



Confidential

Review of USB FFPW PhD Thesis

First name(s), surname, titles of the PhD student: Dalibor Koutnik, Dipl.-Ing.	First name(s), surname, titles of supervisor: Assoc. Prof. Dipl.-Ing. Josef Velíšek, Ph.D.
Title of PhD thesis: The effect of triazine based pesticides and their metabolites on no-target aquatic organisms	
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	
Titles: Assoc. Prof, Ph.D.	E-mail: blahovaj@vfu.cz
Please describe your professional relationship to the PhD student: consultant and supervisor of PhD students at University of Veterinary and Pharmaceutical Sciences Brno	Please describe your field of expertise: changes of selected biomarkers in fish after exposure to various pollutants (e.g. pesticides, drugs, personal care products)

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 very actual topic such as comprehensive evaluation of toxic effects of triazine based pesticides and their metabolites on selected non target aquatic organisms. Author investigated the effects of chronic exposure to selected triazines (prometryne and terbuthylazine) and their metabolites (terbuthylazine-2-hydroxy and terbuthylazine-desethyl) at environmentally relevant concentrations on early-life stages of common carp and two types of crayfish (marbled crayfish and signal crayfish). The effects were assessed with respect to behaviour, mortality, growth, ontogenetic development, histopathological changes and selected indices of oxidative stress. Pollution of aquatic ecosystem by pesticides belongs to the one of the key worldwide issue. Triazine herbicides belong to the most important class of agricultural chemicals



used for sixty decades for weed control in more than 100 countries around the world. Although many of triazine herbicides have been banned in European Union many years ago, they still can be detected in surface and ground waters. Triazine herbicides and their metabolites are present in the aquatic environment at comparatively low concentrations, but these levels are biologically relevant and pose a significant growing risk to various types of aquatic organisms. I really appreciate use of early life stages of aquatic organisms in the toxicity tests, because they are very sensitive, especially at hatching the embryos lose their protective membrane and are fully exposed to contaminants.

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**):

This PhD thesis has good logical structure and is written in English language in the form of commented articles, which are published in relevant scientific journals with impact factor. The thesis is well structured, it consists of ten chapters and is written on 109 pages altogether. It is also completed with English and Czech summary, list of author's publications and author's curriculum vitae. In the first chapter, there are summarized aims of this thesis and general information about triazine herbicides (their fate and occurrence in the aquatic environment, toxicity etc.). This chapter is validated with 64 valuable references. In the next eight chapters, there are scientific studies published in various scientific journals with impact factors (Slovenian Veterinary Research, Pesticide Biochemistry and Physiology – 2x, Neuroendocrinology Letter – 3x, Science of the Total Environment, BioMed Research International). Candidate is first author in three studies, in other studies is as co-author. Most of these studies evaluated results obtained in various toxicity test on aquatic organisms. One article is review dealing with effects of selected triazine herbicides on fish. In the last chapter, author summarizes and discusses obtained results of various markers (behaviour, growth, ontogenetic development, histopathology, oxidative stress and antioxidant parameters). Chapter is validated with 46 valuable references.



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 article, which were published in various scientific journals with impact factor and therefore passed through a severe review procedure. For that reason, my opponent role is facilitated to a large extent. Results of this PhD thesis were published in eight articles. One article is in form of review and other articles are experimental studies describing results obtained in various toxicity test on early life stages of fish or crayfish. The quality of enclosed articles is demonstrated by the fact that all of them have been 59x cited in database Web of Science up to this day. The most cited is an article published in Science of the Total Environment (12x, IF₂₀₁₇ = 4.61), in which authors evaluated effects of terbuthylazine metabolites terbuthylazine-desethyl on common carp embryos and larvae. I have only one short comment on the title of this thesis. It will be better to change the title to: „*The effect of triazine based pesticides and their metabolites on **non**-target aquatic organisms*“.

Overall, obtained results are well presented, their interpretation is at a high scientific level and they have been published in excellent relevant scientific journals with high impact factors as well. The thesis is written in very good English and contains only few grammatical errors, which do not decline overall quality of this PhD thesis. All used sources are cited correctly according citation norm. I highly appreciate that author dealt with evaluation of both triazine herbicides and their metabolites. I also appreciate that candidate studied especially environmentally relevant concentrations of triazine herbicides. It is very current topic.

