RESEARCH ON THE CHANGES OF SOME PHYSIOLOGICAL PARAMETERS IN SEVERAL FISH SPECIES UNDER THE ACTION OF THE TALSTAR INSECTICIDE

Maria Cristina PONEPAL^{*}, Alina PĂUNESCU^{*}, Octavian DRĂGHICI^{*}, Alexandru Gabriel MARINESCU^{*}

^{*}University of Pitești, Faculty of Science, Department of Ecology, Pitești, Romania

Corresponding author: Cristing Maria Ponepal, University of Pitesti, Faculty of Science, Department of Ecology, 2 Targu din Vale, 410087 Pitesti, Romania, tel.: 0040248219709, fax: 0040248219709, e-mail: ponepal_maria@yahoo.com

Abstract. Talstar insecticide is labeled for numerous bugs and many other household pests and lawn pests. Bifenthrin is highly toxic to fish and aquatic arthropods. Bifenthrin LC_{50} values range from 0.0038 to17.8 µg/L and is only slightly toxic to both waterfowl and upland game birds (LD_{50} values range from 1.800 mg/kg to > 2.150 mg/kg). Bifenthrin had no effect on mollusks at its limit of water solubility. This study was carried out to analyze the effects of sublethal and lethal concentrations – from 0.000625 to 0.005 ml Talstar/l water on some physiological parameters (oxygen consumption, breathing frequency, number of erythrocytes) on fish belonging to three species: prussian carp (*Carassius auratus gibelio Bloch*), bleak (*Alburnus alburnus* L.) and perch (*Perca fluviatilis* L.). The acute and subacute toxicity of Talstar insecticide was evaluated in glass aquaria under semystatic conditions. The Tlastar product, under the concentrations from 0.000625 to 0.005 ml/l water, produces, after one week of immersion, a significant decrease of the fish oxygen consumption. The insecticide has changed the fish respiratory rhythm in all investigated concentrations after seven days of exposure. The number of erythrocytes has significantly decrease after seven days of immersion at insecticide concentrations of 0.000625 ml Talstar/l water (bleak and perch) and 0.00125 (prussian carp) ml Talstar/l water. From the three investigated fish species, the perch proved to be the most sensitive to the action of the toxic substance, followed by the bleak and the prussian carp.

Keywords: prussian carp, bleak, perch, bifenthrin, oxygen consumption, respiratory rhythm, number of erythrocytes

INTRODUCTION

Insecticides input take place to protect agricultural crop against damaging caused by insects. However, these chemicals may achieve other ecological compartments as lakes and rivers through rains and wind, affecting many other organisms away from the first target, only 0.1% reaches the specific target [20].

Pyrethroids, synthetic analogues of pyrethrins, belong to the chemical group of nonsystemic insecticides [30].

Talstar (active substance is bifenthrin) is effective for control of insect pests of cotton [2], vegetables [21] and in public health for control of mosquitoes [14]. Bifenthrin, [(2-methyl-1,1-biphenyl-3-yl)-methyl-3-(2chloro-3,3,3-trifluoro-1propenyl)-2,2-dimethyl

cyclopropanecarboxylate] is an insecticide with contact stomach poison. Bifenthrin has some structural similarities to cypermethrin, tetramethrin and permethrin but is characterised by greater photostability and insecticidal activity than previous pyrethroids [8-9, 33].

The environmental fate of bifenthrin is a direct result of its chemical properties and the biotic and abiotic factors which it is exposed; the major biotic pathway of bifenthrin degradation is hydrolysis into 4hydroxy bifenthrin [6]. In aqueous environments bifenthrin is usually absorbed onto sediment and suspended particles.

The assessment of the ecotoxicological risks caused by pesticides to ecosystems is based on toxicity data and effects of pesticide preparations on nontarget organisms (like fish) [30].

Bifenthrin is highly toxic to fish and aquatic arthropods and LC₅₀ values range from 0.0038 to17.8 μ g/L and is only slightly toxic to both waterfowl and upland game birds (LD₅₀ values range from 1.800 mg/kg to > 2.150 mg/kg). Liu et al [10] state the 96-h LC50 value to be 2.08 μ g l⁻¹ and 0.80 μ g l⁻¹ bifenthrin for common carp and tilapia (*Tilapia* spp.), respectively. Bifenthrin had no effect on mollusks at its limit of water solubility.

