HISTOPHYSIOLOGICAL CHANGES INDUCED BY THE ACTION OF MOSPILAN 20SG INSECTICIDE IN PRUSSIAN CARP (*CARASSIUS AURATUS GIBELIO BLOCH*)

Alina Păunescu, Cristina Maria Ponepal, Gabriela Zgurschi, Marinescu Alexandru Gabriel
University of Piteşti, Faculty of Science, Department of Natural Science, Piteşti, Romania
E-mail: alina_paunescu@yahoo.com

Abstract
This study was carried out to analyze the effect of sublethal and lethal concentration (0.1mg/l, 0.2mg/l, 0.4mg/l, 0.8mg/l and 1.6mg/l) of Mospilan 20SG insecticide upon some physiological parameters (oxygen consumption, breathing frequency) and gill histopathology on prussian carp (Carassius auratus gibelio Bloch). The acute and subacute toxicity of this insecticide was evaluated in glass aquaria under semi-static conditions. Mospilan 20SG, is a systemic insecticide from the neonicotinoidic group of products with acetamiprid as active substance. It is used routinely in agriculture and domestic areas against a wide range of insect pests. The toxic has changed the fish respiratory rhythm and oxygen consumption in all investigated concentration after seven days of exposure. We observed that the insecticide had an inhibiting effect on oxygen consumption for the prussian carp. We also reported sign of poisoning in fish, witch included loss of schooling behavior, swimming near the water surface, hyperactivity, erratic swimming, seizures, loss of buoyancy, increased cough rate, increased gill mucus secretions, flaring of the gill arches, head shaking and listlessness before death. The gills showed marked alterations in the epithelia in response to Mospilan 20SG (0.1mg/l water) treatment: dilation of the marginal channel, hyperplasia of the epithelial cells and lifting of the lamellar epithelium, lamellar aneurysm and epithelium rupture with hemorrhage.

Keywords: *Crassius auratus gibelio Bloch,* oxygen consumption, respiratory frequency, gill histology, Mospilan

Submitted: 07.11.2011 Reviewed: 14.11.2011 Accepted: 15.12.2011

1. INTRODUCTION

Mospilan 20SG, systemic insecticide from the neonicotinoidic group of products, is used routinely in agriculture and domestic areas against a wide range of insect pests. Active substance is acetamiprid. Acetamiprid is a broad-spectrum agricultural synthetic insecticide. This pesticide is practiced in agricultural as well as in garden. As it is a broad-spectrum insecticide it is used excessively for getting effective results besides knowing standard concentration doses of insecticides knowingly and unknowingly by framers and peoples. It enters into water and it may have effect on the morphology, physiology and behavior of fresh water organisms. In spite of adverse effects of insecticides and insecticides on the living beings, we are constantly using these biohazardous chemicals.
In the present investigation, Mospilan 20SG insecticide has been used to study its comparative effects of different lethal concentrations on oxygen consumption, breathing frequency and gills histopathology in prussian carp (*Carassius auratus gibelio* Bloch).

2. MATERIAL AND METHODS

Prussian carp fishes (*Carassius auratus gibelio Bloch*) weighing ± 17.1g were collected from local fish ponds and were allowed to acclimatize to the laboratory conditions for 10 days. The healthy specimens were exposed to five concentrations of Mospilan 20SG insecticide (0.1mg/l, 0.2mg/l, 0.4mg/l, 0.8mg/l and 1.6mg/l).
The introduction of fish in solution was done after their mixing and aeration for five minutes. The water temperature was 16-18ºC and the immersion solution was changed every 24 hours. The fish were not fed during experiments to avoid further intervention of this factor.
Oxygen consumption and frequency of respiratory movements have been determinate at intervals of 24, 48, 72, 96, 168 and 336 hours on all samples of these lots. The

energetic metabolism, expressed by the oxygen consumption, was determined by using the closed respiratory chamber method (the oxygen dose in the water was established by using the Winkler chemical method) (Picoş and Năstăsescu, 1988).
After one week of immersion in toxic substance (0.1mg/l Mospilan 20SG), the fish were anesthetized with benzocaine and then sacrificed by decapitation. Pieces of gills were excised, rinsed in physiological saline and fixed in 10% neutral formalin for poikilothermes for 24h and were processed using a graded ethanol series and embedded in paraffin. Paraffin section were cut 5µm-thick slices using a rotary microtome (Slee Maintz Cut 5062) and stained with hematoxylin Mayer (HE) as a general screening method. To avoid differing intensity of staining in different tissues, all sections to be stained by the same method were stained simultaneously.
The sections (10 sections of each fish) were examined and photographed using an Olympus microscope with an attached camera.
The statistical interpretation of the results was performed with ANOVA (LSD) test.

