



machora University of South Bohemia in České Budějovice Faculty of Fisheries and Protection of Waters Institute of Aquaculture

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Please describe your field of expertise:

Toxicity tests, Veterinary Toxicology

Confidential

Titles: doc. MVDr., Ph.D.

PhD student:

none

Please describe your professional relationship to the

Review of USB FFPW PhD Thesis

Surname of the PhD student: Ing. Jana Máchová	Name of supervisor: Prof. MVDr. Zdeňka Svobodová, DrSc.			
Title of PhD thesis: The role of toxicity tests on early life stages of fish in assessing the toxicity of substances and preparations				
REVIEWER:				
Surname: Modrá	Institution: University of Veterinary and Pharmaceutical Sciences Brno			
Name: Helena	Faculty of Veterinary Hygiene and Ecology			
Titles: doc MVDr Ph D	E-mail: modrah@vfu.cz			

QUESTIONNAIRE

Originality, scientific importance, prospects of the PhD thesis and benefits for basic or applied research

The topic of the thesis corresponds with new approaches of environmental toxicology. It is beneficial to assess effects of toxic substances in the water environment. Fish occupy a prominent position in the field of toxicology; they have been widely employed in toxicology studies as model organisms. The thesis points out sensitivity of early life stages of fish and compares results of tests on these stages to results of tests on juvenile and adult fish.

Preparation of the PhD thesis, targets of the work and deliverables

The overall level of preparation of the PhD thesis is high. The thesis includes five articles published in scientific journals with an impact factor, which were refereed by the publisher. Thus my comments apply only on Chapter 1 - General introduction and Chapter 7 - General discussion. The PhD thesis corresponds with its declared purpose.

OVERALL COMMENTARY ON THE PhD THESIS





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Please write comments:

General introduction summarizes requirements of tests on early life stages and describes differences between particular developmental stages. This part contains also the aims of the thesis. However, this chapter includes many typos (for example: ocur/occur, *Oncorhinchus mykiss/ Oncorhynchus mykiss, Oncorhynchus mikyss/Oncorhynchus mykiss*, Sa-fry/Sac-fry, philosophical/philological and so on).

The last part of the first paragraph is identical to the second paragraph. What's more, it contains different citations. Editors of the book "Standard Methods for the examination of water and wastewater" should be quoted directly.

R-phrases for aquatic organisms are:

R 50: Very toxic to aquatic organisms

R 51: Toxic to aquatic organisms

R 52: Harmful to aquatic organisms

A citation of the Annex III EU Directive 67/548/EEC (2001/59/EC) is missing here.

General discussion is of required extent.

I have some questions for the author:

- 1. What was your experience with meeting criteria of maximum 20% decrease in test substances when using semi-static methods of the tests?
- 2. Did you analyze the concentration of diazinon in the test solutions?
- 3. You used the same test solution of diazinon within the 96-h test. Did you consider possible transformation of diazinon to diazoxon in water when comparing your results with results of other authors?
- 4. The results you present in General discussion show that the diazinon concentration 10 μg/l recommended for elimination of zooplankton is not safe for fish. Do you know the NOEC of diazinon for fish?

FINAL RECOMMENDATION

can be recommended for defence of PhD Thesis can be recommended with reservations for defence of PhD Thesis can not be recommended for defence of PhD Thesis		
15/6/2011, Brno	man	
Date and place	Surname and signature	





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Confidential

Review of USB FFPW PhD Thesis

Surname of the PhD student: Ing. Jana Máchová	Name of supervisor: Prof. MVDr. Zdeňka Svobodová, Dr.Sc.			
Title of PhD thesis: The role of toxicity tests on early life stages of fish in assessing the toxicity of substances and preparations				
REVIEWER:				
Surname: Wlasow	Institution: University of Warmia and Mazury in Olsztyr Faculty of Environmental Sciences and Fisheries,			
Name: Teresa	Poland			
Titles: Prof.	E-mail: tewlasow@uwm.edu.pl			
Please describe your professional relationship to the PhD student:	Please describe your field of expertise: Fish toxicology, Fish diseases,			

QUESTIONNAIRE

Originality, scientific importance, prospects of the PhD thesis and benefits for basic or applied research

Evaluate its competitiveness in the international context and compare its level with the current state of the art in the field:

The chosen dissertation topics are currently very topical both from a scientific point of view and for their practical applications. Evaluation of substances that contaminate the aquatic environment and the search for methods that objectively detail the effects of these substances is a priority for environmental protection. In the actual preparation of the thesis the author proceeded with a precise and accurate knowledge of the methods of toxicity tests on fish and with similar knowledge of the issues relating to water contamination by foreign substances. The author's long experience of the water toxicology is evident in the work.

