Bull. Environ. Contam. Toxicol. (2005) 74:250–255

DOI: 10.1007/s00128-004-0577-8

Bulletin of Environmental Contamination and Toxicology

Cholinesterase Activity in Clam *Meretrix casta*: Possible Biomarker for Organophosphate Pesticide Pollution

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Received: 30 June 2004/Accepted: 17 November 2004

Pesticides are used for increasing crop production, but increased usage causes hazardous effects to the environment and human health. In most developed countries, organophosphate (OP) use is restricted because of their toxic effects which results in high morbidity (Garcia et al. 2003). But in developing countries like India, OP usage is still highly prevalent (Balaram 2003). The toxicity of OP's is determined by the exposure level of the pesticide in the environment, the dose observed and by detecting the inhibited levels of choline esterases (ChE) like AChE and BChE when biomonitoring organisms (Hyne and Maher 2003). AChE is widely distributed among vertebrate and invertebrate animals (Bocquene et al. 1997) and inhibition of ChE activity is a specific biological indicator of exposure to agricultural pesticides. Sentinel bivalves like clams, mussels, and oysters are reported to possess AChE in different parts of their body (Kozlovskaya et al. 1993; Porte et al. 2001; Hyne and Maher 2003) and the inhibition of AChE activity has been successfully used as a tool to diagnose OP's and carbamate pesticides in these organisms in western countries. In contrast, only a few studies were conducted in the Asian region, particularly in India (Monirith et al. 2003, Pandey et al. 2003), in connection with marine pollution and biomarkers. Hence, the objectives of the present investigation are (1) to investigate the distribution of AChE in the tissue of the clam *Meretrix casta*, a widely distributed clam in southern parts of India and (2) to scrutinize whether inhibition of AChE by selected pesticides (like methyl parathion, dichlorvos and chlorpyrifos) in these clams could act as a biomarker for detecting pesticide pollution in the marine environment.

MATERIALS AND METHODS

Chemicals for enzyme analysis such as acetylcholine iodide, butyrylthiocholine iodide, 5,5'-DithioBis 92-nitrobenzoate (DTNB) were purchased from Sd Fine Chemicals Ltd and HiMedia Laboratories, India. The commercial grade pesticides, chloropyrifos (Chloroban, Excel Industries Limited, Mumbai, India), dichlorvos (Nuvan, Bharat Insecticides Ltd, Kolkatta, India) and methyl parathion (Metacid, Vikai Agro Chemical Pvt Ltd, India) containing 98.5%, 96% and 82% of the active ingredients respectively were purchased from the local marketplace.

The clams (50 numbers) were collected from back water of Nagatchi, near Uchippuli, Tamil Nadu, India and transported to the laboratory in an aerated tank.

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The samples were maintained in sea water under continuous aeration till analysis. The length and weight of the animals were 3.2 ± 0.29 cm and 2.5 ± 0.87g respectively. The shells of the clams were removed and the whole tissue was dissected, dried in a filter paper and homogenized (1:4 W/V) in ice cold 100mM phosphate buffer, pH 7.4 with a glass homogenizer. The homogenate was centrifuged at 10,000g for 15 min and the supernatants were collected and stored at -20°C till enzyme assay. The enzyme AChE was assayed in the clam by the method of Ellman et al. (1961). Protein was estimated by the method of Lowry et al. (1951).

For *in vitro* inhibition of AChE by pesticides, 400 µL of the tissue homogenates were incubated with five different concentrations of the pesticides (0.02 to 0.1 ppm for chlorpyrifos and 0.2 to 1 ppm for dichlorvos and methyl parathion) for 30 minutes. The levels of AChE were assayed after arresting the reaction with 1.1ml of Tris – HCl, pH 8.0. The concentration of the pesticides which inhibit 50% of AChE levels (IC_{50}) were calculated by Linear Interpolation Method (U.S.E.P.A, 1993)

The experiments were performed in triplicates and the values are presented as Mean ± SD. Statistical analysis was performed by Student's-*t*-test.

RESULTS AND DISCUSSION

The levels of AChE and BChE were evaluated in the whole tissue of the clam *Meretrix casta* using acetylcholine iodide and butyrylthiocholine as substrates. Both enzymes were present at very low levels or below the detectable levels in gills (<0.001 nmol/min/mg protein). Hence the enzymes were assayed in whole tissue of *Meretrix casta*. Similarly, these enzymes were detected at low levels in the gills, adductor muscle, digestive gland, mantle and hemolymph of the clam *T. philippinarum* (Valbonesi et al. 2003). Some organisms have variability in substrate preference for ChE. Normally, the highest AChE activity is observed with the substrate acetylcholine (Ach), whereas AChE of some organisms like the freshwater clam *Corbicula fluminea*, preferentially hydrolyze propionylthicholine iodide (Mora et al. 1999). In the present study both AChE and BChE were observed at detectable levels in whole tissue of *Meretrix casta* when detected with their normal substrates ACh and BCh (Fig 1)

Concerning clams, Hamza-Chaffai et al. (1998) observed inhibitory levels of AChE activity in *Ruditapes decussates* when exposed to xenobiotic compounds. In contrast, in another clam *Macoma balthica,* the organophosphate pesticide malathion had no effect on AChE activity (Lehtonen and Leino 2003). However, no extensive reports are available on the usage of clams as biomarker organisms for detecting AChE responses against pesticide pollution. Hence, the sensitivity of the widely prevalent clam *Meretrix casta* of southern parts of India was investigated for AChE inhibition by the organophosphate pesticides chlorpyrifos, methyl parathion and dichlorvos. AChE activity was measured in the whole tissue extracts, after incubating the tissue *in vitro* with several concentrations of the pesticides.

