



Fakulta rybnářství  
a ochrany vod  
Faculty of Fisheries  
and Protection  
of Waters

Jihočeská univerzita  
v Českých Budějovicích  
University of South Bohemia  
in České Budějovice  
Czech Republic

**Confidential**

### Review of USB FFPW PhD Thesis

<b>First name(s), surname, titles of the PhD student:</b> Alžběta Stará, Dipl.-Ing.	<b>First name(s), surname, titles of supervisor:</b> Dr. hab. Dipl.-Ing. Josef Velíšek, Ph.D.
<b>Title of PhD thesis:</b> The effect of triazine based pesticides on fish	
<b>REVIEWER:</b>	
<b>Surname:</b> Navrátil	<b>Institution:</b> Veterinární a farmaceutická univerzita Brno Fakulta veterinární hygieny a ekologie Ústav ekologie a chorob zvířete, ryb, včel Palackého třída 1/3, 612 42 Brno
<b>Name:</b> Stanislav	
<b>Titles:</b> Prof. MVDr., CSc.	<b>E-mail:</b> navratils@cfu.cz
<b>Please describe your professional relationship to the PhD student:</b> I know the PhD student, but I have not been cooperating with her.	<b>Please describe your field of expertise:</b> Diseases of fish and bees

### 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 increasing pollution with different chemicals is high risk for environment. One of the most important sources of these chemicals are pesticides. The knowledge about their influence to the non-target organisms including aquatic organisms is very important. The presented PhD thesis brings together original results of several studies aimed at this influence. From this point of view the item of the submitted thesis is well-taken and very topical.



### **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 submitted study is written as a collection of seven published papers in scientific journals with impact factor. The review of references corresponds with the needs of the study and contains all necessary data for the orientation in problems. Methods of the investigation are well-taken and standard. The used methods are mostly well described and make repetition possible. Results of all investigations are very valuable and extend the current knowledge on the impact of the influence of triazine pesticides on aquatic non-target organisms.

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### **OVERALL COMMENTARY ON THE PhD THESIS**

**Please write comments in extent of 1-2 pages:**

The submitted study is written as a collection of seven published papers in scientific journals with impact factor. The study is composed of the special chapter of general introduction and subchapters of general discussion, English and Czech summaries, acknowledgements, list of publications, training and supervisors plan during the study and curriculum vitae. It has 113 pages. In my opinion there are some formal imperfections in the study (e.g. bad name of the author – Sobodová - on page 9). I have a critical note on the division of the study. The chapter conclusion is a part of the general discussion. In my opinion, a separate chapter of conclusion is lacking.

The general introduction makes the reason of investigation clear and presents aims of the study. The aims are summarised in two points. On the basis of the evaluation of the results it is possible to state that these points were realised.

The review of references corresponds with needs of the study and contains all necessary data for the orientation in problems.

The methods of the investigation are mostly well-taken, standard and well described.

The results of all investigations are very valuable and extend the current knowledge on the influence of triazine pesticides on non-target organisms, especially on developmental stages of the common carp and on the crayfish *Procambarus clarkii*.

The results of all papers are well documented by tables and figures. The obtained results are discussed with appropriate literature sources.

Unfortunately, a separated conclusion chapter is lacking. In my opinion, practical recommendations are also lacking.

The summary briefly repeats the results.

I have the following notes or questions and recommendations for the general chapters:

1. I recommend the accurate formal control and correction of the general chapters.
2. What explications and practical recommendations do result from the study?



The presented study fulfils the requirements for PhD thesis and therefore I **recommend** it for the defence and after it I **agree** with the conferment of the degree “Doctor”.

### **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

17. 6. 2014 Brno  
Date and place

Prof. MVDr. Stanislav Navrátil, CSc.  
Name and signature



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### **Review of USB FFPW PhD Thesis**

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<b>Title of PhD thesis:</b> The effect of triazine based pesticides on fish	

#### **REVIEWER:**

<b>Surname:</b> Rymuszka	<b>Institution:</b> John Paul II Catholic University of Lublin Institut Biotechnology, Department of Physiology & Ecotoxicology, 14 Al Raclawickie St PL-20950 Lublin Poland
<b>Name:</b> Anna	
<b>Titles:</b> Dr. hab.	<b>E-mail:</b> anrym@kul.lublin.pl
<b>Please describe your professional relationship to the PhD student:</b> none	<b>Please describe your field of expertise:</b> ecotoxicology, fish physiology

### **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 Ph.D. dissertation entitled "The effect of triazine based pesticides on fish" is on important toxicology issues related to assessment of threats of water environment contamination with pesticides. The wide use of triazine herbicides in agriculture as well as in aquaculture (e.g. simasine was used to control and kill submerged aquatic weeds and algae in ponds) resulted in their permeation into and constant presence in water reservoirs. Hence there is a necessity of research on threats to water organisms, mainly fish, which may result from use of these compounds, and such is the aim of the above-mentioned Ph.D. dissertation. Assessment of chronic changes, the effect of long-term influence of triazines at low environmental doses, is particularly important. Although the effects of acute and sub-chronic exposure of fish to s-triazine herbicide have been well-documented, there is a dearth of data on the chronic exposure to these compounds at environmentally realistic concentrations with respect to oxidative stress and antioxidant responses in fish and other aquatic organisms. The dissertation concerns a very important issue in the context of documented triazine toxicity, and especially methods of action required for protection against chronic effects of the



compounds. Therefore information that can be obtained from the conducted research is extremely important for both theoretical consideration and practical use.

