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Histopathological Alterations in Common Carp (*Cyprinus carpio* L.) Gills Caused by Thiamethoxam

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ABSTRACT

This work aimed to study the effect of new neonicotinoid thiamethoxam on the histological structure of common carp (Cyprinus carpio, L.) gills. Three experimental groups of fish were treated with 6.6, 10 and 20 mg/l thiamethoxam in laboratory conditions. Results showed different histological changes in the gill epithelium, which included lamellar lifting, edema, proliferation of the glandular cells and epithelium, covering the gill filament, fusion and degenerative alterations. The blood circulatory system showed mainly vasodilatation. All thiamethoxam concentrations activated compensatory-adaptive mechanisms, which caused pathological changes in the gills. Moreover, there was a tendency towards the enhancement of the gill histological changes, whose degree of expression was proportional to the increasing thiamethoxam concentrations.

Key words: insecticides, histopathology, freshwater fish, gills

INTRODUCTION

Water pollution is the burning issue nowdays all over the world. Pesticides are the class of compounds that despite of their benefits may produce a range of toxic effects that pose potential hazards to the environment. It is well known that their application could present a contaminant source for the aquatic environment (Kolpin et al. 1998; Pathan et al. 2010). Thiamethoxam is one of the neonicotinoids, which is used for controlling insect pests from paddy and cotton fields. This insecticide may reach the surrounding fresh water bodies through irrigation or rainfall. Insecticides cause severe destructive effects on aquatic fauna, particularly fish. They not only affect the respiratory system in the fish organism, but also interfere with the cellular level of respiration (Betoulle et al. 2000). In recent years, thiamethoxam has been increasingly used in agricultural practices. However, its impact on the fish and gill histology structure has not been well studied.

Fish are among the group of non–target aquatic organisms, which represent the largest and most diverse group of vertebrates. A number of characteristics make them excellent experimental models for toxicological research, especially for the contaminants, which are likely to exert their impact on aquatic systems (Law 2003; Raisuddin and Lee 2008). As an indicator of exposure to chemicals, histology represents a useful tool to assess the degree of pollution (Perry and Laurent 1993). According to Wester and Canton (1991), histopathological changes have been widely used as biomarkers for the evaluation of the health of fish, exposed to contaminants in laboratory conditions. One of the important advantages of using histopathological biomarkers in environmental monitoring is that this category of biomarkers allows examining specific target organs, including gills. They are responsible for

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vital functions such as respiration and regulation of osmotic and ionic balance (Gernhöfer et al. 2001; Cengiz 2006).

Common carp (*Cyprinus carpio*, L.) is an economically important fish species. In recent years, it has been increasingly used for food source and sport fishing. Carp has also been proposed as a test organism in many toxicological assays because it is relatively insensitive and as a consequence survives and accumulates contaminants even at heavily polluted sites (Snyder et al. 2004). Thus, carp was selected as a test organism in the present study, which aimed to investigate the histological alterations of common carp exposed to different concentrations of thiamethoxam as well as to present the degree of expression of each lesion.

MATERIAL AND METHODS

Test animals

Forthy healthy common carps were obtained from the Institute of Fisheries and Aquaculture, located in the city of Plovdiv, Bulgaria. They were of the same size-group (mean length 16.3 cm ± 2.7; mean body mass 47.8 g ± 15.2) with no external pathological abnormalities. After transportation, the fish were moved in glass aquaria with 100 L chlorine-free tap water (by evaporation) to acclimatize for four days. The individuals were divided into four groups (n=10). Fish were not fed 48 h prior to the experiment.

Chemicals and experimental setup

Thiamethoxam is a second generation neonicotinoid insecticides, which belongs to the subclass of thianicotinyl and have unique chemical properties (Fishel 2008). Three groups of fish were exposed to the test chemical at concentrations of 6.6 mg/L, 10 mg/L and 20 mg/L, representing 30, 20, 10 times dilution of the stock solution, prepared according to the instructions of the manufacturer. The fourth group served as control and no insecticide was added. The aquaria had a filtration system and the water was kept oxygen saturated. For the entire duration of the experiment, the fish were maintained under a natural light/dark cycle (12:12 h). Physico-chemical characteristics of the aquarium water such as pH, temperature, dissolved oxygen and oxygen saturation were measured once per day, according to standard procedure (APHA 2005), which were as follows: pH (8.1 ± 0.1); temperature (20.5ºC ± 0.9); dissolved oxygen (9.3 mg/L ± 0.2); oxygen saturation (103.8 % ± 0.9). All the 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 purposes according to Directive 2010/63/EU.

