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Effects of Deltamethrin on the South American Toad, *Bufo arenarum*, Tadpoles

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Pyrethroids constitute a new generation of synthetic molecules structurally related to natural pyrethrins, characterized by an intense broadspectrum insecticide activity based on their neurotoxicity. According to their susceptibility to abiotic factors it has been proposed that they be grouped in two categories a) those that were apt to combat and/or to control a wide range of insect pests and mites that affect crops and livestock and, b) those that resulted suitable for household pests. Some pyrethroids resulted equally effective when applied both in out and indoor environments or in integrated pest management programs; that is the case of Deltamethrin, a potent alpha-cyano dibrominated and photostable pyrethroid (Leahey, 1985).

In considering the adverse impact of artificial biocides applied to natural systems by accident or because of natural associated factors such as runoff or spray drift, it is recognized that nearby surface and/or underground freshwater bodies could be considered as the final receptors of complex mixtures of chemicals made by the intact xenobiotics themselves and by their transformation products. The situation is more complicated when the picture is completed with the rest of the ingredients generally present in the commercial formulations i.e. sticking agents, emulsifiers, synergists, solvents, undeclared chemicals, etc. which are constituents of formulated pesticides making their environmental characteristics extremely variable.

It was the purpose of this report to determine the LC50 of pure Deltamethrin solutions and to describe its effects on Bufo arenarum larvae at different age when exposed to the xenobiotics in semi static tests.

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MATERIALS AND METHODS.

Mature female B. arenarum Hensel captured in Buenos Aires Province were used. They were kept in the laboratory at room temperature in tap water for 20-25 - days prior the experiments and fed by force once a week with chopped bovine meat.

The material used in this study was obtained by inducing the ovulation by injection into the dorsal lymphatic sacs of homologous hypophysis. The oocytes were fertilized in vitro; as the embryos reached stage 1-2 blastomers the jelly strings were treated briefly in freshly made 2 % thioglycholic acid solutions in Holtfreter neutralized with NaOH. Free embryos were then washed with Holtfreter and accomodated in covered glass Petri dishes containing the same solution.

Once the embryonic development was completed, larvae were transferred to artificial pond water (see below). At this moment animals began to be fed ad libitum with balanced fish food which was available for 4-6 hr every other day. Incubation media of both embryos and larvae were renewed regularly once or twice a day, removing abnormal and/or dead animals.

Feeding of tadpoles was interrupted 36-48 hr prior to the beginning and along the experiments. Animals were accomodated in polystyrene bowls; they were filled and covered with glass plates in order to get a negligible air-water interface and to minimize evaporation. According to their size there were 5-10 larvae per liter.

Three sets of experiments were conducted simultaneously; two of them served as controls a) animals in pond water with neither insecticide nor its solvent (n, 287) and, b) larvae with absolute ethanol at 2 uL/L (n, 60). The third set corresponded to experimental animals placed in different Deltamethrin solutions.

Experiments were performed on two groups of animals of different fully aquatic stages; tadpoles of comparable body dimensions for each group were selected. The first group corresponded to 658 larvae in stages 26-27 (12-15 mm length) immediately after the end of the embryonic development, characterized by the beginning of the hind limb development; the control of this group was constitued by 182 tadpoles.

The second group of experiments was performed on 421 animals in stages 28-30 (20-25 mm length) when hind limbs development was completed and the first fore limb appeared; the control of this group was run on 105

animals. The stage of larvae was determined following the table of Echeverría and Fiorito de López (1981).

Containers without aeration were placed in a bath with running water. The temperature was kept constant at 20 ± 1 ºC through a Lauda type K2R thermostat. The experiments were conducted at 14 L/10 D photoperiod regimen. Observations were made at 24 hr intervals through 96 hr of exposure. Lethality was the considered endpoint. Every 24 hr a) the number of dead animals was monitored, which were removed from the containers and their external aspect registered, b) the morphological and behavioral characteristics of the alive animals were recorded and, c) all solutions were renewed. Solutions were freshly made immediately before the changes.

The artificial pond water contained (in mM): NaCl, 1.3; $CaCl_2$, 0.8; KCl, 0.1 and $NaCO_3H$, 0.2 (Alvarado and Johnson 1966). All reagents were of analytical grade. Most of the Deltamethrin was kindly provided by Laboratorios Farquimia, Argentina (batches 3L-1605 and 4L-2510, 99.6-99.8 %). In a few experiments there were used standards provided by the USA Environmental Protection Agency (samples E999, F245 and X602, 99 %). The stock pyrethroid solution was prepared dissolving it in absolute ethanol and adequate dilutions were done in artificial pond water in order to reach the following concentrations (in ppb): 0.31, 0.62, 1.25, 2.50, 3.75, 5.00, 7.50, 10.0, 12.5, 15.0 and 20.0. The concentration of Deltamethrin causing 50 % mortality (LC50) at 48, 72 and 96 hr and the 95 % confidence limits were estimated using a probit analysis program based on Finney (1971). All regressions were significatively lineal (χ^2, $p < 0.05$).

