Porto Alegre, 16 September 2020

The Dissertation Committee
St. Petersburg State University

Re: Prof. Allan V. Kalueff's (Kaluev's)
Doctor of Biological Sciences Dissertation

To Whom It May Concern

Dear Dissertation Committee,

As a foreign member of Professor Allan V Kalueff (Kaluev)'s Habilitation Dissertation Committee, with academic rights officially recognized to be at the level of Doctor of Biological Science in Russia, I am pleased to provide this review for his Dissertation. entitled "Biological bases of experimental modeling of CNS processes and human brain disorders using zebrafish (*Danio rerio*)", submitted for the degree of Doctor of Biological Sciences in Physiology at St. Petersburg State University.

I am PhD in Biological Sciences (Biochemistry) and specialize in molecular and behavioral neurobiology, modelling human pathologies and drug effects on variety of animals, including zebrafish. I am well familiar with Dr. Kalueff's long-term research work in the field and am impressed by the quality and breadth of his research and publication foci. He established many collaborative projects around the globe with international scientists, including Brazil, China, Belarus and USA.

Prof. Kalueff's dissertation summarizes his laborious research for the last two decades. It deals with multiple aspects of zebrafish behavior and pathologies, including modelling affective disorders and studying common drug effects on zebrafish. When Allan's started his work on zebrafish, they remained largely understudied and, in my opinion, his pushing, provocative and innovative use of the species helped the field to substantially grow in the last decade. In the dissertation, he summarizes numerous of papers that, for the first time, introduced effects of common and widely spread psychoactive substances on zebrafish, thus characterizing the fish typical behavior in different states and, subsequentially, planting the seeds for successful translation between human, rodents and zebrafish. Furthermore, along with our own lab, Allan was one of the first scientists introducing complex models of neurobehavioral disorders in the field of zebrafish studies and both deepen our understanding of the models, studying behavioral and molecular alterations observed in the model, as well as constantly widen number of the models, developing new methodologies to model human disorders.

The presented dissertation work is organized as grouped chapters, each with a focus on specific theme in modelling psychiatric conditions or drug testing. At the same time, the chapters are in fact interconnected. The first two chapters focus on summary of overall methods used in the work – the first one through standard methods section whereas second chapter develops and validates the methods that

will be further used for behavioral phenotyping of models and drug effects. Similarly, last chapter focuses on testing wide range of psychoactive substances in the zebrafish that allows deep description of common neurobehavioral spectrum, including serotonergic, GABAergic, and NMDAergic systems and associated behavioral alterations. Finally, chapters 3-6 study variety of models, both physical and pharmacological, including three depression/antidepressants related models – chronic unpredictable stress model, reserpine model of depression and serotonin syndrome. Allan also studied effects of proconvulsant drugs and validates test on zebrafish spatial memory using stress as a negative control and nootropic drug piracetam as a positive control.

In summary, Allan's work is a complete, comprehensive, and stand-alone innovative study of a hugely understudied in neuroscience species. Based on my reading of his dissertation, I think he is also an outstanding scientist, both enthusiastic and skilled, and extremely productive. I strongly believe that as Allan's work continues, we will see many other insights in the neurobiology of the zebrafish and successful translation of this work to human neurobiology with development of new therapy methods, drug targets and understanding of evolutionally conservative pathological aspects of the disorders. It is my strong opinion that Dr. Allan V. Kalueff, PhD, fully and undoubtedly deserves awarding him the degree of Doctor of Biological Sciences by St. Petersburg State University.

The dissertation adheres to the ORDER of September 1, 2016 No. 6821/1 "On the Procedure for Awarding Academic Degrees at St Petersburg State University", as specified online at https://spbu.ru/sites/default/files/20160901_6821_1_eng.pdf. The dissertation is compliant with Section 11 of the said Order ("In their dissertation, the seeker of an academic degree is obliged to reference the author and/or the source of the borrowed content or specific results. If the dissertation uses the results of research work performed by the academic degree seeker personally and(or) in collaboration, the academic degree seeker is obliged to indicate this circumstance in their dissertation.")

Sincerely yours,

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