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Human TR146 cells and pig buccal mucosa to assess oral transmucosal passage and buccal toxicity of food-grade titanium dioxide

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Background: Today, the use of titanium dioxide (TiO_2) as food additive (E171) has been banned by the European Commission, due to concerns for human health based on studies showing TiO_2 particles systematically available, tissue accumulation, a genotoxic risk and possible promotion of precancerous lesions. However, E171 is still present in toothpastes and pharmaceutical tablets as a whitening powder mixing nano- and submicronic particles. Risk assessment of TiO_2 intake by oral route is mainly based on the assumption that particles are mainly absorbed by the intestine. However, while the buccal mucosa is the first exposed area, the possibility of an oro-transmucosal passage has not been documented so far. In order to gain insight on possible adverse effects for human health associated to E171 buccal exposure, we analyzed *in vivo* the translocation of TiO_2 (E171) in the buccal mucosa of pigs used as human mouth model. Moreover, we evaluated *in vitro* the particle translocation on human buccal TR146 cell line, and measured cytotoxic and genotoxic effects on proliferative and differentiated epithelial cells.

Methods & Results: Under realistic exposure conditions with 50 µg/ml of food-grade TiO_2 in water suspension (size distribution 20-440 nm; mean size of 105 nm) deposited under the tongue of pigs, TEM-EDX data revealed the presence of small aggregates of TiO_2 particles translocated into the buccal mucosa from 30 minutes of exposure, reaching submaxillary lymph nodes after 4 hours. In human TR146 cells exposed to E171, kinetic analysis using confocal, TEM and SIMS imaging showed progressive and large uptake of isolated or small aggregates of both submicronic and nanosized particles, showing high permeability capacity. At 2h of E171 exposure, cytotoxicity, genotoxicity and oxidative stress were investigated on both proliferative or differentiated TR146 cells, in comparison with two TiO_2 size standards of 115 nm and 21 nm in diameter. All tested TiO_2 particles were reported cytotoxic on proliferative TR146 cells, and this effect was almost abolished following differentiation. Oxidative stress and genotoxicity assessed through γH2AX and 53BP1 foci formation and comet assay were only reported for E171 sample and TiO_2 particles of 115 nm, suggesting the particles above 20 nm responsive of these effects, and mainly on proliferative cells.

Conclusions: Taken together, these results show *in vivo* and *in vitro* that the buccal mucosa is an important absorption route for systemic passage of foodborne TiO_2 (E171) particles. In human cells, uptake

of TiO_2 particles was cytotoxic without size effects, while they generate further oxidative and genotoxic stresses in proliferative buccal cells, that could impair epithelium renewal in the mouth. Altogether, these data emphasize that buccal exposure should be considered for toxicokinetic and risk assessments of TiO_2 in human when used as food additive, including in toothpaste and pharmaceutical formulations.