Fakulta rybářství
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

First name(s), surname, titles of the PhD student: Viktoriia Burkina, M.Sc.	**First name(s), surname, titles of supervisor:** Assoc. Prof. Dipl.-Ing. Vladimír Žlábek, Ph.D.
Title of PhD thesis: Pharmaceuticals in the aquatic environment and their effects in fish	

REVIEWER:

Surname: Fick	**Institution**: Umea University SE-901 87 Umea KBC 6A, Linnaeus väg 6 KB6A11 Sweden
Name: Jerker	
Titles: Dr.	**E-mail**: jerker.fick@chem.umu.se
Please describe your professional relationship to the PhD student: I have no professional relationship with the PhD student, no collaboration projects or publications.	**Please describe your field of expertise:** Pharmaceuticals in the environment, analytical chemistry

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)**:

This thesis adresses an important topic that have received increasing intrest recent years, i.e. potential environmental impact of pharmaceuticals. The thesis is based on 6 articles, three published and three submitted on the PhD student is first author on four of these. These articles adress both bioconcentration and effects of various pharmaceuticals and personal care products.

Relevant methods have been used in these studies, both in the analytical determination and in the measurement of biomarkers. Obtained results are novel and provide needed knowledge about the fate and effects of the pharmaceuticals and personal care products included in the studies. These studies are based on a solid scientific basis, e.g. bioconcentration experiments are based on exceptional long exposure times (up to 42 days), and these studies stand-out in a international context and are close to state-of-the-art in the field.

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, logicality 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 thesis is structured in a logical and comprehensive way and reads well. The chapters general introduction and general discussion are appropriate and covers all necessary topics. Experiments and sampling were conducted using appropriate methods and the analyses were made with a good analytical protocol. Result and discussion sections in the articles reads well, especially the presentation and interpretation of the data in the discussion. The results and the discussion correspond clearly to objectives of the PhD thesis.

References are relevant and cover the addressed field and the abstract provides a clear and to-the-point version of the results and methodology.

All in all, this thesis is based on experimental data obtained with a reproducible and robust methods and are presented in a clear and concise manner. These findings will advance the field of pharmaceuticals in the environment and add more knowledge about the effects of these pollutants.

For a detailed evaluation of the individual publications please see below.

OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:
I consider this to be a strong thesis, based on several vital publications that will have a strong impact on the research in this field. This thesis increases the knowledge regarding the presence and effects of pharmaceuticals in the environment. I strongly recommend this thesis for defence.

A short evaluation of the included articles is presented below;

Article 1. The sub-lethal effects and bioconcentration of the human pharmaceutical atenolol in rainbow trout (*Oncorhynchus mykiss*)

This article focuses on Atenolol and includes three exposure concentrations and exceptionally long exposure times. Relevant number of replicates were used (n=9, in each group). This article presents novel results both on bioconcentration in different compartments and effects, measured with selected biomarkers and histopathology. An extensive study that is novel and

corresponds well with the objectives of the PhD thesis.

Article 2. Verapamil does not modify catalytic activity of CYP450 in rainbow trout after long-term exposure

This article focuses on Verapamil and also includes three exposure concentrations and exceptionally long exposure times. Relevant number of replicates were used (n=10 in each group). This article presents novel, even though they are negative, results about selected biomarkers which corresponds well with the objectives of the PhD thesis.

Article 3. Presence of UV filters in surface water and the effects of phenylbenzimidazole sulfonic acid on rainbow trout (*Oncorhynchus mykiss*) following a chronic toxicity test

This article focuses on three UV-filters, an important group of personal care products. This article includes both a screening and experimental exposure. Again three exposure concentrations and exceptionally long exposure times. Relevant number of replicates were used (n=8 in each group). This article presents the occurrence of UV-filters in the Czech republic and also presents interesting, but difficult to interpret, effect data at relevant exposure concentrations.

Article 4. Clotrimazole in rainbow trout I: distribution, bioaccumulation, depuration and response of cytochrome system after long-term exposure

Article 5. Clotrimazole in rainbow trout II: biological and structural effects after long-term Exposure

Article 6. Clotrimazole, but not dexamethasone, is a potent in vitro inhibitor of cytochrome P450 isoforms CYP1A and CYP3A in rainbow trout.

