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Research Article

ZEBRAFISH EMBRYOS EXHIBITED MORTALITY, ALTERED HATCHABILITY, AND DEVELOPMENTAL ABNORMALITIES FOLLOWING TRIBUTYLTIN EXPOSURE

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ABSTRACT: Tributyltin (TBT), an organotin-endocrine-disrupting substance, is recognized as one of the most important toxic environmental pollutants. The present study was carried out to investigate the developmental toxicity of TBT on zebrafish embryos. Eggs of zebrafish were exposed to 2.5, 5, and 10 µg of TBT per liter of E3 medium for 72 hours to evaluate various parameters of developmental toxicity. The TBT exposure resulted in a dose-dependent negative effect on the hatching rate and increased larval mortality. Different abnormalities in larvae, following exposure to various concentrations of TBT, have also been observed. The tail and spinal deformities, along with dwarfism, were important abnormalities observed in TBT-exposed zebrafish embryos. It has been concluded that tributyltin produced deleterious effects on embryos and larvae during developmental stages, along with multiple deformities that ultimately affected the survival and growth of fish.

Keywords: Tributyltin, Zebrafish, Larvae, Developmental abnormalities.

INTRODUCTION

Various endocrine system regulations, like homeostasis and physiological coordination, alter due to the effects of various xenobiotic chemicals in marine ecosystems [1]. Tributyltin, an endocrine disruptor that poses a threat to the environment, is used as a fungicide in the wood and plastic industry, and as an antifouling agent to be coated on ship hulls [2]. TBT is detected at high levels in some water samples because of its long half-life and extensive unlawful use [3, 4]. Due to the significant negative effects on aquatic life, the possibility of aquatic contamination caused by TBT use has been of great concern [5].

Zebrafish embryos are transparent in the early stages of development, and their eggs are fertilized outside which helps to explore the developmental toxicology [6, 7]. Rapid development and external post-fertilization make embryos more vulnerable to environmental influences and make it easier to observe anatomical malformation, hatching rate, and other behavioral changes [8]. Early stages of organism development frequently respond to external stimuli more frequently than juvenile and adult stages [9]. Bioaccumulation happens when TBT is exposed to fish, whether the fish are embryonic, larval, or juvenile [10]. The transparent embryonic chorion makes it simple to recognize various stages of larvae [11, 12, 13]. Zebrafish at an early stage of life are gaining popularity for testing the toxicity of chemicals and effluents [14, 15].

TBT has also been reported to cause increased mortality and affect larval development [16, 17], decreased hatching [18], and altered population sex ratios [19]. It has been reported that TBT exposure (0.01, 0.1, and 1 nM for 96 hours) produced axial deformity, spinal curve, and pericardial edema in zebrafish larvae [20]. TBT exposure at lower concentrations (0.1, 1, and 10 ng/L) for 144 hours decreased hatchability and produced morphological malformations in cuvier embryos [9]. Zebrafish embryos exposed to TBT have also been

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reported to cause higher mortality and abnormal embryonic development [21]. TBT exposure of 0.2 and 20 µg/L produced harmful effects on the zebrafish larvae [22]. Similarly, neuromuscular and skeletal malformations have also been reported in medaka (Oryziaslatipes) when exposed to TBT [23]. Apoptosis is one of the important mechanisms related to abnormal development due to stress [24]. Zebrafish is used as an animal model to explore the toxicity of various chemicals [25, 26]. Although various studies have been done related to the toxic effects of TBT in male and female animals including aquatic species, there is a need to evaluate the developmental defects in the early stages of life in aquatic animal models like zebrafish following exposure to TBT at various concentrations. Thus, the present study was carried out with a focus on the toxic effect of TBT on zebrafish larvae during the developmental period.

