

Original Research Article

Bisphenol A Disrupt Endogenous Estrone, Estriol and Estradiol Levels in Female Albino Wistar Rats

ABSTRACT:

Bisphenol A (BPA) is a contaminant with increasing exposure and exerts both toxic and estrogenic effects on cells. The general population is potentially exposed to many chemicals that can affect the endocrine system. These substances are called endocrine disruptors (EDs), and among them Bisphenol A (BPA) is one of the most widely used and well-studied. This study investigates the possibility of blood serum endogenous estrogens levels perturbations at prevailing low exposure rates of BPA in albino Wistar rats. Eleven experimental groups each containing five (10) female rats were administered graded doses of BPA/kg body weight (bw)/day. The first control group was given water. Animals blood were collected weekly for twelve weeks and serum sample specimens analyzed by routine diagnostic procedures for estrogens assay using Chemwell Chemical Analyzer. Significantly increased concentrations of estrone and estradiol were observed, alongside significant decreased estriol concentrations, at all concentrations of BPA exposure at different time suggesting that bisphenol A upsets endogenous estrogens and causes perturbation of estradiol concentrations. These findings point to the overall disruption of estrogen metabolism.

Keywords: Bisphenol A, Estrone, Estriol, Estradiol, Hormone,

INTRODUCTION

There is still an ongoing debate whether environmental levels of BPA are harmful for the population or not. Bisphenol A (BPA) is an endocrine disruptor (ED), that still receives attention from the global scientific community and the general public, due to its ubiquity in our environment and uncertainties about its effects on humans. It leaks from polycarbonate plastics, which are used in food and drink containers. BPA enters the body by the ingestion of contaminated food and beverages, through the skin by contact with thermal receipts (Ehrlich et al. 2014,) and inhalation of cigarette smoke or dust (Braun et al. 2011). BPA is a weak estrogen when considering its binding activities to the estrogen receptor (ER) (Welshons et al. 2003). On the other hand, it can act with the same potency as endogenous estradiol (E2) on the non-classical membrane estrogen receptor (Alonso- Magdalena et al. 2012). Its mode of action, however, is much more complex. It may act through other nuclear receptors including the estrogen related receptor (Delfosse et al. 2014), androgen receptor (Teng et al. 2013), thyroid receptor (Moriyama et al. 2002), glucocorticoid receptor (Sargis et al. 2010), peroxisome proliferator activated

receptor γ (PPAR γ) (Pereira-Fernandes et al. 2013, Wang et al. 2010) and pregnane X receptor (Sui et al. 2012). BPA is capable of inducing toxic effect on non-reproductive vital organs; several studies have reported that absorption of BPA has caused extensive damage to the liver and kidney (Ezeonu et al 2015, Oguazu et al 2015), formation of multinucleated giant cells in liver hepatocytes, DNA adduct formation and induced the production of free radicals in hepatocytes in vitro (LaKind et al 2012). Indeed, this chemical compound may be involved in adipose tissue dysfunction, metabolic/endocrine dysfunctions, cancer and fertility problems (Wang et al 2012.), impaired plasma glucose (Teppala et al 2012.), involved in insulin resistance, (Alonso-Magdalena et al 2010), causes permanent chromosomal damage linked to recurrent miscarriage and birth defects (Vom and Hughes., 2005), spur both the formation and growth of fat cells, (Braun et al, 2011). An interaction of BPA with the expression and activity of steroidogenic enzymes has also been reported (Gilibili et al. 2014, Ye et al. 2014). Moreover, BPA exerts a non-monotonic dose response at low physiologically relevant concentrations, with tissue-specific effects (Wetherill et al. 2007). Endogenous estrogens are thought to have an important role in the developing and maintaining female sexual characteristics. The three major endogenous estrogens in females that have estrogenic hormonal activity are; estrone, estradiol, and estriol. They are the three major naturally occurring forms of estrogen in females. Estradiol (**E2 estrogen**) produced in the ovaries primarily by the follicles and corpus luteum, is the most potent and abundant. It is essential for formation of secondary sexual characteristics such as pubic hair, breasts and healthy menstrual cycle. These disturbing facts raise questions about the extent to which current, widespread exposures to BPA are contributing to the burden of infertility and reproductive health challenges. The aim of this study is to unveil/establish the possible effects of Bisphenol A on sex hormones in female wistar albino rats.

