



Confidential

Review of USB FFPW PhD Thesis

First name(s), surname, titles of the PhD student: Mahdi Golshan, M.Sc.	First name(s), surname, titles of supervisor: Prof. Ing. Otomar Linhart, DrSc.
Title of PhD thesis: Environmental Contaminants and Endocrine Associated Male Infertility in Fish	
REVIEWER:	
Surname: Pěknicová	Institution: Biotechnologický ústav AV ČR, v. v. i. Vídeňská 1083 142 20 Praha 4
Name: Jana	E-mail: jana.peknicova@ibt.cas.cz
Titles: doc. RNDr., CSc.	
Please describe your professional relationship to the PhD student: I have no a professional relationship to Mahdi Golshan	Please describe your field of expertise: Reproductive Biology

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 paper is in the context of world science, dealing with the effects of environmental pollutants on the health of animals, including reproduction. In recent years there has been an increase in infertility in the human population (15% of couples of reproductive age have problems with reproduction), which is almost 60% associated with infertility in men.

The situation is similar for other organisms as well as fish. Aquatic ecosystems poses a major risk to aquatic organisms due to the variety of chemicals that are present in water.

Contaminated water and fish in such environments represent a risk to the human population.

Decreased fertility of fish creates a problem in fisheries.



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 paper is well structured, divided into four chapters with a clear division.

The first chapter contains a general introduction, an introduction to fish reproduction, a model organism, general information about the two pollutants, aims of the present thesis and references.

The second and third chapter are focused on the reproductive endocrine system of Goldfish exposed to vinclozolin and DEHP.

In the fourth chapter are all presented results discussed.

Experiments, publications and results are consistent with the objectives of the submitted work.

OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

Many substances of natural and synthetic contaminants be given into the water and affects the health and reproduction of fish and mammals. In the present thesis goldfish was treated by vinclozolin and DEHP and primarily influence on reproductive parameters was evaluated.

The paper is an important contribution to understanding the mechanism of action of selected substances (vinclozolin and DEHP) on male fertility. Both substances differentially effect on the brain and testes and have an impact on fertility.

I have only a few questions and theme for discussion:

1. For all such studies is an important choice of three parameters: an experimental model, the concentration of a substance, exposure to the substance.
Can you tell how these parameters were selected and why?
2. Vinclozolin and DEHP does not act as estrogenic contaminants. Vinclozolin is known anti-androgen. DEHP is also anti-androgen?



3. The oestrogens considered as female hormones (are important for female sexual and reproductive development) play important role also in male mammals, as in fish?
 4. The mouse is an ideal model for studying of multigenerational effect of selected substances in mammals. Is it possible to study such multigenerational effect in fish?
 5. Was published already in print manuscript: Golshan et al., Alternation in transcripts of neuroendocrine genes in goldfish exposed to anti-androgens, testosterone and their combinations?
- Comments:
 - I lacked a shortcut list in the introductory part.
 - I appreciated very clear the final table, page 79

The submitted work has a high level, contributing significantly to the understanding of the knowledge of the molecular mechanism of influence of selected substances on the body and male reproduction.

I recommend this theses for acceptance as a basis for awarding the PhD degree.



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

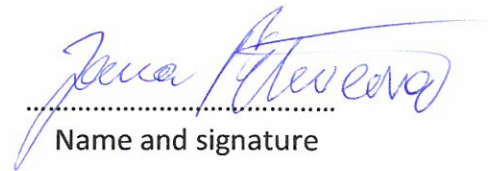
Jihočeská univerzita
v Českých Budějovicích
University of South Bohemia
in České Budějovice
Czech Republic

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

June 15, 2015

.....
Date and place


.....
Name and signature



Confidential

Review of USB FFPW PhD Thesis

First name(s), surname, titles of the PhD student: Mahdi Golshan, M.Sc.	First name(s), surname, titles of supervisor: Prof. Dipl.-Ing. Otomar Linhart, DSc.
Title of PhD thesis: Environmental Contaminants and Endocrine Associated Male Infertility in Fish	
REVIEWER:	
Surname: Ciereszko	Institution: Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Department of Gamete and Embryo Biology Tuwima Olsztyn, Poland
Name: Andrzej	
Titles: Prof.	E-mail: a.ciereszko@pan.olsztyn.pl
Please describe your professional relationship to the PhD student: none	Please describe your field of expertise: Animal reproduction

