



**Confidential**

### **Review of USB FFPW PhD Thesis**

<b>First name(s), surname, titles of the PhD student:</b> Latifeh Chupani, M.Sc.	<b>First name(s), surname, titles of supervisor:</b> Dipl.-Ing. Eliška Zusková, Ph.D.
<b>Title of PhD thesis:</b> Physiological and molecular responses of aquatic organisms to chemical exposure	
<b>REVIEWER:</b>	
<b>Surname:</b> Dobšíková	<b>Institution:</b> University of Veterinary and Pharmaceutical Sciences Brno, Faculty of Veterinary Hygiene and Ecology, Department of Animal Protection, Welfare and Etology
<b>Name:</b> Radka	
<b>Titles:</b> MVDr., Ph.D.	<b>E-mail:</b> dobsikovar@vfu.cz
<b>Please describe your professional relationship to the PhD student:</b> consultant and supervisor of PhD students at UVPS Brno	<b>Please describe your field of expertise:</b> toxicity tests, pesticides, fish haematology and biochemistry, biochemical markers in fish

### **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 present PhD thesis deals with a very current issue, which is the use of nanoparticles in food engineering and agriculture and their potentially adverse effects on the environment (water environment, particularly). The second part of the PhD thesis focuses on the toxicity of paracetamol on selected aquatic organisms.*

*Nanoparticles are the object of an intense scientific research as they have many potential applications in medicine, physics, electronics, and optics. On the other hand, they present a possible danger, both medically and environmentally. They can pass through cell membranes and interact with biological systems. Recent studies on ZnO nanoparticles and its effect on human immune cells have proved different levels of susceptibility to cytotoxicity. I very appreciate author's research in this relatively novel field, based on tissue accumulation and physiological responses, serum protein*

*profile and cell motility and immune system response of the intestine in major fish species of the Czech Republic aquaculture, common carp (Cyprinus carpio L.). The last two articles presented in the PhD thesis aim to evaluate the effect of peracetic acid, a strong oxidant with germicidal properties, which is used in the aquaculture as a sanitizer and an alternative biocide for malachite green and formaldehyde, on non-target water organism, grass carp (Ctenopharyngodon idella) and a signal crayfish (Pacifastacus leniusculus).*

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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 PhD thesis of Latifeh Chupani, M.Sc., evaluating the effect of ZnO nanoparticles and peracetic acid on non-target water organisms, is written in the form of commented collection of scientific articles published (or prepared for publishing/or submitted) in scientific journals with impact factor. The PhD thesis is systematically and logically divided into seven chapters comprised in total 99 pages.*

*In Chapter 1, there is a general introduction into toxicology problematics focused on the use of various biomarkers (i.e. biomarkers of exposure, susceptability, response, oxidative stress etc.) in the evaluation of adverse effects of chemical substances on an organism and a general discussion on the articles presented and their scientific conclusions. Chapter 2 contains an article (prepared for publishing, not given scientific journal title) on tissue accumulation and physiological responses of dietary zinc oxide nanoparticles (ZnO NPs) in common carp concluding that liver and kidneys may be the most sensitive to them. In Chapter 3 and 4, there are given conclusions on the effect of ZnO NPs dietary exposure on common carp juveniles serum protein profile and cell motility and immune system response of the intestine. Dietary ZnO NPs intake could affect the innate and adaptive immune systems (published in Science of the Total Environment) and induce cellular responses (disruption in cell metabolism, limited cell motility in both intestinal folds and muscular layer in treated fish) in carp intestine (not given the journal title intended to be submitted to).*

*Chapter 5 and 6 is focused on the effect of peracetid acid (PAA) on grass carp juveniles (published in*

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*Neuroendocrinology Letters*) and signal crayfish (published in *Fish and Shellfish Immunology*). In grass carp, changes in selected enzymes (AST, CK, and LDH) activities and histopathological changes in gills (fusion, club-shape of primary lamellae tips, and lifting of epithelial cells) were found, but no haematological response was detected. In signal crayfish, changes in SOD and GR activities and disorganisation of gill epithelial cells and also the alteration of hepatopancreas (considered a secondary response to PAA treatment) were found.

Last Chapter 7 comprises general discussion on all articles presented, English and Czech summary, acknowledgements, list of publications, study plan, and author's CV.

### OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

*The PhD thesis is written in the form of commented articles (published – and already 3 times cited, or prepared for the submission to scientific journals). I appreciate the PhD student to be the first author in all scientific publications enclosed. The published articles passed through a peer-review procedure in relevant scientific journals with impact factor, so no formal and scientific mistakes could be expected.*

*The PhD thesis is properly structured and clearly elaborated. List of literature in Chapter 1 comprises more than 60 references and next 41 references is in Chapter 7 – General discussion, which documents student's erudition in the field. As a formal amendment, I would recommend to add a list of abbreviations to facilitate the orientation.*

*To the present thesis, I comment several questions:*

- 1. The concentrations of NPs and PAA used in the experiments were taken from literature data or were tested preliminarily?*
- 2. Nanoparticles are generally very unstable. How was the NPs stability in feed ensured in the experiments?*
- 3. Chapter 2 (Chronic dietary toxicity of ZnO NPs in common carp: tissue accumulation and physiological responses): How can the author explain the changes in biochemical profile (AST, ALT) in the recovery period (when no changes were found during the exposure period)?*
- 4. In Chapter 3 (Evaluation of the toxic effect of peracetic acid on grass carp juveniles), in*

*Table 1, there is extra data on Danio rerio proteins with significant fold changes – but the work is focused on grass carp.*

*5. Chapter 4 (Exposure of dietary ZnO NPs in juvenile common carp affected cell motility and immune system response of the intestine): Table 1 and 3 – there are given significant (I suppose  $p < 0.05$ ) changes in proteins – but as for the protein with accession number Q803X7 and F8W216 (Table 1) and protein B3DIX3 (Table 3), there is the significance  $p > 0.05$  (i.e. 0.054, 0.080 and 0.062, respectively). Could the author explain it?*

*6. What are the „safe“ concentrations of PAA used as a disinfectant in the aquaculture? Could the author specify a therapeutic index of the substance?*

*7. What is the author's opinion on the use of NPs in food industry, agriculture, physics, and in other fields in the context of loading of the environment with relatively poorly tested nanomaterials?*

*8. What is the student's share in individual publications?*


*On the basis of detailed study of the present work, I state that the PhD thesis of Latifeh Chupani, M.Sc., meets all requirements for scientific work. Thesis is elaborated at high professional level and testifies the scientific erudition of the author.*

***In conclusion, I recommend to accept the present PhD thesis for defence and, after successful defence, to award Latifeh Chupani, M.Sc., Ph.D. title.***

## **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

In Brno, June 12th 2017  
Date and place

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



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**Review of USB FFPW PhD Thesis**

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<b>Title of PhD thesis:</b> Physiological and molecular responses of aquatic organisms to chemical exposure	
<b>REVIEWER:</b>	
<b>Surname:</b> Gomulka	<b>Institution:</b> Department of Ichthyology, University of Warmia and Mazury, Olsztyn, Poland
<b>Name:</b> Piotr	
<b>Titles:</b> Dr.	<b>E-mail:</b> pgomulka@uwm.edu.pl
<b>Please describe your professional relationship to the PhD student:</b> No professional relationship between Latifeh Chupani and me exist	<b>Please describe your field of expertise:</b> My field of expertise includes toxicology and diseases of aquatic animals with particular emphasis on the toxicity of therapeutic agents, pesticides and metabolites

**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 refers to a very important issue of fish toxicology, which is the safety of the use of new chemical compounds in aquatic animals. The PhD Candidate has chosen two substances; zinc oxide nanoparticles (ZnO Nps) and peracetic acid (PAA), as examples of new threats, environmental and iatrogenic respectively, which can affect fish. Both compounds were intensively studied in the last decade (according to Scopus, between 2006 and 2016, 3638 and 1331 papers referred to Zn NPs and PAA respectively), however, the number of papers referred to fish was very limited in the same period (25 and 38 papers referred to ZnO NPs and PAA respectively). Moreover, I could not find any publication regarding ZnO NPs where proteomic methods were used. I would like to stress that Chapters 3 and 4 of the Thesis, which regards to changes in carp proteome, are the most valuable part of the Thesis in my opinion. In the conclusions of Chapter 3, The PhD Candidate, based on results of her research, hypothesizes that prolonged exposure to ZnO NPs can affect innate and adaptive immunity of common carp. It is especially important in the context of growing usage of ZnO NPs and following environmental pollution. This hypothesis should be subjected to comprehensive study. In the manuscript which consist on Chapter 4, Latifeh Chupani concluded that dietary ZnO NPs exposure affects immune response, disrupts cell metabolism, limit cell motility in fish intestine and induces increased cell apoptosis. Moreover, authors found increased levels of proteins associated with

cancerous cell survival. Several pathways of above impacts are proposed and discussed. Each of these hypotheses is interesting and needs further investigations.

