Available online at www.sciencedirect.com
ScienceDirect
Journal of Environmental Sciences 19(2007) 1129–1135
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Antioxidant responses to benzo[a]pyrene, tributyltin and their mixture in the spleen of *Sebasticus marmoratus*

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Received 21 November 2006; revised 5 December 2006; accepted 18 January 2007

Abstract

It has been reported that there is an interaction between Benzo[a]pyrene (BaP), a widespread carcinogenic polycyclic aromatic hydrocarbon, and tributyltin (TBT), an organometal used as an antifouling biocide. This study was therefore designed to examine the potential *in vivo* influence of BaP, TBT and their mixture on splenic antioxidant defense systems of *Sebastiscus marmoratus*. The fish were exposed to water containing environmentally relevant concentrations of BaP, TBT and their mixture. Spleens were collected for biochemical analysis after exposure for 7, 25, 50 d and after recovery for 7, 20 d. Cotreatment with BaP and TBT for 7 d potentiated the induction of glutathione peroxidase (GPx) activity by BaP or TBT alone. The cotreatment for 25 and 50 d resulted in inhibition of GPx activity, which was similar to the effect of TBT. Splenic glutathione S-transferase (GST) activities were significantly elevated in *S. marmoratus* exposed to BaP starting from 7 d and remained high up to 25 d. However, no further activity change was found with prolonged exposure. Cotreatment of BaP and TBT primarily inhibited the GST activity, which was similar to the effect of TBT. Cotreatment with BaP and TBT for 25 or 50 d potentiated the depletion of GSH (glutathione) by BaP or TBT alone. MDA (malondialdehyde) contents in spleen of *S. marmoratus* were not significantly altered compared with the control during the test period. Spleen, as an immune organ, is sensitive to exposure of BaP or TBT. It should have an effective mechanism to counteract oxidative damage. Antioxidative defense systems in spleen of *S. marmoratus* should be considered as potential biomarkers. Short-term exposure of BaP or TBT could result in induction of antioxidant defense system. A significant decrease of these indices, such as GSH, GST, GPx might indicate more severe contamination.

Key words: tributyltin; benzo[a]pyrene; antioxidant defense; combined effect; *Sebastiscus marmoratus*

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are produced by any incomplete combustion of organic material and are therefore present worldwide due to anthropogenic activity. Many PAHs, particularly benzo[a]pyrene (BaP), are known to act as potential carcinogens and/or mutagens and are therefore considered as important risk factors in epidemiological and epizootiological cancer (Shaw and Connell, 1994). Results from a previous study showed that the level of BaP in surface water from the Jiulong River Estuary and Western Xiamen Sea was 0.56–3.32 μg/L (Maskaoui *et al*., 2002). Concentrations of BaP varied from 1.0 to 23.4 ng/L in surface seawater of Maluan Bay in Xiamen, China (Tian *et al*., 2004). Organic compounds, particularly tributyltin (TBT) are widely used as biocides in a variety of consumer and industrial products. Besides the acute toxicity of TBT, some studies show that TBT have embryotoxicity (Marin *et al*., 2000), genotoxicity (Jha *et al*., 2000). TBT can also disrupt endocrine functions (Morcillo and Porte, 2000). It was also reported that the levels of TBT in water of the coastal environments of China was below 0.5 ng/L (the detection limit) to hundreds ng/L as Sn (Jiang *et al*., 2001).

It has been reported that both BaP (Carlson *et al*., 2002; 2004) and TBT (Grinwis *et al*., 1998; Schwaiger *et al*., 1994) are immunotoxic to fish. The adverse effects of many chemicals upon animals are related to their capacity for undergoing reactions producing reactive oxygen species (ROS) and lipid peroxidation (LPO). Antioxidant defense systems neutralize chemical reactive intermediates produced by endogenous pathways and/or xenobiotic metabolism (Kappus, 1987; Winston and Di Giulio, 1991). They are also involved in enzymatic reactions eliminating electrophilic chemicals or metabolites, and reducing organic peroxides (Di Giulio *et al*., 1989; Yu, 1994). The conjugation of reduced glutathione (GSH) with a xenobiotic, either spontaneously, or catalyzed by glutathione S-transferase (GST), decreases xenobiotic reactivity. There

Project supported by the National Natural Science Foundation of China (No. 20447001, 40476049) and the Natural Science Foundation of Fujian Province of China (No. D0410007). * Corresponding author.
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are some studies that BaP influenced antioxidant defense systems in liver of fish (Oikari and Jimenez, 1992; Padrós *et al*., 2003), and some studies show that TBT could result in alteration of antioxidant defense systems (Al-Ghais and Ali, 1999; Wang *et al*., 2005). Antioxidant defense systems are impacted by exposure of chemicals, and it is suggested that antioxidant defense systems could be used as potential biomarkers to monitor marine pollution (Van der Oost *et al*., 2003). Spleen, which is an immune organ, is particularly vulnerable to chemical contaminants. It was reported that 3-methycholanthrene induced ethoxyresorufin-O-deethylase (EROD) activity and cytochrome P450 in spleen of Carp (*Cyprinus carpio*) (Marionnet *et al*., 1997). In rainbow trout (*Oncorhynchus mykiss*) exposed to TBT, a concentration-dependent lymphodepletion in the spleen has been reported (Schwaiger *et al*., 1994). However, limited information concerning the effects of PAHs or organotin compounds on antioxidant defense systems in spleen of fish is available.

