Severe effects of neonicotinoid insecticides on *Nitocra spinipes* under different exposure conditions

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Neonicotinoids have been constantly replacing formerly used insecticide groups (e.g. organophosphates, pyrethroids, etc.) in the past few decades. Their physical-chemical properties including lower log K_{OW} and higher water solubility make them readily absorbed by plants and act quickly at low doses. On the other hand, these properties make them susceptible for runoff and subsequent transfer into the aquatic environment. They have been detected in surface waters in the ng L^{-1} to low µg L^{-1} range, and finally in marine waters at concentrations ranging from 0.5 to 10 ng L^{-1}.

In this work, we assessed potential acute and chronic effects of four neonicotinoids (clothianidin CLO, imidacloprid IMI, thiacloprid TCL and thiamethoxam TME) on the brackish copepod *Nitocra spinipes*. Neonicotinoid exposure was performed under three different scenarios. First in a 96h acute lethal toxicity test according to ISO 14669. Second in a recovery experiment based on 96h exposure to two different neonicotinoids (CLO and TCL) followed by transfer to fresh medium and continuous observation to explore the recovery potential of exposed copepods. Finally, we performed a 7-day larval development test with TCL according to ISO 18220.

In the acute tests we found **immobilization to be a more sensitive endpoint than death**, showing EC_{50} (96h, immobilization) values ranging from 0.81 µg L^{-1} (CLO) to 430 µg L^{-1} (TME). Our recovery experiment showed that 69 % of the organisms exposed to 100 mg L^{-1} TCL were able to recover mobility after 24h, while those exposed to 100 mg L^{-1} of CLO remained immobilized.

The larval development test with TCL showed a **clear delay in development** of *N. spinipes* nauplii and the EC_{50} (7d) was calculated at 10.3 µg L^{-1}, while the NOEC (7d) was 1.23 µg L^{-1}.

N. spinipes showed **considerable sensitivity when exposed to neonicotinoid insecticides both in short- and long-term exposure** for adults and nauplii, respectively. Immobilization was a clearly more sensitive endpoint as compared to death in acute testing and locomotion is crucial for copepod survival in terms of feeding behaviour and predator avoidance. Regarding the recovery potential, there seems to be no general agreement for different neonicotinoids. Developmental effects of TCL on copepod nauplii suggested further testing of other neonicotinoids and particularly CLO (lowest acute EC_{50}) under chronic exposure conditions.

Keywords: Neonicotinoid insecticides; Marine copepods; Immobilization; (Ir)reversible effects