EFFECT OF CARBETOX ON METAMORPHOSIS OF *RANA RIDIBUNDA* TADPOLES

INFLUENŢA CARBETOX ASUPRA METAMORFOZEI LA MORMOLOCII DE *RANA RIDIBUNDA*

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Abstract. *In our experiments we investigated the histological modifications induced by the action of the insecticide Carbetox in the Rana ridibunda tadpoles during metamorphosis. Carbetox is known to cause nonreversible acetylcholine inhibition and diminishes activity, growth and development in amphibian tadpoles. The tadpoles used in the experiment were divided in three experimental lots: one lot of control individuals and two experimental lots in which the tadpoles were kept in aquaterrarios with a 0.02 ml/l water and respectively 0.002 ml/l water Carbetox. The water and toxic was changed daily and the tadpoles were fed for the duration of the experiment. After 28 days of exposure to Carbetoxrved significant effects on morphogenetic tissue.*

Key words: Carbetox, insecticide, tadpoles, *Rana ridibunda*

Rezumat. *Exeprimentele au urmărit modificările histologice induse de acţiunea insecticidului Carbetox la mormolocii de Rana ridibunda în timpul dezvoltării embrionare. Carbetox-ul determină inhibiţia ireversibilă a acetilcolinei, diminuează activitatea, creşterea şi dezvoltarea mormolocilor. Mormolocii utilizaţi în experimente au fost împărţiţi în trei loturi: un lot martor şi două loturi de experienţă în care mormolocii erau ţinuţi în acvaterarii cu 0.02ml/l, respective 0.002ml/l Carbetox. Apa şi toxicul erau schimbate zilnic, iar mormolocii au fost hrăniţi pe toată durata experienţei. După 28 de zile de expunere la Carbetox au fost evidenţiate modificări asupra unor teritorii morfogenetice.*

Cuvinte cheie: Carbetox, insecticid, mormoloci, *Rana ridibunda*

INTRODUCTION

The action of chemical pollutants on various species of amphibians is the main objective of the numerous researches carried out in recent years (Ch.Bridges 2000, Ferah Saym 2008, A. Relyea 2004, 2005, 2006, Ponepal et al. 2008, Păunescu et al. 2008). The interest for such a topic is partly explained, by the widespread use of insecticides to combat pests and secondly by the fact that this group of organisms is probably, the most exposed to toxic pesticides (Fulton and Chambers, 1985, Berril et al. 1994, Sparling et al.2001). Larva of amphibians is usually exposed to low levels of pesticides during their development. The studies generally examine, the short-term effects of exposure, but often ignore the importance of individual development cycle during which tadpoles are exposed.

The study has in view the effects of Carbetox insecticide on embryonic development of frog tadpoles.

MATERIAL AND METHODS

In our experiments we have used larvae of *Rana ridibunda*, hatched in the laboratory, the laying of eggs being taken from lakes bordering Pitesti. The larvae were divided into three lots: the first lot includes control individuals of *Rana ridibunda* kept in aquaterrariums with clean water; the second lot consists of larvae of *Rana ridibunda* kept in aquaterrariums with Carbetox in a concentration of 0.02ml/l and the third lot consists of larvae of *Rana ridibunda* kept in aquaterrariums with Carbetox in a concentration of 0.002ml/l. The experiences have been conducted at a temperature of 20-22°C. Water and toxic have been changed daily, and larvae were fed ad libitum. The treatment began on the first day of larval life and lasted four weeks, during which no mortality was recorded for specimens of the third lot; the dose used for the second lot was lethal since the first week. Sampling was done weekly. They were fixed in 8% formalin for poikilotherms, histologically processed, colored with hemalaun-eozine and observed under a microscope.

The toxic used is an organophosphorous insecticide containing melathion as the active substance, which is part of the third group of toxicity. It is used to combat pests in vegetables, field crops, fruit trees, grape moth, defoliating caterpillar, black louse and peas weevil.

RESULTS AND DISCUSSIONS

The results of the lot treated with a solution of Carbetox 0,002ml/l for seven days are shown in figure 1. It has been noticed that, the toxic causes a delay in the development of the neural tube, since its upper edges have not been detached from ectoblast although its typical contour is evident. At this stage it was natural that ectoblast should be restored above the neurectoblast, which has not happened, due to the slow process of differentiation under the action of the toxic. The same phenomenon affects the neural crests, which were not individualized to detach from the neural tube.