TBT and BaP are widespread pollutants that are often found to be present together in many aquatic environments under both dissolved and particulate forms, which could interact in their effects on these biomarkers. Observations (Padrós *et al*., 2000; 2003; Wang *et al*., 2006) about interactions between TBT and BaP reinforce the need to further investigate the combined effects on biomarkers. However, limited information concerning combined effects of TBT and BaP on antioxidant defense systems in fish is available. Accordingly, the present study was designed to expose fish through water to BaP and TBT at environmentally relevant concentrations for a long term.

The aims of this study were: (1) to observe the effects of BaP or TBT on antioxidant defense systems in spleen of fish and to assess effects of combined exposure of BaP and TBT; and (2) to investigate the combined effect of BaP and TBT on these antioxidative indices in spleen of fish. It was expected that the results from these studies will provide experimental basis with regard to whether spleen antioxidants adaptation potentials may serve as a surrogate biomarker to the chemicals exposure. The selection of the test animal species was based on its availability, commercial importance (fisheries and aquaculture), and distribution throughout the coastal waters of China.

1 Materials and methods

1.1 Chemicals

Tributyltin chloride was obtained from Fluka AG, Switzerland, with a purity of greater than 97%. BaP (98% purity) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Other chemicals were of analytical grade and were obtained from commercial sources.

1.2 Experimental species and exposure conditions

Sebastiscus marmoratus weighing 25–50 g were captured from a pristine coast in Xiamen, Fujian Province, China. The fish initially were maintained in tanks containing 60 L of aerated sand-filtered seawater, with natural light-dark cycle for 7 d. Cuvier were exposed to water containing different concentrations of BaP (10, 100, 1000 ng/L, respectively), TBT (1, 10, 100 ng/L), or both in combination of TBT+BaP (ng/L) (0.5+5; 5+50; 50+500); control group received an equal volume of the solvent 98% ethanol (5 μl/L). The maintained density was individual/2 L. The mortality was 5%–15% in each tanks during whole testing period. The water containing different concentrations of the pollutants was exchanged in half every day. The fish were fed with fresh clam (*Meretrix meretrix*) meat for 2 h to satiation before replacing the water. The clams were collected from a pristine coast and maintained in aerated marine water for up to 7 d prior to use. This process was repeated every other day for 3 d before sampling. The water temperature was maintained at 14–22°C, and salinity 22%–24%. The fish were randomly sampled from each treatment group after exposure for 7, 25, 50 d and recovery for 7 and 20 d, respectively. Samples were frozen in liquid N_2 immediately after collection and stored at –80°C before use.

1.3 Biochemical analysis

Homogenate of the spleen was prepared in chilled KCl buffer (1.15% KCl buffered with 0.01 mol/L Tris-HCl, pH 7.4) and centrifuged at 10000×*g* for 20 min at 4°C to obtain post-mitochondrial supernatant, which was used as the source of enzyme (Livingstone, 1988). GST activity was evaluated with 1-chloro-2,4-dinitrobenzene as substrate, following the formation of the conjugate with GSH at 340 nm according to Habig *et al*. (1974). GSH content was determined using a fluorometric assay according to the method of Hissin and Hilf (1976). GPx (glutathione peroxidase) activity was measured according to Hafeman *et al*. (1973) with a slight modification (Zhang *et al*., 2004). One unit of GPx activity is defined as the amount of enzyme that oxidizes 1 μmol/L of GSH per min per mg of protein at 30°C. Levels of malondialdehyde (MDA), a by-product of lipid peroxidation, were determined as described by Ohkawa *et al*. (1979). Protein concentrations in the supernatants were determined by the Bradford procedure (Bradford, 1976) using bovine serum albumin as a standard. All fluorometric assays were determined on a Hitachi F-4010 fluorescence spectrophotometer. The absorbance at UV and visible wavelength was monitored on a Thermo GENESY 2 UV-Visible spectrophotometer.

1.4 Data processing

Results are reported as mean ± SE (standard error). The data were processed by one-way ANOVA and the significance level between data was examined by Dunnet T3 (α = 0.05) post-hoc tests. Two-way ANOVA analysis using SPSS 11.0 software was employed to determine whether there is an interaction between TBT and BaP, with the factors being BaP and TBT.

