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Acute Toxicity of Endosulfan, Nonylphenol Ethoxylate, and Ethanol to Different Life Stages of the Freshwater Snail *Biomphalaria tenagophila* (Orbigny, 1835)

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Biomphalaria tenagophila (Mollusca; Gastropoda) is a tropical freshwater pulmonate snail widely distributed in Brazil where it is one of the intermediate hosts of *Schistosoma mansoni*. It is an hermaphrodite but cross-fertilization occurs as well. At 25 °C, eggs hatch between the sixth and ninth day after oviposition (Paraense 1972). Egg laying generally starts when the snails are about two months old.

Endosulfan is a cyclodiene insecticide used in agriculture and as a wood preservative. It is a potential water pollutant. Residues of endosulfan have been found in water, sediment, rainfall (Laabs et al. 2002), phytoplankton, zooplankton (DeLorenzo et al. 2002), aquatic macroinvertebrates (Leonard et al. 1999) and fish (Kole et al. 2001).

Nonylphenol ethoxylates are nonionic surfactants widely used as emulsifiers in industrial and household cleaning agents, agricultural chemicals, and plastic polymerization. High amounts of nonyphenol ethoxylates have been found in domestic sewage and industrial effluents and, where there are no sewage treatment plants, they are released directly into water bodies (Maguire 1999). Several recent studies have reported relatively high levels of nonylphenol ethoxylates in water bodies (Bennie 1999).

Ethanol is a carrier solvent frequently employed in aquatic ecotoxicity tests to dissolve chemicals that otherwise would not be soluble in water.

Data regarding the toxicity of the foregoing compounds to benthic macroinvertebrates are scarce as compared to data available for other aquatic species. Furthermore, as a rule, toxicity tests are carried out only with adult individuals. Tests to evaluate toxicity to developing embryos within the egg masses and to newly hatched snails are far less frequently conducted.

This study was performed to evaluate acute toxicities of endosulfan, nonylphenol ethoxylate and ethanol to the snail *Biomphalaria tenagophila* at three different life stages to determine the most sensitive stage of this important freshwater organism.

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MATERIALS AND METHODS

Technical grade endosulfan (purity 98.7%) was obtained from Hoeschst-Agrevo. Nonylphenol ethoxylate 9.5 (RENEX 95, nonylphenol with 9.5 ethoxylate units) was obtained from BASF Brazil. Ethanol (*Pro Analysis* grade, purity: 99%) was purchased from VETEC Chemistry.

Nominal concentrations of endosulfan (0.05; 0.1; 0.25; 0.5; 1.0; 2.5; 5.0; 10.0 and 20.0 mg/L), nonylphenol ethoxylate (0.5; 1.0; 3.0; 6.0; 11.0; 26.0; 52.0; 104.0; 262.0; 524.5 and 1049.0 mg/L) and ethanol (0.5; 1.0; 2.5; 5.0; 10.0; 20.0; 40.0; 80.0 % v/v) were dissolved in synthetic soft water (pH 7.1 ± 0.1, water hardness 40-48 mg/L as $CaCO_3$) prepared as recommended by ABNT guidelines (ABNT 2004). Ethanol, up to a maximum concentration of 0.5% v/v, was used to dissolve endosulfan that otherwise is practically insoluble in water.

B. tenagophila snails were from the breeding stock maintained at the Department of Ecology from Brasilia University. Embryos (egg masses 0-24 hours after spawning), newly hatched (shell diameter ± 1mm) and adults (shell diameter 6-8 mm) were exposed to test chemicals for 96 hours. Egg masses (approximately 200 eggs, from 8 to 12 egg masses, per concentration) were collected on cellophane sheets placed overnight in the aquaria where the colony was maintained. Cellophane sheets with the egg masses were transferred to Petri dishes, containing 50 mL of the respective chemical solution, where they were exposed to the test chemicals. Newly hatched snails (20 per concentration, in duplicate) were exposed to test chemicals in 250 mL glass beakers containing 250 mL of solution, and adults snails (20 per concentration, in duplicate) were exposed to test chemicals in 1000 mL glass beakers containing 1000 mL of solution. All beakers were covered with a net to prevent snails from escaping.

Static acute toxicity assays were performed at controlled room temperature (25 ± 2°C) and light/dark cycle (lights on for 16 hours) in the absence of food. Lethality was evaluated at 24, 48, 72 and 96 hours of exposure and the LC_{50}s and their 95% confidence limits were calculated using the Trimmed Spearman-Karber method (Hamilton et al. 1977).

RESULTS AND DISCUSSION

LC_{50} values obtained for the three chemicals are presented in Table 1. Newly hatched snails were more susceptible to the three chemicals than embryos and adult snails. The higher LC50s obtained for embryos could possibly be explained by the protection provided by the jelly mass in which eggs are embedded. Some molluscicides that are also ovicides, such as niclosamide and organotin compounds, apparently easily penetrate this mass killing the developing embryos at concentrations lower than those that are lethal to adult snails (Oliveira-Filho 2003).

Table 1. Acute toxicities of endosulfan, nonylphenol ethoxylate and ethanol to embryos – within the egg masses, newly-hatched and adult snails of *Biomphalaria tenagophila*.

