Whiteout safety assessment of food additive (E171) using in vitro approaches and validation in a human dietary intervention study

Chapter 11

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The research encompassed in this thesis directly addresses critical societal and health concerns regarding the safety of food-grade titanium dioxide (E171) when used as a food additive. Our findings contribute to a better understanding of potential risks arising from dietary E171 exposure, the benefits of protecting and maintaining human health, and possible public health burdens. The findings presented in this thesis underscore a potential health risk associated with dietary exposure to E171, highlighting its role in the deterioration of colorectal health, eventually increasing colorectal cancer (CRC) risk. The growing consumption of ultra-processed foods in industrialized nations, including E171, has been linked to a rise in CRC prevalence. This thesis significantly enhances the understanding of potential health implications by providing human-relevant data demonstrating physiological and molecular changes in the gastrointestinal (GI) tract associated with E171 exposure. It directly addresses consumer concerns about the increased use of food additives, particularly those that do not add any nutritional value to the added products. Preventive actions can be taken by identifying E171 as a potential contributor to increased CRC risk. CRC is one of the most common cancers in the world, contributing to a significant amount of health burden added to society in terms of lost life, but also medical treatment-associated costs. This research emphasizes the importance of rigorous risk assessment to safeguard public health and reduce public health burden in light of societal trends favoring unprocessed and fresh foods that contribute to better health. Besides the addressed health impact, other societal concerns, such as ethical and animal-free testing approaches, have been successfully deployed in this thesis, demonstrating alternative, human-centered, and potentially more sensitive ways to facilitate hazard identification, characterization, and risk assessment.

This thesis furthermore provides scientific evidence and methodological advances grounded in cutting-edge scientific methodologies that extend beyond the conventional deployed toxicology models. Conventional toxicology models often struggle to detect subtle or long-term effects of low-dose exposure. By employing innovative, animal-free approaches, such as iPSC-derived colon organoids and advanced *in vitro* models for GI digestion (TIM-1, TIM-2), this research achieves a high degree of translatability for human conditions. These models enable a precise assessment of physiological and molecular mechanisms that occur in response to toxic exposure and can be deployed beyond the framework of assessing E171's toxicity. The consequently executed studies provide a pipeline for any substance exposure along the oro-gastrointestinal route and cover digestive processes, interactions with the microbiome, and classical toxicology endpoints such as cytotoxicity, genotoxicity, ROS production, or gene expression changes. Complementary human data was generated via a human dietary intervention study to validate the findings *in vitro* and provide much-needed evidence in humans about the potential health implications following the ingestion of E171. This thesis offers robust evidence and extensive datasets that establish a link between E171 exposure

and oxidative stress, DNA damage, and cancer-related transcriptome changes. The summary of the scientific findings presented in this thesis addresses scientific knowledge gaps identified in the past. It advances the scientific basis for assessing food additive toxicity within realistic dietary contexts.

The results from this research are relevant to regulatory decision-making, particularly in support of the European Union ban on E171, based on the precautionary principle (PP). This research strengthens the scientific foundation for this decision by offering comprehensive human-relevant data highlighting potential health risks imposed by dietary consumption of E171. These findings are essential for validating existing regulatory measures and informing global policies in regions that still allow E171 as a food additive without restrictions. Beyond Europe, the generated data can serve as valuable input for regulators and risk assessors, public policymakers, and consumer protection agencies to advocate for stricter measures regarding its use or labeling on products. Pervasive arguments dismissing the potential health implications of dietary E171 exposure, such as the effects of the GI digestive system and the presence of a food matrix on possible adverse effects, have been addressed and incorporated into the experimental designs presented in this thesis. Neither the food matrix nor the GI digestive completely negated any adverse effects in humans; contrary, findings with and without digested E171 show similarities in gene expression changes *in vitro* and humans. The scientific approach in this thesis supports a transition towards next-generation risk assessment (NGRA) by providing a replicable, human-centered framework for evaluating food additives and other nanomaterials exposed to the GI system. Furthermore, the study exemplifies how new approach methodologies (NAMs) can be successfully implemented in the hazard identification, characterization, and risk assessment process by replacing traditional animal testing, aligning with regulatory practices, and modern ethical standards that urge to move away from animal testing and increase scientific standards.

The economic impact of this research extends across multiple industries, particularly the food and, eventually, the pharmaceutical sectors. A potential ban on E171 globally could disrupt a multi-billion-dollar industry, necessitating the reformulation of numerous food products. The transition to alternative whitening and anti-caking agents, such as calcium carbonate or rice starch, presents challenges and opportunities for innovation in safer and more sustainable ingredient developments. While initially, a reformulation will come at substantial costs, the shift could drive a long-term benefit through enhanced consumer trust and compliance with evolving safety standards.

On the other hand, the replacement of E171 in the pharmaceutical industry might pose a more substantial issue. E171 is used as a whitening and UV-protecting agent, highlighting even more complex implications for this industrial sector. If E171 is no longer considered safe as currently used in pharmaceuticals, a reformulation of pharmaceuticals to exclude E171 would require extensive

research and testing to ensure the safety and efficacy of the newly formulated drugs and include the alternative formulations' pharmacokinetics. Such changes could increase production costs, cause supply chain issues, and lead to longer manufacturing timelines. These changes might particularly affect generic drug production since it relies heavily on cost-effective and high-volume productions. Balancing the potential health risk of E171 exposure against these economic considerations will be crucial for stakeholders and requires a thoroughly nuanced risk assessment approach.

This thesis provides critical insights into the health, societal, regulatory, and economic dimensions of E171's use as a food additive. By advancing scientific and methodological approaches, this thesis generated robust human-relevant data. It addresses pressing societal and regulatory needs while aligning with ethical imperatives. These findings strengthen the case for a more stringent regulatory framework regarding E171's use as a food additive and highlight broader implications of food safety as an issue of public health, industrial practice, and consumer trust. This research lays a foundation for future scientific efforts to evaluate food additives, nanomaterials, and other ingested materials, shaping the next generation of toxicological risk assessment.