2 Results

2.1 Influence of ethanol

Ethanol was used as solvent in the present study. Control

groups received an equal volume of ethanol. The results showed that the splenic enzymes activities and GSH content were not affected by ethanol.

2.2 GPx activity

Splenic GPx activities in *S. marmoratus* exposed to 100 ng/L or 1000 ng/L of BaP for 7 d were significantly elevated by 1.36- or 1.08-fold respectively (Fig.1). However, with prolonged exposure to BaP the GPx activities were not significantly changed compared with the matched control. Exposure of 10 ng/L or 100 ng/L of TBT significantly induced GPx activities by 1.25- or 1.19-fold, respectively, and with prolonged exposure to TBT, the activities were significantly inhibited. Cotreatment with BaP and TBT for 7 d potentiated the induction of GPx activity by BaP or TBT alone, GPx activities in fish exposed to the mix of BaP+TBT (ng/L) (50 + 5 or 500 + 50) were elevated by 1.42- or 1.30-fold respectively. The cotreatment for 25 and 50 d resulted in inhibition of GPx activity, which was similar to the effect of TBT, not to that of BaP. Cotreatment with BaP and TBT antagonized TBT-mediated GPx inhibition. There was a significant difference between TBT+BaP groups and BaP or TBT groups for 50 d exposure according to two-way ANOVA analysis. The GPx activities in all exposure groups, transferred to clean recovery tanks for 7 and 20 d, were recovered to the level corresponding to that of the control group.

2.3 GST activity

Splenic GST activities in *S. marmoratus* exposed to BaP for 7 d and to 10 ng/L or 100 ng/L of BaP for 25 d were significantly elevated (Fig.2). The highest elevation reached 2.27-fold relative to the control. However, GST activities were not changed in *S. marmoratus* exposed to BaP for 50 d compared with that of the control group. Splenic GST activities in *S. marmoratus* exposed to 100 ng/L of TBT for 25 d and to the all concentrations of TBT for 50 d were significantly inhibited. GST activities continued to be inhibited in the fish recovered for 7 d at 10 ng/L and 100 ng/L of TBT exposure group. Although cotreatment with the lowest concentration of BaP and TBT for 7 d resulted in induction of GST activity, the cotreatment with BaP and TBT principally inhibited the GST activity, which was similar to the effect of TBT. There was a significant difference between TBT+BaP groups and BaP or TBT groups for 7 and 25 d exposure respectively according to two-way ANOVA analysis. The GST activities in TBT and the mixture exposure groups, transferred to clean recovery tanks for 20 d, were recovered to the levels corresponding to that of the control group.

2.4 GSH content

Splenic GSH levels in *S. marmoratus* exposed to BaP for 50 d were decreased and there was a significant difference between 1000 ng/L group and control group, although GSH levels exposed to BaP for 7 and 25 d were not changed compared with the matched control (Fig.3). The GSH contents in the fish exposed to TBT were decreased with prolonged exposure and there were significant differences between TBT-treated groups and control group for 50 d exposure. Cotreatment with 50 ng/L BaP + 5 ng/L TBT for 7 d resulted in a significant elevation in the GSH levels, while cotreatment with BaP and TBT for 50 d potentiated the depletion of GSH by BaP or TBT alone.

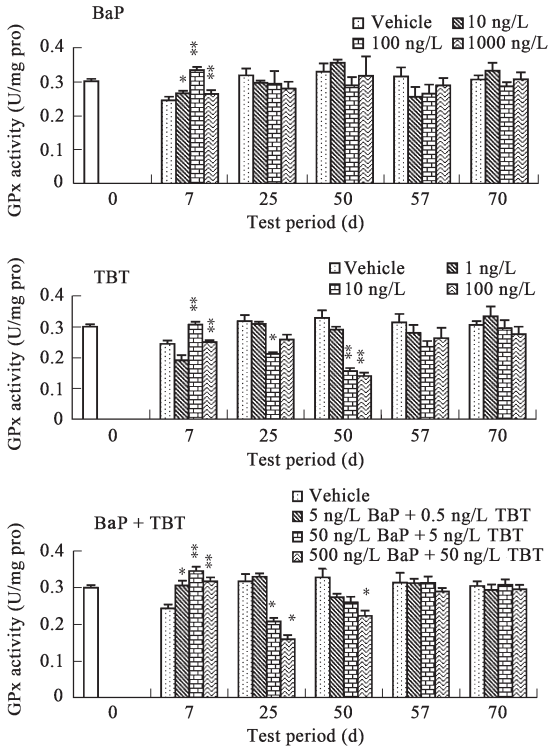

Fig. 1 Effects of BaP, TBT, and their mixture on splenic GPx activity in *Sebastiscus marmoratus* (57, 70 d represent recovered for 7, 20 d, respectively). The results are mean ± SE; n=6–8; * $P < 0.05$; ** $P < 0.01$ vs. the vehicle group.

