Global Veterinaria 1 (1): 45-52, 2007
ISSN 1992-6197

Some Biochemical and Pathological Investigations on Monosex Tilapia Following Chronic Exposure to Carbofuran Pesticide

[1]H. Soufy, [2]M.K. Soliman, [2]E.M. El-Manakhly and [1]A.Y. Gaafar

[1]Veterinary Research Division, National Research Center, Cairo, Egypt
[2]Faculty of Veterinary Medicine, Alexandria University, Alexandria, Egypt

Abstract: Monosex tilapia is widely cultured in Egyptian fish farms. These fish farms are mostly irrigated with agricultural drainage containing vast variety of pesticides which continuously affect fish health. This study concentrated upon the pathologic and clinicopathologic findings due to chronic exposure to the carbamate pesticide, carbofuran on monosex tilapia. Results revealed that 8 weeks exposure to 1/10 LC_{50} led to various respiratory and nervous manifestations of the fish, biochemical changes in some serum parameters including: AST, ALT, ALP, cholinesterase activities, total proteins, total bilirubin and creatinine. Histopathological investigations revealed various degrees of pathological lesions in different organs like gills, hepatopancreas, spleen, kidney, brain, intestine, skin and heart. It was concluded that carbofuran causes a lot of harmful effects to the monosex tilapia fish.

Key words: Carbofuran · *Oreochromis niloticus* · monosex tilapia · carbamate · serum biochemistry · histopathology

INTRODUCTION

Monosex tilapia is commercially worldwide cultured fish, due to its higher productivity and feasible yield. It's produced by selection of all-male tilapia *Oreochromis niloticus* either by hormonal treatment, genetic manipulation, interspecific cross breeding or even manual selection for male individuals [1].

Pesticides are chemicals intentionally introduced to the environment and it's necessary to ensure good harvests in modern agriculture. Since the 1940s, the use of pesticides has grown steadily at about 11% a year, reaching five million tonnes in 1995 [2]. Water pollution due to pesticides is posing intricate problems that need our immediate attention. New chemical formulations are widely used to control pests of agricultural crops. Overspray and/or runoff of pesticides from agricultural fields may easily find their way into the natural water surfaces and adversely affect the quality of water and create hazards for aquatic life resulting in serious damage to non-target species, including fish [3].

Most pesticides ultimately find their way into rivers, lakes and ponds. It has been found to be highly toxic not only to fishes but also to the organisms which contribute to the food chain of fishes [4-5].

Carbofuran is carbamate pesticide that is used mainly for agricultural purposes, Carbamate is a reversible cholinesterase-inhibitor through reversible carbamylation of the enzyme acetylcholinesterase, allowing accumulation of acetylcholine [6]. Carbofuran toxicity for various fish species is estimated in many studies [7-9]. Biochemical alterations due to carbofuran toxicity were reported and included inhibition of acetylcholinesterase (AchE) activity [10-12] and elevation of aspertate transaminase (AST), serum alanine transaminase (ALT) and alkaline phosphatase (ALP) levels [13, 14] and increased total lipids in all the tissues throughout the exposure period with alteration in free fatty acid content in different tissues of *Clarias batrachus* fish [15]. Also many histpathological lesions were observed by [16] in the hepatopancreas *of Channa punctatus* exposed to a dose of 4.5 ppm of carbofuran for 6 months, including cytoplasmolysis, nuclear pyknosis and necrosis. In some regions of the hepatopancreas, extensive degeneration of proliferated hepatocytes, looking like darkly stained debris of hepatomass and induction of tumors were indicative of carcinogenic action of this pesticide. Also [17] described that the long-term exposure of *Channa punctatus* caused hypertrophy, hyperplasia

Corresponding Author: Dr. H. Soufy, Veterinary Research Division, National Research Center, Cairo, Egypt

and degeneration of follicular epithelial cells and reduction in colloid content of the thyroid gland.

The present experiment aimed to investigate some relevant biochemical and pathological parameters to explore the harmful effect of the drained carbofuran pesticide on the non-target aquatic organisms under the Egyptian conditions where the commercially valuable monosex tilapia is raised.

MATERIALS AND METHODS

Carbofuran is one of the carbamate pesticides presented as 10% granular form (Furadan 10% G® FMC Chemical, SPRL). The acute 96 hours LC_{50} value of carbofuran for monosex tilapia was estimated in a previous study [18] and was found to be 0.4 ppm.

