Bull. Environ. Contam. Toxicol. (1991) 46:185–192

Environmental Contamination and Toxicology

Effects of Lindane and Acetone on the Development of Larvae of the Southern King Crab (*Lithodes antarcticus* Jaquinot)

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Among marine organisms, crustaceans have been found to be those that suffer most severely the effects of organochloride pesticides (Epifanio 1971). Several studies have documented high susceptibility of crustaceans to organochlorines in natural areas treated with these pesticides (Cottam and Higgins 1946, Harrington and Bidlingmayer 1958, Springer 1961, Odum et al. 1969). Likewise, laboratory studies have also shown high sensitivity of crustaceans to organochlorines during larval stages (Bookhout and Costlow 1970, Buchanan et al. 1970, Epifanio 1971, Bookhout et al. 1976).

Lithodes antarcticus (southern king crab) is a commercially important species. The present study attempts to describe the effects of an organochlorine pesticide and the most commonly used solvent (acetone) on the early development of this species. The aims of this study were: to determine the effects of lindane on survival, development and moulting during the early larval stages of L. antarcticus, and to determine an incipient lethal level, corresponding to a threshold concentration, at which acute toxicity ceases.

MATERIALS AND METHODS

Two series of experiments were conducted, one aimed at testing the effects of lindane and an other at evaluating the effects of acetone. Lindane used for this study was the crystalized gamma-isomer of 1,2,3,4,5,6 - hexachlorocyclohexane (purity 99.9%) produced by Celamerck. The following concentrations were tested: 0.10, 0.18, 0.32, 0.56, 1.00, 3.20 and 5.60 mg/L of lindane, prepared from a stock solution of 1 g/L. For preparation of the stock solution analytical grade acetone (Baker) was used as pesticide

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solvent. For experiments on the effects of acetone, the following concentrations were tested: 0.75, 1.36, 2.41, 4.22 and 7.54 g/L. Each series of experiments was carried out in duplicate, using 40 larvae in each different concentration (20 larvae per 250 mL). The acetone concentration used in the highest lindane concentration tested (0.75 g/L) was run as solvent control. Seawater controls were also run with 100 larvae distributed in five glass containers.

The experiments were conducted following the recommendations of the American Public Health Association for static bioassay procedures (APHA, AWWA, WPCF 1976). Ovigerous L. antarcticus females were collected from the Beagle Channel. Nine females with embryos almost at birth were selected. The abdominal appendages, which bore embryos, were removed and kept suspended in glass aquaria containing 10 litres 35‰ seawater, at 8 $\pm$0.5^{o}C until hatching. The water was well aerated and was partially renewed every 12 h. Larvae hatched during the night were examined, damaged or deformed ones were discarded and the rest were randomly distributed in glass containers to be used for rearing.

Seawater used for rearing was filtered through a stratified filter (final pore size of 0.22 um). Bowls containing larvae in filtered seawater of 35‰ salinity, were kept in a constant temperature cabinet at 8$\pm$0.5^{o}C under natural photoperiodic conditions. The salinity-temperature combination was selected as the best for succesful rearing according to the literature (Vinuesa et al. 1985). Freshly hatched Artemia sp. nauplii (Tetra Werke), cultivated in filtered seawater, were supplied daily as food.

The experiment was carried out over a period of seven days, which according to Vinuesa et al.(1985) is the maximum time of development for the first zoea stage at 8^{o}C and 35‰ salinity. During the first two days the state of larvae was controlled every 12 h. Presence of exuviae, deaths and prelethal signs were recorded following the criteria established by Lombardo et al.(1982). After the third day, controls were carried out every 24 h. Lindane and acetone solutions as well as seawater, were renewed every 48 h.

The LC50's were estimated by the probit method, including Abbot's adjustment for natural responsiveness. The LC50 confidence limits were calculated by Fieller's theorem and a Chi-square test for departures from lineality was applied (Finney 1971).

RESULTS AND DISCUSSION

As shown in Figure 1, mortality in the seawater controls was lower than 10% during the first seven days of culture, and the acetone controls did not show mortality values above those in seawater during this lapse. The survival rate observed in both cases was similar to that obtained in larval culture by other authors under equal conditions of temperature and salinity (Vinuesa et al. 1985).

The survival rate of larvae exposed to lindane is shown in Figure 2. Higher concentrations produced an abrupt rise in mortality between 48 and 96 h of culture, while lower concentrations produced a more gently sloping mortality curve between 96 and 168 h.