3. RESULTS AND DISCUSSION

In all experimental variants that have been applied on fish, all the stages described by Schäperclaus in the sympthomatologycal scheme for the intoxicated fish (Picoş and Năstăsescu, 1988), have only been observed in the variant with the Mospilan 20 SG in concentration of 1.6 mg/l water, where they have succeeded each other at very short intervals (the fish died in the first 24 hours). For the other variants we have only noticed the first three stages.
For a better comparison between the toxic effects of Mospilan 20 SG in the studied concentrations, the average values of oxygen consumption have been graphically represented in figure 1. Except the variant with Mospilan 20 SG in concentration of 0.8mg/l water, for the insecticide had an inhibiting effect on oxygen consumption for the prussian carp (after shorter or longer periods of time, it has also been noticed a growth of the energetic metabolism, comparing with the values recorded 24 hours ago), the values recorded at the end of experiments, being, in all cases, significantly difference comparing to the control values (for p<0.05). The decrease of oxygen consumption upon the action of some pesticides was observed by Marinescu *et al* (2004) and Ponepal et al (2009a, 2009b, 2009c, 2010).

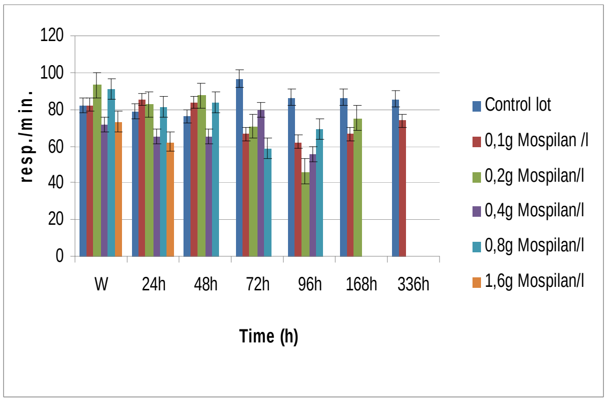

Figure 1. The influence of Mospilan 20 SG insecticide upon breathing frequency on prussian carp

In all studied concentration (0.1, 0.2, 0.4, 0.8 and 1.6 mg /l water), these pesticides modified the values of breathing frequency as shown in figure 2. For the first concentration, the insecticide effect is stimulating at first and then inhibiting of the breathing frequency (in some cases between these two effect, a longer or shorter stationary period of the breathing frequency comes up); for the middle concentrations, the effect is strongly inhibiting first, tending to reestablish the values of this physiological parameter (tendency that has not been kept till the end of the interval for the concentration of 0.1mg/l water, or until the end of the acute test for the concentration of 0.2 mg/l water).
Bradbury and Coats (1989) reported sign of fenvalerate poisoning in fish, witch included loss of schooling behaviour, swimming near the water surface, hyperactivity, erratic swimming, seizures, loss of buoyancy, increased cough rate, increased gill mucus secretions, flaring of the gill arches, head shaking and listlessness before death.

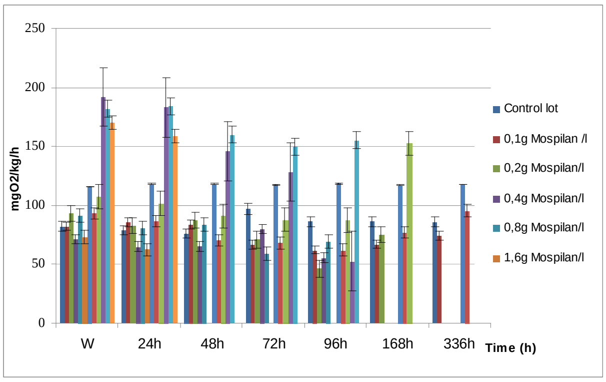

Figure 2. The influence of Mospilan 20 SG insecticide upon oxygen consumption on prussian carp

The use of respiratory stress to monitor sublethal effects of intoxication was previously applied to a variety of toxicants and subjects. Respiratory irregularities are thought to be caused by mucus precipitation on the gill epithelium in response to a toxicant. This may result in a decrease in the dissolved oxygen at the gill surface, initiating the cough reflex which is an attempt to clean the respiratory surface.

