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Inhibition of Glutathione *S*-Transferase Catalyzed Xenobiotic Detoxication by Organotin Compounds in Tropical Marine Fish Tissues

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Organotin compounds, tributyltin (TBT), triphenyltin (TPT) and dibutyltin (DBT) have been extensively used in industry as plastic stabilizers and catalysts, wood and paper preservatives, agricultural pesticides and antifouling agents in paints applied for ships, boats and aquaculture nets (WHO 1990; Morcillo *et al.* 1997). Aquatic pollution resulting from their usage has been of great concern due to their bioaccumulation potential, persistence in sediment up to several years and highly toxic effects on marine organisms including sea mammals at very low concentrations (WHO 1990; Morcillo *et al.* 1997; Kannan *et al.* 1997; Takahashi *et al.* 1997). However, there is a gap in our knowledge regarding the mechanism of action of organotins and their possible interactions with other xenobiotics at the cellular and molecular levels.

Glutathione *S*-transferases (GST) catalyze detoxication of a wide variety of electrophilic xenobiotics and/or their metabolites generated during oxidative metabolism by microsomal cytochrome P450-dependent monooxygenases in hepatic and extrahepatic tissues of fish and other organisms (Mannervik *et al.* 1985; Jimenez and Stegeman 1990; Gallagher and Di Giulio 1992; Al-Ghais and Ali 1995; Al-Ghais 1997). Since xenobiotic metabolizing enzymes exist in multiple forms that exhibit broad substrate specificity and respond rapidly to physiological and environmental stresses, they are potential sites of xenobiotic interactions. Studies conducted in various types of aquatic environment and on different fish species have illustrated the usefulness of detoxication enzymes including GST as molecular biomarkers of chemical pollution and associated toxic manifestations (Jimenez and Stegeman 1990; Collier *et al.* 1992 Otto and Moon 1996; Martinez-Lara *et al.* 1996; Gadagbui *et al.* 1996; Burgeot *et al.* 1996).

Most studies on the responses of cytochrome P450-dependent monooxygenases, GST and other xenobiotic metabolizing enzymes to chemical pollutants in fish have shown selective or differential induction of hepatic enzymes/isoenzymes (Jimenez and Stegeman 1990; Collier *et al.* 1992; Burgeot *et al.* 1996; Martinez-Lara *et al.* 1996). Recently, TBT was shown to have strong inhibitory effect *in vitro* and *in vivo* on hepatic P450-dependent detoxication of xenobiotics in marine

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fish (Fent and Stegeman 1993). Further *in vitro* studies towards elucidation of mechanism of inhibition have demonstrated species-related and selective inhibition of different components of hepatic monooxygenase system by TBT and TPT in freshwater fish (Fent and Bucheli 1994). The present investigation evaluates the comparative *in vitro* effects of TBT, TPT and DBT, which is also a major metabolite of TBT found in high concentrations in marine organisms (Kannan *et al.* 1997; Morcillo *et al.* 1997; Takahashi *et al.* 1997), on hepatic and renal GST activities in two commercially important fish, *Siganus canaliculatus* (Rabbitfish) and *Sparus sarba* (Arabian seabream), collected from the Arabian Gulf coast bordering Abu Dhabi emirate of United Arab Emirates (UAE).

MATERIALS AND METHODS

Tributyltin chloride (96%), triphenyltin chloride (95%), dibutyltin dichloride (96%) and $SnCl_2$ were procured from Aldrich Chemicals Co. Inc., Milwaukee, USA. 1-Chloro-2,4-dinitrobenzene (CDNB), reduced glutathione (GSH), bovine serum albumin and tromethamine (Tris) were obtained from Sigma Chemicals Co., St. Louis, MO, USA. All other chemicals were of analytical grade.

