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Age and Synchronization Influence the Sensitivity of *Daphnia magna* Neonates to the Chitin Synthesis Inhibitor Teflubenzuron

Background

Acute adverse effects of chemicals on aquatic invertebrates (mortality) are assessed using the OECD *Daphnia sp.* acute immobilization test. It stipulates an exposure period of 48h and the use of neonatal *Daphnia sp.* that are less than 24h old. However, adverse effects induced by endocrine disrupting chemicals (EDCs) or substances interfering with endocrine controlled processes that control molting might only become apparent during or after molting. As molting is a periodical process, the absolute age as well as the synchronization of animals might be important factors for the outcome of such toxicity tests. Although not being EDCs *per se*, chitin synthesis inhibitors (CSIs) interfere with an endocrine controlled process that is crucial for the successful completion of molting.
In the present study, we assess the impact of synchronization and age on the susceptibility of *D. magna* exposed to the CSI teflubenzuron (TEF).

Approach

Acute *D. magna* toxicity tests were conducted according to OECD test guideline 202 with slight modifications. In brief, 5 *D. magna* neonates of different age and synchronization windows were exposed to nominal concentrations of 0.5-12 µg/L of TEF and a solvent control (0.01 % DMSO). Exposures were conducted in non-treated 6-well plates at a density of one animal per 2 mL solution (Figure 1). Each treatment consisted of four replicates and molting frequency and survival were monitored at 24, 36 and 48 h post-exposure.

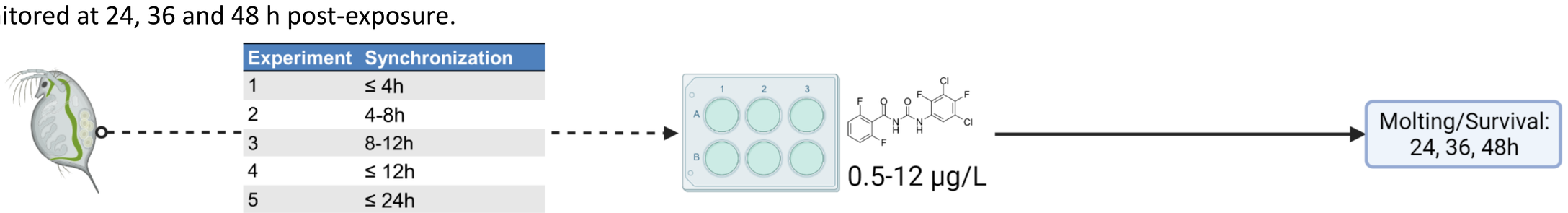

Fig. 1: Schematic overview of experimental design of acute *Daphnia magna* toxicity tests

Results

Effects on Molting

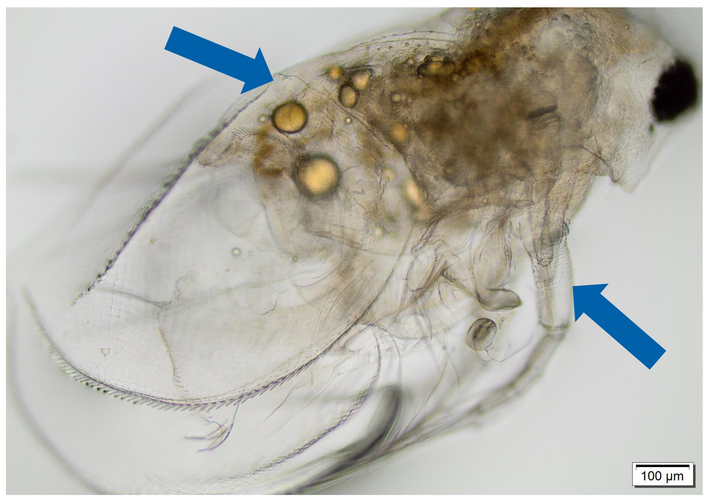

Fig. 2: *Daphnia magna* stuck in its exoskeleton after 48h exposure to 12 ug/L TEF (≤ 4h synchronization).

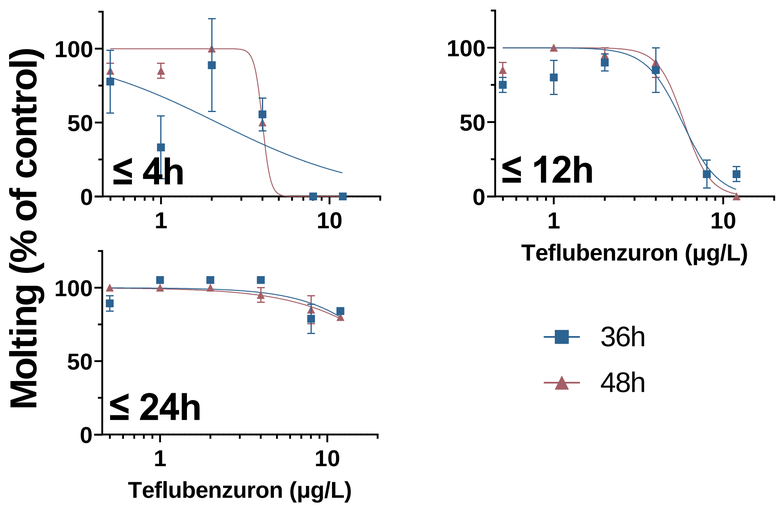

Fig. 3: Synchronization-dependent concentration-response curves for molting disruption in *D. magna* after exposure to TEF for 36h (blue line) and 48h (red line). Molting data from 24h is not show due to high variability. Numbers in the plots indicate the age of neonates at the start of the exposure.

