NEPHROTOXIC EFFECTS OF CHAMPION 50WP FUNGICIDES IN THE MARSH FROG *Pelophylax ridibundus*

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Abstract. The impact of Champion 50WP fungicide on kidney histopathology of the frog *Pelophylax ridibundus* was investigated. The animals were exposed to sub-lethal concentrations ($0.125x10^{-3}$/g of body weight) of Champion 50WP administrated by intraperitoneal shots for 3 weeks at two thermal levels (4-6ºC and 22-24ºC). Light microscopy of kidney revealed morphological changes such as degeneration in epithelial cells of the renal tubule, vacuolization; Perl's stained material, interstitial edema, karyomegalia, degeneration of glomerulus. The most severe changes (interstitial edema, presence of large quantities of iron in renal epithelial cells, congestions of renal capillaries) were observed in animals exposed to Champion 50WP fungicide and kept at 22-24ºC.

Keywords: frog, glomerular degeneration, interstitial edema, cell hypertrophy, Champion 50WP.

Rezumat. Efectele nefrotoxice induse de acţiunea fungicidului Champion 50WP la broasca-de-lac *Pelophylax ridibundus*. În prezentul studiu au fost investigate efectele histopatologice renale induse de acţiunea fungicidului Champion la broasca *Pelophylax ridibundus*. Animalele au fost expuse la concentraţii subletale de fungicid Champion 50WP ($0,125x10^{-3}$/g greutate corporală) administrate prin injecţii intraperitoneale timp de 3 săptămâni la 2 nivele termice (4-6ºC respectiv 22-24ºC). Microscopia optică a indicat prezenţa unor modificări morfologice renale ce au constat în degenerare celulelor epiteliale, vacuolizare celulară, prezenţa materialului Perls reactiv în celule, ederne interstiţiale, kariomegalie, degenerare glomerulară. Cele mai severe dintre modificări amintite (ederne interstiţiale, prezenţa fierului în cantitate mare în celulele epiteliului renal, congestia capilarelor sanguine) au fost înregistrate în cazul animalelor tratate cu fungicidul Champion şi ţinute la o temperatură de 22-24ºC.

Cuvinte cheie: broască, degenerare glomerulară, ederne interstiţiale, hipertrofie celulară, Champion 50WP.

INTRODUCTION

The issue of amphibian population decline has led to an increasing public awareness of the importance of remnant amphibian populations wherever they might occur (MANN et al., 2009).

Chemicals in the form of pesticides and fertilizers are being applied in greater varieties, combinations, and to a greater extent than ever before, and represent a significant suite of pollutants. Agricultural chemicals are receiving increasing attention as a potential cause of amphibian declines, acting singly or in combination with other stressors (RELYEA & MILLS, 2001). Surveys of natural populations have shown correlations between population declines and proximity to agricultural lands (BISHOP et al., 1999; LENOIR et al., 1999; DAVIDSON et al., 2002; HOULAHAN & FINDLAY, 2003; DAVIDSON, 2004).

Champion 50WP is a foliar fungicide with protective action. Copper hydroxide which comprises 77% of this product governs the toxicity of the product. The remaining components have low to negligible toxicity.

Environmental contamination with copper comes from its extensive use in commercial and industrial products, agricultural use in fertilizers and different biocides, animal feed additives and growth promoters, electroplating, textile products, petroleum refining, manufacture of copper compounds (HERKOVITS & PÉREZ-COLL, 2007).

Cu plays a major role as cofactor in haematogenesises (CHIOU et al., 1999). Cu is one of the most critical trace elements in livestock because it is necessary for haemoglobin formation, iron absorption from gastro-intestinal tract and iron mobilization from tissue stores (MPOFU et al., 1999). Ingestion of large doses of copper salts may result progressively in irritation of the gastrointestinal tract, nausea, vomiting, salivation, gastric pain, haemorrhagic gastritis, diarrhoea, capillary damage, liver and kidney damage and central nervous system stimulation followed by depression.

The aims of this study were to define histological and histopathological lesions in the kidneys that were caused by the exposure of the frog to Champion 50WP fungicides at two thermal levels (4-6º, respectively 22-24ºC) and to assess the impact of this toxic on the severity and kind of those lesions.

