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Uncovering Disturbances in Chitin Metabolism after Exposure to Teflubenzuron and linking them to Phenotypical Effects in *Daphnia magna*

Background

Through molting, arthropods shed their cuticles (exoskeletons) periodically. During that process, they depend on the formation of a new, larger cuticle that is stable enough to support muscular contractions during the molting process. Chitin biosynthesis is mediated by a number of different carbohydrate conversions and is crucial for successful molting. Therefore, chitin synthesis has been exploited as a target for control of unwanted arthropods by development of chitin synthesis inhibitors (CSIs), which selectively inhibit chitin synthase, the key enzyme of the chitin biosynthesis pathway. In the present study, we investigated the effects of the prototypical chitin synthesis inhibitor teflubenzuron (TEF) on selected metabolites of the chitin biosynthesis – and degradation pathway as well as on molting and associated mortality. We aimed to identify and quantitatively assess changes of selected metabolites in the pathway and find potential metabolic markers for adverse phenotypical effects upon exposure of *Daphnia magna* to CSIs.

Approach

Acute *D. magna* toxicity tests were conducted according to OECD test guideline 202 with slight modifications. In brief, 40 *D. magna* neonates (≤ 12h old) were exposed to nominal concentrations of 0.1-8 µg/L of TEF and a solvent control (0.01 % DMSO). Exposures were conducted in glass beakers at a density of one animal per 2 mL solution (Figure 1). Each treatment consisted of nine replicates and molting frequency and survival were monitored at 24 and 48h post-exposure. Pools of 30 animals per replicate were sampled for subsequent metabolite extraction after 24h post-exposure.

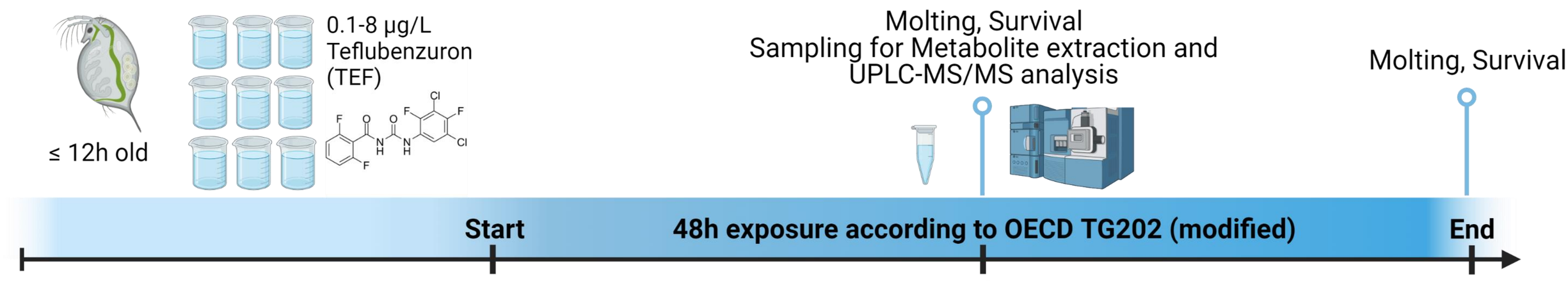

Fig. 1: Schematic overview of the experimental design of the acute *Daphnia magna* toxicity test

