Bull. Environ. Contam. Toxicol. (1993) 51:453–459

Environmental Contamination and Toxicology

Effects of Seasonal Ambient Temperature Variations on Acute Toxicity of Chlordane to an Air-Breathing Indian Catfish, *Heteropneustes fossilis* (Bloch.)

Uma Prasad Rai and Prabhat Kumar Mandal

Department of Zoology, University of Allahabad, Allahabad-211 002, U.P., India

Chlordane, widely used in India, is a potential toxicant against soil pests, non-target organisms and humans. FAO and WHO (1973) have recommended 0.001 mg/kg body weight as the maximum acceptable daily intake (ADI) for chlordane, but no such dose can be recommended safely for fishes.

Temperature and pollution are considered as important limiting factors in fisheries and environmental management. The relationship between ambient seasonal temperature and acute toxicity of pesticides in vertebrates is generally believed to be non-linear (Brown 1980). Unlike homeothermic animals, response of the poikilothermic animals to toxicants at varying ambient temperatures is not well understood. While most of the reports in this regard are from the temperate countries, very few studies have been made in the tropics (Rai and Mandal 1990). The present study was undertaken to evolve a regression model for acute toxicity estimates of chlordane at various temperatures to aid fisheries and environmental management. With this in view the relationship of acute toxicity to seasonal variations in temperature has been investigated in a common Indian table fish, Heteropneustes fossilis.

MATERIALS AND METHODS

Healthy specimens of H. fossilis of length and weight 15.06± 3.00 cm and 12.20±2.35 g, respectively, were acclimated to the laboratory conditions for 15 d and were fed chopped goat's spleen ad libitum. Feeding was stopped 24 hr prior to treatment.

Acute toxicity tests for 48, 96 and 120-hr were conducted employing semidynamic method (Brown 1980) of bath treatment. Water of all the experimental aquaria, including controls was changed every 24 hr. However, water was not changed in the aquaria in which the exposure period was only 24 hr.

For each set of experiment, 60 fishes were selected and

Send reprint requests to Dr. P.K.Mandal at the above adress.

Table 1. Physico-chemical properties of water at different seasonal temperature conditions (means ± SD).

Month	Temperature °C	Dissolved oxygen mg/L	pH	Electrical conductivity µmho	Alkalinity mho	
					CO_3^{--}	HCO_3^{-}
January	22.5 ±1.0	8.10 ±0.20	7.64 ±0.10	680 ±7	15 ±4	265 ±20
February	24.5 ±0.5	8.00 ±0.10	7.70 ±0.06	684 ±12	17 ±2	260 ±10
April	29.0 ±1.5	7.91 ±0.18	7.71 ±0.02	678 ±13	17 ±1	257 ±03
May	33.5 ±0.5	7.78 ±0.20	7.68 ±0.04	686 ±16	18 ±2	256 ±03
June	35.5 ±0.5	7.60 ±0.30	7.73 ±0.02	682 ±16	17 ±2	271 ±22

groups of 10 fishes were transferred to 5 experimental aquaria, each of 40 L capacity, containing 5 different concentrations of commercial grade insecticide, chlordane (Termex, emulsified concentration 20, manufactured by M/s Rallis India Ltd., Bombay). The remaining 10 fishes, regarded as controls were transferred to another aquarium (40 L capacity) maintained under same conditions to which equal aliquot of acetone that was present in the highest concentration, was added as per general practice. Separate experiments were conducted to determine 48, 96 and 120-hr median lethal limits at 29.0±1.5°C. In these experiments the acetone concentrations in controls were 0.20, 0.20 and 0.048-mg/L, respectively, whereas in 24 hr experiments at 5 different temperature conditions, the solvent concentrations were 0.48, 0.40, 0.32, 0.12 and 0.08-mg/L in the experiments conducted at 22.5, 24.5, 29.0, 33.5 and 35.5-°C temperature conditions, respectively. Preliminary bracketing tests were conducted to determine the chosen chlordane concentrations for each set of experiment. No mortality occurred in the control groups. Concentration-mortality data in the range of 10-90 per cent were recorded. All the experiments were conducted in triplicates. The mean values of the concentration-response data were subjected to probit analysis to determine the values ranging from LC_5 to LC_{95} according to Finney (1971).