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 results of the thesis (two papers) have been published in peer-reviewed journal ranked very high by the ISI Web of Knowledge (Journal Citation Reports 2013), Aquatic Toxicology (IF = 3.51) is ranked 6 (of 103) in the category „Marine and Freshwater Biology“ and 17 (of 87) in the category „Toxicology“. For both categories the journal is placed in the first quartile (Q1). As such, the results of the thesis were subjected to a very strict peer-review process and thus were validated according to very rigorous standards of the scientific process. Additionally, the manuscript concerning alternations in transcripts of fish reproductive neuroendocrine hormones and receptors after exposure to xenobiotic substances (vinclozolin, VZ; Di-(2-ethylhexil)phthalate, DEHP) and flutamide (Flu) contains new and original knowledge and certainly will be published in good peer-reviewed journal. In my opinion, this thesis is well focused around the environmental contaminations and their relationship with male infertility in fish. Results of this thesis significantly extend our knowledge concerning the mechanism of interactions between endocrine disruptive substances and brain-pituitary-gonad axis as well as Kiss/Gpr54 system which integrates environmental cues and internal signals for modulation of hypothalamus activity. This thesis also adds valuable information important for evaluation pollutants effects on reproduction in aquatic animals and for improvement of sperm quality control in fish regarding protection



against xenobiotic exposure. Overall, this thesis significantly contributes to the knowledge on male fish reproduction, both regarding basic (reproductive endocrinology, environmental toxicology) and applied (sperm quality, environmental protection) research.

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 main part of the thesis is composed of two chapters (two reprints of published papers and one manuscript). Both paper and the manuscript deal with endocrine-associated disturbances in the reproductive system of goldfish. Chapter 2 contains a reprint of paper and a manuscript. Usually the thesis contains only one paper/manuscript per one chapter and I advise to follow this pattern in this thesis as well. The paper by Golshan et al. (2015) provides novel information concerning VZ-related description of the mechanism involved in disruption of testicular function. Impairments in reproductive function were both related to disturbances to neuroendocrine and endocrine factors, affecting spermatogenesis and testicular development. A model representing neuroendocrine and endocrine disorders following an acute and chronic exposure to VZ was proposed, which in my opinion is valuable part of this paper. The manuscript present in chapter 2 deals with alternations in transcripts of genes related to endocrine regulation (kisspeptin and its receptors, GnRH and androgen receptors). VZ and Flu appear to be capable of interfering with *kiss-1/gpr54* and *gnrh3* functions to control pituitary and testicular hormonal function which results in disturbances of 11-ketotestosterone biosynthesis. Disruption of reproductive endocrine system after exposure of goldfish to DEHP is presented in chapter 3. DEHP exposure was found to interfere with testis and pituitary hormonal functions which resulted in reduction in sperm motility. The scientific part of the thesis is preceded by the introductory chapter 1, in which the author adequately reviews the topics related to environmental pollution at global level, the specificity of male reproduction in fish with special emphasises on neuroendocrine and endocrine regulations and male fertility indicators. Vinclozolin and DEHP are described in detail. General discussion (chapter 4) follows the scientific part; the main achievements of the thesis are summarized and concluded. The thesis consists of three studies concerning endocrine disruption to the reproductive system of male goldfish which resulted in quite an impressive amount of information. In my opinion, the results allow to make general statements regarding reproductive disturbances in fish. The language of the thesis is clear. My concerns (listed below) are of minor importance and can be easily corrected.

OVERALL COMMENTARY ON THE PhD THESIS



Please write comments in extent of 1-2 pages:

As I mentioned above, this thesis covers a set of original topics related to evaluation of the mechanisms of pollutants effects on reproduction in aquatic animals. The results are also important for improvement of sperm quality control in fish. The thesis is written in a clear manner and the results of the thesis were already published in top peer-reviewed journal *Aquatic Toxicology*. Studies on kisspeptin are new and original for fish endocrinology. Basic science is main subject of this thesis but potential applied aspects of fish reproduction and environmental pollution are covered as well.

It needs to be mentioned, that Mr. Mahdi Golshan, in addition to the papers included in the thesis, is the coauthor of 2 papers which confirms his competence in scientific work.

In conclusion, my overall grade of the thesis is excellent and I strongly recommend the thesis for the defense of PhD thesis.

Minor corrections that can be used to improve the text are listed below.

Page 12 and numerous other pages why do substrates (for example cholesterol) and reactions (for example hydroxylation) are written in capital letters?

5th line from the bottom adding a hydroxyl group to C₁₁?

P13, 5th line from the top Ogf-I is not defined nor present in Fig. 2

2nd line from the bottom eSRS22/CA is not defined

P14, 5th line from the top release of sperm from males and ova from female was already stated in L2.

Model organism paragraph vertebrates

P15 please correct the formula for M2 metabolite

P19 2nd paragraph please provide units for the last sentence

3rd paragraph T and 11-KT has already been introduced.

P21 marine medaka

P31 first reference vinclozolin

P75 2nd paragraph first sentence is unclear.

P76 4th paragraph,.....last sentence is unclear

Last paragraph last sentence is unclear

P77

1st paragraph flutamide



3rd paragraph suggests

P78

2nd paragraph, 3rd sentence is unclear

Conclusion and remarks

6th line respectively

9th line flutamide

2nd paragraph please explain "cocktail effect"

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

Date and place

Olsztyn, 14 June 2015.....

Name and signature