MATERIALS AND METHODS

Non-pregnant female rats of age 5 weeks were acclimatized in the laboratory for seven days and randomly divided into eleven groups experimental of 10 rats each and respectively administered 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1 mg of BPA/kg body weight (bw)/day. The first group which served as control did not receive any treatment but distilled water instead. The graded doses of BPA were dissolved in distilled water and administered by oral gavage using intubation canular. Blood were obtained from the tail of the various groups by capillary action weekly, after BPA administration for thirteen (13) weeks. Blood samples were processed for clinical assay.

Animals were housed in aluminum wire-mesh cages in a well-ventilated animal house with a 12 h dark/light cycle and at room temperature and were provided commercial rat pellets (Vital feed from vital group of Company, Nigeria) and water *ad libitum*.

At the end of the experiments serum, Estrone, Estriol and Estradiol were assayed weekly using Chemwell 2910 Auotanalyser. All reagents were commercially obtained as already prepared kits. The kits for Estrone, Estriol and Estradiol were purchased from Egyptian company for Biotechnology (SAE) Cairo Egypt. Individual tests were carried out according to the kit specifications

The data obtained from each set of study were subjected to statistical analysis using the IBM Statistics software, version 20. Differences between obtained values (mean \pm SD) were carried out by one-way analysis of variance (ANOVA) using SPSS software version 20.0 followed by the Tukey-Kramer multiple comparison test. A $P \leq 0.05$ was taken as a criterion for a statistically significant difference.

RESULTS

EFFECT ON ESTRADIOL (ESTROGEN (E2))

It was observed that BPA induced a dose dependent increase in estrogen concentration, which increased with sustained administration as shown in fig 2. The higher concentrations of BPA caused a spike in estrogen concentration which is maintained for all weeks, on the other hand, lower concentration appeared to maintain a slight but steady increase in estrogen concentrations over time, with sustained administration, which did not differ significantly from the estrogen level in the control group for week 1 to 3 for the dose groups that received 0.05mg/kg to 0.5mg/kg of BPA. Across the weeks, there is significant increase ($p \leq 0.05$) in the serum estrogen level. In each week, within the groups there is a significant increase that is dose dependent especially at dose group 0.6mg/kg to dose group 1mg/kg. There is significant increase in estradiol at groups 0.6mg/kg, 0.7mg/kg, 0.8mg/kg, 0.9mg/kg and 1mg/kg at all time interval. The group performance profile revealed that BPA enhanced estrogen production in dose dependent manner. Higher doses of BPA induced higher estrogen production. However, there is noticeable reversion in estrogen profile with time after the BPA dose of 0.8mg/kg group (see fig 1). there was instant estrogen spike, which steadily rise as time progressed for group 0.9mg/kg and 1mg/kg but the observed rise was reversed time dependent.

In all instances, a dose dependent increase was observed for group 0.8mg/kg, 0.9mg/kg and 1mg/kg. The group that received 1mg BPA per kg body weight showed the highest level of estradiol, these were followed by 0.9mg/kg. These groups recorded highest value at week-1 and gradually decrease over time; the concentration of estradiol for these groups was lowest at week-13 (fig 1). Groups 0.6mg/kg and 0.7mg/kg are significantly higher than those of control and week-0, at $p \leq 0.05$. These groups recorded the lowest value at week-1, and showed steady increase over time with their highest value obtained at week-13. Between week-3 to week-13, a significant dose dependent increase was observed for group 0.05mg/kg, 0.1mg/kg and 0.2 mg/kg at $p \leq 0.05$; which was consistent over time. Groups 0.05mg/kg to 0.7 mg/kg recorded their highest value at week-13 and lowest value for estradiol at week-1. While group 0.8mg/kg to 1 mg/kg recorded their highest value at week-1 and lowest value at week-13 (fig 1).

The highest serum concentration of estradiol was recorded in week-13 for groups 0.05mg/kg to 0.7mg/kg; groups 0.8mg/kg to 1mg/kg recorded low estradiol levels at week-13 and high estradiol levels at week-1. In fig 1, a time dependent effect was observed, as the duration of exposure to graded doses of BPA continued, the serum concentration of estradiol continued to rise, up-to group 0.8mg/kg to 1mg/kg where the reverse effect was obtained with the estradiol level decreasing as the duration of the exposure to BPA extends. Between group 0.7mg/kg and 0.8mg/kg was the point of reversal of the trend (fig 1).

