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Acute Toxicity of Thiodan to Catfish (*Heteropneustes fossilis*)[1]

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Endosulfan is widely used as an insecticide in place of endrin. This organochlorine compound is found to be highly toxic to fish (MULLA et al. 1967; MACEK et al. 1969; SCHOETTGER 1970). Thiodan (registered trade name of a formulation containing endosulfan) is extensively used for the control of foliar pests of agricultural crops of this area. So, it has been attempted here to assess the toxicity of Thiodan to H. fossilis, a common edible catfish of Indian freshwaters.

MATERIAL AND METHODS

H. fossilis were collected from unpolluted habitats. They were held for 3-4 weeks in aquaria of 100-L capacity filled with dechlorinated tap water (pH, 7.8 ± 0.001 S.E.; dissolved oxygen, 7.2 ppt ± 0.36 S.E.; total alkalinity, 0.12 ppt ± 0.001 S.E.; free carbon dioxide, 90 ppt ± 3.36 S.E.), which was changed daily. The fish were fed dry shrimp powder.

Toxicity was determined by static bioassay. All experiments were conducted in cylindrical glass aquaria (diam., 23 cm; capacity, 15 L). Each aquarium contained 12 L of dechlorinated tap water, to which the appropriate quantity of Thiodan was slowly added after 1 h of transfer of fish from reservoir tank to experimental aquaria. Addition of toxicant was not found to alter the pH and other chemical characteristics of the medium.

For the purpose of offsetting the effects, if any, of circadian variations and seasonal shifts in photoperiod, the method of MCLEAY & GORDON (1978) was adopted. The experiments were always initiated at a fixed interval of 1-1/2 - 2-1/2 h after sunrise.

Technical grade of Thiodan 35 EC, having endosulfan as its active ingredient, was dissolved in acetone to prepare its stock solution.

All the experiments were conducted at room temperature (18-30°C). Fish were not fed during the experiments. As the test medium was not

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renewed during experiments, only 5 fish were kept in each aquarium. Artificial aeration was not considered necessary because (a) the number of fish in each aquarium was relatively small, (b) oxygen absorption at the exposed water surface was deemed sufficient, and (c) the test fish is air-breathing.

Observations were taken every 12 h; numbers of dead fish and unaffected fish were noted. Fish which lost their equilibrium, floated ventral side up, and did not respond to touch, were considered to be dead, and were promptly removed from the aquaria. Experiments were continued for 96 h for each tested concentration of Thiodan formulation. A minimum of 5 concentrations was used for each experiment, and 15 fish were exposed to each concentration.

The population of H. fossilis, obtained from the collection sites, was seen to comprise individuals of mostly two size ranges as follows:

	Total length (cm)	Body weight (g)
Group I	19.9 (19.6-20.6) ± 0.4	43.8 (40.6-45.8) ± 1.7
Group II	10.0 (9.2-11.6) ± 0.7	11.1 (9.4-12.8) ± 1.2

The toxicity was, therefore, determined separately for the two groups.

The susceptibility of fish to insecticide was measured in terms of 96-h LC50 (concentration of Thiodan in water which kills 50% of the fish population in 96 h), which has been expressed as parts per million (ppm).

The LC50 was determined separately for every month of the year 1980. The concentrations of insecticide tested and percent mortalities observed were subjected to regression, and linear regression equations derived for calculating LC50. The yearly average LC50 (mean of all values recorded monthly for the whole year) was calculated for both group I and II, and 95% confidence intervals were estimated. The difference between the yearly average LC50 for groups I and II was tested for statistical significance by a t-test. For the data on seasonal variation of LC50, curve fitting was done by curvilinear regression involving the principle of orthogonal polynomials, and graphs of polynomials of 4th degree were fitted to the data.

Since larger and heavier fish (group I) were seen to be more tolerant than smaller and lighter fish (group II), attempts were made to find out how the tolerance of fish to insecticide was related to their size and weight. The fish were divided into 4 lots in order of increasing length and weight, and LC50 was determined separately for each lot. These experiments were conducted at 29°C ± 1. The correlation coefficient, r, was then calculated, and tested for statistical significance, for tolerance and length as well as tolerance and weight. Regression of LC50 on length, and LC50 on weight, was also made.

All statistical treatments were made according to methods recommended by SNEDECOR & COCHRAN (1968).

RESULTS

H. fossilis individuals of greater length and weight were seen to be more tolerant to Thiodan. The yearly average LC50 was 0.018 ppm (95% confidence interval, 0.016 - 0.020) and 0.0097 ppm (95% confidence interval, 0.0085 - 0.0108) respectively, for group I and II, the difference between the two averages being statistically very significant ($P < 0.001$). No mortality was observed in controls.

Seasonal variation of LC50 (Table 1, Fig. 1):

Tolerance of the fish to Thiodan showed significant seasonal variation. The fish were seen to be more tolerant to insecticide during the colder months of the year. LC50 was the maximum (0.023 ppm for group I and 0.012 - 0.013 ppm for group II) during December and January, and the minimum (0.014 - 0.015 ppm for group I and 0.0077 - 0.0078 ppm for group II) during June, July and August. The trend of variation for the two groups of fish appeared to be similar, the shape of regression curves being almost the same.