MATERIALS AND METHODS

Experimental design

Zebrafish eggs were obtained by in-house breeding of male and female zebrafish in the ratio of 1:2. Eggs were collected in a petri dish from a breeding tank. After 6 hours, eggs were observed to exclude dead or unfertilized eggs. Eggs were divided into four groups (50 in each group) and kept in 96-well plates (1 egg per well) and enriched with E3 medium. The E3 medium was prepared using the protocol/procedure reported earlier [27]. Untreated eggs were kept as a control group. However, eggs from the other three groups were exposed to 2.5, 5, and 10 µg/L concentrations of TBT in E3 medium for 72 hours. The E3 medium was changed daily, and the concentrations of TBT were maintained for 72 hours. We selected 10 µg/L concentrations as the highest level of exposure, which was based on a previous report of altered morphology and behavior in larvae exposed to TBT at 20 µg/L for 24 hours [22]. Looking at the increased duration of exposure to TBT in the present study, 10 µg/L concentration was the highest level of exposure. Two other levels of exposure were serially half concentrations of the highest level of exposure. Additionally, LC50-96 h of TBT in zebrafish embryos has been reported to be 2.7 µg/L [28], which is approximately similar to the lowest concentration of TBT used in this study.

Evaluation of developmental toxicity

Cumulative mortality percentage was calculated after 24, 48, 72, 96, and 120 hours post-fertilization (hpf), whereas hatching percentage was calculated after 48, 72, and 96 hpf. Larvae were observed for different abnormalities at 96 hpf with the help of a stereo zoom microscope (Model: SZ61TR trinocular stereo zoom microscope with attached MagCam HD Pro camera).

Statistical analysis

The GraphPad Prism (Version 9.4.1.) was used for statistical analyses of all data. Normality and equal variance were checked using the Kolmogorov-Smirnov test and Bartlett's test, respectively. Data with normal distribution and equal variance were analyzed by one-way analysis of variance (ANOVA), followed by Tukey's HSD test. Data without normal distribution or equal variance were analyzed by the Kruskal-Wallis test, followed by Dunn's test. The value of $p< 0.05$(*) was considered statistically significant, and $p< 0.01$(**), $p< 0.005$(***), and $p< 0.001$(****) were considered as highly significant statistically.

RESULTS AND DISCUSSION

Mortality (%) in zebrafish embryos/larvae

Cumulative mortality percentages at 24, 48, 72, and 96 hours post-treatment were significantly higher in the TBT-10 µg/L group as compared to those of the control group. However, the cumulative mortality percentage at 120 hours in all toxicity groups was significantly higher as compared to that of the control group (Fig.1). The TBT exposure for 72 hours resulted in a dose-dependent effect in terms of cumulative mortality percentage.

Hatching rate of zebrafish embryos

The hatching rate in zebrafish embryos in all toxicity groups at 48 hours post-treatment did not differ significantly as compared to that of the control group. However, TBT exposure significantly decreased (dose-dependent) the hatching rate at 72 and 96 hours post-treatment as compared to those of the control group (Fig. 2).

Abnormalities in zebrafish larvae

The majority of abnormalities (%) in zebrafish larvae of all toxicity groups were non-significantly higher as compared to those of the control group. However, TBT exposure significantly increased spinal deformity (%) at a higher exposure level of TBT (10 µg/L) as compared to that of the control group (Fig. 3). Different abnormalities in larvae following exposure to various concentrations of TBT are shown in Fig. 4-6.

