



Confidential

Review of USB FFPW PhD Thesis

First name(s), surname, titles of the PhD student: Pavla Linhartová, Dipl.-Ing.	First name(s), surname, titles of supervisor: Assoc. prof. M.Sc. Sabine Sampels, Ph.D.
Title of PhD thesis: Effects of xenobiotics on oxidative stress, lipid metabolism, DNA integrity and cell viability in human cells and fish spermatozoa in vitro	
REVIEWER:	
Surname: Trattner	Institution: Department of Food Science, SLU, Uppsala, Sweden during jan-feb 2016.
Name: Sofia	Current employer is Nyköpings municipality, Nyköping, Sweden.
Titles: PhD	E-mail: Sofia_fre79@hotmail.com
Please describe your professional relationship to the PhD student: Never met	Please describe your field of expertise: Fatty acid metabolism, gene regulation, fish and animal products , bioactive compounds

QUESTIONNAIRE

Originality, scientific importance, perspectives and impacts of results presented in the PhD thesis for basic and/or applied research

The thesis topic is important, dealing with questions recently raised within food science and aspects of public health. To my understanding, the area of environmental effects on food producing animals e.g. fish and finally the effects on the food quality and human health is an area in need of deeper understanding and more research. The author has made an important contribution in this complex field. Compared with international research this work has a high quality. The methods used has a high standard and are well evaluated. The results from this work, namely how environmental compounds interfere with fish reproduction, lipids and proteins could be used both as a valuable knowledge for further research as well as applied in primary production of fish as food of high nutritional quality for human consumption.



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 design of the thesis is easy to follow and logic. First the author give a brief introduction to the topic and put the research area of this thesis into a larger perspective. Further the reader gets an understanding of the design of the trails and why the hypothesis of the thesis are important. The thesis consists of one accepted paper and four published papers in peer reviewed journals. The results and the content of the thesis corresponds to the scope of the title of the PhD-thesis. Possibly I would have expected some more focus on the metabolism of fatty acids e.g. gene expression or enzymatic activity.

OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

This thesis focus on questions which are word wide important and the authors also refers to reports by for instance EFSA. The author are combining questions of sustainable production of fish as food and the nutritional quality of fish as food in terms of fatty acids and xenobiotic compounds.

As the authors describe there are also cocktail effects of fatty acids and xenobiotics that could be of interest. To my understanding this knowledge can be used to optimize fish survival and fish quality as human food. Farmed fish can be a sustainable approach to produce human food with high quality lipids and proteins for human consumption. As indicated in the thesis, the presence of xenobiotic compounds can interfere with production quantity and quality.

Over all context of the thesis is good, however I have some comments and question as follows:

- Page 106 in the English summary, it is summarized that: *The obtained data in present study gave us new insights in to the effects of fish nutrients and xenobiotics on cell metabolism at different levels with focus on changes in lipid classes and oxidative stress in human heptatoma cell line and fish spermatozoa in vitro.* As a summary of results, it would have been interesting if you indicated which new insights you found.
- Page 17 1.5. Fish of interest it is written that: *N-3 FA are found in every kind of fish, but are*



especially high in fatty fish (for example common carp or salmon, butter fish, eel, mackerel etc.) where EPA and DHA are represented in higher amounts. To my opinion it would be interesting to calculate the content of EFA and DHA in one portion of lean fish (e.g. cod) and compare that to the content of EPA and DHA in one portion of carp.

- Page 38-39, chapter2 it is stated: *another interesting result from our study was that the combined incubation of the FA and Ca²⁺ at the highest level increased the proportion of (off?) DPA in the cells. This indicates either an upregulated metabolism from EPA towards the longer chain products or an increased β -oxidation from DHA due to energy needs of the cells or oxidative stress. Could you please explain the mechanism of how oxidative stress would influence this finding?*
- How do you think that your research results will be applied within aquaculture?

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

6th of June 2016, Nyköping, Sweden

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Date and place

Sofia Trattner

Name and signature



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Title of PhD thesis: Effects of xenobiotics on oxidative stress, lipid metabolism, DNA integrity and cell viability in human cells and fish spermatozoa in vitro	

REVIEWER:

Surname: Todorcevic	Institution: University of Oxford
Name: Marijana	
Titles: Dr.	E-mail: marijana.todorcevic@ocdem.ox.ac.uk
Please describe your professional relationship to the PhD student: I have no professional relationship to the PhD student	Please describe your field of expertise: Molecular and cell biology in fish adipose tissue and human adipose tissue

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**):

Pavla Linhartová have studied and presented two scientifically very important topics in her PhD thesis, namely the effects of pollutants on human liver metabolism and fish spermatozoa using *in vitro* models. I acknowledge the great amount of work that candidate did during her PhD, which resulted in 5 publications of which 4 are published, 3 as the first author and one as the last author. Having 4 articles published during the PhD is a great achievement. The results presented in the thesis are important both for basic and applied 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**):

Publications presented in this thesis are of a great interest for both national and international research community. The results presented in the all 5 articles correspond to the objectives of the thesis. However, the main text needs to be more focused and needs better structuring. Please see bellow my detailed comments on the main thesis text.



OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

The thesis title: should be rephrased to more precisely reflect the focus of the thesis. For example the candidate does not investigate all human cells in her thesis, only HEPG2 cells.

The general introduction (chapter 1): my general opinion about introduction: it is muddled and confused. For example, was there an interest in investigating the effects of different pollutants on the cancer in human liver or on the liver metabolism or both? I assume these cells were used as a model to study liver metabolism and toxicity of xenobiotics, but this needs to be clarified (see comments in the section 1.3.)

