

# Effect Of Azoxystrobin On The Reproductive Hormonal Dynamic In Zebra Fish (Danio Rerio)

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**Abstract:** The existing study investigated the gonadal hormone alternation of Zebrafish brought on by means of sublethal concentration of Azoxystrobin (5ppm and 15ppm). Due to sublethal doses Azoxystrobin exposure leads to endocrine disruption in zebrafish in connection with hormonal reproductive tissues. FSH, LH and testosterone hormone levels were notably reduced ( $P < 0.001$ ) in the treated fish in assessment with manipulate with azoxystrobin attention increased. Reduction within the gonadosomatic index was once evident inside the extend of sublethal dose of Azoxystrobin absorbed their sensitivity to zebrafish intercourse hormone like effects.

**Key words:** FSH, LH, Androgen, Estrogen

## 1. INTRODUCTION

Azoxystrobin could be a highly effective agent created by syngenta in 1992 and the first Bartlett et al. [6] sold out in 1996. According to the data provided by using Phillips, azoxystrobin is hierarchical due to the fact the high antifungal agent on the international fungicides market and additionally the complete income of azoxystrobin had been \$1165 and \$1215 million bucks in 2013 and 2014, severally. As a vast Spectrum, everyday and soil-applied antifungal agent, azoxystrobin is registered to be used in over eighty 5 absolutely distinctive plants round the world [6, 17]. In China, azoxystrobin has been broad wont to combat diseases in soybeans, rice, cereals, vegetables and fruit trees for several years [11]. The liquid photolysis DT50 (time to five hundredth dissipation) of azoxystrobin was once between 8.7 and 13.9 days at pH scale seven, and it had been detected in streams, surface water and ground water in numerous countries Rodrigues et al. [18]. It works with the system to control essential body function. Various body features embrace strength metabolism, replica, increase and development, osmoregulation and equilibrium. The gadget conjointly regulates fruitful approaches and skeletal development [4, 24, 23]. Pituitary gonadotropins vesicle stimulating internal secretion (FSH: Follicle stimulation hormone), Gonadotropins and ICSH (LH: Luteinizing hormone) Gonadotropins are key procreative hormones that are concerned in dominant endocrine development steroidogenesis and biological process [18]. It has been reportable that, gonadotrophic hormone mediate Vitellogenesis and gametogenesis, whereas LH elicited final maturation of gametocyte and spermination in fishes were reported by Choi et al. [8], Yaron et al. [25],

Park et al. [17] and Choi et al. [9] E2 ( $17\beta$  estradiol). The FSH secretion LH cell stimulating hormone gonotrophic hormone and thyrotrophic hormone two belong to a conjugated protein hormone household two hormone family, these hormones are mentioned to hold extremely preserved N-linked glycosylation sites, and hooked up oligosaccharides are fundamental in endocrine bioactivity in teleosts [12, 15, 8]. Follicle development, gametocyte maturation and their physiology in fishes were controlled by hormones, as well as the cyst stimulating endocrine and therefore the ICSH, still as growth factors and hormones created by the ovary [15]. There is a major lack of analysis work the consequences of Azoxystrobin on the fruitful health of fish, notably considering the multiple mechanisms of action of those environmentally relevant compounds. This info is crucial to determine the mechanism of toxicity of Azoxystrobin in decrease vertebrates and can facilitate to supply a lot of complete understanding of the conceivable chance phthalates create to the fruitful fitness of fish inside the settings. A short-term reproduction assay are developed to study the effect on of endocrine disrupting compounds [1, 2] Environmental chemicals that intervene with reproduction in fish have attracted important interest in recent studies [1, 2]. Reproduction in dependent upon the approximate coordination of the complete neural structure – pituitary – gonads (HPG) axis, therefore, chemicals engaging at the any level of this axis via any mechanism will have adverse effect on reproduction [17]. The Zebrafish (Danio rerio) is a captivating model organism for evaluating reproductive toxicity and endocrine disrupting outcomes due to the fact of its small dimension, ease of culture, quick lifestyles cycle and prolific egg production with excessive fertilization and hatching rates [19]. Thus, in the present investigation, Zebrafish Danio rerio was chosen as the model organism to examine and evaluate the spectrum of adjustments precipitated by means of Azoxystrobin in fish.