Monosex tilapia: One hundred and fifty apparently healthy monosex tilapia fish (80±20 g. B.W) were used in the present study; they were obtained from a Private fish farm free from pollutants at Sidi-Salim, Kafer EL-Shiekh province, Egypt. Fish were transported alive in well aerated tanks to the Laboratory of Fish Diseases in the Department of Avian and Aquatic Animal Medicine, Faculty of Veterinary Medicine, Alexandria University. All fish were acclimatized for at least 2 weeks prior to the experiment. During the experiment they were fed commercial dry feed pellets containing 25% protein. The diet was daily provided at 3% of body weight as described by [19]. Fish were kept in clean glass aquaria (90×50×35 cm). These aquaria were supplied with dechlorinated tap water. Oxygen supply was maintained in each aquarium using an electric aerator pumps. The water in the aquaria was renewed every 3 days followed by addition of the tested pesticide to the water.

Experimental design: One hundred and fifty monosex tilapia fish were divided into 2 groups. The first group was 100 fish exposed to $^{1}/_{10}$ LC_{50} of carbofuran (0.04 mg l^{-1}) divided into four (50 liters) aquaria. The second (control) group was 50 fish placed in two (50 liters) aquaria containing water free from any chemicals. The experiment lasted for 8 weeks. Collection of blood samples and scarification of fish were done on weekly interval during the period of experiment.

Blood sampling for biochemical analysis: One ml of blood was collected from the caudal vein of each fish by using disposable syringe into clean dry tube which was left to coagulate, centrifuged and serum was collected to evaluate the following parameters: Serum cholinesterase activity [20] alkaline phosphatase [21], transaminases: aspartate aminotransferase (AST) and alanine aminotransferase (ALT) [22], total serum proteins [24], total serum bilirubin [23] and creatinine [25] by using kits obtained from BioMérieux-France.

Tissue samples: Following necropsy, tissue specimens from the skin with the underlying musculature, gills, liver, spleen, intestine, brain, heart and kidneys were taken weekly from 5 fish of treated and 3 of control group. Tissue specimens were rapidly fixed in 10% neutral buffered formalin and processed through the conventional paraffin embedding technique. Paraffin blocks were prepared, from which 5 microns thick sections were obtained and stained with Hematoxyline and Eosin (H&E) according to the method described by [26].

Statistical analysis: All data were subjected to statistical analysis using a one-way analysis of variance (ANOVA) followed by Duncan's test according to [27] using SPSS version 11 computer program.

RESULTS

Clinical signs: Respiratory manifestations and severe congestion of gills appeared 4 days after exposure, including gasping, rapid opercular movements and collecting at the source of oxygen. Nervous manifestations, including rising of all fins and hyperexcitability appeared after the first day. Excess amount of mucous secretion was evident after 10 days. Darkening of the skin appeared after 2 weeks. Lethargy and disappearance of escape reflex were evident after 7 weeks.

Biochemical parameters: Biochemical analysis of fish chronically intoxicated with carbofuran revealed that AST level significantly ($P<0.05$) increased after the 3rd week then decreased from the 5th, till the 8th weeks. ALT significantly ($P<0.05$) increased during the 1st week and from the 4th till the 6th week and then significantly ($P<0.05$) decreased from the 7th week. ALP level also significantly($P<0.05$) increased after the 3rd week then showed significant($P<0.05$) decrease from the 5th till the 7th weeks. Total proteins significantly($P<0.05$) decreased after the 3rd, 6th, 7th and 8th weeks. Total bilirubin significantly ($P<0.05$) increased during the 8 weeks.

Table 1: Effect of chronic exposure of monosex tilapia (*Oreochromis niloticus*) to 1/10 LC_{50} of carbofuran (CF) on serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, creatinine, total protein and serum cholinesterase for 8 week (Means±SE)