RESULTS AND DISCUSSION.

No mortality occurred throughout the experimental 96 hr period in either control tests or in 0.31 and 0.62 ppb Deltamethrin solutions. In no case 50 or higher percentage of mortality was registered after the first 24 hr period. Data shown in Tables 1 and 2 came from calculations made from results obtained with the remaining nine insecticide solutions.

Table 1. Median lethal concentrations (LC50) for Bufo arenarum larvae (stages 26-27) exposed to aqueous solutions of Deltamethrin.

Hr	LC50	95 % confidence	slope	correlation
48	11.93	9.42 - 16.39	1.69	0.957
72	7.09	6.28 - 8.10	2.59	0.955
96	4.37	3.72 - 5.19	1.58	0.956

Table 2. Median lethal concentrations (LC50) for Bufo arenarum larvae (stages 28-30) exposed to aqueous solutions of Deltamethrin.

Hr	LC50	95 % confidence	slope	correlation
48	16.84	14.25 - 20.99	2.59	0.974
72	12.04	10.59 - 13.98	2.90	0.942
96	4.50	4.02 - 5.00	3.31	0.919

The effects of the insecticide were always graded proportionally according to its concentration and to incubation time being their behaviour a reliable indicator of neurotoxicity. All experimental animals showed four clear cut phases in their responses. At the beginning of the experiments (phase I) toxicity signs were characterized by hyperactivity symptoms: intents of escape, swimming to top of bowls for oxygen, spiral while swimming fast, nervous "flitters" when disturbed and spontaneous arrhythmic "flitters". In phase II most of the tadpoles lay on side or back, showing lateral curve in tail body axis deformities, lateral deflection and link at base of tail. In phase III deformed larvae remained motionless at the bottom of the bowls; death (phase IV) was defined as the lack of response to mechanical stimuli.

The sensitivity to Deltamethrin was higher in younger tadpoles being the differences larger after 48-72 hr of exposure. However, the 96 hr LC50s were quite similar for both groups of tadpoles and fell at 4.4-4.5 ppb which should be taken as a clear indication of the extreme toxicity of the insecticide for B. arenarum larvae under our experimental conditions. Thus we inferred that the real impact of Deltamethrin may be important since sublethal environmental concentrations reached after one single application may render surviving larvae with debilitating abnormalities incapable of coping the environmental stress and more vulnerable to natural predators and affecting, secondarily, other trophic levels of the ecosystem. There is experimental evidence of the insecticide concentration capacity of tadpoles from water (Hall and Kolbe 1980). This fact must be taken into consideration since many species regularly prey on amphibian larvae and in doing so they may ingest considerable amounts of xenobiotics.

We must mention that several physical factors, also present in natural systems, would act simultaneously on our experimental solutions. Such is the case of photolysis that could provoke reduction in the actual concentration of the insecticide thus altering the composition of the solutions in the direction of an

increasing chemical complexity; it was reported that photodegradation of Deltamethrin yields products whose mouse i.p. LD50s were lower that the parent molecule (Ruzo et al. 1977).
The other physical factor that must be considered concerns the adsorption of the insecticide on the walls of the vessels. Several authors have studied the physicochemical aspects of the pyrethroid adsorption-desorption processes on different types of materials (Hasan et al. 1984; Helmuth et al. 1983; Sharom and Solomon 1981 a, b) showing that they rely on various factors such as temperature, volume, agitation, surface of exposure, etc. We did not find information in regard to the adsorption of Deltamethrin on polystyrene; however, if it would exist in considerable extent, our LC50s might be considerably lowered, thus emphasizing the toxicity of the insecticide. Stratton (1986) has shown the importance of solvent-pesticide interaction in bioassays.

We did not forget that ours is an oversimplified closed system that yields information circumscribed to acute intoxication of the test species by exposure to a single neuroactive substance that must be cautiously extrapolated to natural open systems. Nevertheless, our data suggest that caution is required when Deltamethrin is used; its effects might reach at least locally and temporally a harmful threshold for some non-target components of the freshwater biota. The reported field evaluations of the effects of Deltamethrin (Morrill and Neal 1990; Muir et al. 1985; Smith and Stratton 1986) pointed out that a large number of factors such as season, procedure, frequence and doses od application, half life, wind speed, etc. mediate the responses.

We consider tadpoles of B. arenarum as a sensitive indicator organism suitable for aquatic bioassays of pyrethroids. The system we used a) covers partially the requirements established by the FAO (1982) for the evaluation of plaguicides, b) it is relatively inexpensive, c) it allows to observe a large number of animals simultaneously and, d) the end point is easily detectable.

Finally, it is noteworthy to mention that because of its origin the possibility of previous contact of the animals with xenobiotics and consequently a partially masked behaviour could be considered negligible except for that which was in the previous history of the egg donor female.

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