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ANTENNAE REGENERATION OF MARINE AMPHIPOD PARHYALE HAWAIENSIS AS ENDPOINT IN ECOTOXICOLOGY

Otávio J. Diehl*, Patrícia K. Assano, Rhaul Oliveira, Gisela de A. Umbuzeiro.

Abstract

The marine amphipod Parhyale hawaiensis is able to regenerate its appendages, limbs and tissues. The objective of this study was: (i) verify whether the regeneration of P. hawaiensis antenna can be used as an endpoint in a miniaturized toxicity test system (12 well microplates, 5 mL) and, (ii) apply this endpoint in the toxicity assessment of dimethyl sulfoxide (DMSO), a organic solvent commonly used in toxicity tests, and diflubenzuron (DFB), a growth regulator insecticide. The average regeneration time of fifty percent of the population (RT50) in reconstituted saltwater tests was 16.7 days. DMSO induced the regeneration concentrations above 0.02%. As expected for chitin syntesis inhibitor, DFB strogly inhibited the antenna regeneration with a 28 d-EC50 = 0.5 µg/L. Thefore, we conclude that regeneration time can be used as endpoint in a cost effective miniaturized toxicity test, with time < 35 d. Moreover, DMSO should not be used as a solvent.

Key words:

marine toxicology; crustacean; chronic effects; regeneration; growth inhibitors

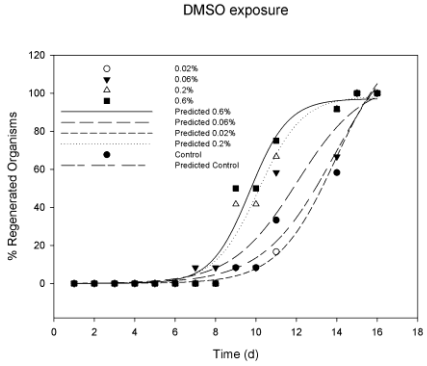
Introduction

Amphipods have been extensively used for toxicity assessment of environmental samples and pollutants worldwide. The marine amphipod *P. hawaiensis* has the ability to regenerate its antennae, limbs and tissues after an injury. The antenna can be involved in sensing food or detecting signals, such as reproduction and excretion. The main objective of this study was to verify whether the regeneration of *P. hawaiensis* antenna can be used as an endpoint in a miniaturized toxicity test system (12 well microplates, 5 mL). Secondaly, apply this endpoint in the toxicity assessment of dimethyl sulfoxide (DMSO), a solvent commonly used in toxicity tests, and diflubenzuron (DFB).

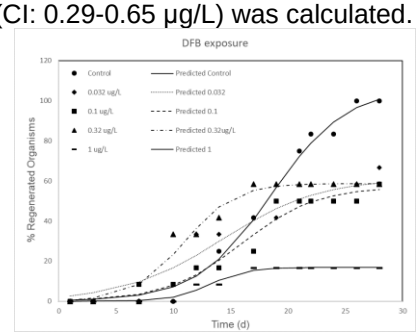
Results and Discussion

Twelve organisms, six months old, six females and six males, with their left antenna amputated, using sterilized tweezers, were individually placed in 12 wells microplates containing 5 mL of reconstituted saline water (Red Sea Salt®). The test was daily monitored until the last organisms had its antenna fully regenerated. During this period, organisms were feed three times a week and seawater was renewed. Four independent experiments were performed. The same procedure was adopted to DMSO and DFB tests.

To verify the DMSO non-effect concentration, amputated organisms were exposed to five concentrations of DMSO (0 – 2%).



As a result, the organisms exposed to DMSO regenerated their antennae faster in concentrations above 0.02% when compared to control group (ANOVA, Tukey test, $p < 0.05$). To verify the effect of diflubenzuron, six concentrations were tested (0 – 10 µg/L). The regeneration time was recorded individually and the number of regenerated organisms by day was predicted by sigmoidal curve, aiming to determine the time that 50% of population were regenerated (RT_{50}). Significant differences from control group were observed for treatment ≥ 1 µg/L (Survival Analysis, Tukey test, $p < 0.05$). Additionally, a 28 d-EC50 = 0.47 µg/L (CI: 0.29-0.65 µg/L) was calculated.



Diflubenzuron inhibited the formation of chitin, altering its structure and also delaying the cycle of molting.[1,2]

Conclusions

The antenna regeneration time can be used as endpoint in a cost-effective miniaturized toxicity test, with time < 35 d. Diflubenzuron inhibited antenna regeneration with a 28 d-EC50 of 0.47 µg/L. Moreover, DMSO is not an appropriate solvent and should be avoided.

Acknowledgement

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