THE EFFECT OF TWO SULPHUR-CONTAINING PESTICIDES, FENITROTHION AND ENDOSULFAN, ON GLUTATHIONE (GSH) CONTENT AND ON GSH S-TRANSFERASE AND γ-GLUTAMYL TRANSPEPTIDASE ACTIVITIES IN MIDGUT GLAND OF THE AMERICAN RED CRAYFISH *PROCAMBARUS CLARKII*

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SUMMARY

The glutathione (GSH) system of *Procambarus clarkii* (P.c.), the American red crayfish, is used as a marker of the effects of Fenitrothion (FT) and Endosulfan (ES), organophosphorus and organochlorinated insecticides, respectively. This system has been shown to be sensitive to different heavy metals poisoning, thus it has a double interest as marker for environmental contamination and as a potential source of xenobiotics or their metabolites to humans, since it is being fished commercially. The GSH content of the organ decreased 24h after treatment with FT. FT promotes a 2-fold induction of GSH S-transferase (GST) activity at 6 h which is followed by a decrease of it at 24 h. The latter coincides with the decrease in GSH content. GST activity increases again over the control values at 48 h. Only the initial increase in GST activity is coupled with an increase in γ-glutamyl transpeptidase (transpeptidase activity) (γ-GT) activity. ES promoted a discrete increase in GSH concentration at the same time as a 2-fold increase in GST activity, again coupled with an increase in γ-GT activity. The GST induction observed at 6 h with FT and 24 h with ES showed concentration dependency up to ¼ of the reported LC_{50} of both pesticides. Higher concentrations showed no further effects on GST activity. The coupled control of the expression of GST and γ-GT in the hepatopancreas of P.c. is discussed.

I. INTRODUCTION

The sulphur-containing pesticides are widely used in agricultural and public health practices, thus becoming an increasing ecological problem. Their use has steadily increased and in view of this, exposure to trace levels of organophosphorus and chlorinated hydrocarbon insecticides is inevitable: their residues are found in potable water /1/ and food /2/.

The Albufera lake in Spain and the surrounding rice fields represent a unique situation since, due to geographical characteristics of the lake, agricultural production and fisheries interact with each other; hence, the interest in a better understanding of the biochemical and physiological processes of bioprotection of organisms against biotic/abiotic stresses. Several biochemical measurements have been

suggested as representative indices of organism self-protection, i.e. activity of enzymes biotransforming xenobiotics (glutathione S-transferases, cytochrome P-450, epoxide hydrolase), or the mechanism by which some of these xenobiotics induce the drug metabolizing enzymes (such as pesticides, PCBs, aromatic compounds) /3,4/.

The american red crayfish, *Procambarus clarkii* (P.c.), appeared in 1978 in lake Albufera and in the surrounding rice fields. This is now a widespread species and has environmental and economical implications. Hence this species fulfills all the criteria suggested by the Committee on Methods for Toxicity Tests with Aquatic Organisms /5/. P.c. participates in food chains and is a human source of xenobiotics, since it is being fished commercially for human consumption. Therefore there exists a double interest in P.c. as a species for environmental analysis; firstly as a biocoenose accumulating pollutants, i.e. as marker of the environmental situation, and secondly, as a source of toxicants to humans. Moreover, the metabolic modifications of the different contaminants may expose humans to even more toxic metabolites.

Glutathione (L-γ-glutamyl-L-cisteinyl-glycine; GSH) is widely distributed in living cells /6/ and is an important component in a number of enzyme reactions, either as a substrate or as a coenzyme /7/. Since GSH is a substrate for the glutathione S-transferase system, the concentration of tripeptide may become rate-limiting when an organism is exposed to a large amount of xenobiotic /8/.

Glutathione S-transferase (GST)-mediated metabolism of organophosphorus insecticides represents a major detoxification pathway *in vivo* in different animals /9-11/. The GST enzymes mediate also the biotransformation of halogenated organic compounds /12-14/, pyrethroid insecticides /15/, and carbamates /16/. An operating glutathione system (GSH content and GSH-related enzymatic activities) has been partially characterized in different organs of P.c. /17/.

