PhD Thesis Acceptance Report
Research Discipline Council of Biological Sciences
Jagiellonian University in Kraków

Candidate's name and surname: Daria Krzysztofik

PhD Thesis Title: *Long-term exposure to high copper. Does it affect the production of pancreatic digestive enzymes?*

Thesis Supervisor: Prof. Grzegorz Dubin

Assistant Supervisor / Second Supervisor/ Co-supervisor (if applicable): Dr Monika Jakubowska

Reviewer: Dr. Roman Polishchuk

1. The topic of the dissertation

Whether the topic of the dissertation is relevant to the development of the discipline of biological sciences (suggested approx. 50-150 words)

This thesis addresses a highly relevant and underexplored area in the field of biological sciences: the physiological and pathological consequences of copper overload on pancreatic function. By integrating knowledge of trace element homeostasis, digestive enzyme regulation, and animal disease models, the thesis contributes to expanding our understanding of how systemic metal imbalances, such as those observed in Wilson disease, affect exocrine pancreas function. This is especially relevant in light of the growing focus on metal toxicology and its implications for human health.

2. The candidate's knowledge

An assessment, with justification, of whether the dissertation demonstrates the applicant's general theoretical knowledge in the discipline of the biological sciences (suggested approx. 50-250 words)

The dissertation demonstrates the candidate's thorough and comprehensive understanding of biological sciences, particularly in physiology, molecular biology, and pathophysiology. The introductory sections present an extensive literature review on copper and calcium homeostasis, relevant transport proteins, and disease models, reflecting a strong theoretical foundation. The candidate successfully outlines the complexity of trace metal regulation and its physiological implications, providing the reader with a clear and detailed background essential for understanding the research objectives. The inclusion of multiple models of copper dysregulation—both genetic (Wilson disease) and induced (chronic exposure)—further highlights the candidate's ability to navigate and connect diverse areas within the biological sciences.

The author critically integrates existing knowledge with her own findings, indicating a solid perception of both conceptual and practical aspects of modern biomedical research. This is evident in the logical progression from hypothesis formulation to experimental execution and data interpretation. The work clearly reflects competence in experimental design, including the choice of appropriate methodologies, statistical analysis, and validation techniques. Furthermore, the ability to contextualize results within broader biological mechanisms demonstrates advanced analytical skills. Overall, the dissertation reveals not only scientific competence but also intellectual maturity and the capacity to contribute meaningfully to the field of biological and biomedical sciences.

3. Independence of the candidate

An assessment, together with a justification, of whether the dissertation demonstrates the applicant's ability to carry out scientific work independently (suggested approx. 50-250 words)

The scope and execution of the research strongly indicate the candidate's capacity for independent scientific work. The thesis demonstrates that the candidate was actively involved in all stages of the project—from conceptual development and hypothesis formulation to the design and execution of experiments, as well as data analysis and interpretation. Her ability to apply diverse methodologies, including histological, biochemical, and molecular techniques, reflects a high level of technical autonomy. Furthermore, the candidate's ability to secure NCN funding is a strong indication of her organizational skills, initiative, and ability to manage complex research tasks independently.
The dissertation reflects careful planning, critical problem-solving, and consistent progress in advancing the research goals. The integration of two complementary experimental models, including both genetic and pharmacological approaches to copper overload, further illustrates the candidate's scientific creativity and strategic thinking. Her active involvement in co-authoring peer-reviewed publications and presenting her work at national and international conferences confirms her growing independence as a researcher and her ability to communicate her findings effectively. Overall, the thesis provides convincing evidence that the candidate is capable of conducting independent, high-quality scientific research.

4. Originality of the dissertation

An assessment, with justification, of whether the dissertation provides an original solution to a scientific problem / an original solution to the application of the results of one's own research in the economic or social sphere (suggested approx. 100-250 words)

The thesis offers a clearly original contribution to the field of biomedical sciences by addressing a novel and clinically relevant research question: how long-term copper overload affects pancreatic function, particularly in the context of digestive enzyme production. While copper metabolism and its role in liver and neurological disorders are relatively well-studied, the effects of copper accumulation on the pancreas remain poorly understood. By employing both a genetic model of Wilson's disease (tx-J mice) and a chronic exposure model using copper chloride injections, the candidate provides a novel contribution to understanding of the pathological consequences of copper overload.
The originality of the research lies not only in its focus on an understudied organ in Wilson's disease but also in the multi-level approach used—integrating histological, biochemical, and molecular analyses with functional assessments of calcium signaling in pancreatic acinar cells. The finding that copper overload disrupts amilase production and calcium homeostasis in the pancreas contributes valuable new insights into the systemic manifestations of Wilson disease. These results may open new avenues for understanding digestive complications (like pancreatitis) in patients and could be helpful for future therapeutic or diagnostic strategies related to metal-induced pancreatic dysfunction.

5. Questions and/or criticisms to which the Reviewer expects the candidate to respond during the defence

1) How might the findings in murine models translate to human physiology and pathology, particularly in Wilson's disease and other copper-overload conditions such as MEDNIK and KIDAR syndromes?

2) How are pancreatic damage mechanisms activated in the absence of detectable copper accumulation in the organ?

3) How might liver and pancreatic damage be interconnected in the context of inter-organ communication during Wilson's disease?

4) How might copper overload affect other pancreatic cell populations, particularly the endocrine cells residing in the islets of Langerhans?

5) Could the observed changes in calcium signaling be secondary to systemic inflammation or metabolic disturbances, rather than a direct effect of copper?

6) Can copper directly influence calcium-regulating proteins such as ORAI1 and STIM1?

7) A specific criticism concerns the description of ATP7A and ATP7B trafficking in the introduction, particularly regarding the role of clathrin in this process. This aspect should be clarified and will be addressed during the defense.

6. Other observations on the content or form of the dissertation (optional)

The thesis is clearly written, well-structured, and effectively supported by relevant figures and tables. The abstract provides a concise and accurate summary of the main objectives and key findings. While minor editorial improvements in grammar and phrasing could enhance the overall readability, they do not detract from the scientific quality or integrity of the work. One technical issue to address is the absence of scale bars in the legends of figures containing microscopy images. This omission should be corrected during the final thesis revision to ensure full clarity of the visual data.

I, hereby, declare that the reviewed PhD thesis by Daria Krzysztofik meets the criteria pursuant to art. 187 of Act of 20 July 2018 The Law on Higher Education and Science (Journal of Laws of 2018, item 1668, as amended) and request that the Research Discipline Council of Biological Sciences of the Jagiellonian University in Kraków accepts Daria Krzysztofik for further stages of doctoral proceedings in the field of exact and biological sciences, in the discipline of biological sciences.

YES/NO

I, hereby, request that the thesis is accepted with distinctions

YES/NO

Justification of the request (if YES is selected)
The thesis presents a comprehensive, original, and technically demanding body of work. The candidate has demonstrated independence, intellectual maturity, and active scientific engagement, including international collaboration and multiple peer-reviewed publications. The research significantly contributes to the understanding of trace metal impact on organ physiology and offers potential translational relevance to human disease. These achievements deserve distinction.

04/06/2025
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date

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Reviewer's signature

INFORMATION FOR THE REVIEWER:

A digital copy should be sent to:
nauki.biologiczne@uj.edu.pl

A duly signed original should be sent to:

Rada Dyscypliny Nauki biologiczne
Dziekanat Wydziału Biologii
Uniwersytet Jagielloński w Krakowie
ul. Gronostajowa 7
30-387 Kraków