Monitoring the Effect of Thiobencarb as an Endocrine Disruptors on Fecundity of Tilapia Cultured Female *Oreochromis niloticus*.

Eissa I. A. M, Nashwa S. Elias , Derwa H. I., Rahma H. Eid

Dept.of Fish Diseases and Mangment , Fac. Of Vet. Medicine , Suez Canal Univ ,Ismailia

Dept.of Fish Diseases , Animal Health Research Institute , Dokki , Giza.

Abstract :

A total of 150 female *O. niloticus* were delivered from a private fish farm and divided into one control group, 2 acute groups (3 and 9 days) and 3 chronic groups (7,14 and 21 days) . The 2 acute and 3 chronic groups were subjected to ½ and 1/10 LC50 (which was calculated as 0.4 ppm) of thiobencarb respectively . The acute effect of Thiobencarb herbicide on *O.niloticus* females was manifested in the form of abnormal skin pigmentation in form of darkening ,excessive mucous secretion on the skin and the most prominent sign was skin ulceration, detached scales, fin and tail rot . Fish also showed some nervous manifestation as gasping air and abnormal swimming behaviour. The postmortem findings revealed congestion and hemorrhages in all internal organs especially ovaries, also autolysis in ovaries and dark congested liver. Wherease *O. niloticus* females in chronic phase showed eye opacity and copied similar clinical signs and postmortem findings as mentioned with the acute in addition to asymmetrical ovaries while the acute was much severe. Females of acute and chronic groups suffered from highly significant drop in both absolute and relative fecundity when compared to control group. Moreover, total protein, sex hormones (estradiol E2 and testosterone T) and AST in chronic 14 and 21 days showed highly significant drop throughout the acute and chronic groups. Oppositely, AST (Aspartate aminotransferase) in acute 3 and 9 days, chronic 7 days and ALT (alanine aminotransferase) in acute 3 and 9 days , chronic 7 ,14 and 21 days showed highly significant increase when compared to control groups. Finally, owing to the drastic effect of thiobencarb on fish fecundity, it is contraindicated to use it for fish farms, water from agriculture sources using Thiobencarb.

Introduction

Pollution is the most worldwide problem creating immense crisis to human and fish health. The environmental pollution exposure not only directly affects fish health, but it also can disrupt the normal steroidogenesis pattern in fish, lead to impaired hormone production in fish, and make disturbance in body function as Estradiol, progesterone, Testosterone, FSH and LH ***(Shoker , 2015)***.he also had proved that low pollution level decrease fish fecundity in spite of showing no illness signs. Moreover, endocrine disrupting chemicals pass adverse reproductive effects in fish onto their off spring up to third generation.

At low concentrations, ***Khan and Law (2005)*** proved that pesticides may act as blockers of sex hormones, causing abnormal sexual development and unusual mating behavior. The chronic exposure to low levels of pesticides may have a more significant effect on fish populations than acute poisoning. Doses of pesticides that are not high enough to kill fish are associated with subtle changes in behavior and physiology that impair both survival and reproduction.

Endocrine disrupting chemicals (EDCs) are silent killers, threatening biodiversity on a huge scale, as well as a foreign substance or mixture that alters functions of the endocrine system, consequently harming an individual life form, its offspring, or populations. ***Bhandari (2015)*** defined that endocrine disruption compounds (EDCS) are chemicals used in a variety of consumer products. Steroid hormones are organic chemical substances secreted by endocrine glands and on ward transported to target organs to maintain reproductive functions.

The present study was aimed to estimate the effect of thiobencarb herbicide (as one of the endocrine disruptors) in female *O. niloticus* fecundity .

Materials and methods

Fish:

A total number of 150 female Oreochromis *niloticus* were collected alive from a private Farm Wadi El-Natroon in ,Autumn 2014 they were transported into large plastic containers supplemented with battery aerators about 4 hours, to Biology Unit - Fish Diseases Dept. at Animal Health Research Institute, Dokki, Their body weight and body length were 100±20 g and 20±2 cm respectively.

Aquaria:

Fish were kept in fully prepared glass aquaria supplied with electric air pumps for continuous aeration for one week for acclimation.

