

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

Branišovská 1645/31a, 370 05 České Budějovice, Czech Republic
☎ +420 389 032 735 e-mail: studijni@frov.jcu.cz VAT: CZ60076658

Confidential

Review of USB FFW PhD Thesis

Surname of the PhD student: M.Sc. Li Zhihua	Name of supervisor: Ing. Tomáš Randák Ph.D.
Title of PhD thesis: Effects of residual pharmaceuticals present in aquatic environment on fish	
REVIEWER:	
Surname: Kloas	Institution: Leibniz Institute of Freshwater Ecology and Inland Fisheries
Name: Werner	
Titles: Prof. Dr.	E-mail: werner.kloas@igb-berlin.de
Please describe your professional relationship to the PhD student: Not personally known, external reviewer of thesis	Please describe your field of expertise: Animal physiology, endocrinology, ecotoxicology, endocrine disruption in lower vertebrates

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:

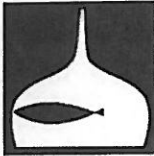
The PhD thesis by Li Zhi-Hua entitled "effects of residual pharmaceuticals present in aquatic environment on fish" deals with an emerging topic of ecotoxicology the potential impacts of residual pharmaceuticals on aquatic organisms, e.g. fish. Mr. Li performed as PhD student in close collaboration with the scientists of RIFCH in Vodnany under supervision of ass. Prof. Dr. Tomas Randak very original research towards impacts of pharmaceuticals on fish. Thus in this field they did a great part of original experiments meeting international standards to contribute essentially to the current state of the art in that context. As deduced by the enormous number of peer-reviewed publications with impact factors the group in Vodnany e.g. Mr. Li could extremely successfully compete in that emerging ecotoxicological topic. In the field of ecotoxicology all research has at least in part greatly an applied component, however, because of its originality the research by Mr. Li also contains at the same level basic research.

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 PhD thesis is cumulative and comprised out of 6 publications/manuscript followed by a dense comprehensive discussion. Thus the thesis is prepared on an extensive scientific basis to target the aim of the work. The methodologies applied spawn from acute toxicity testing over short term and long term testing including various morphological as well as physiological responses to even proteomics. All papers mentioned in the thesis correspond very well with its purpose and as deduced by the list of publications the scientific activities of Mr. Li were even beyond the content of his thesis.

OVERALL COMMENTARY ON THE PhD THESIS



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Please write comments:

However, despite the PhD thesis and the research done by Mr. Li can be acknowledged as being excellent there are some minor topics at least to be mentioned.

Why did he compile the cumulation of papers as a mixture of different fish species and various pharmaceuticals? There would have been a greater benefit for clarity if all papers included would have dealt only with one test organism, trout or carp, focussing on effects caused by only one pharmaceutical or in a comparative analysis by two or more compounds. Thus it is a bit inconcise compilation of a lot of work.

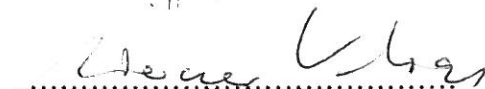
In chapter 2 the verapamil short term exposure consists only of pharmacological rather than environmentally relevant concentrations, which puts any argumentation concerning potential verapamil impacts of environmental concentrations into question. Looking on the Ca-channel blocker verapamil I suggest it would be essential also to analyze electrolytes in plasma. In chapter 3 concentration range for long term exposure of verapamil is fine but the statistical comparison of at least all morphological if not all parameters has been performed comparing all groups at d21 and d42 (C, E1, E2, E3) with control of d0. Here all groups of one sampling point should have been statistically compared between each other. In chapter 5 exposure to propiconazole has been performed for 30 days. Why was the exposure regime for each compound different? Here the potential impact on CF and BW is missing, which should have been mentioned. Why has only intestine investigated instead of further relevant organs? In chapter 6 the interesting data obtained for carp spermatozoa deal only with pharmacologically high concentrations of carbamazepine, here environmental concentrations should have been included and because all other effects are determined in trout that experiment would have been done preferentially also in trout.

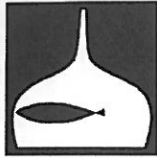
In general it would have been of greater benefit for the scientific community and the author to include more data within one publication instead of dividing one experiment into several papers one dealing with impacts on brain, one on gill, and one on liver or blood parameters in order to enlarge just publication list rather than hitting even higher IF-journals.