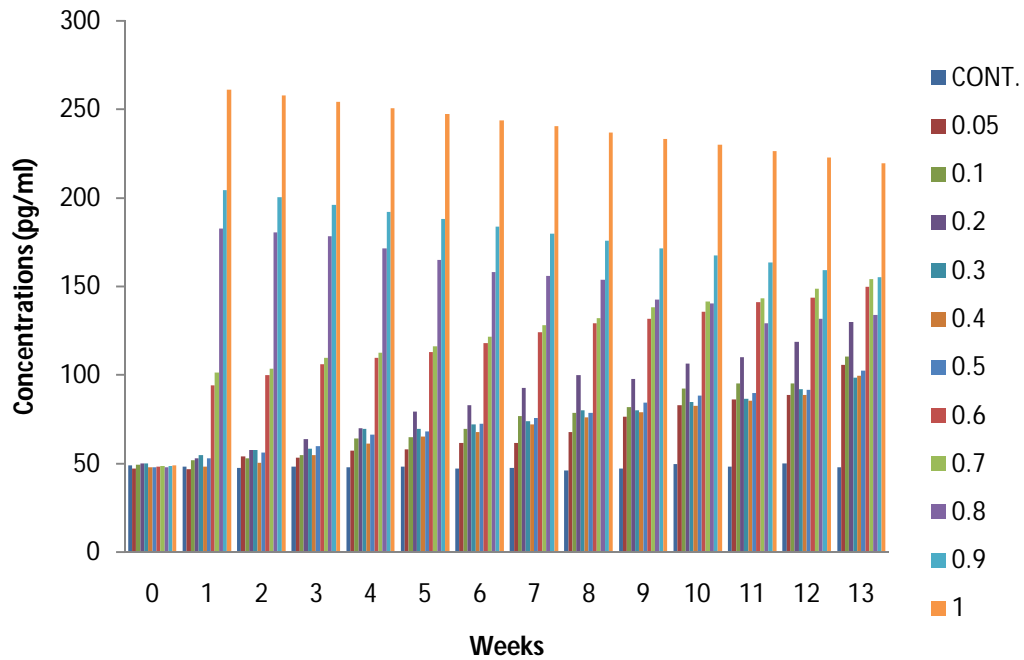


Fig. 1; Chart of concentration against weeks (durations) for change in Estradiol E2 (Estrogen).

EFFECT ON CONJUGATED ESTRADIOL

It was observed that BPA induced a dose a significant decrease in the conjugated estradiol concentration when compared with control throughout the period of exposure (fig 2).

