



Confidential

Review of USB FFPW PhD Thesis

First name(s), surname, titles of the PhD student: Zuzana Richterová, MVDr.	First name(s), surname, titles of supervisor: Prof. Zdeňka Svobodová, DVM, D.Sc.
Title of PhD thesis: The effect of pyrethroid based pesticides on fish	
REVIEWER:	
Surname: Sieroslawska	Institution: The John Paul II Catholic University of Lublin, Poland, Institute of Biotechnology
Name: Anna	
Titles: Dr. hab., Ph.D.	E-mail: ansie@kul.lublin.pl
Please describe your professional relationship to the PhD student: none	Please describe your field of expertise: toxicology

QUESTIONNAIRE

Originality, scientific importance, perspectives and impacts of results presented in the PhD thesis for basic and/or applied research

Evaluate competitiveness of the PhD thesis in the international context and compare its level with the current state of the art in the field (extent ¼ – ½ page):

The main aim of the presented dissertation was to investigate the effects of pyrethroid pesticides on fish, especially the impact of gamma-cyhalothrin and alpha-cypermethrin containing preparations on early life stages of common carp (*Cyprinus carpio* L.). The chosen subject is relevant and not fully explored yet. Therefore presented here results from the experimental studies are an important input into the current state of knowledge on the possible effects of that group of pesticides on aquatic organisms. Moreover, the obtained results show the new areas for the future studies.

Elaboration of the PhD thesis, objectives of the work and deliverables

Evaluate the overall level of elaboration of the PhD thesis (structuring of the main text, comprehensibility, logicity of the chapters and their ordering) and the originality of the selected approaches to solve the objectives; evaluate publications and whether the results described correspond to objectives of the PhD thesis (extent ¼ – ½ page):



The structure of presented thesis fulfils the requirements for PhD dissertations. It consists of five chapters. The first one was conceived as the introduction into the problems connected with the usage of pyrethroids. In this part the Author also indicated the objectives of her thesis. The other three chapters are presented in the form of publications, one as the review paper and two as experimental papers. All these papers were published in journals of the international range, with the impact factor. Chapter 2 contains the literature review on the presence of pyrethroids in the water environment and their toxic impact on fish. The aim of the next two chapters was to accomplish the tasks the Author put in her thesis, ie. to determine the impact of two commercially available formulations containing gamma-cyhalothrin and alpha-cypermethrin on early developmental stages of carp. Both chapters possess the similar structure, with respect to the used experimental model and methodology. The last chapter contains the general discussion on the topics of interest and the final conclusion. Each chapter is provided with the sufficient number of literature sources. From the formal point of view, the dissertation is well written and organized, and meets the requirements of scientific thesis.

OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

Pyrethroids belong to the most frequently used pesticides nowadays. They are generally considered safe, as the acute toxicity to mammals and birds is low. However, fish are much more susceptible to unwanted effects of these compounds. The studies, constituting the presented dissertation, with respect to the present state of knowledge in that subject and the importance of the subject are fully justified and of major significance.

Chapter 1 summarizes data on pyrethroid usage, including in-depth description of two of them, namely cypermethrin and cyhalothrin, being the subjects of the further experiments. Additionally, Chapter 2 is also devoted to the review of the available data and provides detailed description of the current knowledge on the pyrethroids influence on fish. Given here information properly introduces the reader into the subject, comprehensively shows the background of the issue, as well as indicates the deficiencies in the existing data on the possible toxic effects of these pesticides on fish and shows the need for the studies undertaken by the Author. These two chapters confirm good orientation of the Author in the literature data on this issue.



The main aim of the thesis was to assess the sensitivity of early developmental stages of common carp (*Cyprinus carpio* L.) to the alpha-cypermethrin containing formulation Cyperkill 25 EC and gamma-cyhalothrin containing Nexide. This goal was to be achieved by elucidation of the preparations effects on such parameters as hatching and mortality, behaviour, biometric parameters (length, weight, Fulton's condition factor), ontogenetic development, histopathology and parameters of oxidative stress and antioxidant defence. The Author managed to achieve these objectives by conducting the experiments described in Chapters 3 and 4, and by the proper interpretation of the obtained results.

I have also a few critical comments, which however, have no influence on the significance of the whole dissertation. There are some minor shortcomings in the Introduction. For instance, there is one position in the references missing (David, 2005, p. 12), and there is also one position in the references which I cannot find in the text (Ray, 2005). Date of publication given in the text (Pike and Wadsworth, 2000, p. 9) differs from that given in the references. I would also suggest to change the name from "common mirror" to "mirror carp" on p. 11. All these mistakes, however, are probably typing mistakes and are of minor importance.

Overall, presented thesis contains important contribution to our awareness on the possible consequences of the extensive use of pyrethroids.