Herbicide:

Thiobencarb: is a thiocarbamate herbicide used in this study as commercial preparation that contains 50% active ingredient. Thiobencarb is a thiocarbamate one of the EDCs. It is the common name for S-[(4- chlorophenyl)

methyl] diethyl carbamothioate (CAS 28249-77-6). It is a systemic, preemergence herbicide. Thiobencarb is used as a weed killer heavily used in rice cultivation in Egypt. **(Abbas *et al*., 2007).**

Determination of LC50 of Thiobencarb :

A total number of 30 female apparently healthy of *O. niloticu*s were divided into 5 groups, 6 in each group were put into Thiobencarb concentrations (0.1, 0.3, 0.5, 0.7, 0.9 ppm). Fish were observed for 96 hrs. The LC50 was calculated according to

Litchfield and Wilcoxon (1949).

Mortality rate was recorded at the end of the experiment.

Experimental design on 120 fish:

Fish were divided into 3 groups:

- Control group
- Groups subjected to ½ LC50 of thiobencarb (Acute exposure) fish examined after 3days and 9 days.
- Groups subjected to 1/10 LC50 of thiobencarb (Chronic exposure) fish examined after 7, 14 days and 21 days.

Fish Growth Measurements:

For each fish body Length (B. L.) , body Weight (B.W.), ovarian weight (WG) and hepatic Weight (WH) were measured for each fish separately in acute and chronic phases.

Morpho – anatomical Parameters:

For each fish Gonado-somatic Index (I_G) and Hepato-somatic Index (I_H) indices as well as Condition factor (K) were calculated according to ***Sun and Pankhurst (2004).***

Clinical examination:

Ovaries and liver of each fish were examined macroscopically to detect any abnormality and any abnormal behaviour.

Fecundity evaluation:

Absolute fecundity and relative fecundity were calculated for each fish according to ***Elias (2009)***.

blood samples were collected from the caudal blood vessels without anticoagulant after being left overnight in the refrigerator at a sliding position, blood samples were put in the centrifuge at 4000 r.p.m. for 10 minutes. Serum was carefully collected in clean dry Epindorff tubes using micropipette and preserved at -4 ^{O}C until analysis.

Serum was collected from control group, acute groups after 3 and 9 days and chronic groups after 7 , 14 and 21 days (20 fish from each group) .

Biochemical Investigation:

-Serum Total protein (T.P) was estimated for each fish according to ***Young, (1990).***

-Liver enzymes: Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were also measured according to ***Schmidt and Schmidt (1963).***

-Sex hormones levels: concentration of testosterone (T) and estradiol (E2) hormones were measured for each fish by ELIZA kits according to ***Tietz (1995 a and b)*** respectively.

Statistical Analysis: The obtained data were statistically analyzed according to SPSS 14 (2006) by get mean ± S.D by using T test.

Results

Results of LC50 of thiobencarb herbicide: It was revealed that the LC50 of was 0.4 ppm in *O.niloticus* females.

Clinical picture:

The most common clinical and postmortem lesions accompanying the exposure of cultured ***O.niloticus*** to thiobencarb at different concentrations and time of exposure, the fish were exposed to the LC50 for 96 hours to (1/2 LC50) acute or short term exposure for 9 days and to (1/10 LC50) chronic or long term exposure for 21 days . The most recorded clinical signs and postmortem lesions in acute term were manifested as abnormal skin pigmentation in form of darkening (photo 1) ,excessive mucous secretion on skin and the most prominent signs were skin ulceration, detached scales, fin and tail rot (photo 2). Also, fish showed some nervous manifestations as gasping and abnormal swimming behaviour. Postmortem findings revealed congestion and hemorrhages in all internal organs especially ovaries, also autolysis in ovaries and dark congested liver (photo 4). Whereas females *O. niloticus* in chronic phase showed eye opacity (photo 3) and copied similar clinical signs and postmortem finding as mentioned with the acute in addition to asymmetrically in ovaries (photo 4) while the acute revealed much severity.

Fish fecundity:

The results of growth measurements, morpho – anatomical parameters, relative fecundity and absolute fecundity of the acute (3 and 9 days) and chronic (7, 14 and 21 days) phases were tabulated in Tables 1 and 2.

Almost all parameters showed highly significant drop when compared with control group.

Biochemical Investigation:

The level of serum total protein, liver enzymes (ALT and AST) and the steroidal sex hormones (T and E2) were measured in the control , 2 acute and 3 chronic groups. The total protein ,AST (chronic 14 and 21 days) and the two sex hormones showed highly significance drop throughout the experiment , except for the AST (acute 3 and 9 days , chronic 7 days)as well as the ALT registered a highly significance increase were tabulated in (Table 3).