The crustacean hepatopancreas (also called midgut gland) has many of the functions of the vertebrate liver and pancreas. These include synthesis and secretion of digestive enzymes, uptake of nutrients, and accumulation of nutrient reserves /18/. The midgut gland has shown also the capacity to metabolize *in vitro* organophosphorus pesticides such as fenitrothion /19/.

Glutathione S-transferase activity in the hepatopancreas of P.c.

has been reported to be sensitive to environmental pollutants such as cadmium /20,21/ and mercury /22/.

The objective of the present study was to examine the effects of experimental exposure of the crayfish to different sublethal concentrations of an organophosphorus, fenitrothion [O,O-dimethyl-O-(4-nitro-*m*-tolyl)phosphorothioate; FT], and an organochlorinated, endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6, 9-methano-2,4,3-benzodioxathiopin 3-oxide; ES) insecticide, both containing a sulphur atom (see Fig. 1), on the glutathione content and glutathione S-transferase and γ-glutamyl transpeptidase (γ-GT) activities of the midgut gland. The latter activity is important, firstly because it is the only enzyme that can attack the γ-glutamyl bond of GSH and therefore degrade it, and secondly because some effect of environmental mercury has been reported on it (22).

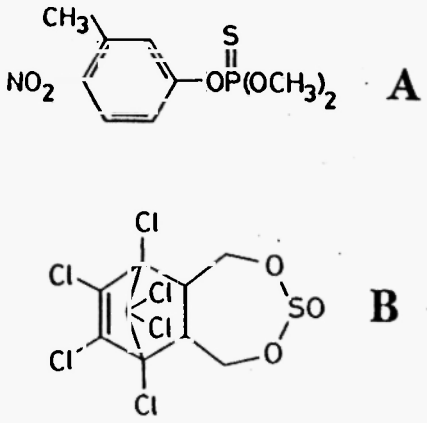

Fig. 1: Chemical structure of Fenitrothion (A) and Endosulfan (B).

II. MATERIALS AND METHODS

Adult intermolt specimens of the crayfish P.c. (Girard, 1852) were collected in the Albufera Lake (Valencia, Spain), transferred for acclimatation into 300 l tanks for 15 days, and maintained at 22°C, in tap water with constant air bubbling /20/. The physico-chemical

parameters of tap water used for the aquaria have been described elsewhere /20/. Control and treated animals were fed a standard diet (pork liver) *ad libitum* and sacrificed 48 h after food-relief in all cases. The LC_{50} of both pesticides for P.c., at this temperature, have been previously reported as 9.8 μg for FT and 0.12 mg for ES per liter surrounding water in both cases /23/. Endosulfan was dissolved initially in acetone due to its low solubility and the necessary amount of this stock solution to attain the desired concentration was added. No effect of the amount of solvent added was observed.

The midgut glands were removed as in ref /20/, by cutting around the periphery of the cephalothorax, and homogenized partly in 0.1 M potassium phosphate buffer, pH 7.0, for protein and enzymatic activities determinations, and in ice-cold perchloric acid (2%), containing 1 mM EDTA for GSH determination.

GSH was determined with 1-chloro-2,4-dinitrobenzene (CDNB) and glutathione S-transferase /24/. GSH S-transferase activity was determined at 25°C with CDNB as substrate according to ref /25/. The transpeptidase activity of γ-glutamyl transpeptidase was determined at 37°C with L-γ-glutamyl-p-nitroanilide and glycylglycine as substrates /26/. Protein was determined by the method of Lowry et al. /27/.

All chemicals were from Merck (Darmstadt, FRG); biochemicals and GSH S-transferase from Sigma Chemie GmbH (Deisenhofen, FRG) and Boehringer (Mannheim, FRG).

The existence of statistically significant differences was established by means of the "Student's t" test.

III. RESULTS

Figure 2 shows the time course of the effect of 2.45 g/l of FT and the effect of 30 g/l of ES, in both cases ¼ of the reported LC_{50} for P.c. /23/, on the GST activity of midgut gland of P.c. ES promoted a 2-fold increase of the activity at 24 h after treatment, whereas FT had a similar effect after 6 h. This initial effect of FT was followed by a decrease in GST activity, which was minimal at 24 h (50% of control activity) before rising again above the initial value at 48 h.

