



**Confidential**

**Review of USB FFPW PhD Thesis**

<b>First name(s), surname, titles of the PhD student:</b> Pavel Šauer, Dipl.-Ing.	<b>First name(s), surname, titles of supervisor:</b> Dipl.-Ing. Hana Kocour Kroupová, Ph.D.
<b>Title of PhD thesis:</b> Environmental pollutants progestins: occurrence, hormonal activities and effects on fish	
<b>REVIEWER:</b>	
<b>Surname:</b> Lutz	<b>Institution:</b> Leibniz-Institute of Freshwater Ecology and Inland Fisheries Mueggelseedamm 301; 12587 Berlin, Germany
<b>Name:</b> Ilka	<b>E-mail:</b> <a href="mailto:ilka.lutz@igb-berlin.de">ilka.lutz@igb-berlin.de</a>
<b>Titles:</b> Dr.	
<b>Please describe your professional relationship to the PhD student:</b> Supervisor for training: "in vivo exposure of fish using a flow-through system"	<b>Please describe your field of expertise:</b> Eco-toxicology, Hormone physiology

**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 Mr. Šauer is closely related to the current conversation regarding the occurrence of natural and synthetic progestins in the aquatic environment and their potential to negatively harm inhabitants. Since no comprehensive data is available it is difficult to estimate possible impacts on wildlife and environment and this makes environmental regulation efforts difficult. This is where the present PhD-thesis provides a fundamental contribution. The competitiveness of the PhD thesis is clearly evident in that papers are all published in highly influential scientific journals.

The basic concept of the research presented in the PhD thesis, is that all progestins that are prescribed in the Czech republic are subjected to examination of their occurrence in the environment. After establishing a method in order to reliably analyse progestins in the nearby environment the progestagenic activities were checked in various water matrices. What makes the thesis so valuable is that a founded chemical analysis was combined with examining the biological activity of the chemicals by using one assay-type.



The outstanding contribution of Mr. Šauer's PhD thesis, is that it presents a project that examines progestins on various levels of their interaction within the environment. With this scientific contribution, Mr. Šauer's work closes an important knowledge gap regarding occurrence and biological impacts of various gestagens and therefore it should be considered to be of high scientific importance. The chemical analysis of the occurrence of progestins in the immediate environment was analysed through a concurrent examination of biological activities using the same assay. Thus, the PhD thesis contains comprehensive data that can be considered as a solid basis for further research on possible effects of progestins on wildlife.

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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 Mr. Šauer is very thoroughly and conscientiously prepared making it enjoyable to read. It is clearly and comprehensively structured and covers six chapters, list of publications, training and supervision plan during the study, his curriculum vitae and a precise statement of his part in the work. The PhD thesis as well as the included articles are characterized by a very clear and logical structure and an excellent use of language. A number of tables were employed to summarise complex data and to provide a very helpful overview of current published data.

The research was intended to examine the occurrence and biological activities of progestins in the local aquatic environment. The applied approaches and the achieved results allowed the author to draw sound conclusions that are of scientific value and significance, not only for the scientific community in general but also for the local one. Furthermore, the results are interesting and clearly presented, the discussion is balanced, based on current literature and the content of the publications compliments the specified objectives of the PhD thesis very well. Finally, each of the published papers is characterized by a sound and reliable study design. Special care was taken to include the appropriate controls and the experimental procedures and analyses were performed thoroughly and conscientiously.

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

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

The PhD thesis of Mr. Pavel Šauer focuses on the occurrence of natural and synthetic progestins in the aquatic environment and their biological potential to adversely affect aquatic inhabitants. Recently, it has been shown that progestins at low concentrations can affect reproductive processes in fish. Therefore, eco-toxicological research on the occurrence and biological activity of progestins



is not only of scientific importance, but also public because the knowledge regarding this topic is limited. In this context Mr. Šauer's thesis is a highly significant contribution to broadening our knowledge about the occurrence of progestins in the aquatic environment and their possible adverse physiological effects in fish. The basic concept of the research presented, is that all progestins that are prescribed in the Czech republic are subjected to examination and not just „model substances“. Therefore, the inclusion of the local environment is of additional value.

The PhD thesis is divided up into six chapters that are ordered in logical sequence. Chapter 1 „General Introduction“ is logically structured beginning with the current situation of a steady increase of chemicals and the urgent requirement to gain information on their occurrence in the environment. And in particular, to determine possible negative effects on wildlife. Consequently, the PhD thesis deals with analytical aspects of progestins and their occurrence in the environment as well as their progestagenic and anti-androgenic activities. Therefore, reliable analytical methods and biological assays are required to allow a high sample throughput. This is the focus of the thesis and Mr. Šauer has summarized accurately the key points of numerous scientific papers. By doing so, a very comprehensive picture is provided in order to explain the scientific background of the topic „progestins in the environment“ which are the chosen objectives of the PhD thesis. The current scientific background and recent data of the topic are examined in depth, reflecting comprehensive research of literature. This results in a well based discussion of pros and cons regarding different aspects of this complex topic e.g. *in vivo* vs *in vitro* assays and also enables the complex theoretical and practical background of the research and the environmental relevance of the topic to be understood well. The following chapter presents the development of an analytical method in order to reliably analyse the occurrence of progestins in various environmental water matrices. This is an important procedure to obtain reliable data for regional environmental authorities in the area of regulative issues. An outstanding result was achieved by discovering a few progestins in the environmental matrices, that have so far not been detected.