Mortality percentage:

Mortality rate of thiobencarb exposure was about 25-30% in acute dose which began high and decreased gradually. While on the contrary, during the chronic period it began low about 25% and increased gradually reaching 90 - 95% after 21 days.

Table (1) *Comparative fecundity of O. niloticus females exposed to acute toxicity with thiobencarb (Mean±S.D.)*

Days post exposure / Reproductive parameters		Control	3 days	9 days
Growth Measurements	B.L. (cm.)	20±2	18±1.2***	20±0.5
	B.W. (g)	106±2	99±2 ***	96.7±3***
	W_G (g)	3.5±0.5	3.3±1 **	2.8±0.2 ***
	W_H (g)	2±0.5	1.3±0.1***	1.2±0.2 ***
Morpho-anatomical Parameters	I_G	3.5 ±0.6	3.2±0.8***	3±0.3***
	I_H	2.2±0.3	2.1±0.2**	1.2±0.1 ***
	K	1.5±0.2	1.6±0.2	1.2±0.1***
Relative Fecundity	FBL	1249±2	1181±2***	1281±6***
	FBW	780±1	728.7±2***	711.5±3***
	FOW	1247±2	971.6±3***	952.8±3***
Absolute Fecundity	T. R. Egg No.	2290±1.3	756±2***	356.7±5***

*N=10 **P < 0.01 ***P < 0.001*

Table (2) *Comparative fecundity of O. niloticus females exposed to chronic toxicity with thiobencarb (Mean ±S.D.)*

Days post exposure / Reproductive parameters		Control	7 Days	14 Days	21 Days
Growth Measuremens	B.L.(cm)	20±2	19±1.8***	19±1***	19±1 ***
	B.W. (g)	106±2	90±1***	82±8***	99±2***
	W_G (g)	3.5±0.5	0.5±0.1***	0.5±0.1***	0.8±0.1***
	W_H (g)	2±0.5	1.1±0.3***	0.8±0.2***	1.1±0.4***
Morpho – anatomical Parameters	I_G	3.5 ±0.6	0.6±0.1***	0.6±0.1***	0.8±0.1***
	I_H	2.2±0.3	1.4±0.3***	1.2±0.1 ***	1.1±0.2 ***
	K	1.5±0.2	1.2±0.3***	1.2±0.1***	1.2±0.1***
Relative Fecundity	FBL	1249±2	1155±2***	1113±1***	1119±2***
	FBW	780±1	686±1***	589±9***	740±1***
	FOW	1247±2	470±3***	460±2***	512±4***
Absolute Fecundity	T. R. Egg No.	2290±1.3	472±4***	500±2***	370±3***

*N=10 ***P < 0.001*

Table (3) ***Comparative biochemical parameters of O. niloticus females following acute and chronic exposure to thiobencarb (Mean ±S.D.)***

Dayspost exposure / Biochemical parameters	Control	Acute stage		Chronic stage		
		3 days	9 days	7 days	14days	21 days
T.P. (g/dl)	5.9±1	3.7±0.6***	7.6±0.8***	2.7±0.8***	2.2±0.7***	2.1±0.4***
AST (µ /ml)	39.7±4	57.2±4***	88.8±1.2***	50±1.5***	22.2±3***	29.4±2***
ALT (g/dl)	18.3±2	81±1***	52.2±2***	62±3.6***	52.2±3***	52.3±4***
T (pg / ml)	4.1±1	1.1±0.3***	0.35±0.1***	0.27±0.2***	2.2±1***	2.6±1***
E_2 (ng / ml)	328±4	51±1***	12±7***	22±5***	88±7***	81±7***

*N=10 **P < 0.01 ***P < 0.001*

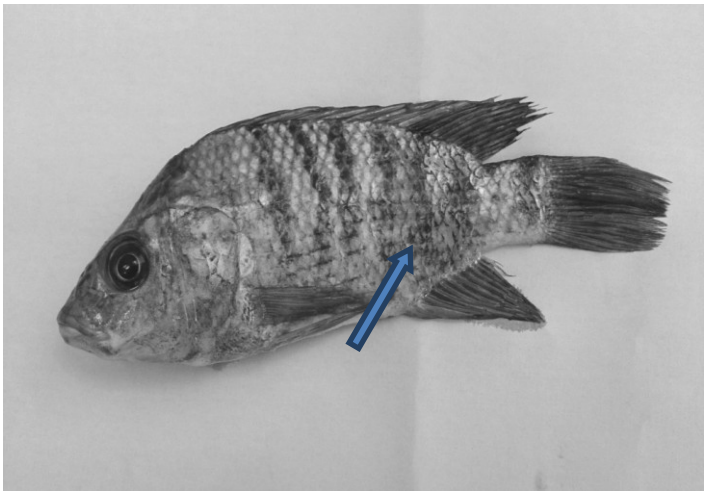

Photo (1):*O.niloticus* *exposed to acute dose of thiobencarb herbicide showing dark and detatched skin with hemoragic patches .*

