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Tributyltin-oxide (TBTO) induced Changes in Plasma Sex Steroid Hormones and Cortisol Level of Korean Rockfish, *Sebastes schlegeli*

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The influence of TBTO (Tributyltin-Oxide) at a series of concentrations (0.52, 1.41 and 3.05 μg/L) for a period of 3 weeks on estradiol-17β, testosterone and cortisol levels in male Korean rockfish, *Sebastes schlegeli* was investigated. Compared to the stable concentration of estradiol-17β, plasma testosterone level was decreased significantly at the 3rd week after TBTO exposure in plasma of fish (>1.41 μg/L). The most profound physiological variation in TBTO exposed fish was a dramatic increase in plasma cortisol level at 3rd week. Conclusively, TBTO exposure caused increase level of cortisol as well as alteration of testosterone in the Korean rockfish. These results suggest that TBTO at environmentally relevant (nanomolar) concentrations disrupt endocrine secretions.

Keywords: Cortisol, Steroid hormones, *Sebastes schlegeli*, Tributyltin-Oxide

Introduction

TBT-based antifoulings were introduced in the mid-1960s and their application gained popularity rapidly. Adverse impact of these compounds on aquatic non-target organisms became apparent in the late 1970s. TBT contamination of marine and freshwater environments is still a global pollution problem (Gibbs et al., 1987). Even though regulations in many industrialized countries have resulted decrease contamination, residues persist at ecotoxicologically relevant concentrations. Recent data illustrates considerable contamination in aquatic compartments of developing countries.

Up to now TBT is known to cause a variety of pathological conditions in animals even at very low environment concentration, in terms of sensitivity, none rivals that of the imposex phenomenon in prosobranchs (Gibbs et al., 1996). Because the analysis of TBT in sea water and tissue is rather difficult, time-consuming and expensive, the determination of an easily detectable physiological and morphological parameters would give better results. For pollution monitoring programs, toxicity tests of TBT on hormone (androgen) are exemplified by the study conducted by Vijayan et al. (1994, 1997a) using marine animals.

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Specific actions of Tributyltin-oxide (TBTO) are evident in marine animals, as indicated by the high susceptibility of certain diseases in aquatic organisms. The following principal modes of action of organotins including TBTO are as follows: (1) perturbation of cellular calcium homeostasis; (2) inhibition of energy production in cells, e.g., the inhibition of mitochondrial oxidative phosphorylation; (3) damage of plasma membrane and inhibition of ion pumps. It also effects on intracellular sulfhydryl-containing proteins. Fent (1996) reported the adverse effect of TBTO on cytochrome P450-dependent monooxygenases and emphasized the coordination of the trisubstituted organotin molecule with the amino acids cystein and histidine.

Although, there are splendid parameters, the limited knowledge on the impact on acute and subacute indicate and an endocrine effects of TBT are rarely reported for fish except for research into imposex or masculinizing effects (Féral and LeGall, 1983). Hence, further studies needed to assess the chronic toxicological potential and effect related to endocrine effects.

This study was intended to assess the possible effects of stress generated by TBTO exposure as an alteration of plasma cortisol and subsequent confinement on reproductive enzymes such as estradiol and testosterone in Korean rockfish. Thus, in the present study, our objective was to investigate a possible changes in plasma steroid hormones and cortisol level in Korean rockfish, *Sebastes schlegeli* exposed TBTO.