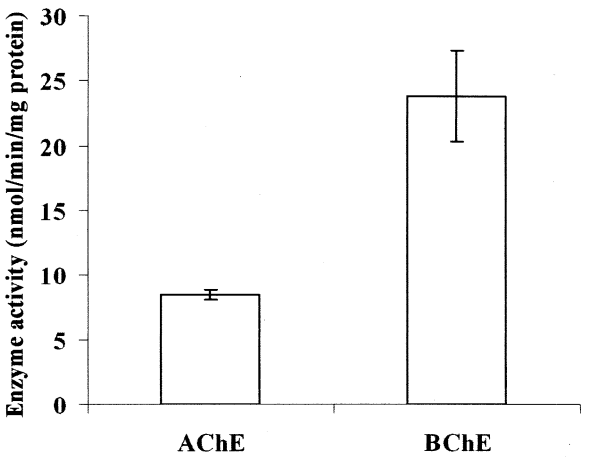

Figure 1. Levels of AChE and BChE in the unexposed clam *Meretrix casta*

Figure 2 (A, B and C) show the effects of the pesticides chlorpyrifos, methyl parathion and dichlorvos respectively on AChE activity from *Meretrix casta*. The IC_{50} for chlorpyrifos was found to be 0.0175 ppm (95% Confidence Limits: Lower 0.0168, Upper 0.0182), dichlorvos- 0.1745 ppm (95% Confidence Limits: Lower 0.1708 Upper 0.1791) and methyl parathion- 0.1958 ppm (95% Confidence Limits: Lower 0.1852 Upper 0.2495)

AChE was inhibited in the clams on exposure to all the pesticides. The inhibition was statistically significant when compared to the respective controls (Fig 2).The toxicity of common OP's like chlorpyrifos, dichlorvos and methyl parathion, involve cytochrome P450 mediated oxidative desulfuration to its oxon metabolites which bind to AChE and reduce its level. Reduced AChE levels prevent the hydrolysis of Ach which further results in paralysis and death (Pope et al. 1991). In the present study, the magnitude of AChE inhibition was greatest in chlorpyrifos.

The order of potency of AChE inhibition in the clam *Meretrix casta* was chlorpyrifos > dichlorvos > methyl parathion. Concerning invertebrates, Barata et al. (2004) reported that chlorpyrifos was almost three-orders of magnitude more toxic to the invertebrate *Daphnia magna,* than other OP's like malathion and carbofuran. Moore et al. (1998) also found that chlorpyrifos was about three-orders of magnitude more toxic to invertebrate organisms such as *Daphnia* and *Hyalella azteca*.

Significant levels of BChE were detected in tissue of the clam *Meretrix casta* (Fig1). Though the activity of AChE was inhibited by the pesticides, the role of BChE in hydrolysis of ACh cannot be ruled out. Since BChE could have a replacing role of AChE, further studies are being carried out concerning the biotransformation of pesticides by BChE.

Fig 2A

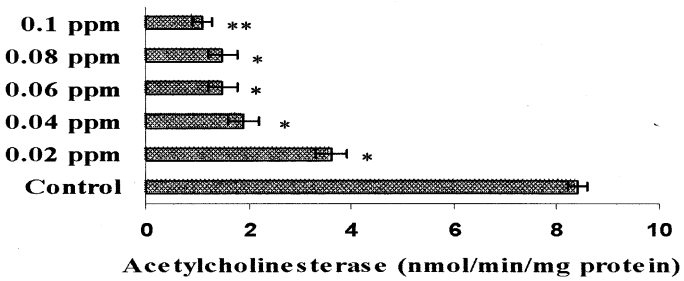

Fig 2B

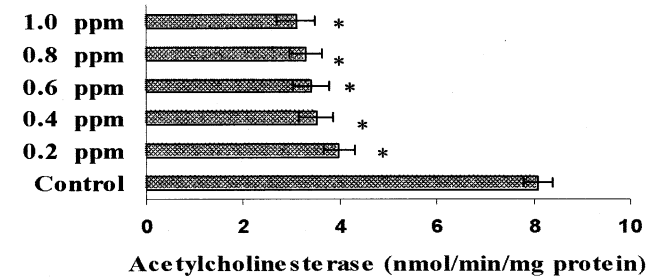

Fig 2C

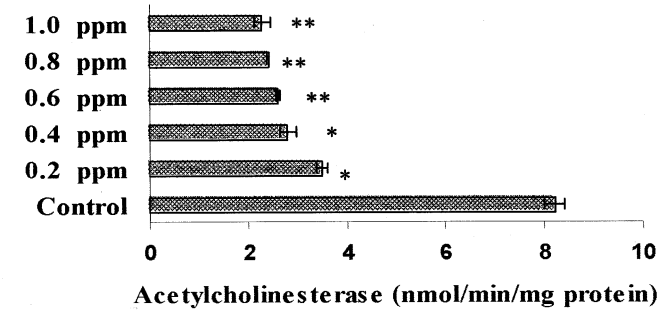

Figure 2. In vitro inhibition of AChE by chlorpyrifos (Fig 2A), methyl parathion (Fig 2B) and dichlorvos (Fig 2C) in the clam *Meretrix casta* (Values are expressed as Mean $\pm$ SD). Comparisons were made between the pesticide treated groups and control; *P<0.05, **P<0.02

To summarize, the results of the present study show that *Meretrix casta* could be used as a suitable bioindicator organism in the coastal area of Tamil Nadu, India, for the early detection of pesticide pollution.

Acknowledgments. The authors are indebted to Prof. T. Vasudevan, Department of Industrial Chemistry, Alagappa University, Karaikudi, India for granting permission to avail instrumental facilities.

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