

# Animal welfare, etológia és tartástechnológia



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**FIBROTIC CHANGES IN THE RABBIT LIVER AFTER PESTICIDE ADMINISTRATION**

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**Abstract**

The aim of our study was to observe fibrotic changes of the rabbit liver tissue treated with bendiocarb, mostly in the area of the central vein and portal tract. Animals were divided into groups (control, days 10, 20, 30 of administration), and each group comprised 14 animals. Rabbits in all experimental groups received carbamate pesticide (bendiocarb) *per os* in a dose 5 mg/kg per day and after day 11 in a same dose per 48 hours. Picro-sirius red staining was used for visualization of collagen fibers and staging of hepatic fibrosis. The central vein wall thickness is increased in thickness at 10 and 30 days, but not significantly. Further, at the same days the fibrous septa of variable lengths are spreading from portal tract into the lobules and perisinusoidal space. In view of the data of the present study, it can be concluded that bendiocarb has moderately toxic effect on the rabbit liver tissue, regardless of gender after short-term (10 days) and long-term (30 days) bendiocarb administration.

**Introduction**

Pesticides are the significant contaminants of environment which are used in agriculture and households. Their widely using cause the question of toxicity pesticides to non-target organisms, their persistence, accumulation and combined effect with other chemicals. The annual application of synthetic pesticides to food crops in the EU exceeds 140,000 tones per year. The wide use of pesticides are mainly organophosphates (OPs), carbamates and pyrethroids. Further, over 25% of fruits, vegetables, and cereals grown in the European Union contain detectable residues of at least two pesticides while their total quantity, which Europe's population is exposed to is unknown (Bjørning-Poulsen a kol., 2008). Carbamate insecticides, like the OPs, inhibit acetylcholinesterase (AChE) and elicit cholinergic hyperstimulation. However, carbamates cause only reversible inhibition of AChE which lasts only minutes or 24 hours (Kamel and Hoppin, 2004).

Bendiocarb is a broad spectrum pesticide belonging to the *N*-methylcarbamate group (Pacioni and Veglia, 2007). Using of bendiocarb can dramatically reduce the risk of insect-borne diseases which are transmitted by arthropod vectors such as mosquitoes (malaria, dengue fever, yellow fever, encephalitis, filariasis, West Nile fever and chikungunya), ticks (e.g. Lyme disease) and sandflies (leishmaniasis; Nauen, 2007). Bendiocarb is degraded in the liver, which has a major role in the biotransformation and excretion of carbamate pesticides from the body. Reactions of detoxication take place in hepatocytes by the enzymatic system situated in the endoplasmic reticulum (Schenck and Kolb, 1990), and bendiocarb is excreted as a sulphate and  $\beta$ -glucuronide conjugates of the phenol derivatives. In a study with rats, 90% of an oral bendiocarb dose was excreted in the urine, 1-3% in expired air, and 3-8% in the feces. Excretion was complete within 24 hours (Challis and Adcock, 1981).

The aim of our study was to observe fibrotic changes of the rabbit liver tissue treated with bendiocarb, mostly in the area of the central vein and portal tract.

**Material and Methods**

In our experiment were used 56 adult rabbits (age = 84 days) of breed HY+, 28 males and 28 females, with average weight 2,5 kg from accredited animal farm (Nitra, Slovakia). Animals

were kept in cages (two per a cage) at standard conditions (temperature 15-21°C, 12 hour light period and relative humidity of 45 %) and fed with granular feed mixture (O-10 NORM TYP, Slovakia). Drinking water was available for all animals *ad libitum*. Animals were divided into groups (control, days 10, 20, 30 of administration), and each group comprised 14 animals. Rabbits in all experimental groups received bendiocarb (96 % Bendiocarb, Bayer, Germany) *per os* in a dose 5 mg/kg per day and after day 11 in a same dose per 48 hours, with respect to the acute oral toxicity of bendiocarb to rabbit, 35-40 mg/kg of the body weight (Petrovova et al., 2011). Animals in control group were not treated and they were killed at 30 day from the beginning of the experimental work. Experimental animals were killed by thiopental (Thiopental Valeant 1g, ICN, Czech Republic; 100 mg/kg of body weight) intravenous administration at days 10, 20 and 30 after bendiocarb treatment. Animal weight was recorded at first and last day of bendiocarb treatment, and calculation of the weight gain was made as the difference between rabbit body weight in experimental group and control group at days 10, 20 and 30 of the bendiocarb treatment. The experimental work on rabbits was performed with approval of the Ethic Committee No. 2647/07-221/5 followed Slovakian protocols for ethical standards for the use of laboratory animals.

