# OPINION

**by Prof. Anelia Veselinova Bivolarska, MD, DSc**
Head of the Department of Medical Biochemistry
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**Regarding:** A doctoral dissertation submitted for the award of the Educational and Scientific Degree of Doctor in the doctoral programme "Biochemistry"

**Dissertation title:**
"Comparative Study of the Biological Effects of Natural Dietary Sources of Sulfur on Human Intestinal Cells"

**Scientific supervisor:** Assoc. Prof. Milka Nashar, PhD

## 1. General Information on the Procedure and the Doctoral Candidate

The procedure has been conducted in accordance with the requirements of the Academic Staff Development Act in the Republic of Bulgaria and the Regulations for its Implementation. By Order No. R-109-320/25 September 2026 of the Rector of the Medical University of Varna, I was appointed as a member of the scientific jury for the procedure for awarding the Educational and Scientific Degree of Doctor to Stefka Marinova Stoeva, a full-time doctoral candidate at the Department of Biochemistry, Molecular Medicine and Nutrigenomics, Faculty of Pharmacy, Medical University of Varna. The candidate was enrolled by Order No. R-109-282/8 July 2021 and was dismissed with the right to defend her dissertation by Order No. R-109-249/20 July 2026.

Stefka Marinova Stoeva is a biochemist at the Medical University "Prof. Dr. Paraskev Stoyanov" – Varna, where she has been working at the Department of Biochemistry, Molecular Medicine and Nutrigenomics since 2001. Her research interests lie in biochemistry, nutrigenomics and phytotherapy, with a particular focus on the biological effects of natural dietary sources of sulfur on human intestinal cells. To date, she has authored six scientific publications, participated in six scientific forums and taken part in four intramural research projects.

## 2. Relevance of the Research Topic

The relevance of the dissertation is determined by the growing scientific interest in the biological effects of natural sulfur compounds and their potential role in regulating inflammatory processes, redox homeostasis and cellular adaptation. The study focuses on a comparative investigation of various dietary sources of sulfur and sulfur-containing mineral waters in human intestinal epithelial cells. Of particular significance is the inclusion of sulfur-containing mineral waters from the Varna Basin, whose biological effects have been relatively little studied at the cellular level.

The experimental approach, which involves assessing cellular responses under basal conditions and following the induction of inflammation, contributes to expanding current knowledge of the biological effects of the investigated substances and provides a basis for further elucidation of the mechanisms through which they influence intestinal cellular homeostasis. In this respect, the research topic is timely and scientifically significant.

## 3. Knowledge of the Research Problem

The literature review demonstrates the doctoral candidate's ability to systematize and analyse current scientific evidence concerning the biological role of sulfur and the effects of natural sulfur compounds on cellular homeostasis. The review systematically addresses the significance of sulfur as a biogenic element, the characteristics of sulfur-containing mineral waters, and the biological effects of selected sulfur-containing compounds of dietary origin, namely sulforaphane, alliin and taurine. Particular attention is paid to their anti-inflammatory and redox-modulating effects, their influence on cell viability and the mechanisms of cellular protection.

The characteristics of the human intestinal epithelial cell line HIEC-6 as an experimental model for investigating the biological effects of dietary components and mineral waters are also discussed. The synthesis of the literature clearly identifies the lack of sufficient comparative studies of the selected substances within a unified cellular experimental system, both under basal conditions and following the induction of an inflammatory response. Based on this analysis, a scientific hypothesis has been formulated to identify similarities and differences in the biological effects of the investigated sulfur sources and the potential mechanisms through which they influence intestinal cellular homeostasis.

## 4. Research Methodology

The experimental methodology involves the study of human intestinal epithelial HIEC-6 cells treated with sulforaphane, alliin, taurine and sulfur-containing mineral water from the Varna Basin. Cell viability was assessed using the MTT assay, and concentrations were selected for subsequent experiments. The effects of the compounds and mineral water were investigated under both basal conditions and conditions of lipopolysaccharide-induced inflammation.