Cengiz (2006) observed histopathological effect of deltamethrin on the gill (desquamation, necrosis, aneurysm in secondary lamellae, lifting of the lamellar epithelium, epithelial hyperplasia and fusion of the secondary lamellae) of common carp after acute exposure at concentrations of 0.029 and 0.041 mg l^{-1}.

The gill arches of *Carassius auratus gibelio Bloch* in the control group show normal arrangement pattern (Figure 3a). The arches contain primary lamellae covered by stratified squamous epithelium. Projecting on the lateral sides of primary lamellae are the secondary lamellae (respiratory lamellae) covered with a delicate layer of a simple squamous epithelium that is the active exchange pillar cells. In the core of the primary lamellae is a rigid mass of cartilaginous tissues around which are traces of vascular channels.

Mospilan 20SG has induced marked pathological changes in the gills. The commonest anomalies found include dilation of the marginal channel, hyperplasia of the epithelial cells and lifting of the lamellar epithelium (Figure 3c).

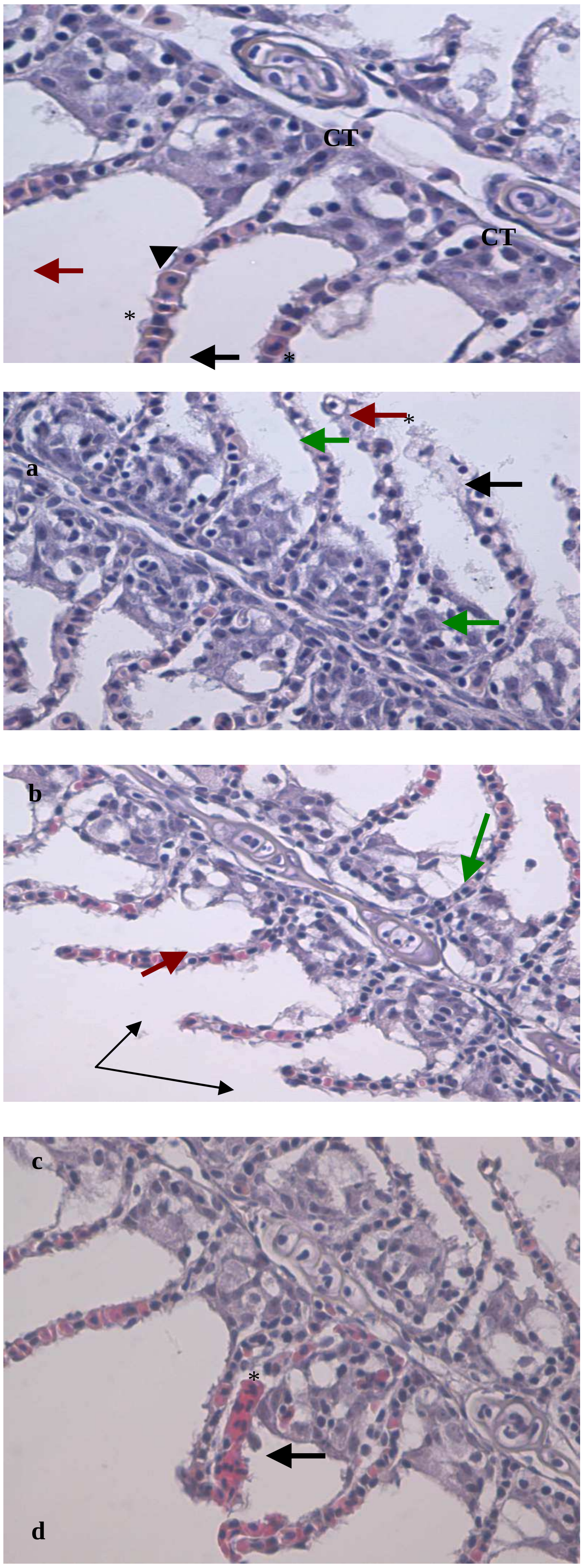

Figure 3. Gills of Prussian carp from the control and experimental group. a- normal aspect of the gill, showing the filament (arrowhead), the lamellae (green arrow), the water channel (*), a pillar cell (black arrow), an epithelial cell (red arrow) and cartilaginous tissues (CT).

