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Effects of the insecticide “Actara 25 WG” on the glycconeogenesis in the liver of common carp (*Cyprinus carpio* L.)

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ABSTRACT

The main goal of the present work is to study the effects of the new neonicotinoid insecticide „Actara 25 WG“ on the intensity of expression of glycogen in the liver of common carp (*Cyprinus carpio* L.) by using PAS-reaction on cryosections. Common carp is an economically important fish species, which is widely used as a bioindicator for the health of freshwater basins since it could also survive at very contaminated sites. We have used 6.6 mg/L, 10 mg/L and 20 mg/L of the test chemical under laboratory conditions. The results demonstrated that the intensity of staining of the PAS-reaction is directly proportional to the increasing concentration of the insecticide. In addition, this indicates that the amount of glycogen in hepatocytes also increased. Conglomerates of accumulated glycogen in certain hepatocytes were found at the highest concentration of the insecticide. Therefore, we consider that under the influence of „Actara 25 WG“ the process of glycconeogenesis in the liver of the studied fish accelerates.

Key words: histochemistry, PAS-reaction, liver, common carp (*Cyprinus carpio* L.), pesticide

Introduction

Nowadays more than 1400 different pesticide formulations are being used in the environment, mostly in agriculture. In the last 50 years, the use of pesticides has greatly increased the quantity and improved the quality of food for the growing world population (Arias-Estévez et al., 2008). However, the widespread use of pesticides in agriculture resulted in a series of toxicological and environmental problems, have received extensive concerns (Li & Randak, 2009). Because most of the pesticides are not completely degraded after application, their metabolites and some unchanged forms of these compounds are excreted and subsequently enter the aquatic ecosystems which constitute the final sink of contamination (Tilak et al., 2007). Furthermore, in water basins pesticides such as insecticides and herbicides and their mixtures often adversely affect non-target organisms such as hydrobiontic species.

At the end of the 19th century pathomorphological tests started to be used in order to verify the effects of the changing environment on the health of different species, particularly that of aquatic organisms. Thus, pathomorphological analysis now becomes a useful tool for assessing the health of fish (Babińska, 2010).

The toxicity of different pesticides on non-target aquatic organisms is assessed by many authors (Singh et al., 1996; Dunkel & Richardsi, 1998; El-Shazly & El-Sharnoubi, 2000; Scott & Kaushik, 2000). According to Scott & Kaushik (2000) the use of insecticides near different water bodies could lead to a significant decrease in the number of the organisms which are an essential link in the food chain.

Pesticides can be passed through the different ecosystems by various agents, such as water, air, food, thus they could also enter into the food chain (Farmer et al., 1972; Weber, 1977). In addition, fractions of pesticides and their relevant

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metabolites have been detected in water and food, which strengthens the increasing concern that they also may have a profound impact on human health (Al-Saleh, 1994; Huber et al., 2000; Van der Oost et al., 2003).

„ACTARA 25 WG“ is a new neonicotinoid insecticide with stomach and contact activity (Yamamoto, 1996; Mason et al., 2000; Shalabey et al., 2010). It interferes with the nicotinic acetylcholine receptor, thereby disrupting the activity of the central nervous system and causing death to the insects. The active substance thiamethoxam has a wide spectrum of activity against aphids, whiteflies and leafhoppers (Senn et al., 1998). Benzidane et al. (2010) reported a trend to reduce the locomotor activity in *Periplaneta americana*, when thiamethoxam was applied to the insect.

Compared to the invertebrates, fish are more sensitive to many toxicants and a convenient test object to identify the status of aquatic ecosystems (Moiseenko & Kudryavtseva, 2002; Moiseenko & Sharova, 2006; Moiseenko et al., 2008). They are key species in the aquatic environment and widely used to study the effects of various toxicants on biological parameters by a large number of biomarkers (Birge et al., 2000).

Fish liver performs many important biological functions such as energy metabolism, which is essential for the functioning of all organisms and it also plays an important role in detoxification of different harmful compounds (Dhalla et al., 2000). Metabolism of glycogen in the liver is the basic energy source for vertebrates, which increases its levels during stress and drastic variation in the components of the environment (Hoffman & Katz, 1998; Oliveira et al., 2004; Bacca et al., 2005). Compared to those of mammals, hepatocytes of fish do not metabolize a large amount of glycogen (Moon et al., 1985).