Parameter	AST (IU/I)		ALT (IU/I)		ALP (IU/I)		Total bilirubin		Creatinine (mg/dl)		Total protein (g/dl)		Cholinesterase (U/I)	
	Treatment													
Time (weeks)	Ctrl*	CF**	Ctrl	CF	Ctrl	CF	Ctrl	CF	Ctrl	CF	Ctrl	CF	Ctrl	CF
1	33.9± 0.66^{ei}	33.49± 0.76^{be}	84.85± 0.66^{b}	141.62± 14.92^{f}	31.03± 0.66^{a}	20.71± 0.73^{k}	0.33± 0.007^{f}	0.58± 0.007^{i}	0.17± 0.007^{be}	0.37± 0.016^{i}	3.92± 0.01^{i}	4.04± 0.2^{i}	57023± 529.52^{l}	57552± 2398.03^{jk}
2	38.39± 0.66^{ei}	34.62± 2.2^{ce}	106.47± 0.66^{e}	85.16± 5.75^{bc}	18.3± 0.66^{ij}	14.55± 0.73^{eg}	0.29± 0.007^{c}	0.59± 0.007^{i}	0.2± 0.007^{eg}	0.21± 0.009^{fh}	2.94± 0.01^{cg}	2.39± 0.31^{ae}	53826± 129.47^{k}	43778± 613.9^{e}
3	28.13± 0.66^{ab}	34.54± 0.27^{ce}	99.76± 0.66^{ce}	97.64± 1.69^{be}	18.72± 0.66^{j}	22.81± 0.73^{l}	0.21± 0.007^{d}	0.3± 0.007^{f}	0.24± 0.007^{hi}	0.24± 0.004^{gh}	3.02± 0.01^{dg}	2.08± 0.11^{ab}	48598± 561.3^{lj}	49154± 11.99^{fh}
4	25.89± 0.66^{a}	30.21± 0.44^{b}	69.2± 0.66^{a}	94.1± 12.25^{be}	16.9± 0.66^{hj}	22.11± 0.73^{kl}	0.1± 0.007^{b}	0.37± 0.007^{g}	0.27± 0.007^{ij}	0.3± 0.005^{ik}	3.02± 0.01^{dg}	2.98± 0.13^{dg}	53014± 940.67^{k}	30827± 851.87^{b}
5	34.71± 0.66^{fi}	30.37± 0.53^{b}	87.83± 0.66^{bd}	95.78± 9.42^{be}	16.2± 0.66^{ij}	13.99± 0.73^{df}	0.17± 0.007^{c}	1.89± 0.007^{r}	0.13± 0.007^{a}	0.31± 0.042^{k}	2.8± 0.01^{cf}	4.1± 0.01^{i}	47095± 865.07^{j}	36712± 741.68^{cd}
6	35.03± 0.66^{gj}	32.45± 1.81^{bc}	87.46± 0.66^{bd}	100.44± 5.75^{de}	12± 0.66^{d}	12.45± 0.73^{de}	0.29± 0.007^{e}	1.37± 0.008^{o}	0.18± 0.007^{cf}	0.13± 0.003^{a}	3.14± 0.01^{fh}	2.36± 0.14^{ad}	41638± 327.39^{h}	31755± 581.51^{b}
7	38.23± 0.66^{jk}	33.01± 1.49^{bc}	91.56± 0.66^{bd}	82.92± 0.87^{b}	15.08± 0.66^{fh}	9.66± 0.73^{c}	0.08± 0.007^{a}	1.39± 0.007^{o}	0.16± 0.007^{ad}	0.17± 0.005^{be}	3.53± 0.01^{gi}	2.66± 0.06^{bf}	53135± 819.87^{k}	35468± 697.29^{e}
8	36.47± 0.66^{ik}	25.64± 1.77^{a}	100.5± 0.66^{de}	70.44± 1.85^{a}	9.49± 0.66^{c}	16.65± 0.73^{gj}	0.18± 0.007^{c}	1.23± 0.007^{m}	0.19± 0.007^{df}	0.15± 0.009^{ab}	3.73± 0.01^{hi}	2.99± 0.1d^{g}	53890± 1263.85^{k}	43578± 414.35^{e}

- *Ctrl = Control fish - **CF = Carbofuran treated fish
- Means with same latter(s) of the same parameter are not significantly different at $p \geq 0.05$.
- Data are represented as Mean±SE - SE = Standard error - Number of observation in each mean = 5

On the other hand creatinine showed fluctuations between significant ($P<0.05$) increase and decrease during the expiremental periods. The activity of cholinesterase was significantly ($P<0.05$) decreased during the entire periods (Table 1).

Pathological examinations

Macroscopical findings: The observed post mortem findings were congestion of the brain, gills, hepatopancreas and kidneys, pale discolored area on the hepatopancreas and severe distention of gall bladder (Fig. 1-A).

