ZooNotes 139: 1-4 (2019)
www.zoonotes.bio.uni-plovdiv.bg
ISSN 1313-9916

Lipid accumulation in *Cyprinus carpio* (Linnaeus, 1785) liver induced by thiamethoxam

STELA STOYANOVA[1], ELENKA GEORGIEVA[1*], ILIANA VELCHEVA[2], PEPA, ATANASOVA[3], VESELA YANCHEVA[2]

[1]Department of Developmental Biology, Faculty of Biology, Plovdiv University, 24, Tzar Assen Str., BG-4000 Plovdiv, Bulgaria, e_tomova@abv.bg*
[2]Department of Ecology and Environmental Conservation, Faculty of Biology, Plovdiv University, 24, Tzar Assen Str., BG-4000 Plovdiv, Bulgaria
[3]Department of Anatomy, Histology and Embryology, Medical University of Plovdiv,15A, Vasil Aprilov Str., BG-4000 Plovdiv, Bulgaria

Abstract. The aim of the present study is to investigate the effects of a thiamethoxam based insecticide on the expression of lipid droplets in Common Carp, *Cyprinus Carpio* (Linnaeus, 1785) liver. The selected concentrations of the test pesticide were 6.6 mg/L, 10 mg/L and 20 mg/L under laboratory conditions for an acute period of 96 h. The Sudan III staining method was applied for detection of fatty degeneration in the fish hepatocytes. Overall, we found that the fat storage in the liver cells increased proportionally with the increased pesticide concentrations. The results demonstrated fat accumulation in the fish liver which in addition, could be used as an easy to perform and relatively inexpensive biological tool for studying the effects of pesticide contamination on fish.

Key words: histochemistry, fish, liver, lipid accumulation, pesticide.

Introduction

Aquatic ecosystems receive a complex mixture of contaminants from anthropogenic sources, including agricultural practices (Zhang & Zhao 2017). One of these chemicals are the neonicotinoids which are the most widely used class of insecticides nowadays and thiamethoxam is one of them (Morrissey *et al.* 2015). In addition, the average residue levels of thiamethoxam in water ecosystems have increased over the past 15 years and its physical-chemical characteristics increase the chances of environmental contamination via surface-runoff or drainage into areas adjacent to the crops reflecting the worldwide trend in usage of this compound (Sánchez-Bayo *et al.* 2016; Iturburu *et al.* 2018).

Fish biomarkers represent an useful tool in order to evaluate the risk assessment process: effect, exposure and hazard assessment, risk characterization or classification, and monitoring the environmental quality of aquatic ecosystems (van der Oost *et al.* 2003). Thus, histopathological studies are conducted to establish fundamental relationships of contaminant exposure and its biological responses (Pathan *et al.* 2010). Moreover, according to Rajini *et al.* (2015) the histopathological evaluation is an important part of the assessment of the adverse effects of xenobiotics on the whole organism.

Accepted: 23 January 2018
Published: 27 January 2018

Based on the above, the main objective of the present work was to study the lipid accumulation in Common Carp liver in order to evaluate the negative effects of a thiamethoxam based insecticide.

Material and Methods

The experiment was conducted in accordance with Directive 2010/63/EU on the protection of animals used for scientific purpose. Three groups of fish (n=15) were exposed to the insecticide at test concentrations of 6.6 mg/L, 10 mg/L and 20 mg/L which were prepared by dilution of the stock solution of the commercial product purchased from a certified agricultural pharmacy. Common Carps were obtained from the Institute of Fisheries and Aquaculture, located in the city of Plovdiv, Bulgaria where fish are reared under strict conditions. They were of the same size-group. The exposure was carried out in static conditions according to APHA (2005). The experiment last for 96 h and no lethal outcome was reported. Cryosections of each specimen were prepared according to standard methodology and the samples were stained with Sudan III according to Pearse (1972). Evaluation of the histochemical changes was carried out using the scale according to Mishra & Mohanty (2008) which we slightly modified: (0) – negative reaction of histochemical staining; (1) – very weak positive reaction of histochemical staining; (2) – weak positive reaction of histochemical staining; (3) – moderate positive reaction of histochemical staining; (4) – strong positive reaction of histochemical staining in the hepatocytes. The results are presented as average. Statistical analysis was performed using Graph Pad Prism 7 for Windows and significant level was set at 0.05.