MATERIAL AND METHODS

The animals used in this study were adult of *Pelophylax ridibundus*, of both sexes, captured in spring (April-May) from the surrounding areas of the city Piteşti (South Romania). The animals were kept in laboratory condition in aquaterrarios filled with tap water for five days to test their health and accommodate them for the experiment. The water was changed daily to avoid the accumulation of toxic substances.

After adaptation in the lab, the frogs were separated in lots, which were used separately for the following experiments: two lots of control individuals, containing animals kept in laboratory at 4-6ºC, respectively at 22-24ºC with no treatment, in running water which was changed every day, (1) one lot containing animals which were subjected to treatment with Champion 50WP in a dose of $0.125x10^{-3}$/g of body weight and kept at 4-6ºC, (2) a second lot containing animals which were subjected to treatment with Champion 50WP in a dose of $0.125x10^{-3}$/g of body weight and kept at 22-24ºC in a thermostatic chamber. Ten animals were used for each lot. The toxic was administered by

intraperitoneal shots, one shot every two days, in a scheme of 3 weeks. The administered dosage of toxic was not lethal as none of the subjects died through the experiment. All frogs were handled in accordance with the standard guide for care and use of laboratory animals. We began sacrificing the animals at the end of 3rd week of treatment; the frogs in each lot were sacrificed after chloroform anaesthesia and kidney were taken to assess histological changes via light microscope examination. Tissues samples were fixed in 8% neutral formalin for poikilotherms for 24h. Samples were then processed using a graded ethanol series and embedded in paraffin. Paraffin section were cut 5µm-thick slices using a rotary microtome (Slee Maintz Cut 5062) and stained with: haematoxylin (H) as a general screening method, Sirius red (JUNCUEIRA et al., 1979) for collagen stain (fibrosis) and Perl's method (PEARSE, 1985) for ferrous iron. The sections were viewed and photographed using an Olympus microscope with an attached camera.

RESULTS AND DISCUSSIONS

The histopathological picture of renal damage caused by Champion 50WP fungicide action in animals kept at a temperature of 4-6ºC, describes the changes both in the renal corpuscles and tubules. Between the visceral and parietal sac, there is a much enlarged Bowman space (Fig. 1a), which characterizes the beginning of glomerular degeneration. Also, the capillaries are dilated and show lymphocytes infiltrates.

In the proximal convoluted tubules, there is a vacuolization of cells in the presence of polymorphic nuclei (Fig. 1c). Some epithelial cells have small pyknotic nuclei, in process of degeneration, while others have hypertrophic nuclei (Fig. 1b, c). Some cells have Perl's positive inclusions in the cytoplasm, and that is not easily explainable. It is known that hemosiderin and/or ferritin are deposited in Kupffer cells (WOLKE, 1992). It was found that in the frog *Rana ridibunda* exposed to cadmium, excess iron was deposited in the liver melano-macrophages, a special type of Kupffer cells (LOUMBOURDIS & VOGIATZIS, 2002). Deposition of ferritin and hemosiderin is the effect of haemolysis of red blood cells, the destroyed cell being phagocytosed by hepatocytes and/or Kupffer cells. The iron trapped in the cells may be similarly the remnants of phagocytosed red blood cells, but the etiology of such a deposition is not clear, unless these epithelial cells are able to phagocytose red blood cells (LOUMBOURDIS, 2003).

