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Can Daphnia behavioral endpoints be used as tool for ecotoxicological assessment of wastewater effluents?

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1. Introduction

Daphnia magna is highly sensitive to a wide range of chemicals. The immobilization test on *Daphnia magna* is one of the most used standardized tests (OECD guideline 202, ISO 6341) for assessing hazard of chemicals and monitoring effluent quality, because this test is simple, rapid and cost effective. However, standard immobilization tests, so-called acute lethal tests do not consider intermediary toxic effects prior to the death of daphnids at the end of the 48 hour exposure. Behavioral tests may provide early and intermediary responses since toxic stress can induce rapid behavioral changes in exposed organisms at concentration below acutely toxic levels [1,2] and are therefore already used to detect potential toxicity in wastewater effluents. However, current image analysis systems available on the market necessary to track and quantify Daphnia behavior offer a limited number measuring cells only and do not allow performing sufficient replicates for an appropriate statistical analysis of the biological variability in behavior. It is currently unclear how to derive robust ecotoxicological endpoints from observation of organism behavior and how to quantify the sensitivity of these endpoints. The aim of the study is (i) to assess how behavioral responses can be used as ecotoxicological endpoints and (ii) to compare the sensitivity of standard versus behavior endpoints in order to assess their usefulness for ecotoxicological assessment of wastewater effluents.

2. Materials and methods

Daphnia magna were cultured at 20°C in 1.5 L vessels of M4 medium and fed daily with *Chlorella sp*. Before the experiment, young neonates (less than 16 hours) were isolated to perform the acute test and the behavioral test. Test compounds were selected with regard to cover different modes of toxic action: 2 narcotics (Ethanol and Isopropanol), 2 Acetylcholine esterase inhibitors (Trichlorfon and Carbofuran), 2 agonists of the nicotinic Acetylcholine receptor and GABAergic receptor (Imidacloprid and Abamectin respectively), 3 modulators of sodium channels (Esfenvalerate, Cypermethrin and Fipronil), 2 psychoactive substances (Caffeine and Sertraline) and a mixed mode of action substance (Copper sulphate). Acute toxicity tests were performed according to the OECD 202 test guideline. Concentration response curves were modelled for the twelve substances and served firstly for selection of concentrations for the behavioral test and later as reference for comparison to behavioral endpoints.

A new image analysis system allowing the simultaneous tracking of up to 200 *Daphnia magna* distributed in 20 exposure chambers (10 daphnids per chamber) was used for behavioral tests. Exposure conditions were designed to cover effect concentrations from the EC_{10} to EC_{100} of the acute test with a dilution factor below 2.3. Five concentrations (3 replicates) for each compound and 5 control replicates were performed. Two behavioral parameters (swimming speed and number of active organisms) were continuously followed during 48 hours. This exposure time was chosen in order to directly compare results with endpoints of the standard tests. Neonate organisms were not fed during the whole experiment to avoid the growth of organisms which is directly related to swimming speed [3]. Time courses of the two parameters (average per hour) were compared to control responses for each chemical in order to quantify behavior changes induced by toxicants and time of effect onset.

3. Results and discussion

The behavior endpoint "swimming speed" has shown higher sensitivity than the number of active organisms. Differences between tested compounds were observed in the time of effect onset, duration and intensity of effects on swimming speed depending of their mode of action. Narcotics showed an intense increase of the swimming speed from the first hour of the experiment followed by a gradual decreased while neurotoxic chemicals induced a slightly but significant increase at different time depending to their mode of action.

As an example, results for Esfenvalerate and Trichlorfon are shown in Figure 1 (swimming speed as function of time) and sensitivities of acute endpoints versus behavior endpoints are summarized in Table 1.

Esfenvalerate induced significant increases of the swimming speed for all tested concentrations from the first hour of the experiment (Figure 1-left). Slightly but significant increase of the swimming speed (21% compared to controls) was observed for a concentration below the EC_5 at the beginning of the analysis for 16 hours (Table 1). An intensive increase of 79% was also detected from the beginning of the analysis for a sub-lethal concentration near EC_{10} during 21 hours. On the other side, Trichlorfon did not show such marked changes of the swimming speed (Figure 1-right). The lowest concentration for which an effect (9% increase of the swimming speed compared to controls) is observed is above the EC_{20} (Table 1). Unlike Esfenvalerate where the strong increases were observed from the first hour of the experiment, Trichlorfon showed slighter and delayed effects. Indeed, increase of the swimming speed appeared later and did not exceed 39% for the highest test concentration (EC_{70}).

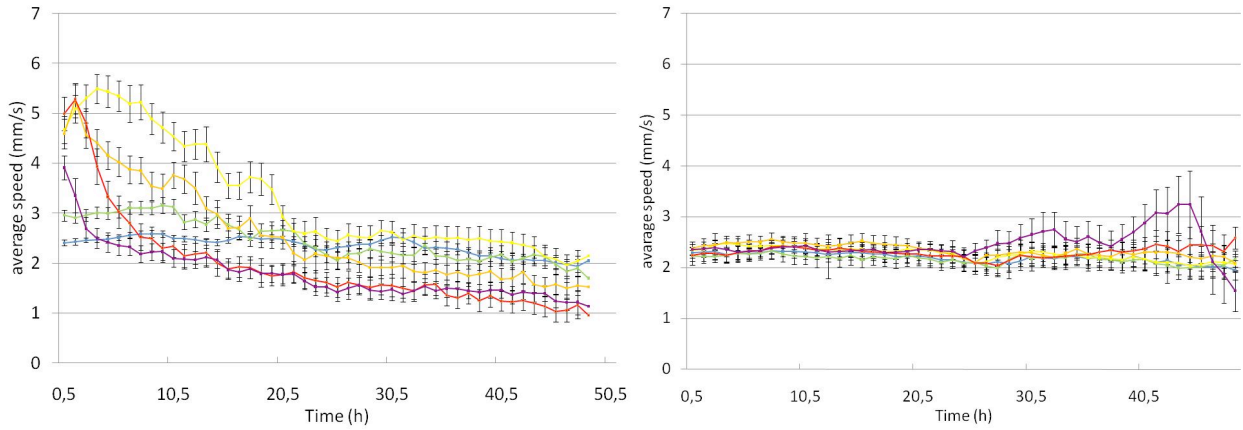


Figure 1: Swimming speed average (per hour) of Daphnia magna exposed to different concentration of Esfenvalerate (left) and Trichlorfon (right) during 48 hours.

(Controls; near EC5; near EC10; > EC20; between EC50-EC70; between EC70-EC100)

Overall, significant behavior responses were observed in the first hours of the experiment well below the acute EC_{50} (based on immobility at 48h) for most tested compounds.

Substance	Acute test	Behavioral Test		
	EC_{50}(48H)	First treshold of significant behavior responses	Sensitivity (% in increase of swimming speed compared to controls)	Time of effect
Esfenvalerate	0.85 µg/L	0.14 µg/L	21%	1h-16h
Trichlorfon	0.21 µg/L	0.13 µg/L	9%	12h-20h

Table 1: Comparison of sensitivities of standard endpoint versus behavior endpoints for Esfenvalerate and Trichlorfon.

4. Conclusions

Differences between tested compounds were observed in the time of effect onset, duration and intensity of effects on swimming speed depending of their mode of action. The exposition in the new image analysis system provides early and sensitive information compared to standard test endpoints and may contribute to wastewater and effluent quality assessment especially in case of chemical spills in order to avoid contamination of surface waters..

5. References

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