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Fenbutatin Acute Toxicity on *Artemia nauplii:* Effects of Sublethal Concentrations on ATPase Activity

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During the last decades, the use of organotin compounds has steadily increased. The numerous applications of these compounds are primarily in industry as stabilizers for PVC, in agriculture as miticides and fungicides and in the marine environment as antifoulants. The increasing annual usage of organotin compounds raises the possibility of environmental pollution.

The toxicity and degradation of organotins in the environment depends strongly on the number and nature of substituents (Muller 1987). In water, triphenyltin compounds decompose slowly (Bock 1981). Persistence of at least 8 months was found when triphenyltin was used against water snails at concentration of 1.0 ppm (Deschiens and Floch 1963). Organotin compounds in the aquatic environment accumulate in the microlayer of the water surface, are readily adsorbed in sediments and on suspended particulates (Chernega et al. 1971, Cooney and Wuertz 1989). The half-life of organotins was found to be up to 5 months in freshwater sediments (Maguire and Tkacz 1985) and up to 2 years in deep and anaerobic estuarine sediments (Waldock et al.1990). Among organotins trisubstituted compounds are generally the most toxic to fish, to microorganisms and to other aquatic inhabitants (Bock 1981, Cooney and Wuertz 1989).

Whereas the adverse effects of tributyltin, the main representative of antifouling agents , on the homeostasis of the aquatic ecosystems has been studied, few data are available concerning the environmental impact of the trisubstituted organotins used in agriculture as miticides.

The aim of this project is to determine the median lethal concentration of the organotins used as miticides, in nauplii of the marine crustacea *Artemia* and to study the effects of sublethal concentrations on the activity of ATPase enzymatic system.

In this study we will present the results from the experimentation with fenbutatin on the survival and ATPase activity of *Artemia nauplii.*

MATERIALS AND METHODS

Both the acute toxicity study and the enzymatic assay were performed with the formulation Vendex 55% SC (fenbutatin 55% w/v), from Shell Chemical Co. Adenosine -5- triphosphate Na_2-salt and bovine serum albumin were obtained

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from Serva Chemical Co. and ouabain from Sigma Chemical Co. All other chemicals were of reagent grade.

The toxicity test was performed according to Vanhaecke *et al.* (1980). *Artemia* cysts (Salt Lake Brine Shrimp, INC.), approximately 250 mg, were placed in cylindroconical tube of 250 ml synthetic sea water at 35‰ salinty and pH=8 (Insant Ocean Sea Salt) and incubated at 25 ± 1 °C under illumination of 1000 lux. All cysts were kept under continuous suspension by gentle aeration. After 24 hours of incubation the moving nauplii were sucked out by pipetting and transferred into a conical flask containing 250 ml of sea water under continuous aeration and illumination at 25 ± 1 °C for 24 hours. At the end of this period the nauplii were collected for the performance of the acute toxicity test or for the study of sublethal effects on ATPase enzymatic system.
The toxicity test was carried out in petri dishes with 10 nauplii in each and 25ml of the respective concentration of the toxicant dissolved in syntetic sea water at 25 °C. The dishes were incubated in darkness at 25 ± 1 °C. During the toxicity tests the nauplii were not fed and the test solutions were not aerated. After 24 hours the number of dead nauplii in each petri dish was counted under dissecting microscope. Five serial concentrations, 0.02, 0.03, 0.06, 0.10 and 0.18 ppm, of fenbutatin were tested in triplicate with parallel controls. Mortality was noted at each concentration level after 24 hours of exposure. The LC_{50} was calculated according to Hamilton (1978).

The effects of fenbutatin on ATPase activity was studied on nauplii 24 hours after hatching. The nauplii were collected from the culture medium with a specially constructed teflon pipette and placed in 100 ml glass tubes with the respective concentration of the toxicant. Two concentrations equal to 1/2 and to 1/5 of the determined LC_{50} were studied and the enzymatic activities were compared to the respective values of the control group. Six groups of 40 nauplii per group were exposed to each concentration level and to the control as well.
All the tubes were placed in water bath at 25 ± 1 °C and equipped with a pipette for the continuous aeration of the solutions for a period of 24 hours. At the end of the exposure period the 40 nauplii from each tube were collected, placed directly to the isolation medium for the ATPase assay and frozen at -20 °C until measurement of enzyme activity.
ATPase activity was assayed according to the method of Ewing et al. (1974) with minor modifications. The frozen nauplii were homogenized in 0.4 ml of 0.1M imidazole buffer, pH 7.2 with an Ultra Turax microhomogenizer. The homogenizer was washed twice with 0.4 ml of a solution of 0.8M NaCl and 0.2M KCl.
Reactions were run in ependorf tubes and started by addition of 0.2 ml of a solution containing 0.125M imidazole, 0.025M $MgCl_2$ and 0.0125M Na_2ATP with and without 0.0025M ouabain, to 0.3 ml portions of the final homogenate.
The reaction mixtures were incubated at 37°C for 30 min in water bath and the reaction was terminated by adding 0.5 of 20% icecold trichloroacetic acid (TCA). After TCA and cooling, the tubes were centrifuged for 2 min in a Beckman table microfuge. Aliquots of 0.5 ml were added to 0.5 ml of a solution of 1% ammonium molybdate in 1.15N H_2SO_4 to which $FeSO_4$, 40 mg/ml, had been added immediately before use. The samples were allowed for color development for one hour and the absorbance of the samples read in a Shimadzu UV - 150 - 02 Double - Beam spectrophotometer at 700 nm. Ouabain is a specific inhibitor of Na^+K^+ATPase and thus the remaining activity was assigned to Mg^{2+}ATPase.