Samples (50-130 g) of *Sigmus canaliculatus* and *Sparus sarba* captured with the help of a trap from the Arabian Gulf coast bordering Abu Dhabi were used in this study. Homogenates of liver and kidney were prepared in chilled buffered KCl (1.15% KCl buffered with 0.01M Tris-HCl, pH 7.4) and centrifuged at 9000 g for 20 min at 4°C to obtain post-mitochondria1 supernatant, which was used as the source of enzyme. The reaction mixture (3 ml) containing 0.1 M acetate buffer, pH 6.5, 2 mM CDNB (in 0.05 ml ethanol), GSH (1 and 2 mM GSH for liver and kidney, respectively) and suitable amount of post-mitochondria1 supernatant was incubated at 28°C to monitor the formation of GSH-CDNB conjugate at 340 nm spectrophotometrically (Al-Ghais 1997). Different concentrations of organotin compounds and $SnCl_2$ in carrier solvent ethanol (maximal volume 0.03 ml) or equal amount of ethanol alone in controls were preincubated with the enzyme for 10 min prior to addition of GSH and CDNB.

RESULTS AND DISCUSSION

Comparison of the constitutive levels of hepatic and renal GST activities towards CDNB, a non-specific substrate, has demonstrated that *Siganus canaliculatus* was approximately 4 and 2 times more efficient than *Sparus sarba* in the GST-dependent detoxication of xenobiotics in the liver and kidney, respectively (Table 1). Species-related differences in cytosolic GST levels in tissues have been reported earlier in freshwater (Lauren *et al.* 1989; Gallagher and Di Giulio 1992; Gadagbui *et al.* 1996) and marine fish (Al-Ghais and Ali 1995; Burgeot *et al.* 1996; Al-Ghais 1997).

As is evident from IC_{50} values of organotin compounds (the concentration of a compound causing 50% inhibition of enzyme activity), all organotins inhibited hepatic GST activity *in vitro* at µM concentrations in both fish species, but the magnitude of inhibition in *Siganus canaliculalus* was 2-3 fold higher than that

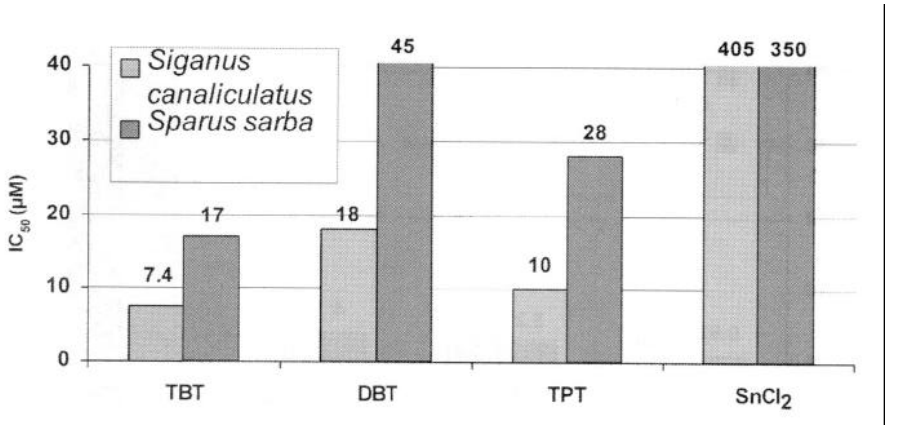

Figure 1. Inhibition of hepatic glutathione *S*-transferase activity by organotin compounds in marine fish. Values represent average of five separate determinations.

Table 1. Glutathione *S*-transferase activity in the tissues of marine fish

Fish species	Specific activity (nmole product/min/mg protein)	
	Liver	Kidney
Siganus canaliculatus (n=6)	271.2 ± 9.8	86.0 ± 2.9
Sparus sarba (n=5)	65.5 ± 3.4	36.8 ± 2.7

Values are the mean ± SE of fish (n=5-6) investigated separately.

observed in *Sparus sarba* (Fig. 1). Notably, the IC_{50} values of $SnCl_2$, a metal present in all the compounds examined, were 405 and 350 µM for *Siganus canaliculatus* and *Sparus sarba* enzymes, respectively. The enzyme inhibitory potency of organotins was in the order of TBT > TPT > DBT. However, preincubation of these organotins or $SnCl_2$ with *Siganus canaliculatus* liver enzyme for 1, 5, 10, 15 and 20 min prior to addition of CDNB and GSH did not cause any significant change in the degree of GST inhibition indicating reversible nature of inhibition. These observations are consistent with the effectiveness and pattern of *in vitro* inhibition of rat liver GST by TBT and TPT, which was shown to be reversible and competitive in nature by dialysis, preincubation and kinetics studies. (Henry and Byington 1976). Recent *in vitro* studies on the interaction of organotins with hepatic microsomal monooxygenases in freshwater fish have shown species-related and selective inactivation of native CYPIA1 and associated monooxygenase activity by TBT and TPT at concentrations ranging between 0.1 and 1 mM, with comparatively lower IC_{50} values of TPT (Fent and Bucheli 1994). Furthermore, TBT was shown to reduce *in vitro* and *in vivo* oxidative detoxication of xenobiotics including its own oxidative dealkylation to DBT and MBT in marine fish liver (Fent and Stegeman 1993).

