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STUDIES ON THE EFFECT OF THIOBENCARB HERBICIDE ON SOME BIOLOGICAL, PHYSIOLOGICAL, BIOCHEMICAL, HISTOLOGICAL AND GENETIC ASPECTS OF NILE TILAPIA, *OREOCHROMIS NILOTICUS*

Hossam H. Abbas[1], Mohammad M. Authman[1], Iman M.K. Abumourad[1], and Ashraf A. El. Badawi[2]

1- Department of Hydrobiology, National Research Centre, Giza, Egypt.
2- Central Lab. For Aquaculture Research-Abbassa, Abo-Hamad, Sharkia, Egypt.

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ABSTRACT

This study was undertaken to evaluate the impact of short-term and long-term exposure to the herbicide thiobencarb, which is largely used for weed control, especially at the rice cultivation in Egypt, on Nile tilapia; *Oreochromis niloticus*. Lethal and sublethal effects of thiobencarb on physiological, biochemical and histological parameters were also studied on the fish as a function of exposure time. Exposure of *O. niloticus* to lethal and sublethal levels of thiobencarb resulted in significantly ($P<0.05$) lower values for liver and muscle glycogen, total protein and total lipids compared with the control group. In contrast, there was a significant increase ($P<0.05$) in muscle ash, water content, glucose, cholesterol, creatinine, uric acid and Lactate dehydrogenase (LDH) in the herbicide-treated group, while aspartate aminotransferase (AST) and alanine aminotransferase (ALT) showed a fluctuated activity in both of acute and chronic exposure. The data indicated that the residue of thiobencarb had higher value in the liver, while the lowest value was in the brain. Histological sections in gills, kidneys, spleen and liver of treated fish revealed pathological alterations. Cytotoxicity assessment were also included. Fish exposed to thiobencarp showed a significant reduction in growth parameters. The genetic investigations showed breaks, gaps, centromeric attenuation, centric fusion and deletion were the common aberrations. The mean percentage of aberrations were time-dependent.

INTRODUCTION

Environmental contamination of air, water, soil and food threatens the continuous existence of many plants and animal communities and may ultimately hinder the survival of humans. The most important "in view" of the environmental pollution is the pollution of water with many contaminants including heavy metals (Abbas, 1998).

Herbicides have contributed by dramatic increase in crop yields and in the quantity and variety of the diet. Also, they have helped to limit the spread of certain diseases. But they have harmful effects since they can cause injury to human health as well as to the environment. The range of these adverse health effects includes acute and persistent injury to the nervous system, lung damage, injury to the reproductive organs, dysfunction of the immune and endocrine systems, birth defects, and cancer (Mansour, 2004).

Problems associated with herbicide hazards to man and the environment are not confined to the developing countries. Developed nations have already suffered these problems, and still facing some problems in certain locations. For many reasons, the severity of herbicide hazards is much pronounced in Third World Countries (Mansour, 2004).

Water pollution due to pesticides is a serious problem; due to their toxicity and persistence in the environment. More and more chemical formulations are widely used to control insect pests of agricultural crops due to lack of suitable substitutes (Westernhagen *et al.*, 1987). As a result of their usage, they find their way into the freshwater resources with the run-off water from agricultural land, or by direct application, spray drift, aerial spraying and by discharge of effluents from factories and sewage (Daabees *et al.*, 1992).

Most environmental problems of concern today are attributed to the production and release of toxic chemicals capable of interacting with the environment and disrupting the ecosystem.

Indiscriminate discharge of herbicides from agricultural run off and other sources into aquatic media affects non target organisms such as fish and prawn which are of great economic importance to humans. In addition, sometimes herbicides are used to control fish diseases; at present, thiobencarb at very low doses is being widely used to control argulus disease and to eradicate the larvae of mosquitoes and milk fishes during pond preparation. Thiobencarb is being used extensively in rice fields to control weeds. Contamination of water bodies adjacent to rice

fields by thiobencarb, mainly through run off, is quite possible. Thus, it can be toxic to aquatic organisms. Fish blood is being studied increasingly in toxicological research and environmental monitoring as a possible indicator of physiological and pathological changes in fishery management and disease investigations (Adhikari, 2004), as the blood in the gill has direct contact with the water medium and any unfavorable change in the water could be reflected in the circulatory system. These studies could be used to indicate the health status of fish.

In recent years, large quantities of pesticides have been produced and discharged into the environment. Herbicides, a distinctive group of pesticides, are considered as selective chemical weed killer; hence they have been intensively used to destroy the unwanted plants, especially in agricultural settings (Dutta & Meijer, 2003).