## 2. MATERIALS METHODS

Exposure of the fish to Di-2-ethylhexylphthalate and determination of  $LC_{50}$  value to determine the median fatal concentration ( $LC_{50}$ ) of Azoxystrobin, ten fishes every were haphazardly elect from the stock and exposed to totally different concentrations of Azoxystrobin 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100ppm) for ninety six hours. Water was replaced daily contemporary Azoxystrobin mixed water

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keep up Constant level of Azoxystrobin throughout exposure amount. At the same time, a bearing was conjointly maintained underneath identical conditions. The concentration at that five hundredth mortality of fish occurred was taken as median fatal concentration ( $LC_{50}$ ), that make up my mind by probit analysis. The  $LC_{50}$  price for Azoxystrobin was fifty ppm. For sub-lethal .study  $1/5^{th}$  and  $1/10^{th}$  of the  $LC_{50}$  value had been chosen.

### 2.1. Exposure experiment

Sexually mature males and females were known visually and were exposed to sublethal concentrations of Azoxystrobin (5ppm and 15ppm) for an amount of sixty days. An impact cluster was maintained at the same time of these experiments were performed in triplicate. Weight of fishes was measured at the top of sixtieth day. At the top of sixteenth day, one fish from every treated cluster was homogenized in 3-fold volume of phosphate buffer (7.4 pH) and centrifuged at 12000 rpm for 15 minutes at 40°C. The supernatant was collected [13].

### 2.2. Quantification of reproductive hormone

The above supernatant was subjected to chemi luminescence assay [10], to calculate approximately the hormone titre Androgen (pg/ml), Luteinizing hormone (MIU/ml), Estrogen (pg/ml), Progesterone (ng/ml), Testosterone (ng/dl) and Follicle stimulating hormone (MIU/ml).

### 2.3. Statistical analysis

Data obtained from the on top of experiment were subjected to one way method analysis of variance (ANOVA) followed by testing exploitation mean, performed with SPSS version 16.0. The info square measure bestowed as  $mean \pm common$  place error of the suggest that.

## 3. RESULTS

### 3.1. Mean LH titre of Zebrafishes exposed to Azoxystrobin

Mean LH hormone titre of male Zebrafish (*Danio rerio*) fishes for the period study was presented in Table 1. Significant ( $F=43.422$ ,  $P<0.001$ ) decrease in the LH hormone was observed in male zebrafish fish expose to Azoxystrobin (5ppm:  $9.45 \pm 0.02$  mIU/ml; 15ppm:  $6.52 \pm 0.04$  mIU/ml) when compared to the control ( $11.34 \pm 0.03$  mIU/ml). In female Zebrafish, significant decreased in the mean LH hormones was evidenced due to Azoxystrobin exposure when compared to the control ( $F=1.432$ ,  $P<0.001$ ). Control group register mean LH titre of  $16.42 \pm 0.05$  mIU/ml, whereas, 5ppm and 15ppm Azoxystrobin treated group showed  $12.35 \pm 0.06$  mIU/ml,  $7.41 \pm 0.09$  mIU/ml respectively (Table-2).

**Table-1.** Mean titre of various reproductive hormones in the testis of male Zebrafish

Groups	LH	FSH	Estrogen	Progesteron
Control	$16.42 \pm 0.05$	$13.78 \pm 0.04$	$28.35 \pm 0.02$	$3.43 \pm 0.01$
5ppm	$12.35 \pm 0.64$	$15.48 \pm 0.07$	$23.47 \pm 0.02$	$2.15 \pm 0.21$
15ppm	$7.41 \pm 0.98$	$19.34 \pm 0.48$	$25.62 \pm 0.15$	$0.45 \pm 0.01$
F values	1.432***	251.345***	590.215***	1.876***

\*\*\*Significant at  $P<0.001$ , DMRT

**Table-2.** Mean titre of various reproductive hormones in the ovary of female Zebrafish

Groups	LH	FSH	Androgen	Testosterone
Control	$11.34 \pm 0.03$	$9.32 \pm 0.04$	$15.2 \pm 0.01$	$8.23 \pm 0.09$
5ppm	$9.45 \pm 0.02$	$14.78 \pm 0.07$	$11.47 \pm 0.21$	$5.42 \pm 0.04$
15ppm	$6.52 \pm 0.04$	$17.32 \pm 0.06$	$7.43 \pm 0.15$	$1.21 \pm 0.01$
F value	43.442***	507.304***	409.321***	3.118***

\*\*\*Significant at  $P<0.001$ , DMRT

### 3.2. Mean FSH titre of Zebrafishes exposed to Azoxystrobin

Follicle stimulating hormones extensively increased on exposure of Zebrafish to Azoxystrobin (Table 1 and 2). Compared to control group ( $9.32 \pm 0.05$  mIU/ml), the Azoxystrobin exposed male zebrafish fishes elicited significant enhance in the mean FSH titre level ( $F=507.304$ ,  $P<0.001$ ). Azoxystrobin treated groups showed increased FSH titre (5ppm:  $14.78 \pm 0.07$  mIU/ml; 15ppm:  $17.32 \pm 0.06$  mIU/ml) in male fish. Azoxystrobin exposed female zebra fish showed significant increase in the mean FSH titre level ( $F=251.345$ ,  $P<0.001$ ). whereas, Azoxystrobin exposed to 5ppm and 15ppm groups showed mean LH titre as  $15.48 \pm 0.07$  mIU/ml and  $19.34 \pm 0.04$  mIU/ml respectively (Table-2).