Changes in the expression of genes encoding inflammatory mediators (TNF-α, IL-1β, IL-6, IL-8 and COX-2), regulators of the inflammatory response (IL1RN and NF-κB), factors associated with redox regulation and cellular adaptation (NRF2, GPx, SIRT1 and FOXO4), caspase-1, and phase II biotransformation enzymes (SULT1A1 and SULT1A3) were assessed by quantitative reverse transcription polymerase chain reaction (RT-qPCR). The results were analysed statistically using one-way analysis of variance and Pearson's correlation analysis.

## 5. Description and Evaluation of the Dissertation and Its Contributions

The dissertation comprises 154 pages and follows the standard structure of a scientific study. It contains all the essential components of a completed research work: introduction, literature review, aims and objectives, materials and methods, results, discussion, conclusion, findings and contributions. The bibliography comprises 298 references, providing the theoretical background for the study.

The dissertation is logically structured. The largest proportions of the text are devoted to the sections "Results" (approximately 38%) and "Discussion" (approximately 19%), which is consistent with the experimental and analytical nature of the research. The literature review accounts for approximately 15% of the total length and presents scientific evidence concerning sulfur, sulfur-containing mineral waters, sulforaphane, alliin and taurine. The "Materials and Methods" section accounts for approximately 7% and describes the experimental approach used to investigate HIEC-6 human intestinal epithelial cells under basal conditions and conditions of lipopolysaccharide-induced inflammation.

The contributions are classified as original, applied and confirmatory. The original contributions include the data obtained on the effects of sulfur-containing mineral water from the Varna Basin on the expression of genes associated with the inflammatory response, redox regulation and biotransformation, as well as the molecular effects of alliin. The applied

contribution consists of the development and implementation of a comparative cellular approach for evaluating the four investigated agents. The confirmatory contribution comprises the findings concerning sulforaphane and taurine, which complement the existing literature on their effects on gene expression.

**6. Evaluation of the Publications and the Doctoral Candidate's Personal Contribution**

The publication activity related to the dissertation includes two scientific articles published in international scientific journals in 2022 and 2026. These publications reflect the main research directions: the effects of sulfur-containing mineral water on the expression of phase II biotransformation enzymes in human intestinal cells, and a comparative assessment of the anti-inflammatory effects of mineral water and sulfur-containing bioactive compounds.

The doctoral candidate has also actively participated in scientific forums, with six contributions to national and international scientific events during the period 2023–2026. The research findings have been presented in the form of posters and oral presentations, including at the international ISM Congress in 2026. Particular recognition should be given to the first prize awarded for Best Doctoral Presentation in the Short Communications session at the Ninth Pharmacy Business Forum and Scientific and Practical Conference held in Varna in 2023.

The thematic consistency of the publications and conference presentations demonstrates the candidate's focused research activity in the field addressed by the dissertation, as well as her active involvement in the development, analysis and presentation of the scientific findings.

**7. Abstract**

The dissertation abstract has been prepared in accordance with the established standards for doctoral dissertation abstracts. It comprises 64 pages and presents the main sections of the dissertation in a condensed form. The research methods, principal findings, conclusions and scientific contributions are presented appropriately.

**CONCLUSION**

The dissertation entitled "Comparative Study of the Biological Effects of Natural Dietary Sources of Sulfur on Human Intestinal Cells" contains original scientific contributions, confirmatory findings and applied research results. It meets the requirements of the Academic

Staff Development Act in the Republic of Bulgaria and the Regulations for its Implementation. The scope and content of the dissertation demonstrate a sustained and focused research effort.

In my opinion, the dissertation is a thorough, precise and logically structured piece of research that demonstrates that doctoral candidate Stefka Stoeva possesses the necessary theoretical knowledge and research skills in the doctoral programme "Biochemistry". On the basis of the above, I give my positive assessment of the dissertation and recommend that the distinguished Scientific Jury award Stefka Stoeva the Educational and Scientific Degree of Doctor in Field of Higher Education 4, "Natural Sciences, Mathematics and Informatics", Professional Field 4.3, "Biological Sciences", in the doctoral programme "Biochemistry".

27 October 2026

Plovdiv

**Prepared by:** ...

Заличено на основание чл. 5, §1, б. „В“ от Регламент (ЕС) 2016/679

Prof. Anelia Bivolarska, MD, DSc