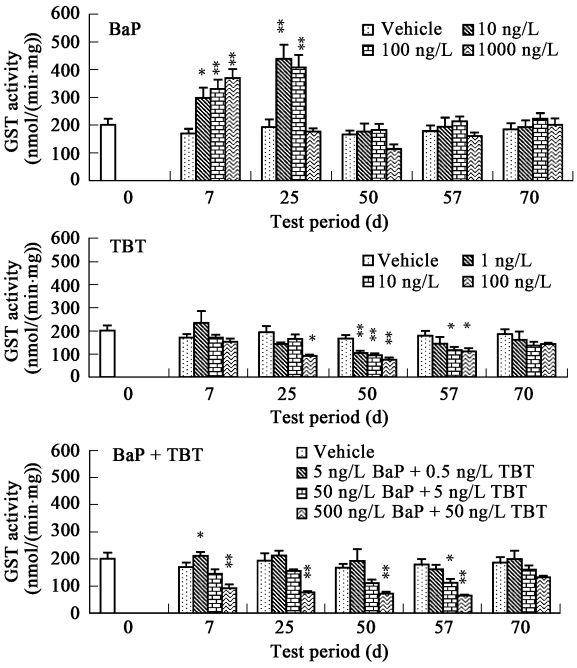

Fig. 2 Effects of BaP, TBT, and their mixture on splenic GST activity in *Sebastiscus marmoratus* (57, 70 d represent recovered for 7, 20 d, respectively). The results are mean ± SE; n: 6–8. *P<0.05, **P<0.01 vs. the vehicle group.

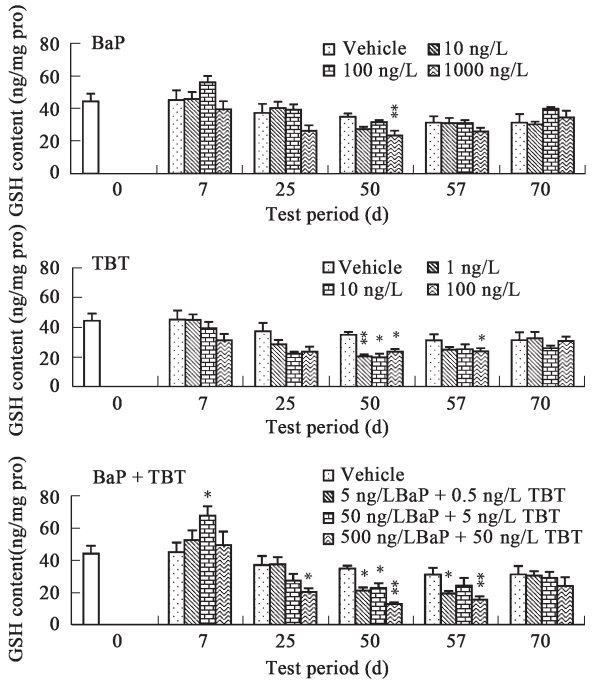

Fig. 3 Effects of BaP, TBT, and their mixture on splenic GSH levels in *Sebastiscus marmoratus* (57, 70 d represent recovered for 7, 20 d, respectively). The results are mean ± SE; *n*: 6–8. * $P<0.05$, ** $P<0.01$ vs. the vehicle group.

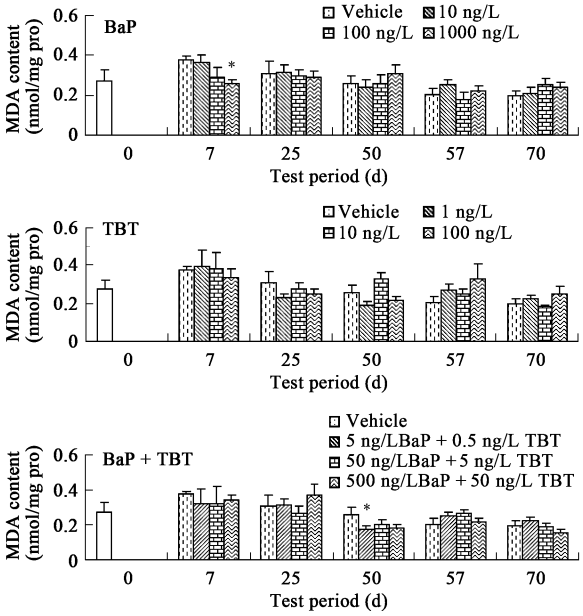

Fig. 4 Effects of BaP, TBT, and their mixture on splenic MDA contents in *Sebastiscus marmoratus* (57, 70 d represent recovered for 7, 20 d, respectively). The results are mean ± SE; *n*= 6–8. * $P<0.05$, ** $P<0.01$ vs. the vehicle group.