The LC50 estimates and their confidence intervals are given in Table 1. These LC50 values are much higher than those recorded for adults of Crangon septemspinosa, Palaemonetes vulgaris and Pagurus longicarpus exposed to lindane, which were found to range between 5 and 10 ug/L for 96 h-LC50 (Eisler 1969). Zoeae I of L. antarcticus appeared to be less sensitive to lindane than adults of this species.

Organochlorine pesticides are generally more toxic to marine crustaceans than organophosphates (Eisler 1969). Previous studies on organophosphate pesticides toxicity in larvae of this species have shown somewhat different results (Lombardo et al. 1982). Lithodes antarcticus zoeae were more sensitive to lindane than to DDVP (dimethyldichlorovinyl phosphate), but less sensitive to this substance than to ethyl parathion. Nevertheless, a comparison of probit line slopes between 72 and 108 hours of exposure indicates that differences are two to three times greater for lindane than for either ethyl parathion or DDVP; this suggests a more acute-tested effect of the organochlorine pesticide when compared to the organophosphates.

An exponential decrease of the LC50 values was observed during the development time of the first zoeal stage (Figure 3). This asymptotic trend with exposure time indicates an incipient lethal level under 94 ug/L; at this concentration acute toxicity ceases.

Mortality during the first larval stage could considerably affect the percentage of individuals completing larval development. In cultures carried out under similar conditions, 93% of larvae reached the second zoea stage and only 29% reached glaucothoe

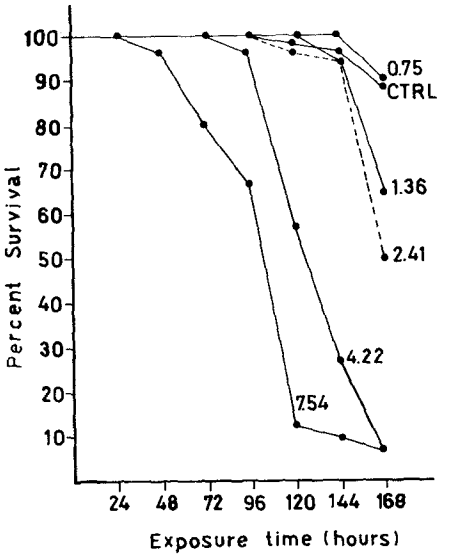

Figure 1. Survival curve of zoeae reared in seawater (CTRL) and exposed to tested concentrations of acetone (in g/L).

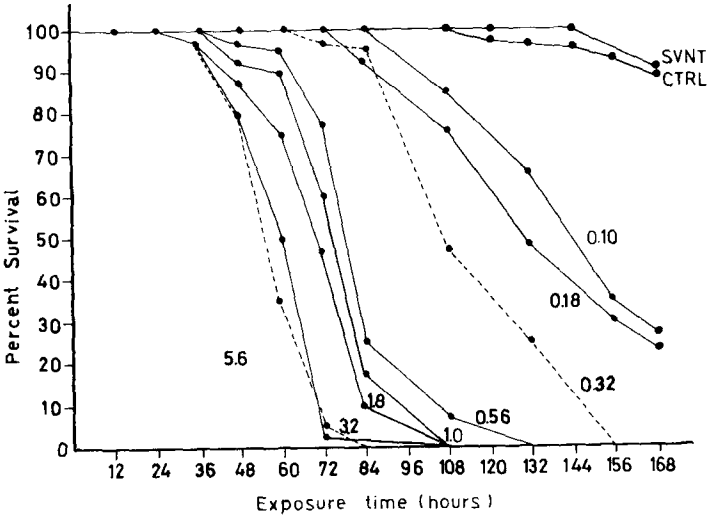

Figure 2. Survival curve of zoeae exposed to tested concentrations of lindane (in mg/L), CTRL: seawater control, SVNT: solvent control.

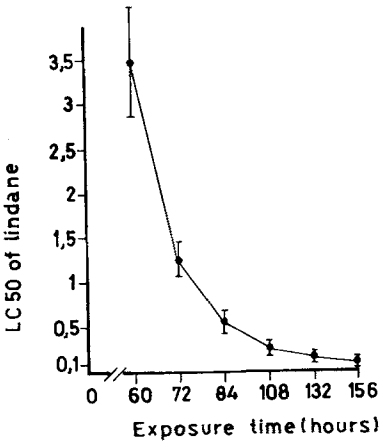

Figure 3. Lindane toxicity curve. LC50 values and 95% confidence limits (mg/L)for each of the observation times.