Pesticides and related chemicals destroy the delicate balance between species, which underlies the functioning of the ecosystem and also cause a number of physiological and biochemical changes, particularly in fish (Oruç & Üner, 1999; Tilak et al., 2007). Black (1958) reported increased levels of lactic acid in the liver, muscle, and blood, and it is assumed that its entry into tissues interferes with the mechanisms that maintain the acid-base balance. It was suggested that the change in the carbohydrate metabolism in fish is a result of stress, which causes hypoxia after excessive treatment of pesticides (Laul et al., 1974).

In general, there is insufficient evidence for the impact of the thiamethoxam on the carbohydrate metabolism of

freshwater fish species. Fujiya (1961), Stonner and Livingston (1978), McLeay and Brown (1979), Saffi (1980, 1981), Oikari and Niittylä (1985), Kumar and Gopal (2001) are among the first authors who provide some of the reports on this issue.

The objective of this work was to study the effects of the insecticide “ACTARA 25 WG” on the intensity of expression of glycogen in the liver of common carp (*Cyprinus carpio* L.).

Materials and Methods

Test organisms

Common carp (*Cyprinus carpio* L.) was the fish species selected for this experiment because it is relative insensitive and as a consequence will survive and accumulate contaminants even at heavily polluted sites (Snyder et al., 2004; Reynnders et al., 2008). In addition, carps are easily maintained under laboratory conditions, thus they have been proposed as test organisms in toxicological assays by many authors (Reynaud & Deschaux, 2005; Oruc & Usta, 2007).

Common carps are wide-spread in Bulgaria and they play a significant ecological role in the Bulgarian freshwater basins. They are also considered as bioindicators for anthropogenic contamination. Unfortunately, it is very often when this fish species become a non-target organism in agricultural areas where pesticides are being intensively used.

Forty healthy common carps were obtained from the Institute of Fisheries and Aquaculture in the city of Plovdiv, Bulgaria. They were of the same size-group (mean std. length \pm SD = 16.3 cm \pm 2.7 cm; mean body mass \pm SD = 47.8 g \pm 15.2 g) with no external pathological abnormalities. After transportation, the fish were divided into four groups (n=10) in glass aquaria (70 cm x 35 cm x 40 cm) with 50 L chlorine-free tap water (by evaporation) to acclimatize for ten days. The fish were not fed during the experiment, which was held in the laboratory at the Department of Ecology and Environmental Conservation, Faculty of Biology, Plovdiv University, Bulgaria.

Chemicals and experimental setup

The insecticide “ACTARA 25 WG” was used in the experiment. It contains thiamethoxam, 3-(2chloro-thiazol-5-ylmethyl)-5-methyl-1,3,5-oxadiazinan-4-ylidene-N-nitroamine and was provided by Syngenta Bulgaria, Inc.

Three groups of fish were exposed to insecticide at concentration of 6.6 mg/L, 10 mg/L and 20 mg/L,

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respectively. The stock solution was prepared according to the instruction of the insecticide package. After the treatment, observations were made every hour for changes in the fish behavior patterns or morphological features. The experiment last for 96 h and no lethal outcome was established. It was also planned in such a way that the fish from all the groups were sacrificed on the same day. Furthermore, the fourth fish group served as a control and the fish were kept in a tank with no added insecticide.

All the aquaria had a filtration system and the water was kept oxygen saturated. For the entire duration of the experiment, the animals were maintained under a natural light/dark cycle. Physico-chemical characteristics of aquarium water such as pH, temperature, dissolved oxygen; oxygen saturation were measured three times per day using a portable system Multi 340i (WTW) according to a standard procedure (APHA, 2005). They were as follows: pH (8.1 ± 0.1); temperature (20.5 ± 0.9); dissolved oxygen (103.8 ± 0.9); oxygen saturation (9.3 ± 0.2).

Histochemical analysis

Fish dissection was performed according to the international standard procedures given in the EMERGE Protocol (Rosseland *et al.*, 2003).

Fish were decapitated and fish liver was then immediately isolated and stored at -25°C before analysis began. The histochemical analysis was carried out in the laboratory at the Department of Anatomy, Histology and Embryology at Medical University of Plovdiv, Bulgaria. Cryostat (Leica, Jung Frigocut 2800 N) was used to cut the samples. Multiple sections of each specimen were prepared according to standard methodology and ten slides were used for observation for PAS-reaction (Periodic acid-Schiff stain) (Volkova & Eletzki, 1991). Slices were fixed in absolute alcohol (for 20 min), transferred in periodic solution (for 10 min), then in Schiff's reagent (for 20 min) for the detection of glycogen in hepatocytes. The nuclei of cells were stained with Harris's haematoxylin (for 5 min), dehydrated in a graded series of ethanol concentrations, cleared in xylene and prepared for light microscopy analysis.