Acute toxicity (24 hr) was recorded in the winter and summer months, viz., January, February, May and June representing the two extremes of annual temperature variations and also in

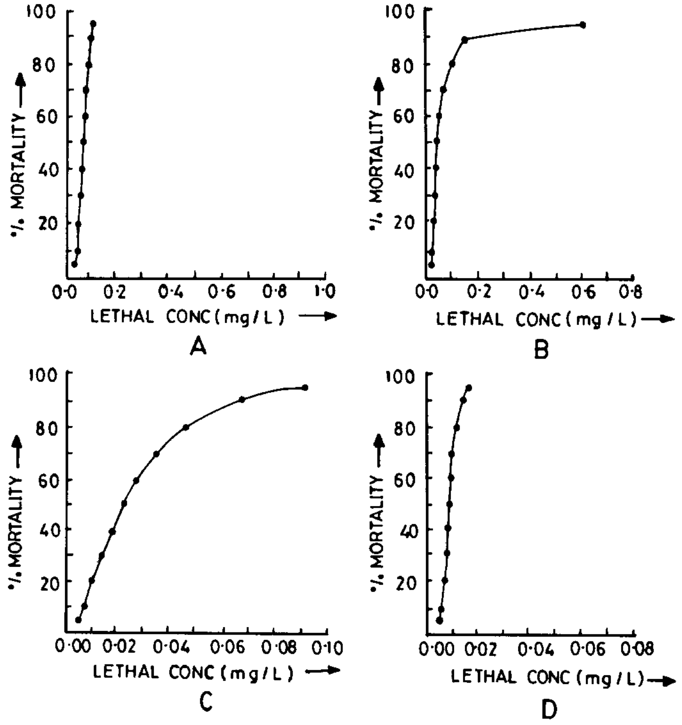

Figure 1. Showing toxicity range (LC_5 - LC_{95}) at different experimental durations.(A-24 hr, B-48 hr, C-96 hr and D-120 hr).

April when temperature was in the intermediate range. The LC_{50} values were analysed for testing the significance of season related variability using ANOVA technique (single - classification) and single-tail t-test. The temperature and LC_{50} data were further analysed for correlation between the two variables. Temperature was considered as independent and LC_{50} value as dependent variable. The regression linearity was tested using t-test and ANOVA with one way classification.The statistical methods employed were as described by Snedecor (1956).

The physico-chemical characteristics of the water (Table 1) were recorded according to APHA (1971). Free carbon dioxide and alkalinity in terms of hydroxide ions were not present.

RESULTS AND DISCUSSION

The intermediate lethal concentration values between 5-95% estimated at four experimental durations are plotted in Fig.1 A-D. The range between LC_5-LC_{95} was narrowest at 24 hr and widest at 48 hr exposure which tended to narrow down thereafter. At 96 hr exposure, the range was intermediate between 48 and 120-hr.

Table 2. Acute toxicity (24 hr) of Chlordane-exposed *H. fossilis* at different ambient temperature conditions.

Ambient water temperature °C	LC_{50} mg/L	95% Fiducial limits mg/L	Probit regression line	Slope	Standard error of slope
22.5 ±1.0	0.089	0.081-0.097	Y=-12.666+9.073.X	9.0726	2.7430
24.5 ±0.5	0.070	0.046-0.106	Y= 0.886+2.229.X	2.2294	0.7620
29.0 ±1.5	0.060	0.052-0.069	Y=- 5.883+6.126.X	6.1260	1.9193
33.5 ±0.5	0.018	0.016-0.021	Y=- 0.363+4.261.X	4.2614	1.0560
35.5 ±0.5	0.014	0.012-0.016	Y=- 2.405+6.476.X	6.4757	2.5711

Apparently the narrower range at 24 hr is due to the high toxicity of chlordane to the test fish. Wider range at 48 hr may be due to the intermediate dehydrogenation product of chlordane which oxidizes into the ultimate metabolite oxychlordane (Matsumura 1975). Narrower toxicity range at 96 hr and onwards than at 48 hr is probably due to the combined toxicity of chlordane and oxychlordane. Cyclodienes are known to be epoxidized into equally or more toxic metabolites.

