L-II1

Effects of exposure to an organophosphorus pesticide on the ciliary activities of a marine bivalve

Victor AXIAK and Carmelo GALEA

Department of Science, The University of Malta, Msida (Malta)

The gills (ctenidia) of lamellibranch bivalves play a dominant role in controlling the interaction between the animal and its environment. However, not much is known about the responses of these bivalve organs to environmental stressors including chemical pollutants. Organophosphorus pesticides are known to be potent neurotoxins and may be expected to exert an effect on neurophysiological activities of such cilia. The present paper presents some preliminary results on the effects of exposure to MALATHIONE on the activities of the frontal cilia of Venus verrucosa.

Frontal ciliary activities were investigated using a procedure described in detail by Axiak and George (1987). Each bivalve had its left valve and mantle removed, allowed to recover for at least 2 h in running sea water and then introduced with its exposed half upwards, in a glass cubicle measuring 8 cm by 7cm and 6cm high and holding 300 ml of sea water maintained at 20°C at a salinity of 37 ppt and pH 8.4. Each preparation was observed under a binocular microscope at magnifications up to 75X. The activity of frontal cilia was investigated by measuring the rates of transport of Latex spheres, 8.06 µm in diameter, along the plical grooves of the inner demibranch by means of an eyepiece micrometer and a stopwatch. The mean rate of transport as measured in mm sec was calculated from a minimum of 10 observations at any one particular time. For any one animal, all observations and measurements were made on the same area of gill surface. In all cases, only particles unbound to mucus had their rates of transport measured. Measurements of particle transport were made for two hours prior to exposure and then 1, 2 and 3 hours after exposure. Animals were exposed to a nominal concentration of 4.75 ppm of MALATHIONE suspension in sea water.

Effects of exposure to MALATHIONE upon frontal ciliary activities as measured by rates of transport of particles along the frontal gill surface in half animal preparations are shown in the figure. The mean rate of frontal transport of eight preparations (ie. half animals) on exposure and in controls were calculated. The results are expressed as percentage of the rate of transport immediately prior to exposure for a particular animal. In the exposed preparations, the rate of transport decreased to 66 % of the initial values within 2 h of exposure. On the other hand, in the controls there was an increase in the rates of frontal particle transport up to the second hour. Such velocities were never found to be less than those reported prior to the water change at least up to 6 hours in the control runs.

T-test for paired comparisions indicated that the mean rate of frontal particle transport after 2 h of exposure was significantly lower than that immediately prior to exposure at $P < 0.05$ level of significance.

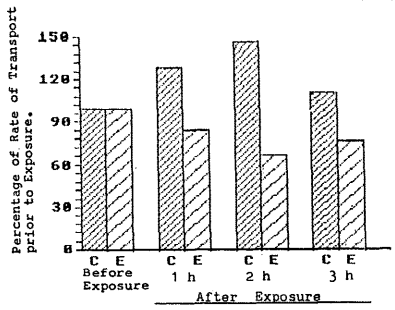

Figure 1. Venus verrucosa. Effects of exposure to 4.75 ppm of MALATHIONE on the frontal ciliary activities of the gills as shown by changes in the rates of transport of particles along their frontal surfaces. Results are expressed as percentage of the rate of transport immediately before exposure for a particular animal. Means of eight replicate runs for Exposed animals (E) and for controls (C).

The activities of frontal cilia of the gills of Venus verrucosa were found to be supressed on exposure to 4.75 ppm of MALATHIONE, as evident from decreased rates of particle transport across the gill's frontal surface. In a previous study (Axiak and George, 1987), the activities of these cilia were found to be enhanced on exposure to low levels of petroleum hydrocarbons.

Any decrease in the activity of the frontal cilia is expected to lead to a decrease in the rate of ingestion of food particles filtered by the gill of the exposed bivalve. The biological significance of this response is self evident especially if it is maintained over relatively long periods of time. Moreover, any altered ciliary activities may imply a direct or indirect effect of exposure to organophosphorus pesticides on the nervous control and/or on some other aspect of the physiological and/or biochemical processes linked with ciliary movement (e.g. on membrane bound factors responsable for ciliary membrane polarization, ATP production, etc.).

As yet, such a biological response has only been detected on exposure to relatively high levels of organophosphorus pesticides. Further investigations on effects of exposure to lower levels of of such contaminants, is in progress.

Reference:

AXIAK, V., and GEORGE, J.J. (1987) Effects of exposure to petroleum hydrocarbons on the gill functions and ciliary activities of a marine bivalve. Mar. Biol. 94, 241-249.

Acknowledgements

This work is part of a research project on the effects of organophosphorus pesticides on marine invertebrates within the framework of the MEDPOL Long-term Research and Monitoring Programme.

L-II2

Inhibition of Sardine liver esterases by organophosphate and carbamate pesticides in vitro

M. KRAJNOVIC-OZRETIC and B. OZRETIC

Center for Marine Research, Rudjer Boskovic Institute, 52210 Rovinj (Yugoslavia)

To assess the potential hazard of organophosphate and carbamate pesticides on fish, the inhibitory effects on liver esterases of sardine (Sardina pilchardus Walb.) have been investigated in vitro.

Esterases belong to a very complex and polymorphic group of species and tissue specific hydrolizing enzymes. They are highly involved in organophosphate and carbamate intoxication, but little efforts have been spent on the assessment of the differential inhibition of their multiple molecular forms (2).