Materials and Methods

Exposure

Sexually mature Korean rockfish, *S. schlegeli* were chosen as the test organism for this study. Korean rockfish were commercially available, relatively easy to maintain in the laboratory, and large enough to provide tissue and plasma volumes sufficient for multiple analyses. Korean rockfish were obtained from the fishery is located in the Tongyeong, Korea. Fish were randomly housed in each of six tanks (520×360×300 mm) tanks. Fish in all tanks were allowed at least three weeks for acclimatization. The tanks were maintained under controlled condition of temperature (10-13°C) and water quality parameters like: DO, COD, pH, NH_4, NO_2, NO_3 and PO_4 monitored every other day to maintain the similarity in all the tanks (Table 1). No difference was measured in physical and chemical parameters of water in all tanks. The fish were fed daily with approximately 20 g rock chow (Jeilfeed Co., Korea) per tank. Unconsumed food were removed daily. Fish were exposed to bis-tributyltin oxide (TBTO. $C_{24}H_{54}OSn_2$. Fluka Co., USA) for 3 weeks using a flow through system. The dose of experimental tanks were treated with the different concentration (0.52, 1.41 and 3.05 μg/L) of TBTO. The two tanks served control without TBTO exposure.

Blood samples

At the end of each period (at first, second and 3th week) fish were anaesthetized in 3-aminobenzoic acid ethyl ester methanesulfonate (Sigma Chemical, St. Louis, MO, USA) and blood was taken from caudal vein using the heparinized-syringe. Anaesthesia, measurement (weight and length) and blood withdrawal generally took less than 3 min/fish with minimum disturbance. The blood was allowed to clot at room temperature (22°C) for 1-2 h and kept at 4°C overnight. Plasma was collected after centrifugation 3,000 g for 5 min at 4°C (MIKRO 22R, Hettich, Germany) and divided into 200 μl aliquots and stored at -80°C until analyzed.

Table 1. Chemical analysis of the seawater used in bioassays

Item	Value
Temperature (°C)	20±1
Salinity (‰)	32.3~33.3
pH	8.0~8.2
Dissolved oxygen (mg/L)	6.9~7.4
NH_4^+-N (μg-at *N*/L)	3.37±0.4
NO_2^- -N (μg-at *N*/L)	0.95±0.1
NO_3^- - N (μg-at *N*/L)	2.18±0.3
PO_4^{3-} - P (μg-at *N*/L)	0.83±0.1
COD (mg/L)	1.02±0.1

Radioimmunoassay (RIA)

Plasma cortisol, estradiol-17β (E_2) and testosterone (T) levels were measured by radioimmunoassay (RIA), in the National Fisheries Bioscience Information Center (NFRDI), according to the methods of Lou et al. (1984). Rabbit anti-Cortisol-3-CMO-BSA, Rabbit anti-E_2-6-CMO-BSA and Rabbit anti-T-3 (E)-CMO-BSA sera were purchased from Cosmo-Bio Co. Ltd. (Tokyo, Japan). Non-radioactive steroids used as standards were purchased from Steraloids Inc. (Wilton, NH, USA). Radiolabeled steroids ([1,2,6,7-^{3}H]-Cortisol), ([2,4,6,7-^{3}H]-E_2) and ([1,2,6,7-^{3}H]-T) were purchased from Amersham Life Science (England). The sensitivities of the assay were 22.5 pg/ml, 12.5 pg/ml and 10 pg/ml for cortisol, E_2 and T, respectively. The intra- and inter-assay coefficient of variations at the 50% binding were 2.8 (n=5) and 8.1% (n=6) for cortisol, 3.4 (n=5) and 11.5% (n=6) for E_2, 2.3 (n=5) and 12.5% (n=6) for T, respectively. The cortisol antibody cross-reacted with 11-deoxycortisol (16.3%), cortisone (2.9%) and corticosterone (3.3%). Cross-reactivities of other steroids in the cortisol assay were less than 0.01%. The E_2 antibody cross-reacted with estron (0.5%), estriol (0.9%) and T (0.01%). All other steroids tested showed less than 0.01%. The T antibody cross-reacted with dihydrotestosterone (2.7%), androsten-3, 17-dione (0.5%), 11-ketotestosterone (0.5%) and androstenedione (0.35%). All other steroids tested showed less than 0.001%.