Histopathological observations

Hepatopancreas: after the 1st week till the end of the experiment, the hepatopancreas showed congestion of hepatic sinusoids and diffuse vacuolar degeneration of the hepatocytes with necrotic focal areas allover the experimental period (Fig. 1-B). The severity of the lesions was progressed with the progression of the experimental period.

Gills: After the 1st week till the end of the 8th weeks of treatment, the mostly recorded lesions were congestion, separation between surface epithelium and capillary beds and telangiectasis. Then after the 3rd week filamentous clubbing of tips of primary gill filaments due to hyperplasia and fusion of the secondary lamellae (Fig. 1-C) and edema and epithelial hyperplasia at the base of secondary lamellae (Fig. 1-D) were observed. After the 4th week there were marked shortening of secondary lamellae with fusions and hyperplasia with desquamated necrotic tissues in between the gill filaments.

Kidneys: Alterations exhibited in the posterior kidney of monosex tilapia during the 8 weeks of the experiment and directly after the 1st week were congestion and diffuse cloudy swelling of renal tubules. Moreover, the anterior kidney revealed activation of melanomacrophage centers with mild reduction of hemopoietic tissue. Then after the 3rd week decrease of the interstitial hemopoietic tissue and some focal hyaline droplet degeneration and activation of melanomacrophage centers were observed.