Results

Effects on Phenotypical Endpoints

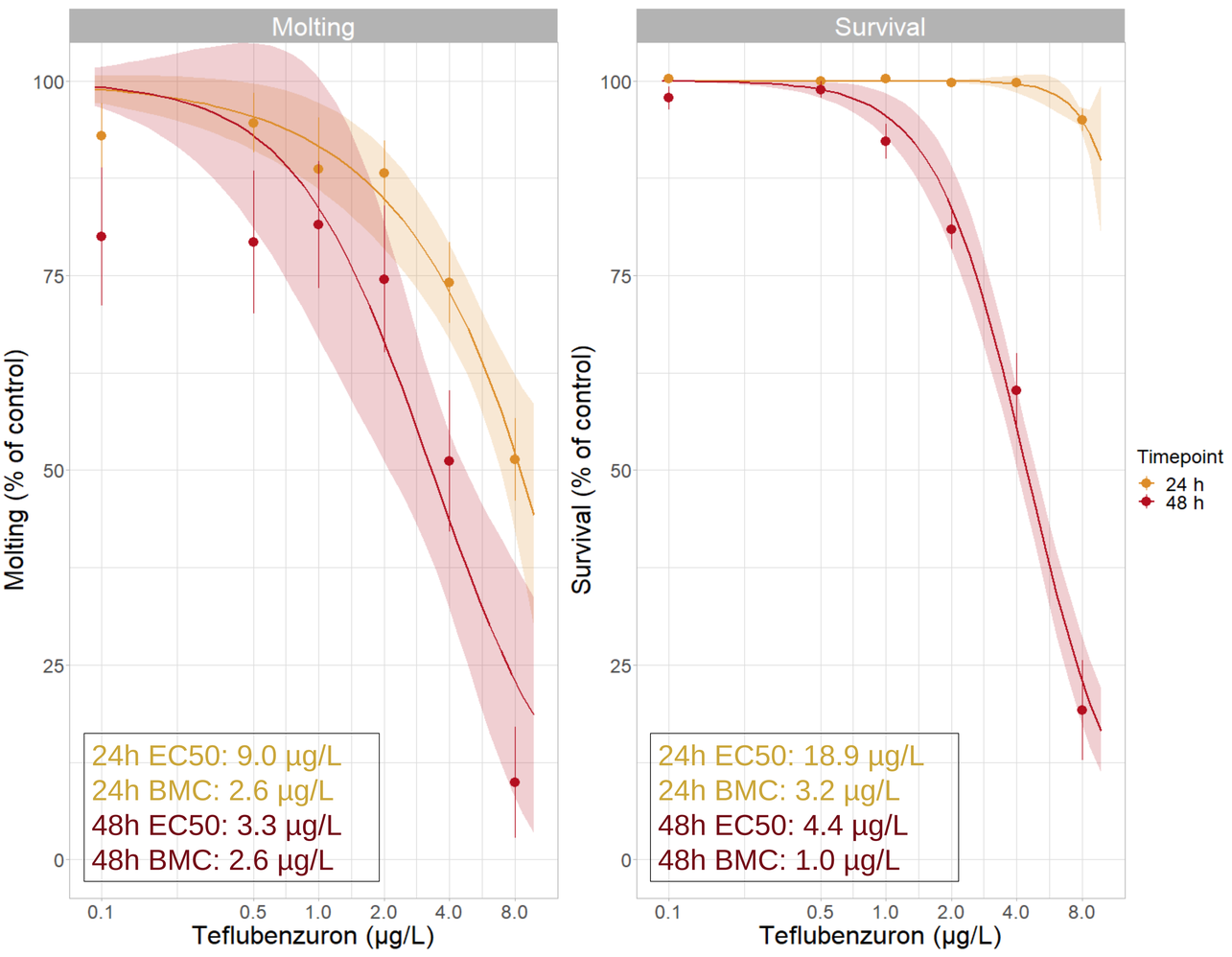

Fig. 2: Concentration-response curves for molting disruption (left panel) and survival (right panel) in *D. magna* after exposure to teflubenzuron for 24h (orange line) and 48h (red line). Bands around the fitted curves represent 95% confidence intervals. Data points with whiskers represent the mean ± standard error of the mean. Text boxes in the plots indicate the median effect concentration (EC50) and the benchmark concentrations (BMCs) for the respective endpoint. Values in orange are for observations made after 24h and values in red are for 48h.