To the present PhD thesis, I have following questions to the candidate and they could be subsequently used in discussion:

1. Could you summarize which triazine herbicides are still applied in the Czech Republic? What are their annual consumptions and for which commodities are they use?
2. Could you summarize which aquatic organism used in your studies is the most sensitive? What biomarkers are the most reliable?



3. What is the candidate's share in individual publications? Does candidate plan to continue with this current topic (e.g. use other aquatic organisms for toxicity tests, expand spectrum of biomarkers)?

In conclusion, this PhD thesis fulfils all the requirements given to doctoral thesis and I recommend to accept it for defence, and after successful defence, to award the degree of Doctor of Philosophy (PhD.) to Dipl. Ing. Dalibor Koutník.

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

17/12/2018 Brno

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Date and place

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Assoc. Prof. Jana Blahová, Ph.D.

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Title of PhD thesis: The effect of triazine based pesticides and their metabolites on no-target aquatic organisms	
REVIEWER:	
Surname: Rymuszka	Institution: John Paul II Catholic University of Lublin Institut Biotechnology, Department of Animal Physiology & Toxicology, 14 Al Raclawickie St PL 20-950 Lublin Poland
Name: Anna	E-mail: anrym@kul.pl
Titles: dr hab.	
Please describe your professional relationship to the PhD student: none	Please describe your field of expertise: 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 doctoral dissertation by Dalibor Koutnik entitled "The effect of triazine based pesticides and their metabolites on non-target aquatic organisms" concerns a very important issue - the contamination of environment with various chemical compounds. The research topic has many aspects, which are systematically studied in the recent years.

Pesticides are one of the main organic components contributing to the pollution of agricultural soils as well as ground and surface waters. As a consequence of the extensive use of these compounds, residual amounts of pesticides and their metabolites are found in all components of ecosystems leading to the risk for the environment and, consequently, for humans. Among pesticides, the most frequently reported in the environment are residuals of triazine herbicides. Although most of triazines (e.g. prometryne) were banned in Europe in last years, these substances and their metabolites in residual concentrations are still present in water, soil, food and various components of the environment. In the literature, the effects of acute and sub - chronic exposure of fish to triazine herbicides have been well documented. There is still a dearth of data on chronic



toxicity of triazines and their metabolites at environmentally realistic concentrations on non-target aquatic animals, especially freshwater fish and crayfish. Therefore, I found the topic of the Thesis very interesting and scientifically important. Moreover, information that were obtained from the conducted research is important for both theoretical consideration and practical use.

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 dissertation is organized in a standard way and is divided into ten chapters with numerous subsections, making it easy to navigate in the text. The first introductory chapter outlines the research problem and main goals of the thesis. Next chapters (from 2 to 9) comprise the subject-specific cycle of 8 original publications from the years 2014-2017. The last chapter includes: General Discussion, English and Czech Summary, Acknowledgements and other detailed information on scientific achievements, Training and Supervision Plan during Study and Curriculum Vitae.

The introduction chapter is very condensed but it contains necessary information crucial to follow the Author's work and discussion. The context of the research problem and the objectives of the thesis, the hypothesis was correctly presented. Dalibor Koutník realized all of the aims of the study. As a result, PhD thesis consisting of eight original publications, presents the description of numerous, well-planned, and logically designed experiments allowing the precise examination the effects of chronic exposure to selected triazines and their metabolites on non-target aquatic organisms. Author took an advantage of multiple toxicology tools and methods, which allowed monitoring several important parameters: behaviour, mortality, biometric, biochemical blood, histopathology, oxidative stress and antioxidants. I consider the results to be valuable and of high scientific significance. The fact that the obtained results were accepted and published in high - quality journals, also confirms their strong scientific merit.

OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

The research presented in the thesis was conducted at the Research Institute of Fish Culture and Hydrobiology, Faculty of Fisheries and Protection of Waters, University of South Bohemia in České Budějovice, under the supervisor Assoc. Prof. Dipl.-Ing. Josef Velíšek.