There was a significant difference between TBT+BaP groups and TBT groups for 7 d, and between TBT+BaP groups and BaP groups for 50 d exposure respectively according to two-way ANOVA analysis. The GSH levels in TBT and the mixture exposure groups were not recovered to the level corresponding to that of the control group until transferred to clean recovery tanks for 20 d.

2.5 MDA contents

MDA contents in spleen of *S. marmoratus* exposed to BaP, TBT and their mixture were not significantly changed compared with the matched control during the test period (Fig.4), although there was a significant decrease at 1000 ng/L of BaP exposure for 7 d or 5 ng/L BaP + 0.5 ng/L TBT exposure for 50 d. There was a significant difference between TBT+BaP groups and BaP groups for 50 d exposure according to two-way ANOVA analysis.

3 Discussion

Spleen is an immune organ, which is one of the targets attacked by contaminants. It is known from mammalian as well as fish studies that the immune system is sensitive to the toxic effects of pollution (Anderson and Zeeman, 1995). Ethoxyresorufin-O-deethylase (EROD) activity and cytochrome P450 contents and GST activities in spleen were induced in carp (*Cyprinus carpio*) exposed to 3-methylcholanthrene (3MC) (Marionnet *et al*., 1997, 2006). This provides new information on the existence of detoxification potential in extra-hepatic organs. However, few information on antioxidant defense system in spleen of fish is available. No significant increase of MDA levels in the present study suggest that the exposure of BaP, TBT and their mixture would not result in lipid peroxidation, however, antioxidant defense system in spleen, such as GPx, GST activities and GSH levels, were significant altered, which was an interesting phenomena. As an immune organ, spleen contains a large number of macrophages. One of the bactericidal mechanisms employed by fish and mammalian macrophages is the conversion of molecular oxygen into a variety of ROS, including superoxide anion O_2^- and hydrogen peroxide H_2O_2 (Chung and Secombes, 1988; Secombes *et al*., 1992). *In vitro* exposure of fish phagocytes to pollutants, including TBT, has resulted in a variety of effects on release of ROS including stimulation (Cossarini-Dunier, 1987; Rice and Weeks, 1989), inhibition (Wishovsky *et al*., 1989; Rice and Weeks, 1989) or no significant effect (Cossarini-Dunier, 1987), with the same chemical having opposite effects depending on the concentration. No alteration of MDA levels in the present study was consistent with some previous studies. There was no effect on hydrogen peroxide production in spleen macrophages from fish exposed to BaP, BaP injection completely inhibited the respiratory burst in spleen macrophages (Lemaire-Gony *et al*., 1995). Secombes *et al*. (1991, 1992) found that ROS and bactericidal activity in spleen cells were not affected following dab (*Limanda limanda*) exposed to sewage sludge. The results in the present study implicate that spleen has an effective mechanism to counteract oxidative damages.

Splenic GST activities in *S. marmoratus* exposed to BaP alone for 7 d and to the lower concentrations of BaP for 25 d were significantly induced, which is consistent with some previous studies (Burgeot *et al*., 1996; Van der Oost *et al*., 1996, 1998). Strangely, the GST activities in fish exposed to 1000 ng/L of BaP for 25 d and to BaP for

50 d had no significant alteration. Some previous studies reported that no significant difference between fish from control and polluted site were observed (Collier *et al*., 1992; Fenet *et al*., 1998; Stephensen *et al*., 2000; Vigano *et al*., 1995). Our previous study showed that GST activities in liver of *S. marmoratus* ip injected with 0.5–10 mg/kg BaP had not a significant alteration (Wang *et al*., 2006). This could be a consequence of loss or decline of response due to the inability of the organ to adequately respond to xenobiotic insult after extended periods of time. This may be one of the reasons that most studies did not demonstrate any significant alterations after laboratory exposure of fish to BaP (Collier and Varansi, 1991; Lemaire-Gony and Lemaire, 1992; Van Schanke *et al*., 2002; Wang *et al*., 2006). Splenic GST activities in *S. marmoratus* exposed to TBT were inhibited, this result is similar to some previous observations, Al-Ghais and Ali (1999) reported that organotin compounds inhibited *in vitro* GST activities in liver and kidney of *Siganus canaliculatus* and *Sparus sarba*, GST activity in arctic charr (*Salvelinus alpinus*) exposed to 0.3 mg TBT/kg was significantly inhibited (Padrós *et al*., 2003). However, the results presented here are different from our previous observations in which hepatic GST activities were induced 4 d after ip injected with 19.3 μg/kg of TBT (Wang *et al*., 2005) or 7 d after Intraperitoneal injection with 1 mg/kg of TBT (Wang *et al*., 2006). The difference might result from different exposure methods and different sampling tissues. The alteration of GST activities in fish exposed to the mixture in the present study was similar to the effect of TBT. Cotreatment with TBT antagonizes BaP-mediated GST induction. These results suggest that effect of TBT on splenic GST activity would override that of BaP at environmentally relevant concentrations.