Table 1 shows the time course of GSH content and γ-glutamyl

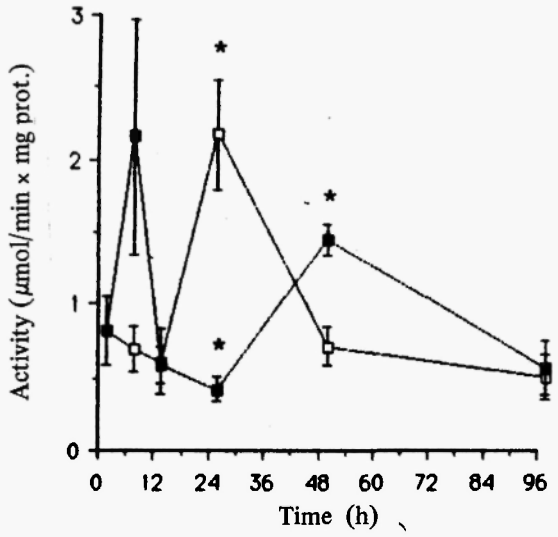

Fig. 2: Time course of the GST activity of hepatopancreas of P.c. in the presence of 2.45 μg/l of FT (■) or 30 μg/l of ES (□). *p < 0.005.

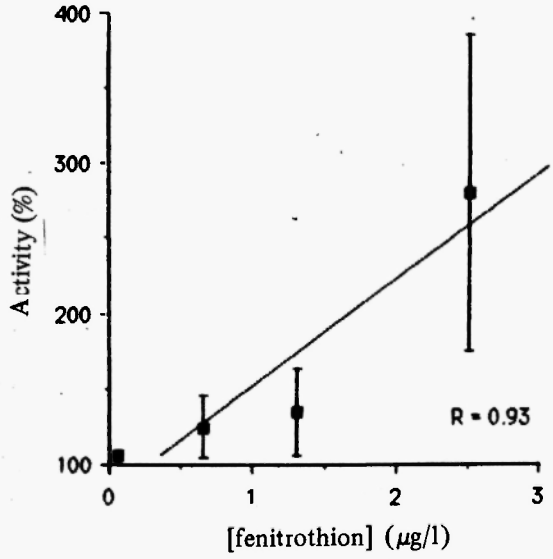

Fig. 3: Effect of the concentration of FT on the GST activity in hepatopancreas of P.c. (6 h after starting the treatment). Control values (100%) for GST activity: 761.71 ± 233.45, N = 38 (nmol/min x mg protein). All the data are means ± S.D. of at least 6 replicates. R: linear correlation coefficient.

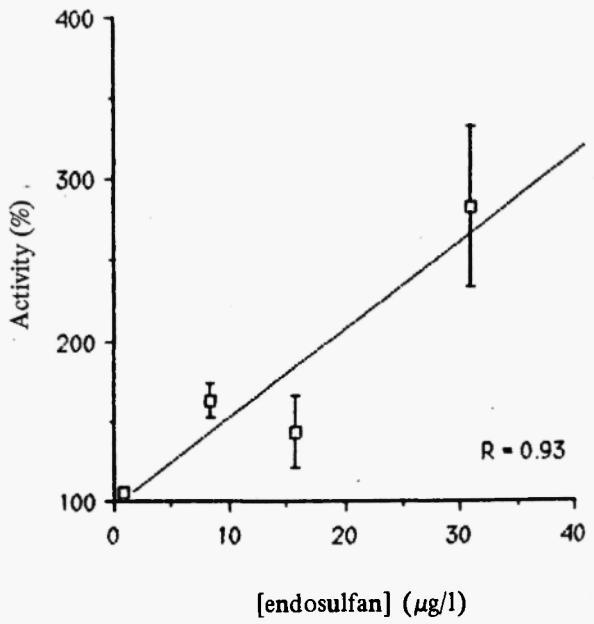

Fig. 4: Effect of the concentration of ES on the GST activity in hepatopancreas of P.c. (24 h after starting the treatment). Control values (100%) for GST activity: 761.71 ± 233.45, N = 38 (nmol/min x mg protein). All the data are means ± S.D. of at least 6 replicates. R: linear correlation coefficient.

transpeptidase activity in midgut gland of P.c. when treated as in Fig. 2. FT decreased GSH concentration 24 h after treatment without affecting γ-GT activity, whereas ES promoted a small increase in GSH concentration and γ-GT activity.