Bifenthrin is a type I (noncyano) pyrethroid [24] that affects the central and peripheral nervous system and cause synaptic discharge, depolarisation and ultimately death [7, 22]. 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 [25].

Bifenthrin 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 [13].

The aim of this study is to analyze the influence of Talstar insecticide upon some physiological parameters (oxygen consumption, breathing frequency, number of red blood cells) in freshwater fishes: prussian carp (*Carassius auratus gibelio* Bloch), bleak (*Alburnus alburnus* L.) and perch (*Perca fluviatilis* L.).

MATERIALS AND METHODS

Determinations were made between September and November 2008 on fish belonging to three species: prussian carp (*Carassius auratus gibelio* Bloch), bleak (*Alburnus alburnus* L.) and perch (*Perca fluviatilis* L.), having an average weight of 26 ± 1.28 , 18 ± 0.5 an respectively 32 ± 1.4 g, caught in the surrounding lakes and rivers of Pitești city. We choose this species of fish because these are the most frequent in Arges River. After 10 days of adaptation in the lab, when they were fed *ad libitum*, the fish were separated in lots (of ten fish), which were used separately for the following experiments:

The first experiment was carried out with prussian carps individuals separated in five lots subjected to Talstar insecticide in concentrations of 0.000625, 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

 $0.00125,\ 0.0025$ and $0.05\ ml/l$ water and the control lot.

The second experiment was carried out with bleak individuals, separated in three lots which were subjected to Talstar concentrations of 0.000625 and 0.00125 ml/l water and the control lot.

The third experiment was carried out with perch individuals, separated in three lots which were subjected to Talstar concentrations of 0.000625 and 0.00125 ml/l water and the control lot.

There have been made determinations of oxygen consumption and frequency of respiratory movements at intervals of 24, 48, 72, 96, 168 and 336 hours on all samples of these lots (depending on survival).

The fourth experiment was carried out to determine the number of erythrocytes, after one week of exposure to Talstar insecticide at 0.000625 (prussian carp, bleak, perch) and 0.00125 ml/l (prussian carp) comparative to control groups.

Talstar P (7.9% bifenthrin) concentrations were determined by preliminary tests of survival. The introduction of fish in solutions was done after their mixing and aeration for ten minutes. The test was performed semi statically with the bath exchange every 24 hours in 100 l aquaria. Water temperature in the test ranged from 16.2 to 17.8°C, oxygen saturation of water ranged between 92 and 96% and pH ranged from 8.1 to 8.4.

The fish were not fed during experiments to avoid further intervention of this factor [15]. 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) [15]. 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 [15]. Fish were not anaesthetised prior to blood sampling, as they were calm due to low temperature and there was no handling stress.

The statistical interpretation of the results was performed with ANOVA test (SPSS 16.0 software for Windows) [34].

RESULTS

Talstar has changed the respiratory rhythm of prussian carp, bleak and perch in all investigated concentrations (Fig. 1 & 2). At the concentration of 0.000625 ml/l water, the insecticide had no significant effect on respiratory rhythm on prussian carp and bleak (for p<0.05) in the first 48 hours. The values of this physiological index recorded after 96 hours, one and two weeks of exposure are significantly lower compared with control values between 86.42% and 52.51% of the values recorded prior to fish exposure to insecticide.

The oxygen consumption was found to be significantly influenced by the species and the concentration of the Talstar insecticide into the water (Fig. 3 & 4). The insecticide Talstar had an inhibitory effect on the energy metabolism of prussian carps, bleak and perch except the first two concentration on prussian carp (0.000625 and 0.00125 ml Talstar/l water).

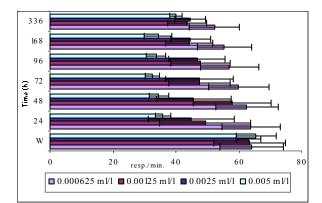


Figure 1. The influence of Talstar insecticide upon breathing frequency on prussian carp.

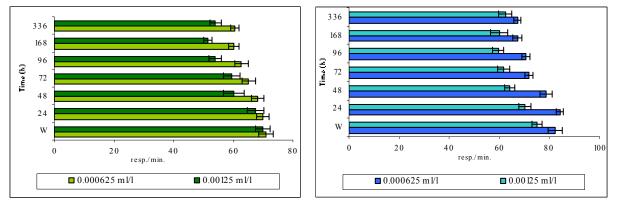


Figure 2. The influence of Talstar insecticide upon breathing frequency on bleak (left) and perch (right).