### 3.3. Mean Estrogen hormone titre of female Zebrafish exposed to Azoxystrobin

Compared to control group ( $28.35 \pm 0.02$  pg/ml), all the Azoxystrobin treated group female fishes significantly ( $F=596.215$ ,  $P<0.001$ ) declined in the mean estrogen hormone. 5ppm and 15ppm Azoxystrobin group showed decreased mean estrogen hormones of  $23.47 \pm 0.02$  pg/ml and  $25.62 \pm 0.15$  pg/ml, respectively (Table-2).

### 3.4. Mean Androgen hormone of male Zebrafish exposed to Azoxystrobin

Significantly ( $409.321$ ,  $P<0.001$ ) decreased mean androgen hormone titre of exposed male Zebrafish was evident when compared to untreated ones ( $15.21 \pm 0.01$  ng/ml). As the concentration of Azoxystrobin increased, the mean androgen hormones was found to decrease in Zebrafish (5ppm:  $11.47 \pm 0.21$  ng/ml; 15ppm:  $7.43 \pm 0.15$  ng/ml) (Table 2)

### 3.5. Mean progesterone hormone of female Zebrafish exposed to Azoxystrobin

Progesterone hormone level was found to decline significant ( $F=1.876$ ,  $P<0.001$ ). Azoxystrobin at higher concentration (15ppm) registered mean progesterone titre of  $0.45 \pm 0.01$  ng/ml, whereas, at 5ppm, mean progesterone level was  $2.15 \pm 0.21$  ng/ml, respectively (Table 2) where control group showed  $3.43 \pm 0.01$  ng/ml. Thus a Specific dose based relationship was once experimental between the awareness of Azoxystrobin and progesterone hormone titre in the ovary of female Zebrafish (*Danio rerio*).

### 3.6. Mean Testosterone hormone of male Zebrafish exposed to Azoxystrobin

In Male zebrafish, significantly ( $F=3.118$ ,  $P<0.001$ ) declined mean testosterone titre was registered by the all the treated groups (5ppm:  $5.42 \pm 0.04$  ng/dl, and 15ppm:  $1.21 \pm 0.01$  ng/dl) when compared to the control ( $8.23 \pm 0.09$  ng/dl) (Table-2). The mean testosterone hormone level significantly decreased (Table 2).

#### 4. DISCUSSION

United Nations agency have determined no vital variations in weight, length and gonadosomatic index of 3 spined sticklebacks (*Gasterosteus aculeatus*) exposed to fifty and 100µg Di-n-butylphthalate (DBP) for a amount of twenty-two days in comparison to unexposed ones. The results of this study reveal that sublethal doses of endocrine disrupting chemicals will considerably decrease concentration of LH, oestrogen, Androgen, androgen, progesterin and increase in others cyst stimulating endocrine (FSH), in Zebrafish *Danio rerio*. Azoxystrobin ablated egg production, the fertilization rate and therefor the GSI altered the E2 and T concentrations: ablated the quantity lately vitellogenic oocytes and mature spermatocytes and down regulated lhb, lhr, fshb and lhb levels at two hundred mg/L. studies have shown that follicle stimulating hormone and gonadotrophin have a serious influence on the rules of steroidogenesis and improvement through binding to FSHR and LHR [13]. Pronounced that nonylphenol compound causes a number of dysfunction in the male and female reproductive systems, editing the ranges of luteinizing hormone and Follicle stimulating hormone. Also, our results are not in good accord with Van Baal et al. [22] results; In male fish, gonadotrophin plays a key role within the early stages of gametogenesis and gonadotropic hormone is concerned in spermatozoa maturation [19] at the same time, E2 and T, that may well be stirred up by gonadotrophin and LH, regulate ontogenesis, germ cell maturation and copy, and therefore the disturbance of sex steroid hormones would adversely have an effect on fish copy. Reported that insignificant minimize in FSHβ stages in the fish uncovered to higher an NP dose in evaluation with control. Also it has been pronounced that plasma FSH ranges used to be appreciably impaired with the aid of long term publicity to Npin Female rainbow trout and in tilapia [26].

#### 5. CONCLUSION

Zebrafish *Danio rerio* exposed to two concentrations of azoxystrobin induced hormonal disturbance leads to many changes in reproduction which suggesting the estrogenic impact of this chemical pollutant.

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