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Influence of Thermal Prehistory on Endosulfan Susceptibility of *Oziotelphusa senex senex*, a Freshwater Crab

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Water temperatures in aquatic environments vary both seasonally and diurnally and it is well documented that differences in water temperature affect the susceptibility of fauna to toxicants. The effects of temperature regime on the susceptibility of fish (Schoettger 1970; Cairns et al. 1978; Sprague 1985; Keller et al. 1988) and crustaceans (Sanders 1969; McLeese et al. 1980; Mayer and Ellersieck 1986; Sogorb et al. 1988) to pesticides have been studied extensively, but the effects on the susceptibility of freshwater crabs have rarely been examined.

Oziotelphusa senex senex (Fabricius) is an edible freshwater crab inhabiting burrows in paddy fields and irrigation canals of south India. Endosulfan [Thiodan®] (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9,methano-2,4,3-benzodioxathiepine-3-oxide), an organochlorine compound, used extensively to kill insect pests of rice, secondarily contaminates freshwater ecosystems and thus affects non-target species such as O. senex senex. Endosulfan effects on oxygen consumption (Subhadra Devi 1985), energy reserves (Kallapur and Yadway 1986), oxygen transportation (Vijayakumari et al. 1987), hydromineral balance (Rajeswari et al. 1988) and glutathione content and glutathione-S transferase activity (Yadwad 1989) of O. senex senex have been examined. However, the influence of thermal prehistory on the susceptibility to endosulfan, or any other pesticide, of O. senex senex has not previously been examined.

MATERIALS AND METHODS

O. senex senex were collected from paddy fields and irrigation canals around Tirupati, India (13.39N, 79.25E) (water temperature 26±1°C), before spraying with endosulfan began to ensure they were not contaminated. Uninjured, male, intermoult (C4) crabs of approximately equal size (28±1g) were transferred to large, uncrowded holding tanks at 28°C with ad libitum food. The physicochemical properties of the well water (Table 1), changed daily, were maintained throughout the acclimatization and experimental periods. Crabs acclimatized to these conditions for 7 d were used in acute toxicity testing and respiration measurements.

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Table 1. Physicochemical parameters of the test medium used during acclimatization and the experimental exposure of Oziotelphusa senex senex to endosulfan.

Parameter	Range
Light regime	12 hr light:12 hr dark
Dissolved oxygen	7.8 to 8.0 $mg.L^{-1}$
Salinity	0.19 $g.L^{-1}$
Chlorinity	0.11 $g.L^{-1}$
Sodium	128 m $moles.L^{-1}$
Potassium	30.2 m $moles.L^{-1}$
Calcium	4.28 m $moles.L^{-1}$
Carbon dioxide	2.08 $mg.L^{-1}$
Specific conductivity	212 μS
Alkalinity	102 $mg.L^{-1}$ (as $CaCo_3$)
Hardness	112 $mg.L^{-1}$ (as $CaCo_3$)

Groups of crabs were conditioned to test water temperatures (12±1°, 28 ±1° and 38 ±1°C) in separate tanks with loadings not exceeding 1 $g.L^{-1}$ for 3 d prior to acute toxicity testing. A stock solution of technical grade (95%) endosulfan (Parry and Co., Madras, India) was prepared in acetone (Gupta and Gupta 1979) each day to make the required dilutions. Acute toxicity tests were simultaneously conducted at each of the three test temperatures over the range 0, 8, 12, 16, 20, 24 and 28 $mg.L^{-1}$ endosulfan. Ten crabs were exposed to each concentration at each of three temperatures and the mortality recorded daily for 96 hr. The unweighted regression method of probit analysis was used to calculate 96-hr LC50 and 95% confidence limits (Finney 1971).

Groups of O. senex senex (n=10) were exposed for 96 hr to the range of acetone concentrations (0.5-2.0 $mL.L^{-1}$) used in preparing the stock solutions of endosulfan and survivorship recorded hourly.

Crabs collected during the last weeks of January 1989 (temperature 22±1°C) (Group 1) and June 1989 (temperature 39±1°C) (Group 2) were acclimatized for 3 mon to 22±1°C and 39±1°C, respectively. These two collecting periods represent the extremes of the annual temperature regime in Tirupati. After acclimatization, both groups (n=10) of crabs were transferred to 28°C and after 3 d endosulfan susceptibility measured.

The basal (resting) respiration rates of O. senex senex were measured at 12°, 28° and 38°C. Individual crabs (six per temperature) were placed in respiratory chambers (500 mL), allowed to stabilize for 12 hr and oxygen consumption over 6 hr measured following Winkler's iodometric technique (Mackereth et al. 1978).