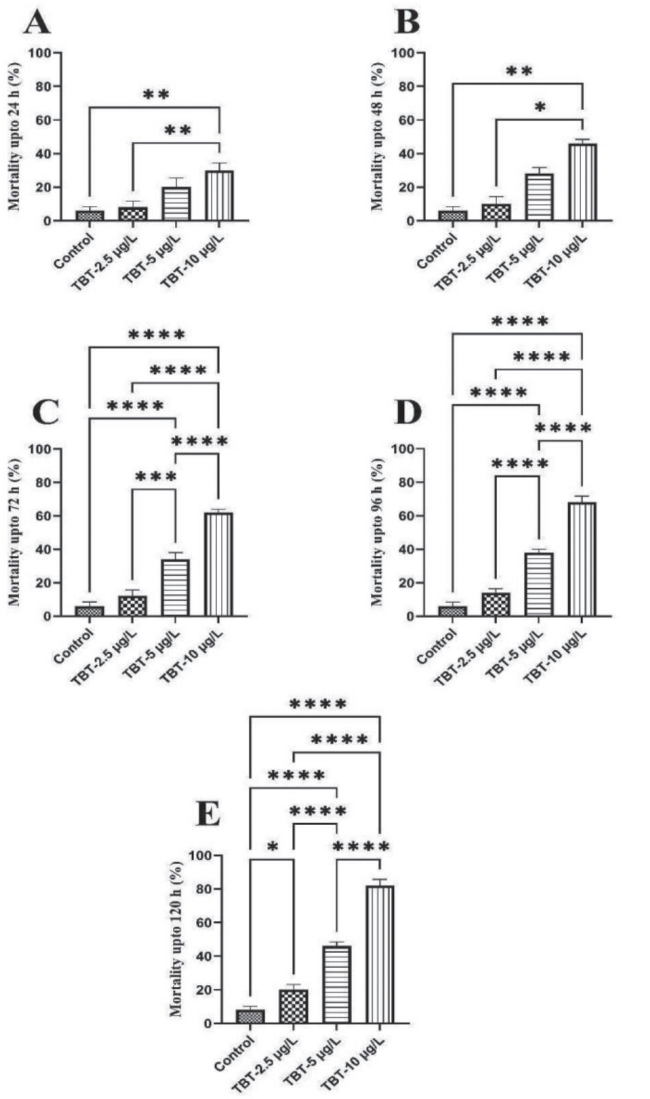

Fig. 1. Mortality (%) in zebrafish embryo/larvae following TBT exposure for 72 hours. [(A, C, D, E): Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's HSD test. (B): Data were analyzed by the Kruskal-Wallis test followed by Dunn's test. Where * indicates p<0.05, **indicates p<0.01, *** indicates p<0.005, **** indicates p<0.001]

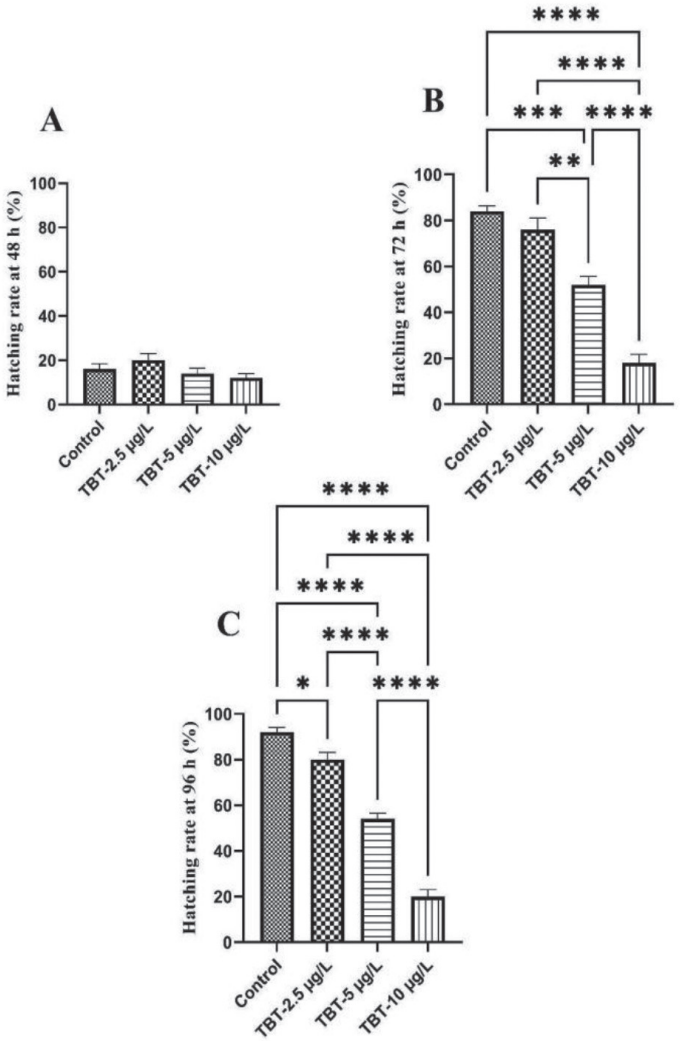

Fig. 2. Hatching (%) of zebrafish embryo following TBT exposure for 72 hours. [(A, B, C): Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's HSD test. Where * indicates p<0.05, **indicates p<0.01, *** indicates p<0.005, **** indicates p<0.001].