The methods for tissue extraction and electrophoretic separation of esterases by starch gel have been previously described (1). After separation of water soluble extracts the liver esterases of adriatic sardine were found to be multilocus variable. At least five esterase zones were identified (ES-I; ES-II^1; ES-II^2; ES-III; ES-IV) and among them only the ES-IV zone was monomorphic (Fig. 1). The other consisted of one or more bands that were distinguishable due to their electrophoretic mobility, substrate specificity and sensitivity to various inhibitors. After electrophoresis, gels were split into several horizontal slices and each one was incubated for 30 min. with different concentrations of pesticides. The slices were than stained with 1- and 2-naphtyl acetate and the intensity of the bands were compared to a control zymogram. Results are summarized in Table 1.

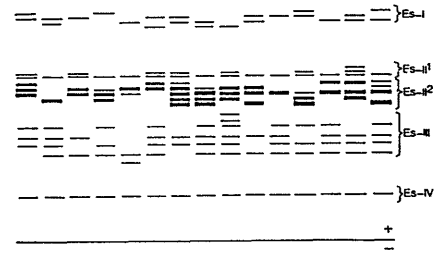

Figure 1. Esterase patterns in liver extracts from Adriatic sardine. The five zones of esterase bands are indicated.

Paraoxon, in the range of 10^{-3} to 10^{-6} M, was the most effective inhibitor to all liver esterases. On the contrary phosalone, a frequently used insecticide, did not show inhibitory effects on any of the esterase fractions. The other pesticides exibited different inhibition patterns, specific to various esterase fractions and dependent to the concentration. Esterases from the ES-I fraction were particularly sensitive to dichlorvos and carbaryl, while only the highest concentrations of malathion and guthion exibited evident inhibitory effects. This esterase fraction was not affected by baygon. The ES-II^1 fraction was inhibited by the highest concentrations of dichlorvos, bromphos, carbaryl and baygon but no effects were displayed by phosalone, malathion and guthion. The ES-II^2 and ES-IV esterases were the most resistant. The former were slightly affected only by the highest concentrations of bromphos, guthion and baygon, and similarly ES-IV were completely inhibited only by the highest adopted concentrations of dichlorvos and baygon and slightly depressed by guthion. On the contrary the ES-II^1 esterases were the most sensitive and, with the exception of phosalone, they were substantially affected by all pesticides, particularly by dichlorvos, malathion, carbaryl and baygon.

Table 1. Esterase activity in liver extracts from Adriatic sardine and the inhibitory effects* of various pesticides.

Pesticide	Conc. (M)	ES-I	ES-II^1	ES-II^2	ES-III	ES-IV
Control	-	+++	++	+++	++	+++
Dichlorvos	10^{-3}	-	-	+++	-	-
	10^{-4}	-	-	+++	-	+++
	10^{-5}	-	+	+++	-	+++
	10^{-6}	-	++	+++	-	+++
Bromphos	10^{-3}	-	-	++	+/-	+++
	10^{-4}	+	+	+++	+/-	+++
	10^{-5}	+++	++	+++	+/-	+++
	10^{-6}	+++	++	+++	+/-	+++
Phosalone	10^{-3}	+++	++	+++	++	+++
	10^{-4}	+++	++	+++	++	+++
	10^{-5}	+++	++	+++	++	+++
	10^{-6}	+++	++	+++	++	+++
Para-Oxon	10^{-3}	-	-	-	-	-
	10^{-4}	-	-	-	-	-
	10^{-5}	-	-	-	-	-
	10^{-6}	-	-	+	+/-	-
Malathion	10^{-3}	-	++	+++	-	+++
	10^{-4}	-	++	+++	-	+++
	10^{-5}	+	++	+++	-	+++
	10^{-6}	++	++	+++	+	+++
Guthion	10^{-3}	-	++	+/-	+	++
	10^{-4}	-	++	+	+	++
	10^{-5}	++	++	++	++	+++
	10^{-6}	+++	++	+++	++	+++
Carbaryl	10^{-3}	-	-	+++	-	+++
	10^{-4}	-	+/-	+++	-	+++
	10^{-5}	-	+	+++	-	+++
Baygon	10^{-3}	+++	-	+/-	-	-
	10^{-4}	+++	+/-	++	+/-	+
	10^{-5}	+++	+	+++	+/-	++
	10^{-6}	+++	++	+++	+/-	++

* The inhibitory effects were scored according to the control zymogram as follows: (+++ and ++) no inhibition; (++ and +) slight; (+/-) low and (-) complete inhibition.

The obtained results demonstrated that the inhibition of esterase isozymes by organophosphate and carbamate pesticides is more complex than it was expected and additional information are requested.

1. Krajnović-Ozretić M., R. Žikić (1975). Esterase polymorphism in the Adriatic sardine (Sardina pilchardus Walb).1. Electrophoretic and biochemical properties of the serum and tissue esterases. Anim. Blood Grps Biochem Genet. 6, 201-213.
2. Krajnović-Ozretić M., W. de Ligny (1978) Effects of organophosphate pesticides on fish esterases: a case for an isozyme approach. In Marine Animals, B. Battaglia and J. Beardmore Eds. Plenum Publishing Corporation pp 667-678.

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