RESULTS AND DISCUSSION

As the crabs exposed to acetone (0.5-2.0 $mL.L^{-1}$) for 96 hr showed 100% survival, mortality exhibited by O. senex senex in the acute toxicity tests can be attributed to the effects of endosulfan. Crabs collected at 26°C and acclimatized to 28°C and subsequently exposed to a range of endosulfan concentrations at different temperatures (Table 2) showed a significant decrease in LC50 with increase in temperature. The relative change in susceptibility to endosulfan showed an increase of 2.3 times between 12° and 38°C, 1.6 between 12° and 28°C and 1.4 between 28° and 38°C.

Table 2. LC50 values ($mg.L^{-1}$) with 95% confidence limits of Oziotelphusa senex senex acclimatized to 28°C and exposed to endosulfan at 12°, 28° or 38°C (n=10 for each concentration at each temperature).

	Temperature		
	12°C	28°C	38°C
LC50	28.6	17.4	12.2
95% CL	26.7-30.8	16.0-18.3	10.9-13.6

Crabs collected from the field at different times, and thus with different thermal prehistories, but tested at 28°C showed significant differences ($p<0.05$) in susceptibility with crabs acclimatized to 22°C having a significantly higher LC50 than those acclimatized to 39°C (Table 3).

Table 3. LC50 values ($mg.L^{-1}$) with 95% CL of Oziotelphusa senex senex at 28°C for groups (n=10 for each concentration at each temperature) of crabs with different thermal prehistories of 22°C and 39°C.

Thermal Prehistory	22°C	39°C
LC50	17.2	15.1
95% CL	15.9-18.2	13.9-15.4

The basal (resting) oxygen consumption (Table 4) showed significant increases ($p < 0.05$) with temperature. The relative increase in oxygen consumption between 12° and 38°C was 3.3, between 12° and 28°C it was 2.6 and between 28° and 38° it was 1.4.

Table 4. Basal (resting) oxygen consumption (mL O_2.g.hr^{-1}) (mean ± S.D.) of Oziotelphusa senex senex exposed to 12°, 28° or 38°C (n=6 for each temperature).

	Temperature		
	12°C	28°C	38°C
Oxygen Consumption	0.071 ± 0.008	0.167 ± 0.026	0.234 ± 0.031

An increase in susceptibility to a toxicant with increase in temperature has frequently been reported, and thus the increase in susceptibility of O. senex senex to endosulfan over the range of 12°-38°C was expected. Decrease in LC50 with increase in temperature suggests that the accumulation of endosulfan increases proportionally more than that metabolized by the crabs, as demonstrated for fish by Schoettger (1970).

The endosulfan LC50 of O. senex senex (17.4 mg.L^{-1} at 28°C) (Table 2) compared with that recorded by Vijayakumari et al. (1987) (15.1 mg.L^{-1}) is significantly different ($p<0.05$) . However, except for the time of collection from the field, all acclimatization and experimental parameters used in this study and that of Vijayakumari et al. (1987) were exactly the same, including water temperature, time of exposure, size (weight), sex and nutritional status of the crabs.

Although it is impossible to document all the differences in prehistory between animals collected from the field at different times, the most overt difference between the crabs collected in July (Vijayakumari et al. 1987) and those collected in February in this study is the thermal prehistory. All other environmental parameters including food abundance were apparently similar. This conclusion is supported by the data from O. senex senex collected at different times of the year with different thermal prehistories and then tested at 28°C (Table 3). Crabs having experienced a mean water temperature of 22°C over the preceding 3 mon had a significantly ($p<0.05$) higher LC50 (17.2 mg.L^{-1}) than crabs that had experienced a mean water temperature regime of 39°C and had a LC50 of 15.1 mg.L^{-1}. Thus, tested at the same ambient temperature (28°C) differences in thermal prehistory significantly affected endosulfan susceptibility of O. senex senex. This study demonstrates that for animals similar in all other respects that differences in LC50 result from differences in the thermal regime experienced prior to experimental acclimation.

Macek et al. (1969) speculated that the mechanism involved in the increased susceptibility of fish to pesticides was a higher rate of pesticide uptake at higher temperatures. Murphy and Murphy (1971) supported this and showed a positive correlation between respiration

and DDT uptake by Gambusia affinis. As gills are the main respiratory organs for both fish and crabs, it is possible that the gills of O. senex senex constitute the main route of endosulfan uptake from the water. Although basal respiration and susceptibility to endosulfan of O. senex senex increases with temperature (Tables 2 and 4), analysis of the relative increase in oxygen consumption and the relative increase in susceptibility shows that there is no direct linear relationship between the two parameters.

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