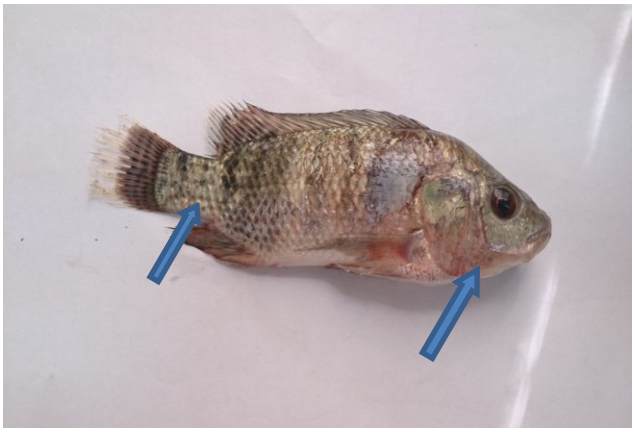

Photo (2): ***O.niloticus*** *exposed to acute dose of thiobencarb herbicide showing skin ulceration with fin and tail rot.*

Photo (3): ***O.niloticus*** exposed to chronic dose of thiobencarb herbicide showing opacity in eyes .

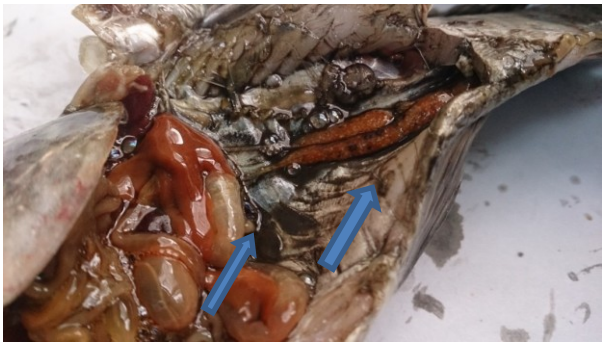

Photo (4) : ***O.niloticus*** exposed to chronic dose of thiobencarb herbicide showing hemorrhage and deformity in shape of ovaries .

Discussion

Pollution of soils and water resources by pesticides is pressing worldwide problem in aquatic environment resulting from the indiscriminate use of these pesticides in agricultural lands and consequently supplying fish farms through agriculture drainage.

Lethal and sublethal effects of pesticides may occur from one exposure over a short time (acute) or from small amounts over a longer period (chronic) ***(Massmer and Dahl 1991).***

Doses of pesticides that are not high enough to kill fish are associated with subtle changes in behaviour and physiology which impair survival and fecundity.

Experimentally, the LC50 in this research on female *O. niloticus* of body weight 106±16 g was found to be 0.4 ppm for 96 hrs which was far less than that recorded by ***Abbas et al (2007)*** which was 720 ug/L on Nile Tilapia of body weight (15-20 g). Inspite of being the same species the difference in LC50 was referred to the difference in body weight. In addition, ***Shalaby et al (2007) and Ghada Ahmed (2009)*** had proved the LC50 of butachlor herbicide on Nile Tilapia for 96 hrs as 3.85 ppm and 2.1 ppm respectively.

O. niloticus Females exposed to ½ LC50 (acute dose) and 1/10 LC50 (chronic dose) of thiobencarb herbicide was characterized by excessive mucus secretion, dark coloration, skin ulceration, opacity in eyes, detached scales and fins and tail erosion (photo 1). These findings appeared similar to ***Ghada Ahmed (2009)*** resulting from Butachlor exposure on *O. niloticus*.

In addition, the authors registered highly nervous manifestations, abnormal swimming behaviour of *O. niloticus* exposed to thiobencarb with much severity with acute dose that came with ***Hassan (2005)*** who approved that short and long term exposure of *O. niloticus* to carbofuran herbicide were manifested by abnormal swimming, darkening of skin with excessive mucous secretion. On the contrary ***Abbas et al (2007)*** registered that all clinical signs and nervous manifestation severity were confirmed with the long term exposure.