Table 1. LC50 values, 95% confidence limits, slope values and correlation coefficient of probit lines for larvae exposed to lindane.

Hours	LC50(mg/L)	confidence limits	slope	correl
60	3.51	(2.84 - 4.63)	2.21	0.99
72	1.27	(1.08 - 1.49)	2.90	0.96
84	0.53	(0.46 - 0.61)	3.52	0.90
108	0.26	(0.22 - 0.30)	3.55	0.96
132	0.16	(0.14 - 0.19)	3.49	0.97
156	0.09	(0.06 - 0.01)	3.23	0.87

stage (Vinuesa el al. 1985). It would therefore seem that low concentrations of lindane severely affect juvenile recruitment.

Survival rates of larvae exposed to acetone are shown in Figure 1. The mortality curve of larvae exposed to 0.75 g/L acetone (acetone controls) did not differ from that of seawater controls. In both cases, all the stage I zoeae reached the second larval stage during the expected lapse according to results of previous experiences (Vinuesa et al. 1985). In seawater controls the moulting process began after the third day and in acetone controls larvae started to moult 24 hours later. With a 1.36 g/L acetone solution, only 5% of the larvae had reached zoea

stage II by the seventh day and 10% were found to die during moulting by the eighth day. In higher acetone concentrations the first dead larvae appeared after the third day and toxicity curves presented steep slopes (LC50 and slope values, are given in Table 2). At these concentrations no larvae had reached the second zoeal stage by the end of the experiment. It would therefore seem that acetone has an inhibiting effect on moulting which is more marked with increasing acetone concentrations. With respect to lindane, a strong inhibition on the moulting process was observed; no larvae succeeded in moulting to second zoeae under any of the tested lindane concetrations.

Table 2. LC50, 95% confidence limits, slope values and correlation coefficients of probit lines for larvae exposed to acetone.

Hours	LC50 (mg/L)	confidence limits	slope	correl
120	4.66	(4.13 - 5.24)	6.14	0.96
144	3.88	(3.40 - 4.37)	5.95	0.87
168	2.33	(1.91 - 2.77)	3.40	0.94
192	1.01	(0.71 - 1.26)	3.12	0.62

The effect of prolonged zoeal intermoult periods of decapod larvae has been described as sublethal for other organochlorine pesticides (Epifanio 1979, Bookhout and Costlow 1974). The occurrence of an extra larval stage was also described for decapod larvae as a sublethal effect of organochlorines (Bookhout et al. 1972, Bookhout et al. 1976). The increment in length zoeal intermoult periods has been cited as an effect of a carbamate (Buchanan el al 1970) and of a organophosphate (Bookhout and Monroe 1977).

The relation between this delay in moulting and sublethal concentrations of pesticides could be due to the fact that organochlorines, organophosphates and carbamates are all neurotoxic substances, and moulting is a neuroendocrine process. Nevertheless, it has also been found that heavy metals and petroleum hydrocarbons affect moulting, as does saline or thermal stress. Epifanio (1979) has suggested that the delay in moulting could be a response of crustaceans to generalized stress.

In this experiment the response to acute lethal

concentrations of lindane was anecdysis. No larvae exposed to the different lindane concentrations reached the second zoeal stage, in spite of the fact that some of them lived through a period of eight days and that all the zoea I reared as controls reached the second larval stage in an equal period of time.

A large quantity of larvae died before or during the ecdysis. A common prelethal sign was found to be carapace swelling. Premoult mortality seems to be strongly related to an increase in pesticide penetration during water intake through the gastric epithelium in the premoult phase. Toxicity of this lipophilic organochlorine compound could also be magnified during the premoult period by the high metabolic activity associated with recovery of organic and inorganic material of the exoskeleton, including active mobilization of lipids. Similar sublethal effects were described for larvae of the same species exposed to ethyl parathion and DDVP (Lombardo et al 1982).

Acknowledgments. We wish to thank Lic. Victoria Lichtschein for their careful reading of the manuscript. This research was supported by a National Research Council (CONICET) grant.

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Received November 6, 1989; accepted September 13, 1990.