EROD ACTIVITY IN TILAPIA (*Oreochromis mossambicus*) EXPOSED TO A BLEACHED KRAFT MILL EFFLUENT

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Introduction

Alteration of the MFO (mixed function oxygenase) system in fish is a hallmark of biological characteristics of bleached kraft pulp and paper mill effluents (BKMEs). For examples, the 7-resorufin O-deethylase (EROD) activity, a sensitive indicator of CYP1A enzyme activity, in carp (*Cyprinus carpio*) was elevated when exposed to a treated pulp and paper mill effluent[1]. EROD activity in juvenile whitefish (*Coregonus lavaretus* L. *s.l.*) could be induced to 12-fold by exposed to 3.5% of a BKME[2]. White sucker sample from the downstream of several pulp mills also revealed increases in hepatic EROD activity comparing to the levels in fish from the reference locations[3]. Dioxins compounds are known to be present in BKMEs, and are partially, if not fully, responsible for MFO elevation in fish exposed to BKMEs, as reviewed by Hodson[4]. There were only few investigations on the impact of BKMEs in Taiwan freshwater ecosystem. Earlier studies showed that this type of industrial wastewater exhibited toxicity toward different aquatic animals. In a study of toxicity of a BKME in Taiwan, it was found that 5% dilution of the effluent significantly reduced oyster sperm viability, as well as increased the numbers of deformed oyster larvae[5]. The same study also reported that 25% dilution of the effluent was acutely toxic to daphnia[5]. In an earlier investigation (data unpublished), we have found that a BKME was not acutely toxic to juvenile fishes, Japanese medaka (*Oryzias latipes*) and tilapia (*Oreochromis mossambicus*), but could affect medaka embryo's survival and hatch. There was no report on the physiological or biochemical effect of BKMEs to fish in Taiwan. In the current report, we used hepatic EROD assay to evaluate the MFO inducibility of a BKME to tilapia.

Methods and Materials

Different sizes of sexually immature tilapia, weighing from 10 to 50 g, were separated into different experiment groups. One group of fish, designated as the field experiment group, was removed from our laboratory to a fishpond (4m x 4m x 1m) located at the outlet of a pulp/paper mill. The fish were retained in a 0.3m x 0.3m x 0.5m gridiron cage, which submerged completely in the water. The fishpond received a whole BKME without dilution, and contained other fish raised by the mill's personnel. Another group of fish was caged, in the same manner as the field group, in a fishpond (7m x 5m x 1.5m) in our college campus served as the field control. The third group (lab experiment group) of fish were exposed to 100% of the BKME. The BKME was collected at the same day for conducting field experiment. The fourth group was the laboratory control group, and was exposed to filtered, dechlorinated tap water only. Flow-through exposure systems for both laboratory groups were used in the study, and the flow rate was set at 10 mL/min.

Each fish was kept in a 30 L tank full of 20 L of water. The setup was kept in a dark room with the temperature of 25~26°C, and a photoperiod of light:dark=16:8. Three experiments have been conducted. Each group contained at least two fish, except the laboratory control, in which the numbers varied. Animals were not fed during the experiments. The exposure time was one week.

By the end of each experiment, fish body weighs were measured, and their livers were removed and weighed. Preparation of microsomal fractions and the EROD assay were based on the method described by Peince[6]. Briefly, 0.5g of liver was homogenized and added with 1 mL of TES buffer (0.05M Tris-HCl, 1.0nM EDTA, 0.25M sucrose, pH 7.2 at 25°C). The homogenate was centrifuged at 15600 x g for 5 minutes, and supernatant was re-centrifuged at 213000 x g at 4°C for 13 minutes. The resultant pellet was re-suspended with 1 mL of TES buffer. EROD activity was measured by monitoring the production of fluorometrical resorufin from ethoxyresorufin added in the microsome. Protein contents were determined by the BioRad® protein assay. The statistical significance of the differences for the observed responses between treated and control animals were determined by Student *t*-test or ANOVA (analysis of variance). The significant level was set at p=0.05.

Results and Discussion

In the first experiment, two fish in the laboratory experiment group had EROD induction up to 6- and 4-fold, respectively, comparing to that of the laboratory control fish (16.7 pmol/min/mg protein)(Figure 1a). The field experiment group showed a much higher elevation in EROD activity, with an increase up to 22-fold in one of the fish, and 7~8-fold for the other two (Figure 1a). It should be mentioned that the EROD activity between the laboratory and the field experiment groups were not significantly different. We did not have the field control group in this experiment due to the pond at our campus not being available to us during that time period.

In the second experiment, the same results as the first one was observed, with the highest EROD induction being in the field experiment group. The maximal induction was up to 15-fold (Figure 1b). Two fish exposed to the 100% effluent in the laboratory also resulted in 7-fold increases in EROD activity (Figure 1b). By comparing the EROD activity in two experimental groups to that in the field control group (fish caged in the college pond), we have also noted that the difference was significant (Figure 1b). The ANOVA results of three groups, not including the laboratory control, also showed that the differences in EROD activity were significant.