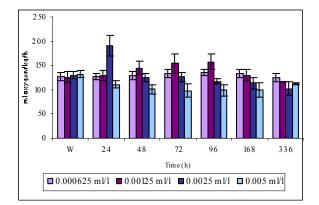


Figure 3. The influence of Talstar insecticide upon oxygen consumption on prussian carp.

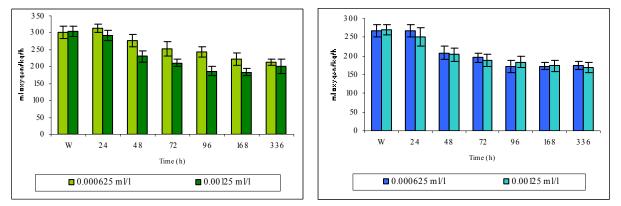


Figure 4. The influence of Talstar insecticide upon oxygen consumption on bleak (left) and perch (right).

After one week of exposure to the Talstar concentration of 0.000625 ml/l water, number of erythrocytes in bleak and perch decrease significantly compared to the control groups (Fig. 5). Compared to

the control prussian carps value, those after the acute (one week) exposure to Talstar insecticide at the concentration of 0.000625 ml/l water had no effect on RBC.

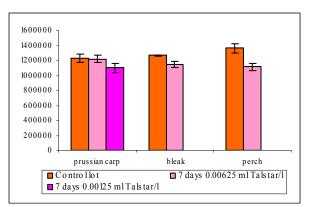


Figure 5. Number of erythrocytes of prussian carp, bleak and perch after seven days of exposure Talstar insecticide.

Table 1. Lethal effect of the insecticide Talstar on prussian carp, bleak and perch.

Experimental variants	Talstar (ml/l water)	The number of living specimens Immersion time (hours)					
		I. Prussian carp	0 (control lot)	10	10	10	10
0.000625	10		10	10	10	10	10
0.00125	10		10	10	10	10	10
0.0025	10		10	9	8	7	6
0.005	10		10	8	6	5	3
II. Bleak	0 (control lot)	10	10	10	10	9	9
	0.000625	10	10	10	10	8	7
	0.00125	10	10	10	10	8	7
III. Perch	0 (control lot)	10	10	10	10	10	9
	0.000625	10	10	10	10	8	7
	0.00125	10	10	10	8	7	6

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

Table 1 shows the data on fish mortality during the experiments. The perch proved to be the most sensitive to the action of the Talstar insecticide, followed by the bleak and the prussian carp.

DISCUSSIONS

Clinical symptoms observed during bifenthrin exposure of prussian carp, bleak and perch correspond to observations by other authors reporting on the toxicity of pyrethroid pesticides [19, 28-30]. Bradbury and Coats [3] 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. Sublethal effects of pyrethroids on fish include gill damage and behavioural changes [30].

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. The use of respiratory stress to monitor sublethal effects of intoxication was previously applied to a variety of toxicants and subjects [23, 31]. Respiratory irregularities are thought to be caused by mucus precipitation on the gill epithelium in response to a toxicant [23]. 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.

Pyrethroids affect the sodium ion channel in both the peripheral and central nervous system of insects, initially stimulating nerve cells and eventually causing paralysis [32].

In the Talstar EC10 exposure, the following clinical symptoms were observed: increased respiration, loss of coordination and fish (common carp) lying on their flank and moving in this orientation [30]. Subsequent short excitation stages with convulsions, jumping above the water surface and moving in circles alternated with resting [30].

Cengiz [4] observed histopathological effect of deltamethrin on the gill (desquamation, necrosis, aneurysm in secondary lamellae, lifting of the lamellar epithelium, oedema, epithelial hyperplasia and fusion of the secondary lamellae) of common carp after acute exposure at concentrations of 0.029 and 0.041 mg I^{-1} .

Decreased oxygen consumption under the action of some pesticides and changes in respiratory rate (Dithane M 45, Reldan, Tilt) has also been noticed by Marinescu [11] and Ponepal [16-18].

Haematological studies in fishes 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. Haematological analysis can provide important information about the internal environment of the organism [12]. Other stress effects, such as transport and handling, also appear to cause changes in fish haematological indices [1]. Svobodova et al. [27] reported significantly lower values of common carp RBC, Hb and PVC, but no changes in the white blood profile, after acute exposure to delthametrin. Sopinska and Guz [26] observed a decrease in total leukocyte count and neutrophile granulocyte count in carp following acute poisoning with permethrin. The decrease in RBC after seven days of exposure of some pesticide in fish was observed by Dhembare and Ponha [5], Ponepal [17].