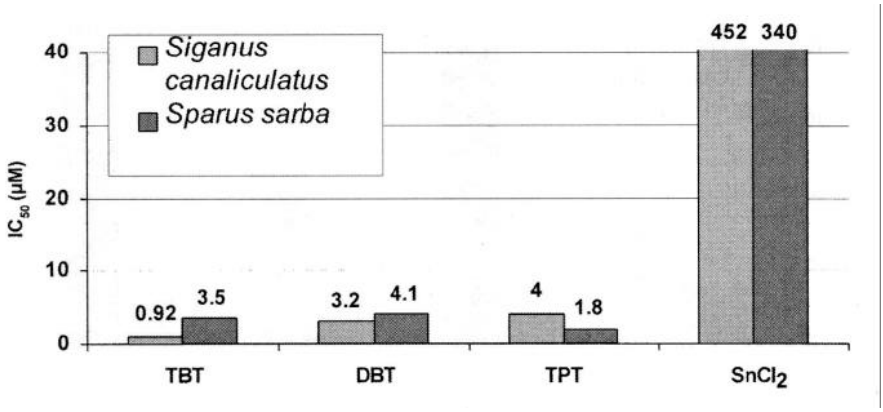

Figure 2. Inhibition of renal glutathione *S*-transferase activity by organotin compounds in marine fish. Values represent average of five separate determinations.

Several fold lower IC_{50} values of organotins recorded in this study for kidney GST, as compared to liver enzyme, in both species indicate remarkably higher sensitivity of renal isoenzyme(s) to inhibition by organotin compounds (Fig. 2 and 3). Maximal inhibition of renal enzyme was elicited by TBT in *Siganus canaliculatus*. The IC_{50} values of $SnCl_2$ for kidney enzyme, like liver GST, were found to range between 0.3 and 0.5 mM and not related to tissue, species or the inhibitory pattern of organotins. Little information is available on the effects of organotins on xenobiotic detoxication in kidney or other extrahepatic tissues. On the basis of physical, structural and immunological properties, substrate specificity and sensitivity to inhibitors, mammalian cytoplasmic GST isoenzymes have been identified and classified into three major families called, alpha (α), mu (μ) and pi (π) (Mannervik *et al.* 1985; Martinez-Lara *et al.* 1996). All members of these GST families conjugate CDNB to GST but display differential specificity towards other substrates. Class mμ, which was represented by a major isoenzyme of rat kidney, exhibited highest sensitivity to inhibition by TPT with IC_{50} values in μM range, whereas approximately 10 and 50 fold higher IC_{50} values were obtained for GST of classes alpha and pi, respectively (Mannervik *et* al. 1985). Further studies on the characteristics of purified forms of rainbow trout kidney GST have demonstrated selective substrate specificity of renal forms for CDNB, as there was no detectable activity towards other potential GST substrates namely 1,2-epoxy-3-(p-nitrophenoxy) propane, ethacrynic acid, p-nitrobenzylchloride or p-nitrophenylacetate (Nimmo and Spalding 1985; Lauren *et* al. 1989). In the light of these observations and our findings it may be suggested that glutathione conjugation to xenobiotics in fish kidney is predominantly catalyzed by GST form(s) that can be classified with highly organotins-sensitive isoenzymes of mammalian class mu GST, whereas in the liver by a heterogeneous mixture of different GST classes as indicated previously in channel catfish liver cytosol (Gallagher *et al.* 1996).

