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Acute Toxicity of the Pesticides Endosulfan and Ametryne to the Freshwater Prawn *Macrobrachium rosenbergii* De Man

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The freshwater prawn culture plays an important role in aquaculture development, particularly in some countries such as Taiwan, Thailand, India, Ecuador and Brazil. *Macrobrachium rosenbergii* has been one of the main species used in freshwater prawn farming. The success of this activity is directly related to the water quality in the culture ponds. The use of pesticides in agriculture has undoubtedly contributed to increase the crop-yield, but it has also produced adverse effects in the natural environment. Pesticides can be washed downstream affecting non-target aquatic organisms, including those commercially important fish and crustaceans which are produced in aquaculture farms.

Endosulfan is an organochlorine insecticide and acaricide used extensively in agriculture throughout the world. The acute toxicity of this pesticide has been reported for many species of fish. However, relatively little is known about its toxicity to freshwater prawns (Omkar and Murti 1985; Berbet et al. 1989; Natarajan et al. 1992).

The triazine chemical group is used in the formulation of a large number of herbicides, including the ametryne compound which is widely used especially in Brazil. A few studies on toxicity of ametryne have been conducted on fish (Johnson and Finley 1980; Hartley and Kidd 1987), however its toxicity to freshwater prawns has not been investigated so far.

The objective of this study was to determine the acute toxicity of endosulfan and ametryne to the freshwater prawn *Macrobrachium rosenbergii*, according to static and renewal tests.

MATERIAL AND METHODS

Macrobrachium rosenbergii postlarvae were produced under rigorously controlled conditions in the Instituto de Pesca's hatchery – SP – Brazil. Fifteen days after the metamorphosis, prawns were transferred to the

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bioassay laboratory, and acclimatised for 48 hours in a 100-liter glass aquarium. During this period, postlarvae were fed with dry commercial food (pellets with 35% of protein content).

Reconstituted standard water was used for acclimatising prawns and conducting tests. It was prepared according to the standard methods (APHA et al. 1989), which state the composition of soft waters by adding the required salts ($NaHCO_3$, $CaSO_4.2H_2O$, $MgSO_4$ and KCl) to deionized fresh water. The temperature was controlled by air conditioning and adjusted to 28°C, and the photoperiod was 12 hr L:D cycle.

The test pesticides used in this study were: the organochlorine endosulfan (Thiodan CE™) – Hoechst do Brasil Quim. e Farm. S.A., and the herbicide ametryne (Gesapax 500™) – Ciba Geigy Ltd. The stock solutions of these pesticides were prepared by dissolving a calculated amount of active ingredients in distilled water. A series of five concentrations (toxic range determined by preliminary tests) of each pesticide was prepared by adding a calculated volume from the stock solution into test containers. Therefore, toxic nominal concentrations were: 0.35, 0.70, 1.40, 2.80, 4.20 $\mu g\ L^{-1}$, and 5.0, 10.0, 20.0, 25.0, 30.0 $mg\ L^{-1}$ for testing endosulfan and ametryne, respectively. One container was maintained as the unexposed control group. Test containers were constituted by placing 5 liters of test solution in rectangular 16-liter glass aquaria. Each aquarium was covered with a plastic film to prevent volatilization. Air pumps and individual stone air diffusers provided the aeration. Twenty postlarvae (mean weight of 0.020 ± 0.009 g) were randomly distributed into each test container (4 postlarvae L^{-1}, n=20). No food was supplied during the experiments.

The bioassays were carried out according to two different methods: static and renewal tests (APHA et al. 1989). In the static tests, there were no solution replacements during the 96 hours of exposure. In the renewal tests, solutions were replaced by fresh ones of the same respective concentrations after every 24 hours until 96 hours of testing. A parallel aquaria battery was maintained with the fresh solution. Prawns were carefully transferred from the "in use" aquaria (with the 24 hr used solution) to the new aquaria battery (with the fresh solution in the same initial nominal concentration). Aquaria batteries were replicated four times in the static method and three times in the renewal one.

Dissolved oxygen ($mg\ L^{-1}$), temperature (°C), pH and electric conductivity ($\mu S\ cm^{-1}$) were recorded individually in each test container at exposure times of 24, 48, 72 and 96 hours. Hardness and alkalinity ($mg\ CaCO_3$) and total ammonia ($mg\ L^{-1}$) were determined by standard methods (APHA et al. 1989) only after ending the experiments.

