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Toxicity of Ethyl-parathion and Carbaryl on Early Development of Sea Urchin

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Organophosphorates and carbamates are extensively used pesticides, both in agricultural practices and in domestic pest control. Since these chemicals eventually reach the sea, increasing attention is being paid to their effects on non-target marine organisms (Schmidt el al. 1971; Tanabe et al. 1983).

Sea urchins are potentially important marine resources (Kato 1972). In some South American countries they are exploited for human consumption (e.g., in Chile, about 15,000 ton/year were captured in 1981; SERNAP 1981). These organisms have been proposed as a test system in toxicity studies (Hagstrm and Lnning 1973; Kobayashi 1971 et seq.). They were used in biological experiments testing heavy metals (Castagna el al. 1981; Pagano et al. 1983), antiseptics (Rosenkranz et al 1980), polychlorinated biphenyls (Tjerdema and Jacobs 1987), polynuclear aromatic hydrocarbons (de Angelis and Giordano 1974) and pesticides (Ferrari et al. 1989).

The aims of this study were: 1) to analyze the effects of two intensively used pesticides, ethyl-parathion (DNTP) and carbaryl (CARB), on early developmental stages of the sea urchin Pseudechinus magellanicus (Philippi); 2) to improve sea urchin laboratory manipulation in order to avoid developmental abnormalities, such as polyspermy and exogastrulation (Kobayashy 1973, 1974), which might interfere with the evaluation of toxic effects of xenobiotics. Sea urchin from Ushuaia (Tierra del Fuego, Argentina) were used as test organisms because that area is not exposed to local polluting sources. At present however, the region is being promoted as an industrial agricultural and cattle raising center.

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MATERIAL AND METHODS

Adults of Pseudechinus magellanicus were collected from natural populations in the Beagle Channel (54° 47´S, 68° 36´O, Ushuaia, Tierra del Fuego, Argentina). Gametes were obtained by the 0.5 M ClK method in filtered sea water (33%. salinity; pH: 7.4; dissolved oxygen: 7.8 mg/L).).

In order to minimize the individual variability, eggs and sperm were pooled after fertilization using 14 females and 7 males. The pooled eggs and sperm were rinsed three times with filtered sea water.

Fertilization with different sperm concentration was tested to select the sperm/oocyte ratio, which minimizes polyspermy and at the same time maximizes fertilization. Egg density was controlled according with Pagano et al. (1983) and sperm density was checked using a Neubauer camera (W. Schreck Hofreim, Federal Germany Republic) (Castagna et al. 1981). Fertilized eggs were rinsed three times with filtered sea water to remove sperm excess; then they were diluted to $50^{+}5$ eggs/mL; 250 mL of the egg suspension were dispensed into glass vials of 400 mL volume.

A DNTP 1 mg/L stock solution was made from technical grade ethyl-parathion (95% purity active principle), obtained from Malthius Labs. (Buenos Aires, Argentina). CARB 1 mg/L stock solution was made from 99% purity active substance, obtained from Union Carbide (Buenos Aires, Argentina). CARB was added to the sea water in analytical grade acetone (Merck, Germany), so that the final concentration of solvent in test medium did not exceed 1.8 ug/L of acetone. In both, DNTP and CARB assay concentrations were: 18, 32, 56, 100 and 180 ug/L. Sea water and acetone controls were also run. All experiments were conducted in duplicate.

The effects of the pesticides on early development of Pseudechinus magellanicus were examined in the following stages: blastula (Bl), gastrula (Gs), prism (Pr) and pluteus (Pl). Mass cultures of fertilized eggs in filtered sea water were carried out previously in order to determine the time after fertilization at which the maximum relative proportion of a given stage (i.e., Bl: 12 h, Gs: 36 h, Pr: 48 h and Pl: 96 h after fertilization) was obtained. Samples were taken from the test vials during those periods; the material was fixed in 5% buffered formalin and the following data were

recorded: a) percentage of individuals reaching each stage and b) percentage of abnormals individuals (embryos with altered cleavage, in terms of number and shape of blastomeres; larvae with slight malformations and abnormal shapes, alterations in shape and size of the skeleton and in differentiation of the digestive tract). From each sample 200 organisms were observed at random.