After establishing the proper analytical method the author researched progestagenic and anti-progestagenic activity not only in influent and effluent local waste water treatment plants, but also in receiving waters. Additionally, the biological significance was examined by using an adequate *in vitro* system. The fourth chapter includes the publication presenting results about the anti-androgen potential of progestins in the aquatic environment. To my knowledge it is the first time that anti-androgenic potencies of relevant progestins were examined so thoroughly using the same



*in vitro* bioassay. Finally, an *in vivo* experiment was performed using one model progestin that had not been tested so far, examining its physiological effects on different endpoints for reproduction in guppies. The corresponding paper is already submitted and reports on very interesting effects on reproductive behaviour. In the final chapter „General discussion“ the topics and results presented in the various previous chapters are thoroughly discussed in a well-balanced manner with reference to the appropriate literature and finally a brief and precise summary is given.

I consider Mr. Šauer's PhD thesis to be of very good scientific quality, presenting very interesting and novel results based upon a sound experimental design. Mr. Šauer published a substantial amount of publications in highly regarded peer-reviewed journals (with a high impact factors) that are characterized by carefully and precisely planned experiments and sound practical procedures. His training- and supervision plan shows that Mr. Šauer not only visited international conferences but also improved his knowledge by learning new methods in laboratories in foreign countries which are also useful for establishing his scientific network. In general, Mr. Šauer's PhD thesis reflects a very well educated and highly motivated scientist.

### **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

18th January 2019, Berlin  
Date and place

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

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<b>Title of PhD thesis:</b> Environmental pollutants progestins: occurrence, hormonal activities and effects on fish	
<b>REVIEWER:</b>	
<b>Surname:</b> Hilscherová	<b>Institution:</b> RECETOX, Faculty of Science Masaryk University Brno
<b>Name:</b> Klára	<b>E-mail:</b> hilscherova@recetox.muni.cz
<b>Titles:</b> doc. Mgr., Ph.D.	
<b>Please describe your professional relationship to the PhD student:</b>	<b>Please describe your field of expertise:</b>

### **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 dissertation thesis by Pavel Šauer submitted for PhD defence focuses on the study of progestins as aquatic pollutants. These compounds are frequently used pharmaceuticals, but the knowledge of their occurrence as well as effects in the aquatic environment is rather scarce. The topic of these theses is highly relevant to the field of aquatic toxicology, since these compounds belong among important emerging water pollutants. The studies summarized in these theses present development of the method for the analyses of progestins as well as characterization of their in vitro toxic potencies and contribution to the effects of environmental samples. One comprehensive study also describes effects of selected progestin on fish mating behaviour and reproduction. The research presented in the thesis is of very good quality, the studies are well designed and conducted and bring important new knowledge. The presented research reflects the current state of knowledge in the field of aquatic ecotoxicology and contributes important new findings to the toxicological profiles of studied progestins as well as characterizes their environmental levels. The results are original and novel and significantly contribute to our understanding of the occurrence and relative importance of the studied compounds in both waste and surface waters.



### ***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 based on four scientific papers (3 of them already published), with included Introductory and Discussion and Conclusion section. It is logically structured into six main chapters, contains Czech and English summary and further required information (list of publications, training plan and CV). The aims of the thesis are clearly described and addressed in the included scientific publications based on the conducted research. First Chapter contains the general introduction, Chapters 2-5 present the research papers and the Chapter 6 contains the discussion and conclusions. Pavel Šauer is the first author on two published articles, his % contribution to all papers is clearly defined. The papers were published in several of the most prestigious international journals in the field of aquatic environmental chemistry and ecotoxicology (Science of the Total Environment, Environmental Pollution, Water Research), which confirms the quality of the conducted research since they had to undergo a scientific review by international experts prior to publication. Pavel is also a co-author on two additional published review papers, which are not part of his thesis.

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

The thesis are very well written. They present interesting and highly relevant topic, which is addressed in several well designed studies. There is also good quality of English. There were only a few grammatical errors in the Discussion part. The Introductory part is based on very extensive literature review. It includes large tables summarizing the current knowledge on the physico-chemical properties of progestins, their detected levels, in vitro and in vivo effects. The Introductory part itself contains about 7 pages of text and another 15 pages with the summary tables. The list of references for the Introductory section spans over pages 13 pages. It includes close to 200 references, which confirms very good orientation of the student in the current literature relevant for the topic. The study designs, results and interpretation are well presented in the included papers. They are interlinked, summarized and put into context with current state of knowledge in Chapter Discussion and Conclusions (10 pages), which includes around 50 references. Even though the thesis are in general well structured, the table of contents on p.4 does not present this structure very well. Between Chapter 1 and Chapter 6, for which titles of subchapters are listed, thus the content is obvious, there are Chapters 2 – 5, which span across pages 42 – 97, but the list of Content does not provide any information on what is included in these chapters. They should have titles demonstrating what is in these Chapters in the Content. Moreover, even the Chapter 1 and 6 do not show as Introduction and Discussion and Conclusion in the Content on p. 4.