The research addressed to PAA toxicity has more practical sound as in conclusions two therapeutic concentrations of PAA are proposed,  $1 \text{ mg L}^{-1}$  and  $10 \text{ mg L}^{-1}$  for grass carp and signal crayfish respectively. In both cases, PhD Candidate stressed that PAA affected antioxidant system of treated animals and caused some pathological changes in gills, however, in her opinion, these alterations are reversible and PAA can be recommended for treatment against fish and crayfish parasites.

In summary the Thesis of Latifeh Chupani is an original contribution with high enough scientific value to be promoted to a PhD in fisheries sciences. The Thesis brings new important data regarding mechanism of toxic action of ZnO NPs and PAA which will certainly be the source of new scientific hypotheses, having a direct impact on the development of knowledge in field of fish toxicology.

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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  $\frac{1}{4}$  –  $\frac{1}{2}$  page**):

The Thesis consisted of 7 Chapters constituting the typical structure of the PhD Thesis based on the already published manuscripts. First Chapter is the General Introduction with comprehensive description properly explaining the main research problem undertaken in the view of relevant scientific literature. This Chapter is ended with the Thesis objectives, which were presented as overall aim of the study and supplemented with the concise indication of the specific objectives. The latter are later addressed by the next five Chapters, being the main body of the Thesis. In Chapters 3, 5 and 6 original articles are incorporated, which were already published in scientific journals with current Impact Factor 3.976, 0.946 and 3.025 (Chapters 3, 5 and 6 respectively). Chapters 2 and 4 include ready to publish manuscripts addressed to dietary toxicity of ZnO NPs. In my opinion, these manuscripts will be easily published in reputable scientific journals.

The Thesis is ended with the General Discussion (Chapter 7) which is closed with conclusions highlighting the importance of insight into the possible mechanism involved in ZnO NPs toxicity and need for further investigations.

In general, the Thesis is well structured treatise. All parts are meeting the general criterions expected to be met by a PhD Candidate. Although clearly divided into two separate parts addressed to ZnO NPs and PAA toxicity, the entire Thesis constitute the logic whole with properly determined objectives followed by respective chapters and properly concluded. The use of integrated biomarkers approach is a clamp that holds two separate parts of the work.

### **OVERALL COMMENTARY ON THE PhD THESIS**

**Please write comments in extent of 1-2 pages:**

The Thesis of M.Sc. Latifeh Chupani is consisted of 3 original research articles published in reputable scientific Journals and two ready to publish manuscripts. The Thesis is supplemented by brief and concise introduction (Chapter 1), where all the relevant information concerning the justification of the undertaken study were presented. In this Chapter clear information on biomarkers used in toxicology as well as chemicals included in the research were provided in relation to the research objectives of the entire Thesis, i.e. evaluation of fish response to ZnO NPs and assessment of PAA safety for aquaculture applications. The entire thesis is closed with comprehensive General Discussion (Chapter 7) ended with brief conclusions highlighting the importance of insight into the possible mechanism involved in ZnO NPs toxicity provided with the study included in the Thesis. No conclusions regarding PAA application in aquaculture is provided in this paragraph. This reflects probably the lesser importance PhD Candidate is giving to this part of her work. Besides the main body of the Thesis, M.Sc. Latifeh Chupani provided clear enough Summary (in English and Czech) and an overview over her research experiences, PhD courses and seminars, and Curriculum vitae as well.

In general, the Thesis is well structured treatise and its content is relevant scientific contribution. The main strength of the Thesis is exploring nature of the research which has resulted in several interesting scientific hypotheses regarding the pathways of toxic action of ZnO NPs. It needs to be highlighted that M.Sc. Latifeh Chupani used a very wide range of research techniques to complete the Thesis objectives. She was the first author in all the manuscripts consisting on the main body of the Thesis, and in case of two published articles also the corresponding author, which proves her most important contribution to these papers. Also, this indicates that PhD Candidate have already twice went trough the publication process, including manuscript preparation, revision as well as open discussion with the Reviewers of those papers.

M.Sc. Latifeh Chupani did not avoid several lingual clumsiness and ambiguous sentences in her Thesis, however, these imperfections did not hinder the understanding of the text and should be considered as a really minor issue.

I feel obliged to mention that I found an mistake; in Chapter 7, in the part regarding PAA safety evaluation, PhD Candidate reported that no mortality had been observed in grass carp exposed to 1 mg L<sup>-1</sup> nad 3 mg L<sup>-1</sup> of PAA. Howevder earlier, in Chapter 5, in the Results section, high mortality (above 70%) is reported in case of fish exposed to 3 mg L<sup>-1</sup>.

I recommended candidature of M.Sc. Latifeh Chupani for futher evaluation process and deffence

**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

2017.06.14 Olsztyn  
.....  
Date and place

Piotr Gornatko  
.....  
Name and signature