

Confidential

Review of USB FFPW PhD Thesis

First name(s), surname, titles of the PhD student: Christoph Steinbach, Dipl.-Biol.	First name(s), surname, titles of supervisor: Dipl.-Ing. Hana Kocour Kroupová, Ph.D.
Title of PhD thesis: Effect to selected cardiovascular pharmaceuticals found in aquatic environment on fish	
REVIEWER:	
Surname: Dobšíková	Institution: VFU Brno Palackého tř. 1/3 612 42 Brno
Name: Radka	E-mail: dobsikovar@vfu.cz
Titles: MVDr., Ph.D.	
Please describe your professional relationship to the PhD student: PhD thesis opponent	Please describe your field of expertise: aquatic 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**):

PhD thesis of Christoph Steinbach, Dip.-Biol., focused on the effect of selected cardiovascular pharmaceuticals found in aquatic environment on fish is very important as pharmaceuticals are increasingly used in human and veterinary care and thus found in higher concentrations in aquatic environment. In fish, in most cases non-target organisms, residues of drugs in water can affect various metabolic pathways and act as toxicants rather than therapeutic agents. That is why the PhD thesis, aimed to evaluate the effect of selected group of cardioactive pharmaceuticals, is of very scientific importance in national as well as international context.

The PhD thesis is focused on the acute and sub-chronic effects, bioconcentration, half-time and metabolism of verapamil, diltiazem and atenolol in selected fish species.

The results indicate the negative effects of even environmental concentrations of the drugs on a non-target organism.



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 PhD thesis text is comprehensibly structured as a compilation of seven chapters; chapter 1 – General Introduction to the problematics of cardiovascular drugs divided into subchapters focused on beta-blockers and calcium channel blockers with some general conclusions and the outlines and aims of the thesis.

Chapter 2 and 3 are created of two peer-reviewed articles named Toxic effects, bioconcentration and depuration of verapamil in the early life stages of common carp (*Cyprinus carpio*) and The sub-lethal effects and tissue concentration of the human pharmaceutical atenolol in rainbow trout (*Oncorhynchus mykiss*) both published in Science of the Total Environment.

Next three chapters contain manuscripts of articles focused on the effect of diltiazem on rainbow trout, diltiazem metabolit pathways in fish and a proposal for the assessment of histopathological alterations of the cardiovascular system in fish.

Chapter 7 consists of general discussion, English and Czech summaries and other formal requirements for PhD thesis.

The structure of the PhD thesis is intelligible and logically divided. Author's scientific approach to solve the aims of the work is very professional. Results of the thesis correspond to the objectives formulated as the aims of the thesis.

OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

Chapter 1:

- General Introduction:

1.1 Cardiovascular pharmaceuticals: more recent information on pharmaceutical use, sales, approvals, uptake would be convenient (in the study, years 2000, 2004 are mentioned); 2nd paragraph - not only a long-term exposure is largely unknown, but also synergistic/antagonistic effects of various drug mixtures present in aquatic environment are scarcely studied;

1.1.1 Beta-blockers: Conclusion: not true that „Acute toxicity of beta-blockers is very low“, as e.g. LC50 of propranolol for the fish is even less than 10 mg/l – i.e. propranolol is an acutely toxic substance.

1.1.4 The outlines and aims of the thesis: well described and logically structured to the chapters

Chapter 2 and 3:

- no comments as the articles are peer-reviewed and published in IF journal (Science of the Total Environment)

- importance: atenolol is not metabolised in rainbow trout and environmentally relevant concentration of the drug leads to changes in haematological and biochemical profile in the fish species tested; verapamil appeared to bioconcentrate at a low level and its environmental levels caused no effects on studied endpoints in common carp.



Chapter 4:

- the objective was to assess the effect of sub-chronic exposure to diltiazem on rainbow trout. At an environmentally relevant concentration, diltiazem exposure led to altered activity of antioxidant enzymes in the liver and gill, to the elevation of TBARS level in the gills as well as to the increase in CK activity in plasma.
- a question: could author explain the differences between the SOD, GR, and CAT activities in liver tissue of control rainbow trout in the article manuscript and the IF article „The sub-lethal effects and tissue concentration of the human pharmaceutical atenolol in rainbow trout (*Oncorhynchus mykiss*)“ published in Sci Tot Envi? There are differences „in orders“ (for example: 3 $\mu\text{mol}/\text{min}/\text{mg}$ versus 0.24/0.09 $\mu\text{mol}/\text{min}/\text{mg}$ for SOD, 4 $\mu\text{mol}/\text{min}/\text{mg}$ versus 0.04 $\mu\text{mol}/\text{min}/\text{mg}$ for CAT).

Chapter 5:

- a rapid and sensitive LC-HRMS method for the measurement of diltiazem metabolites in fish tissues was developed using hybrid quadrupole-orbital trap mass spectrometer.
- a question: why no standards were required for the identification of diltiazem metabolites in fish tissues?
- a question: what part of the analytical method/article was processed by the PhD thesis student?

