DIFFERENTIAL EFFECTS OF ORGANOPHOSPHATE DIAZINON ON MEMBRANE AND SOLUBLE ACETYLCHOLINESTERASE IN HONEYBEE HEAD AND THORAX

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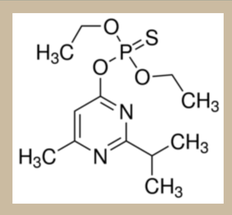
Introduction

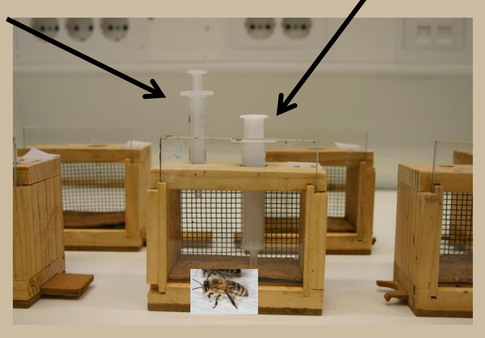
Honeybee is an important pollinator threatened by different xenobiotics, such as organophosphate insecticides. Organophosphates specifically inhibit acetylcholinesterase (AChE), which is responsible for the rapid hydrolysis of the neurotransmitter acetylcholine. Thus, the activity of AChE is often used as an important biomarker of neurotoxicity after exposure to pesticides. AChE in honeybees occurs in two major forms, membrane and soluble. Recently, *in vitro* experiments suggested that the membrane form of AChE is mainly neuronal whereas the role of soluble form is largely unknown, but some suggestions of their protective role against xenobiotics have been given[1]. Therefore, in regard to neurotoxicity, monitoring of only soluble or only membrane form of AChE is not sufficient. The aim of this study was to investigate *in vivo* effects of AChE inhibitor diazinon on the activity of soluble and membrane AChE in honeybee head and thorax.

Methods & Materials



- **Organophosphate diazinon** (0.2; 0.5; 1; 2.5; 5 mg/L)
- **Control**: sucrose feeding (1.5 M)

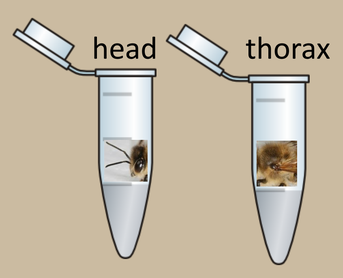
Dechlorinated water



Summer honeybees *Apis mellifera carnica*
Number of bees per treatment: 40; 20 bees/cage

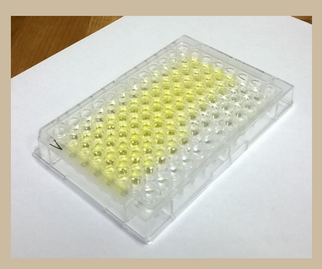
10 days incubation at 34 °C, 80% relative humidity
Daily inspection of feeding and survival

Abdomen-discharged



- Homogenisation
- Separation of membrane and soluble fraction
- Centrifugation (15 min, 4° C, 16000 g)

Acetylcholinesterase measurement[2]



Results

MORTALITY AND FEEDING RATE

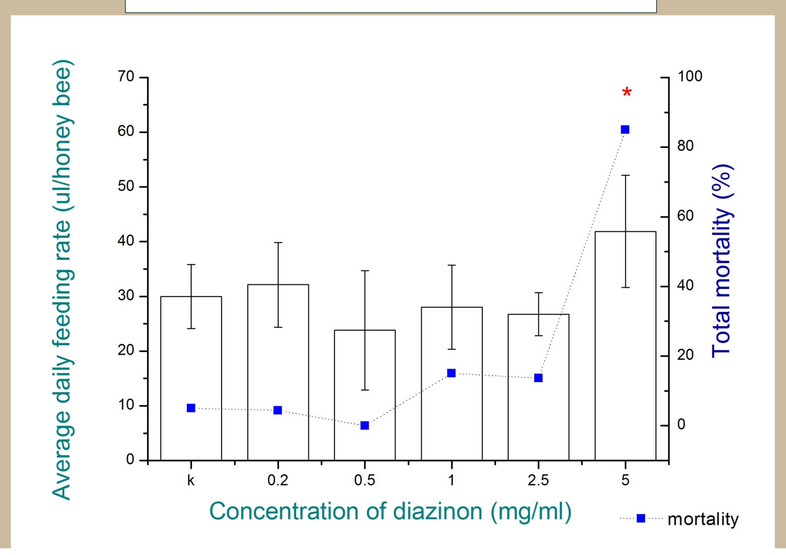


Fig. 1. The feeding rate and mortality of honeybees during 10 days oral feeding exposure to diazinon (0, 0.2; 0.5; 1; 2.5; and 5 mg/L).

