
Maynard, L.A. and J.K. Loosli. 1969. Animal nutrition. 6th edition. McGraw-Hill, New York. 613 pp.
Pickering, P.H. and C. Henderson. 1966. Acute toxicity of some important petrochemicals to fish. Journal of the Water Pollution Control Federation 38: 1419-1429.
Razani, H., K. Nanba and S. Murachi. 1986a. Chronic toxic effect of phenol on zebrafish *Brachydanio rerio*. Bulletin of the Japanese Society of Scientific Fisheries 52: 1553-1558.
Razani, H., K. Nanba and S. Murachi. 1986b. Acute toxic effect of phenol on zebrafish *Brachydanio rerio*. Bulletin of the Japanese Society of Scientific Fisheries 52: 1547-1552.
Snedecor, G.W. and W.G. Cochran. 1973. Statistical methods. 6th edition. Iowa State University Press. Iowa. 593 pp.
Sprague, J.B. 1973. The ABC's of pollutant bioassay using fish. In: Biological methods for the assessment of water quality. (ed. J. Cairns, Jr. and K.L. Dickson), pp. 6-30. ASTM STP 528, American Society for Testing and Materials, Philadelphia.
Stephan, C.E. and D.I. Mount. 1973. Use of toxicity tests with fish in water pollution control. In: Biological methods for the assessment of water quality. (ed. J. Cairns, Jr. and K.L. Dickson), pp. 164-177. ASTM STP 528, American Society for Testing and Materials, Philadelphia.
Stoner, A.W. and R.J. Livingston. 1978. Respiration, growth and food conversion efficiency of pinfish (*Lagodon rhomboides*) exposed to sublethal concentrations of bleached kraft mill effluent. Environmental Pollution 17: 207-217.
Webb, P.W. 1978. Partitioning of energy into metabolism and growth. In: Ecology of freshwater fish production (ed. S.D. Gerking), pp. 184-214. Blackwell Scientific Publications, London.
Webb, P.W. and J.R. Brett. 1972. The effects of sublethal concentrations of whole bleached kraft mill effluent on the growth and food conversion efficiency of under-yearling sockeye salmon (*Oncorhynchus nerka*). Journal of the Fisheries Research Board of Canada 29: 1555-1563.
Webb, P.W. and J.R. Brett. 1973. Effects of sublethal concentrations of sodium pentachlorophenate on growth rate, food conversion efficiency and swimming performance in under-yearling sockeye salmon (*Oncorhynchus nerka*). Journal of the Fisheries Research Board of Canada 30: 499-507.

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