In this study, we evaluated the developmental defects in the larvae produced by the TBT. The TBT exposure for 72 hours resulted in a dose-dependent increase in the cumulative mortality percentage and a decreased hatching rate at 72 and 96 hours in larvae. Different abnormalities like dead coagulated embryos and larvae, spinal deformity, tail deformity, yolk sac edema, pericardial edema, and dwarfism in larvae following exposure to various concentrations of TBT have also been observed. Similarly, to the present study, tin chloride has been reported to cause tail deformity in embryos at 28 hpf following exposure to 100 µM concentration, while at 96 hpf, larvae exposed to 50 µM exhibited altered growth, small-sized head and eyes, twisted body, pericardial edema, and a smaller caudal fin [29]. Tin levels in embryos have been reported to be linked with dorsal curvature percentages, implying that excessive tin accumulation in embryos produced a deleterious impact on axial development and led to dorsal curvature, which is an indication of defective embryogenesis due to stress-induced apoptosis [24].

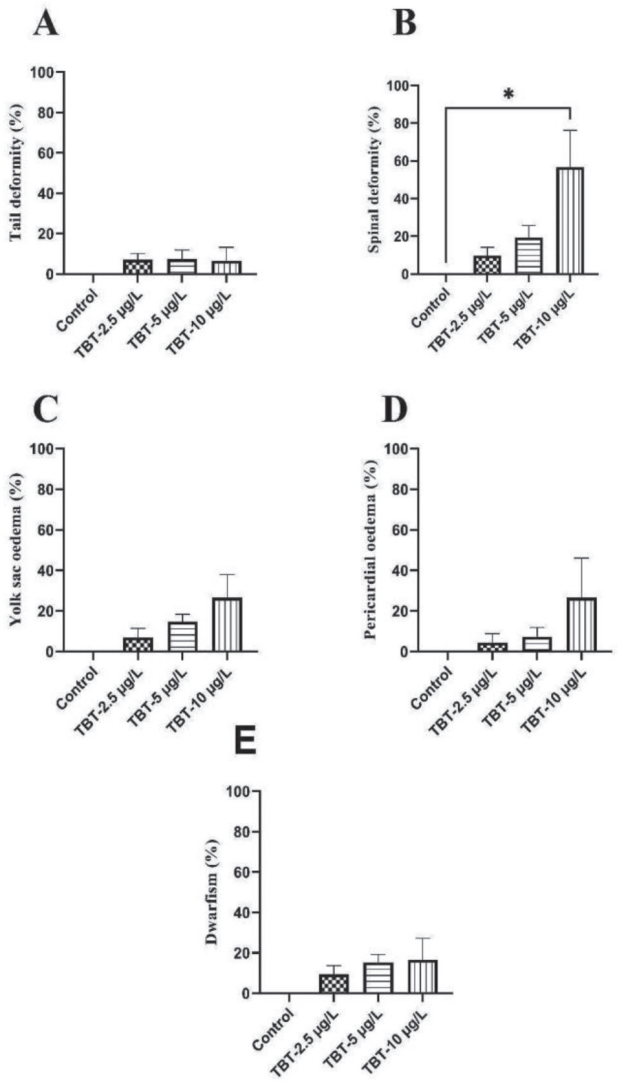

Fig. 3. Different abnormalities (%) in zebrafish larvae following TBT exposure for 72 hours. [(A, B, C, D, E): Data were analyzed by Kruskal - Wallis test followed by Dunn's test. Where * indicates $p<0.05$].