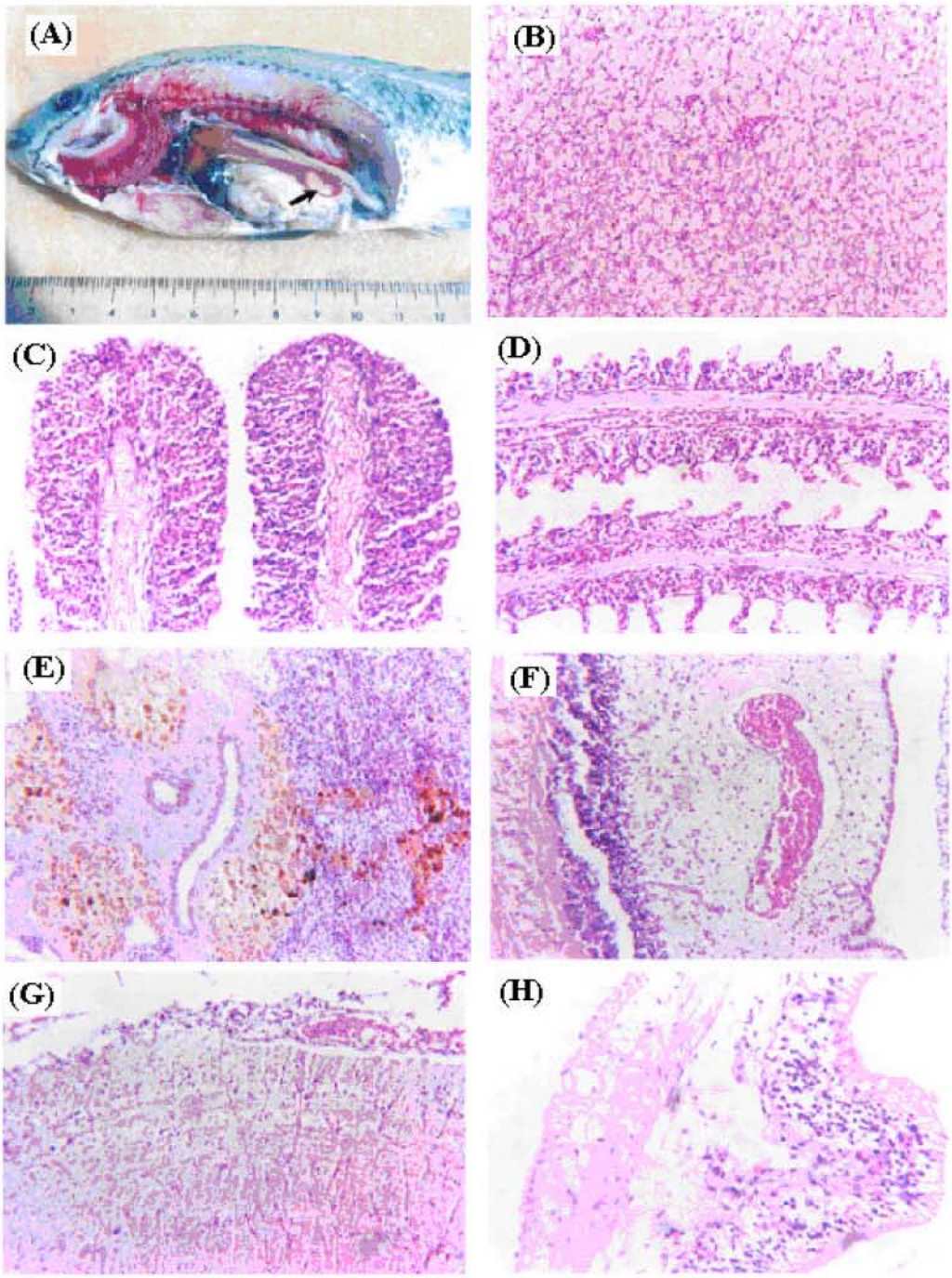

Fig. 1: A monosex tilapia after chronic exposure to carbofuran toxicosis (**A**) showing congestion of gills and presence of white area in the hepatopancreas (arrow) with distended gallbladder after 3 weeks. (**B**) hepatopancreas showing diffuse vacuolar degeneration with congestion of the hepatic sinusoids after 3 weeks. (H&E X100). (**C**) Gills showing filamentous clubbing due to hyperplasia and fusion of secondary lamellae after 3 weeks (H&E X200). (**D**) Gills showing edema and epithelial hyperplasia at the base of secondary lamellae after 3 weeks (H&E X250). (**E**) Spleen showing activation of melanomacrophage centers associated with heavy metal concentration of melanin pigments after 2 weeks (H&E X250). (**F**) Brain showing congestion of blood vessles after 2 weeks (H&E X160). (**G**) Brain showing congestion of meningeal blood vessles after 5 weeks (H&E X250). (**H**) Intestine showing epithelial degeneration leading to villus atrophy and blunting as well as submucosal edema after 2 weeks (H&E X400)

Spleen: The histopathologic examination from the beginning of the 2nd till the end of the 8th week revealed activation of melanomacrophage centers with reduction of the spleenic haemopoietic tissues. From the 3rd week dilatation of spleenic ellipsoid (Fig. 1-E) and multifocal necrotic areas surrounded by the activated melanomacrophage centers were common.

Brain: The brain revealed congestion of major blood vessels after the 2nd week till the end of the experimental period (Fig. 1-F), with concurrent congestion of meningeal blood vessels (Fig. 1-G).

Skin: The encountered lesions in the skin were mild vacuolar degeneration of epidermal cells with proliferation of club cell and hyperactivation of the melanophores after the 2nd week of treatment and during the rest of experimental period.

Intestine: After the 1st week of treatment, the histopathologic examination revealed esinophilic granular (EG) cell infiltration in the submucosa, then after the 2nd week epithelial degeneration led to villus atrophy and blunting as well as submucosal edema were observed till the end of the 8 weeks (Fig. 1-H).

Heart muscles: The histopathologic examination of heart muscles revealed congestion in between muscle fibers with (EG) cell infiltration from the 2nd week till the last week of exposure.

DISCUSSION

In the present study, the fish were exposed to carbofuran concentration equal to $^{1}/_{10}$ 96 hours LC_{50} (0.04 ppm). The clinical signs and post mortem changes were in the form of nervous manifestations, such as deviated swimming activity, abnormal social interactions and behavioral responses. Moreover, respiratory manifestations reflected by severe congestion of gills and severe congestion of most internal organs with prominent darkness of skin were observed. The same results were recorded by [28, 29] in gold fish *Carassius auratus*.

Results of serum biochemistry revealed significant increase in ALT, AST and ALP after the 3rd, 4th and 3rd weeks, respectively. Increased serum transaminases may reflect hepatic toxicity which leads to extensive liberation of the enzymes into the blood circulation [30]. Moreover, serum ALT and AST activities are considered as a sensitive indicator to evaluate hepatocellular and myocardial damage [31]. Then after the 5th, 7th and 5th weeks, respectively, the level of enzymes showed significant ($P<0.05$) decrease. This decrease was also reported by different authors [14, 32]. The increased activity of ALP in fish linked to the increased catabolic tissue breakdown in melanomacrophage centers [33]. The decrease in liver transaminases activity may be due to the decrease in protein content in serum and tissues resulting to hepatic necrosis of fish exposed to pesticides [34]. Also the severe hepatic necrosis leads to lack of cells from which the enzymes are produced. These findings indicated that decrease in liver function occurred may lead to major dangerous sequelae in body metabolism.