Statistical analysis

Statistical analysis were performed using SPSS/PC+statistical package. Significant differences between groups were determined using the Student's *t*-test for two groups, and level of significance was established at $P<0.05$.

Results

During the 3 weeks exposure to TBTO, there were few differences in exposure condition among treatment tanks which might be expected to confound the effects of TBTO treatment. Water quality conditions were stable throughout the exposure period and no marked differences among treatment tanks were observed (Table 1).

Although there was substantial variability in the size of the fish exposed, there were no significant differences in mean fish length or mass among treatment tanks. Mean length was

17.4±2.1 cm. The minimum and maximum fish lengths were 16.0 cm and 19.9 cm, respectively. The mean fish mass was 100 g, with minimum and maximum masses of 80 g and 120 g, respectively. There was no mortality during the exposure period. The fish was acclimated and examined. Externally all fish appeared healthy throughout the exposure. No differences in hepatosomatic index (HSI) detected among treatment groups. HSI ranged from 2.92±0.44%, with a mean of 2.25%. HSI was calculated relative to body weight after blood collection.

The effects of TBTO exposure on plasma cortisol levels are illustrated in Figure 1. Exposure of TBTO for different duration caused increased level of cortisol when compared to the reference group. No significant change in 1st week and 2nd week was observed over the duration of the experiment, except in 3rd week, the cortisol level reached to its maximum for the concentration of 1.41 and 3.05 μg/L TBTO (Fig. 1). The effects of TBTO exposure on plasma E_2 and T levels are illustrated in Figure 2 and 3. respectively. T levels did not have significat changes compared with control group during 2 week exposure in all treated group with TBTO. On the other hand, T concentrations were considerably reduced in 0.51, 1.41 and 3.05 μg/L TBTO compared with the control after 3rd week of exposure ($P<0.05$). Differ to cortisol and T levels investigation of the plasma E_2 concentrations by radioimmunoassay did not revealed a significant differences during 3rd week of exposure in all treated group with TBTO. E_2 levels were increased from baseline levels of 0.18±0.034 to 0.26±0.05 ng/ml E_2 but didn't have a significance in 0.52 μg/L TBTO after first week exposure ($P>0.05$). During 1st week of TBTO exposure E_2 level increased in a concentration-dependent manner. However, in the rest of exposure duration (i.e. 2nd and 3rd week) did not show and gradual increment in does-dependent manner.

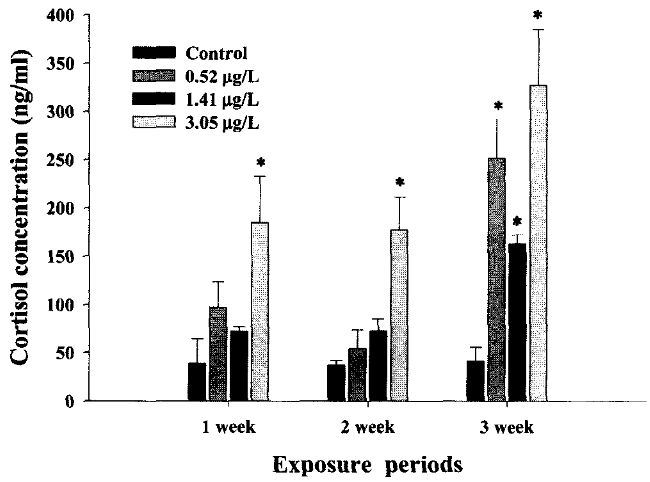

Fig. 1. Changes of plasma cortisol level in *Sebastes schlegeli* exposed to various concentrations of TBTO for 3 weeks. Each column represents the mean±S.E. (n=4). *Significantly different from control ($P<0.05$).