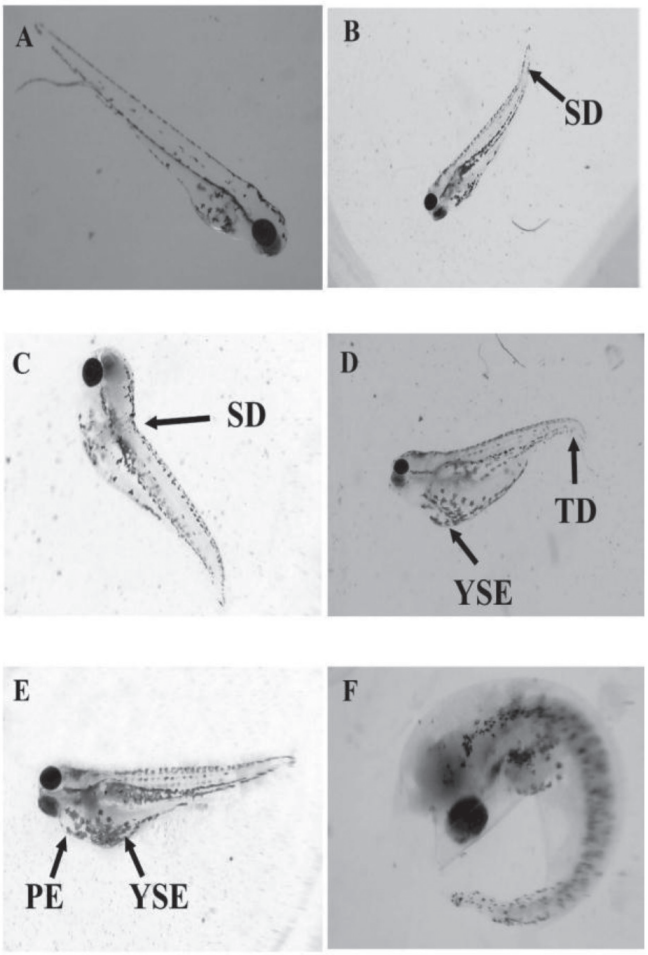

Fig. 4. Zebrafish larvae exposed to TBT at a concentration of 2.5 µg/L for 72 hours. [A: Normal larvae, B and C: Larvae showing spinal deformity (SD), D: Larvae having tail deformity (TD), yolk sac edema (YSE) and dwarfism, E: Larvae having yolk sac edema (YSE), pericardial edema (PE) and dwarfism (less severity) and F: Dead coiled larvae].

In the present study, the toxic effect of tributyltin was studied on different developmental stages of zebrafish embryos. The changes in hatching time are a substantial stress reaction for fish larvae [30]. At the early stage of larval development, aromatase expression is higher [31], and defects in the development following TBT exposure might be due to the inhibition of aromatase expression in the developing age [22]. There have been examples of prematurely born eggs having developmental issues such as reduced growth, spine curvature, and yolk sac edema [32, 33, 34]. TBT has shown a deleterious impact on mortality and hatching time, which are linked to decreased oxidative respiration [35]. TBT exposure in Medaka females caused lower fertility, fewer hatched eggs, and poor egg quality [36, 37]. TBT exposure has been reported to produce smaller yolk sacs, delayed eye development, and hemorrhage along with a significantly decreased cardiac beat [21]. In addition to this, TBT exposure has been reported to reduce the energy metabolism in Chinese rare Minnow (*Gobiocypris rarus*) larvae, which might be responsible for abnormal behavior [38]. However, Liang *et al.* (2017) reported that TBT has no effect on oxygen uptake in zebrafish larvae, but alters the behavior and timing of hatching [20]. Ultimately, the exposure of TBT to eggs or larvae of aquatic animals may be harmful as it causes oxidative stress-mediated changes in developing organs.