Histochemical alterations in fish liver were observed and photographed by using Nikon microscope model SE mounted with digital camera DCE-2, AMCAP, software version 1.0.2. Assessing the intensity of the accumulation of glycogen in hepatocytes was represented by the gradation of purple-magenta staining of the positive PAS-reaction.

All experiments were conducted in accordance with

national and international guidelines of the European Parliament and the Council on the protection of animals used for scientific purpose (Directive 2010/63/EU)

Results

We found discreet purple-magenta staining in single sections of the investigated slides in 10% of the test-organisms from the control group (Figure 1a). We also observed a tendency towards an increased intensity the staining of positive PAS-reaction in the other three experimental groups, suggesting an increase in the amount of accumulated glycogen in hepatocytes.

There was an increase of PAS-reaction in all slides (100%) of the investigated fish group in the tank with 6.6 mg/L "ACTARA 25 WG", i.e. more-intense purple-magenta staining compared with the control group. Furthermore, the noticed glycogen in the cells was diffusely scattered (Figure 1b).

We detected intense purple-magenta staining (100%) of the investigated fish from the experimental group from the aquarium with higher concentration of the insecticide (10 mg/L) compared to the previous group, treated with 6.6 mg/L of the insecticide. This indicates an increase in the accumulation of glycogen levels in the liver cells analyzed along with the increased levels of insecticide (Figure 1c).

Also, we found the highest intensity of periodic acid-Schiff staining (Figure 1d) in the final test group of fish, which were treated with the highest concentration of the insecticide (20 mg/L).

This proved the larger quantity of glycogen levels in the hepatocytes investigated in these organisms compared with the previous two groups. In addition, in 80% of the slides we detected accumulated glycogen, which formed conglomerates in single hepatocytes (Figure 2). They were not observed in the other two experimental fish groups.

Our results indicate that under the influence of pesticides such as "ACTARA 25 WG" the process of glycogenesis in the liver of common carp (*Cyprinus carpio* L.) is rapidly being increased.

Discussion

Our study presents data for glycogenesis in the liver after treatment of common carp (*Cyprinus carpio* L.) with the insecticide "ACTARA 25 WG". In the literature there are few similar studies, but most of them include other pesticides.

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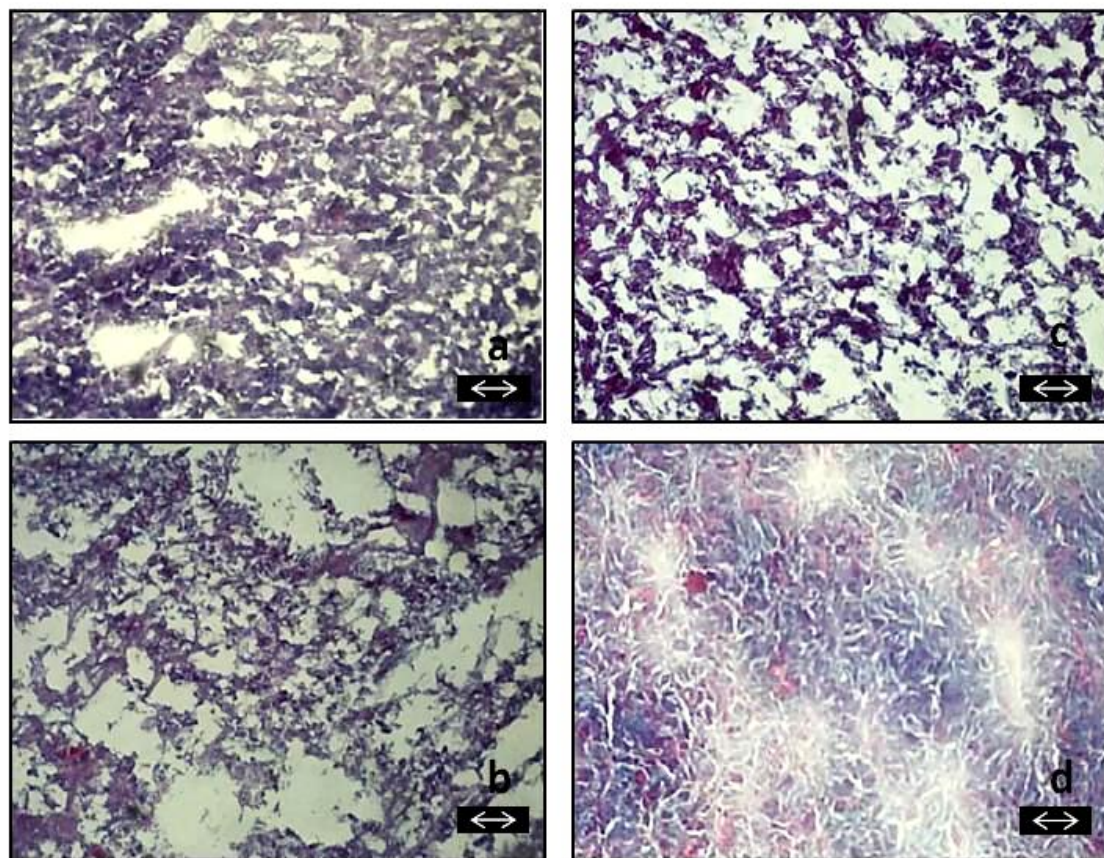


