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TOXICOLOGICAL EFFECT OF BUTATAF HERBICIDE ON SOME PHYSIOLOGICAL ASPECTS AND THE REPRODUCTIVE PERFORMANCE OF NILE TILAPIA *OREOCHROMIS NILOTICUS*

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ABSTRACT

This study was carried out to assess the effects of butataf herbicide on the physiological state and reproductive endocrine function of Nile tilapia "*Oreochromis niloticus*". The 96 hour half lethal concentration (96 hr LC_{50}) of the butataf herbicide (N-Butoxymethyl-2-chloro-2,6-diethylacetanilide) determined for the adult *Oreochromis niloticus* was 0.2 ppm. The field concentration of this herbicide was 0.004 ppm which equals to the $^1/_{50}$ of the 96 hr LC_{50}. The present experimental assay was evaluated on the 30th day of exposure to the $^1/_{100}$ LC_{50} ; $^1/_{50}$ LC_{50} ; $^1/_{25}$ LC_{50} and $^1/_{10}$ LC_{50} in comparison with control group. During determination of the 96-hr LC_{50}, the fish exhibited erratic swimming movements. The mucus secretion was increased and accumulated on the gills, so the fish exhibited a respiratory disorder with surfaced swimming, opening their mouth with rapid and frequent exhalation.

The red blood corpuscles (RBCs), haemoglobin (Hb) and haematocrit (Ht) were decreased significantly in fish groups exposed to butataf herbicide compared to the control one. The plasma aspartate amino transferase (AST) and alanine amino transferase (ALT) enzymes activities were also decreased significantly in fish exposed to the herbicide. On the other hand, the uric acid and creatinine recorded

high levels in fish groups exposed to the high concentrations of the herbicide, also, glucose and total plasma lipids were increased significantly in all treated groups. Meanwhile the total plasma protein was strongly decreased and the effects were dose-dependent, as well as the fecundity, gonado-somatic index and hepato-somatic index. Estradiol and testosterone hormones showed severe changes in females and males specially those exposed to the high concentrations of butataf herbicide. Also, T_3 and T_4 recorded the same trend.

INTRODUCTION

The aquatic environment is not only the ultimate recipient of pollutants, but also the place where some chemicals (herbicides included) are applied directly. Consequently, aquatic organisms; including fish are subjected to different toxic agents which adversely affect the physiological state and/or reproduction in fish through multiple pathways within the reproductive tract and hypothalamic–pituitary–gonadal axis. (Wester, 1988). The study of toxicity should be more concerned with sublethal effects and sublethal studies has been forced because of the need is to find the safe concentration of the pollutants (Johnson,1968). Tooby (1971) recorded many differences in the toxicities of different herbicides even between different forms of the same herbicide. Pollutants stress apparently lead to increased secretion of catecholamine (Pickering, 1981) and corticosteroids (Mazeaud *et al.*, 1977). This produces an alteration in the metabolic rate which in turn reduces metabolic reserves and affects the physiological status as well as, the growth rate of fish. Moreover, this increase in energy demand may interfere with the enzymatic system of the metabolic pathway as well (Abo-Hegab *et al.*, 1990).

The environmental contaminants that act as estrogen receptor agonists have received much attention in terms of possible effects on reproduction and development in fish and wildlife (WHO, 2002). However, chemicals can adversely affect endocrine function through many other biologically relevant pathways within the reproductive tract and the hypothalamic– pituitary–gonadal axis (HPG). There is an increasing evidence that environmental contaminants that interact with androgen receptors could cause significant adverse effects on individuals and populations (Gray *et al.*, 2005). Several studies indicate that androgen receptor agonists are affecting fish exposed to some types of discharges (Howell *et al.*, 1980 ; Larsson *et al.*, 2000 ;

Parks *et al.*, 2001 ; Orlando *et al.*, 2004). In addition to chemicals that interact directly with receptors, function of the HPG axis can be affected by xenobiotics that affect the metabolism of sex steroids. Recent studies suggest that altered steroid-genesis is associated with adverse effects observed in fish from the field (Noaksson *et al.*, 2003 : Lavado *et al.*, 2004).