Mortality rate of thiobencarb exposure was ranged from 25-30% in acute dose which began high and decreased gradually. While on the contrary , during the chronic phase it began low about 25% and increased gradually reaching 90 - 95% after 21 days. In case of ***Abbas et al (2007)*** mortality rate was 25% among acute and chronic periods.

Depending on the negative correlation proved by ***Mahmoud and Allam (2002)*** between fish gonads and their length, thus the researcher referred the highly significance decrease in body length (B.L) and accordingly the fecundity related to body length (F.B.L) among both acute and chronic periods. The significance

correlation proved by ***Khallaf et al (2003)*** between females gonado-somatic index and pollution is an explanation for the highly significance decrease in females *O. niloticus* body and ovarian weight followed by highly significance drop of gonado-somatic index in this research during acute and chronic phases.

Concerning relative fecundity the authors found that female *O. niloticus* suffered from highly significance drop of fecundity related to body length (F.B.L), body weight (F.B.W) as well as that related to ovarian weight (F.O.W) through the acute and chronic phases which agreed with results of ***Ghada Ahmed (2009)*** in *O. niloticus* exposed to acute and chronic doses of butachlor herbicide.

These findings also came in accordance with ***Nashwa Elias (2007)*** who proved that the highly significance decrease in ovarian weight, gonado-somatic index and (F.O.W) of *O. niloticus* polluted with heavy metals to the extent that ovaries became rudimentary .

Absolute fecundity (Total ripen egg number) in *O. niloticus* exposed to thiobencarb for acute and chronic doses showed highly significance drop in this research , this was approved by the belief of inhibited pronounced influx of protein yolk from liver to ovary resulting from both liver and ovaries injuries due to pollution , ***(Hanna et al ., 2005*** *and* ***Nashwa Elias 2007) .***

Susca et al (2001) had defined liver as vitellogenin (yolk protein) precursor. Moreover, ***Hanna et al (2005)*** proved that the major role of teleosts hepatic tissue being a marker for pathological damages as liver reabsorbs toxic compounds. The highly significance hypoproteinaemia among the acute and chronic phases in this research appeared in accordance with ***Abbas et al (2007)*** who proved the presence of thiobencarb residues in fish tissues after acute and chronic exposure with the highest values in liver.

In addition, ***Abbas et al (2007)*** added that the penetrable thiobencarb with chronic treatment was more than acute through fish skin and gills. They added that the chemical structure of thiobencarb caused its accumulation in tissue because of its lipophilic nature. The increase in glucose level in pollutants-exposed fish may also be attributed to degranulation and vacuolization of the pancreatic alpha cells in the initial stages and damage of beta cells in later stages ***(Abbas, 1998; Abbas et al, 2002).***

Determination of enzyme activity in plasma or serum and tissues has proven to have diagnostic application in fish health studies ***(Bouk et al, 1978).*** Many pollutants have been shown to act specifically by inhibiting certain enzymes, thus interfering with metabolic processes in development ***(Weis et al, 1981).***

Transamination represents one of the principal pathways for the synthesis and deamination of amino acids, thereby allowing an interplay between carbohydrate, fat and protein metabolism during fluctuating energy demands of the organism in various adaptive relations ***(Waarde & Henegaurven, 1982).*** Therefore, attention has been focused on the changes in the aminotransferases, (AST) and (ALT) which promotes gluconeogensis from amino acids and relates changes in their activities to the liver condition ***(Marie, 1994).*** AST and ALT are normally found in low concentrations in blood; so if liver cells are damaged, they may leak them into the plasma causing an increase in catalytic activity ***(Heath, 1987).*** However, liver cells are particularly rich in transaminases because this organ is the major site for interconversion of food stuff.