Figure 1. Intensity of the positive PAS-reaction in common carp liver tissue (magnification x200): **a** - discreet staining in control group; **b** - more-intense purple-magenta staining (6.6 mg/L of the insecticide); **c** - intense purple-magenta staining (10 mg/L of the insecticide); **d** - highest intensity of periodic acid-Schiff staining (20 mg/L of the insecticide).

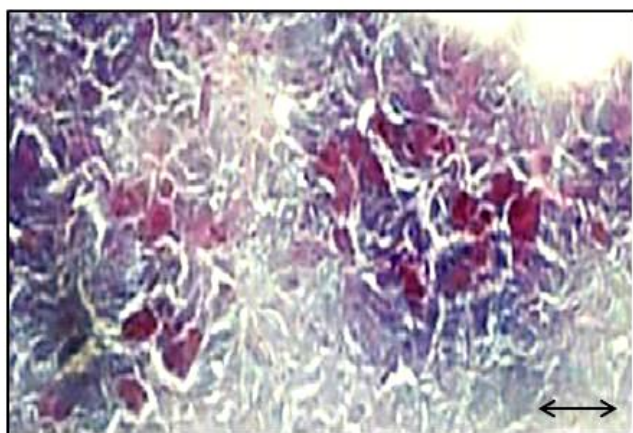


Figure 2. Conglomerates of accumulated glycogen in single hepatocytes in common carp liver tissue (20 mg/L of the insecticide), PAS-reaction (magnification x200).

McLeay & Brown (1979), Sancho et al. (1998), Sakr and Jamal (2005), Yadav et al. (2007) found a decrease in the levels of glycogen due to the effects of different toxicants. For instance, a drastic reduction of glycogen content in fish tissues proved the negative effect of accumulated pollutants from industrial wastewater (Roger, 1980). In addition, Nivedhitha et al. (1998) studied also the effects of the carbamate fungicide ziram on blood, liver, muscle and heart tissues of *Sarotherodon mossambicus*. Based on the results obtained, the authors concluded that the fish showed adaptive utilization of stored glycogen, particularly in liver tissue and adaptive accumulation of glycogen in muscle and heart tissues, probably by glycogenesis. The observed effects of lindane on carbohydrate metabolism in fish have been discussed in relation to decreased liver glucose levels.

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Ferrando and Andreu-Moliner (1991) and Oruç and Üner, (1999) reported a reduction in liver glycogen levels in herbicide-exposed fish compared to the control fish.

Wolf & Wolf (2005) consider that exposure to toxic agents of the fish liver could lead to accumulation of glycogen in the hepatocytes. We also determined accumulation of glycogen in the carp hepatocytes exposed to the increasing concentrations of the test insecticide. Similar to our results, Anandhi & Murthy (1997) also reported for increased levels of glycogen in the liver cells of *Glossogobius giuris*, but they investigated different periods of the reproductive cycle of this fish species under the influence of pesticides. Rao & Rao (1984) investigated the effects of methyl parathion on *Oreochromis mossambicus* and determined induced glyconeogenesis and the diversion of acetyl-CoA to cholesterol synthesis in relation to toxication.

In conclusion, on the basis of these results as well as our findings we suggest that having in mind the increasing of the glycogen deposition in fish liver tissue could be result of the stress caused by the insecticide.

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