These articles focus on the fungicide Clotrimazole and provide a comprehensive overview of the fate and effect of this pharmaceutical. Again three exposure concentrations and exceptionally long exposure times. Relevant number of replicates were used in articles 4 and 5 (n=12 respectively n=8, in each group), however a somewhat limited experimental set-up was used in article 6 (n=3). These extensive studies that include partioning and depuration, as well as measurements of biomarker and histological investigations are comprehensive and correspond well with the objectives of the PhD thesis.

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FINAL RECOMMENDATION

X PhD Thesis can be recommended for defence

☐ **PhD Thesis can be recommended with reservations for defence**

☐ **PhD Thesis can not be recommended for defence**

Umeå 23/6 2014

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Date and place Jerker Fick

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First name(s), surname, titles of the PhD student: Viktoriia Burkina, M.Sc.	**First name(s), surname, titles of supervisor:** Assoc. Prof. Dipl.-Ing. Vladimír Žlábek, Ph.D.
Title of PhD thesis: Pharmaceuticals in the aquatic environment and their effects in fish	

REVIEWER:

Surname: Hilscherová	**Institution:** Centrum pro výzkum toxických látek v prostředí Masarykova univerzita Kamenice 753/5, pavilon A29 625 00 Brno
Name: Klára	
Titles: Mgr., Ph.D.	**E-mail:** hilscherova@recetox.muni.cz
Please describe your professional relationship to the PhD student: none	**Please describe your field of expertise:** Ecotoxicology, environmental 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 dissertation thesis submitted for PhD defence of Burkina Viktoriia focuses on the study of the effects of several representatives of pharmaceuticals and personal care products (PPCPs) that belong among important emerging pollutants. The topic of the submitted work is very relevant to the field of environmental toxicology. The author has evaluated potential effects of PPCPs that can contaminate aquatic ecosystems on fish, which is an important issue widely discussed in recent scientific literature. The studies included in the thesis are of very good quality and bring important new knowledge. Combination of different methods including study of biomarkers, histopathological investigation and chemical analysis have been used to provide very comprehensive outlook into the uptake and effects of studied pharmaceuticals in fish. The results are original and significantly contribute to our understanding of effects of selected pharmaceuticals atenolol, verapamil, dexamethasone and clotrimazol as well as UV filter 2-phenylbenzimidazole-5-sulfonic acid (PBSA) on fish. The quantity of actual work and quality of science in the thesis is at very good level. The research presented in the thesis corresponds to the current state of the art in the field of aquatic eco/toxicology and contribute important information to the toxicological profiles of studied PPCPs.

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, logicality 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 thesis has been submitted as a commented compilation of previously published/submitted papers. It is logically structured into 3 chapters. First is the general introduction, second chapter consists of the research papers and the third chapter contains the discussion and conclusions, summary, list of publications and CV. Burkina Viktoriia is the first author in two published articles. She is also the first author on two papers submitted for publication and contributed to one published and one submitted paper. The papers were published in well established international journals (Chemosphere, Ecotoxicology and Environmental Safety) which confirms the quality of the conducted research since they had to undergo a scientific review by international experts prior to publication. I appreciate that the actual role (and contribution) of the candidate in preparation of the papers is clearly defined. Five of the papers are based on *in vivo* studies, one uses *in vitro* approach. The general exposure design was similar in all *in vivo* studies, exposing juvenile fish for 42 days to various PPCPs. The set of studied endpoints was adapted according to the specific goals of each study. They always included selected set of biomarkers, some studies included also chemical analysis and histopathology. No mortality was observed up to the highest tested concentrations for any of the tested PPCPs, which is also very important and relevant information for their risk assessment.

I miss clear specification of the overall aims of the whole thesis possibly broken down to specific aims. There is a subchapter Direction of the present study, which summarizes what has been done within the thesis, but specification of the aims would be useful to evaluate if the aims of the research have been fulfilled.

OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

The first part of the thesis introduces the studied compounds, their environmental concentrations, mechanism of action, their known effects on fish. It also includes subchapters focused on different types of biomarkers that could be affected by PPCPs. The Introductory part includes about 150 references which indicates good orientation in the field of study. The second chapter formed by the compilation of the published or submitted papers is of the best quality compared to the other parts. Third chapter contains on five pages General Discussion and Conclusions synthesizing and discussing all major findings presented in individual papers (cited 50 references).

List of publications demonstrates that next to the mentioned papers author also contributed to 13 posters/presentations at conferences (5 of them international, 4 contributions as the fist author). The listed Training Plan demonstrates that author has international research experience, since she has realized several research stays abroad during her PhD studies - in the Department of Food Science, Swedish University of Agricultural Sciences, Uppsala, Sweden (total 1 month) and in CESAM & Department of Biology, University of Aveiro, Portugal (total 3 months).

