



HAL
open science

Validating intestinal effects of food-grade titanium dioxide using a murine gut organoid model as alternative to in vivo models

Yann Malaisé, Lauris Evariste, Aurélie Pettes-Duler, Eva Casale, Christel Cartier, Eric Gaultier, Natália Martins Breyner, Bruno Lamas, Eric Houdeau

► To cite this version:

Yann Malaisé, Lauris Evariste, Aurélie Pettes-Duler, Eva Casale, Christel Cartier, et al.. Validating intestinal effects of food-grade titanium dioxide using a murine gut organoid model as alternative to in vivo models. The XVith International Congress of Toxicology, Sep 2022, Maastricht, Netherlands. hal-03775518

HAL Id: hal-03775518

<https://hal.inrae.fr/hal-03775518v1>

Submitted on 12 Sep 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Validating intestinal effects of food-grade titanium dioxide using a murine gut organoid model as alternative to *in vivo* models

Y. Malaisé¹, L. Evariste¹, A. Pettes-Duler¹, E. Casale¹, C. Cartier¹, E. Gaultier¹, N. Martins Breyner¹, B. Lamas¹, E. Houdeau¹

¹ *Toxalim UMR1331 (Research Centre in Food Toxicology), Toulouse University, INRAE, ENVT, INP-Purpan, UPS, Toulouse, France*

Background: Nanoparticles (NPs) found in the human diet mainly originate from inorganic food additives, often used *quantum satis* in common foodstuff, which raises public health concerns due to daily exposure. The whitener and opacifying agent titanium dioxide (TiO₂, E171 in EU) is one of the most studied nanomaterial, evoking inflammatory responses and precancerous lesions in the rodent intestine. Investigating the potential hazards of chronic oral exposure to NPs is often time-consuming and requires animal models, specific spaces and skills. However, recent technical advances in stem cells and three-dimensional cultures allowed the use of organoids as an alternative model to *in vivo* experiments. Herein we used murine intestinal organoids to characterize intestinal impacts of food-grade TiO₂ in comparison to already reported *in vivo* data, and to validate organoids as a reliable model for studying the effects of foodborne NPs in the gut.

Methods: Three different wild-type C57bl/6 mice were used for small intestine collection. Intestinal crypts were purified, dissociated, and cells were cultured for organoid growth. After 4 passages, organoids were dissociated and seeded as a 2.5D culture, then exposed to 0.1, 1, 10 or 100µg/ml of E171 for 24h. Supernatants were collected, and cytotoxicity assessed by LDH release quantification. Total RNA was extracted from samples and analyzed for cell proliferation and differentiation, genotoxicity, antimicrobial peptides, permeability, oxidative stress, Toll Like Receptors (TLR), NFκB, cytokine and chemokine gene expressions by qPCR. Cell apoptosis was also evaluated by cleaved Caspase-3 quantification using immunofluorescence.

Results: Gut organoids exposed to E171 showed a dose-dependent up-regulation of the cell proliferation marker *Mki67* together with increased protein expression of cleaved-Caspase-3, suggesting epithelium renewal or restructuring. This occurred in parallel to a decreased expression of the enterocyte differentiation markers *Alpi* and *Krt20* as well as up-regulation of the neuroendocrine marker *Chga*. Moreover, food-grade E171 decreased gene expression of antimicrobial peptides (*Lyz*, *Reg3b*, *S100a8*) and tight junction proteins (*F11r*, *Tjp1*, *Ocln*, *Cldn7*, *Cldn15*), suggesting altered epithelial secretion and permeability. We also showed that the TLR4-NFκB pathway was negatively impacted in a dose-dependent manner, while oxidative stress, cytokine and chemokine gene expressions remained unaltered. Although E171 exposure was not cytotoxic, TiO₂ increased expression of *gadd45a* at low dose (i.e. 1µg/ml), suggesting DNA damage.

Conclusions: Taking together, a 24h-exposure of murine intestinal organoids to food-grade TiO₂ impacts epithelial barrier integrity (cell proliferation and differentiation, gut permeability, genotoxic effect) and antimicrobial defenses as reported *in vivo* in rodent models, hence validating the use of intestinal organoids for toxicological studies of foodborne NPs.