Total serum proteins were useful in diagnosis of fish diseases [35]. In the present study, there was hypoproteinaemia after 3 weeks of exposure to carbofuran which may be due to liver damage where most plasma protein synthesis usually occurs, this result agreed with that of [7].

Total bilirubin showed significant increase during the experimental periods. This result may be attributed to the great damage of hepatocytes, obstruction of bile duct; note the enlargement of gallbladder or a resultant hemolysis. Similar result was noticed by [36] in *O. mossambicus* treated with phosphamidon.

Creatinine also showed some sort of significant increase after the 1st week. This result supported that carbofuran exerts harmfull effects on kidney tissues.

Acetyle cholinesterase activity significantly ($P<0.01$) decrease during the experimental periods and it may be attributed to the potent anticholinesterase of the used insecticide and accumulation of acetylecholine at the synapsis of neurons leading to nervous manifestations. So, decreased ACHase activity may be used as boindicator of pollution by such pesticides in the environment. The obtained results agreed with [37].

The observed histopathological changes during the chronic toxicosis of carbofuran were various. The hepatic tissue showed congestion with various degrees of degenerative changes starting firstly with granular degeneration then vacuolar degeneration with progression towards hepatic cell necrosis after 2 weeks of exposure. These changes may be attributed to direct toxic effect of carbofuran on hepatocytes since the hepatopancreas is the site of detoxification of all types of toxins and chemicals [38].

Congestion and various degrees of pathological alterations were evident in the gills. The firstly observed lesion was lamellar edema which is frequent following exposure to chemical pollutants. Ultimately complete

edematous separation of the respiratory epithelium of primary and secondary lamellae with necrosis of lamellar epithelial cells and severe, often lethal, respiratory and osmoregulatory distress may supervene [39]. Also, severe epithelial proliferation of secondary gill lamellae, which resulted as a response of the malpighian cells to chemical irritation, as they migrate distally, often in the early stages, resulting in an accumulation of cells at the leading edge of the secondary lamella, progression of this migration leads to lamellar fusion and terminal lamellar clubbing [38]. This may be attributed to that carbofuran has a direct effect on gill filaments as cytotoxic and irritating substance which resulted in proliferation and fusion of secondary lamellae. Moreover, gills are important not only for gaseous exchange but also for osmoregulation and excretion of toxic waste products [38], thus any harm in the gills leads to impairment of such vital functions revealing respiratory distress, impaired osmoregulation and retention of toxic wastes. Hyperplasia may in some situations represent an adaptation by the organism to protect underlying tissues from any irritant. However, increased thickness of the epithelial layers including mucous cell hyperplasia and fusion of adjacent secondary lamellae as the result of hyperplasia will not only decrease the surface area available for oxygen extraction, but also will increase the oxygen diffusion distance between water and blood [40]. Also exposure to pollutants, including pesticides can cause rupture of the retaining pillar, or pilaster cells, which normally join the dorsal surface of secondary lamellae to the ventral one. The result will be dilation of the lamellar capillary and pooling of the blood, thrombosis and eventually fibrosis. Fusion with adjacent lamella, leads to the telangiectasis which is a characteristic pathological change of the gill associated with physical or chemical trauma [38].

The renal tissue of posterior kidney exhibited congestion, diffuse granular and vacuolar degenerative changes and focal hyaline droplet degeneration after 1 week of exposure and the marked depletion in haemopoietic elements which was evident in spleen and anterior and posterior kidney were probably caused by direct cytotoxic effect of carbofuran.

The activation of melanomacrophage centers either in spleen, hepatopancreas or anterior and posterior kidney was a prominent and constant lesion. It is quite known as an unusual sequel to infection or irritation in fish belonging to fish immune response [38].

In conclusion, carbofuran should be listed under the highly toxic pollutants to monosex tilapia fish even at sublethal dose (0.4 ppm) where it may cause toxicity or death, not only to rice pests but also to fish. Prolonged exposure of monosex tilapia to low doses of carbofuran pesticide (0.04 ppm) caused damage in kidney, liver, spleen and gills tissues.

