EFFECTS OF TRANSGENERATIONAL EXPOSURE TO LOW CONCENTRATIONS OF NONYLPHENOL ETHOXYLATE ON THE REPRODUCTIVE PERFORMANCE OF THE SNAIL *Biomphalaria tenagophila*

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Introduction

Nonylphenol ethoxylates (NPEs) are nonionic surfactants widely used as emulsifiers in industrial and household cleaning agents, agricultural chemicals, and plastic polymerization process. Several studies have reported relatively high levels of nonylphenol ethoxylates in aquatic ecosystems (Bennie, 1999) and sub-lethal concentrations of NPEs have been reported to impair the reproductive performance of aquatic species, an adverse effect that has been attributed to the estrogenic activity of these environmental contaminants (Servos, 1999). The present study was undertaken to investigate the effects of nonylphenol ethoxylate (NPE) on the reproductive performance of the freshwater snail Biomphalaria tenagophila in successive generations.

Material and Methods

2.1. Test organism

Biomphalaria tenagophila (Orbigny, 1835) is a freshwater pulmonate snail (Mollusca, Gastropoda) found in Brazilian water bodies where it is one of the intermediate hosts of Schistosoma mansoni. Since Biomphalaria snails are also easily breed and kept under laboratory conditions, their use in ecotoxicity assays has been suggested by several authors (Ravera, 1977; Bellavere and Gorbi, 1981; Münzinger, 1987; Oliveira-Filho et al, 2004; 2005).

2.2. Assay water, test substance and nominal concentrations

The nonylphenol ethoxylate 9.5 (RENEX 95, nonylphenol with 9.5 ethoxylate units) (NPE) used in this study was supplied by BASF, Brazil. All snails were kept in a synthetic softwater (pH 7.2 ± 0.1, hardness 40-48 mg/L as CaCO3) prepared as recommended by guidelines of the Brazilian Association for Technical Standardization (ABNT, 2004). NPE was dissolved directly in the assay water at the following nominal concentrations: 0.01, 0.1 and 1 mg/L.

2.3. Experimental design

2.3.1. Effects on the fecundity of the parental generation (F0) Three-month-old snails were individually exposed to NPE (10 snails per concentration) in glass vessels of 300 mL and kept under controlled environmental conditions (25 ± 1°C and light/dark cycle 16/8 hours). The assay water was renewed twice a week and snails were fed with fresh lettuce leaves without pesticides plus 1 mg of a laboratory-made chow (Freitas et al., 1997).

2.3.2. Developmental toxicity and effect on the fecundity of the first generation (F1)

Egg-masses laid by parental snails (F0-generation) after an eight-week exposure period, were separated to be exposed to the same concentrations of NPE during the embryonic development and post-hatching growth of the subsequent (F1) generation of snails. A first group was separated for the evaluation of developmental toxicity (F1 embryos) and the remaining ones continued to be exposed during post-hatching growth until reproductive maturity when they were about three-month old. Mature F1 generation snails (10 per concentration) were used for the evaluation of the effects on egg-production (fecundity) and were also the source of egg-masses used to investigate the developmental toxicity on the subsequent generation (F2 embryos). Developmental toxicity was evaluated in 4 to 5 egg-masses (100 embryos) per concentration. The endpoints for developmental toxicity were lethality, malformations and delay of hatching.

2.3.3. Developmental toxicity in the second generation (F2)

Evaluation of developmental toxicity in F2 embryos (egg-masses laid by F1 snails) was performed as previously described for the F1 embryos.

2.4. Statistical Analysis

Differences in the number of eggs and egg-masses produced, as well as proportions of dead embryos, malformed embryos and embryos that did not hatched were evaluated by one-way ANOVA followed by Dunnett's multiple comparison test. Data shown as proportions were transformed (arc sine square root) before performing the parametric analysis.

Results

3.1. Effects on the reproductive performance of F0 generation

The effects of 8-week exposure to NPE on the fecundity of parental generation (F0) are shown in Figures 1 and 2.

3.2. Effects on the F1 generation: Developmental toxicity and fecundity

The effect of NPE on the embryonic development of the F1 generation was evaluated in a sub-group of egg masses produced by the parental F0 generation. We found no statistically detectable difference between unexposed controls and NPE exposed groups regarding the incidence of deaths and the proportion of hatchings (Figures 3 and 4). Nevertheless, an augmented incidence of malformed individuals, as compared to the incidence in unexposed controls, was found in snails from the egg-masses exposed to the highest concentration of NPE (Figure 5). The effect of exposure to NPE on the reproduction of the F1 generation was examined in individuals originated from another sub-group of egg-masses produced by the F0 generation. After hatching, snails were grown in collective beakers for additional 12 weeks and then, 10 individuals per concentration group, were separated and transferred to individual glasses for a new 8 week evaluation of snail eggs and egg-masses-production (Figures 6 and 7).