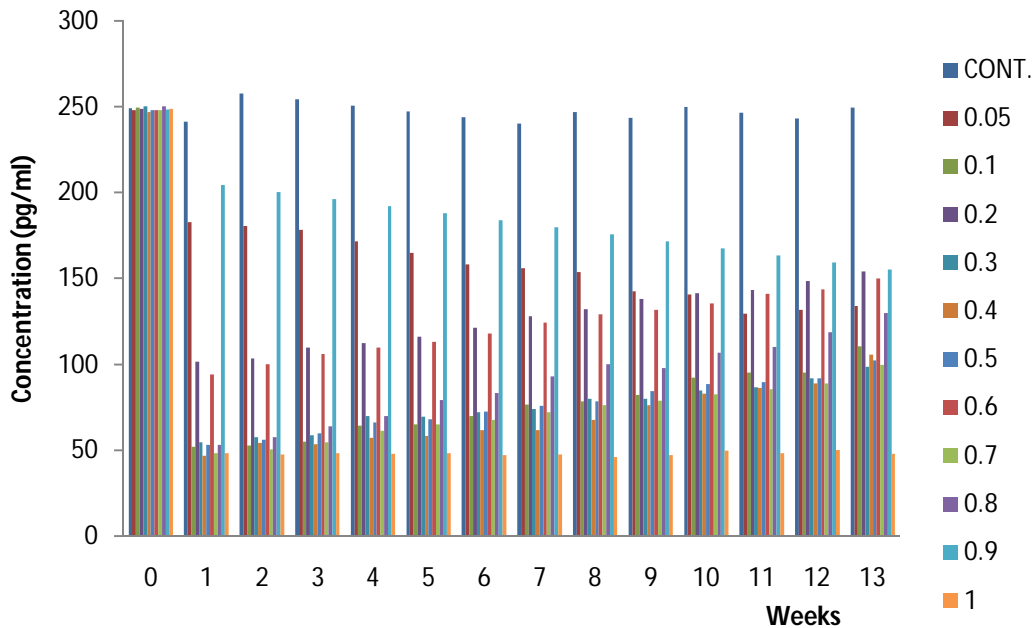


Fig. 2; Chart of concentration against weeks (durations) for change in conjugated Estradiol E2 (Estrogen).

EFFECT ON ESTRONE

At the onset of the experiment, it was observed that the estrone serum concentration were increase across the weeks (fig. 3) . Estrone levels were higher in week 1 for groups 0.8mg/kg to 1mg/kg. There is significant rise in estrone level when compared with the control at $p \leq 0.05$. Groups 0.05mg/kg to 0.5mg/kg showed a non significant difference at weeks-1 to week-3. As the duration of exposure increased, the level of estrone increased correspondingly (fig 3). At groups 0.8mg/kg to 1mg/kg, there is a turn around with the week 13 having the least rise in estrone level. The group performance profile reveal a dose dependent increase which is more pronounced at higher doses 1mg/kg, it was observed that the estrone serum concentration showed slight increase over time for group 0.05mg/kg to 0.5mg/kg, this was followed for a spike rise. Again from group 0.8mg/kg, there is decline in sterone in the time (see fig. 3) that is,. Estrone levels were higher in week 13 for groups 0.05mg/kg to 0.7mg/kg, but the reverse was the case in groups 0.8mg/kg to 1mg/kg.

In all instances, a dose dependent increase was observed for group 0.8mg/kg, 0.9mg/kg and 1mg/kg. The group the received 1mg BPA per kg body weight showed the highest level of estrone, these was followed by 0.9mg/kg. These groups recorded highest value at week-1 and gradually decrease over time; the concentration of estrone for these groups was lowest at week-13 (fig 3). Groups 0.6mg/kg and 0.7mg/kg are significantly higher than those of control and week-0, at $p \leq 0.05$. These group recorded the lowest value at week-1, and showed steady increase over time with their highest value obtained at week-13. Between week-3 to week-13, a

significant dose dependent increase was observed for group 0.05mg/kg, 0.1mg/kg and 0.2mg/kg at $p \leq 0.05$; which was consistent over time. Groups 0.05mg/kg to 0.7mg/kg recorded their highest value at week-13 and lowest value for estrone at week-1. While group 0.8mg/kg to 1mg/kg recorded their highest value at week-1 and lowest value at week-13 (fig 3).

The highest serum concentration of estrone was recorded in week-13 for groups 0.05mg/kg to 0.7mg/kg; groups 0.8mg/kg to 1mg/kg recorded low estrone levels at week-13 and high estrone levels at week-1. In fig 2, a time dependent effect was observed, as the duration of exposure to graded doses of BPA continued, the serum concentration of estrone continued to rise, up-to group 0.8mg/kg to 1mg/kg where the reverse effect was obtained with the estradiol level decreasing as the duration of the exposure to BPA extends. Between group 0.7mg/kg and 0.8mg/kg was the point of reversal of the trend (fig 3).

