



Confidential

Review of USB FFPW PhD Thesis

First name(s), surname, titles of the PhD student: Sidika Sakalli, M.Sc.	First name(s), surname, titles of supervisor: Assoc. Prof. Dipl.-Ing. Vladimír Žlábek, Ph.D.
Title of PhD thesis: Bioactive compounds in the aquatic environment and their effects on fish – a special focus on piscine cytochrome P450	

REVIEWER:

Surname: Gang	Institution: Institute of Quality Standards and Testing Technology for Agro-products Chinese Academy of Agricultural Sciences (CAAS) Address: 12, South Street of ZhongGuanCun, Beijing 100081, P.R.China
Name: Chen	E-mail: gang_chen2012@126.com
Titles: Dr.	
Please describe your professional relationship to the PhD student: The student carried on research about contaminants metabolism via CYP450 enzymes and AhR receptor, which is close to my research area about aryl hydrocarbon compounds metabolism and physiological function.	Please describe your field of expertise: In food safety and toxicology. Especially on chemical contaminants analysis and toxicological mechanism study.

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 studied the synergistic effect of bioactive compounds with chemical contaminants to the fish CYP enzymes system. The study included the comparison of in vitro, in vivo, and in-situ experiment. Before studying, the influence of organic solvents to enzymatic activity was validated. In general, the originality of these studies is new, and the scientific importance is good. The author already has had very good publication in peer reviewed journal. The structure of the manuscript is logic and clear, and the results are convincing. Competitiveness of this thesis can reach top level in the toxicological research 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, 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 logic of this manuscript is clear. The author first evaluated the carrier solvent to the enzyme activity, and determined the optimal condition for further enzyme incubation. Then the author designed in vitro, in vivo, and In-situ experiment, to study the influence of chemical contaminants to CYP enzyme system. The effect of phytochemicals under the interference by contaminants to the enzyme activity was also studied. The topic of synergistic effect of chemical contaminants to carp is new and meaningful to the fish industry. The selected chemicals have not been studied before. The results and the experimental design are convincing. The novel finding in the thesis gives scientific evidence about the mechanism how chemical contaminants affect fish. The publications are good in relevant field, and the topics are closely related to the main sketch of the thesis. The English writing is clear.

OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

As mentioned above, the overall evaluation of the thesis is very good, and reached top level in relevant research field. The topic for studying chemical contaminants influence to fish via the aquatic environment is important and meaningful. The structure of the thesis is logic and the writing is clear. I have the following thinking to the author.

1. The thesis has studied the enzymatic influence of chemical contaminants to CYP enzymes comprehensively. There are still other important topics that the study should be carried on in the future. E.g. the residue levels of chemical contaminants in fish, which is directly related to the safety of fish products. Since the fish is related to human consumption, the risk assessment should be taken. The ADI (average daily intake) can be obtained, MRL (maximum residue limit) can be calculated, this is to ensure the residue of certain chemical will not threat human health.

2. The CYP enzymes play key role in metabolism and elimination of chemical contaminants. The key CYP type which is related to certain contaminants metabolism is worth to be identified. The information of toxic effect of contaminants e.g. carcinogenic, endocrine disruption, etc. is also of concern. The reaction of related enzymes other than CYP enzymes after exposure to contaminants is also worth to be 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

30-June-2018
.....
BEIJING, CHINA
Date and place

Gang Chen GANG CHEN
.....
Name and signature



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Jihočeská univerzita
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Title of PhD thesis: Bioactive compounds in the aquatic environment and their effects on fish – a special focus on piscine cytochrome P450	
REVIEWER:	
Surname: Hilscherová	Institution: Masarykova univerzita Brno
Name: Klára	
Titles: Assoc. Prof. Mgr., Ph.D.	E-mail: hilscherova@recetox.muni.cz
Please describe your professional relationship to the PhD student: no direct relation	Please describe your field of expertise: ecotoxicology and toxicology, environmental 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):

The submitted thesis studies an important environmental problem related to the wide spread exposure of fish populations to pharmaceuticals from sewage treatment plants, also in potential co-exposure to natural compounds. It specifically focuses on their impact on metabolism, namely cytochrome P450 enzymes. The research included in the thesis is based on the current state of knowledge and brings novel findings of scientific importance. The whole thesis is built around five original papers published in quality international journals, which present five studies using in vitro, in vivo and in situ approaches. The quality of the methodology of the published studies and results interpretation is high and the studies are certainly of interest for other international experts in this field. The published papers also have a good quality and understandability of English, which unfortunately is not the case for some other parts of the thesis (namely Introduction and Discussion), which is the major drawback of the submitted thesis that should be corrected for better utility of the overall thesis for international readers.



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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 thesis is written on 104 pages in English, it is logically structured into 5 chapters. First is the general introduction, second chapter consists of two research papers related to in vitro approaches, the third chapter presents two studies using in vivo approaches. Chapter 4 presents in situ study and chapter 5 contains the discussion and conclusions, summary, Training and Supervision Plan, list of publications and CV. The objectives are listed as several points at the end of the Introductory section. The published papers included in the thesis directly address the thesis objectives using in vitro, in vivo and in situ methods. The applied methodology reflects the current state of art and is sufficiently described in the papers. The comprehensibility of some parts is unfortunately complicated by problems with English grammar that need to be corrected.

OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

The thesis brings interesting novel information regarding the effects of pharmaceuticals and natural compounds on the metabolic enzymes and other endpoints in fish. The first part of the thesis (General Introduction on 5 pages) briefly introduces the studied compounds, xenobiotic metabolism, CYP enzymes and in vitro, in vivo and in situ approaches applied in the studies. The Introductory part includes over 90 references, which indicates good orientation in the field of study. Three following chapters are prepared as a compilation of five published papers, where Sidiki Sakalli is the first author on three of them. All papers were published in very good per-review international journals (e.g. Chemosphere, Science of the Total Environment), which confirms the quality of the conducted research, since they had to undergo a scientific review by international experts prior to publication.

I miss clear specification of the actual role (and contribution) of the candidate in preparation of the papers. It should be added, it is good to specify the contribution namely in case where there is an extensive research team and the student is one of e.g. 13 authors such as in the case of paper Burkina et al. 2016.

According to the list of publications, Sidika Sakalli during her PhD studies also contributed to 5 more published papers and several manuscripts in preparation that are not included in her thesis.

Last part of thesis contains on five pages General Discussion and Conclusions synthesizing and discussing major findings presented in individual papers (cited 53 references). The results of individual studies are also thoroughly discussed in each paper.

While English quality is good in the published papers, it needs improvement in the parts that were not previously published, namely in the Introduction and Discussion some sentences are hard to understand and there are some misspellings and grammatical errors and frequent mistakes in verb forms.

The listed Training Plan demonstrates that author has international research experience, since she has realized two research stays abroad during her PhD studies - in the Department for Ecosystem Analyses, Institute of Environmental Research at the RWTH Aachen University (5 months) and in Department of Molecular Sciences, Swedish University of Agricultural Sciences, Uppsala (2 months).



Specific comments:

1. The version of writing titles for the committee members and opponents on p.3 should be unified, Czech version „doc.“ (docent) equals to Assoc.Prof. in English. I am not sure about the current titles of all the committee members, but I think Tomáš Randák is a full professor and not associated professor as is written there. This should be corrected.
2. Formatting of *In vivo*, *in vitro* and *in situ* should be unified throughout the thesis, it is sometimes with hyphen, sometimes not in italics.
3. Next to the grammatical problems mentioned above, there are some incomplete sentences, some sentences even missing verbs. There are also grammatical errors (especially in the use of verbs, prepositions, conjunctions and commas) that make some sentences hard to understand, e.g. on p.9: this particularly happen when orally ingested or intestinally applied pharmaceuticals use
p.88: CYP2E1 activity in human hepatocytes inhibited more that 50% at 1% acetone
p.91: However, drug-drug or food-drug interactions observed when two compounds combined in *in vitro*.
p.89: Dexamethasone was included in the list of environmental risk assessment (Roos et al., 2012) and predicted critical environmental concentration of pharmaceuticals (Fick et al., 2010) where, clotrimazole increased the concerns of scientist due to its toxicity towards aquatic organisms...
4. Figure 1 is mixing different things together – while it lists groups of compounds of plant, animal and synthetic origin, it lists the producers of the compounds for microorganisms. It should be revised to clearly present the information in the same structure for the listed origins

Questions:

1. What do you mean by this sentence (p.4): Moreover, wide variety of plant leaves, seeds, extracts are poisonous to fish even so, it has been using as a fishing method since the ancient times.
2. On p.9 in Introduction you state: Phase I metabolism involves three reactions: oxidation, reduction and/or hydrolyses of the xenobiotics. However, this statement is incomplete. What other reactions can be part of phase 1 metabolism? The sentence should be rewritten to reflect that also other reactions can play role.
3. What do you mean by this sentence (p.10): The numbers of genes vary between and within the species. It relates to CYPs in fish, whose number certainly differs among species, but how do the numbers of genes vary within species?
4. How long are the exposures in the *in vitro* studies? In methodology for the first paper you state that you have either added the solvents simultaneously with the substrate or preincubated the microsomes with the solvents for 10-30 min. How is this design relevant for the environmental exposure situation? What trends of responses would you expect with longer exposure duration?
5. What is your interpretation of the unclear relation of the observed effects on enzymatic activities with exposure dose and duration in the *in vivo* studies reported in the two papers by Burkina et al. (2015, 2016)?
6. Can you please explain this sentence from p.88?: Because microsomes are rich in CYPs, they are commonly used for identifying xenobiotic metabolites, potential drug-drug or food-drug interactions, and as a biomarker after exposure to environmental contaminant.
7. You have often observed no enzymatic responses at longer term exposures with the interpretation that the fish adapted to the exposure. Do you think that is a general situation in STP exposed water bodies that the fish adapt to the exposure?
8. In your summary you write regarding the *in situ* study: Both, EROD and BFCOD activities were affected by the PPCPs that are present in the exposed fish. How do you know that the PPCPs were driving this effect? Could not that be some other compounds from the environment related or unrelated to the STP effluent?



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Overall evaluation

In conclusion, Sidiki Sakalli demonstrated through her research work on several published studies good expertise and research potential. Her work made significant contribution to the understanding of the effects of PPCPs and a few natural compounds on fish. Unfortunately, the problems with English language in important parts of the thesis undermine the good scientific quality of the thesis and should be corrected prior to final publication and defence of the thesis. After these language problems are solved I can 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
 PhD Thesis can not be recommended for defence

July 25, 2018, Brno

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Date and place

Klára Hilscherová

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Name and signature