3.3 Effects on the F2 generation: Developmental toxicity The effect of a trans-generation exposure to NPE on the embryonic development was investigated in egg masses laid by F1 snails, i.e. in F2 snail embryos. NPE had no lethal effect on F2 generation embryos. It induced, however, a concentration-dependent augment of hatching delays, and a higher incidence of malformed embryos at the highest concentration tested (Figures 8 and 9).

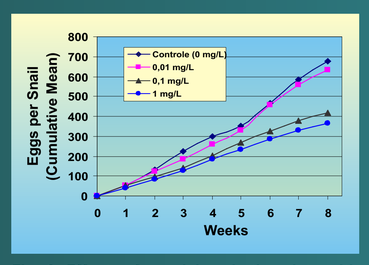

Fig. 1. Effects of nonylphenol ethoxylate with 9.5 units (0, 0.01, 0.1 and 1.0 mg/L) on the number of eggs laid by mature B. tenogophila snails (F0 generation). Data are shown as cumulative means of number of eggs laid per snail during an eight-week exposure. An asterisk (*) indicates that the mean differ ($p<0.05$ ANOVA and Dunnett`s multiple comparisons test) from that of unexposed controls (0 mg/L) at the eighth week.

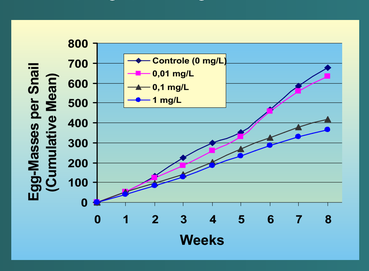

Fig. 2. Effects of nonylphenol ethoxylate with 9.5 units (0, 0.01, 0.1 and 1.0 mg/L) on the number of egg-masses laid by mature B. tenogophila snails (F0 generation). Data are shown as cumulative means of number of egg-masses laid per snail during an eight-week exposure. An asterisk (*) indicates that the mean differ ($p<0.05$ ANOVA and Dunnett`s multiple comparisons test) from that of unexposed controls (0 mg/L) at the eighth week.

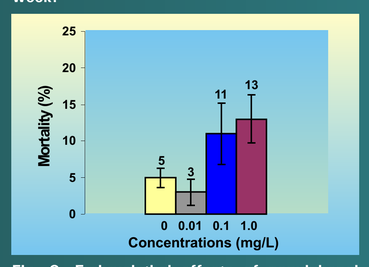

Fig. 3. Embryolethal effects of nonylphenol ethoxylate with 9.5 units (0, 0.01, 0.1 and 1.0 mg/L) on B. tenagophila embryos (F1 generation) recorded at the tenth day after spawning. Proportions are means (SE) of embryo deaths [(No. of deaths / No. of eggs) x 100] per egg-mass. No difference was found ($p<0.05$ ANOVA and Dunnett`s multiple comparisons test) between any group exposed to NPE and unexposed control group (0 mg/L).

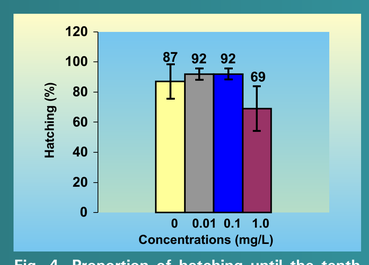

Fig. 4. Proportion of hatching until the tenth day after spawning (F1 generation embryos) exposed to nonylphenol ethoxylate with 9.5 units (0, 0.01, 0.1 and 1.0 mg/L). Values are means (SE) of percentages of hatching [(No. of successfully-hatched snails / No. of eggs) x 100] per egg-mass. No difference was found ($p<0.05$ ANOVA and Dunnett`s multiple comparisons test) between any group exposed to NPE and unexposed control group (0 mg/L).

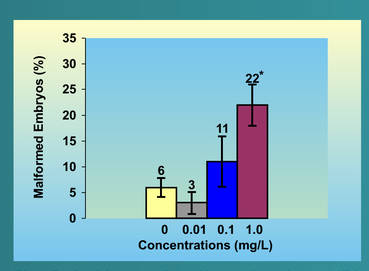

Fig. 5. Incidence of malformed embryos (F1 generation) exposed to nonylphenol ethoxylate (0, 0.01, 0.1 and 1.0 mg/L). Proportions are means (SE) of malformed embryos [(No. of malformed embryos / No. of live embryos) x 100] per egg-mass recorded at the tenth day after spawning. An asterisk (*) indicates that the mean differ ($p<0.05$ ANOVA and Dunnett`s multiple comparisons test) from that of unexposed controls (0 mg/L).