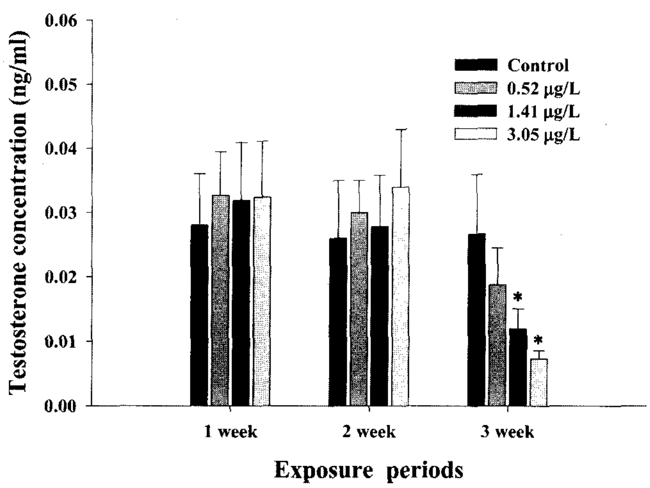

Fig. 2. Changes of plasma testosterone level in *Sebastes schlegeli* exposed to various concentrations of TBTO for 3 weeks. Each column represents the mean±S.E. (n=4). *Significantly different from control ($P<0.05$).

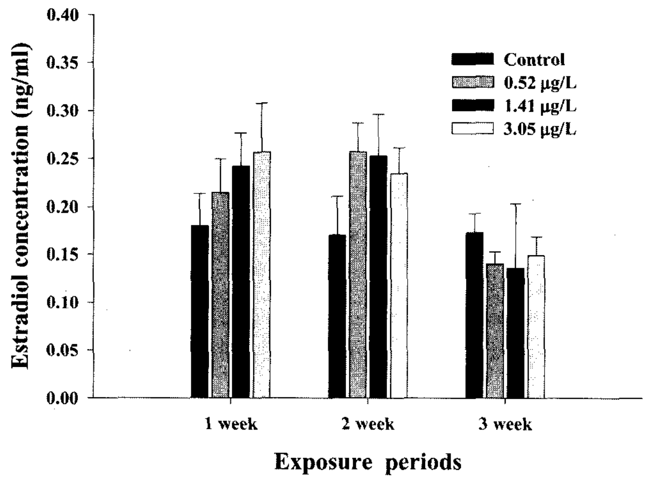

Fig. 3. Changes of plasma estradiol level in *Sebastes schlegeli* exposed to various concentrations of TBTO for 3 weeks. Each column represents the mean±S.E. (n=4). *Significantly different from control ($P<0.05$).

Discussion

Our results demonstrate that chronic exposure of Korean rockfish to TBTO, resulted in higher plasma cortisol concentration during the 3 weeks immersion. This increase in cortisol levels may be either due to increased production and/or decreased clearance of the steroid from circulation (Vijayan et al., 1997b). Previous studies of hepatic P-450 inducers showed an increased clearance of plasma steroid hormone from the circulation (Hansson and Rafter, 1983; Förlin and

Haux, 1985; Snowberger and Stegeman, 1987), arguing against the possibility of plasma steroid hormones as a factor for the higher TBTO concentration exposure in the present study. Also, our experiment for plasma biochemical studies indicated that the potential for negative effects to TBTO exposure in the rock fish.

These steroid, namely testosterone, estradiol-17β and cortisol were selected for monitoring in this study in order to determine, if any changes in steroid metabolism occur in response to TBTO exposure. The concentrations of T, E_2 and cortisol in the plasma, were measured throughout experimental by RIA. This technique was used as it facilitates the rapid and accurate quantitative analysis of large sample numbers. Ideally, RIA is able to accurately quantify the substance in question without extensive prior purification. In addition to the relatively well-definded actions during stress, cortisol is a major regulator of intermediary metabolism and normal physiological parameters in fish (Mommsen et al., 1999).