Preparation of the PhD thesis, targets of the work and deliverables

Evaluate the overall level of preparation of the PhD thesis and the originality of the selected approaches; evaluate publications and whether the targets set in the PhD thesis correspond with the declared purpose of the thesis:

The presented dissertation has an accumulated total of 109 pages consisting of five separate scientific papers published in journals with IF. This work begins with a chapter – General Introduction and the results are summarized and discussed in a charter General Discussion. The work contains a full appendix and is well presented. The aim of this study was to assess the sensitivity of particular tests for acute and subchronic toxicity on the juvenile stages of fish.

OVERALL COMMENTARY ON THE PhD THESIS





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Please write comments:

The paper summarizes the results of the toxicological assessment of the effects of the application of chemical substances and products which are/or were applied to the water environment, in order to improve water quality. One such product is Diazinon 60 EC, which in appropriate cases, was used as a biocide to suppress the extensive development of large *Daphnia* zooplankton. Another product was PAX-18, which as a coagulation agent was mainly used to precipitate phosphates, to decrease the concentration of phosphorus, to prevent surface water eutrophication and incidences of cyanobacteria in the aquatic environment.

The next test substance was sodium nitrite (NaNO₂ p.a.), which was used to test for environmental effects of sub-lethal concentrations of nitrite in the early developmental stages of fish. The last test substance was dimethylsulfoxide (DMSO), which among others is an auxiliary substance used as a solvent in the preparation of solutions of substances insoluble in water, which are used in toxicity tests on aquatic organisms.

The basis of the work consists of five scientific publications that have already undergone a successful review process, and therefore I have decided to concentrate mainly on comments and questions relating to the following issues:

- What do you think are currently the most endangered of surface water?
- What importance should be attached to testing the toxicity of the early life stages of fish in the overall context of water toxicology?
- What role should these tests play in the evaluation of the effects of residues that are currently the subject of interest of other experts (e.g. residues of drugs, metabolites, surfactants, residues of pesticides, musk compounds, etc.)?

You are a co-author of the methodology of Diazinon use in fishing practise. Do you have a response from fishermen, as to what extent, this preparation has proved its worth in practise?

- How from your point of view, has the use of Diazinon compared with the previous use of Soldep?

One section of the work evaluates dimethylsulfoxide in terms of its toxicity to fish in realation to its possible use as a solvent in toxicity testing. What do you think in general of the use of auxiliary substances (solvents) in toxicity tests on fish and other aquatic organism?

In the work, which deals with the toxicity of nitrite for the early developmental stages of carp, it is clear that negative effects were observed at nitrite concentrations that are significantly lower than concentrations that are commonly found in surface waters. Do you think that stock carp (and other fish) in natural conditions, including rivers and lakes are threatened by increased concentrations of nitrite?

Preparation PAX based polyaluminium chloride is used in the Czech Republic to reduce eutrophization aquatic environment and to reduce one of the consequences of eutrophication (the elimination of turbidity caused by the growing cyanobacteria and algae). What are the current experiences with this product and how widespread is it use?

I have a number of formal observations to the work: in the introductory chapter (p. 9), repeated lines 9-15 and lines 16-21, modify and unify the Latin name for the rainbow trout *Oncorhychus mykiss*, on page 12, line 6 is missing in c Word CAC-Fry, p. 73, R. 9 geills fix on the gills.

The above remarks commenting on and confirm the timeliness matters and suggests that addressing the issue is not currently available in the Czech Republic, but can be used in conditions of the Polish fishing and water protection.





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Conclusion

Based on a detailed study I can clearly claim that submitted a doctoral thesis "The role of toxicity tests on early stages of fish in assessing the toxicity of substances and preparations" by Ing. Jana Máchová is an important contribution to the assessment of risks arising from the targeted application of substances and preparations into the aquatic environment. Furthermore, I stated that the objectives of the doctoral thesis has been fully achieved, and therefore strongly recommends the thesis for defence and the successful defence of the Ph.D. degree award.

FINAL RECOMMENDATION

x can be recommended for defence of can be recommended with reserva can not be recommended for defended	tions for defence of I	PhD Thesis
16.06.2011. Olsztyn, Poland	Teresa Własow	T. Wasaw
Date and place	Surm	ame and signature

Surname and signature