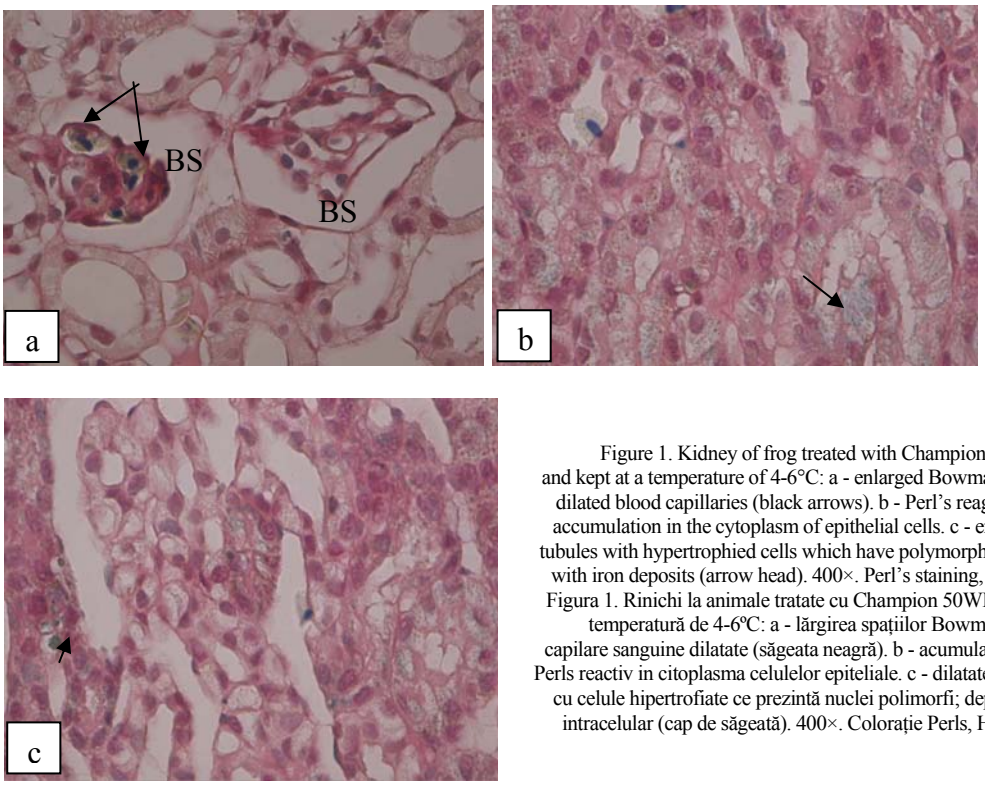

Figure 1. Kidney of frog treated with Champion 50WP and kept at a temperature of 4-6°C: a - enlarged Bowman space (BS); dilated blood capillaries (black arrows). b - Perl's reagent material accumulation in the cytoplasm of epithelial cells. c - enlarged renal tubules with hypertrophied cells which have polymorphic nuclei; cells with iron deposits (arrow head). 400×. Perl's staining, H-Sirius red.
Figura 1. Rinichi la animale tratate cu Champion 50WP şi ţinute la o temperatură de 4-6ºC: a - lărgirea spaţiilor Bowman (SB); capilare sanguine dilatate (săgeata neagră). b - acumulare de material Perls reactiv in citoplasma celulelor epiteliale. c - dilatatea tubilor renali cu celule hipertrofiate ce prezintă nuclei polimorfi; depozite de fier intracelular (cap de săgeată). 400×. Colorație Perls, H-Sirius red.

Due to the presence of interstitial edema, the renal tubules do not have spaces between them. Some tubules are completely degenerate, their cells showing hyaline material in the cytoplasm while the lumen is completely obstructed.

Administration of the same concentrations of toxic to animals kept at a temperature of 22-24ºC causes similar but less profound histological changes. There is a dilation of capillaries and an enlargement of the Bowman space in the renal glomerulus indicating glomerular degeneration (Fig. 2a). Areas of edema occur in the renal parenchyma with extravasated vascular elements, and even bleeding areas (Figs. 2a, b).

Nephrotoxic effects can also occur in the renal tubules resulting in their degeneration by cell hypertrophy, the presence of small pyknotic or polymorphic nuclei (Fig. 2c). Vacuolated epithelial cells are present in the cytoplasm as iron deposits in the form of hemosiderin (Figs. 2c, d), resulting from haemolysis, which may occur at this level.

Hemosiderin and/or ferritin is the only other well-characterized system for the intracellular storage and sequestration of metals apart from metallothionein and glutathione (ROESIJADI & ROBINSON, 1994), but it should be emphasized that metallothionein and glutathione are mainly bound to cadmium, copper, mercury and zinc.