So, the present work was undertaken to find out to what extent the changes in some physiological and biochemical functions which take parts as indicators for metabolite mobilization which in turn affect the production and reproduction of the studied fish. Since butataf herbicide is used to eliminate unwanted weeds in the rice fields, unsuspected side effects on fish are to be expected (Svobodova *et al.*, 1993).

MATERIALS AND METHODS

Apparently healthy Nile tilapia "*Oreochromis niloticus*" specimens with an average body weight of 100_ 5 g obtained from Abbassa fish farm, Abbassa, Abou-Hammad, Sharkia were acclimated in laboratory conditions for two weeks prior the experiment. The 96 hour half lethal concentration (96 hr LC_{50}) of the butataf herbicide (N-Butoxymethyl-2-chloro-2,6-diethylacetanilide) was determined according to Behreus and Karbeur (1953) as 0.2 ppm, however this herbicide was applied the field with a concentration of 0.004 ppm.

Five groups of female and male of the studied fish were holded for 30 days to evaluate the effects of different sublethal concentrations of butataf herbicide on the physiological and biochemical assayes. Each group consisted of 24 fish was divided into three replicates of eight fish (6 females and 2 males), then maintained in glass aquaria supplied with dechlorinated aerated tap water at a temperature of 26_2 °C , pH 7.2_0.2 and dissolved oxygen 5.5 _ 0.5 mg/l. The first group was kept as control. Other groups were exposed to the $^1/_{10}$ LC_{50} , $^1/_{25}$ LC_{50}, $^1/_{50}$ LC_{50} and $^1/_{100}$ LC_{50} (0.02, 0.008, 0.004 & 0.002 ppm). respectively. The feeding rate was holded at the percent of 3% of the body weight dialy.

At the end of the experiment, blood samples were taken from the caudal vein of non anaesthetized fish by sterile syringe. 0.5 ml of the blood containing EDTA as an anticoagulant was used for erythrocyte count (Dacie and lewis 1984), haemoglobin content (Vankampen, 1961) and haematocrit value (Britton, 1963).

Plasma was obtained by centrifugation at 3000 rpm for 15 min and nonhaemolyzed plasma was stored in deep freezer for further steroid analyses. The gonads and liver of all fish specimens were removed, wet weighed then the gonado-somatic index (G.S.I) and hepato-somatic index (H.S.I) were calculated derived by assessing the gonad and liver weights as a percentage of the total body weight. Egg numbers in the ovaries of the female were counted to determine the fecundity (Munkittrich and Dixon 1988).

Aspartate amino transferase (AST) and Alanine amino transferase (ALT) were determined according to the method of Reitman and Frankel (1957). The uric acid and glucose were estimated according to the method of Trinder (1969), while creatinine was determined according to that of Henry (1964). Total protein was determined according to Henry (1964) and total lipids according to Schmit (1964). All of these parameters were measured using specific reagent kits purchased from Diamond Diagnostic Company.

Estradiol 17-_ (E_2) was determined by the radio-immune assay method according to Xing *et al.* (1983) using kit Immunchem provided by Diagnostic products corporation, Los Angeles. Total testosterone (T) was determined by radio immuno assay method according to Abraham (1977) using kit (Coat-A-count) provided by Orion Diagnostic Spectria , Fin land. Estimation of Triiodothyronine (T_3) and Throxin (T_4) levels was down by using radio-immuno assay technique using Coat-A-Count total T_3 and T_4 kits (Los Angeles, USA) according to Murphic and Pattie (1964), using gamma counter in Animal Production Research Institute.

The data were statistically analyzed using Duncan's multiple range test to determine differences in means (Duncan, 1955).

