CHANGES OF SOME PHYSIOLOGICAL PARAMETERS IN PRUSSIAN CARP (*Carassius auratus gibelio* BLOCH 1782) UNDER THE ACTION OF THE FOLPAN 80 WDG

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Abstract. The massive use of xenobiotics in agriculture has stimulated the research of those compounds in the environment. Folpan 80 WDG is a fungicide used on a broad range of phytopathogenic fungi. This study was carried out to analyze the effects of sub lethal and lethal concentrations – from $25*10^{-5}$g Folpan 80 WDG to $1*10^{-3}$g Folpan 80 WDG /l water on some physiological parameters (oxygen consumption, breathing frequency, number of erythrocytes) on Prussian carp (*Carassius auratus gibelio* BLOCH 1782). The acute and subacute toxicity of Folpan 80 WDG fungicide was evaluated in glass aquaria. The Folpan 80 WDG product, under the concentrations from $25*10^{-5}$g Folpan /l water to $1*10^{-3}$ Folpan 80 WDG g/l water, produced, after one week of immersion, a significant decrease in the fish oxygen consumption. The fungicide changed the fish respiratory rhythm in all investigated concentrations after 14 days of exposure. The number of erythrocytes significantly decreased after 14 days of immersion at fungicide concentrations.

Keywords: Prussian carp, Folpan 80 WDG, oxygen consumption, respiratory rhythm, number of erythrocytes.

Rezumat. Modificările unor parametri fiziologici la caras (*Carassius auratus gibelio* BLOCH 1782) sub acţiunea Folpanului 80 WDG. Utilizarea masivă a xenobioticelor în agricultură a stimulat cercetarea acestor compuşi în mediul înconjurător. Folpan 80 WDG este un fungicid folosit asupra unui spectru larg de ciuperci fitopatogene. Acest studiu a fost efectuat pentru a analiza efectele concentraţiilor subletale şi letale - de la $25*10^{-5}$g Folpan 80 WDG la $1*10^{-3}$g Folpan 80 WDG /l de apă asupra unor parametri fiziologici (consumul de oxigen, frecvenţa respiratorie, numărul de eritrocite) la caras (*Carassius auratus gibelio* BLOCH 1782). Toxicitatea acută şi subacută a fungicidului Folpan 80 WDG a fost evaluată în acvarii de sticlă. Produsul Folpan 80 WDG, în concentraţiile de la $25*10^{-5}$ Folpan g/l de apă până la $1*10^{-3}$ Folpan 80 WDG g/l de apă, produce, după o săptămână de imersiune, o scădere semnificativă a consumului de oxigen la peşte. Fungicidul a schimbat ritmul respirator al peştelui în toate concentraţiile studiate, după 14 zile de expunere. Numărul de eritrocite s-a redus în mod semnificativ după 14 de zile de la imersiune, la concentraţiile fungicidului.

Cuvinte cheie: caras, Folpan 80 WDG, consum de oxigen, ritm respirator, număr de eritrocite.

INTRODUCTION

Pesticides are recognized as environmental pollutants, particularly in case of ground and water (FIELDING *et al*., 1992). The presence of pesticides has also been noted in the air by scientists and addressed by regulatory authorities (ECOBICHON, 1996). They can enter water bodies directly or indirectly, resulting in contamination of various aquatic ecosystems. The effects of insecticide pollution on non-target organisms in the environment can be studied by detecting changes in organisms at the physiological, biochemical, or molecular levels, providing "early warning" tools in monitoring environment quality (CRANE *et al.,* 1991; MIREN *et al*., 2000). These sensitive early warning biomarkers can measure interaction between environmental xenobiotics and biological effects. Inhibition and induction of these biomarkers is a good approach to measure potential impacts of pollutants on environmental organisms (RENDO´N-VON OSTEN *et al.*, 2005).

Folpan 80WDG (active substance is folpet, (N-[(trichloromethyl)thio)phthalimide]) is a contact fungicide belonging to the dicarboximide family, a protective leaf-fungicide. Its mode of action inhibits normal cell division of a broad spectrum of microorganisms. It is used to control cherry leaf spot, rose mildew, rose black spot, and apple scab.

Folpan is a pyrethroid (SHAN *et al*., 1997) that affects the central and peripheral nervous system and causes synaptic discharge, depolarization and ultimately death (ROBERTS & HUDSON, 1999). Pyrethroids, synthetic analogues of pyrethrins, belong to the chemical group of non-systemic insecticides (VELISEK *et al.,* 2009). Like most pyrethroids, is also an ATP-ase inhibitor. Because they are highly lipophilic, pyrethroids are likely to be strongly absorbed by the gills, even from water containing low levels of pyrethroids (SMITH & STRATTON, 1986).

Studies with typical end-use products indicate that folpet is highly toxic to both rainbow trout and bluegill sunfish. Rainbow trout were the most sensitive species and the folpet product tested was classified in the very highly toxic range of toxicity for his species. The 96-hours LC50 for bluegill sunfish is 675 ppb and the 96-hours LC50 for rainbow trout is 185 ppb (FOLPET, http://pmep.cce.cornell.edu/profiles/extoxnet/dienochlor-glyphosate/folpet-ext.html). Folpet is characterized as being "highly toxic" to both cold water and warm water fish.