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***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 Ph.D. dissertation's arrangement is typical of works for the scientific degree applied for. The first chapter is the Introduction. Next chapters (from 2 to 8) comprise the subject-specific cycle of 7 original publications from the years 2011-2014. The last chapter includes: General Discussion, English and Czech Summary, Acknowledgements and other detailed information on scientific achievements: List of other published scientific papers and indicator of scientific achievements, Training and Supervision Plan during Study and Curriculum Vitae.

The Introduction is excellent. It concisely reviews about mechanism of selected triazine action and used this pesticides. The Introduction section is a concise description of the mechanism of action of triazine compounds (especially prometryne, simazine, terbutryn), the range of their occurrence in water environments and level of toxicity to water organisms. Moreover, it describes the effect of triazines on non-target aquatic organisms. The author logically presents the nature of the problem and explains the aim of the research. Methods are well described and referenced. Methods used in the research ensured reliable and objective results. Results are concisely presented; figures and tables support the text discussion. The discussion puts the results into a larger context and identifies how they can be applied. Overall, there are a well done studies that focus on a specific question with useful applications. The factual content of the dissertation is clear and correct.

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**OVERALL COMMENTARY ON THE PhD THESIS**



**Please write comments in extent of 1-2 pages:**

Environmental pollution caused by pesticides, especially in aquatic ecosystems has been well- documented worldwide and constitutes a serious global problem. Pesticides are chemical compounds which are deliberately introduced into environment in large quantities in order to produce intended biological effects. Due to high biological activity and a wide range of toxic effects, pesticides may be a reason for many irregularities and disturbances in functioning of living organisms. Therefore the scientific subject of the Ph.D. thesis entitled “The effect of triazine based pesticides on fish “ is very interesting.

A monothematic cycle of publications, subject of the review of Alžběta Stará, consists of 7 papers published in journals included in the JCR list, which IF is from 1.29 to 2.88 (total points= 12.47). The Ph.D. student is the first author of 4 papers and the second author of the other three. The subject of the doctoral dissertation at first was to investigate the effects of chronic exposure to selected triazines (prometryne, simazine, terbutryn) at concentrations commonly occurring in the environment on developmental stages of common carp (*Cyprinus carpio* L.). In the second stage of the experiment, the effects of triazine (prometryne) on crayfish (*Procambarus clarkia*) as a non-target organism were also investigated. Pursuing such a direction of study is substantiated by prevalent use of triazine herbicides, and their relative stability in the environment, easy permeability to water and a potential carcinogenic effects, which poses a threat to health of water organisms.

It should be emphasized that both the selection of research model and the appropriate doses of pesticides used in the experiments were deeply considered. In aquatic toxicology, fish are important indicators of the impact of toxic substances. For the majority of studies, the common carp (*Cyprinus carpio* L.) has been chosen. Carp is one of the most common fish species typical of Central Europe. In addition, a red crayfish (*Procambarus clarkii*) as a model species has been chosen. Crayfishes are ecologically important organisms and they are considered as a good indicator of environmental pollution in both standing and flowing waters. For several years ecotoxicologists have been noting threats associated with exposure of living organisms to chronic pesticide doses, i.e. doses which do not cause clearly noticeable symptoms of poisoning, while having an effect at the cellular level. Under natural conditions, very high, lethal concentrations of xenobiotic are rare. Generally, fish are exposed to low doses of these compounds.

The Ph.D. student's papers presented as special scientific achievements include a sequence of well-designed studies. The first part is about monitoring the long-term effects of triazines on the early development stages of carp. Another part of the work has included long-term effects of low concentrations of triazines on biochemical, physiological and morphological parameters and oxidative stress and antioxidant response in common carp. The last part of the study



examined the chronic effect of prometryne on red swamp crayfish. The research methods were properly selected and used to obtain reliable and statistically well-analyzed results. The Ph.D. student employed techniques requiring considerable experience in laboratory work. The high scientific level of the conducted research and papers as well as practical importance of the obtained results are reflected also in the titles of the journals which published them: Pesticide Biochemistry and Physiology Neuroendocrinology Letters, BioMed Research International, Environmental Toxicology and Pharmacology. The dissertation submitted for the review is written with appropriate language and correct use of professional terminology. Extensive literature collection presented both in the introduction, discussion and as enclosed publications proves substantial qualifications of the Ph.D. student for accomplishing the established aim.

Scientific achievements of Alžběta Stará, after including the cycle of monothematic publications, comprise 11 papers published in journals of the JCR database. Many of the 11 publications were issued in recognized journals with high IF, such as e.g. BioMed Research International, Ecotoxicology and Environmental Safety, Aquaculture. Also this part of scientific achievements confirms the high level of competence, meeting international standards of designing and carrying out scientific work and publishing its results.

Alžběta Stará confirmed high scientific activity in the years 2011-2012 by active participation in numerous national and international conferences where she presented the obtained results in the form of posters. Improved qualifications and scientific development are reflected in her participation in numerous scientific trainings, courses and workshops during study.

After learning the scientific achievements of Alžběta Stará, I am stating that the scientific research activity, and especially the scientific achievement of the monothematic cycle of papers, but also the rest of the publications about toxicity of xenobiotics, are very valuable. The results of the research presented in the dissertation significantly expand knowledge of the issue of potential chronic influence of triazines on fish and crayfish. The papers published by the Ph.D. student significantly contribute to development of science, mainly fish toxicology.



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### **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

12.06.2014 , Lublin

Date and place

Anna Rymuszka

Name and signature