Mass culture glass containers as well as control and test vials were incubated in a Blue-MX bath (Cole Parmer, Illinois, U.S.A.) at $13^{+}1^{o}C$.
When only 50% of the organisms reached a given stage showing no abnormalities a 50% effective concentration (EC50) was considered.

The EC50 for each series was estimated using the probit method including Abbot's adjustment for natural responsiveness and their confidence limits by Fieller's theorem (Finney 1971).

Acetone test data were analyzed one-way ANOVA in conjunction with Dunnett's test (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

To obtain a 90 - 100% fertilization, the best sperm-per-egg ratio in P. magellanicus was 50:1. With this ratio, percentage of fertilized eggs is maximum (99%), percentage of polyspermy is null and percentage of unfertilized eggs is minimum (1%) (Figure 1). This ratio is similar to that observed in Strongylocentrotus droebachiensis (Müller) (Pennington 1985), but smaller than the estimated for Paracentrotus lividus (Lamarck) and Psammechinus microtuberculatus (De Blainville) (Hagstrm and Lnning 1973).

In acetone test no significant difference (Dunnett's test, $p < 0.05$) with control experiments was found (Table 1).

Results of toxicity tests are shown in Table 1. All regressions are significantly linear (X , $p < 0.05$). Neither polyspermy nor the presence of exogastrula was recorded in any case; these phenomena are frequently observed in the development of embryos exposed to xenobiotics (Kobayashi 1971). A remarkable toxic effect of the pesticides during the early development stage in sea-urchin was observed: all the EC50 values ranged from 157 ug/L to 2.8 ug/L.

Concerning sensitivity of the analysed stages, a

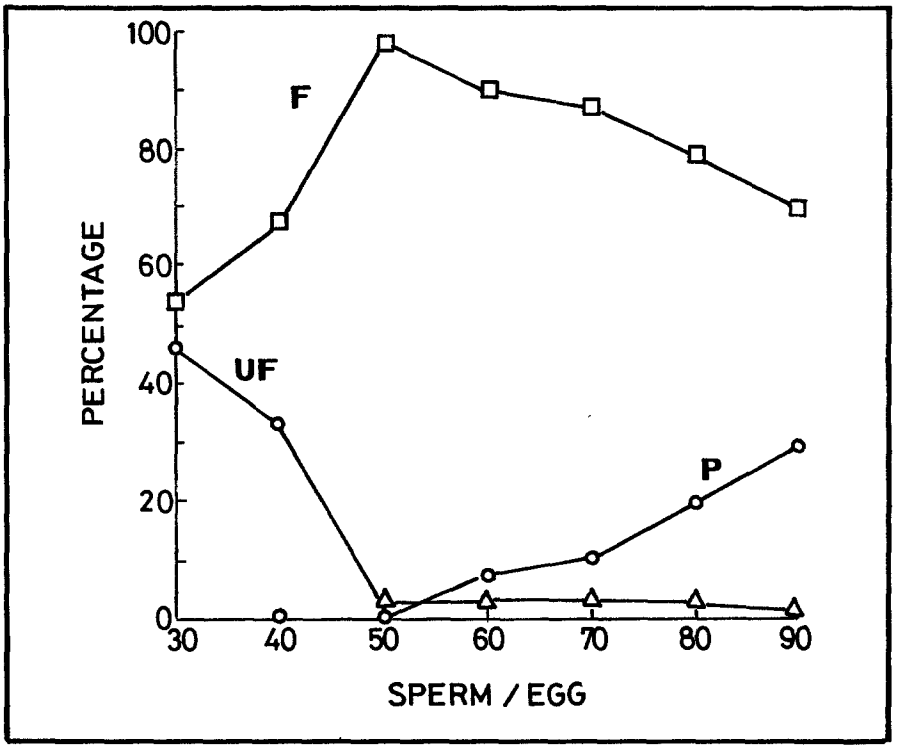

Figure 1. Percentage of fertilized (F), unfertilized (UF) and polyspermic eggs (P) in relation to the sperm/eggs ratio.

difference between DNTP and CARB toxic effect was noticed (Table 2).

