Bull. Environ. Contam. Toxicol. (1988) 41:597–603

Environmental Contamination and Toxicology

In vivo and *in vitro* Effect of Triclorfon on Esterases of the Red Crayfish *Procambarus clarkii*

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The red American crayfish Procambarus clarkii (Girard, 1852), from Louisiana, (USA), was introduced into the marshes of the Guadalquivir river (south of Spain) in 1974 and into the inland marine lake of Valencia (east of Spain) in 1979. This decapod crustacean has adapted perfectly to both habitats, so that its capture has meant a new source of riches in both zones.

Great expansion and excessive proliferation of the red crayfish has had tremendous ecological impact on the ricefields. The evident damage to the crops has caused some farmers to apply very high concentrations of the pesticides normally used in the ricefields, in an attempt to eliminate them.

O,O-dimethyl(1-hydroxy 2,2,2-trichloroethyl)phosphonate (TCF) is one of the most used compounds. It is an organophosphorous pesticide quite soluble (14%) and moderately toxic. Although the clinical symptoms in poisoned humans are easily reversible when produced by low dosage, it is necessary to take into account, that in normal condition of use it is very quickly hydrolysed to form diclorvos (O,O-dimethyl 2,2-dichloro-vinyl phosphate) which is much more toxic.

Organophosphorous compounds in general produce a specific inhibition of acetylcholinesterase, which in some cases is accompanied by the inhibition of NTE (neuro target esterase). The modification of the activity of NTE (esterase activity at pH 8) is responsible for the apparition of the syndrome of delayed neurotoxicity induced by some organophosphorous compounds (Johnson 1982). Although we have not found any reference to its effect on hens, Triclorfon, due to its chemical phosphonate structure, is a potential producer of delayed neurotoxicity (Johnson 1975).

The absence of data in the literature on the effect of

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TCF and its metabolites in crayfish, led us to set up an in vivo and in vitro study. The purpose of our study was to establish the lethal concentration of TCF at 96 h and to initiate a biochemical study of the extent of its effect on muscle and hepatopancreas. This required the setting up of the method of detection of NTE in muscular tissue of crayfish. We also measured the activity of AChE under the effect of different concentrations of TCF and exposure times. At the same time, the modification of some energy-transfer mechanisms, were analysed , by assessing the evolution of the reserves of glycogen in muscle and hepatopancreas.

MATERIAL AND METHODS

100 adult intermoult red crayfish of both sexes from the Guadalquivir marshes, weighing 21.8 ± 3.4 g, were used. They were kept in quarantine for five weeks in aquaria with constantly aerated and filtered tap water, and fed with commercial meat for dogs.

The in vivo tests were carried out individually in 1 L glass aquaria maintained at 20 ± 0.1º C, with constant aeration, photoperiod: 12:12 L:D after 36 h of previous acclimatization. The exposure was semistatic, with daily renewal of the test solution. This test solution was prepared daily from a stock solution of TCF in water (20 mg/ml, TCF solubility in water 15.4 10 µg/ml)

The lethal concentration of TCF at 96 h was established by observing the rate of survival of crayfish on exposure to different concentrations of the pesticide.

The in vitro tests were carried out by incubation for 10 min. at 37º C of 0.4 mL of tissue homogenates in phosphate buffer 0.1 M at pH 8.0 with 10 µL of 10, 7, 3, 1 and 0.1 mM TCF solutions.

Enzyme activities were measured in whole muscle homogenate in phosphate buffer 0.1 M pH 8.0.

The activity of AChE was measured according to Ellman et al (1961) as µ moles of acetylcholine hydrolysed/min/mg protein. Glycogen (µg/mg of tissue) was determined by the Seifter et al (1950) method modified by Seay and Rosenkrantz (1965). The Johnson method was used to assay NTE which was estimated in µ moles of phenol/mg protein/min. The protein content of the extracts was established by the Lowry et al (1951) method.

The statistic significance of the data was estimated by means of a Student t test for $p < 0.05$.

RESULTS AND DISCUSSION

As can be deduced from figures 1 and 2, the results of the evaluation of the lethal effect of TCF on the red crayfish indicated the low toxicity of this substance in this animal species.

The approximate LC 50 concentration of TCF on Procambarus clarkii at 96 h of exposure was 5 mg/L at 20º C.