The work carried by Dalibor Koutník covers an interesting area of contemporary research in ecotoxicology - the assessment of environmental pollution caused by pesticides in aquatic ecosystems. The thesis makes distinct and well-defined contribution to the knowledge base of this broad subject. It specifically deals with the effects of chronic exposure to triazines on aquatic animals.

A monothematic cycle of publications, subject of this review, consists of 8 papers published in journals included in the JCR list, which IF is from 0,222 to 3.976 (total points= 14.177). 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, such as e.g. Science of the Total Environment, Pesticide Biochemistry and Physiology, BioMed Research International, Neuroendocrinology Letters. The Ph.D. student is the first author of three papers and the second or/third author of the other five.

The Author in his thesis makes a thorough investigation of the two experimental setups. The first one considers the effects of chronic exposure to triazines (prometryne and terbuthylazine) and their metabolites (terbuthylazine-2-hydroxy and terbuthylazine-desethyl) at real environmental concentrations on developmental stages of common carp (*Cyprinus carpio* L.) and marbled crayfish (*Procambarus fallax* f. *virginalis*). The second one addresses recordings the effects of triazine metribuzine on signal crayfish (*Pacifastacus leniusculus* Dana).

The published works provide answers to the questions posed by Dalibor Koutník. In the publications the research methodology is clearly described. Because the papers were reviewed by 2-3 substantial specialists designated by journals' editors, therefore I will refrain from assessing these works. The fact that the manuscripts were accepted and published confirms that they were highly rated.

In the reviewer's opinion the short description of applied methods in the chapter "Introduction" would be very helpful for understanding of the results and discussions. Thanks to this, the reader would receive a guide to the Chapter 2 to 9 with reference to the individual papers.

It should be emphasized that both the selection of research model and the appropriate doses of pesticides used in the experiments were deeply considered. Fish are good animal models for the study of aquatic contamination, since altered water chemistry affects not only their behaviour and histology but also their physiology and biochemical processes. Crayfish are widely used as biological markers of environmental effects of anthropogenic pollutants because they are easily identified, widespread, and provide sufficient tissue for individual biochemical and chemical analyses.

The thesis presents numerous relevant findings. I consider the obtained results to be valuable and of high scientific significance as well as have the practical implications. For example they show that chronic exposure to metabolites of triazines affected growth,

ontogenetic development, and the antioxidant system and caused pathological changes in the organs of early life stages of carp and crayfish. These results confirmed that metabolites of triazines as more polar than the parent substance may poses a greater potential risk for aquatic animals.

In the "General discussion" Dalibor Koutnik critically analysed the results of the study. This part of the dissertation proves that The Author is mature scientist able to analyse results of his study and also the available data and present it in comprehensive but concise manner.

As a reviewer I would expect a few bullet points summarizing the main obtained results.


Generally, it can be said that Dalibor Koutnik, during performing researchers, showed quite broad range of skills and knowledge. The dissertation is written in a very clear language and correct use of professional terminology. Although, the reviewed text has some minor typing errors but they do not decreases the level of understanding and the value of dissertation (e.g. title page "no-target"). 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.

The thesis ends with an overview of research dissemination by Dalibor Koutnik in the years 2014-2018 during the course of the study. This dissemination was quite broad and includes presentations at international and national conferences. The original papers which were published in internationally recognized journals as well as attendance in a number conferences show his great interest in participation scientific life. The improved qualifications and scientific development are reflected in his participation in scientific seminars, courses and foreign stays during study.

Overall, **in my opinion the reviewed dissertation fulfills all requirements for the PhD degree.** The results of the research presented in the monothematic cycle of papers significantly expand knowledge of the issue of potential chronic influence of triazines on fish and crayfish. It presents significant data and opens up new perspectives of further research.

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



Fakulta rybnářství
a ochrany vod

Faculty of Fisheries
and Aquaculture
in Vodňany

Jihočeská univerzita
v Českých Budějovicích

University of South Bohemia
in České Budějovice
Faculty of Science

Lublin, 14.12.2018

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Date and place

Jan Zymuška
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Name and signature