The impact of chemical environmental contamination on fish health, consequently fish productivity is of economical relevance for fishes as well as aquaculture. Environmental pollutants have been reported to accumulate in fish and have threatened human health either directly or indirectly through the food chain. Accumulations of toxic compounds which may be carcinogenic or mutagenic were manifested as hazards (Porte & Albaiges, 1994; Jacobs *et al.* 2002). However, the proper handling and use of herbicides in aquatic areas are especially critical, accidental spills or over dose can kill fish or cause other damage to its habitats that may lead to reduction in the fish population.

The concept of cytotoxicity assessment induced by the chemical pollutants is receiving a wide spread attention and requires sensitive tests to establish the maximum allowable chemical concentrations prior to release to the environment and affect other organisms. There is also a global interest concerning the problems of polluted ecosystem which comprise hazards on fish health consequently human health (Shakoori *et al.*, 1996; Ulrich *et al.*, 2004).

The study of DNA damage at the chromosome level is an essential part of genetic toxicity because chromosomal mutation is an important event in carcinogenesis. The chromosomal aberration is one of the preferred methods for assessing the chromosome damage (Chauhan *et al.*, 2000).

Knowledge of the sublethal effects of toxic compounds on biochemical, genetic and histopathological levels is very important for delineating fish health status and for understanding future ecological impact.

Thiobencarb is used as a weed killer which is heavily used in rice cultivation in Egypt, where tilapia fish is frequently cultured in, although there are few reports on long-term effects of this herbicide.

In this respect, the present study aimed to investigate the biochemical, genetic and histopathological alterations in Nile tilapia in response to short (4 days) and prolonged (60 days) exposure to sublethal concentrations of thiobencrb.

MATERIALS AND METHODS

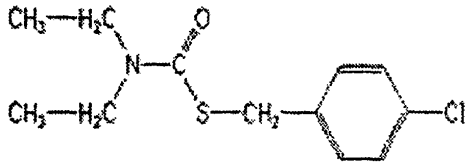
1-Experimental animals:

The present study was carried out on the Nile tilapia *Oreochromis niloticus* (family, Cichlidae). Adult fish were collected from Abbassa fish farm, Central Laboratory of Aquaculture (CLAR), Sharkia Governorate, Egypt. The fish were acclimated to laboratory conditions for at least one week prior to the experiment by holding them in aerated tap water in 2500 litre tanks. Fish were not fed for 48 hours prior to and during the experiments. Mortality was less than 2% during acclimation period. Fish were individually examined and the healthy ones were used in the study. The average body weight and total body length were determined to the nearest mg and 0.1 cm respectively. *O.niloticus* body weight and body length were ranging from 15-20 g and 8-10 cm respectively.

2-Aquaria:

Glass aquaria (75X50X50 cm) were designed to be self cleaned with a system of removable baffles and provided with air pumps for aeration. Water quality characteristics were measured by the methods described by APHA (1985).

3-Chemicals:



Thiobencarb: is a thiocarbamate herbicide used in this study as commercial preparation that contains 50% active ingredient.

4-Experimental design:

A total of 120 fish sample of tilapia with an average weight of 20±3g were collected , and acclimated to laboratory conditions in aerated, and dechlorinated tap water in 150 L aquaria for 1 week, at 26±1 °C, under a natural photoperiod (light/dark hours = 12). Fish were divided into three

groups, the first was the control group (60 fish/three replicates aquaria/group); the second group (60 fish/three replicates aquaria/group) was subjected to 100% of 96 hours L_{C50} for 4 days and the third group (60 fish/three replicates aquaria/group) which is subjected to 1/10 96 hours L_{C50} for 60 days. Fish samples were kept under observation along the period of experiment, samples were taken daily in case of acute exposure (2^{nd} group) and every two weeks intervals in chronic exposure (3^{rd} group).

5-Determination of 96 hours lethal concentration dose (L_{C50}) for thiobencarb

Preliminary screenings were carried out to estimate the concentration of the used herbicide which is most likely to cause 50% mortality (as L_{C50}) for 96 hours exposure to determine the appropriate testing range of concentrations for thiobencarb. This task was done according to the procedure described by Johnson & Finley (1980) and EPA (1985), where 3 doses of thiobencarb were used and given to 3 groups of fish in addition to control, each contained 20 tilapia and reared in glass aquaria that contained dechlorinated tap water and aerated continuously. A total of four groups were used with a total number of eighty tilapia fish that were equally distributed between groups. The fourth group served as a control. The selected dose of thiobencarb was added to the corresponding group and fish were kept under observation for 96hrs.

6-Pathological investigation and post mortem examination:

The exposed fish were kept under proper observation during the period of experiment for any external pathological signs, PM lesions or deaths according to Amlacher (1970).

7-Extraction and clean up of pesticides:

The extraction procedure adopted by AOAC (1990), dealt with pesticide extracts that were evaporated at 30°C to dryness. After clean up and dissolution in 1 ml methanol they were directed to HPLC analysis. These extracts were chromatographed with UV spectrophotometer detector and C18 stem less column 25 mm.

