Molting inhibition in *Calanus finmarchicus* after exposure to the chitin synthesis inhibitor teflubenzuron

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Introduction and objective

Chitin synthesis inhibitors (CSIs) are widely used in marine aquaculture to control parasitic salmon lice. However, these compounds may also harm non-target keystone species in Arctic and boreal ecosystems. With growing environmental stress from climate change and pollution, it is critical to understand the toxicity mechanisms and adverse impact of such compounds to improve ecological hazard and risk assessments.

This study experimentally explored molecular responses, phenotypical and adverse effects relevant for molting disruption in juvenile *Calanus finmarchicus* (copepodite stage C2) after 7-days waterborne exposure to teflubenzuron (TEF). Data generated was used to evaluate different molecular initiating events (MIE)s, key events (KE) and adverse outcomes (AOs) in an Adverse Outcome Pathways (AOPs) for molting disruption (fig. 1).

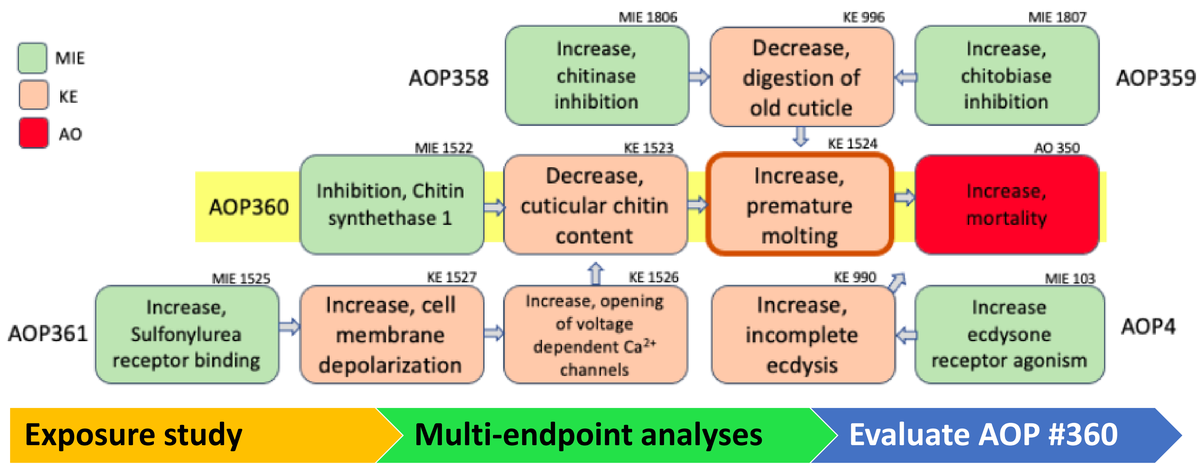

Figure 1. Exposure study with C. finmarchicus to control , solvent control and waterborne TEF (0.001-3 ug/L) for 7-days. Gene expression, phenotypical effects (lifestage distribution, protosome length, morphology) and mortality were determined and compared to AOP #360 "Chitin synthase 1 inhibition leading to mortality".

Multi-endpoint effects analysis

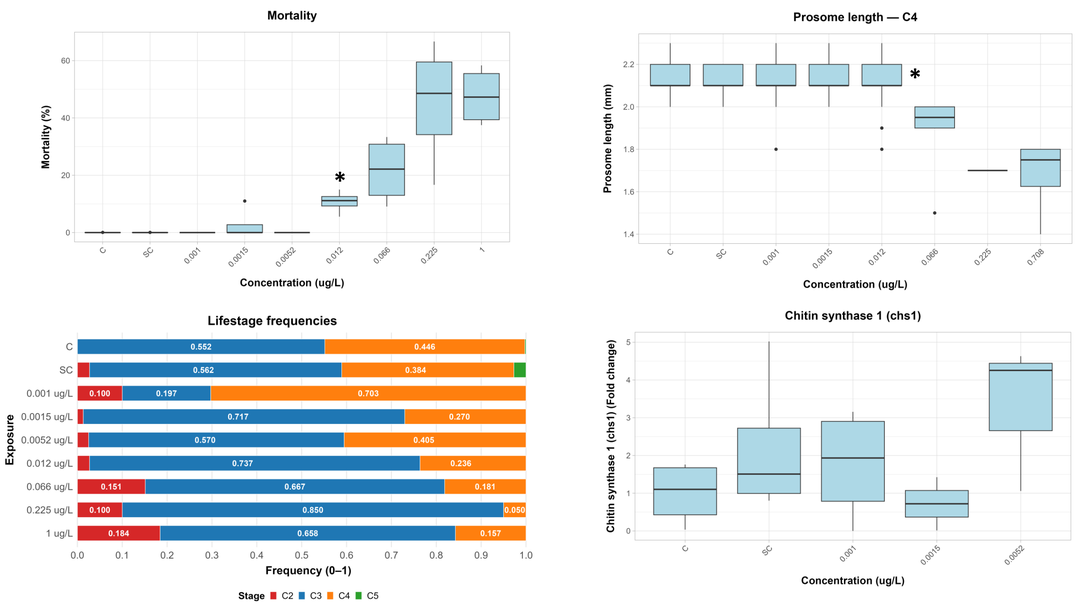

Figure 2. Mortality, phenotypical (life stage frequency, prosome length) and gene expression of one key AOP#360 marker gene (chs1) in C. finmarchicus after 7 days exposure to waterborne TEF. * p≤0.05

Points of Departures (PODs)

Toxicity	Biological organisation	Endpoint	Concentration TEF (ug/L)
Mortality	Population	NOEC	0.0052
		LOEC	0.012
Shift in lifestage frequency (development)	Population	NOEC	0.012*
		LOEC	0.066*
Prosome length (growth, development)	Individual (C4 stage)	NOEC	0.012
		LOEC	0.066
chitin synthase 1 (chs1)	Molecular (gene expression)	NOEC	> 0.0052**
		LOEC	

* Apparent shift in frequency (stats pending). ** No statistical difference to solvent control at highest concentration analysed.

Table 1. Determination of Points of Departure (POD) from normality for molecular, phenotypical and adverse outcomes in C. finmarchicus after 7 days exposure to TEF.

Summary and outlook

Summary

The AOP-informed exposure study with TEF and *C. finmarchicus* revealed several key results :

1) Molting-related mortality, inhibited growth/development, and induced deformities occurred for concentration ≥12 ng/L (Table 1).
2) Expression of molting- & chitin-related genes affected, but chitin synthase 1 (chs1) remained unaffected at low concentrations (Fig. 2).
3) Findings not conclusive for supporting a chs1-based mechanism proposed by AOP #360.

Outlook

1) Develop enzyme-associated methods that can determine CSI inhibition directly to support MIE assessment.
2) Develop additional bioassays to characterize role of alternative toxicity pathways for molting disruption in crustaceans.
3) Determine relevance of findings in terms of contribution to risk of CSIs in the environment.

Co-funded by the European Union

Norwegian Environmental Toxicology Symposium (NETS) Stavanger, Norway, 2025

With funding from The Research Council of Norway

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This work was carried out in the framework of the European Partnership for the Assessment of Risks from Chemicals (PARC) and has received funding from the European Union's Horizon Europe research and innovation programme under Grant Agreement No 101057014, Research Council of Norway project EXPECT (RCN-315969) and NIVA's Computational Toxicology Program, NCTP (RCN-342628). Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the Health and Digital Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.