REFERENCES

1. Guerrero, R.D. and L.A. Guerrero, 1988. Feasibility of commercial production of Nile tilapia fingerlings in Philippines. Pages 183–186. in R.S.V. Pullin, T. Bhukaswan, K. Tonguthai and J.L. Maclean, eds. The Second International Symposium on Tilapia in Aquaculture. ICLARM Conference Proceedings 15, Department of Fisheries, Bangkok, Thailand and International Center for Living Aquatic Resources Management, Manila, Philippines.
2. Carvalho, F.P., D.D. Nhan, C. Zhong, T. Tavares and S. Klaine, 1998. Tracking pesticides in the tropics. IAEA Bulletin, 40(3): 24-28.
3. Bondarenko, S., J. Gan, D.L. Haver and J.N. Kabashima, 2004. Persistence of selected organophosphate and carbamate insecticides in waters from a coastal watershed. Environmental Toxicology and Chemistry, 23(11): 2649-2654.
4. Anees, M.A., 1975. Acute toxicity of four organophosphorus insecticides for a fresh-water teleost *Channa punctatus* (Bloch). Pakistan Journal of Zoology, 7(2): 135-141.
5. Berny, P., 2007. Pesticides and the intoxication of wild animals. Journal of Veterinary Pharmacology and Therapeutics, 30(2): 93-100.
6. Haddad, L.M. and J.F. Winchester, 1983. Clinical Management of Poisoning and Drug Overdosage. Philadelphia, PA: W.B. Saunders Co. 711.
7. Singh, R.K. and B. Sharma, 1998. Carbofuran-induced biochemical changes in *Clarias batrachus*. Pesticide Science, 53(4): 285-290.
8. Hong, W., B. Shan, G. Cai and S. Hu, 2000. Studies on the toxicities of five types of organophosphates to the embryonic and larval development of *Nibea miichthioldes*. Marine sciences; Haiyang Kexue Qingdao (Marine Sciences; Haiyang Kexue), 24(1): 43-45.
9. Bretaud, S., 2001. Neurotoxic effects of pesticides in goldfish *Carassius auratus* L. Rennes France Universite - Rennes, 1: 167.
10. El-Kasabani, S.A., 1994. Effect of some pesticides on in vivo activities of certain enzymes in the tissues of *Tilapia zilli*. Mansoura University Journal of Agricultural Sciences, 19(5): 1851-1856.