8-Genetic study:

Oreochromis niloticus was injected intrapretonialy with 0.01% cholchicine at a dose of 1 ml /100 gm body weight. After three hours, fishes were sacrificed and chromosome preparation was carried out according to Danial & Andre (1986).Only well-spread chromosomes were selected for scoring. Five hundred metaphases were investigated microscopically for control and exposed fish.

9-Apoptosis analysis:

DNA was separated in 1.5% agarose gel, visualized by UV Transilluminator after ethidium bromide staining, and photographed by a Polaroid camera.

10-Histopathological study:

Tissue specimens from gills, liver, kidneys and spleen were taken from tilapias that were exposed to thiobencarb by the end of chronic exposure. The specimens were fixed in 10% buffered formalin. They were processed by conventional method, sectioned at 4 *μm* and stained with Haematoxylin and Eosin, (Bancroft *et al.*, 1996).

11-Biochemical analysis:

Serum glucose: The concentration of serum glucose was measured using the GOD-PAP method (Enzymatic Colorimetric method) according to Trinder (1969).

Liver and muscle glycogen: Glycogen was determined using the anthrone reagent according to the method of Handle & Van (1965).

Muscle total protein: was determined using the semi-micro-kjeldahl method, as reported by Josyln (1950).

Muscle total lipids: were determined by the standard method reported in AOAC (1990). Extraction was carried out in Sockslet using petroleum ether.

Serum cholesterol: Cholesterol forms a coloured complex with acetic anhydride and concentrated sulfuric acid. Serum cholesterol was measured colorimetrically according to Watson (1960).

Serum aspartate aminotransferase(AST) and alanine aminotransferase(ALT): The serum AST and ALT activities were determined colorimetrically by transaminases kits according to Reitman and Frankel (1957).

Serum Alkaline phosphatase: Serum alkaline-phosphatase (AP) was determined according to the method described in Bergmeyer (1972).

Plasma lactate dehydrogenase (LDH): The activity of plasma lactate dehydrogenase (LDH) was determined by the enzymatic reaction described by Bergmeyer (1974).

Serum Creatinine: Creatinine in alkaline solution reacts with picrate to form a colored complex then measured colorimetrically as described by Henery (1974).

Serum uric acid: The serum uric acid was measured using enzymatic determination (after deproteinization) according to Barham & Trinder (1972).

Muscle water content: Muscle and liver samples were rapidly transferred directly after decapitation to a weighing bottle and accuratley weighed. The bottles were then placed in drying oven thermostatically regulated at 105°C for 72 hours. The loss in weight was taken as equivalent to the weight of water content of the samples.

Muscle ash: was determined by burning the samples in a muffle furnace for 16 hours at 550°C (Sidwell *et al.*, 1970).

Growth indices:

12-Weight gain and growth rate: The body weight was recorded every 2 weeks and weight gain was determined as the difference between the initial and final weights of fishes at the end of the experimental period (12 weeks).

13-Statistical analysis:

The results were statistically analyzed using analysis of variance and Duncan's multiple comparison tests to evaluate the comparison between means at $P< 0.05$ (Duncan,1955). Moreover, percentage of change has been calculated compared to control fish to evaluate changes in parameters levels among the species due to effect of the metal exposure and because the initial values of every parameter were different according to the species.

RESULTS AND DISCUSSION

Many biochemical and physiological variables show specific responses to certain type of environmental stressors such as herbicides. Such biochemical and physiological responses make them particularly useful in fisheries management and resources protection as a mean of identifying possible courses of environmental deterioration. However, the possibility to set a standard as a diagnostic tool is still not definitive (Abbas, 1998).

LC_{50} and tissue residue in tilapia fish:

The recorded L_{C50} of thiobencarb for 96hrs was 720µg/l. The HPLC conditions for thiobencarb was recorded in Table (1).

The recorded pathological signs in this study were more or less similar in both acute and chronic herbicide exposed groups; severity was confirmed with the long term exposure in chronic case. Pathological signs were manifested in the form of nervous manifestations, abnormal swimming behavior in the form of erratic swimming, abnormal skin discoloration. Mortality rate was 25% for thiobencarb-exposed fish. The

postmortem findings revealed congestion and haemorrhages in all internal organs in addition to pale anemic gills.

Data in Table (2) indicated the presence of thiobencarb residues in fish tissues after acute and chronic exposures, with higher value recorded in liver and lower value in the brain for two groups. The high uptake and penetration within tissues of pesticides via integument of tilapia fish was observed by El-Shemy *et al.*(1991). The results Also indicated that the penetrable thiobencarb with chronic treatment was more than acute treatment through fish skin and gills. The compounds were added to active ingredient to make thiobencarb formulation that caused high penetration and penetrability leading to higher residue levels of thiobencarb (Radwan & El- Said, 2006). Moreover, these results may be attributed to the chemical structure of the tested herbicide thiobencarb that may its accumulation in the tissue because of its lipophilic nature (El-Said & Radwan, 2004).