400× b- lamellar disorganization (black arrow), partial fusion of some lamellae (green arrow) and hypertrophy of the lamellar epithelium (red arrow); c- lamellae with the marginal channel dilated (black arrows), hyperplasia of the epithelial cells (red arrow) and epithelial lifting (green arrow); d- lamellar aneurysm (black arrow) and epithelium rupture with hemorrhage (*). 100×. H.E

There were some cases where the hyperplasia was more severe, resulting in the fusion of some secondary lamellae (Fig. 3c). Hyperplasia in some situations represents adaptations by the organism to protect underlying tissues from any irritant (Meissner and Diamandopoulous, 1977). Thus, while hyperplasia may indeed be having a protective function it may also inhibit the respiratory, secretory and excretory functions of the gills. It was observed to lower circulation at the gills, widen the blood spaces and contract the pillar cells (Fanta *et al.*, 2003). Hyperplasia and epithelial lifting are part of the pathological features observed by Cardoso *et al.* (1996) in Pacama, *Lophiosilurus alexandri.*
Kumaraguru et al. (1982) reported fusion of adjacent secondary lamellae as a result of hyperplasia in the gill of rainbow trout *Salmo gairdneri* exposed to permethrin.

Frequently, alterations such as blood congestion (Fig. 3c), hypertrophy of epithelial cells and lamellar disorganization (Fig. 3d) were also observed. More severe lesions found in the gill were lamellar aneurysms and hemorrhages with rupture of the lamellar epithelium (Fig. 3e).
A number of pathological changes have been reported in fish exposed to different pesticides. Butchiram *et al.* (2009) observed histopathological changes in gill of fresh water fish (*Channa punctatus*) exposed to sublethal concentration of a chloroacetanilide herbicide. These changes include: necrosis, vacuolar degeneration, fusion and atrophy of primary and secondary gill lamellae.
African catfish, *Clarias gariepinus* fingerlings were exposed to sub lethal concentrations of herbicide, glyphosate (0, 0.05, 0.1%, v/v) over 42 days period. The gills showed marked alterations in the epithelia in response to glyphosate treatment. There was fusion in adjacent secondary lamellae resulting in hyperplasia, with profound oedematous changes, characterised by epithelial detachment (Olurin *et al*., 2006).
In juvenile african catfish (*Clarias gariepinus*) exposed to glyphosate, the major changes observed in the gills were oedema, epithelial lifting and thickening of the primary lamellar epithelium and fusion of secondary lamellae (Ayoola, 2008).
The gills, which participate in many important functions in fish, such as respiration, osmoregulation and excretion, remain in close contact with the external environment, and particularly sensitive to changes in the quality of the water, are considered the primary target of the contaminants (Poleksic & Mitrovic-Tutundzic, 1994; Mazon *et al.*, 2002; Fernandes & Mazon, 2003).
The histological changes observed in the gills of *Carassius auratus gibelio Bloch* indicate that the fish were responding to the direct effects of the contaminants. Such information confirms that histopathological alterations in gills tissues are good biomarkers for field assessment.

4. ACKNOWLEDGMENT

This work was partially supported by the strategic grant POSDRU 107/1.5/S/77265 (2010) of the Ministry of Labor, Family and Social Protection, Romania, co-financed by the European Social Fund – Investing in people.