Chemicals	Exposure	Life Stage		
		Embryo	Newly hatched	Adult
Endosulfan mg/L	24-h	15.52 (11.50-20.95)	0.48 (0.19-1.22)	8.80 (6.92-11.21)
	48-h	9.58 (7.92-11.61)	0.34 (0.14-0.86)	1.77 (0.93-3.41)
	72-h	7.71 (6.63-8.96)	0.20 (0.10-0.41)	1.41 (0.85-2.34)
	96-h	5.81 (5.03-6.71)	0.12 (0.06-0.23)	0.89 (0.46-0.81)
Nonylphenol Ethoxylate mg/L	24-h	640.25 (444.77-921.64)	11.22 (7.42-16.97)	35.49 (21.65-58.21)
	48-h	322.22 (205.75-504.62)	7.13 (4.52-11.26)	24.33 (13.89-42.64)
	72-h	78.43 (54.91-112.05)	4.42 (2.59-7.54)	12.56 (7.79-20.24)
	96-h	29.23 (25.86-33.04)	1.68 (0.88-3.21)	6.39 (4.16-9.82)
Ethanol % (v/v)	24-h	40.91 (32.45-51.57)	15.87 (8.79-28.65)	33.63 (18.18-62.22)
	48-h	27.54 (25.50-29.74)	14.14 (8.88-22.51)	18.66 (12.61-27.62)
	72-h	24.80 (23.47-26.20)	4.45 (2.77-7.18)	8.12 (4.95-13.34)
	96-h	23.82 (22.66-25.04)	1.99 (1.28-3.10)	2.94 (1.91-4.53)

Data are presented as LC_{50} (mg/L) with, in brackets, their respective 95% confidence limits. Ethanol concentrations are expressed as % (v/v) dilution.

Published data with other species report that immature stages are more sensitive than adult stages. This has been observed with other freshwater snails such as *Physa gyrina* (Wier and Walter 1976) and *Lymnaea stagnalis* (Coeurdassier et al. 2004), and insects as *Chironomus riparius* (Williams et al. 1986). On the other hand for life stages of the grass shrimp *Palaemonetes pugio* adults were more sensitive to endosulfan than larvae (Key et al. 2003).

B. tenagophila, at the three life stages, seemed to have been less susceptible to endosulfan than other freshwater species that have been tested so far. It was

reported that fish are much more susceptible to endosulfan with 96-h LC_{50} values ranging from 0.014 to 42 µg/L (Sunderam et al. 1992). The estuarine amphipod *Gammarus palustris* was also more susceptible to endosulfan than *B. tenagophila* with a 96-h LC_{50} as high as 0.43 µg/L (Leight and Van Dolah 1999), but the freshwater crab *Oziotelphusa senex senex*, with a 96-h LC_{50} = 15.14 mg/L (Vijayakumari et al. 1987), was somewhat more resistant than this snail.

Toxicity of nonylphenol with 9 ethoxylate units to different aquatic species was investigated by Dorn et al. (1993) They reported a 96-h LC_{50} of 4.5 mg/L for the fish *Pimephales promelas*, a 48-h EC_{50} of 13 mg/L for the microcrustacean *Daphnia magna*, and a 5-min EC_{50} of 60.6 mg/L for the marine bacterium *Photobacterium phosphoreum* (Microtox System). In the present study *B. tenagophila* (newly hatched snails) was more susceptible to nonylphenol ethoxylate than *D. magna* (48-h) and *P. promelas* (96-h).

Data on the toxicity of ethanol to aquatic species are difficult to compare because, in this particular case, different exposure systems (open or closed) have been used. Since in the present study static assays were carried out using open containers (glasses or Petri dishes), and ethanol is volatile, some ethanol could have been lost during the 96 hour test period. Geraldino et al. (2004) found, for *B. glabrata* embryos exposed into closed Petri dishes, an ethanol 96-h LC_{50} of 0.31M (i.e. 14,291 mg/L or 1.43%). In this study, for *B. tenagophila* embryos exposed into open Petri dishes, ethanol 96-h LC_{50} was as high as 23.82%. Although a difference of susceptibilities between the two species of *Biomphalaria* snails cannot be entirely ruled out, the use of a closed system in the case of *B. glabrata* embryos - preventing the loss of the solvent - could have enhanced the toxicity of ethanol. As noted with endosulfan, in the case of ethanol, newly hatched snails were also more susceptible than embryos and adult organisms (Table 1).

Two other aquatic species data on the toxicity of ethanol are available in the literature. Ethanol was toxic, for instance, to the fish *Pimephales promelas* with a 96-h LC_{50} of 14,200 ppm (or 1.42 %) (USEPA 1995) and to the marine alga *Skeletonema costatum* with a 96-h EC_{50} of 10,943 mg/L (or 1.09 %) (Cowgill et al. 1989). The LC50s obtained for the foregoing organisms are very similar to those obtained for newly hatched snails in the present study (96-h LC_{50} = 1.99%).

In conclusion, results from this study indicated that *B. tenagophila* snails, at the three life stages, were more susceptible to endosulfan than to nonylphenol ethoxylate and ethanol-induced toxicities. It should be noted that toxicity increased significantly at longer exposures (96 hours), suggesting that the persistence or continuous presence of these compounds in water is likely to drastically affect snails communities.

Data presented here also suggested that, as far as the three substances are concerned, newly hatched snails are more vulnerable to the toxic effects of test

chemicals and could be a suitable life stage to evaluate adverse effects of pollutants in aquatic ecosystems.

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