In the present study, AST activity showed a general trend to increase during the acute (3 and 9 days) and chronic (7 days) periods whereas at the end of the chronic period (14 and 21 days) AST showed a general trend to decrease when compared to the control values. On the other hand, ALT showed highly significance increase during both acute and chronic phases. This fluctuation in AST and ALT activities could be attributed to a number of factors such as leakage from liver and muscle into the blood; liver enzyme inhibition by the effect of pollutant, and/or disturbances in kreb's cycle ***(Salah El-Deen, 1991).***

Endocrine disrupting chemicals were classified by ***Mills et al (2001)*** into two categories, some as DDT having an estrogenic effect that showed depression of plasma testosterone (T) and vitellogenesis induction. Oppositely, others as Octylphenol being an anti-androgenic causes increase estradiol without vitellogenin production. Steroidal hormonal values T and E2 among both phases (acute and chronic) registered highly significance drop throughout the acute and chronic phases in this study which was approved by ***Singh and Canario (2004)*** in response to pesticide Y-hexachorocyclohexane. Also, ***Singh and Singh (2007)*** had proved that 40 days exposure of DDT in catfish resulted in 17-β estradiol levels declination. Exposure of Butachlor herbicide in *O.niloticus* also eqistered same result, ***(Ghada Ahmed, 2009).***

Finally, owing to its drastic effect on fish fecundity, it is contraindicated to use for fish farms water from agriculture sources using Thiobencarb.

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رصد تأثير الثيوبنكارب علي إختلال الغدد الصماء و خصوبة اناث البلطى النيلى

إسماعيل عبدالمنعم عيسى ، نشوى سمير الياس ، حسن إبراهيم محمد دوره
رحمة حسين عيد حسن

أستهدف هذا البحث قياس تأثير مبيد الحشائش الثيوبنكارب علي إناث البلطى النيلي المستزرع . تم تجميع عدد ١٥٠ أناث بلطى نيلي من مزرعة خاصة وتقسيمهم الي مجموعات مجموعة ضابطة – عدد ٢ مجموعة تسمم حاد فحص بعد (٣ و ٩ أيام) – عدد ٣ مجموعات تسمم مزمن فحص بعد (٧ و١٤ و٢١ يوم) . وقد تم تعرض أسماك المجموعتين الحادة والثلاث مجموعات المزمنة إلى ½ و١٠/١ من الجرعة النصف مميتة للثيوبنكارب على التوالى والتى تم تقديرها ٠,٤ جزء في المليون.
وفي نهاية التجربة تم قياس طول السمكة ووزنها ووزن الكبد والمبيض و أخذ عينات دم لفصل المصل لقياس البروتين الكلي والهرمونات الجنسية (الأستراديول والتستستيرون) وانزيمات الكبد (اسبارتات امينوترانسيفيراز و الانين امينوترانسيفيراز).
ولقد ظهر تأثير التعرض للثيوبنكارب في المجموعتين الحادة علي شكل ظهور لون داكن غير طبيعي علي جلد السمكة ، زيادة في الأفرازات المخاطية وكانت العلامات المميزة هي تقرحات الجلد وتساقط القشور وتأكل الزعانف والذيل . كما ظهر على الأسماك بعض العلامات العصبية مثل القفز لأعلي والحركة بطريقة غير طبيعية .
وقد تبين من الصفة التشريحية وجود إحتقان وبقع نزفية في جميع الأعضاء وخاصة المبايض وتحللها وكذلك إحتقان الكبد .
بينما أظهرت اناث البلطى النيلى في مجموعات التسمم المزمن عتامة في العين .وكانت العلامات الأكلينيكية مماثلة للتسمم الحاد بالأضافة إلي عدم تماثل فى فصى المبايض .
الأناث في مجموعات التسمم الحاد والمزمن كانت تعانى من إنخفاضا معنويا عاليا في الخصوبة النسبية والكلية مقارنة بالمجموعة الضابطة .
وعلاوة علي ذلك ظهر إنخفاضا معنويا شديدا في نسبة البروتين الكلي والهرمونات الجنسية (الأستراديول والتستستيرون) في جميع مجموعات التسمم الحاد والمزمن وفي إنزيم (اسبارتات امينوترانسيفيراز) مجموعتي التسمم المزمن (١٤ و٢١ يوما).
وعلي العكس سجل انزيم الكبد (اسبارتات امينوترانسيفيراز) في مجموعتي التسمم الحاد (٣ و٩ يوما) والمجموعة الاولي في التسمم المزمن (٧ أيام) وكذلك إنزيم الكبد (الانين امينوترانسيفيراز) في كل مجموعات التسمم الحاد والمزمن ارتفاعا معنويا واضحا .
أخيرا ، نظرا لتأثيره المدمر علي خصوبة أناث البلطي النيلي فإنه يجب عدم إستعمال مياه في المزارع السمكية من مصدر زراعي يستخدم الثيوبنكارب .