5. REFERENCES

[1]Ayoola S.O., Histopathological Effects of Glyphosate on Juvenile African Catfish (*Clarias gariepinus*). American-Eurasian J. Agric. & Environ. Sci., 2008; 4 (3): 362-367.
[2]Bradbury S.P., Coats J.R., Comparative toxicology of the pyrethroid insecticides. Bulletin of Environmental Contamination and Toxicology, 1989; 108: 134- 177.
[3]Butchiram M.S., Tilak K.S., Raju P.W., Studies on histopathological changes in the gill, liver and kidney of *Channa punctatus* (*Bloch*) exposed to Alachlor. Journal of Environmental Biology, 2009; 30(2): 303-306.
[4]Cardoso E.L., Chiarini-Garcia H., Frreira R.M.A., Poli C.R., Morphological changes in the gills of *Lophiosilurus alexandri* exposed to un-ionized ammonia. J. Fish Biol., 1996; 49: 778-787.

[5]Cengiz E.I., Gill and kidney histopathology in the freshwater fish *Cyprinus carpio* after acute exposure to deltamethrin. Environmental Toxicology and Pharmacology, 2006; 21: 1093-1096.
[6]Fanta E., Saint Anna Rios F., Romao S., Vianna A., Fieiberger S., Histopathology of the fish *Corydoras paleatus* contaminated with sublethal levels of organophosphorus in water and food. Ecotoxicol. Environ. Safety, 2003; 54:119-130.
[7]Kumaraguru A.K., Beamish F.W.H., Ferguson P., Direct and circulatory paths of Permethrin causing histopathological changes in gills of *S. gairdneri.* J. Fish Biol., 1982; 20: 87-91.
[8]Marinescu A.G., Drăghici O., Ponepal C., Păunescu A., The influence of fungicide Dithane M-45 on some physiological indices in the Prussian carp (Carassius auratus gibelio Bloch). International Association for Danube Reserch, Novi Sad, 2004, 35: 209-214.
[9]Mazon A.F., Pinheiro G.H.D., Fernandes M.N., Hematological and physiological changes induced by short-term exposure to copper in the freshwater fish, *Prochilodus scrofa*. Brazilian Journal of Biology, 2002; 62 (4A): 621-631.
[10]Olurin K.B., Olojo E.A.A., Mbaka G.O., Akindele A.T., Histopathological responses of the gill and liver tissues of *Clarias gariepinus* fingerlings to the herbicide, glyphosate. African Journal of Biotechnology, 2006; 5 (24): 2480-2487.
[11]Ponepal M.C., Păunescu A., Drăghici O., Marinescu A.G., The chamges of some physiological parameters in Prussian carp under the action of the Tilt fungicide. Bulletin USAMV Cluj, 2009a, 66(1): 47-52.
[12]Ponepal M.C., Păunescu A., Drăghici O., Marinescu A.G., Effect of the fungicide chlorothalonil (Bravo) on some physiological parameters in Prussian carp. Lucrări ştiinţifice USAMV Iaşi, seria Horticultură, 2009b, 52: 1157-1162.
[13]Ponepal M.C., Păunescu A., Drăghici O., Marinescu A.G., Research on the changes of some physiological parameters in several fish species under the action of the Talstar insecticide. Analele Universităţii din Oradea, 2010, tom XVII(1): 175-179.
[14]Meissner W.A., Diamandopoulos G.Th.,. Neoplasia. In. Pathology (WAD Anderson, JM Kissane, Eds). Pp. 640-691, 1977;
[15]Fernandes M.N., Mazon A.F.,. Environmental pollution and fish gill morphology. In: Val, A. L. & B. G. Kapoor (Eds.). Fish adaptations. Enfield, Science Publishers, pp. 203-231, 2003.
[16]Picoş, C.A., Năstăsescu, Gh., Lucrări practice de fiziologie animală. University of Bucharest Press, pp. 107, 122-123, 192-195, 1988
[17]Poleksic V., Mitrovic-Tutundzic V., Fish gills as a monitor of sublethal and chronic effects of pollution.. In: Müller, R. & R. Lloyd (Eds.). Sublethal and Chronic effects of pollutants on freshwater fish. Oxford, Fishing News Books, Pp. 339-352, 1994.
[18]Ponepal M.C., Păunescu A., Drăghici O., Marinescu A.G., Research on the changes of some physiological parameters in Prussian carp under the action of the Reldan insecticide. In: Proceedings of the Second International Conference "Reserch people and actual task on multidisciplinary sciences", Lozenek, Bulgaria, 2009c, 1: 209-212.