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### ► To cite this version:

William Dufefoi, Kristy Moniz, Emma Allen-Vercoe, Marie-Hélène Ropers, Virginia K. Walker. Testing food grade and nano-TiO<sub>2</sub> on a defined human intestinal community. 11. International Conference on the Environmental Effects of Nanoparticles and Nanomaterials (ICEENN 2016), Aug 2016, Golden, United States. 2016. hal-02793405

**HAL Id: hal-02793405**

**<https://hal.inrae.fr/hal-02793405v1>**

Submitted on 5 Jun 2020

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# Testing food grade and nano-TiO<sub>2</sub> on a defined human intestinal community

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Titanium dioxide is a white metal oxide, which is commonly used as a pigment in coatings of candies and chewing-gum. Food-grade TiO<sub>2</sub>, referred to as E171 in Europe and INS171 in North America, includes a nano-sized fraction, representing up to 44% of the particles. Due to concerns about TiO<sub>2</sub> nanoparticles (NPs) as potentially hazardous, at least by inhalation, the toxicity of ingested TiO<sub>2</sub> NPs are currently under investigation. However, the impact of confectionary titania have yet to be determined.

We used a defined gut bacterial community, MET-1 (microbial ecosystem therapeutic-1), as a model human intestinal community. The anaerobic consortium containing 33 bacterial species was batch cultured (n=30) for 48 h at 37°C in a starch-based medium. Food-grade TiO<sub>2</sub> from several suppliers were used to amend the cultures at two realistic concentrations (based on a single unit of gum; 100-250 ppm). In addition, purchased TiO<sub>2</sub> NPs (25 nm; P25) were used. The impact of the additives was assessed with physiological, biochemical and molecular assays. Gas production was monitored using gas chromatography, and fatty acid methyl ester (FAME) analysis used the MIDI Sherlock Microbial Identification System protocol. DNA analysis included polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) and 16S ribosomal RNA gene fragment 454-pyrosequencing.

Our results showed that TiO<sub>2</sub> particles had no impact on gas production nor on fatty acid composition. Only a food grade sample (n°1) induced a small variation in culture gas composition, when tested at 250 ppm (p<.05) and this, as well as P25 particle controls, resulted in a limited shift in the saturated fatty acid composition (12:00 and 14:00, p<.05). PCR-DGGE profiles and phylogenetic distributions obtained from 454 pyrotag 16S rRNA gene sequencing confirmed a modest impact on the bacterial community (food grade n°1 and P25), with a significant decrease in sequences corresponding to the dominant *Bacteroides ovatus* (-10%) in favor of *Clostridium. cocleatum* (+10%; p<0.05).

Despite these minor shifts in the relative abundance of two members of the model gut consortium, taken together, we believe that food grade titania and TiO<sub>2</sub> NPs particles do not have a major impact on the human gut microbiota when tested at realistic concentrations.

## References

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## Acknowledgements

This work has been carried out in the framework of the Labex Serenade (ANR-11-LABX-0064) and of the

A\*MIDEX project (ANR-11-IDEX-0001-02), funded by the «Investissements d’Avenir» French Government program managed by the French National Research Agency (ANR). VKW was funded by the Natural Sciences and Engineering Research Council (Canada).

We thank G. Cairns (Analytical Services Lab, Queen’s University), C. Carlucci and P. Das, for providing the initial help with the gas chromatography, the MET-1 community, and helpful assistance.

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