Histopathological analysis

Fish dissection was performed according to the procedures given in the EMERGE Protocol (Rosseland et al. 2003). All the samples were placed in vials with 10% neutral buffered formaldehyde solution (pH 7.0). They were rinsed in tap water, dehydrated in a graded series of ethanol concentrations, cleared in xylene, embedded in paraffin wax with melting point of 54–56ºC, sectioned to a thickness of 5–7 µm using a semi-automated rotary microtome (Leica RM 2245) and mounted on sterilized glass slides. Sections were then deparaffinised, stained with hematoxylin and eosin (H&E) for histological examinations and prepared for light microscopy analysis (Romeis 1989).

Semi–quantitative Scoring

From the gills of each specimen, ten paraffin sections were produced. Each section was taken from a different location from the paraffin block, instead of in series. The main purpose was to follow the histological changes in a maximum part of the sample. Histological alterations in the gill structure of each paraffin section were analyzed by observing the whole gill surface. The degree of expression of histological changes in each of them was studied, including corresponding changes on the gill surface in relation to the normal histological structure. Histological alterations in the gill epithelium were determined semi–quantitatively by using the grading system of Peebua et al. (2006), with a slight modification. The system enabled an objective assessment of the histological integrity of fish gill in histopathological analyses and clearly distinguished between the normal gill structure and gill pathology. Each grade represented specific histological characteristics and was categorized as follows: (-) – no histological alterations, which represented normal histological structure; (+/-) – mild histopathological alterations; (+) – moderate histopathological alterations; (++) – severe histopathological alterations; (+++) – very severe

histopathological alterations of the gill surface architecture.

RESULTS AND DISCUSSION

Fish respond to environmental toxic changes with adapting of their metabolite functions (Mishra and Shukla 2003). They have been successfully used as a model to study the negative effects of various pesticides to the environment (Wenderlaar Bonga and Lock 1992). Gills of most teleost fish are typically composed of four pairs of gill arches, which are supported by a bone skeleton. Filaments come from the gills arches, supported by cartilage (primary lamellae), from which the secondary lamellae exit. The secondary lamellae are constituted by a simple epithelium, where gas exchanges occur (Laurent and Perry 1995). No histopathological changes were observed in the control fish gills. Gill structural details of the control carps are shown in Figure 1A. With regard to the grading system, proposed as the control common carp histological characteristics, these were evaluated as relatively normal (-).

Histopathological results indicated that the different concentrations of thiamethoxam caused morphological alterations in the gill epithelium and the blood circulatory system. Lamellar epithelium lifting, edema, proliferation of the stratified epithelium and glandular cells, fusion, and degeneration were found in the gill epithelium. In addition, mainly vasodilatation in the blood circulatory system of the common carp gills was instituted. There were no aneurysms, which in fact represent the most severe disturbances in the blood vessels (see Table 1).

Pronounced lamellar epithelium lifting at concentration of 6.6 mg/L thiamethoxam was observed (Table 1, Fig. 1B). Edema and proliferation of stratified epithelium were found in a moderate degree of expression (Fig. 1C). In contrast, proliferation of glandular cells and fusion at the lowest thiamethoxam concentration were not observed. Gill epithelium degeneration was present in a mild degree in single areas of the gill surface. Santhakumar et al. (2001) found similar histopathological effects, such as degeneration of epithelial cells of gill filament, but following monocrotophos insecticide exposure. Furthermore, relatively slight changes in the blood circulatory system were also observed in the present study. They were presented in vasodilatation, which was detected along the length and at the apical part of the blood vessels of the secondary lamellae (Table 1).

Table 1 - Histopathological alterations in common carp gills, exposed to different concentrations thiamethoxam.

Histopathological alterations	**Concentration thiamethoxam**			
	Control group	**6.6 mg/L**	**10 mg/L**	**20 mg/L**
Lamellar lifting	+/-	++	+++	+++
Edema	-	+	+++	+++
Proliferation of stratified epithelium	-	+	++	+++
Proliferation of glandular cells	-	-	+/-	+/-
Fusion	-	-	+/-	+/-
Degeneration of gill epithelium	-	+/-	+/-	+
Vasodilatation of secondary lamellae:	-	+/-	+	+
Along the length of blood vessel	-	+/-	-	+/-
At basal part of the blood vessel	-	+/-	+/-	+/-
At apical part of the blood vessel	-	+/-	+/-	+/-
Vasodilatation of central venous sinus	-	-	-	-
Aneurysms				

(-) – no histological alterations, which representing normal histological structure; (+/-) – mild histopathological alterations; (+) – moderate histopathological alterations; (++) – severe histopathological alterations; (+++) – very severe histopathological alterations in the gill surface architecture.