Similar researches have been carried out by Mary Jordan, K. Rzehak and Anna Maryanska (1977), studying the effects of Miedzian 50 and Gesagard 50 pesticides on the development of tadpole *Rana temporaria*. The water slurries matter of the two pesticides induced changes in the digestive tract, brain and muscles; Miedzian 50 caused a partial cytolysis of intestinal epithelium cells an liver parenchyma. Gesagard 50 pesticide caused significant degenerative changes of the alimentary canal and the brain, which consisted in a partial inhibition of growth and delay in the adjustment process of the opercula.

Our experiments also recorded a late development of the nothocord which, under normal circumstances, it should have been outlined. The related research made by M. Pilar Honrubia et al. (1993) have demonstrated that CarbamatZZ- Apfax ® insecticide induce structural changes of gills, liver, gall-bladder, heart and notochord to *Rana perezi* tadpoles in a dose of 0.02‰ and 0.14‰ for nineteen weeks.

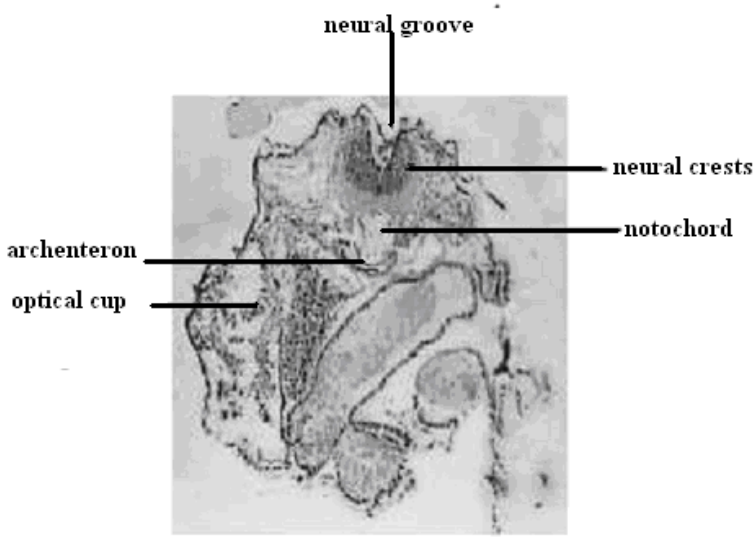

Fig. 1. Cross section through the frog tadpole treated with Carbetox in a concentration of 0,002ml /l, HE coloration, (4X)

The research also shows that after fourteen days of treatment, the same dose of Carbetox of 0,002ml/l, induces changes similar to those observed after seven days of treatment.

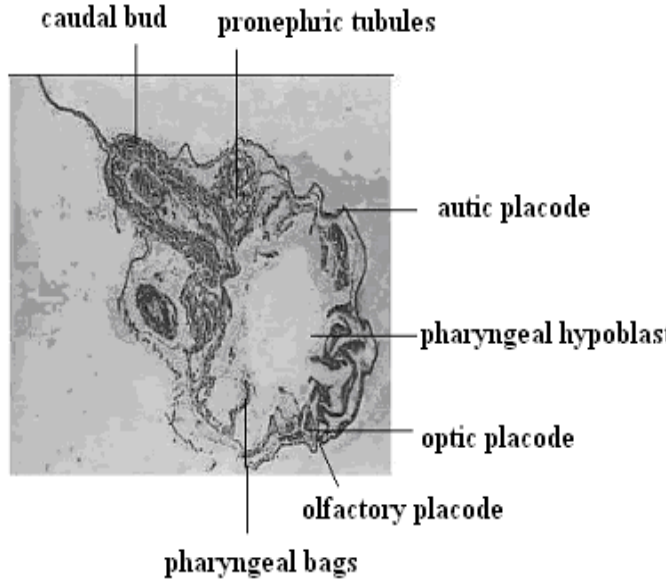

Fig. 2. Cross section through the frog tadpole treated with Carbetox in a concentration of 0,002ml/l, HE coloration, (4X)

Therefore, as seen in figure 2, the primary sense organs which begin to appear are least apparent while the development of the excretion organs causes deficiencies manifested through the incomplete structure organisation of the mesonephros.

It shoud be emphasized that after 21 days of treatment, degenerative changes induced by the harmful effect of Carbetox are illustrated by delays in the development of the mesoblast that have not developed somites, the intermediate parts and intestines (fig.3).