Results and Discussion

The results of the control group showed that the intensity of Sudan III staining was very weak. It was expressed in a discreet yellow staining in the hepatocytes. At the lowest concentration of 6.6 mg/L, a mild positive histochemical reaction was found and it was expressed in yellow-orange staining. This showed a slight accumulation of lipid droplets in the hepatocytes cytoplasm. At the higher insecticide concentration of 10 mg/L an increase in the intensity of Sudan III staining was observed and according to the proposed semi-quantitative scale it was evaluated as a moderate positive histochemical reaction. At the highest concentration of 20 mg/L strong positive Sudan III staining was found which indicated the largest amount of lipid droplets accumulated in the liver, and it was expressed as intense orange staining (Table 1, Fig. 1).

Table 1. Intensity of Sudan III staining in Common Carp liver, n=15 for each group. (0) – negative reaction of histochemical staining; (1) – very weak positive reaction of histochemical staining; (2) – weak positive reaction of histochemical staining; (3) – moderate positive reaction of histochemical staining; (4) – strong positive reaction of histochemical staining in the hepatocytes. * - significantly different ($p<0.05$).

Insecticide concentration, mg/L	**Control group**	**6.6**	**10**	**20**
Intensity of Sudan III staining	1*	2	3	4*

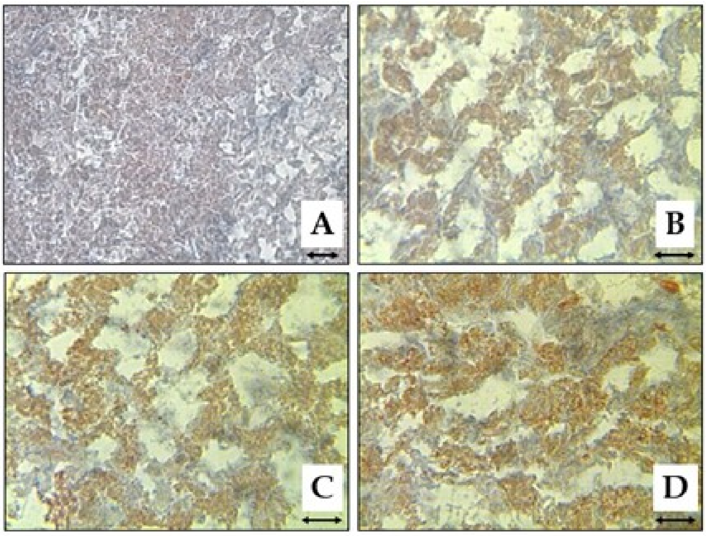

Fig. 1. Intensity of Sudan III staining in Common Carp liver: A – control group, x200; B – 6.6 mg/L insecticide, x400; C – 10 mg/L insecticide, x400; D – 20 mg/L insecticide, x400.

In regards to our previous study (see Stoyanova et al. 2012) we found that thiamethoxam induces also accumulation of glycogen which formed conglomerates in single hepatocytes. Hence, we determined induced gluconeogenesis in Common Carp. In addition, we found that the tested insecticide affected liver lactate dehydrogenase (LDH), enzymatic activity, as well as, aminotransferases aspartate (ASAT) and alanine (ALAT) activity (unpublished data). In this study, we found fatty degeneration in the fish liver along with an increase in the accumulation of glycogen levels in the liver cells. Similarly to us, Namita *et al.* (2007), Rajini *et al.* (2015) and Javed *et al.* (2016) also found fatty degeneration in the fish liver due to different toxicants, such as pesticides and heavy metals. Therefore, we consider that the observed changes in the lipid metabolism could be used as a biomarker for a non-specific response of the fish organism to various toxicants but not only pesticides. Bechmann et al. (2012) stated that hepatic lipogenesis includes de novo synthesis of fatty acids from acetyl-CoA or malonyl-CoA and further processing to triglycerides. According to the authors, these changes showed that the impaired carbohydrate and lipid metabolism could be due the toxicant activity and its chemical characteristics. We think that the observed fatty infiltration in the liver cells could be associated with the absence of the enzyme glucose-6-phosphatase and the inability to release glucose in the blood which leads to hypoglycemia in the organism. On the other hand, this could be due to increased amounts of pyruvate in the liver, and hence by the pyruvate dehydrogenase complex of excess amounts of acetyl-CoA which is used for the synthesis of fatty acids and cholesterol. Therefore, the increased fatty acid synthesis results in increased triglyceride synthesis and hyperlipidemia associated with fatty infiltration of hepatocytes.