Once the LC 50 was established, the effect on muscular cholinesterase, muscular glycogen and hepatic glycogen of different concentrations of TCF at 24 hours exposure was studied. The results of these experiments appear in figures 3 and 4. In this acute phase an increase in AChE was observed, followed by progressive reduction as the concentration of TCF in the environment increased. A narrow concentration-response relationship existed at over 0.1 mg/L. At doses below those which produced the increase in activity of AChE (1 mg/L) an increase in glycogen was observed in hepatopancreas accompanied by a decrease in muscular reserves. At higher doses the hepatic glycogen remained slightly above normal values, and the muscular glycogen dropped gradually below basal values.

The effects observed with low concentrations of TCF (0.1 mg/L) were also studied as they evolved through time. The results of this study appear in figures 5 and 6. The activity of AChE (Fig. 5) increased slowly with an abrupt decrease at 4 days and recuperation later. This strange behaviour requires futher investigation. The glycogen in hepatopancreas maintained the increase observed at 24 hours whilst muscular glycogen suffered an abrupt fall as from the 5th day of exposure (fig. 6). It can be deduced that concentrations higher than 0.1 mg/L or exposure times of 3 or 4 days at lower concentrations are necessary for the toxic effects of TCF to appear (inhibition of AChE and decrease of muscular glycogen without significant mobilization of hepatic reserves). With sublethal concentrations the activity of AChE was rapidly recuperated.

Initially (first 24 hours) the hepatic and muscular glycogen behaved in exactly the opposite way; strong decrease in muscle accompanied by an increase in hepatopancreas. One could postulate that a reduction of glycolisis took place in hepatopancreas with an increase in the synthesis of glycogen which it would be necessary to investigate.

The evolution of the activity of NTE in function of

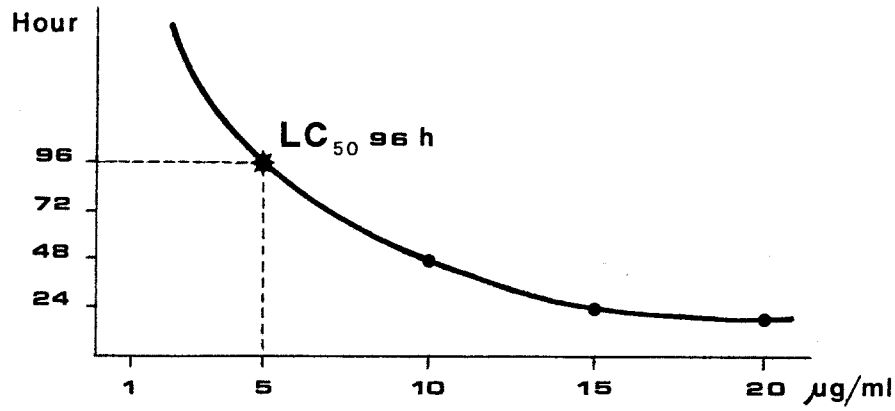

Figure 1.- Survival time of crayfish at different concentrations of Trichlorfon.