Biochemical results:

Analysis of serum constituents has been proved to be useful in the detection and diagnosis of metabolic disturbances and disease incidence processes (Aldrin *et al.*, 1982). Serum glucose appeared to be a sensitive and reliable indicator of environmental stress in fishes (Salah El-Deen, 1991).

Fish exposed to acute and chronic concentrations of thiobencarb showed a significant increase ($P<0.05$) of serum glucose concentration during the exposure time (Table 3). This increase was accompanied with significant decrease ($p<0.05$) in liver and muscle glycogen content (Table 4). This decrease of liver glycogen content indicated an extensive utilization of the energy stores. Perhaps, this increased utilization was to meet the expected extra demand of energy necessitated by the fish at the first hours of exposure. Fall in the liver glycogen content may be due to rapid turnover of the glycogen synthesis (Abbas & Mahmoud, 2003). The decrease in liver and muscle glycogen and elevation of blood glucose are indicative of increased rate of glycogenolysis (Ghazaly, 1992). Another reason for glycogen decrease in liver and muscle may be due to decrease in the rate of glycogenesis and/or gluconeogenesis (Salah El-Deen *et al.*, 1995).

Abu El Ella (1996) related the change in carbohydrate concentration to the indirect response to the internal hypoxia and the great mobilization of liver glycogen into blood glucose.

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 promote gluconeogensis from amino acids and relate 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 experiment, AST activity (Table. 3) showed a general trend to increase during the acute and chronic exposure time but at the end of the two experiments AST showed a general trend to decrease when compared to the control values.

On the other hand, ALT activity showed a similar effect like AST in case of acute exposure to thiobencarb, while it showed a significant increase ($P<0.05$) in fish exposed to chronic concentration of thiobencarb.

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).

Moreover, Abbas & Mahmoud (2003) pointed out that toxicants act on carboxyl, amino, sulfhydryl, phosphate and other similar groups of the cell components. They further summarized the mode of action as: (i) disruption of the enzyme system by blocking active sites; (ii) formation of stable precipitates or chelation by essential metabolites and immobilizing; (iii) catalytic decomposition of essential metabolites; (iv) combination of the substances with the cell membranes, thus affecting their permeability;

and (v) replacement of the structurally or electrochemically important elements in the cells which then fail in function.

The concentration of cholesterol in control group was 127.09±4.56, when fish exposed to lethal and sublethal concentrations of thiobencarb, cholesterol values increased significantly ($P<0.05$) till reaching 198.1±5 and 179.90±4.6 in acute and chronic exposure respectively (Table. 3).

The hypercholesterolemia could be attributed to the large amount of cholesterol produced by liver or less excretion of cholesterol to the bile duct as a result of stress. Similar rises in serum cholesterol were reported in fish following exposure to different pollutants (Goel & Garg, 1980; Ghazaly, 1992).

Plasma creatinine and uric acid can also be used as a rough index of the glomerular filtration rate (GFR) and kidney dysfunction (Hernandez & Coulson, 1967). Increasing levels of creatinine and uric acid above normal values indicate several disturbances in the kidney (Maxine & Benjamin, 1985).

In the present study, *O. niloticus* showed a significant increase ($P<0.05$) in creatinine and uric acid concentrations and Lactate dehydrogenase activity (Table. 3) with increasing the time of exposure in both acute and chronic experiment when compared to control group.

The increase of plasma creatinine and uric acid may be attributed to the action of thiobencarb herbicide on the glomerular tissues as well as deficiency of oxygen on the glomerular filtration rate which cause pathological changes in kidneys, due to the accumulation of herbicide in kidneys (Jacobs *et al.*, 2002 ; Shalaby *et al.*, 2005)

Muscle chemical composition and body weight gain:

Data concerning the effect of thiobencarb exposure on *O. niloticus* for 8 weeks (chronic experiment) on body weight and length gain are shown in Table (5). The results revealed that, fish exposed to thiobencarb showed a reduction in body weight gain compared to the control group during the entire experimental period.

The measurements concerning the muscle chemical composition of the studied fish groups were carried out at the end of the acute and chronic experiments (4 days and 8 weeks).

Table (6) revealed a significant increase ($p<0.05$) in muscle water content and muscle ash of fish exposed to thiobencarb with increasing the time of exposure in both acute and chronic experiment, while muscle total protein and total lipids (Ether Extract) showed a significant decrease in

fish exposed to thiobencarb during the entire experimental period of acute and chronic exposure.