LC50 for the different months of the year, when considered in relation to the corresponding monthly water temperature, displayed a marked tendency to be lower at higher temperatures. A definite inverse proportion between LC50 and water temperature could be established. The correlation coefficients of -0.975 and -0.962 for groups I and II, respectively, were statistically very significant ($P < 0.001$). Regression analysis revealed that LC50 decreased at the rate of 0.0008 and 0.0004 ppm per °C for group I and II, respectively.

LC50 in relation to size and weight of fish (Table 2, Fig. 1):

Tolerance of fish to Thiodan was directly proportional to their length and weight. LC50 for lot 1 (smallest and lightest individuals) was the minimum, and that for lot 4 (largest and heaviest individuals) the maximum. The coefficient of correlation between LC50 and body

Table 1. Seasonal variation of Thiodan toxicity.

Month	Water temp. (°C)	No. of fish	96-h LC50 in ppm Group I	Group II
Jan.	18	75	0.0228	0.0127
Feb.	20	75	0.0222	0.0100
Mar.	22	75	0.0199	0.0098
Apr.	24	75	0.0169	0.0107
May	25	75	0.0170	0.0091
Jun.	30	75	0.0151	0.0077
Jul.	29	75	0.0141	0.0077
Aug.	29	75	0.0143	0.0078
Sep.	28	75	0.0141	0.0074
Oct.	24	75	0.0181	0.0103
Nov.	22	75	0.0203	0.0106
Dec.	18	75	0.0228	0.0124
Yearly average			0.0181	0.0097
S.D.			± 0.0034	± 0.0018
95% confidence interval			0.0160 - 0.0203	0.0085 - 0.0108