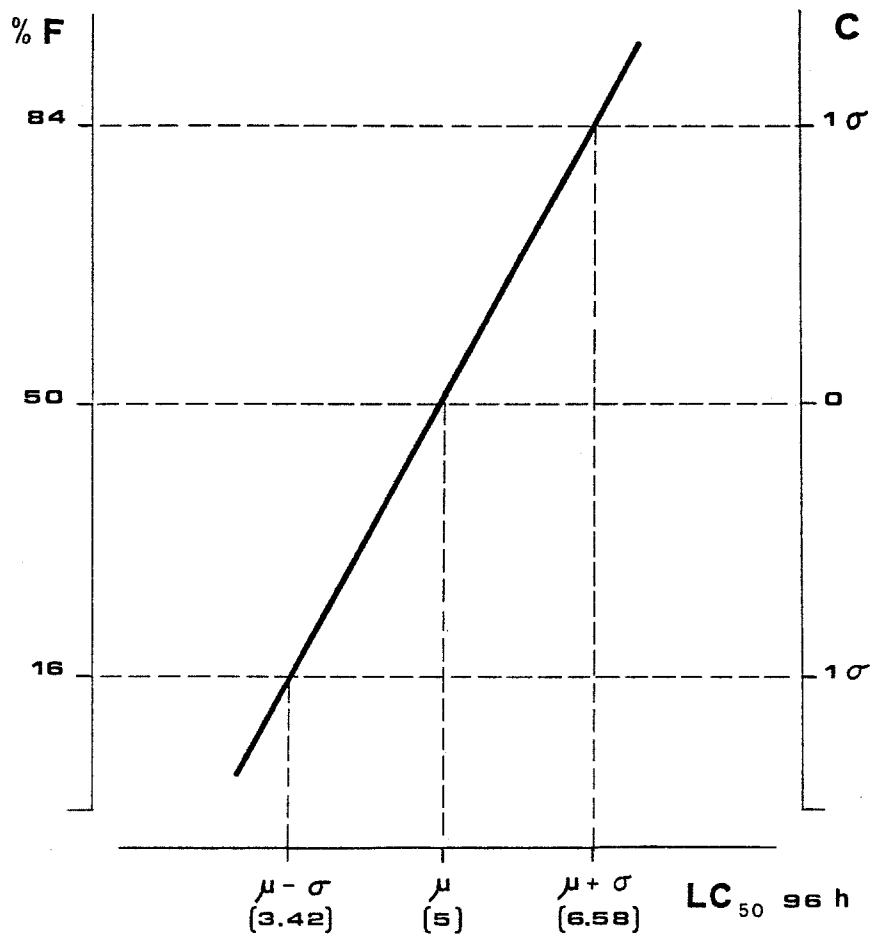

Figure 2.- Log probit curve of LC_{50} at 96 hours of Trichlorfon.

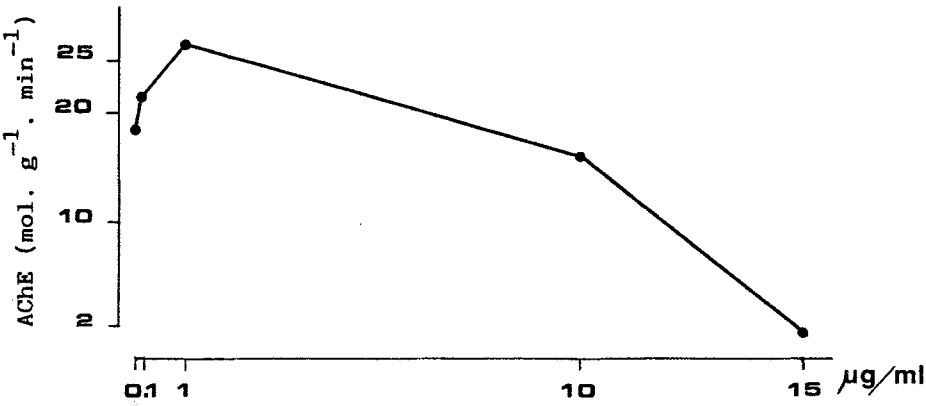

Figure 3.- AChE activity in crayfish muscle after 24 h of exposition to different concentrations of Trichlorfon.

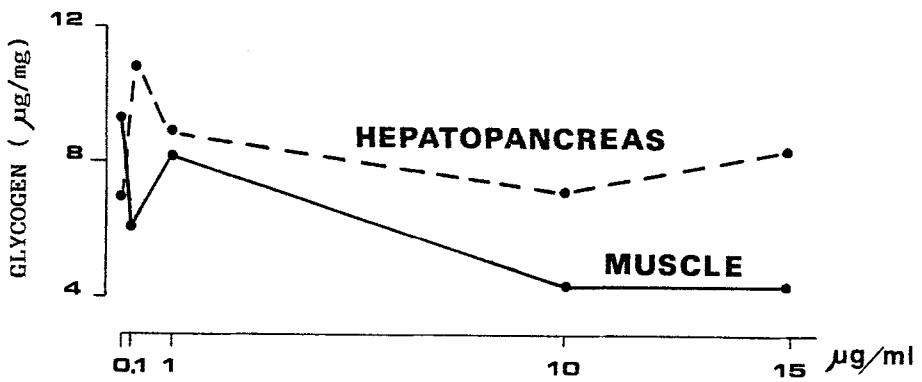

Figure 4.- Glycogen content in muscle and hepatopancreas of crayfish after 24 h of exposition to Trichlorfon.

time and at low concentration of TCF (0.1 mg/L) was also determined in muscle.

We consider the detection of NTE activity in muscular tissue of great interest. It widens the possibilities of study of NTE activity to other tissues besides the nervous system, where it has always been determined up to now.

