STATEMENT

by Prof. Yoana Dimitrova Kiselova-Kaneva, PhD

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Member of the Scientific Jury for the procedure for awarding the educational and scientific degree of "Doctor" (PhD) to Stefka Marinova Stoeva at Medical University – Varna, in accordance with Order No. R-109-320/25.09.2026 issued by the Rector of Medical University "Prof. Dr. Paraskev Stoyanov", Varna.

1. Information about the Candidate

Stefka Marinova Stoeva has been a full-time PhD student in Biochemistry, within Higher Education Area 4. Natural Sciences, Mathematics and Informatics, Professional Field 4.3. Biological Sciences, at the Department of Biochemistry, Molecular Medicine and Nutrigenomics, Medical University – Varna, during the period 2021–2026, under the scientific supervision of Assoc. Prof. Milka Asparuhova Nashar, PhD.

She has completed her doctoral studies and has been granted the right to defend her PhD thesis in accordance with Order No. R-100-390/08.07.2026 of the Rector of Medical University "Prof. Dr. Paraskev Stoyanov", Varna.

2. Structure of the PhD thesis

The PhD thesis entitled *"Comparative Study of the Biological Effects of Natural Dietary Sources* of Sulfur on Human Intestinal Cells" comprises 154 pages, illustrated with 73 figures and 6 tables, and includes the following sections: Introduction – 2 pages; Literature Review – 20 pages; Aim and Objectives – 1 page; Materials and Methods – 10 pages; Results – 59 pages; Discussion – 29 pages; Conclusion – 2 pages; Conclusions – 1 page; and Scientific Contributions – 1 page.

The reference list occupies 18 pages and includes 298 cited sources. A list of frequently used abbreviations is provided before the literature review, facilitating reading and understanding of the information presented in the PhD thesis. At the end, a list of scientific publications related to the PhD thesis and conference presentations reporting results obtained within the PhD thesis research is also included.

The PhD thesis is structured in accordance with the relevant requirements and contains all necessary sections, presented with an appropriate level of detail and completeness.

3. Evaluation of the Study Rationale and Literature Review

The literature review is well structured and provides comprehensive information on various natural sources of sulfur. A detailed analysis of the available scientific literature concerning the selected sulfur sources—mineral water from the Varna basin, sulforaphane, alliin, and taurine—has been conducted, addressing various aspects, including biotransformation, safety, molecular mechanisms of action, and others.

Data on their biological effects obtained from *in vitro* studies using cell cultures, animal models, and human intervention studies are presented. A comprehensive analysis of the available evidence regarding their role in regulating processes such as inflammation and maintaining cellular redox homeostasis, as well as their systemic cytoprotective and metabolic effects, has been performed.

Information on their effects on the intestinal epithelium and the gastrointestinal system is also included, supporting the rationale for the PhD thesis topic. In addition, the suitability of the HIEC-6 cell line as a model for screening the biological effects of foods and waters has been evaluated.

The section is illustrated with informative, well-designed summary figures. It concludes with a summary and the formulation of a scientific hypothesis.

4. Evaluation of the Aim and Objectives

The aim of the PhD thesis is clearly defined, and the specific objectives are consistent with it. They are formulated clearly and precisely.

5. Evaluation of the Materials and Methods Used

The description of the materials and methods employed is detailed and clear. The selected analytical techniques are informative and appropriate for addressing the stated objectives. Modern experimental and analytical methods have been applied. The statistical analysis of the obtained results has been performed using appropriate methods.

6. Evaluation of the Results and Discussion

The obtained results are described in detail and illustrated with relevant figures and tables.

Data have been obtained on the effects of the investigated substances and mineral water on cell viability (HIEC-6 cell line) and the expression of target genes in a model of lipopolysaccharide (LPS)-induced inflammation.

Genes associated with various biological processes have been investigated, including the inflammatory response (NF-κB, TNF-α, IL-6, IL-8, and COX-2), including the Caspase-1/IL-1β axis and the endogenous inhibitor IL1RN; antioxidant defense (Nrf2 and GPx); cellular adaptation (Sirt1 and FOXO4); and biotransformation (SULT1A1 and SULT1A3).

Correlation analyses have been performed to assess the relationships between gene expression profiles both in unstimulated cells and under conditions of induced inflammation.

The discussion is comprehensive and structured according to the different groups of results.

7. Evaluation of the Conclusions and Scientific Contributions

Based on the conducted research, the PhD candidate has formulated 8 conclusions, which correspond to the stated objectives and accurately reflect the data obtained from the experimental results.

The scientific contributions are divided into three categories: contributions of an original nature, contributions of an applied nature, and contributions of a confirmatory nature.

8. Fulfilment of the Criteria for Awarding the Educational and Scientific Degree of "Doctor" (PhD)

The PhD candidate is a co-author of two full-text scientific publications in peer-reviewed journals, thereby fulfilling the requirement of 30 points necessary for awarding the educational and scientific degree of "Doctor" (PhD), in accordance with the Academic Staff Development Act of the Republic of Bulgaria and the Regulations for the Development of Academic Staff at Medical University – Varna.

9. Conclusion

The PhD thesis submitted for my evaluation, entitled *"Comparative Study of the Biological Effects of Natural Dietary Sources of Sulfur on Human Intestinal Cells"*, represents a complete scientific investigation addressing a relevant contemporary research topic and presenting significant conclusions and scientific contributions.

In my capacity as a member of the Scientific Jury, appointed by Order No. R-109-320/25.09.2026 of the Rector of Medical University "Prof. Dr. Paraskev Stoyanov", Varna, and in view of the above considerations, I express my support for awarding Stefka Marinova Stoeva the educational and scientific degree of "Doctor" (PhD) in Biochemistry, within Higher Education Area 4. Natural Sciences, Mathematics and Informatics, Professional Field 4.3. Biological Sciences.

06 November, 2026
Varna

Prepared by: .....
(Prof. Yoana Dimitrova Kiselova-Kaneva, PhD)

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