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117 - SUBLETHAL EFFECTS OF CAFFEINE IN *Daphnia magna* LIFE CYCLE: USING A NEW-TERM ENDPOINT

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Keywords: Daphnia magna; Molting cycle curve; Molting inhibition/induction index; Caffeine; Offspring

INTRODUCION

Bioassays using macroinvertebrates are utilized to evaluate water quality and ecotoxicological effects of substances. Caffeine is an emerging substance broadly consumed as stimulant drug and found in food and beverages and its presence in water bodies has been measured from 0.005 to 127 $\mu g.L^{-1}$. As standardized, ecotoxicity tests usually access *Daphnia magna* classical endpoints and any disturbance in the organism life-cycle might increase their sensitivity and affect the aquatic biota, we suggest new endpoints to assess the effects of Caffeine in sublethal concentrations.

METHODS

D. magna was obtained from the Ecology of Aquatic Environments Laboratory (LEAA) in the University of São Paulo. The health status of the organisms was tested in sensitivity bioassays using copper sulfate pentahydrate ($CuSO_4.5H_2O$) as reference substance. The chronic test used 10 replicates with one *D. magna* offspring (aged less than 24-hours). *D. magna* offspring were exposed to four concentrations (0 – control, 5, 30 and 127 $\mu g.L^{-1}$ of Caffeine) for 21 days in a semi-static condition. Three times a week we replaced the solution-test and the organisms were fed. During the test, number of molt, aging, mortality/immobility and number of offspring were recorded daily. The molting-cycle curve was plotted in OriginPro 8.0® software using Logistic equation according to fitting adjustments (R2). After the exposure, the molting data measured was used to calculate the percentage inhibition or induction of caffeine (%I) relative to the control solution-test. The equation used was %I = [(A2substance - A2control) /A2control] x 100, where A2substance = maximum molting data for each Caffeine solution-test and A2control = maximum molting data for control solution-test. When induction of molt occurs, the index is positive and when inhibition of molt occurs, the index is negative.

RESULTS AND DISCUSSION

The organism presented an average sensitivity rate (EC50=0,44 $mg.L^{-1}$) and its use in ecotoxicity test can be validated. This test used reconstituted water at controlled temperature (20±2°C) and photoperiod of 16 hours light/ 8 hours dark. The chemical characteristics of reconstituted water were controlled, and the pH remained between 7.6 and 8.0 and hardness between 175 and 225 mg $CaCO3.L^{-1}$. The average conductivity along the test was 426.5 $\mu s.cm^{-1}$ for control and 507.7 $\mu s.cm^{-1}$ for all Caffeine concentrations. All results were plotted in boxplot graph and analyzed

individually. As predicted, by lethal endpoints, Caffeine in these concentrations did not induce mortality/immobility responses and did not influence the life cycle of *D. magna*. Consequently, the mean age of the organisms at all concentrations of Caffeine presented approximate values when compared to the control condition. However, when sublethal effects were analyzed in two new-term endpoints, Caffeine presented the capacity of stimulating the organism's reproduction. *D. magna* exposure to Caffeine produced a high number of offspring, 66 for all concentrations versus a range between 28 to 65 for control condition. Despite the presence of this compound, no significative difference of intermolting time (number of day between two molting processes) was observed comparing to control condition. Analyzing the molting number for 127 $\mu g.L^{-1}$ of Caffeine, an induction in the total number of molts was evaluated (ranging from 7 to 10), while for 5 and 30 µg.L-1 no significative difference compared to control condition was observed (ranging from 7 to 8). Concurrently, the molting cycle curve was calculated using a Logistic model adjustment ($R2 > 0.99$) and all concentrations of Caffeine presented a typical curve similarly to the control condition. However, in daily measurements, the molt numbers in the presence of Caffeine presented higher values than in control condition. This curve was characterized as a molting induction curve. To evaluate the induction or inhibition effects of Caffeine in organism's life-cycle, we used the maximum molt measured data (A2) and calculated the induction/inhibition index (%I). The results show an %I of 0, +4.28 and +15.71 for 5, 30 and 127 $\mu g.L^{-1}$, respectively. These findings indicate that even in low concentrations, Caffeine can modify the life-cycle of *D. magna* and might affect negatively the aquatic biota equilibrium.

CONCLUSION

This study shown that Caffeine in low concentration, usually found in water bodies, did not cause mortality/immobility effect on *D. magna*. On the other hand, the application of new sublethal endpoints revealed an indirect effect of this compound in the organism life-cycle, increasing the number of offspring and causing molting induction. These results confirm that the organism's molting cycle curve and molting induction or inhibition index (%I) endpoints are useful in evaluating the Caffeine emerging substance.

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