



Review of USB FFPW PhD thesis

First name(s), surname, titles of the PhD student: Marie Eugenie Sancho Santos, M.Sc.	First name(s), surname, titles of supervisor: Prof. Dipl.-Ing. Tomáš Randák, Ph.D.
Title of PhD thesis: Psychoactive compounds in aquatic environment and their effects on fish	

REVIEWER:

Surname: Pestana	Institution: University of Aveiro, Portugal
Name: João Luís Teixeira	
Titles: Dr.	E-mail: jpestana@ua.pt
Please describe your professional relationship to the PhD student: none	Please describe your field of expertise: Aquatic Ecotoxicology

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**):

Maria Eugenia Santos PhD thesis presents original work and interesting results dealing with the occurrence and effects of psychoactive compounds in fish. The results presented in three already published scientific articles are sound and present new knowledge for the assessment of ecological effects of these emerging contaminants.

There are several innovative aspects I would highlight: i) first the fact that Maria Eugenia has focused on two not so used endpoints, behaviour and histopathology. In fact, both can be sensitive and early warning indicators and are ideal to assess some of the effects of neuroactive compounds; ii) also the fact that methamphetamine (and its main metabolite) and tramadol, two not so well studied compounds concerning their ecotoxicological effects, were analysed in fish brain so concentrations can be related with observed effects is an important aspect that strengthens the results and contributes to their accurate interpretation; and finally, iii) the fact that some of the results are interpreted under the “read across hypothesis” is also a plus of the thesis since much more data are needed for different pharmaceutical compounds in order to achieve the ultimate goal of predicting their environmental and ecological impacts from information obtained during the drug development process.

All considered this is a good PhD thesis and results here presented will be of interest for ecotoxicologists and environmental scientists.



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**):

Maria Eugenia Santos PhD thesis is generally well written and clear. The chapters are organized in a logic order. Main conclusions are correctly drawn by the results obtained.

I agree with the structuring of the thesis (general introduction followed by chapters in the form of published or submitted scientific papers and general discussion). The general introduction and the discussion/conclusion sections are informative but brief. In my opinion a more in depth coverage of some aspects would be of interest within the introduction and the discussion would greatly benefit from the inclusion of a developed “further research” section. Experimental chapters are already published or in press and it’s good to see the supplementary material of each one presented.

Moreover, appropriate methods were used and data analyses were done competently to address the specific objectives. The outcomes of the thesis are published /submitted to well respected journals within environmental sciences and, as stated, offer important data and approaches that can be used for a better understanding of the ecological effects of methamphetamine, tramadol and other psychoactive compounds.

Nevertheless, I would say that from the four objectives presented in the Introduction, the thesis addressed only objective 2 and 3. Concerning objective 1, I would not state that performing experiments was an objective of the work. Moreover, this work did not obtain information about psychoactive drug consumption in Central Europe in an organized and comprehensive manner.

Also, *underlining the current problematic regarding the use of the histopathological method in ecotoxicological studies* stated as objective 4 seems a bit like an “a posteriori” objective that is only present in the thesis based on a comment paper. The inclusion of this letter to the editor (chapter 4) in the thesis, although relevant per se as a publication, is somewhat poorly justified.

OVERALL COMMENTARY ON THE PhD THESIS

Please write in the box specific comments concerning the PhD thesis in extent of 1-2 pages:



As stated, Maria Eugenia Santos PhD thesis is focused on the effects of two psychoactive compounds, Methamphetamine and tramadol in fish. These drugs are considered emerging contaminants of aquatic ecosystems and thus data on its effects as well as the approaches used here to assess their behavioural and histological effects are new and will elicit interest from researchers and regulators. I think that the presented document is of sufficient quality to be recommended for defence and I only have some minor comments and questions for the candidate:

General introduction: This section is short but overall manage to give a good summary of the problematic around the contamination of aquatic ecosystems with due to neuroactive and psychoactive substances. Occurrence, usage and main sources of Methamphetamine and Tramadol are listed. I think that maybe the section about the effects of these two compounds might have been developed maybe extending it to effects observed in other aquatic organisms. I would suggest that when presenting “ Effects in human and animal models” the text should be made clear on what organisms are those effects reported (Human, vertebrate models, fish)

Chapter 2 – This chapter is composed by two scientific articles. One dealing with histopathological changes induced by Methamphetamine in trout and the other dealing with behavioural effects after exposure to Methamphetamine in the same species. I particularly liked the experimental design and approach used to assess fish behaviour and in this way address withdrawal/addition.

In the first paper fish were divided into six aquaria which gives two replicates per concentration. Was individual aquarium considered in the statistical analyses? The experiment was done in duplicate meaning that a random factor (replicate/tank) could be considered. Aren't individual measures (in each fish) sub replicates? In the second paper only one fish tank was used per treatment, why? Why the different exposure periods (between first and second paper, 35 vs 56 days?)

If the parent compound and its main metabolite appeared in great concentration in kidney, why weren't the histopathological effects assessed in this organ?

In my opinion biochemical markers would be a great add on to the results presented. Why weren't they performed? If it was possible which one would you choose to complement the analyses?

Chapter 3 – This chapter deals with effects of tramadol in several behavioural traits of chubs and presents sound results given the association of tramadol concentrations in brain and behavioural effects. I would argue that using more than one concentration could strengthen results and interpretation: Why only one concentration was tested?

Maybe not in the article itself but the problematic of behavioural syndromes when addressing ecotoxicological effects could be developed in the introduction/discussion sections.

Chapter 4 – is composed a letter to the editor, basically a comment directed at a different study. Although I see merit in pointing to some errors or deficient data treatment of published studies I have some doubts concerning the inclusion of this letter to the editor as a chapter of the thesis. I would rather prefer to have read a review focused on what authors (and the candidate) argues in that letter, i.e. the need for standardize protocols and methods within ecotoxicological testing to adress histopathological effects on fish and other models.

General discussion: This section summarizes the results but is a bit short considering that it could have been used to explore the personal views of the candidate concerning several topics:

- Behavioural syndromes in fish in the ecotoxicological context
- Need for standardization of methods/ protocols for histopathological studies



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Czech Republic

- Validity and sensitivity of fish species and behavioural endpoints towards psychoactive drugs
- Future research including usage of different endpoints in fish
- Ecological effects of psychoactive substances in aquatic environments (including ecological relevant scenarios such as occurrence in combination with other compounds and under concomitant exposure to natural stressors)

FINAL RECOMMENDATION

PhD thesis can be recommended for defence

PhD thesis can be recommended for defence with reservations

PhD thesis cannot be recommended for defence

Aveiro, 07 July 2021

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Date and place

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Name and signature