Chapter 6:

- a method for a standardised assessment and evaluation of histopathological alterations in the fish heart (modelled on rainbow trout) was developed and heart tissues of other orders fish species were used for the testing the adaptability of the protocol.
- necessary to review the text: several linguistic/formal mistakes (e.g. Fig. 1: *Oncorhynchus myciss*)
- a question: on the basis of what the author fixed the importance factor (I) of the parameters monitored?
- a question: is atenolol itself the representative drug for the evaluation of the changes in heart tissue?
- explain the abbreviation of CFWH in „The heart protocol...“, the paragraph not aligned to „block“.

Chapter 7:

- studied cardioactive drugs (atenolol, diltiazem, and verapamil) do not intensively bioaccumulate in fish; environmental levels of atenolol and diltiazem could have negative effects in fish (verapamil not);
- very beneficial general discussion and erudite commentaries and explanations of the topic studied.

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

Brno, 6/12/2015
Date and place


MVDr. Radka Dobšíková, Ph.D.
Name and signature



Fakulta rybnářství
a ochrany vod
Faculty of Fisheries
and Protection
of Waters

Jihočeská univerzita
v Českých Budějovicích
University of South Bohemia
in České Budějovice
Czech Republic

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Title of PhD thesis: Effect to selected cardiovascular pharmaceuticals found in aquatic environment on fish	
REVIEWER:	
Surname: Kloas	Institution: Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB), Dep 5 - Ecophysiology and Aquaculture, Müggelseedamm 310, 12587 Berlin, Germany
Name: Werner	E-mail: werner.kloas@igb-berlin.de
Titles: Prof. Dr.	
Please describe your professional relationship to the PhD student: none	Please describe your field of expertise: Ecotoxicology, endocrinology, aquaculture

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 PhD thesis of Christoph Steinbach contains very original research being of great importance for ecotoxicologists investigating the potential impacts of pharmaceuticals on aquatic organisms e.g. fishes. He performed ground breaking studies with the cardiovascular pharmaceuticals verapamil, diltiazem and atenolol on fishes, which is of great importance as basic studies for applied research because Christoph Steinbach investigated also various endpoints of acute and sub-chronic effects. The thesis presented due to its content fulfills fully requirements of international peer-reviewed journals and thus can be seen as a marked contribution to international research for ecotoxicological risk assessment concerning an important threat for our aquatic ecosystems presented by pharmaceuticals.



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 PhD thesis of Christoph Steinbach is very well structured, starting with an informative general introduction followed as a cumulative thesis by five articles and ending with a general discussion including also a respective summary. The overall thesis is presented in a technically very sound English and the aims of the study have been achieved by a very logic approach concerning methodology, endpoints, and experimental designs. It has to be acknowledged that Christoph Steinbach used several important methods to approach successfully the overall goals in a very broad manner. The resulting publications are in highly recommended peer-reviewed journals concerning ecotoxicology and the results obtained correspond very well to the objectives of the PhD thesis.

OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

The PhD thesis of Christoph Steinbach represents very original research dealing with cardiovascular pharmaceuticals being a potential threat for aquatic organisms. The pharmaceuticals chosen, verapamil, diltiazem, and atenolol, are used for humans to regulate the cardiovascular system. Therefore also in fishes, comparable effects can be expected. The knowledge about effects of these compounds on various fishes has been rather limited and the thesis presented unravels many uncertainties concerning ecotoxicological modes of action. The thesis is presented as a cumulative work of high quality and contains five original articles in well recognized international journals.

The overall organization of the thesis written in sound English is clear and logic and the general introduction is providing an excellent information about the appearance, significance, and effects of pharmaceuticals in aquatic ecosystems. The objectives of the thesis are clearly introduced and the arrangement of the publications is very well done. The methods applied are very well chosen to approach the objectives adequately. It is noteworthy that Christoph Steinbach applied a very broad range of methods from classical histology over biochemistry



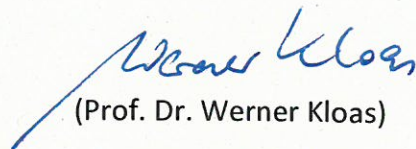
to detailed chemical analytical assessment, which has to be appreciated. The general discussion covers in an excellent manner the results obtained and are providing a good comprehensive knowledge with sound conclusions including up-to-date references. Therefore the presented thesis by Christoph Steinbach in summary is an excellent presentation of sound ecotoxicological research and provides very new insights into the effects of cardiovascular pharmaceuticals on fishes. The presentation is arranged in a scientifically sound way and the publications put forward represent original research at an internationally competitive level. That excellent research adds important knowledge concerning ecotoxicological effects for the international scientific community and thus I can only highly recommend the PhD thesis by Christoph Steinbach without any reservations for defence.

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

Berlin, 14th June 2015

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Date and place


(Prof. Dr. Werner Kloas)
.....
Name and signature