**Behavioral and Physiological Responses of *Daphnia magna*to Fluoxetine and Propranolol Exposure**

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Fluoxetine and propranolol are neuroactive human pharmaceuticals that occur as pollutants in surface waters. The potential effect of such pharmaceuticals on aquatic organisms including invertebrates has raised some concern but many adverse effects are not well characterized. In this study, 6 behavioral and physiological parameters in the freshwater Cladoceran *Daphnia magna* were compared for their responses to fluoxetine and propranolol exposure: mobility (dichotomous response), active swimming time, swimming distance, swimming velocity, swimming acceleration speed, and survival in the absence of food (starvation-survival). Changes in swimming behavior of *D. magna* were quantified by video tracking of single organisms followed by image analyses. Active swimming time and swimming distance appeared to be more responsive behavioral endpoints than swimming velocity and swimming acceleration. The EC50s for fluoxetine and propranolol determined from swimming time and swimming distance were comparable (1-2 mg/L). At low sublethal exposure concentrations (µg/L), nonmonotonic responses in swimming behavior were observed in *D. magna.* Behavior profiling estimated from multiple behavioral parameters showed that fluoxetine and propranolol stimulate swimming activity at 1-10 µg/L.

EC50 values for fluoxetine and propranolol estimated from survival time in the absence of food (starvation-survival) were much lower than EC50 values estimated from changes in swimming behavior. Starvation-survival is strongly affected by energy metabolism and we suggest that this parameter can be a potential sensitive endpoint for determining adverse effects of pharmaceutical to *D. magna*. Combining behavioral and physiological responses to high and very low exposure concentrations should be considered in models predicting adverse effects of pharmaceuticals to non-target organisms.

**Keywords:** *Daphnia magna*, fluoxetine, propranolol, video tracking, behavior, survival,

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1.3 Behavioral Toxicology: Putting behavioral toxicology in a regulatory perspective