Section 1.1 needs to be re-written because the ideas are not well organized. The subtitle does not reflect the text. A big part of the text is missing references. Most of the references used in this paragraph are from the late 90s and from 2000s. Why did not candidate refer to the older literature?

The first paragraph in this section should be written in a more scientific language.

The second paragraph needs to be presented as a table and to include references for each pollutant mentioned; otherwise it is very hard to follow. The examples given in the text have to be better explained in order to make them relevant. For example: "Metal ions and other xenobiotics can be concentrated in aquatic organisms to a level that affects their physiological state". To which other xenobiotics does candidate refer to? To which exactly aquatic organisms does candidate refer to and what were the consequences seen in each of the aquatic organisms when exposed to different xenobiotics?

The last paragraph needs to be either deleted or re-written so that it shows the information about each pollutant studied in the thesis. Mercury and lead might be interesting in general but do not give any information related to the work presented in this thesis.

Section 1.2 DQ, TBBPA and BPA should have been mentioned in the Section 1.1. It is difficult to see why are they chosen as they did not appear (except for the cadmium) in any of the pollutant classifications presented in 1.1. The candidate missed very important information, what are the concentrations of cadmium found in different fish organs and different fish species? How much of cadmium-polluted fish would we need to eat in order to see harmful effects on humans?

It is not clear how any of the studied pollutants end up in water?

The following status is very confusing: "The study, where more than 344 food samples from the fish and other seafood products were examined, concluded that the dietary exposure to TBBPA in the European Union does not raise a health concern." "TBBPA is acutely toxic to fish at low concentrations". These 2 sentences contradict each other and I suggest that candidate make them clear and use appropriate references.

In the last paragraph it is not clear when the candidate refer to fish and when to humans, and a lot of references are missing.

Section 1.3 Half of the first paragraph is general knowledge and it should be shortened. This is the most confusing paragraph and in order to make it clear and focused candidate needs to firstly demonstrate why HEPG2 cells were chosen as a model system. Why primary human hepatocytes were not used (see comments in the chapter 7)? What are the advantages/disadvantages of using cell line over primary cells? What are the advantages/disadvantages of using *in vitro* vs *in vivo* models and these explanations are very important since candidate used *in vitro* systems in all 5

publications (see comments in the chapter 7)? Has the cadmium been shown to be accumulated in human liver and at what concentrations? As already mentioned it is not clear if HEPG2 cells were used to study the effects of cadmium on liver cancer or on liver metabolism. In order to avoid further confusion candidate needs to clarify the main reason for using this *in vitro* model. For example, HEPG2 cells, due to being able to express a wide variety of liver-specific metabolic functions have been shown to be a relatively good model for studying the central role of the liver metabolism. In addition HEPG2 cells have been shown, by other research groups, to be a good model system for studies of liver metabolism and toxicity of xenobiotics. Why is the Fig 1. taken from the web site and not from candidates own work?

Section 1.4 the subtitle is not informative and should be changed. The whole paragraph contains a lot of general knowledge and it needs to be shortened down. Most of the text does not represent results of this thesis. For example why is n-6 pathway presented in Fig.3 when only the effects of EPA and DHA were studied in this thesis?

Section 1.5 the subtitle does not represent the text in this paragraph and it should be changed to for example: "Fish and its role in human nutrition".

Chapter 2 will not be commented as it is already published.

Chapter 3 candidate is strongly advice to change the title of the chapter 3 to better reflect the study and change human hepatocytes to HEPG2 cells. In introduction section candidate should refer more to the chapter 2 as I believe results presented in the chapter 2 were basis for the results presented in the chapter 3.

Section 2.2.4 the uptake of cadmium and FAs has to be tested in each experiment and it is not enough to refer to previous study.

Section 2.3 why were cells centrifuged several times in PBS/FBS? Why the cell pellets were not stored in appropriate storage buffers for each analyses candied performed later on?

2.4.1 why was the pellet defrosted on the room temperature? Could this have an effect on enzyme analyses performed?

Section 3.1 the uptake of FA should be changed to FAs trough out the whole chapter as the candidate studied both EPA and DHA. The uptake of FAs into the cells has to be shown for each experiment and this is why it cannot be discussed in the previous publication.

Figures 1, 2, 3, 4 the results should be presented per mg of proteins or total DNA not per number of cells seeded. The number of cells seeded can change depending on many factors (e.g. increased proliferation or increased apoptosis during experiment), so using the number of cells can give misleading results. Figure 2 what is CP related to?

Chapter 4, 5, 6 will not be commented in details as they are already published. However, the question for the candidate is to speculate if the ATP, CP, TBARS, SOD data would be different if presented per mg of total proteins or DNA.

Chapter 7 would benefit from more connection and cross-referencing across the chapters. The candidate needs not provide stronger arguments why she thinks the research questions were important to investigate. Firstly, candidate needs to discuss why it is important to study the effects of different pollutants in human and fish. Why the effects of cadmium were studied in human liver cells (e.g liver is one of the organs mostly affected by cadmium). Then it is important to discuss the reason for choosing HEPG2 as a model system (e.g primary liver cells are hard to obtain and *in vitro* models offer to study cellular and molecular pathways in details which cannot be studied using the whole organism). Then candidate should discuss the role of EPA and DHA in liver metabolism. The clear statement explaining that HEPG2 cell were used to study liver metabolism and toxicity of xenobiotics is needed because candidate showed increased oxidative stress and apoptosis in HEPG2 after cadmium treatment; while EPA and DHA had a protective effect. As presented it is not clear if the effects of EPA and DHA are positive or negative, as apoptosis has become a promising therapeutic target in cancer research. However, these effects are very important in non cancerogenic cells.



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

01/07/2016, OXFORD

Date and place

MARIJANA TODORCEVIC 

Name and signature