RESULTS AND DISCUSSION

After exposure of Nile tilapia; *Oreochromis niloticus* to different concentrations of the butataf herbicide, in order to estimate the 96 hour half lethal concentrations (96 hr LC_{50}) that was 0.2 ppm, the field concentration (0.004 ppm) was found to equal $^1/_{50}$ of the half lethal concentration (Table1). Consequently, according to Bathe *et al.* (1974), the butataf herbicide is considered highly toxic to *Oreochromis niloticus*. During determination of the 96-hr LC_{50}, fish exhibited erratic swimming movements. The skin had a mottled appearance and mucus secretion increased and accumulated on the gills, so the fish exhibited

a different respiratory manifestation such as surfaced swimming, opening their mouth with rapid and frequent respiratory movement . The erratic swimming and surfaced fairly frequently movements may be due to hyper-contraction of the muscles due to cholinesterase inhibition as previously reported by Ferguson (1989), while Atallah *et al.* (1997) attributed such changes to the extraordinary need for the oxygen due to the thick coating of the gills with profuse mucus together with congestion and hyper-plastic epithelium of the secondary lamellae. The mottled appearance of skin and heavy mucus secretion are due to the melanosis (aggregation of melanocytes) happened by the stimulation of α -melanonine stimulating hormone (α-MSH) secretion in fish subjected to stressors and irritation of the gills by the pollutants (Satchell,1984 and Ferguson, 1989). Data given Table (2) showed significant decrease in the red blood cell counts (RBCs), hemoglobin (Hb) and haematocrit (Ht) in the adult Nile tilapia treated with the sublethal concentrations of butataf herbicide, the effects were dose-dependant. The decreases in RBCs, Hb and Ht were the most common effects usually expected in all animals exposed to chemical and toxicological metabolites in which the haemopoietic tissues are the target organs of their effect. The most of toxic substances including herbicides, suppress the processes of erythropoiesis and Hb synthesis. Also, the decrease in the RBCs, Hb and Ht may be due to the elimination of the RBCs from the circulation as a result of butataf-induced extravasations of blood (Jordan *et al.*, 1977 & Mousa, 2004). These results are in agreement with those of Shalaby *et al.* (2006), they indicated that the reduction of these blood parameters in Blue tilapia "*Oreochromis aureus*" at sublethal levels of chromium might be due to the destruction of mature RBCs and the inhibition of erythrocyte productions due to reduction of haemosynthesis that affected by pollutants.

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes are frequently used to diagnose the sublethal damage to certain organs specially the liver (Benedeczky *et al.*, 1984). Results in Table (3) showed that the fish plasma AST and ALT enzyme activities were decreased at the 30th day of exposure of butataf. These results were attributed to the hepato-cellular damage and inhibition of enzymes synthesis as a result of toxic effect of the herbicide (Nesckovic *et al.*, 1996 and Mousa, 2004).

The uric acid and creatinine levels are indicators for kidney function and considered as important variables predicting to which limit the kidney is adversely affected. In the present study, creatinine and uric acid (Table 3) showed a significant increase in fish exposed to butataf sublethal concentrations. These results may be due to the action of this herbicide on glomeruli filtration rate (Abbass *et al.*, 2002) and/or it may be cause pathological changes of resulting in kidney dysfunction.

Alteration of blood sugar level revealed a stress response of fish (Nemcsok *et al.*, 1987). In the present study, the blood glucose was increased significantly in fish groups treated with the highest two doses (Table 4). The increase in blood glucose might be resulted from an increase in plasma catecholamines and corticosteroid hormones (Pickering, 1981). Moreover, the hyperglycemia induced by any toxicant might be explained by the inhibition of the neuro-effector sites in the adrenal medulla leading to hyper secretion of adrenaline, which stimulates the breakdown of glycogen to glucose (Gupta, 1974).

The quantitative determination of the total plasma protein reflects the liver capacity of protein synthesis and denoted the osmolality of the blood and the renal impairments. So it is of valuable effect in the diagnosis of the toxicity of the fish. In the present study, the total plasma protein was decreased significantly and the effect was dose-dependent (Table 4). The decrease was attributed to either a stage of hydration and change in water equilibrium and/or disturbances in the liver protein synthesis (Salah El-Deen and Rogers, 1993). On the other hand, total plasma lipids showed significant increase in all treated fish groups during the exposure time (Table 5). The intensity of hyper-lipemic state may reflect the degree of stress imposed on the animal under the influence of toxic reagents and environmental pollutants (Saeed,1989). The increase of total plasma lipids may be due to the increase of lipids peroxides formation induced by the effect of butataf herbicide as previously reported by Arias (1990) and Mousa (2004). Otherwise, the destruction of the liver cells and other organs due to the effect of the butataf herbicide increase the levels of total lipids in the plasma (Inui, 1968 & Mousa, 2004).