Results of the third experiment was similar to the previous two experiments. One fish in the field experiment group died during the experiment for unknown reasons. The other two fish showed a much higher EROD activity than the average of the three laboratory control fish (5.79 ± 1.99 pmol/min/mg protein). The induction was about 13-fold (Figure 1c), and the difference was statistically significant. Laboratory experiment fish also showed significantly higher EROD activity than the laboratory controls, and the increase was 10-fold (57.16 vs. 5.79 pmol/min/mg protein). In this experiment, however, the EROD activity of the field control group was increased to an unexpectedly high level (Figure 1c). If comparing to the laboratory control group, the highest EROD induction was about 57-fold (331.62 vs. 5.79 pmol/min/mg protein), the highest value observed in this study. Other two fish in this group also have very high EROD activity, which were 90.93 and 47.54 pmol/min/mg protein, respectively (Figure 1c). This was quite unexpected and not observed in the first two experiments, so we could only speculate that during

the exposure, some environmental factors altered their hepatic biochemical process resulting in such a dramatic change. It may be owning to runoff carrying contaminants into the fishpond, or even a not-well-maintained water pump with lubricant leaking from it.

Although our study was the first one to characterize biochemical responses of fish exposed to Taiwan BKMEs, our results were not surprised, since there have numerous reports been documented regarding changes of EROD activity in fish exposed to BKMEs either in fields or in laboratories. For instance, using a laboratory exposure system, Martel and his colleagues diluted different pulp/paper mill effluents up to 40% and found that a percent dilution as low as 0.75% in one of the effluent could result in a significant EROD induction in sexually immature rainbow trout (*Oncorhynchus mykiss*)[8]. White sucker caught immediately and 95 km downstream from a BKME revealed 10-fold and 5-fold increases in hepatic AHH (aryl hydrocarbon hydroxylase) activity, respectively, relative to fish caught 10 km upstream[9]. It should be mentioned that in most of the studies, diluted BKMEs were assayed in stead of the whole effluent used in our study. The outcomes of this study are three-fold. Firstly, the strength of the whole BKME in eliciting the biochemical response of tilapia was determined by using different exposure systems, either field or laboratory. Secondary, the field-caging exposure system was the most sensitive in detecting fish responses, but may not be suitable for future field studies on the receiving water due to experimental difficulty and multiple pollution sources in most of the rivers in Taiwan. The flow-through system may not truly mimic the conditions occurred at field, but is more feasible for further studies. Thirdly, although tilapia are widely distributed in Taiwan freshwater system, application of this species to investigate its responses to environmental changes are limited [10,11]. Nevertheless, the results of this study indicated the sensitivity of the EROD assay in detecting subtle changes of organisms to BKMEs, and could provide some basic information for future studies.

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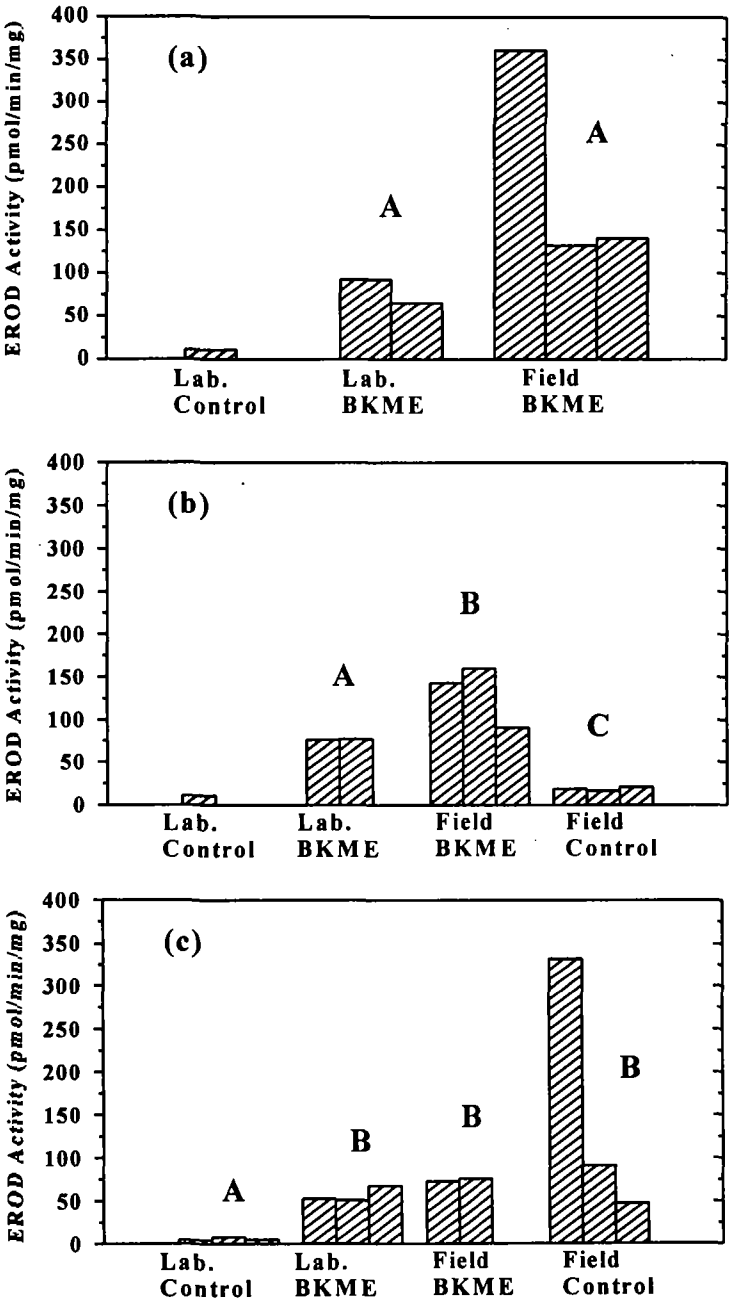

Figure1. EROD activity in tilapia exposed to the BKME in three experiments conducted at different time periods. Different labels indicate that the averages of the two groups are significantly different.