Folpan 80 WDG is more toxic at lower temperatures, and thus more toxic to cold – than warm – water fish, but the toxicity of pyrethroids is little affected by pH or water hardness (MAUCK *et al*., 1976).

The aim of this study is to analyze the influence of Folpan 80 WDG fungicide upon some physiological parameters (oxygen consumption, breathing frequency, number of red blood cells) in Prussian carp (*Carassius auratus gibelio*).

MATERIAL AND METHODS

The concentrations of Folpan 80 WDG used in all experiments and treatments were established by conducting preliminary survival tests. Since the toxic substance (the folpet) is highly soluble in water, the fish individuals were introduced into water after only 5 minutes of mixing and aeration. The water temperature was maintained at 18°C-20°C, and the "immersion" solution was permanently aerated and changed every 24 hours.

Determinations were made between February and April 2011 on fish belonging to the species: Prussian carp (*C. auratus gibelio*) having an average weight of 26 g±1.28 g, 18g±0.5 g and respectively 32 g±1.4 g, caught in the surrounding lakes and rivers of Piteşti city. We choose this species of fish because it is the most frequent in the Argeş River. After 10 days of adaptation in the lab, when they were fed *ad libitum* once a day, the fish were separated in lots, which were used separately for the following experiments: the first experiment was carried out with ten Prussian carp individuals having an average weight of 26 g±1.28 g, which were subjected to folpet concentrations of $25*10^{-5}$ Folpan 80 WDG g/l water. The second experiment was carried out with ten Prussian carp individuals having an average weight of 18g ±0.5 g, which were subjected to Folpan concentrations of $5*10^{-4}$ Folpan 80 WDG g/l water.

The third experiment was carried out with ten Prussian carp individuals having an average weight of respectively 32±1.4 g, which were subjected to Folpan concentrations of $1*10^{-3}$ Folpan 80 WDG g/l water.

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 & NĂSTĂSESCU, 1988). These determinations were made at intervals of 24, 48, 72, 96, 168 and respectively 336 hours.

The number of erythrocytes was microscopically determined with a Thoma cells numbering chamber, by using a small amount of blood collected from the caudal artery (PICOŞ & NĂSTĂSESCU, 1988).

RESULTS

For a better comparison between the toxic effects of Folpan 80 WDG in the concentrations investigated, the average frequency of respiratory movements and oxygen consumption were represented graphically in figure 1 and figure 2.

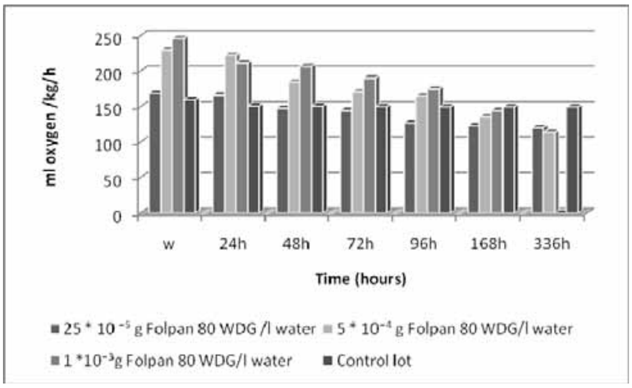

Figure 1. The influence of Folpan 80 WDG fungicide upon oxygen consumption on Prussian carp (*Carassius auratus gibelio*). / Figura 1. Influenţa fungicidului Folpan 80 WDG asupra consumului de oxigen la caras (*C. auratus gibelio*).

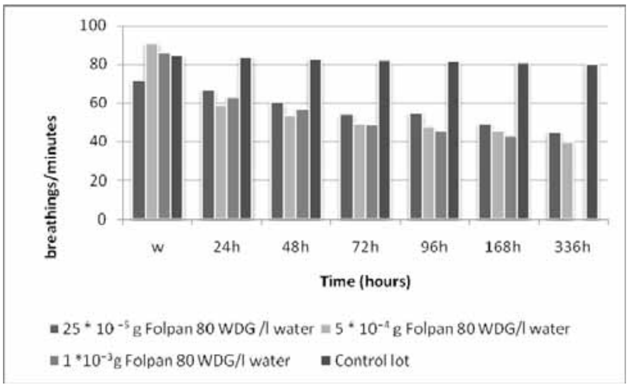

Figure 2. The influence of Folpan 80 WDG fungicide upon breathing frequency on Prussian carp (*Carassius auratus gibelio*). / Figura 2. Influenţa fungicidului Folpan 80 WDG asupra frecvenţei respiratorii la caras (*C. auratus gibelio*).