Lamellar epithelium lifting and edema in all the specimens treated with 10 mg/L thiamethoxam was observed with a very severe degree of expression. Proliferation of the stratified gill epithelium was severe and also included areas along the length of particular gill filaments. Fusion as a more severe form of proliferation of the filamentous stratified epithelium was in a mild degree, as well as increasing amount of glandular cells was observed (Fig. 1D). Similarly Neskovic et al. (1993) and Oropeca et al. (2005) found hyperplasia of gill epithelial cells after pesticide exposure. Degenerative changes in the gills were also presented in a mild degree of expression. Vasodilatation could be seen more frequently in the blood vessels in the secondary lamellae as well as in single sections of the central venous sinus of the gill filament. With regard to the secondary lamellae, this histological alteration was in a

moderate degree, located mostly along its length. Moreover, vasodilatation was presented in a mild degree at the apical part of the blood vessels (Table 1).

At the highest thiamethoxam concentration of 20 mg/L, the most pronounced histological alterations were lamellar lifting, edema and proliferation of filamentous epithelial cells, which were expressed in a very severe degree. Rao et al. (2005; 2006) also reported histopathological alterations in the gills of the mosquito fish, *Gambusia affinis* under the effect of high concentrations monocrotophos insecticide. As reported by Pane at al. (2004) and Schwaiger et al. (2004) increased number of glandular cells among the epithelium covering the gill filament was observed in the present study (Table 1). Fusion in a mild degree was found (Fig. 1E). In parallel, with hyperplasic alterations, degenerative changes, which were expressed in a moderate degree of expression, were also observed. Vasodilatation affected mainly the secondary lamellae (Table 1), which was in a moderate degree, and was along the length of the blood vessel. Vasodilatation at the basal and apical part of the blood vessels was present in a mild degree of expression (Fig. 1F).