In my opinion, the dissertation fulfils requirements posed on theses aimed to obtain PhD degree.

FINAL RECOMMENDATION

- PhD Thesis can be recommended for defence
 PhD Thesis can be recommended with reservations for defence
 PhD Thesis can not be recommended for defence

15.06.2016 Lublin
Date and place


Anna Sierostawska
Name and signature



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Review of USB FFPW PhD Thesis

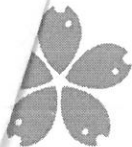
First name(s), surname, titles of the PhD student: Zuzana Richterová, MVDr.	First name(s), surname, titles of supervisor: Prof. Zdeňka Svobodová, DVM, D.Sc.
Title of PhD thesis: The effect of pyrethroid based pesticides on fish	
REVIEWER:	
Surname: Blahová	Institution: University of Veterinary and Pharmaceutical Sciences Brno, Faculty of Veterinary Hygiene and Ecology, Department of Animal Protection, Welfare and Etology
Name: Jana	E-mail: blahovaj@vfu.cz
Titles: Dipl.-Ing., Ph.D.	
Please describe your professional relationship to the PhD student: consultant and supervisor of PhD students at University of Veterinary and Pharmaceutical Sciences Brno (Czech Republic)	Please describe your field of expertise: study of biochemical markers in fish

QUESTIONNAIRE

Originality, scientific importance, perspectives and impacts of results presented in the PhD thesis for basic and/or applied research

Evaluate competitiveness of the PhD thesis in the international context and compare its level with the current state of the art in the field (extent ¼ – ½ page):

The present PhD thesis deals with the very topical issue, which is a monitoring of effects of pyrethroid based pesticides on fish. Pyrethroids are an important group of pesticides with worldwide use. Unfortunately, due to their stability and abundant use in agriculture, they may subsequently enter aquatic ecosystem where they affect adversely physiological functions of non-target aquatic organisms such as e.g. fish. Even though available scientific data concerning effect of pyrethroid based pesticides on fish are relatively extensive, there is still ample space to add further information on negative effects thereof. I really appreciate use of embryolarval toxicity tests within this work, since early developmental stages belong among very sensitive ones. Furthermore, I appreciate that not only basic indicators such as mortality, influence on developmental and morphological parameters were evaluated within conducted toxicity tests,



OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

The PhD thesis is written in the form of commented articles, which passed through a severe review procedure in relevant scientific journals with impact factor. Therefore, my opponent role is facilitated to a large extent. I also appreciate that student MVDr. Zuzana Richterová is stated as the first author in all scientific publications enclosed. The quality of enclosed published articles is demonstrated by the fact that two of them have been 8x cited in Wos database (out of which review 7x) up to this day.

The PhD thesis is well structured, clearly elaborated and brings interesting topical information. List of literature comprises more than 150 references, which documents erudition of the student who got acquainted with the large scope of information related to studied issue. Not quite consistently, in my opinion, one list of references is stated behind the first chapter and another list of references is stated behind the last chapter. Furthermore, I would recommend, for clear arrangement, to complete the thesis with a list of abbreviations to facilitate orientation.

To the present thesis, I take liberty to state several questions, which could be subsequently used in discussion:

- 1. Is it possible to use monitoring of oxidative stress markers in fish for field studies assessing loaded localities? Alternatively, which biomarkers monitored in fish are most frequently used in field studies due to their high information value?*
- 2. Could student state an overview and consumption of all pyrethroid based pesticides approved and applied in the Czech Republic? What is the tendency in the use of these pesticides in recent five years?*
- 3. In the Introduction, you state environmental concentrations of cypermethrin in surface water in the Czech Republic. What is the situation in other pyrethroids in our surface water? What is the ability of wastewater treatment plants to remove these contaminants in course of purifying procedures?*



4. Author used TBARS method when monitoring lipid peroxidation. Could she state both advantages and disadvantages of the stated method? Or which other analytic methods can be used when evaluating degree of lipid peroxidation?
5. What is the student's share in individual publications?

On the basis of detailed study of the present work, I state that the PhD thesis of MVDr. Zuzana Richterová meets all requirements of scientific work, is elaborated at high professional level and testifies the scientific erudition of the author. The thesis includes original and highly topical data and is clearly elaborated from the formal viewpoint.

In conclusion, I recommend to accept the present PhD thesis for defence and, after successful defence, to award MVDr. Zuzana Richterová Ph.D. title.

FINAL RECOMMENDATION

- PhD Thesis can be recommended for defence
 PhD Thesis can be recommended with reservations for defence
 PhD Thesis can not be recommended for defence

13/6/2016, Brno

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Date and place

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Ing. Jana Blahová, Ph.D.