At the same time, conjugation of GSH with a xenobiotic results in the depletion of the GSH. The protective effect of GSH was attributed to its ability of scavenging the free radicals and thereby blocking the LPO. Thus the decrease in GSH levels can be interpreted as being a result of BaP or TBT exposure in the present study. Cotreatment of BaP and TBT potentiated the depletion of GSH, although GSH levels in fish exposed to the mixture for 7 d were induced. Both GST activity and GSH contents in TBT and the mixture groups in the present study continued to decrease in fish recovered for 7 d. This suggests that metabolism and elimination of TBT in spleen would be slow. Inhibition of the GST activity and depletion of GSH by the cotreatment would reduce the capacity of spleen to detoxify other chemicals and increase the vulnerability to oxidative stress.

Limited information on the effects of BaP on GPx activity in fish is available. An increased hepatic GPx activity was observed in *Dicentrarchus labrax* exposed to 20 mg/kg of 3MC for 5 d and in *Limanda limanda* for 11 d respectively, while a decrease was observed in 3MC-treated *D. labrax* for 1 d (Lemaire *et al*., 1996). In the present study, initially significant induction of splenic GPx activities were observed in fish exposed to BaP. However, with prolonged exposure to BaP the GPx activities were not significantly changed, which is similar to the response of GPx to BaP in our previous study (Wang *et al*., 2006). Cotreatment with BaP for 50 d in the present study appeared to antagonize TBT-mediated GPx inhibition. This result indicates that interactions between BaP and TBT could influence GPx altertion. This result may have relevance for the use of GPx activities as indicators of aquatic pollution by PAHs or organtins. This index should be interpreted with caution in biomonitoring studies.

4 Conclusions

As an immune organ, spleen is sensitive to exposure of BaP or TBT. Exposure of contaminants could induce antioxidant potential of this organ. Spleen has an effective mechanism to protect itself from oxidative damage. Antioxidant defense systems in spleen of *Sebastiscus marmoratus* should be considered as potential biomarkers. Although the results present here demonstrate interactions between these two widespread aquatic pollutants, these indices could still give some indications to monitor these pollutions. Short-term exposure of environmentally relevant concentrations of BaP or TBT could result in induction of antioxidant defense system. A significant decrease of these indexes, such as GST, GPx activities and GSH contents appear to imply more severe contamination.

References

Al-Ghais S M, Ali B, 1999. Inhibition of Glutathione S-transferase catalyzed xenobiotic detoxication by organotin compounds in tropical marine fish tissues[J]. Bull Environ Contam Toxical, 62: 207–213.

Anderson D P, Zeeman M F G, 1995. Immunotoxicology in fish[M]. In: Fundamentals of aquatic toxicology: Effect, environmental fate, and risk assessment (Rand G., ed.). Washington: Taylor and Francis. 371–404.

Bradford M M A, 1976. Rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding[J]. Analytical Biochem, 72: 248–254.

Burgeot T, Bocquéné G, Porte C *et al*., 1996. Bioindicators of pollutant exposure in the northwestern Mediterranean Sea[J]. Mar Ecol Prog Ser, 131: 125–141.

Carlson E A, Li Y, Zelikoff J T, 2002. Exposure of Japanese medaka (*Oryzias latipes*) to benzo[a]pyrene suppresses immune function and host resistance against bacterial challenge[J]. Aquat Toxicol, 56: 289–301.

Carlson E A, Li Y, Zelikoff J T, 2004. Suppressive effects of benzo[a]pyrene upon fish immune function: Evolutionarily conserved cellular mechanisms of immunotoxicity[J]. Marine Environmental Research, 58: 731–734.

Chung S, Secombes C J, 1988. Analysis of events occurring within teleost macrophages during the respiratory burst[J]. Comp Biochem Physiol B, 89: 539–544.

Collier T K, Singh S V, Awasthi Y C *et al*., 1992. Hepatic xenobiotic metabolizing enzymes in two species of benthic fish showing different prevalences of contaminant-associated liver neoplasms[J]. Toxicol Appl Pharmacol, 113: 319–324.

Collier T K, Varanasi U, 1991. Hepatic activities of xenobiotic metabolizing enzymes and biliary levels of xenobiotics in

English sole (*Parophrys vetulus*) exposed to environmental contaminants[J]. Arch Environ Contam Toxicol, 20: 462–473.

Cossarini-Dunier M, 1987. Effects of the pesticides atrazine and lindanc and of manganese ions on cellular immunity of carp, *Cyprinus carpio*[J]. J Fish Biol, 31A: 67–73.