Mortalities were recorded at 24, 48, 72 and 96 hours of exposure and the dead organisms were removed regularly from the test solutions. The criterion for death was taken to be the total lack of movement, observed when prawns did not respond to gentle touching with a glass rod. The obtained data was analyzed through the Multi-method LC_{50} and Gwbasic 3.10 software, according to the four statistical methods for estimating Median Lethal Concentrations (LC_{50}): Binomial (Stephan 1977), Moving Average Interpolation (Bennett 1952; Stephan 1977), Probit (Finney 1971) and the Trimmed Spearman Karber (Hamilton et al. 1977). Since the estimated data showed no statistical differences, the lower LC_{50s} were used in order to provide large security margins. The median values of LC_{50s} obtained in static and renewal tests were analyzed by student *t*-tests. Safe concentration levels were estimated by the quotient of LC_{50}(96hr) / 100, according to the same methodology used by Natarajan et al. (1992).

RESULTS AND DISCUSSION

The LC_{50s} determined for endosulfan ranged from 1.64 to 2.08 $\mu g\ L^{-1}$ at 24 hr of exposure, and from 0.93 to 0.20 $\mu g\ L^{-1}$ at 96 of exposure in the static and renewal bioassays, respectively, while for ametryne LC_{50s} ranged from 27.81 to 19.62 mg L^{-1} at 24 hr of exposure, and from 7.54 to 6.25 mg L^{-1} at 96 hr of exposure. The variations between static and renewal tests were statistically significant (Table 1), except for endosulfan (24 hr of exposure), which was expected, since solution was not changed during this period. Despite this, the statistical test pointed out a significant difference at 24 hr of exposure for ametryne.

The different values of toxicities determined for static and renewal tests may be related to several factors. The toxic action of pesticides may have been altered during static tests due to high instabilities of their active ingredients in the water. According to Worting and Hance (1991), endosulfan is very unstable in alkaline media which provides slow hydrolysis to diol and sulfur dioxide. This transformation seems to have occurred in the static test, reducing the toxicity of this pesticide during the 96 hours of exposure. The same statement may also be made for ametryne, since the hydrolysis of this pesticide produces an inactive 6-hydroxy analogue (Worthing and Hance 1991). In addition, the physico-chemical properties of toxicants, as well as other biological aspects should be considered for explaining those discrepancies of toxicity (e.g., vapor pressure, volatilization, adsorption, lipophilicity, bioaccumulation, etc.).

The physical and chemical parameters analyzed during the bioassays showed no differences among the range of 5 concentrations, neither between concentration ranges and control groups, therefore it was possible to calculate the respective averages for these parameters (Table 2). Similarly, there were no significant variations between the

Table 1. Comparative LC_{50} values for static and renewal acute toxicity tests with endosulfan and ametryne to *M. rosenbergii.*

Exposure time (hr)	LC_{50} static	LC_{50} renewal	Student *t*-test *t*	d.f.
Endosulfan ($\mu g\ L^{-1}$)				
24	1.64 (1.11-2.43)[a]	2.08 (1.45-2.99)	-1.905[ns]	19
48	0.99 (0.53-2.45)	0.57 (0.21-0.74)	6.354**	26
72	0.96 (0.53-2.10)	0.31 (0.06-0.65)	5.988**	26
96	0.93 (0.69-1.21)	0.20 (0.04-0.38)	6.309**	17
Ametryne ($mg\ L^{-1}$)				
24	27.81 (20.42-38.14)	19.62 (15.29-25.38)	5.650**	18
48	22.47 (15.62-27.77)	11.94 (7.06-16.84)	6.298**	25
72	11.30 (3.71-17.61)	7.21 (2.73-10.74)	4.057**	25
96	7.54 (2.40-12.62)	6.25 (3.13-8.80)	2.242*	25

a = 95% confidence limit; ns = non significant; * ($P< 0.05$); ** ($P< 0.01$);
d.f.= degrees of freedom

Table 2. Physical and chemical parameters of water used in bioassays (± standard deviation).

Parameter	Endosulfan static	Endosulfan renewal	Ametryne static	Ametryne renewal
Temperature (°C)	26.88 ± 0.31	27.60 ± 0.80	23.85 ± 0.38	23.81 ± 0.84
pH	7.48 ± 0.13	7.42 ± 0.18	7.33 ± 0.20	7.47 ± 0.19
Electric conductivity ($\mu S\ cm^{-1}$)	174.65 ± 2.74	169.21 ± 8.66	171.06 ± 3.61	171.96 ± 5.58
Dissolved oxygen ($mg\ L^{-1}$)	7.06 ± 0.13	7.42 ± 0.17	7.56 ± 0.17	7.62 ± 0.22
Hardness ($mg\ CaCO_3\ L^{-1}$)	44.79 ± 1.50	46.80 ± 0.78	48.89 ± 1.55	45.87 ± 2.22
Alkalinity ($mg\ CaCO_3\ L^{-1}$)	27.33 ± 1.10	33.33 ± 2.57	35.98 ±1.56	32.97 ± 3.09
Total ammonia ($mg\ L^{-1}$)	0.38 ± 0.14	0.32 ± 0.10	0.74 ± 0.12	0.24 ± 0.18