Of the various experimental conditions, temperature has been regarded as the most important factor modifying the toxicity of pollutants. Reports in this regard are, however, varied. Ambient temperature may bear direct relationship with the toxicity of the pollutant or toxicity may increase at lower temperatures or ambient temperature may have no effect on the toxicity (Sprague 1970). Though majority of workers have observed increase in the toxicity of pollutants to fish with increase in the ambient temperature, it is inappropriate to make a generalization. It is highly likely that toxicity as influenced by temperature is a toxicant specific phenomenon.

How temperature modifies toxicity of a pollutant is not well understood and various explanations have been put forward in this regard. Miller (1977) observed that increased sensitivity of fish to toxicants and disease at higher temperatures is basically due to the decrease in the oxygen concentration. Ferguson et al. (1964) and Adelman and Smith (1972)

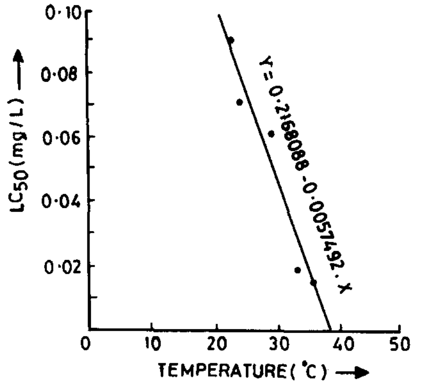

Figure 2. Scatter diagram showing rate of correlation between LC_{50} (24 hr) and seasonal ambient temperature; $r^2=0.9609$.

regarded both, the higher temperature and lower dissolved oxygen are capable of modifying toxicity. Many studies have shown that teleosts experience respiratory stress at higher temperatures due to hypoxic condition which probably makes the fish more vulnerable to toxicants.

Increased chlordane toxicity in the warmer months noted in the present study was apparently due to higher temperatures and lower oxygen levels (Tables 1 and 2). However, H. fossilis is capable of supplementing aquatic respiration by breathing air directly. Increase in the surfacing rate observed by the authors indicated respiratory stress resulting in increased aerial respiration. Our studies strongly emphasize that temperature is the major factor modifying toxicity and reduced availability of oxygen is a minor one. Since the physiological state of the fish is known to be effected by temperature (Gardner and Yewich 1969), variations in the seasonal temperature might have brought about changes in the rhythmic physiological state of H. fossilis too. Hence it is plausible that toxicity data of the present study represent the net response of the fish to seasonal variations in temperature.

The 24 hr LC_{50} values of chlordane at different temperature conditions are given in Table 2. The values were influenced by all the temperature conditions, i.e., in the range of 22.5 - 35.5°C. The median lethal concentration of chlordane was found consistently lower at higher temperatures. The analysis of variance (Table 3) rejected the hypothesis $H_o:\mu_1=\mu_2=\mu_3=\mu_4=\mu_5$. However, comparison of means of LC_{50} at two subsequent temperatures revealed that for testing $H_{01}:\mu_1=\mu_2$ against $H_{11}:\mu_1>\mu_2$, the calculated value of t is 14.97; for testing $H_{02}:\mu_2=\mu_3$ against $H_{12}:\mu_2>\mu_3$, t is 9.95; for testing $H_{03}:\mu_3=\mu_4$ against $H_{13}:\mu_3>\mu_4$, t is 43.84; for testing $H_{04}:\mu_4=\mu_5$ against $H_{14}:\mu_4>\mu_5$,

Table 3. Analysis of variance of LC_{50}.