Some cells are completely disorganized being replaced by a hyaline amorphous mass (Fig. 2c).

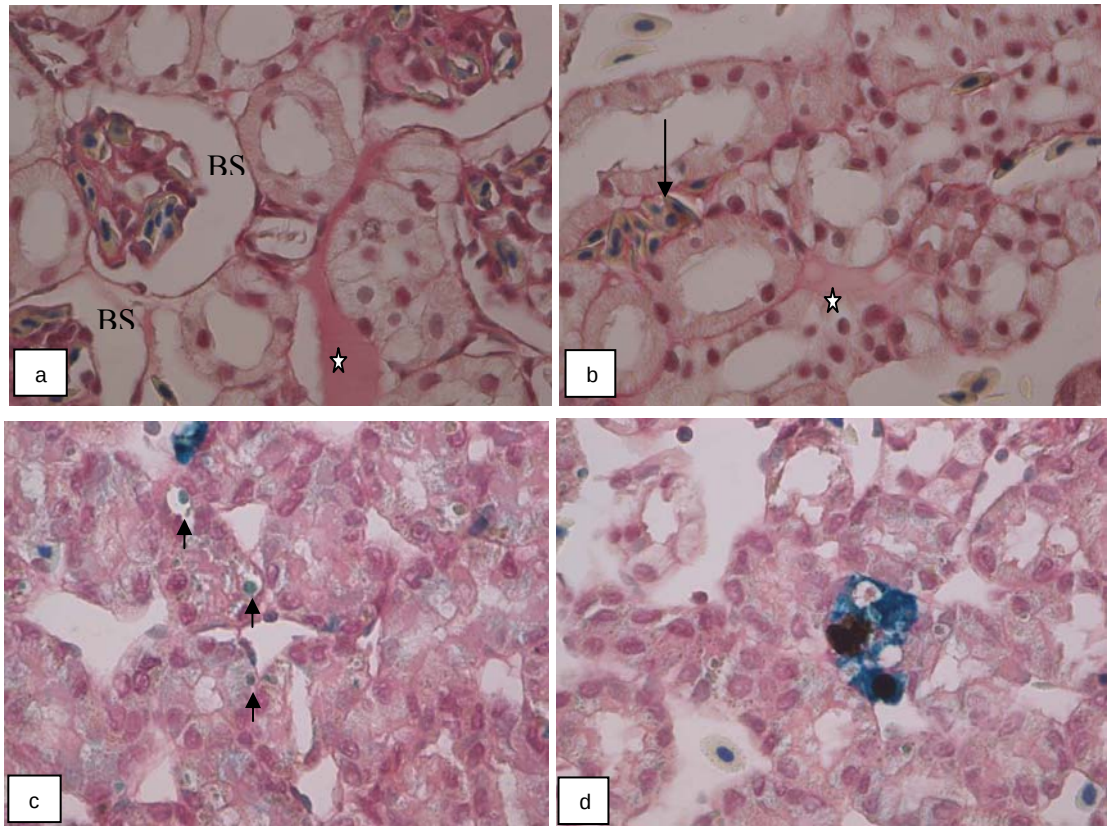

Figure 2. Kidney of frog treated with Champion 50WP and kept at a temperature of 22-24ºC. a - enlarged Bowman space (BS); dilated blood capillaries; bleeding areas (star). b - interstitial edema with extravasated blood elements (black arrow). c - cells with iron deposits (arrow head); renal tubular degeneration. d - Perl's positive material deposits. 400×. Perl's staining, H-Sirius red.
Figura 2. Rinichi la animale tratate cu Champion 50WP şi ţinute la o temperatură de 22-24ºC. a - dilatarea spaţiilor Bowman (SB) şi a capilarelor sanguine; zone hemoragice (steluţa). b - edeme interstiţiale cu extravazarea elementelor sanguine (săgeata neagră). c - depozite de fier în celule (cap de săgeată); degenerare tubulară. d - depozit de material Perls pozitiv. 400×. Colorație Perls, H-Sirius red.