Examination of histological tissue on common carp after 96 h of exposure to Talstar EC10 revealed teleangioectasiae of secondary gill lamellae and degeneration of hepatocytes [30]. Exposure to Talstar EC10 (57.5 μ g l⁻¹) had no effect on the erythrocyte profile on common carp; values recorded for RBC were comparable between the experimental and control groups [30].

Fish sensitivity to pyrethroids may be explained by their relatively slow metabolism and elimination of these compounds [28-29].

The Talstar EC10 insecticide is toxic to prussian carp, bleak and perch, because it change the level of some physiological indices (oxygen consumption, breathing frequency, number of erythrocytes). From the three investigated fish species, the perch proved to be the most sensitive to the action of the toxic substance, followed by the bleak and the prussian carp.

REFERENCES

- Acerete, L., Balasch, J.C., Espinosa, E., Josa, A., Tort, L., (2004): Physiological responses in Eurasian perch (*Perca fluviatilis* L.) subjected to stress by transport and handling. Aquaculture, 237: 167-178.
- [2] Ali, M.I., Karim, M.A., (1993): Biological efficacy of some chemical insecticides against the cotton jassid, *Amrasca devastans* (Dist.), Bangladesh Journal of Zoology, 21: 161-167.
- [3] Bradbury, S.P., Coats, J.R., (1989): Comparative toxicology of the pyrethroid insecticides. Bulletin of Environmental Contamination and Toxicology, 108: 134-177.
- [4] Cengiz, E.I., (2006): Gill and kidney histopathology in the freshwater fish *Cyprinus carpio* after acute exposure to deltamethrin. Environmental Toxicology and Pharmacology 21: 1093-1096.
- [5] Dhembare, A.J., Pondha, G.M., (2000): Haematological changes in fish *Punctius sophore* exposed to some insecticides. Journal of Experimental Zoology, India, 3(1): 41-44.
- [6] Fecko, A., (1999): Environmental fate of bifenthrin. Environmental Monitoring and Pests Management Branch, Department of Pesticide Regulation, Sacramento, 10 p.
- [7] Hayes, K., (1994): Principles and methods of toxicology. Raven Press, New York, 1468 p.
- [8] Hougard, J.M., Zaim, S.D., Guillet, P., (2002): Bifenthrin: a useful pyrethroid insecticide for treatmen of mosquito nets. Journal of Medical Entomology, 39: 526-533.
- [9] Laskowski, D.A., (2002): Physical and chemical properties of pyrethroids. Reviews of environmental contamination and toxicology 174: 49-170.
- [10] Liu, T.L., Wang, Y.S., Yen, J.H., (2005): Separation of bifenthrin enantiomers by chiral HPLC and determination of their toxicity to aquatic organism. Journal of Food and Droug Analysis, 12: 357-360.