Di Giulio R T, Habig C, Wolfe T, 1989. Phase I and phase II biotransformation enzyme activities in channel catfish exposed to contaminated sediments[J]. Toxicologist, 9: 43.

Fenet H, Casellas C, Bontoux J, 1998. Laboratory and field-caging studies on hepatic enzymatic activities in European eel and rainbow trout[J]. Ecotoxicol Environ Safe, 40: 137–143.

Grinwis G C M, Boonstra A, van den Brandhof E J *et al*., 1998. Short-term toxicity of bis(tri-*n*-butyltin)oxide in flounder (*Platichthys flesus*): Pathology and immune function[J]. Aquat Toxicol, 42: 15–36.

Habig W H, Pabst M J, Jakoby W B, 1974. Glutathione S-transferases: the first enzymatic step in mercapturic acid formation[J]. J of Biol Chem, 249(22): 7130–7139.

Hafeman D G, Sunde R A, Hoekstra W G, 1973. Effect of dietary selenium and erythrocyte and liver glutathione peroxidase in the rat[J]. J Nutr, 104: 580–587.

Hissin P J, Hilf R A, 1976. fluorometric method for determination of oxidized and reduced glutathione in tissues[J]. Anal Biochem, 74(1): 214–226.

Jha A N, Hagger J A, Hill S J *et al*., 2000. Genotoxic, cytotoxic and developmental effects of tributyltinoxide (TBTO): an intergrater approach to the valuation of the relative sensitives of two marine species[J]. Mar Environ Res, 50: 565–573.

Jiang G B, Zhou Q F, Liu J Y *et al*., 2001. Occurrence of butyltin compounds in the waters of selected lakes, rivers and coastal environments from China[J]. Environ Pollut, 115: 81–87.

Kappus H, 1987. Oxidative stress in chemical toxicity[J]. Arch Toxicol, 60: 144–149.

Lemaire P, Förlin L, Livingstone D, 1996. Responses of hepatic biotransformation and antioxidant enzymes to CYP1A-inducers (3-methylcholanthrene, β-naphthoflavone) in sea bass (*Dicentrarchus labrax*), dab (*Limanda limanda*) and rainbow trout (*Oncorhynchus mykiss*)[J]. Aquat Toxicol, 36: 141–160.

Lemaire-Gony S, Lemaire P, 1992. Interactive effects of cadmium and benzo[a]pyrene on cellular structure abd biotransformation enzymes of the liver of the European eel *Anguilla anguilla*[J]. Aquat Toxicol, 22: 145–160.

Lemaire-Gony S, Lamaire P, Pulsford A L, 1995. Effects of cadmium and benzo[a]pyrene on the immune system, gill ATPase and EROD activity of European sea bass *Dicentrarchus labrax*[J]. Aquat Toxicol, 31: 297–313.

Livingstone D R, 1988. Responses of microsomal NADPH-cytochrome c reductase activity and cytochrom P-450 in digestive glands of *Mytilus edulis* and *Littorea* to enviromentl and experimental exposure to pollutants[J]. Marine Ecology Progress Series, 46: 37–43.

Marin M G, Moschino V, Cima F *et al*., 2000. Embryo toxicty of butyltin compounds to the sea urchin *Paracentrotus livius*[J]. Mar Environ, 50: 231–235.

Marionnet D, Taysse L, Chambras C *et al*., 1997. 3-Methylcholanthrene-induced EROD activity and cytochrome P450 in immune organs of carp (*Cyprinus carpio*)[J]. Comp Biochem Physiol C, 118(2): 165–170.

Marionnet D, Deschaux P, Reynaud S, 2006. Possible implication of macrophages in the regulation of cytochrome P450 activities in carp (*Cyprinus carpio*)[J]. Fish & Shellfish Immunology, 21: 80–91.

Maskaoui K, Zhou J L, Hong H S *et al*., 2002. Contamination by polycyclic aromatic hydrocarbons in the Jiulong River Estuary and Western Xiamen Sea, China[J]. Environ Pollut, 118: 109–122.

Morcillo Y, Porte C, 2000. Evidence of endocrinedis tribution in clams-Ruditapes de cussata-transplanted to a tributyltin polluted environment[J]. Environ Pollut, 107: 47–52.

Ohkawa H, Ohishi N, Yagi K, 1979. Assay for lipid peroxides in animals and tissues by thiobarbituric acid reaction[J]. Anal Biochem, 95(2): 351–358.

Oikari A, Jimenez B, 1992. Effects of hepatotoxicants on the induction of microsomal monooxygenase activity in sunfish liver by b-naphtoflavone and benzo[a]pyrene[J]. Ecotoxicol Environ Safe, 23: 89–102.