The kidney is a common target organ for toxic compound since it forms a major pathway for excretion of these from the body (VOGIATZIS & LOUMBOURDIS 1998, 1999; PAPADIMITRIOU & LOUMBOURDIS, 2003), and has a high metabolic activity with a number of sensitive metabolic processes (LOUMBOURDIS, 2003). OSMAN et al., (2009) describe the nephrotoxic effects similar to those we observed in kidneys of the frog *Pelophylax ridibundus* for *Oreochromis niloticus* fish species after applying concentrations of 0.5, 1.0 respectively 2.5 mg Cu/l water. They refer to tubular degeneration, necrosis in the renal parenchyma, lymphocytic infiltrates and decrease in the number of melano-macrophages.

The histological studies performed on *Abramis brama* and *Aspius aspius* species living in polluted water containing copper, KOPONEM et al. (2001) indicated the presence of renal damage described by cell injury, dilated glomerular capillaries or interstitial edema. Because the fish kidney receives the largest amount of post-gill blood, the possible kidney lesions may be considered a good indicator of aquatic pollution (ORTIZ et al., 2003).

In addition to liver and intestine, the kidney is the organ that accumulates the largest amount of copper (PAPADIMITRIOU & LOUMBOURDIS, 2003). It seems that in the increased accumulation of copper, over a long period of time, the *Rana ridibunda* species has a new way of kidney detoxification when the liver is overloaded. This has also been observed by GROSSEL (1998) in other vertebrates.

CONCLUSIONS

These observations lead us to conclude that Champion 50WP fungicide in a dose of $0.125x10^{-3}$/g of body weight determinates morphologic modifications in the kidney of *Pelophylax ridibundus* in both thermic variants (at 4-6º and at 22-24ºC). Under sublethal concentration of this fungicide, the renal tissue of the frogs showed marked pathological changes such as: degeneration in epithelial cells of the renal tubule, vacuolization, Perl's stained material, interstitial edema, karyomegalia, and degeneration of glomerulus. Highly degenerative changes in the renal tissue were evident in animals that was treated with toxic and kept at 22-24ºC. These results could be used as indicator of renal toxicity for a variety of chemical, including pesticides.

REFERENCES

BISHOP C. A., MAHONY N. A., STRUGER J., NG P., PETTIT K. E. 1999. *Anuran development, density and diversity in relation to agricultural activity in the Holland River watershed, Ontario, Canada (1990–1992).* Environmental Monitoring and Assessment. SpringerLink. **57**: 21-43.

CHIOU P. W. S., CHEN C. L., WU C. P. 1999. *Effects of high dietary copper on the morphology of gastro-intestinal tract in broiler chickens.* Asian-Austalasian Journal of Animal Science. Publisher AAAP and KSAST. **12**: 548–553.

DAVIDSON C. 2004. *Declining downwind: amphibian population declines in California and historical pesticide use*. Ecological Applications. ESA Publications. **14**: 1892-1902.

DAVIDSON C., SHAFFER H. B., JENNINGS M. R. 2002. *Spatial tests of the pesticide drift, habitat destruction, UV-B, and climate-change hypotheses for California amphibian declines*. Conservation Biology. Wiley-Blackwell. **16**: 1588-1601.

GROSSEL M. H. 1998. *Renal Cu and Na excretion and hepatic Cu metabolism in both acclimated and non acclimated rainbow trout (Oncorhynchus mykis).* Aquatic Toxicology. Elsevier B.V. **40**: 275-291.

HERKOVITS J. & PEREZ-COLL C. S. 2007. *Acclimation to Low Level Exposure of Copper in Bufo arenarum Embryos: Linkage of Effects to Tissue Residues.* International Journal of Environmental Research and Public Health. www.mdpi.com/journal/ijerph. **4**(2): 166-172.

HOULAHAN J. E. & FINDLAY C. S. 2003. *The effects of adjacent land use on wetland amphibian species richness and community composition*. Canadian Journal of Fisheries and Aquatic Sciences. NRC Research Press. **60**: 1078-1094.

JUNCUEIRA L. C. U., BIGNOLAS G., BRENTANI R. R. 1979. *Picrosirius staining plus polarization microscopy, a specific method for collagen detection in tissue section.* Histochemical Journal. Kluwer Academic Publishers **11**: 447-455.