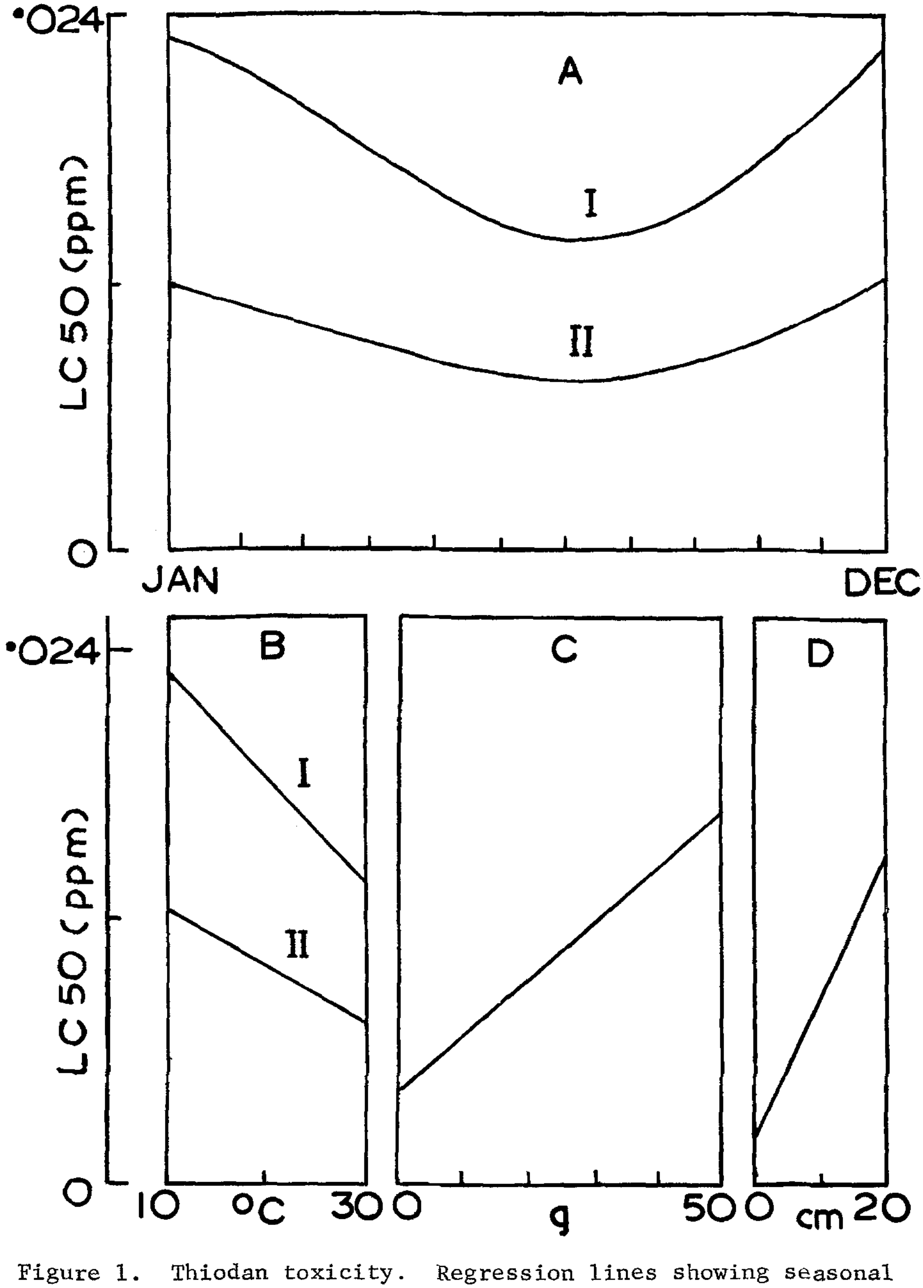

Figure 1. Thiodan toxicity. Regression lines showing seasonal variation for fish of group I and II (A), and its relation to environmental temperature (B), and the relation of toxicity with weight (C) and length (D) of stressed fish.

weight was 0.998 and that between LC50 and total length 0.995, both the coefficients being statistically very significant ($P < 0.01$). Regression analysis showed that LC50 increased at the rate of 0.0003 ppm per g weight and 0.0007 ppm per cm length of fish.

Table 2. Thiodan toxicity in relation to fish size and weight.

Lot No.	No. of fish	Length (cm)	Weight (g)	LC50 (ppm)
1.	75	6.2 (5- 7) ± 0.5	4.8 (4- 6) ± 0.7	0.0050
2.	75	10.2 (10-12) ± 0.5	11.3 (9-14) ± 1.6	0.0073
3.	75	14.3 (13-15) ± 0.7	23.2 (18-26) ± 2.7	0.0100
4.	75	19.7 (18-20) ± 0.8	41.8 (35-48) ± 4.7	0.0147

DISCUSSION

Susceptibility of *H. fossilis* to Thiodan showed marked seasonal variation, being greater during the warmer months; LC50 exhibited an indirect relation with environmental temperature, and varied accordingly through the year. Such an interrelationship between temperature and susceptibility to toxicants appears to be common for fish. A wide range of insecticides have been found to increase in toxicity at higher temperature (BRIDGES 1965; MACEK et al. 1969; MUIRHEAD-THOMSON 1971).

The mechanism involved in the increase of susceptibility of fish to toxicants with rise in temperature is not well understood, though effect on general metabolism and respiration rate could be involved (MACEK et al. 1969; WEDEMEYER et al. 1976; GORDON & MCLEAY 1977). Rise in water temperature reduces the solubility of oxygen in water. This could affect fish physiology. It could increase the metabolic rate (oxygen demand) of fish (DAVIS 1975), limiting the effectivity of blood oxygenation and haemoglobin affinity for oxygen (Bohr effect), thus resulting in low dissolved oxygen levels and greater accumulation of waste products and lowering the resistance of fish to stress. Reduced solubility of oxygen in water at higher temperatures could also increase the ventilation at gills and the respiration rate (JONES et al. 1970), causing a larger quantity of water to move across the gill epithelium, thus increasing the possibility of greater uptake of contaminant(s) from the medium and intensifying the pollutional stress. In case of Thiodan toxicity, lethal concentrations have been shown to raise oxygen consumption of fish like *Mystus vittatus* (REDDY & GOMATHY 1977). Whatever be the physiological mechanism(s) underlying the lowering of resistance of *H. fossilis* to stress of Thiodan toxicity at higher temperatures, the general effect of temperature is evident and, as suggested by MACEK et al. (1969), emphasises the need for an enquiry into the interaction between pesticides and ecofactors to determine safe concentrations of pesticides in aquatic habitats.

However, while considering the seasonal variation in susceptibility of *H. fossilis* to Thiodan, the biological state of the fish through the course of the year might also be taken into account. Studies by SUNDARARAJ (1959, 1960) have shown that the annual reproductive cycle of *H. fossilis* comprises 4 phases, i.e., preparatory (Feb. - Apr.), prespawning (May - Jul.), spawning (Aug. - Oct.), and postspawning (Nov. - Jan.). It is, therefore, evident from the present study that *H. fossilis* is more sensitive to Thiodan during that part of the year (Jun. - Sep.) which corresponds more or less to the spawning period of the fish. Increased susceptibility to stress from pollutants during spawning condition has also been observed in other fish, e.g., Pacific herring exposed to benzene toxicity (KORN et al. 1976). It is, therefore, felt that functional state of fish, particularly with reference to its breeding cycle, could be a significant biological factor affecting the resistance of fish to environmental contamination.

Fish sensitivity to toxicants is, in general, inversely proportional to size (WEDEMEYER & YASUTAKE 1978), and *H. fossilis* seems to be no exception with regard to its susceptibility to Thiodan; there was an indirect relationship of LC50 with length and weight of stressed fish. However, while size would be a general measure of age, and older fish would be expected to be more capable of physiological adjustment to stress (WEDEMEYER et al. 1976), it stands to reason that increase in tolerance with increase in size and weight, as observed in the present study, cannot be extrapolated indefinitely.

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