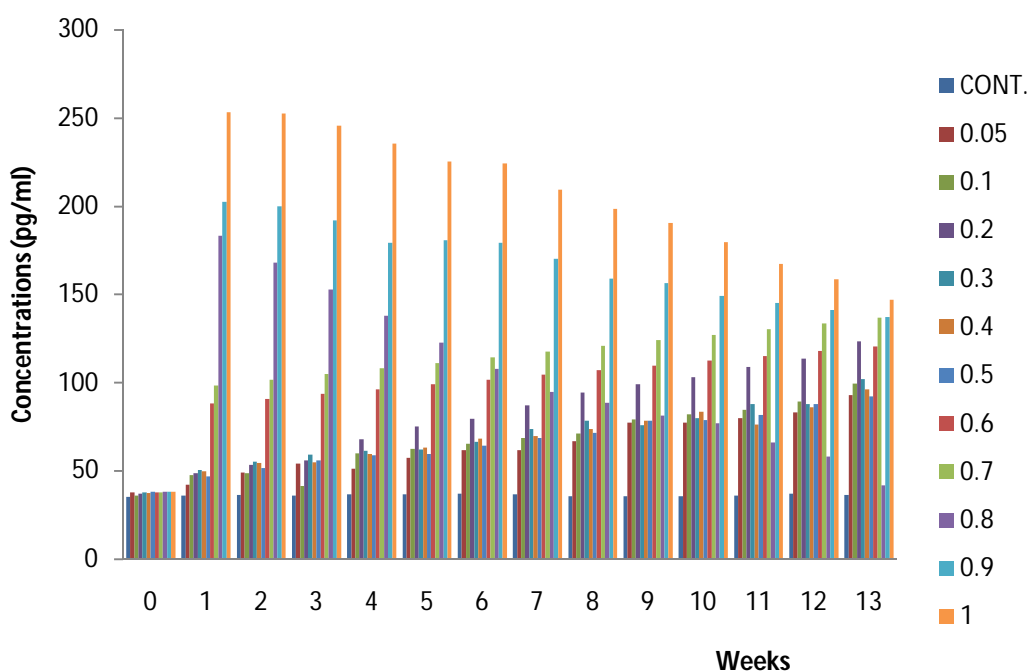


Fig. 3; Chart of concentration against weeks (durations) for change in serum estrone level.

EFFECT ON ESTRADIOL

There is a significant decrease in serum estradiol level when compared with the control at $p \leq 0.05$ (see fig. 4). As the estradiol decreases from group 0.05mg/kg to 0.3mg/kg, at group 0.4mg/kg, there is a rise peak; group 0.8mg/kg showed the lowest levels of estradiol (fig.4). As the estradiol decreases from group 1(0.05mg/kg) to 4(0.3mg/kg), at group 5(0.4mg/kg), there is a rise peak (fig.4); group 0.8mg/kg showed the lowest levels of estradiol; the week effect observed was mild, as it appeared to be superimposed on each other (fig. 4). The trend observed for estradiol and estrone levels were almost similar. But the trend observed for estradiol was different, in that there was a drastic and significant ($p \leq 0.05$) decrease in the estradiol level in all the treated groups.

The BPA group 0.4mg/kg, gave a rise (peak) effect in the estriol which is still significantly lower than that of the control. There is an instant decrease in serum estriol level of all the treated rats within the first week(fig 4). This decrease which is significant ($p \leq 0.05$) is sustained virtually consistently at the same level throughout the thirteen (13) weeks of the study (fig 4). The decrease in estriol level appear to be dose dependent except for group 0.4mg/kg (400 μ g/kg) and group 0.7mg/kg (700 μ g/kg). The result suggests that the reduction in serum estriol level is influenced by dose of BPA administered but not by time.

In fig. 4, it was observed that following the administration of BPA, a pattern of behaviour was established by the dose groups throughout the duration of the experiment. There was a dose dependent decrease in estriol levels from 0.05mg/kg to 0.3mg/kg, then a spike increase by 0.4mg/kg, which decrease at 0.5mg/kg to 0.6mg/kg, another rise was observed at group 0.7mg/kg, this was followed by a decrease at group 0.8mg/kg from which dose dependent rise in estriol through 0.9mg/kg to 1mg/kg (fig 4). The test dose groups showed a time dependent effect which was mild. All the weeks peaks at test dose 0.4mg/kg group (fig. 4).