A change in body weight would be accompanied by a change in the amounts of the main constituents in muscle tissue (Weatherley & Gill, 1987). Bioaccumulation of thiobencarb in fish tissue may critically influence the growth rate and the quality (muscle protein) of fish (Hodson *et al.*, 1978). The transformation, storage and utilization of carbohydrates, protins and fats are regulated to a certain extent by hormones (Khangarot & Tripathi, 1991).

The increase of oxygen consumption, resulted from hypoxia induced by the herbicide is considered as a reflection of the total metabolism and the metabolic state of the fish, suggesting the acceleration of the oxidative metabolism which lead to decrease in meat quality (Abbas, 1998).

The decrease of muscle total protein in thiobencarb-exposed fish could be attributed to the reduction in food consumption and/or decrease in gross food conversion or the decrease in insulin level, detected by the observed higher plasma glucose level in thiobencarb-exposed fish; insulin has greater effect on protogenic and lipogenic pathways (Zaghloul, 1997).

Lipids may be very transient body materials, but they are an important source of potential chemical energy and their presence or absence reflects the physiological capacity of fish (Schreck & Moyle, 1990). The reported decrease of muscle total lipids in fish exposed to acute and chronic concentrations of thiobencarb may be of value for energy production to satisfy the increasing demand of energy in the studied fish on exposure to thiobencarb (Jacobs *et al.*, 2002).

Weatherley & Gill (1987) reported that the depletion of muscle total protein results in tissue hydration (increase in muscle water content) and adverse dynamic relationships between protein and water content in the muscle. In addition, the increase in muscle ash could be attributed to the bioconcentration of herbicide in fish as previously reported by Jyothi & Nrayan (1999) and Jacobs *et al.*(2002).

Genetic results:

The *in vivo* effects of thiobencarb, an organocarbamate, on chromosomes of the *Oreochromis niloticus* revealed different pictures of structural chromosomal aberrations due to chronic exposure of the fish (72µg/l for 30 and 60 days). Breaks, gaps, centromeric attenuation, centric fusion and deletion were the common aberrations recorded. The mean percentage of aberrations were time-dependent, where the total

means of aberrations after 30 days was 18.8 and after 60 days was 34.2 (Table.7). Many herbicides were found to have the ability to induce chromosomal aberrations, sister chromatid exchange (SCE) and micronuclei (MN) on fish (Krane *et al*, 1998).

Babu *et al.* (1989), in their study on the in vivo and in vitro effects of thiobencarb on acetylcholinestrase activity in the brain of *Sarotherodon mossambicus* revealed an inhibition of the acetylcholinestrase activity.

In a study of thiobencarb mitotic toxicity and sister chromatid exchange using a Chinese hamster cell line V79, this herbicide decreased the mitotic index and the second mitosis index in a dose - dependent manner.

A decreased activity of $Mg2^{+}$ and $Ca2^{+}$-ATPase were observed in a study of the adverse effects (in vivo and in vitro) of thiobencarb on developing rat brain (Srinivas *et al.*, 1989). This study suggests an impairing in energy synthesis and utilizing process in developing CNS of the rat during thiobencarb poisoning. From these and our study, it could be concluded that thiobencarb has a cytotoxic effect and a DNA damaging activity.

Histopathological observations:

The above biochemical investigations were confirmed by the histological study. Gills are the first target organ of pollutants because of their large interface area between external and internal fish environment, performing vital functions such as gas exchange and ion osmoregulation, the gills are particularly sensitive to adverse environmental conditions. In this study, gills were seen with clear edema of the lining epithelium of secondary lamellar cells and separation of the lining epithelial cells of their capillary beds (Fig. 2).

The fish kidney is composed of three distinct systems: endocrine, hematopoitic, and excretory. Lesions that develop in the kidney may involve one or all of the three tissue systems, thus it is essential to study the changes that may occur in different cell types. Fig. (3) shows the posterior kidney of *O. niloticus* with diffusing tubular vascular degeneration and necrosis.

The spleen of thiobencarb-exposed fish showed hyperactivation of melanomacrophage centers (Fig. 4) and dilatation of spleenic ellipsoid (Fig. 5).

The liver is generally regarded as central organ of metabolism in fish; structural damage from the effects of pollutants in liver metabolism have been supported by the results of biochemical studies, which provide

early warning indicators of toxicological responses. In addition, the liver of the treated fish showed vascular degeneration with congestion of main hepatic blood vessel (Fig. 6) and diffuse vascular degeneration (Fig. 7).

The parenchymatous organs showed vacuolar degeneration, necrosis and nuclear pyknosis of the spleen lymphocytes, in accordance with our results, Gingerich (1982) found that the most frequently encountered types of degenerative changes are those of hydropic degeneration, vacuolization and focal necrosis that were reported in case of severe intoxication. The vacuolization of hepatocytes might indicate an imbalance between the rate of synthesis of substances in the parenchymal cells and the rate of their release into the circulation system (Gingerich, 1982). Because the excretion of the divalent ions is a major function of the renal tubular epithelium, pollution with pesticides would be highly likely to affect these cells. The defused tubular necrosis appeared in the kidney exposed to the used herbicide has been suggested to be an indicator of renal toxicity for a variety of chemicals, including pesticides and herbicides (Jiraungkoorskul *et al.*, 2003).