In the case of DNTP, the EC50 values at the BL and G1 stages are similar, while at the Pr stage the EC50 is significantly lower (Table 2). This remarkable increase of DNTP toxic effect could be attributed to a greater penetration and to the pesticide mode of action. Penetration is favored by the following morphological events, that take place between the 36 and 48 h: 1) the formation of the mouth, 2) the formation of the primary pore canal and 3) the differentiation of the digestive tract.
Once gastrulation has taken place there is an increase in acetylcholinesterasic activity (Ozaki 1976).

Due to its mode of action, DNTP produces an accumulation of acetylcholine (ACh). The high concentration of ACh impairs the development of sea urchin embryos, inhibiting processes of cellular division and morphogenesis (Mc Mahon 1974). This fact would also account for the significant decrease (up

Table 1. Percentage of embryos and larvae of Pseudechinus magellanicus at different times after fertilization in acetone and control experiments. Results are the mean of independent determinations (n=200) from each duplicate. Bl: blastula; Gs: gastrula; Pr: prism; Pl: pluteus.

Stage	Time (h)	Control	Acetone (1.8 ug/L)
Bl	12	97.0 + 1.4	95.8 + 1.2
Gs	36	96.0 + 1.4	94.8 + 1.1
Pr	48	96.8 + 2.5	94.0 + 1.4
Pl	96	95.6 + 1.0	95.3 + 1.0

to 14 fold) of the EC50 values recorded 48 h after fertilization. The Pl showed a lower sensitivity than that of other stages; its EC50 value was twice as high (Table 2).

In the case of CARB the developmental stages with active clevage and cellular mobilization (Bl and Gr) turned out to be more sensitive. The EC50 values for Pr and Pl are significantly higher than those for Bl and Gl (Table 2).

An important detoxification mechanism of CARB involves the combined action of cytochrome-oxidase system, which requires the presence of NADH, NADPH and oxygen (Kulkarni and Hodgson 1980). Besides respiratory chain, an additional electron transport system in which NADH is oxidized has been described for sea urchin. Moreover, b-type cytochrome in mitochondrial and microsomal fraction, a- and c-cytochrome, cytochrome-oxidase, NADH-cytochrome c-reductase and NADH cytochrome b5- reductase were also described (Okabashi and Nakano 1983).

An increase of carbohydrate and lipid metabolism was found after gastrulation had taken place augmenting NADPH and NADH levels (Lovtrup-Rein and Lovtrup 1980). Consequently, once gastrulation has been accomplished, the presence of NADPH and NADH could favor cytochrome oxidase activity (Peterson and Prough 1986). This fact and the exponential increase of respiration (Lovtrup-Rein and Lovtrup 1980) suggest that the decrease of toxicity of CARB after gastrulation could be attributed to an increase in detoxification processes by the cytochrome oxidase system; therefore, EC50 values for Pr and Pl were about 8 to 26 times higher than those for the other stages (Table 2).

Table 2. Toxicity of DNTP and CARB to early developmental stages of PM. (r: correlation coefficient). Bl: blastula; Gs: gastrula; Pr: prism; Pl: pluteus.

Chemical	Stage	Time (h)	EC50 (ug/L)	95% Conf.Lim. (ug/L)	Slope	r
DNTP	Bl	12	31.2	(31.2 - 52.0)	1.46	0.97
	Gs	36	34.6	(27.2 - 48.4)	1.07	0.94
	Pr	48	2.8	(1.0 - 6.5)	0.46	0.89
	Pl	96	73.7	(46.7 - 92.2)	0.88	0.89
	Bl	12	6.3	(4.0 - 8.4)	1.18	0.90
	Gs	36	10.7	(5.3 - 16.5)	0.61	0.91
	Pr	48	157.4	(77.2 -190.9)	0.77	0.90
	Pl	96	92.5	(50.6 -106.4)	0.87	0.95

In the case of DNTP, this cytochrome-oxidase system acts as inductor mechanism of toxicity (Kulkarni and Hodgson 1980). Therefore the EC50 value for Pr stage was the lowest one in the DNTP experiment (Table 2).

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