#### **Specific comments**

1. In Table 1 on p.7 it would be good to refer to paper Golovko et al., which includes also MW and structures of these compounds. Alternatively, at least MW should be included in this table.



2. Table 6 on p.17. I think the columns showing androgenic and antiandrogenic activities should be separately labelled to clearly show which column shows what. Readability would improve if the column with flutamide equivalent is in different units to avoid too long numbers.
3. In subchapter 3.2.3 Mating study it is written: "Both groups of exposed males (3.2 and 320 ng·l<sup>-1</sup>) showed significantly shorter duration and lower frequency of mating attempts compared to the controls". But the results in Figure 2 document that also in both cases with unexposed males, but exposed females, the mating frequency was strongly decreased. This should be emphasized in the results.
4. Discussion – in subchapter 6.2 you should include the info from which organism and organ are the cells and also receptors of which species are built into the used models.
5. P.103, last paragraph – there it is written: "progestins were stronger than reference compound flutamide" and "progestins are much stronger than flutamide" - it needs to be specified in which regard are they "stronger", what specifically is meant here.
6. Summary: I would suggest to modify the first sentence of the summary, since in its current form it sounds like if many chemicals commonly pass through the WWTPs without any change, which is not true, at least for Czech WWTPs.
7. P.113 – I would suggest to modify the wording related to relative potencies in the Czech version. The wording "stanovili relativní progestagenní, androgenní a antiandrogenní síly všech progesterinů" sounds strange.

#### General comments and discussion questions

1. On page 6 you write: "EDCs interfere with biological actions of endogenous hormones directly by binding to receptors or indirectly by altering synthesis of hormones, enzymes or receptors."  
What are other mechanisms how endocrine disruptors can interfere with endocrine system?
2. On page 6 you write: In vitro reporter gene bioassays are typically based on a tumorous cell line that in an ideal case does not contain any measurable amount of receptors. Why is this the ideal case?
3. On page 16: Table 5 – last row – what does the listed concentration correspond to? What are the units? How comes it is so much greater than in case of the other studies? Since different reference compounds are used in different studies, how is it possible to compare the bioassay responses from different studies? Would it be possible to translate them into comparable equivalent values? In relation to this, why is the artificial compound ORG 2058 used as a standard in your studies rather than the native ligand for the PR progesterone? Is some simple recalculation to progesterone equivalent possible?
4. Chapter 2 - Paper 1 (Golovko et al.), Table 5 – samples from the WWTP Vodňany seem to contain the greatest levels of most progestins. Why did not you include some data for potentially affected surface waters, where the effluent goes to, or the upstream/ downstream design as in the other cases? Could the observed cases where levels in the effluent from this WWTP were greater than in influent be besides potential biotransformation affected by some other factors?
5. Chapter 5 – Paper 4 (Steinbach et al.): Why did you select specifically etonogestrel for the in vivo study, when it was not detected as relevant in any of your field studies? Why did not you



choose some more environmentally relevant compound? In the paper you state: "The fish were exposed to etonogestrel at environmentally relevant (E1: 3.2 ng·l<sup>-1</sup>) concentrations", but no such levels were shown as environmental relevant in any of your other studies. Actually, in the Introductory part you write: "etonogestrel and its precursor desogestrel have not been detected to date in waste and surface waters." Thus, can you provide some references that would document the environmental relevance of such levels? If there are no such studies, you should change the wording in the methodology section, Abstract and throughout the paper, since you cannot state that this exposure is environmentally relevant.

6. In Paper 4. Introduction: "progestins mostly do not bind to fish progesterone receptor". How homologues/different are the ligand binding domains of mammalian/human and fish PR? How good is the predictability from mammalian/human in vitro assays for fish effects of progestins?
7. Page 102, last paragraph: Based on your research results and other available information is it possible to conclude as you write that all the studied progestins are "relevant for the aquatic environment", especially for surface waters?

#### Overall evaluation

In conclusion, Pavel Šauer succeeded through his research work on several studies in demonstrating good expertise, research potential, creativity and independence. His work made significant contribution to the advancement in the field of understanding of the occurrence and effects of progestins in aquatic environment. List of publications demonstrates that next to the mentioned papers he also contributed to 6 presentations at conferences (3 of them international). The listed Training Plan documents his international research experience, since he has realized two research stays abroad during his PhD studies, 2 months at Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB), Berlin, Germany and one month at INERIS, France. Given the high quality of the thesis and included research papers I can without any reservation 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

1.2.2019 Brno

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Date and place

*Marta Hrbáčková*

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Name and signature