Picro-sirius red staining was used for visualization of collagen fibers and staging of hepatic fibrosis. Deparaffinized liver sections were washed in water, and stained in Picro-sirius red (0.1 % Picro-sirius red in saturated aqueous picric acid; Sigma-Aldrich, USA) for 1 hour at room temperature. Sections were rinsed twice in 0.5 % acetic acid and stained in Hoechst (2 µl / 200 ml 0.1% Triton/H<sub>2</sub>O). Subsequently, histological sections were rinsed in distilled water, dehydrated in absolute ethanol and xylene, and mounted in Depex (VWR International GmbH, Austria). Microscopic evaluation of fibrosis was analyzed using a Olympus BX46 microscope equipped with a camera DP70 (Olympus, Hamburg, Germany) and objective with 20 x magnification. Five central veins, as a general term (Danko et al., 2011) and areas of portal tracts in each animal were studied, for a total of 260 central veins and portal tracts.

## Results

Figures 1 and 3 illustrate the histological features of central veins portal tracts with and without extension of collagen fibers into the perivenous parenchyma, perisinusoidal and pericellular space, particularly at 10, 20 and 30 days.

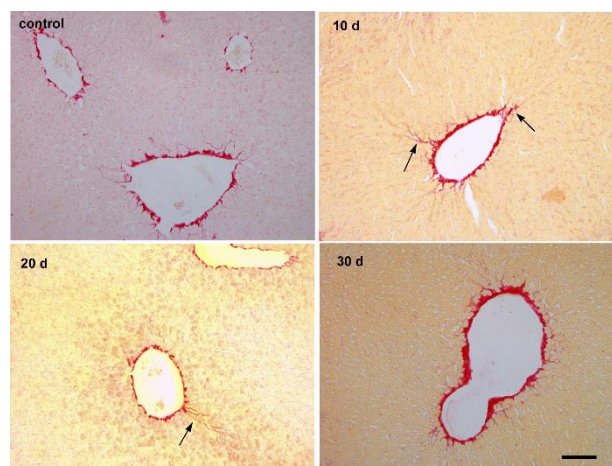
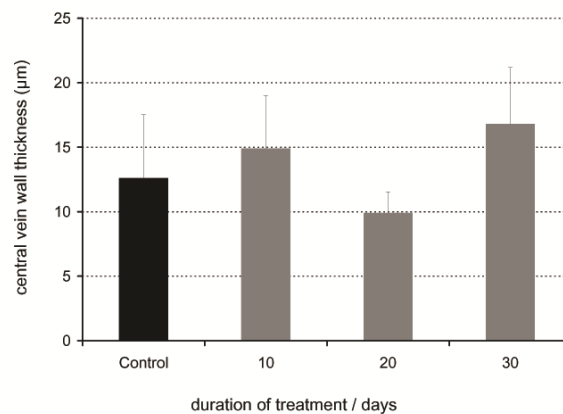


Fig. 1

Control samples show a thin-walled central vein with fine fibrous extension by Sirius red staining of collagens.

At 10 and 30 days of bendiocarb treatment the wall of central vein increased in thickness. Furthermore, fibrous strands emerge from the central vein into the parenchyma and perivascular space, particularly at 10 and 20 days (arrows). Sirius Red staining, magnification 20 x, Scale bar = 100  $\mu\text{m}$  (Petrovova et al., 2013).