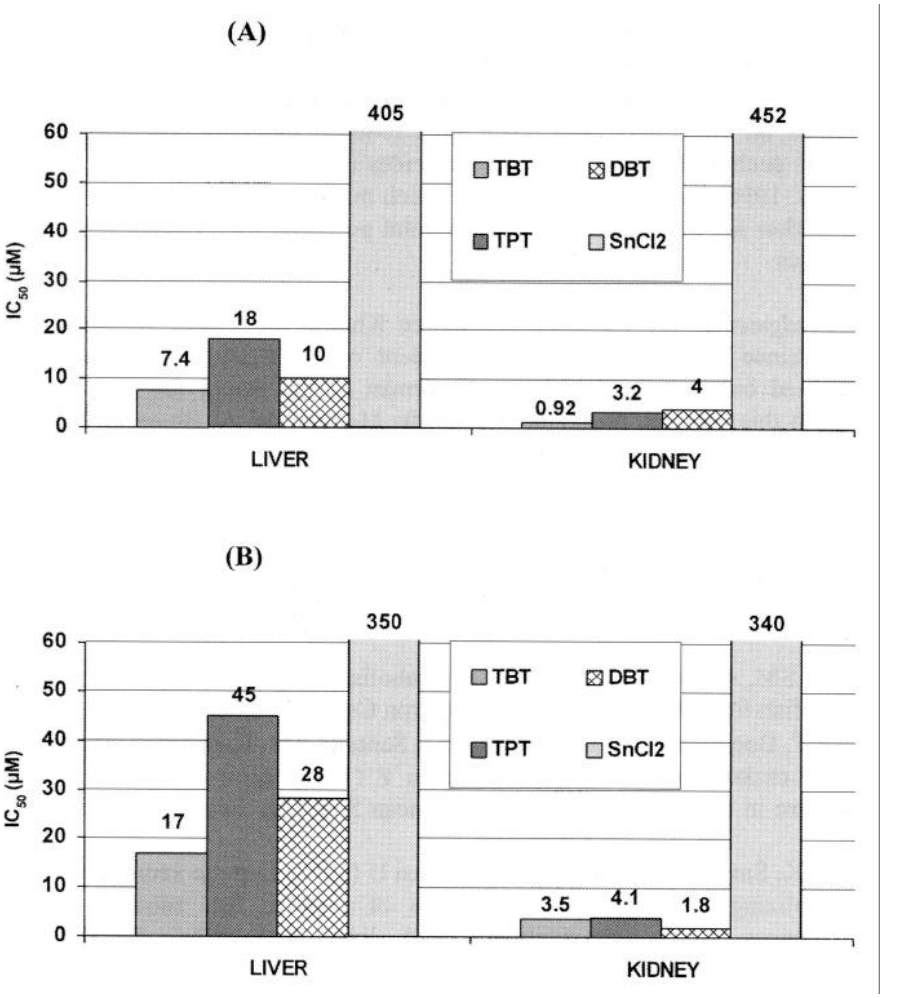

Figure 3. Tissue variation in glutathione *S*-transferase inhibition in (A) *Siganus canaliculatus* and (B) *Sparus sarba*. Values represent mean of five separate determinations.

In summary, the present study shows that organotin compounds, TBT, TPT and DBT are potent inhibitors of GST-mediated detoxication of xenobiotics in the liver and kidney of tropical marine fish, *Siganus canaliculatus* and *Sparus sarba* and, hence, may aggravate the toxicity of other chemical pollutants present in the marine environment. There were marked species, tissue and organotins structure-related differences in the magnitude of GST inhibition. Several fold greater susceptibility of renal GST to organotins inhibition, as compared to that of liver enzyme, noted in this study provides evidence that major forms of fish kidney GST are the representatives of mammalian mu class isoenzymes. These results further suggest the importance of fish tissue GST as molecular biomarker for organotin pollution. *Siganus canaliculatus*, which was more susceptible to

organotins inhibition, appears to be a better bioindicator species. Moreover, kidney GST isoenzyme(s) could be of value as more specific and responsive tissue biomarker for exposure to this group of pollutants. The strong inhibitory action of organotins on GST may also result in a reduced induction response to other pollutants such as PAHs, PCBs and pesticides (Otto and Moon 1996; Martinez-Lara *et al.* 1996; Gadagbui *et al.* 1996), which may be relevant for the use of GST as biomarker for evaluation of environmental pollution by a complex mixture of xenobiotics.

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