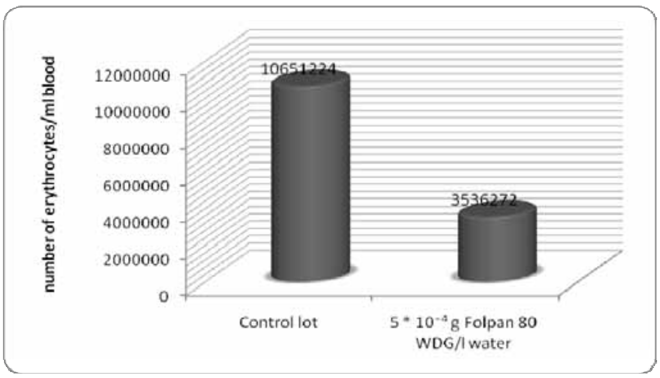

Figure 3. Number of erythrocytes of Prussian carp (*Carassius auratus gibelio*) after 14 days of exposure to Folpan 80 WDG fungicide. / Figura 3. Numărul eritrocitelor la caras (*C. auratus gibelio*) după 14 zile de expunere la fungicidul Folpan 80 WDG.

DISCUSSIONS

MARINESCU *et al.,* (2004) and PONEPAL *et al.*, (2009a, 2009b) also noticed decreased oxygen consumption under the action of some pesticides and changes in respiratory rate. For all the concentrations investigated (Fig. 1), Folpan 80 WDG produced, since the first hours of exposure, a significant reduction in the oxygen consumption of the carp, as an index of their energy metabolism, reducing toxic directly proportional to the concentration and duration of exposure to its action. Folpan 80 WDG produced, in all organized experimental variants (Fig. 2), a decrease in the respiratory frequency in the case of Prussian carp, the more powerful the higher the concentration of the toxic was.

When Folpan solution was replaced every time, *C. auratus gibelio* (BLOCH 1782) was vivacious at the beginning and then relaxed. Thirty minutes later, Prussian carp became nervous, and the gills, abdomen turned red because of blood congestion; some of them were jerked, up floated and back stroked. *C. auratus gibelio* breathed quickly and their gills turned red, too because of blood congestion.

Clinical symptoms observed during Folpan exposure of Prussian carp correspond to the observations made by other authors reporting on the toxicity of pyrethroid pesticides (PRASHANTH *et al*., 2005; VELISEK *et al*., 2006, 2009). BRADBURY & COATS (1989) reported signs of fenvalerate poisoning in fish, which 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. Sublethal effects of pyrethroids on fish include gill damage and behavioural changes (VELISEK *et al.,* 2009). In fish, direct contact between the aquatic environment and the gill epithelium may cause these surfaces to become sensitive to environmental alteration in the presence of toxic materials or other irritants.

Folpan effects on Prussian carp are positively related to the concentrations of the chemical substances, suggesting an obvious dose-response relationship. As for the mortalities of $25*10^{-5}$g Folpan 80 WDG, only one piece of Prussian carp died on the 10^{th} day. In $5*10^{-4}$g Folpan 80 WDG concentration the mortality of Prussian carp was 1 on the 12^{th} day and increased to 3 on the 14^{th} day. The mortality of $1*10^{-3}$g Folpan 80 WDG was 100% on the 14^{th} day. The experimental results showed that mortality was very small in the case of lower concentrations of Folpan 80 WDG, and was raised significantly as the concentrations of Folpan 80 WDG increased; when the concentrations of Folpan 80 WDG reached a higher value, all Prussian carp individuals were seriously toxic and died in a very short period of time. It is suggested that water organisms can be poisoned by Folpan 80 WDG such as Prussian carp, and toxicity relies on the chemical concentrations and the exposure duration.

The toxic effect of Folpan was proven to be more powerful in the first 24 hours from the fish immersion.

Respiratory irregularities are thought to be caused by mucus precipitation on the gill epithelium in response to a toxicant (SCHAUMBURG *et al*., 1967). The use of respiratory stress to monitor sublethal effects of intoxication was previously applied to a variety of toxicants and subjects (SCHAUMBURG *et al*., 1967; WALDEN *et al*., 1970). 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.

Haematological studies in fish have assumed greater significance because these parameters were to be used as an effective and sensitive index to monitor physiological and pathological changes induced by natural or anthropometric factors (DHEMBARE *et al.,* 2000). Prussian carp anaemia could be due to hypoxia that was induced by injuring the gills, as the red-pink colour of the gills became red-white, and at high concentrations the gills completely lost their red colour, while abundant secretions of mucus and even mucosal detachment with abundant bleeding could be observed (Fig. 3).

CONCLUSIONS

Folpan 80 WDG produced, in all organized experimental variants a decrease in respiratory frequency and consumption of oxygen in the case of Prussian carp, the concentration is higher as the more powerful of the toxic was.

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 & NĂSTĂSESCU, 1988), were only observed in the variant with the Folpan 80 WDG in concentration of $1*10^{-3}$g/l water, where they succeeded each other at very short intervals (the fish died in the first 24 experimental hours). For the other variants we only noticed the first three stages.

After two weeks of exposure to $5*10^{-4}$g Folpan 80 WDG the number of erythrocytes in Prussian carp decreased significantly compared to the control groups.

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