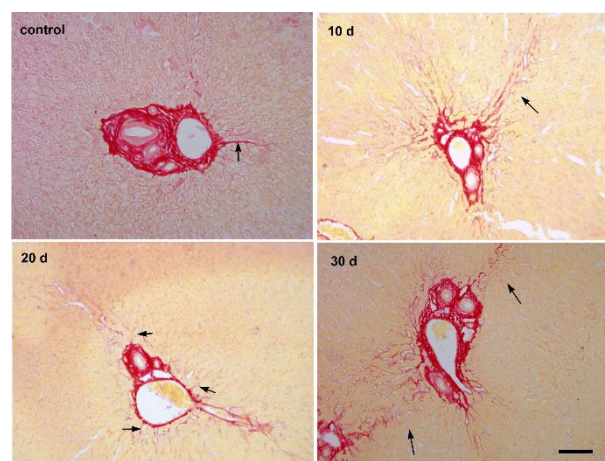
The vein wall thickness ranged from 4 to 27  $\mu\text{m}$ . As shown in Fig. 1, the vein wall thickness is increased in thickness at 10 and 30 days, but not significantly (Fig. 2). The control samples show a thin-walled central vein with fine fibrous extension by Sirius red staining of collagens. Fibrous extension was not detected in association with the wall thickness of the central vein (Petrovova et al., 2013).



*Fig. 2*

**The central vein wall thickness in bendiocarb-treated rabbit liver (Petrovova et al., 2013).**

Portal tract (PT) shows increased collagen staining in the matrix. Further, at 10 and 30 days the fibrous septa of variable lengths are spreading from PT into the lobules and perisinusoidal space. The perisinusoidal fibrosis is focal, which is marked by increased collagen staining along the sinusoidal borders. Fine fibrous strands extend from PT for a short distance into the lobules at 20 day of bendiocarb treatment (Fig. 3; Petrovova et al. 2013).



*Fig. 3*

**Portal tract shows increased collagen staining in the matrix.**

Fibrous septa of variable lengths are spreading from portal tract into the lobules and perivascular space (arrow). Sirius Red staining, magnification 20 x, Scale bar = 100  $\mu$ m (Petrovova et al., 2013).

### Discussion

Agrochemicals have been in use since the early days of modern agriculture. Their widely using cause the question of toxicity pesticides to non-target organisms, their persistence, accumulation and combined effect with other chemicals. Pesticides are biologically active and this renders them intrinsically more hazardous than most other classes of industrial products.

Chronic hepatic injury is a common disorder defined pathologically by ongoing hepatic necrosis and inflammation of the liver, often accompanied by fibrosis. Liver fibrosis represents the final common outcome of chronic liver injury and is often progressive, eventually evolving into cirrhosis. Liver fibrosis is a complex process involving production and deposition of insoluble components that constitute the extracellular matrix. Chronic hepatitis is classified by histology based on activity of inflammation and degree of fibrosis; extent of fibrosis relates to likelihood of developing cirrhosis (Frazzetto et al., 2012).

Chronic liver disease is a major risk of pesticides exposure. The laboratory tests have a variety of potential uses in the diagnosis of liver diseases. Routinely performed tests (e.g., serum bilirubin, albumin, and prothrombin time) may be of prognostic value in liver disease but they are not accurate in identifying early fibrosis. The most promising markers of fibrosis relate to collagen deposition, such as serum P-III-P and tissue inhibitor of metalloproteinase. Currently available laboratory tests are of value in excluding various types of liver diseases. Commonly performed serum liver enzymes are of limited value in monitoring liver diseases (Malaguarnera et al., 2012). The development and application of laboratory tests that can identify early fibrosis and chronic hepatic disease have the potential of reducing healthcare costs and suffering associated to chronic liver diseases.

Miranda et al., (2008) studied the bioaccumulation of chlorinate pesticides and PCBs in the tropical freshwater fish *Hoplias malabaricus*, and they found the most important alterations in the liver were lesions such as fibrosis, large necrosis area and leukocyte infiltration.

In our study, the weak increase of the fibrosis was observed around the central vein and PT in treatment groups after short-term (10 day) and long-term (30 day) bendiocarb administration in comparison to control. It can be concluded that bendiocarb has moderately toxic effect on the rabbit liver tissue, regardless of gender. The case with the poisoned animals in this study, human beings would not develop any systematic poisoning observable at the level of the whole organism.

### Acknowledgement

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