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

30th May 2011, Berlin
Date and place


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Surname and signature



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Review of USB FFPW PhD Thesis

Surname of the PhD student: M.Sc. Li Zhihua	Name of supervisor: Ing. Tomáš Randák Ph.D.
Title of PhD thesis: Effects of residual pharmaceuticals present in aquatic environment on fish	
REVIEWER:	
Surname: Bláha	Institution: Masaryk University, Faculty of Science, Research Centre for Toxic Compounds in the Environment (RECETOX)
Name: Luděk	
Titles: doc. RNDr. Ph.D.	E-mail: blaha@recetox.muni.cz
Please describe your professional relationship to the PhD student: - no relationship	Please describe your field of expertise: Aquatic ecotoxicology, In vitro and molecular toxicology, mechanisms of toxicity

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:

Very good quality, highly competitive, significant contribution to the field - see my comments below

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:

Very good quality, a lot of work presented in a number of publications - see my comments below

OVERALL COMMENTARY ON THE PhD THESIS

1. Research subject and quality of science

The dissertation thesis submitted for PhD defence of Zhi-Hua Li addresses the current issue of side effects of trace organic contaminants on aquatic biota. Trace bioactive chemicals contaminating surface waters such as pharmaceuticals that are specifically addressed in the evaluated PhD thesis, are of great environmental concern. They are likely to have various adverse effects on fish as well as other components of the ecosystems.

Work presented in the thesis significantly contributed to our understanding of acute and chronic effects of selected pharmaceuticals such as verapamil or propiconazol on traditional toxicological endpoints as well as less explored parameters in fish such as intestine-related responses or spermatozoa. To my opinion, the quality of science as well as quantity of actual work presented in the thesis is at outstanding level, and it substantially contributes to the current state of the art in the field of fish toxicology and general aquatic ecotoxicology.



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It should be pointed out that besides the five chapters (original papers) presented in the thesis, Zhi-Hua Li, has an outstanding additional publication record presented in his CV. In total, he contributed to 32 papers published in peer-reviewed international impacted journals; and he is the first author of 25 of these contributions.

2. Dissertation thesis - content and formal issues

The thesis has been submitted as a commented compilation of previously published (or submitted) papers. It is structured into 7 chapters. The thesis is submitted in English, which is at excellent level. While studying the thesis, I have had a problem to understand what was the actual role (and contribution) of the candidate to presented work. Zhi-Hua Li is the first author at all discussed publications (as well as at many others listed in his CV) but in all cases - as it is common practice - the papers were collaborative work of several authors. It would be highly beneficial to have an overview of the candidate's role at individual papers, and this should be clarified (e.g. during the thesis defence).

Chapter 1 has been published in peer-reviewed journal (*Veterinarni medicina*) as a review, and it provides a comprehensive introduction and overview of the most studied pharmaceuticals in the aquatic ecosystems. Toxic as well as pharmacokinetic properties are summarized and presented in a clear and comprehensive way.

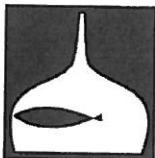
Chapter 2-6 are original papers published/submitted in well established international journals such as *Comparative Biochemistry and Physiology*, *Journal of Hazardous Materials*, *Molecular and Cellular Proteomics* (submitted manuscript), *Chemico-Biological Interactions*, *Chemosphere*. In all papers, the candidate is the first author, which clearly demonstrate his active role in the preparation of published papers.

After reading all chapters 2-6, I can honestly declare that each individual paper is a self-standing complete piece of work, which is clearly presented and very well discussed. Enormously high number of the author's publications initially brought me some doubts about the "spagetti-like series" but the clear and concise presentation (and especially quality discussions within individual papers) convinced me about the quality of the work.

Referee's questions: Most of the work has been peer-reviewed before, and thus I have only minor methodological questions to the chapter 4 (submitted MS): In the method section, it was not fully clear to me, what was the experimental design and how authors dealt with variability. How many fish were actually exposed per treatment? How many fish were used for protein extraction (individual?, pooled samples?)? How many samples per fish were repeatedly analyzed (three? single?)? How were the data of repeated measurements manipulated (averaged?)? Was the experiment repeated independently? (or only one experiment has been conducted?). I would like to have this clarified during the thesis defence because standard errors (or standard deviations?) presented in Figure 2 were very low (which is quite unexpected when considering variability between individual fish).

Chapter 7 provides a thorough General Discussion synthesizing all major findings presented in individual papers. I have no specific questions here because this part is very well elaborated. (Further sections of the Chapter 7 are summaries in Czech and English, CV etc.)

Formal issues: Only minor typo errors were rarely found in the text (e.g. title on p. 29 - "Ecotoxocological effects ...). To my experience, author devoted substantial work to the finalization of the thesis, and the formal quality is outstanding.



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3. General comments and discussion questions

1) What may be the consequences of observed toxic effects (at biochemical/individual level) at higher levels of biological organization (population/community/ecosystem) ? Are there some direct evidences from the field studies that pharmaceuticals may have such effects?

2) What is author's opinion on the use of biomarkers such as TBARS or Carbonylated proteins in the field? Can they be used/applied to monitor e.g. for pharmaceutical effects ? What is their selectivity in response to different chemical classes ?

4. Overall evaluation

Submitted work clearly demonstrate excellent expertise, research potential, creativity and independence of Zhi-Hua Li. I recommend the thesis for the defence at the Examination Committee of the University of South Bohemia, and after successful defence, candidate can be awarded by "PhD" degree according to current legislation.


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

Brno 31.5.2011
.....
Date and place

BLAHA 
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Surname and signature