CONCLUSION

It could be concluded that thiobencarb; a carbamate herbicide, has harmful effects on the physiology, biochemistry, histology and genetics structure of fish which in turn affect the growth rate and reproduction and leads to deterioration of meat quality of the exposed fish to the point that it can be hazardous to humans at certain levels in water.

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LEGEND AND FIGURES

Fig. (1): Chromosomal aberrations induced by thiobencarb toxification of *Oreochromis niloticus* (C.A: centromeric attenuation; C.F: centric fusion; F: frgment.

Fig. (2): Gills of *O. niloticus* showing edema of the lining epithelium of secondary lamellar cells with separation of the lining epithelial cells of their capillary beds H&E X400

Fig. (3): Posterior Kidney of *O. niloticus* showing diffuse tubular vascular degeneration and nicrosis H&E X400

Fig. (4): Spleen of *O. niloticus* showing hyperactivation of melanomacrophage centers H&E X400.

Fig. (5): Spleen of *O. niloticus* showing dilatation of spleenic ellipsoid H&E X400.

Fig. (6): Hepatopancreas of *O. niloticus* showing diffuse vascular degeneration H&E X400.

Fig. (7): Hepatopancreas of *O. niloticus* showing vascular degeneration with congestion of main hepatic blood vessel. H&E X400.

Table (1): The HPLC standardization for the determination of thiobencarb.

Pesticides	Mobile phase	Flow rate	Retention time
Thiobencarb	Methanol 90/10AN	1 ml/min	3.106

Table(2): Residues analysis of short-term (L_{C50}96 hours) and long-term (1/10 L_{C50}96 hours) exposure to thiobencarb herbicide in fish tissues after 1, 2, 3 and 4 days and after 15, 30, 45 and 60 days, respectively.

Treat. / organs	Short-term (µg/g)				Long-term (µg/g)			
	1 day	**2 day**	**3 day**	**4 day**	**15 day**	**30 day**	**45 day**	**60 day**
Brain	34.44	25.98	26.88	27.12	36.56	39.39	40.44	46.46
Kidney	63.94	48.65	39.03	31.42	36.69	41.53	43.34	51.96
Liver	44.61	47.02	49.86	47.36	56.11	71.37	78.84	77.38
Muscle	49.70	22.77	31.49	42.38	49.67	53.88	66.45	78.20
Gills	76.60	74.58	62.35	23.44	65.20	77.29	81.94	83.12

Table (3): Changes of some plasma constituents in Nile tilapia; *Oreochromis niloticus* after short-term (Lc_{50} 96 hours) and long-term (1/10 Lc_{50} 96 hours) exposure to thiobencarb herbicide.

Short-term								Long-term							
	Glucose (mg/dl)	Cholesterol (mg/dl)	AST (U/l)	ALT (U/l)	Creatinine (mg/dl)	Uric acid (mg%)	LDH (U/l)		Glucose (mg/dl)	Cholesterol (mg/dl)	AST (U/l)	ALT (U/l)	Creatinine (mg/dl)	Uric acid (mg%)	LDH (U/l)
	T								T						
Control	68.40 ±4.34^a	124.01 ±3.62^a	32.13 ±0.82^a	10.32 ±0.93^a	0.899 ±0.016^a	22.76 ±2.01^a	1347.98 ±37.85^a	Control	68.40 ±4.34^a	124.01 ±3.62^a	32.13 ±0.82^a	10.32 ±0.93^a	0.899 ±0.016^a	22.76 ±2.01^a	1347.98 ±37.85^a
1 day	81.20 ±4.05^b	142.12 ±4.23^b	38.74 ±1.68^b	12.89 ±0.53^b	0.986 ±0.031^b	26.16 ±0.82^b	1382.93 ±34.38^a	2 weeks	76.20 ±4.19^{ab}	131.65 ±2.73^{ab}	33.87 ±1.85^a	11.85 ±0.53^a	0.976 ±0.023^b	24.92 ±1.48^b	1362.60 ±24.13^a
2 days	91.80 ±2.84^b	152.1 ±4.72^{bc}	47.12 ±2.81^c	15 64 ±0.82^c	1.02 ±0.18^b	29.59 ±0.83^c	1517.74 ±18.22^b	4 weeks	84.40 ±4.25^b	136.46 ±3.07^b	44.14 ±2.98^b	13.97 ±0.76^b	1.01 ±0.07^b	27.16 ±0.44^c	1497.74 ±25.39^b
3 days	107.6 ±3.53^c	166.56 ±8.28^c	38.56 ±1.60^b	16.25 ±0.43^c	1.07 ±0 36^c	34.59 ±2.69^d	1566.56 ±19.87^{bc}	6 weeks	99.20 ±3.57^c	150.24 ±3.01^c	34.55 ±1.57^a	14.49 ±0.36^b	1.05 ±0.079^c	28.60 ±0.61^c	1546.54 ±22.07^{bc}
4 days	126.2 ±5.08^d	198 1 ±5.0^d	24.05 ±2.34^d	9.11 ±0.43^a	1.14 ±0.25^d	35.64 ±3.60^d	1639 54 ±15.45^c	8 weeks	116.60 ±2.32^d	179.90 ±4.6^d	26.19 ±1.53^c	17.65 ±0.71^c	1.11 ±0.019^d	31.86 ±0.35^d	1617.47 ±17.07^c