The thesis is submitted in English. The English in the published papers is of a very good quality. Unfortunately, the parts that have not been previously published or submitted for publication suffer many

problems with English grammar, which undermine the good scientific quality of the thesis. Author should have devoted more attention to the finalization of the thesis to bring the formal quality up to the quality of presented science. The grammar and typo errors namely in the Chapter 1 and 3 unfortunately in some sentences make the text difficult to understand.

Specific comments

1. Probably it is not necessary to include overlapping sets of references in the thesis. Of course each paper has its own set of references, but besides the papers there could probably be just one general set of references for the whole thesis at its end and not individual sets of references after chapters 1 and 3 which overlap in many references.
2. List of Abbreviations would be useful. Abbreviations should be used consistently throughout the thesis e.g. WWTP (text in Introduction) vs. STP (Table 1).
3. Numerous grammar and typo errors which complicate the understandability of the text occur in Chapters 1 and 3. These include incorrect word order, repeating or missing words, unclear/strange wording, problems with using prepositions, verb forms and tenses, non separated words, inconsistency in spelling (e.g. defence x defense, hematological x heamatological). Number of these mistakes could be corrected just by using spell check. There are even repeating mistakes in the names of institution that the author wants to receive the PhD from.
4. The curriculum vitae is kind of brief with several grammar mistakes including two in the name of institution where the student is applying for the PhD title.
5. In Table 1 on p. 12-13 there is unclear continuation of the table on top of the p. 13. Decimal points should be used in all numbers. Why is there sometimes below LOQ, sometimes N.D. in this table?
6. on p. 16 you write about "CYP19, involved in aromatase synthesis". This is incorrect and should be rewritten.
7. The unit in column Duration in Table 3 should be united to days.

General comments and discussion questions

After reading this interesting thesis I would like to raise several questions for further discussion:

1. What is the current status of the three papers submitted for publication?
2. on p.11 you write about situation with pharmaceuticals consumption specifically in India and USA. What is the situation in the Czech Republic?
3. Why were specifically juvenile rainbow trout and 21 and 42 days exposure selected for the experiments?
4. In the experiments juvenile fish were used which are randomly allocated into treatment and control groups. Could sex of the fish affect any of the studied parameters, e.g. could there be any sex-related differences? How was this taken into account?
5. In the paper Grabicova et al. (2013) you have focused on UV filters. The results demonstrated that

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swimming people using sunscreen introduce these compounds into waters. What can be the half-life of the studied compounds in aquatic environment in summer?

6. Much greater activity in MROD and also EROD were observed in control groups at 42 days compared to 21 days in the paper Grabicova et al. (2013). No such difference (Burkina et al. 2012) or actually an opposite trend with greater EROD activity at 21days (Burkina et al. submitted) was observed in your other studies. What could be the reason for the differences among the trends of EROD/MROD activities in controls between 21 and 42 days in your studies using juvenile rainbow trout from the same hatchery?

7. In paper Burkina et al. (2012) you investigate the possibility of using CYP450 enzymes as biomarkers to monitor verapamil contamination in aquatic environment. In general, what is the specificity of the biomarkers used in your studies for studied pharmaceuticals or PPCPs in general? Could any of them be used as tools in monitoring of PPCPs?

Overall evaluation

In conclusion, Burkina Viktoria succeeded through her research work on several published and submitted studies in demonstrating good expertise, research potential, creativity and independence. Her work made significant contribution to the advancement of the field of understanding of the effects of PPCPs on fish. Unfortunately the problems with English language in important parts of the thesis undermine the good scientific quality of the thesis and thus should be corrected prior to final publication and defence of the thesis. After these language problems are sorted out I can without any reservation recommend the acceptance of this dissertation for the defence at the Examination Committee of the University of South Bohemia as part of the fulfilment of requirements to obtain the title of Doctor of Sciences. After successful defence, candidate can be awarded PhD degree according to current legislation.

FINAL RECOMMENDATION

☐ **PhD Thesis can be recommended for defence**
☒ **PhD Thesis can be recommended with reservations for defence**
- can be recommended after the problems with the English language are corrected
☐ **PhD Thesis can not be recommended for defence**

16/6/2014
Date and place

Hilscherova
KLÁRA HILSCHEROVÁ
Name and signature