We consider that the observed histochemical alterations could be proposed as a biological tool for the effects of water contamination in monitoring programs and assessment of aquatic pollution.

Acknowledgements. The National program "Young Researches and Postdocs, 2018" financed by the Ministry of Education and Science, Bulgaria is highly appreciated. The authors also thank the Ministry for Education and Science, Bulgaria and The Scientific Research Fund for the financial support of project M26/6 (Scientific Fundamental Research for Young Scientists and Postdocs).

References

APHA. (2005) *Standard methods for examination of water and wastewater, 21st Ed.* – American Public Health Association, Washington, DC, 541 pp.

Bechmann, L.P., Hannivoort, R.A., Gerken, G., Hotamisligil, G.S., Trauner, M. & Canbay, A. (2012) The interaction of hepatic lipid and glucose metabolism in liver diseases *Journal of Hepatology,* 56: 952-964.

Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes. *Official Journal of the European Union*, 46 pp.

Iturburu, F.G., Bertrand, L., Mendieta, J.R., Amé, M.V. & Menone, M.L. (2018) An integrated biomarker response study explains more than the sum of the parts: Oxidative stress in the fish *Australoheros facetus* exposed to imidacloprid. *Ecological Indicators,* 93: 351-357.

Javed, M., Ahmad, I., Usmani, N. & Ahmad, M. (2016) Studies on biomarkers of oxidative stress and associated genotoxicity and histopathology in *Channa punctatus* from heavy metal polluted canal. *Chemosphere,* 151: 210-219.

Mishra, A.K. & Mohanty, B. (2008) Acute toxicity impacts of hexavalent chromium on behaviour and histopathology of gill, kidney and liver of the freshwater fish, *Channa punctatus* (Bloch). *Environmental Toxicology and Pharmacology*, 26: 136-141.

Morrissey, C.A., Mineau, P., Devries, J.H., Sanchez-Bayo, F., Liess, M., Cavallaro, M.C. & Liber, K. (2015) Neonicotinoid contamination of global surface waters and associated risk to aquatic invertebrates: a review. *Environment International,* 74: 291-303.

Namita, J., Dharmlata, P. & Sahu, A.P. (2007) Histopathological changes in liver of *Heterpneustes fossilisilis* exposed to cypermethrin. *Journal of Environmental Biology,* 28(1): 35-37.

Pathan, T.S., Shinde, S.E., Thete, P.B. & Sonawane, D.L. (2010) Histopathology of liver and kidney of *Rasbora daniconius* exposed to paper mill effluent. *Research Journal of Biological Sciences,* 5(5): 389-394.

Pearse, A.G.E. (1972) *Histochemistry: Theoretical and Applied*, *3rd Ed.* Churchill Livingstone, London, 1518 pp.

Rajini, A., Revathy, K. & Selvam, G. (2015) Histopathological changes in tissues of *Danio rerio* exposed to sub lethal concentration of combination pesticide. *Indian Journal of Science and Technology,* 8(18), DOI: 10.17485/ijst/2015/v8i18/68323.

Sánchez-Bayo, F., Goka, K. & Hayasaka, D. (2016) Contamination of the aquatic environment with neonicotinoids and its implication for ecosystems. *Frontiers in Environmental Science,* 4: 1-14.

Stoyanova, S., Georgieva, E., Velcheva, I. & Yancheva, V. (2012) Effects of the insecticide "Actara 25 WG" on the glyconeogenesis in the liver of common carp (*Cyprinus carpio* L.). *Journal of Bioscience and Biotechnology*, 1(3): 249-254.

van der Oost, R., Beyer, J. & Vermeulen, N.P.E. (2003) Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environmental Toxicology and Pharmacology,* 13: 57-149.

Zhang, H. & Zhao, L. (2017) Influence of sublethal doses of acetamiprid and halosulfuron-methyl on metabolites of zebra fish (*Brachydanio rerio*). *Aquatic Toxicology*, 191: 85-94.