Effects on Metabolite Levels

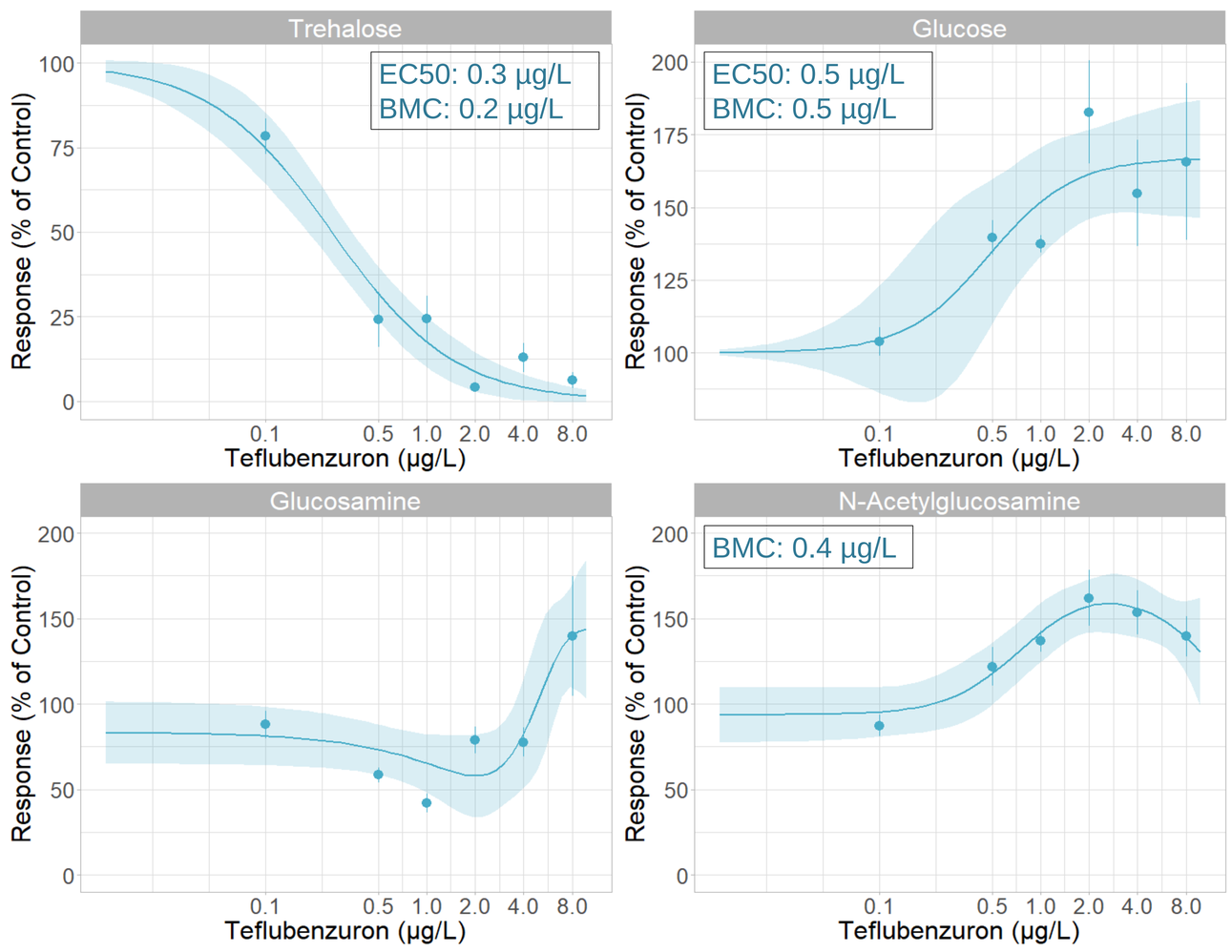

Fig. 3: Concentration-response curves for changes in levels of Trehalose (upper left panel), Glucose (upper right panel), Glucosamine (lower left panel), and N-Acetylglucosamine (lower right panel) after 24h of exposure to teflubenzuron. Bands around the fitted curves represent 95% confidence intervals. Data points with whiskers represent the mean ± standard error of the mean. Measurements were made after 24h of exposure to TEF. Text boxes in the plots indicate the median effect concentration (EC50) and the benchmark concentrations (BMCs) for the respective endpoint.

Conclusions

- We observed **concentration dependent** effects for carbohydrates involved in chitin synthesis pathway
- Effects on **metabolites** occurred **earlier** and at **lower concentrations** than phenotypical effects
- Metabolic changes indicate that most likely both, **chitin synthesis and degradation are affected**

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