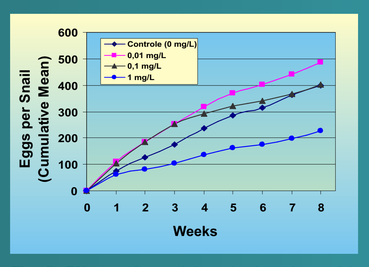

Fig. 6. Effects of nonylphenol ethoxylate with 9.5 units (0, 0.01, 0.1 and 1.0 mg/L) on the number of eggs laid by mature B. tenagophila snails (F1 generation). Data are shown as cumulative means of number of eggs laid per snail during an eight-week exposure. An asterisk (*) indicates that the mean differ ($p<0.05$ ANOVA and Dunnett`s multiple comparisons test) from that of unexposed controls (0 mg/L) at the eighth week.

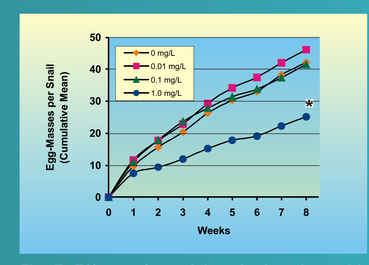

Fig. 7. Effects of nonylphenol ethoxylate with 9.5 units (0, 0.01, 0.1 and 1.0 mg/L) on the number of egg-masses laid by mature B. tenagophila snails (F1 generation). Data are shown as cumulative means of number of egg masses laid per snail during an eight-week exposure. An asterisk (*) indicates that the mean differ ($p<0.05$ ANOVA and Dunnett`s multiple comparisons test) from that of unexposed controls (0 mg/L) at the same week.

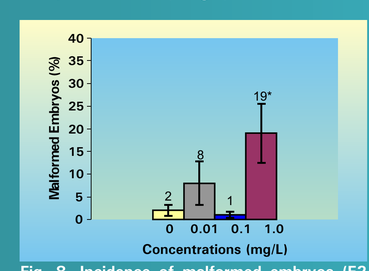

Fig. 8. Incidence of malformed embryos (F2 generation embryos) exposed to nonylphenol ethoxylate (0, 0.01, 0.1 and 1.0 mg/L). Proportions are means (SE) of malformed embryos [(No. of malformed embryos / No. of live embryos) x 100] per egg-mass recorded at the tenth day after spawning. An asterisk (*) indicates that the mean differ ($p<0.05$ ANOVA and Dunnett`s multiple comparisons test) from that of unexposed controls (0 mg/L).

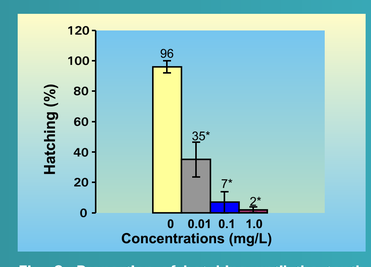

Fig. 9. Proportion of hatching until the tenth day after spawning among B. tenagophila snails (F2 generation embryos) exposed to nonylphenol ethoxylate with 9.5 units (0, 0.01, 0.1 and 1.0 mg/L). Values are means (SE) of percentages of hatching [(No. of successfully-hatched snails / No. of eggs) x 100] per egg-mass. An asterisk (*) indicates that the mean differ ($p<0.05$ ANOVA and Dunnett`s multiple comparisons test) from that of unexposed controls (0 mg/L).

Conclusions

Under the conditions of this study, NOECs (no observed effect concentration) for reproductive toxicity of nonylphenol ethoxylate to the freshwater snail B. tenagophila were as follows: for fecundity, F0 = 10μg/L, F1 < 10 μg/L, for developmental toxicity, F1 = 100 μg/L, F2 < 10 μg/L. The NOEC for effects on reproduction obtained in the present study (<10 μg/L) was lower than NOECs found for nonylphenol ethoxylate with 9 units in chronic assays performed with other aquatic species such as 96-h assay with Selenastrum capricornutum (8000 μg/L), 7-day survival and reproduction assay with Daphnia magna (10000 μg/L) and 7-day assay with Pimephales promelas (1000 μg/L) (Dorn et al., 1993). This relatively low NOEC for nonylphenol ethoxylate, found for a freshwater snail seems to support the view that mollusks are highly vulnerable to endocrine disruption, and B. tenagophila seems to be a suitable bioindicator of water contamination by endocrine disruptors with estrogen-like activity.

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