11. Rendo´n-von, J., A. Osten, L. Ortý´z-Arana, A.M. Guilhermino and V.M. Soares, 2005. *In vivo* evaluation of three biomarkers in the mosquitofish (*Gambusia yucatana*) exposed to pesticides. Chemosphere, 58: 627-636.
12. Yi, M.Q., H.X. Liu, H.X., X.Y. Shi, P. Liang and X.W. Gao, 2006. Inhibitory effects of four carbamate insecticides on acetylcholinesterase of male and female *Carassius auratus* in vitro. Comparative Biochemistry and Physiology, Part C, 143: 113-116.
13. Shalabi, A.A., 1997. Carbosulfan toxicity to the Nile catfish *Clarias lazera* and its persistence at different water pH levels. Zagazig Journal of Agricultural Research, 24(6): 1087-1102.
14. Begum, G., 2004. Carbofuran insecticide induced biochemical alterations in liver and muscle tissues of the fish *Clarias batrachus* (linn) and recovery response, Aquatic Toxicology, 66: 83-92.
15. Begum, G. and S. Vijayaraghavan, 2001. Carbofuran toxicity on total lipids and free fatty acids in air breathing fish during exposure and cessation of exposure - *in vivo*. Environmental Monitoring and Assessment, 70(3): 233-239.
16. Ram, R.N. and S.K. Singh, 1988. Carbofuran-induced histopathological and biochemical changes in liver of the teleost fish, *Channa punctatus* (Bloch). Ecotoxicology and Environmental Safety, 16(3): 194-201.
17. Ram, R.N., 1988. Carbofuran-induced histophysiological changes in thyroid of the teleost fish, *Channa punctatus* (Bloch). Ecotoxicology and Environmental Safety, 16(2): 106-113.
18. Gaafar, A.Y., 2005. Pathologic and clinicopathologic effect of carbofuran and edifenphos on *Nile tilapia* and *Monosex tilapia*. M.V.Sc. Thesis, Faculty of Veterinary Medecine, Alexandria University.
19. Eurell, T.E., S.D.H. Lewis and L.C. Grumbles, 1978. Comparison of selected diagnostic tests for detection of motile *Aeromonas* septicemia in fish. American Journal of Volume Research, 39(8): 1384-1386.
20. Knedel, M.Y. and R. Böttinger, 1967. Eine kinetsche Methode. zur Bestimmung der Aktivitat der Pseudocholinesterase. Klin. Wzeitscr., 45: 325-327.
21. Belfield, A. and D.M. Goldberg, 1971. Colorimetric determination of alkaline phosphatase activity. Enzyme, 12: 561.
22. Reitman, S.M.D. and S. Frankel, 1957. "A colorimeter method for determination of serum glutamic oxaloacetic acid and glutamic pyruvic acid transferases." Am. J. Clinical Pathology, 28: 56-63.
23. Henary, R.J., D.C. Cannon and J.W. Winkleman, 1974. "Clinical Chemistry Principles and Techniques." 2nd Ed. Harper and Roe, New York.
24. Walter, M. and H. Gerade, 1970. Bilitubiu assay. Microchemical Journal, 15: 231-236.
25. Houot, O., 1985. "Interpretation of Clinical Laboratory Tests." Ed. by Siest, G., Henny, J.; Schiele, F.; and Young, D.S. Biochemical publications, pp: 220-234.
26. Culling, C.F., 1983. Handbook of Histopathological and Histochemical Staining Techniques. 3rd Ed.; Butterworth - London.
27. Armitage, P., 1971. "Statistical Methods in Medical Research." Blackwell Scientific Publications, London.
28. Saglio, P., S. Trijasse and D. Azam, 1996. Behavioral effects of waterborne carbofuran in goldfish. Archive of Environmental Contamination and Toxicology, 31(2): 232-238.
29. Bretaud, S., P. Saglio, C. Saligaut and B. Auperin, 2002. Biochemical and behavioral effects of carbofuran in goldfish (*Carassius auratus*). Environmental Toxicological Chemistry, 21(1): 175-81.
30. Daabees, A.Y., El-N.A. Damiaty, S.A. Soliman and M.Y. El-Toweissy, 1992, Comparative action of three synthetic pesticides on serum, liver and brain of the freshwater fish *Clarias lazera*. Journal of Egyptian German Society for Zoology. 9(A). Comparative Physiology, 105-119.
31. Abo-Hegab, G.A., M.S. Ibrahim, M.H. Bahnajawy and S.I. Abdel-Baky, 1992. Toxic effects of some water pollutants, Gallant and Mercury on blood parameters of catfish (*Clarias lazera*). Journal of Egyptian German Society for Zoology, 16(A): 201-209.
32. El-Boushy, M.A., 1994. The effect of molluscicides polluted on the blood picture and serum biochemical parameters in *Clarias lazera*. M.V.Sc. Thesis, Faculty of Veterinary Medecine, Suez-Canal University.
33. Agius, C. and W. Coushman, 1986. Induction of enhanced alkaline phosphatase activity in the melano-macrophage centers of *Oreochromis aureus* (Steindachner) through starvation and vaccination. Journal of Fish Biology, 28: 87-92.
34. Saeed, R.M.A., 1983. Biochemical and Physiological Studies on the Effect of some Pesticides on the Nile fish *Clarias lazera*. Ph.D. Thesis, Faculty of Girls Ain Shams University.
35. Mulcahy, M.F., 1967. Serum protein changes in diseased Atlantic Salmon. Nature Land, 215: 143-144.

36. Jayantha, R.K., B. Azhar and K. Ramamurthy, 1984. Effect of a systemic pesticide phosphamidon on some aspects of freshwater fish *Tilapia mossambica*. Indian Journal of Environmental Health, 26: 60-64.
37. Gosseline, R.E., R.P. Smith and H.C. Hodge, 1984. Clinical Toxicology of Commercial Products. 5th ed. Baltimore, London: Williams and Wilkins, II-305.
38. Robert, R.J., 2001. Fish Pathology. 3rd Ed., Bailliere Tindall, London, Philadelphia, Sydney, Tokyo, Tornonto.
39. Yang, C.Z. and L.J. Albright, 1992. Effects of the harmful diatom *Cheatoceros concavicornis* on respiration of rainbow trout *Onchorynchus mykiss*. Disease of Aquatic Organisms, 19: 51-55.
40. Kumaraguru, A.K., F.W.H. Beamish and H.W. Ferguson, 1982. Direct and ciyculatory paths of Permethrin (NRDC-143) causing histopathological changes in the gills of rainbow trout, Salmo gairdneri Richardson. Journal of Fish Biology, 20: 87-91.

(Received: 25/10/2007; Accepted: 27/11/2007)