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: Kateřina Grabicová, B.Sc., M.Sc.	**First name(s), surname, titles of supervisor:** Assoc. Prof. Dipl.-Ing. Tomáš Randák, Ph.D.
Title of PhD thesis: Effects of chemicals present in sewage treatment plants´ effluents on fish	

REVIEWER:

Surname: Cvačka	**Institution**: Ústav organické chemie a biochemie AVČR, v.v.i. Flemingovo nám. 2 166 10 Praha 6
Name: Josef	
Titles: Assoc. Prof. RNDr., Ph.D.	**E-mail**: cvacka@uochb.cas.cz
Please describe your professional relationship to the PhD student: none	**Please describe your field of expertise:** analytical chemistry, mass spectrometry, chromatography

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 work summarized in the PhD thesis of Kateřina Grabicová is an important contribution to the research on the chemicals released by sewage treatment plants and their effects on fish. Numerous pharmaceutical compounds are known to pass through STPs and contaminate aquatic environment. The risks associated with the presence of pharmaceuticals are not fully understood. Unlike to pesticides the consumption of pharmaceuticals cannot to be easily restricted because of their beneficial health effects. The work of Kateřina Grabicová brings new data on the concentration of selected pharmaceuticals and illicit drugs in aquatic environment in the Czech Republic, shows their presence in benthic fauna and evaluates concentrations of antidepressants in various tissues of fish. The work represents high quality research supported by reliable data. It is undoubtedly scientifically sound work with clear results and conclusions, which is also evident from high-quality journals where the papers appeared.

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)**:

PhD thesis of Kateřina Grabicová is a compilation consisting of short introduction based on more than 100 references, list of specific aims, scholarly publications, general discussions and summary in English and Czech language, followed by a list of publications, training plan and CV of the author. There are five publications included; two published papers, two either accepted or in press papers and one unpublished manuscript. I would appreciate a short summary of the specific contributions of the student to these papers. The overall level of elaboration of the PhD thesis meets the academic standards and requirements for a concise style of thesis. The text is structured logically. The approaches used are mostly based on state of the art methods, often analytical mass spectrometry. The publications were published or accepted in high quality international peer-reviewed journals (Sci. Total Environ., IF=3.26; Ecotox. Environ. Safe, IF=2.20). The results in published papers correspond to the objectives of the PhD thesis.

OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

This work brings new and interesting data on the pharmaceuticals and illicit drugs in the aquatic environment of the Czech Republic (and Sweden in part). Pharmaceuticals were studied in water samples and aquatic fauna. Their effects on fish were investigated mostly in the areas of municipal STP effluents and also in other areas. The research does not encompass only studies of various compounds in fish, their organs and food sources, but also acute toxicity tests like lethal concentrations and effects on fish heart rate or enzyme assays. The work also touches biology of aquatic organism, e.g., brings an interesting information on the preference of *Erpobdella* leeches to polluted sites. Behind the data and findings reported in the PhD thesis stand advanced methods of sampling and analyzing water and tissue samples. As the expertise of the reviewer is mostly in the field of analytical chemistry, this review is mostly focused on these aspects of the work.

As regards analytical methodology, the concentrations of the analytes were determined by high performance liquid chromatography coupled to mass spectrometry. Two approaches of quantitative analysis were used in this work; quantification based on MS/MS in a triple quadrupole instrument and quantification based on exact masses in orbitrap. Both methods are commonly accepted as a proper way of quantitative analysis. They were used for analyzing few compounds (e.g., UV filters project) or for large-scale measurement of up to 70 compounds (benthic fauna project). To achieve meaningful results the samples have to be

sampled and treated correctly. In this work highly sophisticated sampling approaches were used, e.g., Polar Organic Chemical Integrative Samplers (POCIS) which requires proper calibration. I appreciated high level of quality control and validation of the experimental conditions. For instance, the concentrations of the tested chemicals in the experimental aquaria were not only calculated from the added amount of the chemicals, but aquarium water was regularly sampled and subjected to quantitative analysis. The analytical methods were properly validated. The quality of data was assured by blank samples, duplicates, fortified samples and matrix-matched standards. The results were subjected to relevant statistical treatment. Data shown are based on solid analytical chemistry and reflect correct concentrations in the samples investigated.