KOPONEN K., MYERS M. S., RITOLA O., HUUSKONEN S. E., LINDSTRÖM-SEPPÄ P. 2001. *Histopathology of feral fish from a PBC-contaminated freshwater lake.* Ambio. Published by Royal Swedish Academy of Sciences. **30**: 122-126.

LENOIR J. S., MCCONNELL L. L., FELLERS G. M., CAHILL T. M., SEIBER J. N. 1999. *Summertime transport of current-use pesticides from California's Central Valley to the Sierra Nevada mountain range, USA*. Environmental Toxicology and Chemistry. Wiley Knowledge for Generation. **18**: 2715-2722.

LOUMBOURDIS N. S. 2003. *Nephrotoxic effects of lead nitrate in Rana ridibunda.* Archives of Toxicology. SpringerLink. **77**: 527-532.

LOUMBOURDIS N. S. & VOGIATZIS A. K. 2002. *Impact of cadmium on liver pigmentary system of the frog Rana ridibunda.* Ecotoxicology and Environmental Safety. Elsevier Inc. **53**: 52-58.

MANN R. M., HYNE R. H., CHOUNG C. B., WILSON S. C. 2009. *Amphibians and agricultural chemicals: Review of the risks in a complex environment.* Environmental Pollution. Elsevier Ltd. **157**: 2903-2927.

MPOFU I. D. T., NDLOVU L. R., CASEY N. H. 1999. The Copper, Cobalt, Iron, Selenium and Zinc Status of cattle in the Sanyati and Chinamhora small holder grazing areas of Zimbabwe. Asian-Australasian Journal of Animal Science. Publisher AAAP and KSAST. **12**: 579-584.

ORTIZ J. B., DE CANALES M. L. G., SARASQUETE C. 2003. *Histopathological changes induced by lindane (gamma-HCH) in various organs of fishes*. Scientia Marina. Published by CSIC. **67**(1): 53-61.

OSMAN M. M., EL-FIKY S. A., SOHEIR Y. M., ABUR A. I. 2009. *Impact of Water Pollution on Histopathological and Electrophoretic Characters of Oreochromis niloticus Fish*. Research Journal of Environmental Toxicology. Taylor & Francis. **3**(1): 9-23.

PAPADIMITRIOU E. & LOUMBOURDIS N. S. 2003. *Copper kinetics and hepatic metallothionein levels in the frog Rana ridibunda after exposure to* $CuCl_2$. Biometals. Kluwer Academic Publishers. Printed in the Netherlands.**16**: 271-277.

PEARSE A. G. E. 1985. *Histochemistry, theoretical and applied*. Churchill Livingstone. London. 4th Edition. **2**: 441-1055.

RELYEA R. A. & MILLS N. 2001. *Predator-induced stress makes the pesticide carbaryl more deadly to gray treefrog tadpoles (Hyla versicolor)*. Proceedings of the National Academy of Sciences of the United States of America. David B. Wake. University of California, Berkeley, CA. **98**: 2491-2496.

ROESIJADI G. & ROBINSON W. E. 1994. *Metal regulation in aquatic animals: mechanisms of uptake, accumulation and release*. In: Malins DC, Ostrander GK (eds) Molecular biology and biochemical approach to aquatic toxicology. Lewis, Boca Raton FL: 387-420.

VOGIATZIS A. K. & LOUMBOURDIS N. S. 1998. *Cadmium accumulation in liver and kidneys and hepatic metallothionein and glutathione levels in Rana ridibunda after exposure to* $CdCl_2$. Archive of Environmental Contamination Toxicology. Springer Verlag. **34**: 64-68.

VOGIATZIS A. K. & LOUMBOURDIS N. S. 1999. *Exposure of Rana ridibunda to lead-I. Study of lead accumulation in various tissues and hepatic d-aminolevulinic acid dehydratase activity*. Journal of Applied Toxicology. John Wiley & Sons Ltd. **19**: 25-29.

WOLKE R. E. 1992. *Piscine macrophage aggregates: a review*. Annual Review of Fish Diseases. Pergamon Press, NY. **2**: 337-343.

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