SENSITIVITY OF ACETYLCHOLINESTERASE FROM THE TISSUES OF COMMERCIALLY IMPORTANT BIVALVE SPECIES WARTY VENUS *VENUS VERRUCOSA* (LINNAEUS, 1758) AND NOAH'S ARK SHELL *ARCA NOAE* (LINNAEUS, 1758) TO ORGANOPHOSPHOROUS PESTICIDES

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Introduction:

Organophosphorous pesticides (OP's) are widely applied in agriculture and represent a considerable threat to cultivated and wildlife populations of marine non-target organisms in the near-shore coastal areas. These compounds selectively inhibit cholinesterases (ChE), serine hydrolases essential for transmission of nerve signals. Biological effect of exposure to OP's has been routinely evaluated by the measurement of ChE activity in the tissues of marine organisms, most commonly in bivalve species such as mussels *Mytilus galloprovincialis*. Recently, it was suggested that use of bivalve species of different ecology and physiology, could increase the usefulness of environmental monitoring (Valbonesi et al, 2003; Bonacci et al, 2008). In this work, the possibility of ChE activity measurement was examined in the tissues of two commercially important bivalve species: warty venus *Venus verrucosa* and Noah's ark *Arca noae*, abundantly distributed and harvested for consumption along the eastern Adriatic coast.

The aim of the study:

1. To establish the main properties of ChE, using acetylthiocholine (ASCh), considered so far as the most specific substrate for ChEs from the tissues of bivalves (Valbonesi et al, 2003; Brown et al, 2004)
2. To evaluate the *in vitro* and *in vivo* sensitivity of *V. verrucosa* and *A. noae* ChE to a widely applied OP trichlorfon (TCF), and to compare it with the data obtained for *M. galloprovincialis*.

Materials and methods:
Specimens of *A. noae* and *V. verrucosa* were collected from sites along the coast of north – eastern Adriatic. Mussels *M. galloprovincialis* were obtained from aquaculture farm. The animals were immediately dissected, the tissues frozen in liquid nitrogen and stored at – 80 °C. The sample homogenates for *in vitro* analyses were prepared from the pool of tissues extracted from five animals (five pools were used for analysis). Samples from individual mussels were used for *in vivo* analysis. The tissue was homogenised with ice-cold 0.1 M Tris-HCl buffer pH 7.4 (1:4 w/v) and centrifuged at 10000g for 30 min at +4 C. The supernatant was collected and used for subsequent analysis.

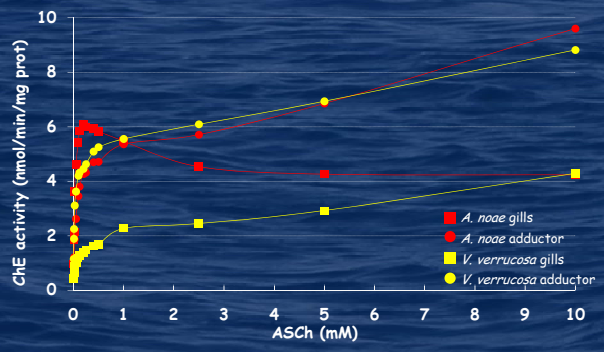
Determination of enzyme activity
ChE activity in the tissue homogenates was determined according to the method of Ellman (1961) adapted to microtitar plates (Bocquené and Galgani, 1998). ASCh concentration ranged from 0.005-10 mM for determination of enzyme activity with increasing substrate concentrations. *In vitro* and *in vivo* tests were performed using 1mM ASCh except for *A. noae* gills (0.2 mM). The enzymatic activity was expressed as nmol of hydrolysed substrate per min per mg of protein (specific activity). The protein concentration in the samples was determined by the method of Lowry (1951) using bovine serum albumin as standard.

***In vitro* effect of specific inhibitors and trichlorfon on ChE activity**
The sensitivity of tissue ChE was examined by incubation of samples with eserine as total inhibitor of all ChEs and 1,5-bis-(4-allyldimethyl-ammoniumphenyl)-pentan-3-one dibromide (BW284C51) considered as selective inhibitor of AChE in vertebrates. Inhibition reactions were performed at 22-24 C, for 30 min (Valbonesi et al, 2003).
***In vivo* effect of trichlorfon on ChE activity:**
Bivalve individuals (n=10) were exposed in tanks containing 30 l of seawater, to 0.4 and 1 mg/ml of trichlorfon for 24 hours. The concentrations of TCF selected for the study corresponded to those previously found to induce lethal and sub-lethal effects in other aquatic organisms of different trophic level (Coelho et al, 2011). Enzyme activity was determined in the gills. Samples were prepared and assayed as described above.