I have few remarks to this work. I am a bit puzzled how the internal standards (IS) were selected for the particular analytical methods. IS usually have structures and properties very similar to the analyte in order to efficiently compensate matrix effects. The applicant used diltiazem as an IS for verapamil, but structures of these two compounds cannot be considered similar. A short explanation of the I.S. selection strategy would be helpful. I was also surprised by several details in analytical methodology, which could perhaps be also commented. In the verapamil study the dried fish samples were stored at 20 °C before analysis; substantially lower temperatures are usually used to avoid decomposition. I was also curious about the stability of the analytes in the samples allowed to evaporate the solvent overnight, presumably at room temperature (UV filters project). The composition of a solvent used for final preparation of samples for LC/MS (5% acetonitrile in water) seems to me too much aqueous for efficient dissolution of the analytes (project on tissue-specific bioconcentrations of antidepressants). The last remark concerns metabolism of pharmaceuticals in fish. The experiments targeted only pharmaceuticals, but not their metabolites, which are formed by fish organisms. Analyzing the metabolites should be feasible with the instrumentation available and the results would help us to better understand fish metabolism and fate of the pharmaceuticals in the aquatic environments.

In conclusion, the work of Kateřina Grabicová is a valuable contribution to our knowledge on the pharmaceuticals in aquatic ecosystems. The results were published in reputable scientific journal. The applicant adequately demonstrated her readiness for an independent scientific work.

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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**

Prague, June 19, 2014 Cvacka

…… ……

Date and place Name and signature

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Title of PhD thesis: Effects of chemicals present in sewage treatment plants´ effluents on fish	

REVIEWER:

Surname: Moazzami	**Institution:** Swedish University of Agricultural Sciences P.O. Box 7015 SE-750 07 Uppsala SWEDEN
Name: Ali	
Titles: Assoc. Prof., Ph.D.	**E-mail**: ali.moazzami@slu.se
Please describe your professional relationship to the PhD student: There is no professional relation.	**Please describe your field of expertise:** Analytical chemistry, metabolomics, food and environmental analysis.

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 thesis has addressed major environmental problems with regard to pharmaceutical and personal care products (PPCPs). The findings are of both scientific and practical importance. The work is original and benefices from robust experimental procedures. The data presented is original.

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 findings presented are in coherence with objectives defined in the thesis. The design of the experiments and their order of presentation are logical. The thesis is using well established

methodologies which are relevant for the experiments objectives.

OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages: The defendant in the present thesis has investigated the biological effects and presence of the environmental pollutants associated with pharmaceuticals and personal care products (PPCPs) using robust and well established methodologies.
The worked has added novel and important pieces to the present knowledge regarding PPCPs. In addition, the work has generated new knowledge which can be used in policy making. The thesis includes a comprehensive and clear literature review. However, this part could have been improved by further explanation/motivation regarding the choice of pollutants investigated. The analytical approach is robust and includes enough biological and analytical replicates. The analytical approach includes the application of internal standard and the report of method specification where it is necessary. The statistical approach is also robust and reliable. The thesis includes four accepted/published manuscripts and one manuscript which are sufficiently covering the original objective of the thesis. Overall, I consider the present work with regard to international competitiveness of the state-of-the-art within the top 20%.
The overall design of experiment and addressing to the problems defined in introduction are logical and in right order.
The general discussion is clear and in logical order. In addition the defendant has presented the limitation of her work clearly and critically. However, the future prospective presented is very limited.

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**

20140623
Date and place

Name and signature