static and renewal test methods for any parameter, except for the total ammonia concentration, which was statistically higher ($P<0.01$) only in the static test carried out with ametryne. Although ammonia concentration had been higher in that test, it did not exceed 1.0 mg L^{-1}, reported by Sandifer et al. (1983) as the critical level to postlarvae of *M. rosenbergii*. Anyway, renewal test seems to be the best method for carrying out bioassays with no toxic interference of ammonia, especially for tests with ametryne.

A comparison of the acute toxicity values among pesticides indicated that the organochlorine endosulfan is 8108 and 31250 times more toxic than ametryne, in 96 hours of static and renewal exposures, respectively.

Different levels of endosulfan sensitivity were determined for some macrocrustaceans (Table 3). Berbet et al. (1989) and Natarajan et al. (1992) found higher LC_{50s} of endosulfan to *M. rosenbergii* than those determined in this study. However, they used larger organisms (1.4 ± 0.3 g) and higher alkalinity (200 ± 20 mg $CaCO_3$ L^{-1}), which can produce different levels of susceptibility.

The toxicity of ametryne to freshwater prawns has not been reported in the literature so far. Data reported for acute toxicity of ametryne to fish (Table 4) are in the same order of magnitude to those estimated in the present study for *M. rosenbergii*.

Table 3. Acute toxicity of endosulfan to macrocrustaceans.

Species	Exposure time (hr)	LC_{50} (µg L^{-1})
Barytelphusa guerini	96	0.018[a]
Crangon septemspinosa	96	0.20[b]
Heteropanope indica	96	31.0*[,c]
Macrobrachium dayanum	96	4.10[d]
Macrobrachium rosenbergii	72	5.40[e]
Macrobrachium rosenbergii	96	6.0[f]
Macrobrachium rosenbergii	**24**	**1.64 – 2.08[g]**
Macrobrachium rosenbergii	**48**	**0.57 – 0.99[g]**
Macrobrachium rosenbergii	**72**	**0.31 – 0.96[g]**
Macrobrachium rosenbergii	**96**	**0.20 – 0.93[g]**
Nanosesarma batavicum	96	31.0*[,c]
Neoepisesarma mederi	96	31.0*[,c]
Oziotelphusa senex senex	96	0.019[h]
Oziotelphusa senex senex	96	0.029[i]

* = larval stage; a = Reddy et al. (1995); b = Mcleese and Metcalfe (1980); c = Selvakumar et al. (1996); d = Omkar and Murti (1985); e = Berbet et al. (1989); f = Natarajan et al. (1992); g = present study; h = Rajeswari et al. (1988); i = Reddy et al. (1992)

Table 4. Acute toxicity of ametryne to aquatic macroorganisms.

Species	Exposure time (hr)	LC_{50} (mg L^{-1})
Macrocrustaceans		
Macrobrachium rosenbergii	**24**	**19.62 – 27.81[a]**
Macrobrachium rosenbergii	**48**	**11.94 – 22.47[a]**
Macrobrachium rosenbergii	**72**	**7.21 – 11.30[a]**
Macrobrachium rosenbergii	**96**	**6.25 – 7.54[a]**
Fish		
Carassius auratus	96	14.10[b]
Lepomis macrochirus	96	3.70[c]
Mugil cephalus	96	0.68[d]
Salmo gairdneri	96	3.20[c]

a = present study; b = Hartley and Kidd (1987); c = Johnson and Finley (1980); d = USEPA (1984)

Safe concentration levels for endosulfan and ametryne were estimated to be 0.002 μg L^{-1} and 0.063 mg L^{-1}, respectively, whereas Natarajan et al. (1992) reported safe levels of endosulfan for *M. rosenbergii* of 0.06 μg L^{-1}.

Tan and Vijayaletchumy (1994) found 24 water samples contaminated with endosulfan among 25 samples analyzed from the major rivers in Peninsular Malaysia (natural habitat of *M. rosenbergii*), and the maximum contamination level was 0.12 μg L^{-1}. Maximum concentrations of ametryne (0.1 and 450 μg L^{-1}) were reported in the USA by USEPA (1988) for surface and ground waters, respectively. Therefore, its is important to be concerned about the environmental levels of these pesticides, since they may be a risk to the natural populations of freshwater prawns and also a serious problem for the farming producers.

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