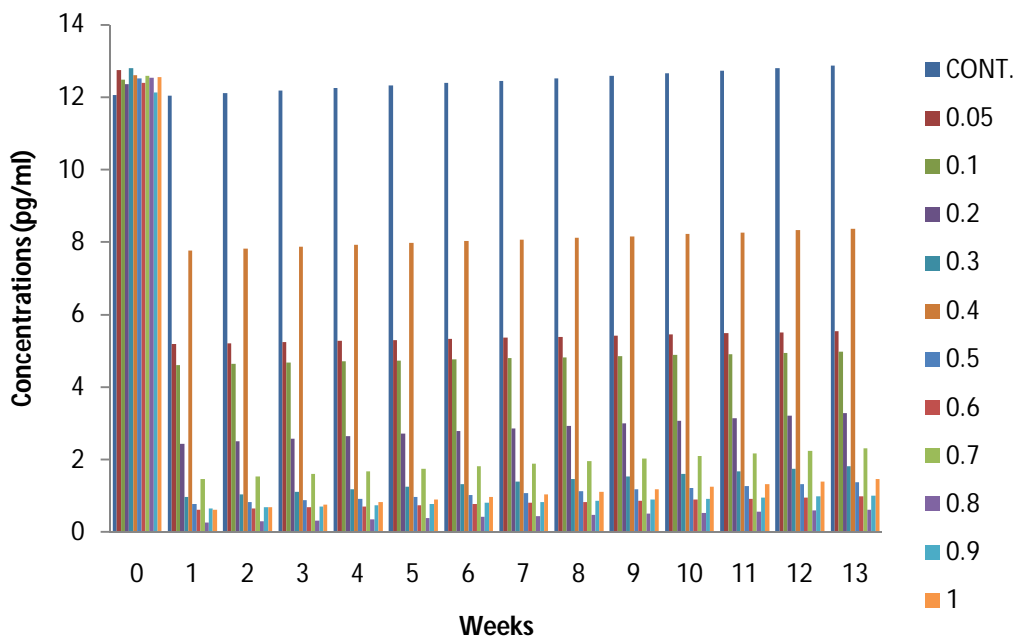


Fig. 4; Chart of concentration against weeks (durations) for serum estriol concentration.

DISCUSSION

The present study examined the effect of BPA exposure on serum estrone, estradiol, conjugated estradiol and estriol hormones concentrations. The result obtained from the study revealed significant high estrone and estradiol concentrations; and significant low estriol and conjugated estradiol concentration. A significant positive association was found between BPA level and serum PROG, testosterone, androstenedione and endogenous estrogens concentration.

Endogenous estrogens are thought to have an important role in the developing and maintaining female sexual characteristics. The three major endogenous estrogens in females that have estrogenic hormonal activity are; estrone, estradiol, and estriol. And they are the three major naturally occurring forms of estrogen in females. All of the different forms of estrogen are synthesized from androgens, specifically testosterone and androstenedione, by the enzyme aromatase.

Low dose BPA decreased progesterone levels in adult mice during early pregnancy (Berger et al. 2008 and Zhou et al. 2008) but tends to increase serum progesterone level in non-pregnant wistar rats (oguazu et al 2021). Additionally, in adult rats, low dose BPA (below 0.1 mg/kg/day) decreased estradiol (Lee SG et al. 2013) which contravene the finding of this study.

The results of in vitro studies on the effects of BPA on steroidogenesis are also equivocal. BPA exposure inhibited estradiol, testosterone, androstenedione, estrone, dehydroepiandrosterone, and progesterone production in cultured intact murine antral follicles (Peretz et al. 2011; Ziv-Gal et al. 2013), However, BPA exposure caused an increase in estradiol, testosterone, androstenedione, estrone, and progesterone production female rats (oguazu et al 2021 and oguazu et al 2020) with decrease in estriol, conjugated estradiol concentration. Also, in isolated rat theca-interstitial cells, BPA (100 nM to 100 μ M) had an increasing testosterone synthesis effect (oguazu et al 2020; Zhou et al. 2008). In a separate study using porcine granulosa cells, 0.1 μ M BPA increased estradiol levels (Grasselli et al. 2010) aligned the findings of this present study.

Further, if all of the different forms of estrogen are synthesized from androgens, specifically testosterone and androstenedione, by the enzyme aromatase and if low dose BPA decreased expression of estrogen and progesterone receptors (Berger et al. 2010), this might be the reason for observed high estradiol concentration and account for the observed low conjugated estradiol concentration and implicative to the high testosterone and androstenedione concentration observed in a separate study (oguazu et al 2020).

Conclusion

These studies indicate that BPA adversely affects on estrogenic hormonal activity, but the effects depend on BPA concentration, and that different species model used (intact follicles, isolated cells in cultures, mice or rats).

Ethical Approval

Animal ethical clearance was obtained from Nnamdi Azikiwe University animal ethics committee.

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