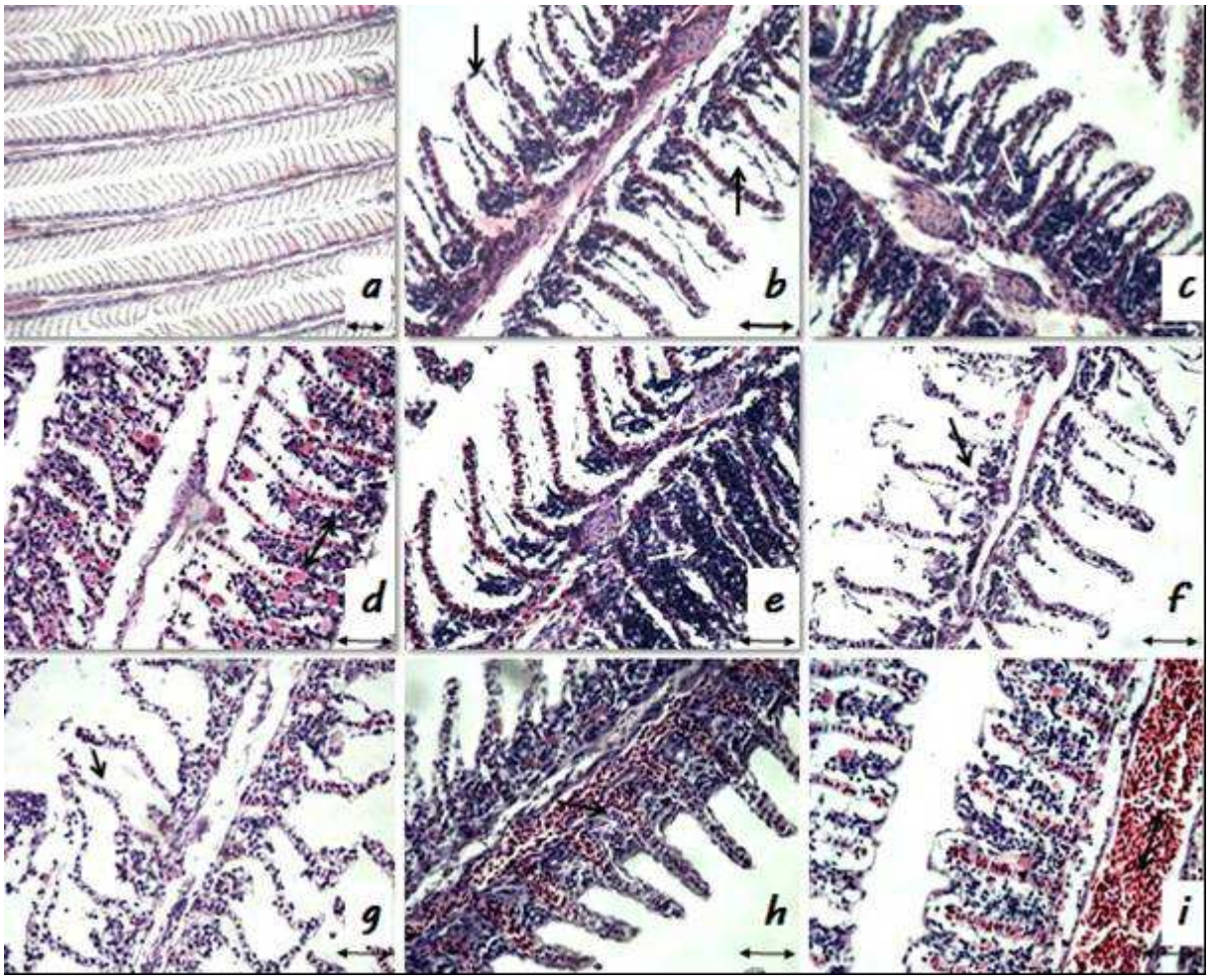

Figure 1 - Histological alterations in common carp gills, caused by thiamethoxam, (H&E): **a** - normal gill histological structure, 200X; **b** - lamellar lifting in gills, exposed to 6.6 mg/L thiamethoxam, 400X; **c** - edema in gills, exposed to 6.6 mg/L thiamethoxam, 400X; **d** - proliferation of glandular cells and filamentous epithelium (↔) in gills, exposed to 10 mg/L thiamethoxam, 400X; **e** - fusion in gills, exposed to 20 mg/L thiamethoxam, 400X; **f** - degenerative alterations in gills, exposed to 10 mg/L thiamethoxam, 400X; **g** - degenerative alterations in gills, exposed to 10 mg/L thiamethoxam, 400X; **h** - vasodilatation in blood vessels of secondary lamellae in gills, exposed to 6.6 mg/L thiamethoxam, 400X; **i** - vasodilatation in gill filament blood vessels, exposed to 6.6 mg/L thiamethoxam, 400X.

Lamellar lifting, edema and filamentous epithelial proliferation are histopathological alterations, which have been described by other authors who investigated the effects of pesticide contamination on aquatic species (Cengiz and Unlu 2002; Cengiz 2006; Velmurugan et al. 2009). Similarly to the present results, they found that these changes were the most frequent in the gill histological structure under the influence of different pesticides. These pathological alterations could be due to a two-way process. On one hand, the histological changes in the gill structure could be a reaction of the fish to toxicants intake or an adaptive response in order to prevent the entry of the pollutants through the gill surface. Therefore, they could serve as defense mechanism. This results in the increase of the distance between the external environment and the blood, which also serves as a barrier to the entrance of different contaminants (Mohamed 2009). On the other hand, the increased distance between the blood vessel and the gill epithelium limits the gas exchange and delays the oxygen access to the blood circulatory system. Hence, this could lead to oxygen deficiency and stress for the fish. For this reason, vasodilatation mainly in the blood vessels in the secondary lamellae was observed in this study. Degree of expression of this histological alteration was also increased proportionally to the thiamethoxam concentrations. The limit of gas exchange induces a compensatory increasing of the blood cells in the circulatory system. Probably the increasing internal pressure in the blood vessels in parallel with the changes in the endothelial cells caused the pathological growing of the lumen of the blood vessel. Aneurysms, which present more severe changes in the blood vessels, were not found. Weak expression of degenerative changes in the gill epithelium at all used concentrations and exposure duration was observed, suggesting that a higher dose of the test insecticide probably could activate necrotic processes in the epithelial cells. Overall, all the tested concentrations of thiamethoxam induced the processes, which were mostly associated with epithelial proliferation rather than degenerative processes.

CONCLUSION

It could be concluded that all the used concentrations of thiamethoxam negatively affected the histological structure of common carp gills and also activated compensatory-adaptive mechanisms, resulting in pathological changes. These histological lesions affected the gills by disrupting their functions. There was a tendency towards enhancing the morphological alterations and their degree of expression was proportional to the increasing concentration of thiamethoxam.

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