At the same time the finding of a possibly more ubiquitous activity of NTE introduces a new point of view which could help to elucidate its biological function, at present unknown, In figure **7** we observe that the activity of NTE was inhibited as the time of exposure to TCF progressed, thus showing the typical

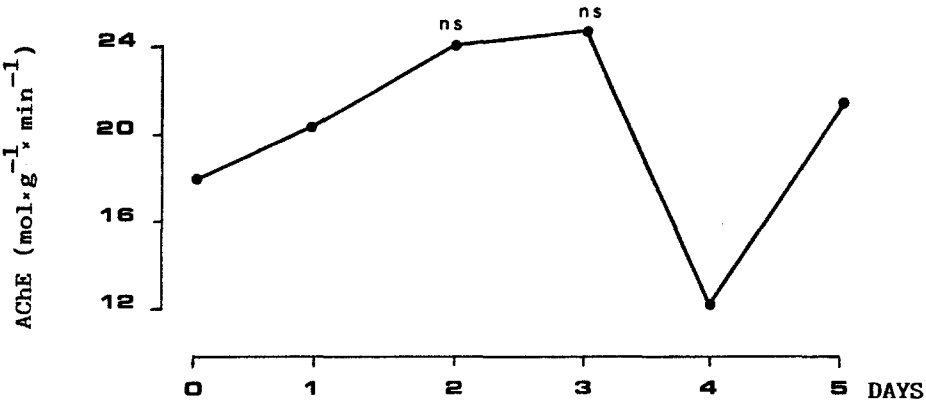

Figure 5.- Effects of daily exposure to 0.1 µg/ml Trichlorfon on AChE activity in crayfish muscle.

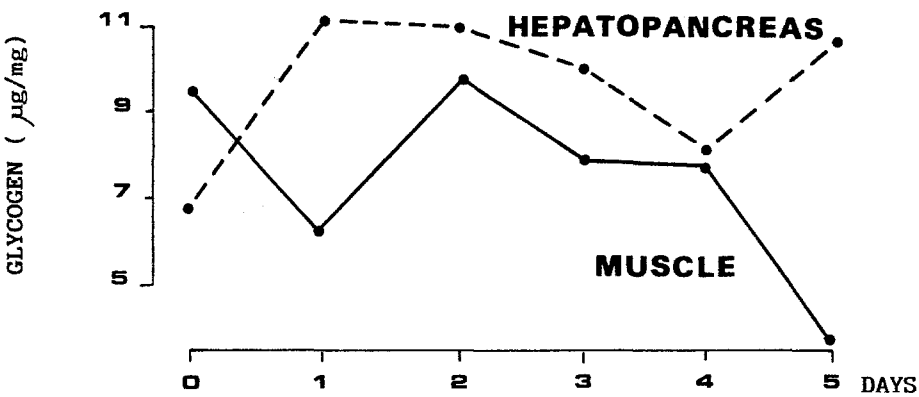

Figure 6.- Effects of daily exposure to 0.1 µg/ml Trichlorfon on glycogen in crayfish muscle and hepatopancreas.

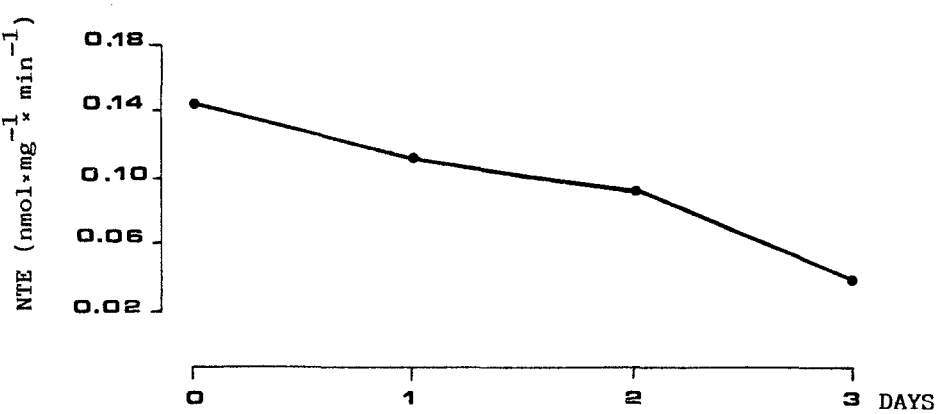

Figure 7.- Effects of daily exposure to 0.1 µg/ml Trichlorfon on NTE activity in crayfish muscle.

effect of some organophosphates. The question still stands as to whether or not this inhibition is produced irreversibly, which would settle whether it is a delayed neuropathy or no.

Acknowledgments. We thank S.A. Owens and I. Flores for their colaboration in the preparation of this manuscript.

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Received February 23, 1988; accepted March 18, 1988.