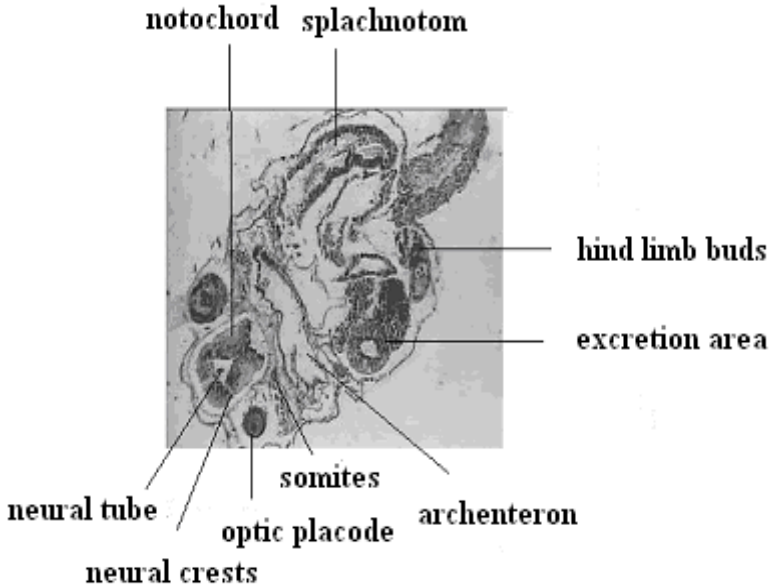

Fig. 3. Cross section through the frog tadpole treated with Carbetox in a concentration of 0,002ml/l, HE coloration, (4X)

Similar effects were observed in the research conducted by H.P.Gurushankara et al. (2007) who followed the effects of malathion on survival, growth and feeding of *Limnonectus limnocharis tadpoles*. They found out that the number of tadpoles that survived the increasing doses of malathion (from 500μ/g to 3000μg/g) decreased, their food consumption also decreased and slowed the tadpoles growth and development. Similarly, in his studies on the larvae of *Rana ridibunda* treated with malathion, Ferah Sayim (2008) has observed some signs of toxicity such as: loss of balance, delay of growth, deformation of the tail, abnormally coiled intestine and generalized edema.

We point out that the deformation of the tail and edema were observed in our research as well, for larvae treated with Carbetox in a dose of 0,002ml/l which had no lethal effect, and a dose of 0.02ml/l, respectively. This latter dose appears

to have completely blocked all the processes of differentiation and development of morphogenetic territories (fig.4). This concentration of Carbetox is likely to have inhibited the development of the hypoblast which formed pharyngeal bags in the pharyngeal regions. The toxic effect was lethal.

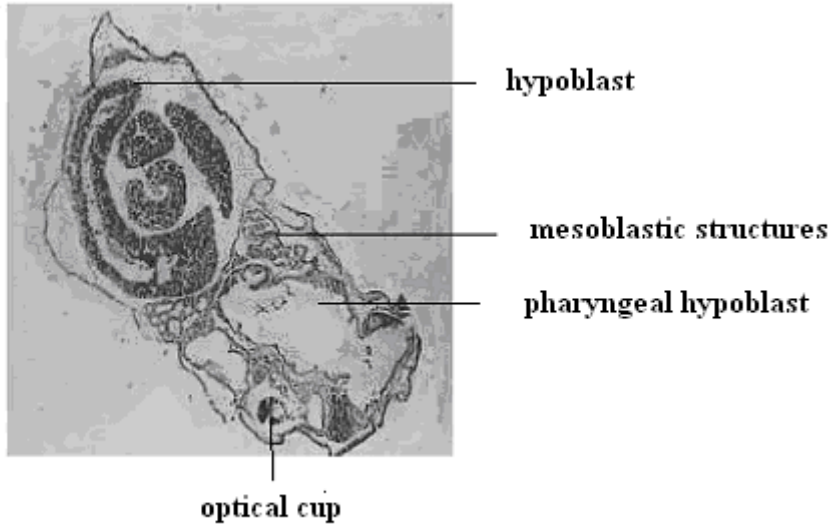

Fig. 4. Cross section through the frog tadpole treated with Carbetox in a concentration of 0.02ml/l, HE coloration, (4X)

It can be concluded that the harmful effect of Carbetox, in which malathion is the active substance, is manifested by deformation of the tail and the entire larval body.

CONCLUSIONS

A dose of 0.02 ml/l Carbetox causes a noticeable slowing larval development in *Rana ridibunda*, its effect being lethal after seven days of treatment. A dose of 0,002ml/l Carbetox causes disorders of histogenesis and organogenesis, which are clear in the neural stage throughout the treatment. This dose is not lethal, but causes delays in the development and evolution of the neurectoblast, chordoblast, mesoblast and hypoblast. After 28 days of treatment there appear some malformations with deformities of the tail and the whole larval body, delays of histogenesis and organogenesis.

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