Source of variation	Sum of squares	Degrees of freedom	Mean sum of squares	F
Between temperatures	0.01317	4	0.0032925	1703.017
Error	0.0000193	10	0.0000019	
Total		14		

Table 4. Analysis of variance of regression line.

Source of variation	Sum of squares	Degrees of freedom	Mean sum of squares	F
Regression	0.00416	1	0.00416	70.864
Error	0.00018	3	0.00006	
Total		4		

H_o:b=0;H_1:b<0; t=-8.42, H_1 is accepted at 0.01 l.s. (**)

t is 4.22. Since tabulated t values (4 d.f.) at 0.05 and 0.01 significance levels are 2.78 and 4.60, respectively, we observe that in all the cases (T_1 to T_5) the alternative hypothesis (H_{11} to H_{14}) is accepted. Increase in the ambient temperature by 2.0, 6.5, 11.0 and 13.0 - ^oC induced 20.86, 32.49, 79.52 and 84.28 - % decrease in the LC_{50} values, respectively. Pearson's coefficient of correlation was -0.9803 which showed high degree of correlation at 0.05 and 0.01 significance levels between temperature and the LC_{50} values, whereas the negative value indicated inverse correlation between the two variables. The regression coefficient (b) was found highly significant (t=-8.42**, n-2=3 d.f.). Analysis of variance of the regression line is summarized in Table 4. High F value further confirms the good fits of the regression line. Our findings thus clearly show a linear relationship between the seasonal temperature changes and LC_{50} values in chlordane-treated H. fossilis and do not support the contention of Brown (1980) that temperature-toxicity response is non - linear, for this species of fish. However, variability at each temperature may make it difficult to establish a non-linear relationship.

Fisheries and environmental managers particularly in the tropical countries find it difficult to make predictions in terms of hazard assessment of pesticides to fishes in season related changes in temperature as there are no set guidelines in this regard. In the present study, 0.006 mg/L decrease in the LC_{50} value of chlordane has been predicted following every $1^{o}C$ rise in the ambient temperature between 20-36^{o}C. LC_{50} estimates derived from the regression equation are close to those obtained in this study and hence the equation can be used to predict LC_{50} values at any specified temperature (Fig.2) for this species of fish.

Acknowledgments. Authors are grateful to Dr.C.B.L.Srivastava, Head of the Department of Zoology, University of Allahabad for providing laboratory facilities and Dr.Anoop Chaturvedi for his valuable assistance in statistical analysis.

REFERENCES

Adelman IR, Smith LL (1972) Toxicity of hydrogen sulphide to goldfish (Carassius auratus) as influenced by temperature, oxygen and bioassay techniques. J Fish Res Board Can 29: 1309-1317

APHA (1971) Standard methods for the examination of water and wastewater. American Public Health Association, 13th ed. New York

Brown VK (1980) Acute toxicity in theory and practice. John Wiley and sons, Chichester

FAO/WHO (1973) Evaluation of some pesticide residues in food. FAO/AGP/1973/M/9/1 (1974)

Ferguson DE, Culley DD, Cotton WD, Dodds RP (1964) Resistance to chlorinated hydrocarbon insecticides in three species of freshwater fish. Bioscience 14:43-44

Finney DJ (1971) Probit analysis. Cambridge University Press, London

Gardner GR, Yewich PP (1969) Studies on the blood morphology of three estuarine cyprinodontiform fishes. J Fish Res Board Can 26:433-446

Matsumura F (1975) Toxicology of insecticides. Plenum Press, New York

Miller S (1977) The impact of thermal effluents on fish. Environ Biol Fish 1:219-222

Rai UP, Mandal PK (1990) Seasonal temperature related aldrin toxicity to an air-breathing catfish, Heteropneustes fossilis (Bloch.). J Freshwat Biol 2:305-312

Snedecor GW (1956) Statistical methods. The Iowa State University Press, Ames

Sprague JB (1970) Measurement of pollutant toxicity to fish. II. Utilizing and applying bioassy results. Wat Res 4:3-32

Received March 11, 1992; accepted March 1, 1993.