Gonad growth is an easily measured endpoint for assessing the potential for the chemical effects on gonad development and thus impact on the fish reproductive performance. Data in Table (5) revealed that all groups of fish exposed to butataf sublethal

concentrations, exhibit an apparent decrease in gonad (ovaries & tests) size recorded significant decrease in the gonado-somatic index (G.S.I) compared to those in the control group. It is concluded that butataf herbicide inhibits gonad growth and consequently, the fecundity was significantly decreased in female fish exposed to butataf herbicide (Table 5). These results are in agreement with those of (Makynen *et al.*, 2000) who found that the fecundity and gonado- stomatic index were decreased significantly in female fathead minnows (*Pimephales promelas*) after exposure to vinclozolin fungicide. Hepato-somatic index (H.S.I) showed the same trend indicated the alterations in nutrient contents and normal absorption as well as metabolism in treated fish.

The present study showed that levels of estradiol were significantly elevated in mature male and female *O. niloticus* (Table 6). The estrogenic like effects may be produced as result that butataf binds to estrogen receptors and exhibits estrogenic activity (Stephen , 2001) , or by direct exposure to sertoli cells resulting in decreased 3-hydroxyl-steroid – dehydrogenase activity that change estradiol to androgen thus raising estradiol levels (Colborn *et al* ., 1993). Moreover, the significant elevation of estradiol in male *O. niloticus* which received high dose of butachlor could be attributed to increase the incidence of hypertrophy and / or vacuolation (empty cavities) of adrenal cortex that enhanced the steroidogenic activity (Pandian and Sheela, 1995). Meanwhile, the significantly decreased testosterone in *O. niloticus*, specially the male may be as a results of direct damage of butataf on the leydig cells. Also, butataf may alter androgen biosynthesis mediated by cytochrome P-450 system of interstitial cells of the a testis which is required for function of 17 &-hydrolase and 17-20 layse (Flodstrom *et al.*, 1990), or induces a variety of hepatic bio-transforming enzymes, which are capable of metabolically transforming androgens into products with low androgen receptor binding activity. Further research in this area would be desirable, with particular attention given to the status of enzymes involved in steroid metabolism (Gerald *et al.*, 2005).

The levels of thyroid hormones (T_3 & T_4) in plasma of female and male *O. niloticus* were recorded in Table (7). There were significant decrease in tri-iodothyronine T_3 and Thyroxine T_4 in fish groups treated with sublethal concentrations of butataf herbicide except the group treated with the lowest concentration in which the

levels of T_3 and T_4 showed insignificant decrease compared to the normal fish. Hotz *et al.* (1977), Henery and Gasiewicez (1987) and El-Kashoury *et al.* (2005) reported that pesticides increased deiodination and biliary excretion of thyroid hormones which increased the rate of T_3 or T_4 elimination from the blood. Mahgoup (1992) claimed that, the mechanism of thyroid action may be due to the continuous demand and need of body to ATP needed for the production of energy and direct effect of thyroid hormones on the production ATP in the mitochondria. The decrease in T_3 levels may be resulted in or inducing toxic goiter which is manifested by increased metabolic reactions or may be secondary to a pituitary insufficiency (Kaneko, 1997). Generally, pesticides induce alteration in nutrient contents and metabolism in animals (CAST, 1989).

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Table (1): Different applied sublethal concentrations ($^1/_{10}$ LC_{50} , $^1/_{25}$ LC_{50}, $^1/_{50}$ LC_{50}& $^1/_{100}$ LC_{50}) of butataf herbicide for adult *Oreochromis niloticus*

Concentrations Fish species	Field conc. (ppm)	96 h LC_{50} (ppm)	$^1/_{10}$ LC_{50} (ppm)	$^1/_{25}LC_{50}$ (ppm)	$^1/_{50}$ LC_{50} (ppm)	$^1/_{100}$ LC_{50} (ppm)
Oreochromis niloticus	0.004	0.20	0.02	0.008	0.004	0.002