Effects on Survival

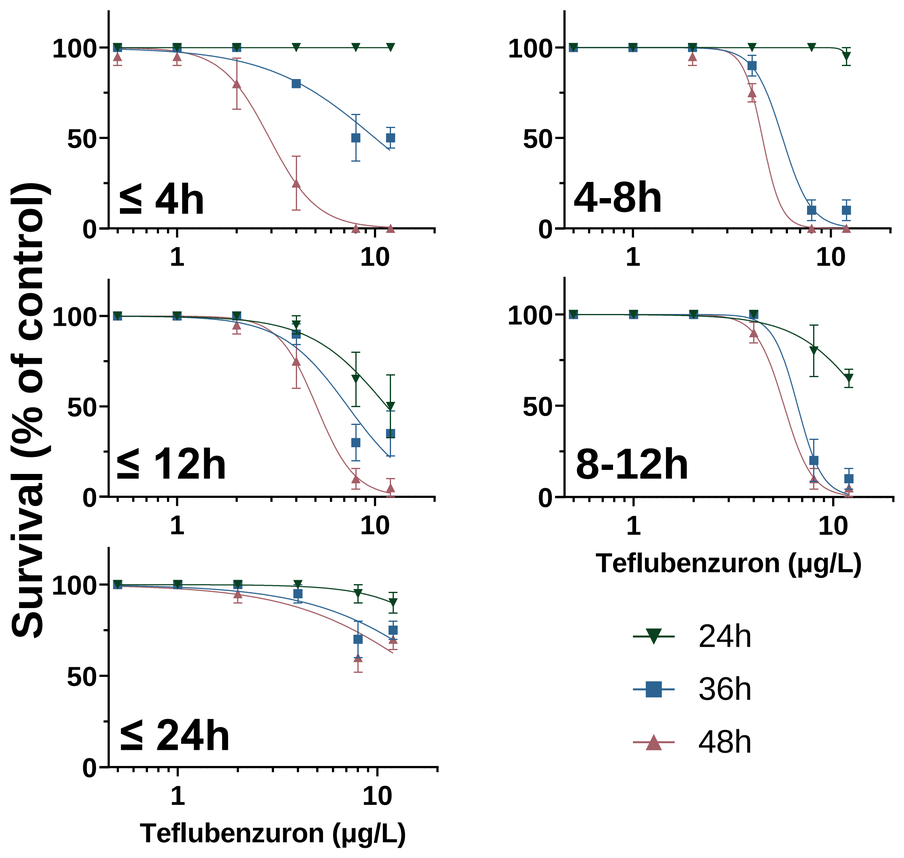

Fig. 4: Synchronization-dependent concentration-response curves for mortality in *D. magna* after exposure to TEF for 24h (green line), 36h (blue line) and 48h (red line). Numbers in the plots indicate the age of neonates at the start of the exposure.

Table 1: Summary of LC50 values with 95% confidence intervals for different observation timepoints and synchronization windows.

Age	24h LC50 (ug/L) (95% CI)	36h LC50 (ug/L) (95% CI)	48h LC50 (ug/L) (95% CI)
≤ 4h	-	10.0 (7.8-12.3)	2.9 (2.4-3.5)
4-8h	-	5.7 (5.1-6.3)	4.5 (3.6-5.5)
8-12h	15.2 (10.1-20.2)	6.6 (5.6-7.7)	5.7 (5.2-6.1)
≤ 12h	11.5 (8.2-14.7)	7.3 (5.9-8.7)	5.1 (4.3-5.8)
≤ 24h	31.7 (0.0-79.3)	22.2 (6.8-37.5)	17.7 (8.3-26.9)

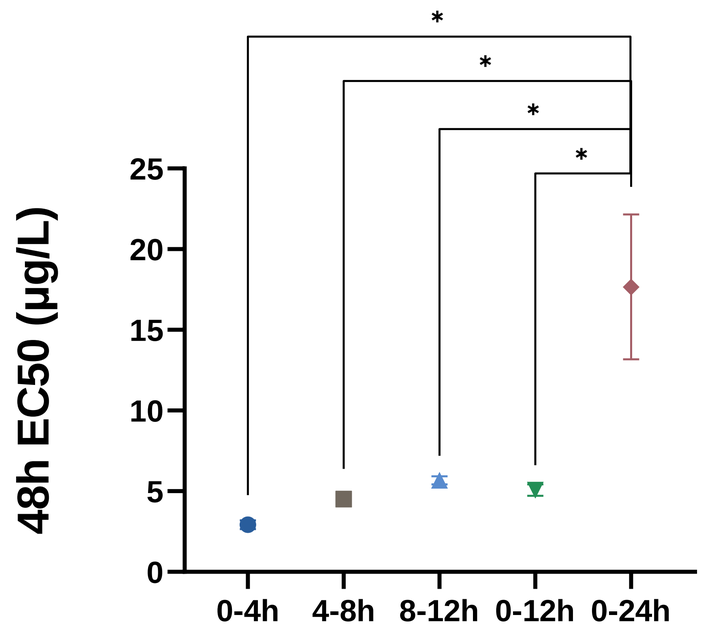

Fig. 5: Comparison of 48h LC50s of different synchronization windows. Statistically significant differences are indicated with asterisks ($p < 0.05$).

Conclusions

- We demonstrate the importance of synchronization and age in *D. magna* acute toxicity tests with CSIs.
- We show a tendency to higher susceptibility to TEF with narrower synchronization windows and younger animals.
- Our results indicate that the synchronization window stipulated in the OECD 202 test guideline might not be narrow enough to fully uncover adverse effects of CSIs, which might lead to the underestimation of adverse effects.

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