-T=Thiobencarb -The data are represented as means ± standard error
-Number of fish used for each group= 5
-Means with the same letter for each parameter are not significantly different, otherwise they do (Duncan's multiple range test, 1955).

Table (4): Changes of liver and muscle glycogen (mg/g fresh weight) in Nile tilapia; *reochromis niloticus* after short-term (Lc_{50} 96 hours) and long-term (1/10 Lc_{50} 96 hours) exposure to thiobencarb herbicide.

Short-term exposure			Long-term exposure		
Treatment	**Muscles**	**Liver**	**Treatment**	**Muscles**	**Liver**
Control	2.17±0.32^a	20.73±1.77^a	Control	2.17±0.32^a	20.73±1.77^a
1 day	2.11±0.21^a	16.90±0.81^b	2 weeks	2.08±0.12^a	20.52±2.84^b
2 days	1.83±0.29^a	15.17±1.05^b	4 weeks	1.82±0.15^{ab}	20.41±0.43^b
3 days	1.48±0.18^b	14.71±1.38^b	6 weeks	1.55±0.12^{ab}	18.75±1.18^b
4 days	1.35±0.37^b	11.71±0.40^c	8 weeks	1.37±0.18^b	16.62±0.72^c

-T=Thiobencarb -The data are represented as means ± standard error
-Number of fish used for each group= 5
-Means with the same letter for each parameter are not significantly different, otherwise they do (Duncan's multiple range test, 1955)

Table (5): Changes of growth rates in Nile tilapia; *Oreochromis niloticus* after short-term (Lc_{50} 96 hours) and long-term (1/10 Lc_{50} 96 hours) exposure to thiobencarb herbicide.

Time	Zero time		2 weeks		4 weeks		6 weeks		8 weeks	
Treatments	C	T	C	T	C	T	C	T	C	T
Fish Length (cm)	8.87 ±0.29^{a}	9.41 ±0.20^{a}	9.22 ±0.32^{a}	9.23 ±0.16^{a}	9.29 ±0.31^{a}	9.06 ±0.29^{a}	9.76 ±0.40^{a}	8.95 ±0.45^{a}	9.78 ±0.39^{a}	8.75 ±0.39^{a}
Fish Weight (g)	10.22 ±0.86^{a}	10.60 ±0.63^{a}	10.60 ±1.08^{a}	9.97 ±0.41^{a}	11.84 ±1.45^{a}	9.67 ±1.16^{b}	13.45 ±1.61^{a}	9.14 ±1.33^{b}	13.84 ±1.53^{a}	7.74 ±1.15^{b}

C=control T=Thiobencarb

Table (6): Changes of muscle chemical composition (% of fresh weight) in Nile tilapia; *Oreochromis niloticus* after short-term (Lc_{50} 96 hours) and long-term (1/10 Lc_{50} 96 hours) exposure to thiobencarb herbicide.

Short-term					Long-term				
Parameter	Total protein	Ash	Water content	E.E.	Parameter	Total protein	Ash	Water content	E.E.
Treatment	T				Treatment	T			
Control	16.75 ±0.22^{a}	1.18 ±0.02^{a}	76.24 ±1.28^{a}	3.22 ±0.09^{a}	Control	16.75 ±0.22^{a}	1.18 ±0.02^{a}	76.24 ±1.28^{a}	3.22 ±0.09^{ab}
1 day	16.10 ±0.13^{ab}	1.25 ±0.07^{ab}	77.18 ±0.19^{b}	3.33 ±0.09^{ab}	2 weeks	16.29 ±0.13^{a}	1.20 ±0.04^{a}	77.10 ±1.26^{b}	3.32 ±0.08^{ab}
2 days	15.75 ±0.20^{bc}	1.33 ±0.04^{abc}	77.58 ±0.31^{bc}	3.55 ±0.12^{c}	4 weeks	15.94 ±0.21^{ab}	1.27 ±0.03^{ab}	77.50 ±1.30^{b}	3.48 ±0.08^{b}
3 days	15.01 ±0.39^{c}	1.38 ±0.04^{bc}	78.34 ±0.27^{cd}	3.08 ±0.10^{ad}	6 weeks	15.35 ±0.38^{b}	1.32 ±0.03^{b}	77.98 ±0.24^{bc}	3.10 ±0.09^{bc}
4 days	13.65 ±0.30^{d}	1.47 ±0.06^{c}	78.76 ±0.12^{d}	2.82 ±0.04^{d}	8 weeks	14.43 ±0.36^{c}	1.37 ±0.05^{b}	78.45 ±1.27^{c}	2.89 ±0.08^{c}

