

Maciej Serda, PhD, DSc, University Professor

Katowice, 02/10/2025

Institute of Chemistry

University of Silesia in Katowice

REVIEW

of the doctoral dissertation by **Mr. Sattibabu Merugu (MSc)** entitled “*Advancing transcriptomics-based and AOP-anchored predictive models for carbon nanotube inhalation: The case studies on acute phase signaling–driven inflammation*” submitted to the Scientific Council of the Department of Chemistry, University of Gdansk, for the purpose of obtaining the degree of Doctor of Chemical Sciences.

Over the past two decades, the rapid development of nanotechnology has transformed the chemical sciences: in laboratories and daily life alike, we encounter structurally modified nanomaterials engineered with precisely defined physicochemical properties. Within this context, carbon-based nanomaterials have found expanding applications, particularly in nanomedicine and molecular biology. Advanced transfection agents, brain-imaging modalities within near infrared region II, and the photoinactivation of microorganisms have become standard experimental approaches employing carbon nanotubes, fullerenes or carbon dots. This naturally raises a question—familiar from medicinal chemistry—whether we can systematically model their physicochemical properties and evaluate their toxicity in selected biological systems. The doctoral dissertation submitted by Mr. Sattibabu Merugu seeks to address these issues using cheminformatics methodologies.

Formal assessment of dissertation

The reviewed PhD dissertation follows a traditional structure and comprises 142 pages. It opens with a theoretical introduction to the applications of carbon nanotubes and an overview of the mechanisms underlying their toxic effects. The subsequent chapters address the scientific problem

that constitutes the dissertation's central research question and describe chemoinformatic methods used to model the toxicity of various types of carbon nanotubes (single and multiwalled). The work concludes with succinct research findings and a presentation of the key scientific literature (146 citations).

The doctoral dissertation submitted for review contains 19 figures and 12 tables. The figures and charts are carefully prepared; however, the labels could be larger to enhance readability. The PhD dissertation ends with a supplement, containing a summary of the candidate's principal scientific achievements. Mr. Merugu (MSc) documents that he is a co-author of three research articles, two of which appear in journals indexed in *Scopus* and *Web of Science* databases. The studies conducted as part of his doctoral research were published in the highly prestigious journal *Small*, where Mr Merugu is the first author. However, the dissertation supplement does not include co-author contribution statements. Nevertheless, the author-contribution section within the published article in *Small* confirms the candidate's substantial role in the study, which is reflected in his position as first author, as well as contribution statement (main role in: *conceptualization, designing the experiments, performing calculations and writing the manuscript*).

Substantive Assessment of the Doctoral Dissertation

In the first chapter of the dissertation, Mr. Merugu describes the structure and physicochemical properties of various types of carbon nanotubes, including multi-walled (MWCNTs) and single-walled (SWCNTs) forms, which underpin the further applications of the resulting nanomaterials. The author effectively prepares the reader to understand the potential key molecular descriptors that influence the toxicity of carbon nanotubes. It is worth emphasizing that the author navigates biomedical topics competently, indicating plausible causes and mechanisms of carbon nanotube toxicity. In the final two subsections of the introduction, the author presents chemoinformatics methods used to model the biological properties of nanomaterials, including the fundamentals of the NANO-QSAR approach, the Adverse Outcome Pathway (AOP) framework, and the descriptors employed to characterize the modeled nanomaterials.

Subsequently, the author outlines the detailed aims of the study, formulated around three research

hypotheses supported by three research objectives. These include the premise that the structural properties of MWCNTs determine their biological effects, whereas metallic impurities may exacerbate inflammatory states and thereby increase pulmonary toxicity. One of the key objectives of the PhD dissertation is to identify the structural features of multi-walled carbon nanotubes that, upon inhalation, perturb the acute-phase response (AR) signaling pathway across a panel of 14 MWCNTs—responses associated with inflammation that may lead to lung fibrosis and/or cardiovascular pathologies.

In Chapter 3 of the doctoral dissertation (Research Methodology), the author details the sources from which the experimental data for the *in vivo* studies were obtained, as well as the transcriptomic datasets—commendably sourced from well-recognized international research teams in Denmark and Canada. The author also specifies the computational tools and software libraries employed in the simulations. However, I did not find information regarding the carbon nanotubes themselves, including the synthesis method and the analytical techniques used to determine metallic impurities.

The Results and Discussion section opens with a description of the physicochemical parameters of the multi-walled carbon nanotubes (MWCNTs), linked to the toxicity metric BMDL (benchmark dose lower confidence limit). Based on these experimental data, the author defines training and validation sets, and in a subsequent table compares experimentally observed toxicity with model-predicted values, including relevant statistical parameters. The author discusses each result as it is presented, drawing inferences about the critical descriptors (or descriptor groups) that govern the toxicity of the investigated nanomaterials. The dissertation concludes with rather concise final remarks that, importantly, acknowledge the limitations of both the data and the developed model.

My review of the dissertation prompts the following points, offered as a starting point for discussion with the Doctoral Candidate:

- In the introduction section, the dissertation lacks a description of the protein corona that forms on carbon nanotubes, including its structure and composition. Which proteins are most prominently adsorbed? Conceptually, if intracellular carbon nanotubes are uniformly coated with proteins, how valid or informative are the reported calculated/tested descriptors?

- The author should at least briefly discuss the rapidly advancing methodologies for dispersing carbon nanotubes in aqueous media, for example by complexation with oligonucleotides.
- Only 14 MWCNTs were used to model their activity; manual 80/20 split with $n=3$ in validation might be a fragile and susceptible to optimistic estimates. Did the author consider replacement with nested cross-validation or repeated K-fold plus a leave-one-material-out analysis?
- In the Methodology and Research chapter, the author stated an intention to compare the toxicological properties of the studied multi-walled and single-walled carbon nanotubes (MWCNTs/SWCNTs). However, the exact composition of the nanotube mixtures is not specified (it is a complex “soup” of differing chiralities and numbers of walls).
- The doctoral candidate relies exclusively on data provided by the research groups from Canada and Denmark. Why were the results not compared with *in vivo* findings reported in the scientific literature? Such a comparison might have enabled the construction of a larger dataset.
- The scientific literature describes numerous studies of separating chiral single-walled carbon nanotubes. Are there any differences in their toxicity also reported? For example, (6,5)-SWCNTs vs (7,5)-SWCNTs?
- In what units is the concentration of metallic impurities expressed (Table 7)?

The results presented by the Doctoral Candidate are highly interesting and innovative, constituting a significant step toward the optimization of carbon nanotube structures and a deeper understanding of their toxicity. These findings are particularly significant, especially as nanomedicine research is entering clinical studies; consequently, computational modeling of nanomaterial toxicity is becoming a critical concern not only for the scientific community but also from the patient perspective. The research problem articulated in the dissertation, the methodological framework employed, and the Candidate's capacity for analyzing the obtained data attest to the work's high scholarly standard and originality. The doctoral dissertation submitted by Mr. Sattibabu Merugu **meets the requirements** set out in Article 192(2) of the Act of 20 July 2018—Law on Higher Education and Science (consolidated text: Journal of Laws of 2023, item 742, as amended).

Accordingly, I request that the Scientific Council of the Faculty of Chemistry University of Gdańsk, **admit** Sattibabu Merugu (MSc) to the further stages of the proceedings for the conferment of the doctoral degree.