Figure 3 shows the concentration effect of GST activity in the presence of FT and Figure 4 in the presence of ES. A linear correlation could be established ($r = 0.93$ in both cases; see Figures) up to ¼ of the LC_{50} (2.45 and 30μg/l, respectively). A higher dose (½ of LC_{50}) promoted no further increase (data not shown).

Interestingly, a coupled increase in GST and γ-GT activities can be observed for FT 6 h after treatment, which also appears for ES after 24 h. The decrease in GST activity observed with FT at 24 h is associated with a significant ($p \leq 0.05$) decrease of GSH concentration (see Table 1).

TABLE 1

Time course of glutathione (GSH) concentration and γ-glutamyl transpeptidase (γ-GT) in midgut gland of *Procambarus clarkii* (P.c.) treated with 2.45μg/l Fenitrothion or 30 μg/l Endosulfan.

	Fenitrothion (FT)		Endosulfan (ES)	
Time (h)	GSH concentration (nmol/mg protein)	γ-GT activity (nmol/min x mg prot)	GSH concentration (nmol/mg protein)	γ-GT activity (nmol/min x mg prot)
6	15.62±0.60 (4)	30.96±4.68 (5)*	17.99±1.80 (4)	20.35±2.24 (5)
12	14.88±2.30 (5)	24.62±4.72 (5)	21.83±4.39 (5)	20.83±2.79 (6)
24	12.57±2.09 (8)	24.22±5.26 (5)	25.21±6.99 (3)	31.90±7.44 (7)
48	17.18±2.72 (5)	22.51±3.30 (6)	23.46±2.62 (5)	20.83±2.38 (6)
96	13.45±2.45 (5)	25.77±2.67 (5)	20.60±2.88 (4)	17.43±3.48 (8)

Results are expressed as means ± S.D. with the number of observation.
Control values for GSH concentration: 17.99±5.49 N=38 (nmol/mg protein) and γ-GT activity 22.33±3.83 N=8 (nmol/min x mg protein). *$p \leq 0.005$

IV. DISCUSSION

The delayed effects of FT and ES, might be explained by their different lipid solubility /28/. FT, as less lipid soluble, might interact directly with GST, whereas ES or its metabolites might dissolve in the lipid phase for a somewhat longer period until it reaches the necessary concentration to promote the induction.

As mentioned above, no significant modification was observed in GSH concentration with both pesticides. This is believable since, in the case of a direct bimolecular reaction between ES or FT with GSH, the decrease in GSH concentration would be less than 1%, which would not be detectable. Moreover, GSTs of midgut gland of P.c. are, as other well characterized GSTs, thiol-dependent enzymes (Romero and Almar, in preparation). Thus, in the case of a direct interaction of these sulphur-containing substances with GST, it would lead to a covalent inactivation of the latter in a concentration-dependent fashion, and in any case to the induction observed. This interaction has been shown to happen when the cytosolic GST of P.c. is incubated in the presence of Hg^{++} /22/ but not with FT or ES (data not shown).

A coupled increase of γ-GT and GST was observed with both pesticides at different times, though only significant enough in the case of FT (for ES only $p < 0.05$). So it is proposed in this report the hypothesis of nuclear sensitive receptors responsible for these coupled effects (increase of GST and of γ-GT). In order to clarify the mechanisms of enzyme induction in this organ, the concentration dependency of these effects was studied (Fig. 3 and 4). It could be established that GST induction proceeds in a linear manner depending on the xenobiotic concentration only up to ¼ of the LC_{50}, whereas this effect could not be observed for γ-GT. These facts allow the proposal of the existence of different receptors controlling the expression of both protein families independently. Whether the isozyme/s induced here are different is under current investigation in our laboratory, as well as the recognition of the isozyme/s induced 6 and 48h after FT treatment.

The clarification of all these aspects of the glutathione system in the midgut gland of P.c. may contribute to a better understanding of the phenomena implicated in the toxicity of these xenobiotics.

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