As cortisol is a gluconeogenic steroid hormone in trout (Vijayan et al., 1994), one would expect an increase in plasma cortisol concentration with elevated glucose levels (It did not present a result from this dissertation). The results showed increase of cortisol concentration in TBTO-exposed fishes (Fig. 1), which might result in cortisol being unavailable for normal physiological actions (Vijayan et al., 1997c). Cortisol, synthesized in the interrenal cells of teleost head kidney, has a major role in the physiological response to stressors. Exposure to xenobiotic alter cortisol secretion. Chemicals such as DDT, TCDD or PCBs activate cortisol secretion following acute exposures (Hontela, 1997). Cortisol also plays an important role in maintaining physiological homeostasis through effects on growth and osmoregulation (Hontela, 1997; Wendelaar, 1997).

Tributyltin-Oxide (TBTO) is extremely lipophlic, readily bioaccumulated and possess the potential ability of modulating the endocrine systems, and thereby interfere with reproduction and developmental processess in aquatic organisms. Imposex phenomenon, has been suggested disruption witch in there acts on target tissues to regulate gametogenesis, reproduction, sexual phenotype and behavioral characteristics of sex steroids. Elevations in tissue concentrations of testosterone (but not progesterone or estradiol) have been reported in the female dogwhelk, *Nassarius lapillus* exposed to TBT (Spooner et al., 1991). In the present experiment, illustrated in Fig. 2, the TBTO-0.52, 1.41 and 3.05 μl/L at 3 weeks exposure resulted considerable decreased with 0.02, 0.012 ($P<0.05$) and 0.007 ($P<0.05$) ng/ml of testosterone in plasma comparison to 0.028 ng/ml of the control group individually. This is agreement with the increased testosterone level in the TBT-Cl exposed snails, *Ilyanassa obsoleta* (Oberdorster et al., 1998, 2002) and TBT exposed periwinkle, *Littorina littorea* (Ronis and mason, 1996).

The numerous studies that include plasma estradiol concentration as a variable have demonstrated with few exceptions the reduction of circulating estradiol in the presence of organic contaminations both in the field (Johnson et al., 1998, 1993; Casillas et al., 1991), and in the laboratory exposures (Snowberger and Stegeman, 1987; Thomas, 1990; Singh, 1989; Pajor et al., 1990; Thomas and Budiantara, 1995; Thomas et al., 1999). The understanding of the processes by which organic contamination affect estradiol levels in not clear. It has been established that the drop in plasma estradiol in the presence of TBTO, when it occurs in clam, *Ruditapes decussata* is associated with an increase of the excretion of estradiol metabolites. And it could be due to indirect effects such as down-regulation of the expression of the enzyme (Morcillo et al., 1998). However, in this study, estradiol level did not differ significantly between fish from each treatment group (Fig. 2, $P<0.05$).

Toxicological studies have demonstrated the disruptive effects of xenobiotics on adrenal sterodidogenesis in teleosts (Ilan and Yaron, 1983; Hontela, 1997) as well as in birds and mammals (Lund, 1994; Mgbonyebi et al., 1994). In this study, the exposure of the Korean rockfish to TBTO revealed stable plasma cortisol level stable, in all groups exposed for 14 days, except 3.05 μg/L group. Results concerning showed a remarkable in cortisol concentration the influence of TBTO exposure at 21 days (Fig. 3). As seen in Fig. 3, cortisol levels were increased (by 2.5-3.5 fold) in all treatment fish at 21 days ($P<0.05$). Fishes exposed to TBTO show an cortisol rise with the passing of the time and TBTO concentration. This change can be interpreted as acclimation to the chemical stressors (Fu, 1990) or as an exhausion stage of the hypothalamo-pituitary-interrenal axis (James and Wigham, 1986).

In conclusion, TBT exposure caused increase in plasma cortisol as well as an alteration of testosterone in the Korean rockfish. These results suggest that TBTO at environmentally relevant (nanomolar) concentrations could disrupt endocrine secretions, and might be suitable for toxicological studies, particularly in view of the steroid hormones in marine organisms exposed to TBTO.

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