Protein concentrations were determined in the final homogenate according to Bradford (1976). ATPase specific activity units are given in µmoles PO^{3-}_4 per hour per mg protein. The results were statistically analyzed with ANOVA from Statgraphics.

RESULTS AND DISCUSSION

Fenbutatin was found to be very toxic to *Artemia* nauplii and the LC_{50} was equal to 0.05 ppm (0.04-0.06) at 95% confidence limits after 24 hours exposure.
At the concentration of 0.02 ppm no mortalities were observed (NOEC).

Enzyme activities were determined at 0.01 and 0.025 ppm of fenbutatin. The effects on ATPase activity at the respective dose levels after 24 hours of exposure are shown at the Table 1.
Protein concentrations in the final homogenate varied from 32.1 to 44.4 µg/ml homogenate.
Total ATPase activity, the sum of the two enzyme systems, and both Mg^{2+} and Na^+-K^+ dependent ATPases activities were inhibited in all nauplii exposed to both toxicant concentrations.
The inhibition of Mg^{2+} ATPase was statistically significant at both concentration levels and ranged from 19.2 at 0.01 ppm to 66.04% at 0.025 ppm as compared to controls. In addition, a statistically significant increase in the degree of enzyme inhibition was observed at the higher concentration level of toxicant as compared to the lower concentration.
The inhibition of Na^+-K^+ ATPase was statistically significant at both concentration levels and ranged from 32.66 to 30.07% as compared to controls. No significant differences were observed in enzyme activity between the two concentrations.
Under all conditions Na^+-K^+ dependent ATPase activity was lower than Mg^{2+} ATPase. General depression of total ATPase and Na^+-K^+ ATPase activity was observed at both tested concentration levels. The inhibition of Mg^{2+} ATPase activity was more specific and reached higher degrees of inhibition correlated directly to the toxicant concentration.

The results from this study concerning Mg^{2+} ATPase are in agreement with the findings of other investigators. Generally the Mg^{2+} ATPase shows higher activity than Na^+-K^+ ATPase, the process of inhibition seems to be more specific and most often highest inhibition values have been associated with the ouabain sensitive Mg^{2+} ATPase (Davies et al. 1972, Jowett et al. 1978).
The ATPase enzyme system catalyses the conversion of ATP to ADP with release of energy. This energy is considered to be used in the process of active transport. Na^+-K^+ ATPase is generally accepted to be related to osmoregulation and therefore to be more active at extreme salinities in which the animal hyperosmoregulates (Holliday 1988, Jowett et al. 1978). The Mg^{2+} ATPase is associated with ATP formation in mitochondria through coupling the process of oxidation to phosphorylation and therefore respiration (Ulrich 1963). Effects of various pesticides on mitochondrial electron transport has been shown by Pardini et al. (1980) and this may be one of the reasons for Mg^{2+} ATPase inhibition after exposure to the test compound.

From the present study the following can be concluded:
a. Fenbutatin is highly toxic to *Artemia* nauplii and at sublethal concentrations it

Table 1. ATPase activity of *Artemia* nauplii after 24 hours exposure to sublethal concentrations of fenbutatin.

Fenbutatin Concentration (ppm)	Total ATPase Activity[a]	Mg^{2+} ATPase Activity	Na^{+}-K^{+} ATPase Activity	% of Na^{+}-K^{+} ATPase Inhibition	% of Mg^{2+} ATPase Inhibition
0	13.50 ± 1.026	9.10 ± 1.117	4.39 ± 0.560	–	–
0.01	10.14*+ ± 0.755	7.36*+ ± 0.600	2.78* ±0.319	32.66	19.12%
0.025	6.15*+ ± 0.713	3.09*+ ± 0.961	3.07* ±0.496	30.07	66.04%

a: Enzyme activity is given in µmoles PO_4^{3-} per hour per mg protein.
*: Denotes a statistically significant difference from the control.
+ : Denotes a statistically significant difference between the dose of 0.01 and 0.025ppm.

may interfere with cellular functions and hinder energy production.

b. LC_{50} is the less sensitive end point for the risk - hazard evaluation of a potential environmental pollutant and very often much lower concentrations may have high physiological significance.

c. Mg^{2+} ATPase activity of a well standardized strain of *Artemia* when it is measured under standard conditions, may be a very important bioindicator. In combination with other physiological parameters may give useful information for the ecotoxicological evaluation of possible environmental pollutants or be used as a criterion for water quality.

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