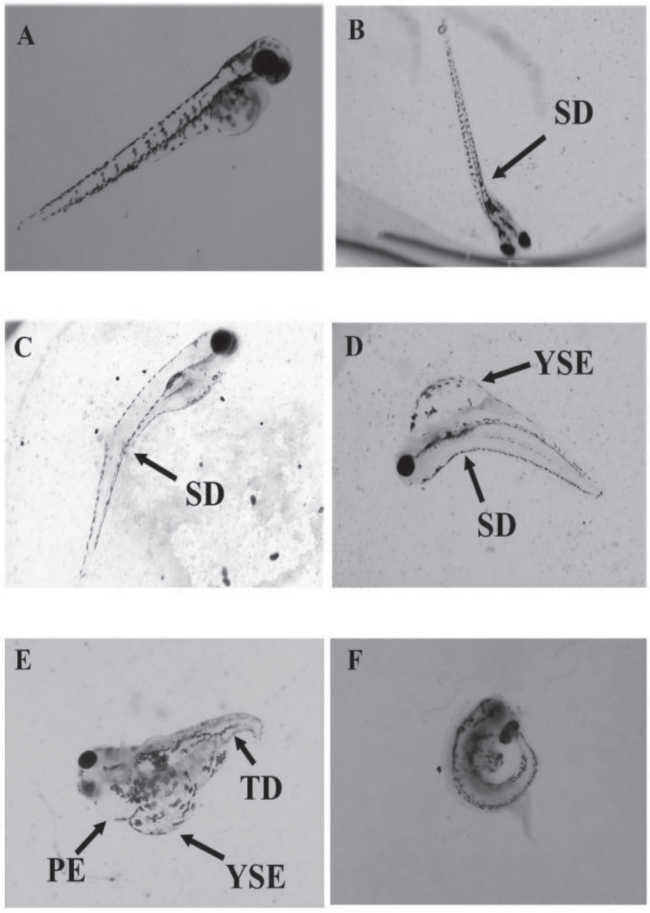

Fig. 5. Zebrafish larvae exposed to TBT at a concentration of 5 µg/L for 72 hours. [A: Normal larvae, B and C: Larvae showing severe spinal deformity (SD), D: Larvae having spinal deformity (SD), yolk sac edema (YSE), and dwarfism, E: Larvae having tail deformity (TD), yolk sac edema (YSE), pericardial edema (PE) and dwarfism and F: Dead supercoiled larvae].

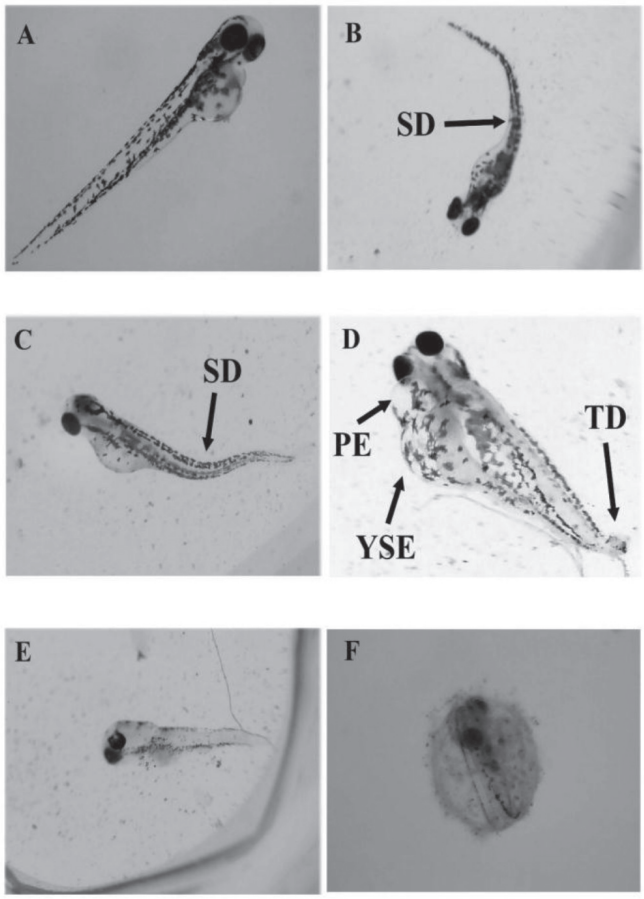

Fig. 6. Zebrafish larvae exposed to TBT at a concentration of 10 µg/L for 72 hours. [A: Normal larvae, B and C: Larvae showing highly severe spinal deformity (SD), D: Larvae having tail deformity (TD), yolk sac edema (YSE), pericardial edema (PE) and dwarfism, E: Dead coagulated larvae and F: Dead coagulated embryo]

CONCLUSIONS

Zebrafish embryos have been adversely affected by exposure to various concentrations of tributyltin in terms of both survival and hatching percentage. Zebrafish larvae exposed to tributyltin exhibited multiple developmental abnormalities, typically tail and spinal deformity, along with dwarfism, which may ultimately affect the survival of fish.

Farmers being unaware of the adverse consequences of using chemicals and aqua-medicines are fully dependent on private aquaculture consultants for time-to-time advice, which may have augmented their indiscriminate use. Initiative for the implementation of better management practices by creating awareness among farmers and adopting strict aquaculture policy guidelines might improve the scenario.

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