- [11] Marinescu, Al.G., Drăghici, O, Ponepal, C., Păunescu,A., (2004): The influence of fungicide (Dithane M-45) on some physiological indices in the prussian carp (*Carassius auratus gibelio Bloch*). International Association for Danube Research, Novi Sad, 35: 209-214.
- [12] Masopust, J., (2000): Clinical biochemistry. Karolinum, Prague, 832 p.
- [13] Mauck, W.L., Olson, L.E., Marking, L.L., (1976): Toxicity of natural pyrethrins and five pyrethroids to fish. Archives of Environmental Contamination and Toxicology, 4: 18-29.
- [14] Mittal, P.K., Adak, T., Subbarao, S.K., (2002): Relative efficay of five synthetic pyrethroids against four vector mosquitoes, *Anopheles culicifacies, Anopheles stephensi, Culex quinquefasciatus* and *Aedes aegypti*. Indian Malaria Journal, 39: 34-38.
- [15] Picoş, C.A., Năstăsescu, Gh., (1988): Lucrări practice de fiziologie animală. University of Bucharest Press, pp. 107, 122-123, 192-195.
- [16] Ponepal, M.C., Păunescu, A., Marinescu Al. G., Drăghici, O., (2009): The Changes of Some Physiological Parameters in Prussian Carp Under The Action of the Tilt Fungicide. Bulletin UASVM, Cluj, 66(1-2): 47-52.
- [17] Ponepal, M.C., Păunescu, A., Marinescu Al. G., Drăghici, O., (2009): Effect of the Fungicide Chlorothalonil (Bravo) on Some Physiological Parameters in Prussian Carp. Lucrări ştiințifice USAMV Iași, seria Horticultură, 52: 1157-1162.
- [18] Ponepal, M.C., Păunescu, A., Marinescu, Al. G., Drăghici, O., (2009): Research on the changes of some physiological paramerers in prussian carp under the action of the Reldan insecticide. Proceedings of the second international conference "Research people and actual tasks on multidisciplinary sciences", Lozenek, Bulgaria, 1: 209-212.
- [19] Prashanth, M.S., David, M., Mathed, S.G., (2005): Behavioural changes in freshwater fish, *Cirrhinus mrigala* (Hamilton) exposed to cypermethrin. Journal of Environmental Biology, 26: 141-144.
- [20] Rand, G.M., Petrocelli, S.M., (1984): Fundamentals of Aquatic Toxicology Methods and Applications. McGraw-Hill International Book Company, 666 p.
- [21] Reddy, D.J., Rao, B.N., (2002): Efficacy of selected insecticides against pests of grapevine. Pesticide Research Journal, 14: 92-99.
- [22] Roberts, T., Hudson, D., (1999): Metabolic pathway of agrochemicals. Part 2: insecticides and fungicides, 1st edn. The Royal Society of Chemistry, Cambridge, United Kindom, pp. 594-596.
- [23] Schaumburg, F.D., Howard, T.E., Walden, C.C., (1967): A method to evaluate the effects of water pollution on fish respiration. Water Research 1: 731-737.

- [24] Shan, G., Hammer, R.P., Ottea, J.A., (1997): Biological activity of pyrethroid analogs in pyrethroid-susceptible and-resistant tobacco budworms, *Heliothis virescens* (F.). Journal of Agricultural and Food Chemistry, 45: 4466-4473.
- [25] Smith, T.M., Stratton, G.W., (1986): Effects of synthetic pyrethroid insecticides on nontarget organisms. Research Reviews, 97: 93-119.
- [26] Sopinska, A., Guz, L., (1998): Influence of permethrin on phagocytic activity of carp. Med Welt, 54: 126-128.
- [27] Svobodova, Z., Luskova, V., Dratischov, J., Svoboda, M., Zlabek, V., (2003): Effect of deltamethrin on haematological indices of common carp (*Cyprinus carpio* L.). Acta Veterinaria (Brno), 72: 79-85.
- [28] Velisek, J., Dobsikova, R., Svobodova, Z., Modra, H., Luskova, V., (2006): Effect of deltamethrin on the biochemical profile of common carp (*Cyprinus carpio* L.). Bulletin of Environmental Contamination and Toxicology, 76: 992-998.
- [29] Velisek, J., Wlasow, T., Gomulka, P., Svobodova, Z., Dudzik, M., (2006): Effects of cypermethrin on rainbow trout (*Oncorhyinchus mykiss*). Veterinarni Medicina, 51: 469-476.
- [30] Velisek, G., Svobodova, Z., Machova, J., (2009): Effects of bifenthrin on some haematological, biochemical and histopathological parameters of common carp (*Cyprinus carpio* L.). Fish Physiology and Biochemistry, 35: 583-590.
- [31] Walden, C.C., Howard, T.E., Froud, G.C., (1970): A quantitative assay of the minimum concentration of kraft mill effluents which affect fish respiration. Water Research, 4: 61-68.
- [32] Ware, G.W., (2000): The pesticide book, 5th edition. Thompson Publications, Fresno, California, 415 p.
- [33] Yadav, R.S, Srivastava, H.C., Adak, T., Nanda, N., Thapar, B.R., Pant, C.S., Zaim, M., Subbarao, S.K., (2003): House-scale evaluation of bifenthrin indoor residual spraying for malaria vector control in India. Journal of Medical entomology, 40: 58-63.
- [34] http://www.brothersoft.com/downloads/spss-16.0.html accessed in 11 April 2010

Submitted: 13 April 2010 Accepted: 3 May 2010

Analele Universității din Oradea – Fascicula Biologie http://www.bioresearch.ro/revistaen.html Print-ISSN: 1224-5119 e-ISSN: 1844-7589 CD-ISSN: 1842-6433