Table (2): Effect of sublethal concentrations of butataf herbicide on erythrocyte count (RBCs), haemoglobin content (Hb) and haematocrit value (Ht) of adult Nile tilapia;*Oreochromis niloticus*

parameters Exp. Groups	RBCs(cell x10^6/mm^3)	Hb (g/dl)	Ht %
Control (0.0 ppm)	2.21 _ 0.44^A	8.62 _ 0.18^A	16.75 _ 0.32^A
$^1/_{100}$ LC_{50} (0.002 ppm)	1.70 _ 0.35^B	7.52 _ 0.27^B	14.00 _ 0.25^B
$^1/_{50}$ LC_{50} (0.004 ppm)	1.46 _ 0.16^C	4.56 _ 0.76^C	13.21 _ 1.55^C
$^1/_{25}$ LC_{50} (0.008 ppm)	1.40 _ 0.17^C	4.59 _ 0.57^C	12.70 _ 0.45^C
$^1/_{10}$ LC_{50} (0.02 ppm)	1.15 _ 0.22^D	3.34 _ 0.77^D	12.75 _ 0.67^C

Data are represented as means _ S.E.Means with the same letter in the same column are not significantly different ($P \leq 0.05$)

Table (3): Effect of sublethal concentrations of butataf herbicide on plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT), uric acid and creatinine of adult Nile tilapia, *Oreochromis niloticus*

parameters Exp. Groups	AST /l	ALT /l	Uric acid mg/dl	Creatinine mg/dl
Control (0.0 ppm)	16.23 _ 1.21^A	14.35 _ 1.01^A	1.54 _ 0.04^D	0.85 _ 0.01^C
$^1/_{100}$ LC_{50} (0.002 ppm)	15.63 _ 2.14^A	14.61 _ 0.64^A	1.56 _ 0.01^D	0.88 _ 0.01^C
$^1/_{50}$ LC_{50} (0.004 ppm)	12.42 _ 1.07^B	11.54 _ 0.35^B	1.66 _ 0.05^C	0.95 _ 0.03^B
$^1/_{25}$ LC_{50} (0.008 ppm)	9.78 _ 0.50^C	6.58 _ 1.61^C	1.85 _ 0.01^B	1.01 _ 0.01^B
$^1/_{10}$ LC_{50} (0.02 ppm)	9.36 _ 1.31^C	5.16 _ 1.31^C	1.98 _ 0.01^A	1.23 _ 0.02^A

Data are represented as means _ S.E.
Means with the same letter in the same column are not significantly different ($P \leq 0.05$)

Table (4): Effect of sublethal concentrations of butataf herbicide on plasma; glucose, total protein and total lipids of adult Nile tilapia, *Oreochromis niloticus*

parameters / Exp. Groups	Glucose mg/dl	Total protein g/dl	Total lipids g/dl
Control (0.0 ppm)	56.14 _ 2.17^{C}	3.15 _ 0.71^{A}	3.82 _ 0.51^{C}
$^{1}/_{100}$ LC_{50} (0.002 ppm)	56.48 _ 2.43^{C}	2.31 _ 0.52^{B}	3.48 _ 0.90^{C}
$^{1}/_{50}$ LC_{50} (0.004 ppm)	57.41 _ 2.86^{C}	2.09 _ 0.35^{B}	4.28 _ 0.11^{B}
$^{1}/_{25}$ LC_{50} (0.008 ppm)	60.96 _ 1.23^{B}	2.14 _ 0.78^{B}	4.67 _ 0.24^{B}
$^{1}/_{10}$ LC_{50} (0.02 ppm)	76.68 _ 1.83^{A}	1.75 _ 0.34^{C}	5.34 _ 0.74^{A}

Data are represented as means _ S.E.
Means with the same letter in the same column are not significantly different ($P \leq 0.05$)

Table (5): Effect of sublethal concentrations of butataf herbicide on fecundity, gonado-somatic index (G.S.I) and hepato-somatic index (H.S.I) of adult Nile tilapia, *Oreochromis niloticus*