The data are presented as mean values (± SD). IC_{50} of trichlorfon was determined by probit analysis. Statistical comparison of experimental data was performed by Mann Whitney non-parametric test, with significance level of p<0.05 (*).

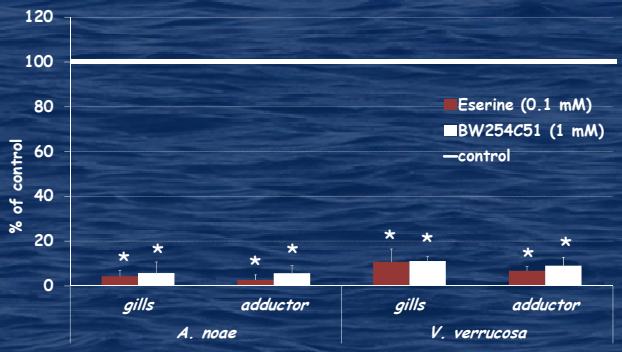
ChE characterisation

Specific ChE activity with ASCh as substrate



Two patterns of response to increasing ASCh concentration were observed:
- A "bell-shaped" curve showing substrate inhibition at concentrations > 0.2 mM (*A. noae* gills)
- No substrate inhibition at higher concentration of substrate (*A. noae* adductor, *V. verrucosa* gills and adductor)

ChE Activity following exposure to eserine and BW254C51

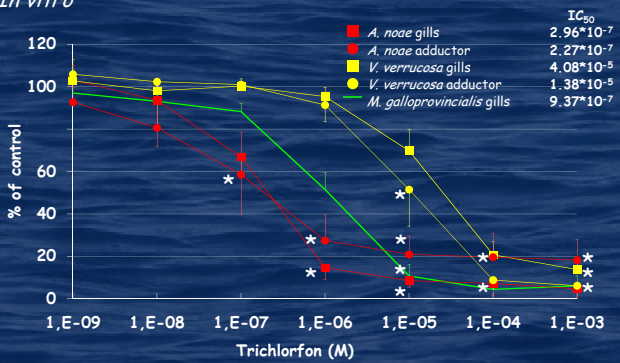


Significant reduction (up to 90%) of ChE activity was detected in both tissues of bivalves indicating:
- The absence of non-specific esterases that might hydrolyse ASCh
- The prevalence of ChE with preference for ASCh

* - significant difference from control (p<0.05)

Trichlorfon exposure

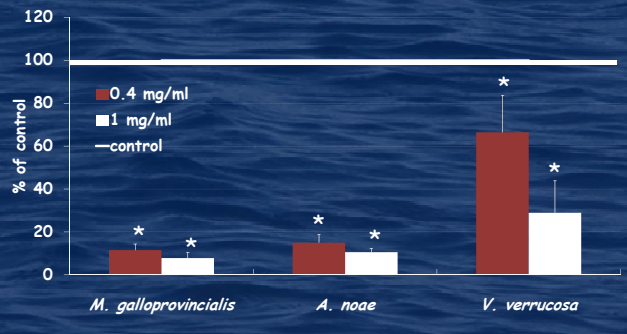
In vitro



In vitro exposure of bivalve species to trichlorfon resulted in a concentration-dependent inhibition of ChE. Values of IC_{50} increase in order *A. noae*<*M. galloprovincialis*<*V. verrucosa* indicating highest sensitivity of *A. noae*.

* - significant difference from control (p<0.05)

In vivo



ChE activity was significantly inhibited after 24-hour *in vivo* exposure to trichlorfon in all bivalve species. The inhibition was most pronounced in *A. noae* and *M. galloprovincialis* gills (> 80%) but no mortality was observed.

* - significant difference from control (p<0.05)

CONCLUSION: *A. noae* displayed the potential as indicator of exposure to OP's in marine environment, in particular within the areas not inhabited by other common bioindicator species such as mussels. In contrast, *V. verrucosa* seems less suitable for this purpose.

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