Padrós J, Pelletier É, Reader S *et al*., 2000. Mutual *in vivo* interactions between benzo[a]pyrene and tributyltin in brook trout (*Salvelinus fontinalis*)[J]. Environ Toxicol Chem, 19: 1019–1027.

Padrós J, Pelletier É, Ribeiro C O, 2003. Metabolic interactions between low doses of benzo[a]pyrene and tributyltin in arctic charr (*Salvelinus alpinus*): a long-term *in vivo* study[J]. Toxicol Appl Pharmacol, 192: 45–55.

Rice C D, Weeks B A, 1989. Influence of tributytin on *in vitro* activation of oyster toadfish macrophages[J]. J Aquat Anim Health, 1: 62–68.

Schwaiger J, Falk H F, Bucher F *et al*., 1994. Prolonged exposure of rainbow trout (*Oncorhynchus mykiss*) to sublethal concentrations of bis(tri-nbutyltin) oxide: effects on leucocytes, lymphatic tissues and phagocytosis activity[M]. In: Sublethal and chronic effects of pollutants on freshwater fish (Mler R., Lloyd R., ed.). Cambridge, MA: Blackwell. 113–123.

Secombes C J, Fletcher T C, O'Flynn J A *et al*., 1991. Immunocompetence as a measure of the biological effects of sewage sludge pollution in fish[J]. Comp Biochem Physiol C, 100: 133–136.

Secombes C J, Fletcher T C, White M J *et al*., 1992. Effect of sewage sludge on immune responses in the dab *Limanda limanda* L.[J]. Aquat Toxicol, 23: 217–230.

Shaw G R, Connell D W, 1994. Prediction and monitoring of the carcinogenicity of polycyclic aromatic compounds (PACs)[J]. Rev Environ Contam Toxicol, 135: 1–62.

Stephensen E, Svavarsson J, Sturve J *et al*., 2000. Biochemical indicators of pollution exposure in shorthorn sculpin (*Myoxocephalus scorpius*), caught in four harbours on the southwest coast of Iceland[J]. Aquat Toxicol, 48: 431–442.

Tian Y, Zheng T L, Wang X H, 2004. Concentration, distribution and source of polycyclic aromatic hydrocarbons in surface sediments of Xiamen western harbor[J]. Oceanologia et Limnolgia Sinica, 35(1): 15–20.

Van der Oost R, Goksøyr A, Celander M *et al*., 1996. Biomonitoring aquatic pollution with feral eel (*Anguilla anguilla*): II. Biomarkers: pollution-induced biochemical responses[J]. Aquat Toxicol, 36: 189–222.

Van der Oost R, Lopes S C C, Komen H *et al*., 1998. Assessment of environmental quality and inland water pollution using biomarker responses in caged carp (*Cyprinus carpio*); use of a bioactivation: detoxication ratio as biotransformation index (BTI)[J]. Mar Environ Pollut, 46: 315–319.

Van Schanke A, Boon J P, Aardoom Y *et al*., 2002. Effect of a dioxin-like PCB (CB 126) on the biotransformation and

genotoxicity of benzo[a]pyrene in the marine flatfish dab (*Limanda limanda*)[J]. Aquat Toxicol, 50: 403–415.

Van der Oost R, Beyer J, Vermeulen N P E, 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review[J]. Environ Toxicol Pharmacol, 13: 57–149.

Vigano L, Arillo A, Melodia F *et al*., 1995. Hepatic and biliary biomarkers in rainbow trout injected with sediment extracts from the river Po (Italy)[J]. Chemosphere, 30: 2117–2128.

Wang C G, Chen Y X, Li Y *et al*., 2005. Effects of low dose tributyltin on activities of hepatic antioxidant and phase II enzymes in *Sebastiscus marmoratus*[J]. Bull Environ Contam Toxicol, 74(1): 114–119.

Wang C G, Zhao Y, Zheng R H *et al*., 2006. Effects of tributyltin, benzo[a]pyrene, and their mixture on antioxidant defense systems in *Sebastiscus marmoratus*[J]. Ecotoxicol Environ Safe, 65: 381–387.

Winston G W, Di Giulio R T, 1991. Prooxidant and antioxidant mechanisms in aquatic organisms[J]. Aquat Toxicol, 19: 137–161.

Wishkovsky A, Matthews E S, Weeks B A, 1989. Efffect of tributyltin on the chemiluminescent response of phagocytes from three species of estuarine fish[J]. Arch Environ Contam Toxicol, 18: 826–831.

Yu B P, 1994. Cellular defenses against damage from reactive oxygen species[J]. Physiol Rev, 74: 139–162.

Zhang J f, Shen H, Wang X R *et al*., 2004. Effects of chronic exposure of 2,4-dichlorophenol on the antioxidant system in liver of freshwater fish *Carassius auratus*[J]. Chemosphere, 5: 167–174.