Parameters / Exp. Groups	Fecundity	G.S.I %		H.S.I %	
		Female	Male	Female	Male
Control (0.0 ppm)	1173 _ 65.34^{A}	4.06 _ 0.83^{A}	1.54 _ 0.16^{A}	1.26 _ 0.21^{A}	1.34 _ 0.11^{A}
$^{1}/_{100}$ LC_{50} (0.002 ppm)	989 _ 45.78^{B}	2.80 _ 0.30^{B}	1.23 _ 0.02^{B}	1.22 _ 0.23^{A}	1.27 _ 0.17^{A}
$^{1}/_{50}$ LC_{50} (0.004 ppm)	839 _ 55.20^{C}	2.73 _ 0.60^{B}	0.66 _ 0.10^{C}	0.93 _ 0.10^{B}	0.78 _ 0.28^{B}
$^{1}/_{25}$ LC_{50} (0.008 ppm)	647 _ 59.49^{D}	1.46 _ 0.51^{C}	0.58 _ 0.02^{C}	0.91 _ 0.18^{B}	0.67 _ 0.10^{C}
$^{1}/_{10}$ LC_{50} (0.02 ppm)	624 _ 74.35^{D}	1.20 _ 0.17^{C}	0.49 _ 0.12^{D}	0.66 _ 0.08^{C}	0.60 _ 0.04^{C}

Data are represented as means _ S.E.
Means with the same letter in the same column are not significantly different ($P \leq 0.05$)

Table (6): Effect of sublethal concentrations of butataf herbicide on Estradiol and Testosterone hormones of adult female and male Nile tilapia, *Oreochromis niloticus*

parameters / Exp. groups	Estradiol (pg/ml)		Testosterone (ng/ml)	
	Female	Male	Female	Male
Control (0.0 ppm)	340.03 _ 8.82^{D}	303.09 _ 4.21^{D}	13.66 _ 2.47^{A}	28.07 _ 3.12^{A}
$^{1}/_{100}$ LC_{50} (0.002 ppm)	438.44 _ 5.13^{C}	313.47 _ 5.87^{D}	10.93 _ 1.45^{B}	22.61 _ 2.01^{B}
$^{1}/_{50}$ LC_{50} (0.004 ppm)	552.12 _ 7.58^{B}	623.29 _ 7.14^{C}	6.98 _ 0.11^{C}	12.52 _ 1.24^{C}
$^{1}/_{25}$ LC_{50} (0.008 ppm)	603.86 _ 7.13^{A}	2312.94 _ 14.51^{B}	6.40 _ 1.07^{C}	7.91 _ .65^{D}
$^{1}/_{10}$ LC_{50} (0.02 ppm)	619.29 _ 9.45^{A}	5373.17 _ 0.45^{A}	5.44 _ 1.02^{D}	6.46 _ 0.78^{D}

Data are represented as means _ S.E.

Means with the same letter in the same column are not significantly different ($P \leq 0.05$)

Table (7): Effect of sublethal concentrations of butataf herbicide on Tri-iodothyronine (T_3) and Thyroxine (T_4) hormones of adult female and male Nile tilapia, *Oreochromis niloticus*

parameters / Exp. groups	T_3 (ng/ml)		T_4 (ng/ml)	
	Female	Male	Female	Male
Control (0.0 ppm)	62.11 _ 2.17^{A}	74.03 _ 5.41^{A}	8.79 _ 2.24^{A}	13.49 _ 1.24^{A}
$^{1}/_{100}$ LC_{50} (0.002 ppm)	63.72 _ 7.06^{A}	72.21 _ 5.42^{A}	6.71 _ 2.01^{B}	6.53 _ 1.12^{B}
$^{1}/_{50}$ LC_{50} (0.004 ppm)	57.36 _ 2.43^{B}	67.36 _ 2.23^{B}	4.80 _ 1.06^{C}	5.93 _ 1.03^{B}
$^{1}/_{25}$ LC_{50} (0.008 ppm)	56.23 _ 3.71^{B}	59.85 _ 2.53^{C}	4.78 _ 1.54^{C}	3.32 _ 1.01^{C}
$^{1}/_{10}$ LC_{50} (0.02 ppm)	49.81 _ 4.06^{C}	58.92 _ 3.18^{C}	4.56 _ 0.78^{C}	1.82 _ 0.43^{D}

Data are represented as means _ S.E.

Means with the same letter in the same column are not significantly different ($P \leq 0.05$)