-T=Thiobencarb -The data are represented as means ± standard error

-Number of fish used for each group= 5

-Means with the same letter for each parameter are not significantly different, otherwise they do (Duncan's multiple range test, 1955)

Table (7): Number and mean percentage of metaphases with different types of chromosomal aberrations in *Oreochromis niloticus* kidney cells induced by long-term (30 and 60 days) exposure to thiobencarb herbicide.

Doses µg/l	Total examined metaphase	Total No.of abnormal metaphase	Abnormal metaphases					% mean ± S.E
			gap	Break	Centric fusion	Centromeric attenuation	Deletion	Total
Control	500	27	2	8	3	5	9	5.4±0.31
72µg/l (30day)	500	94	11	19	34	22	8	18 8±0.83
72µg/l (60day)	500	171	29	39	47	32	24	34 2±0 42

T-test < 0.05

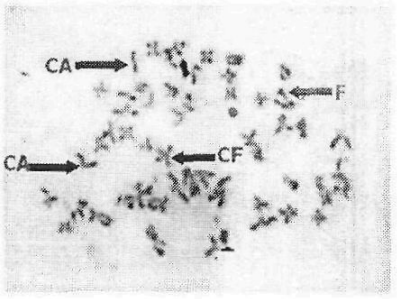
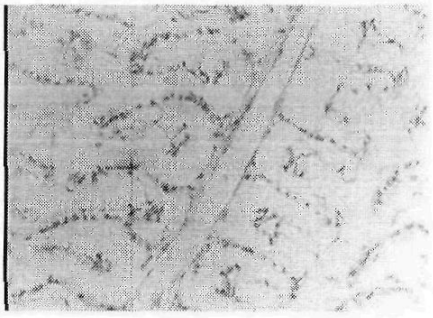


Fig. (1)



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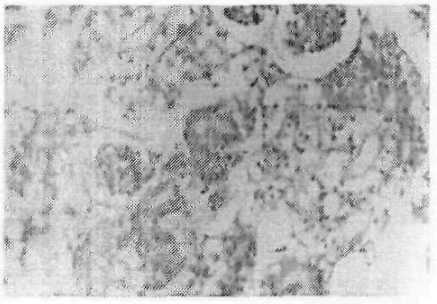

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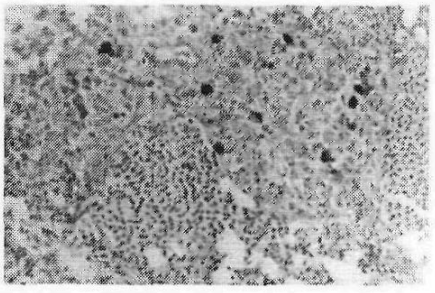

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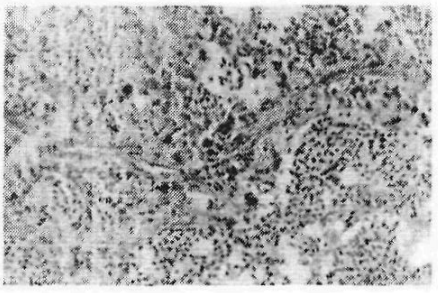

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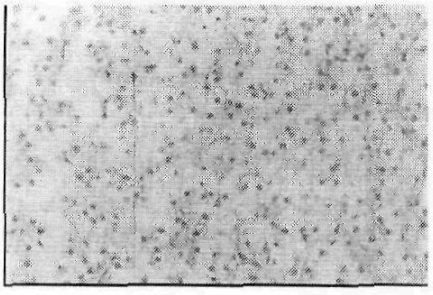

Fig. (6)

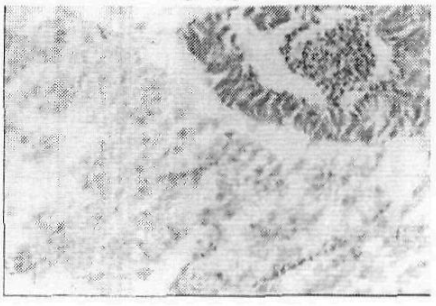

Fig. (7)