ACETYLCHOLINESTERASE ACTIVITIES

MEMBRANE BOUND

SOLUBLE FRACTION

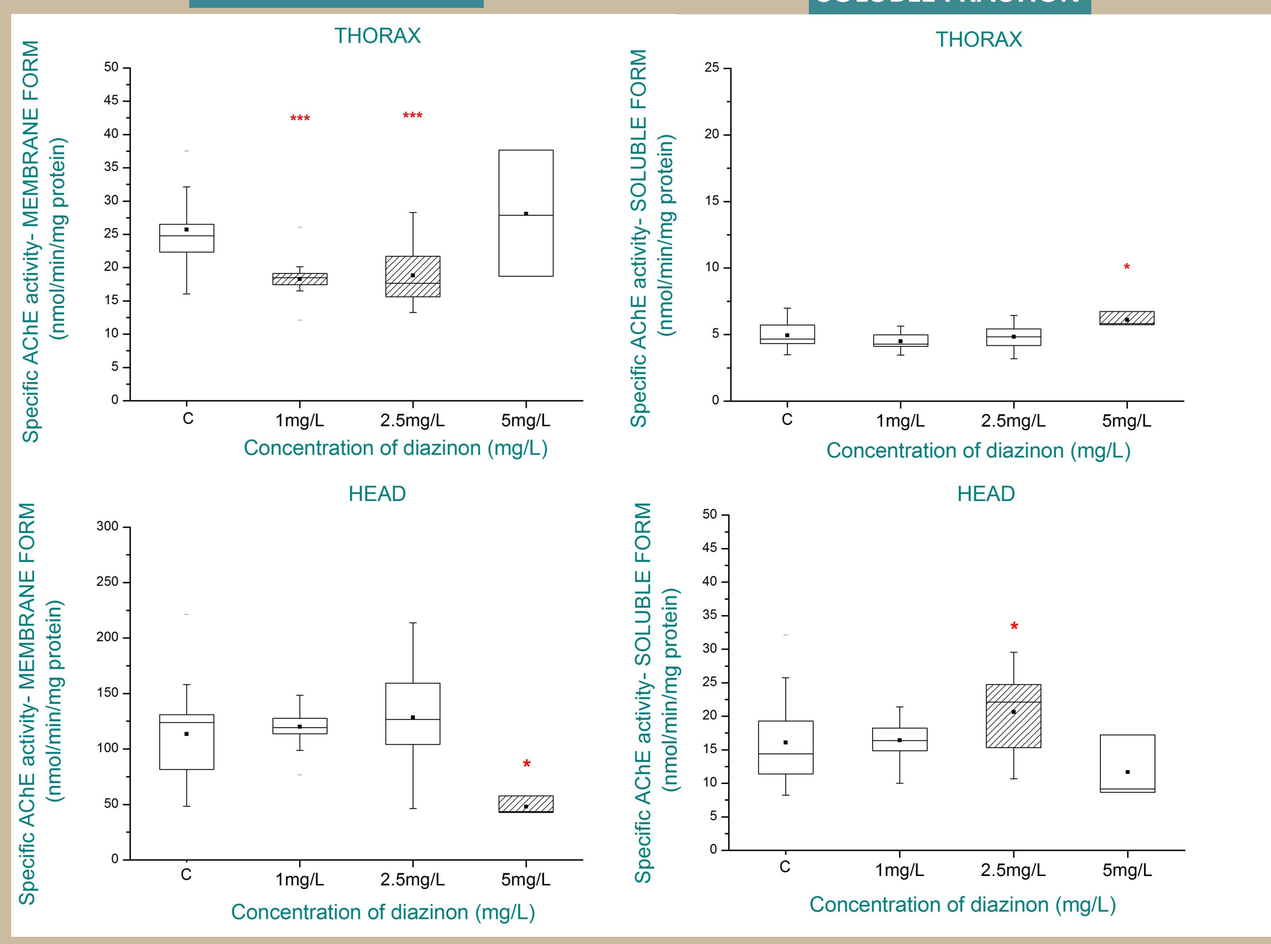


Fig. 2. Different fractions of acetylcholinesterase activity (membrane, soluble) in head and thorax of honey bees exposed to diazinon (0, 0.2; 0.5; 1; 2.5; and 5 mg/L) for 10 days.

Discussion

Chronic 10 days exposure to diazinon caused increase in feeding rate and mortality of honeybees at the highest concentration of diazinon in comparison to control. The activity of membrane AChE in the head of control untreated honey bees was much higher than the soluble confirming results of *in vitro* experiments showing that the membrane form is probably neuronal. In the thorax this ratio was much lower. The chronic exposure to diazinon diminished the activity of membrane AChE in the head and thorax, but elevated the soluble AChE in both body parts. These differential effects on AChE activity were shown for higher concentrations of diazinon tested. The elevation of the activity of soluble AChE demonstrated in this study might be predictable for its detoxifying function whereas the changes in the activity of membrane AChE could be the result of the compensatory effect of nervous system or direct inhibition by diazinon. However, the role of the soluble AChE needs to be further investigated.

Acknowledgements
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References:
1 Kim et al., 2012. PLoS ONE 7(11): e48838.
2 Ellman et al. 1961. Biochem. Pharmacol. 7: 88-95.