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Recent academic publications on adverse effects of E171 and/or TiO_2 nanoparticles via oral exposure

11 September 2019

Below is a (non comprehensive) list of the most recent scientific articles in addition to those taken into account by ANSES in its "**Opinion on the risks associated with ingestion of the food additive E171"** published in April 2019.

- **Gender difference in hepatic toxicity of titanium dioxide nanoparticles after subchronic oral exposure in Sprague-Dawley rats, Chen Z et al., *Journal of Applied Toxicology*, 2019**

  The study examined female and male Sprague-Dawley rats administrated with TiO2 NPs orally at doses of 0, 2, 10 and 50 mg/kg body weight per day for 90 days ; it found **significant hepatic toxicity** that could be induced by **subchronic oral exposure to TiO2 NPs**, which was **more obvious and severe in female rats** and caused through indirect pathways

- **Hepatic and Renal Toxicity Induced by TiO2 Nanoparticles in Rats: A Morphological and Metabonomic Study, Valentini, X et al., *Journal of Toxicology*, 2019 :**

  Rats were exposed to different doses of TiO_2nanoparticles and sacrificed, respectively, 4 days, 1 month, and 2 months after treatment. Dosage of TiO_2 in tissues was performed by ICP-AES and revealed **an important accumulation of TiO_2 in the liver**. The nanoparticles induced **morphological and physiological alterations in liver and kidney.** In the liver, these alterations mainly affect the hepatocytes located around the centrilobular veins. These cells were the site of an oxidative stress evidenced by immunocytochemical detection of 4-hydroxynonenal (4-HNE). Kupffer cells are also the site of an important oxidative stress following the massive internalization of TiO_2nanoparticles. Enzymatic markers of liver and kidney functions (such as AST and uric acid) are also disrupted only in animals exposed to highest doses. The metabonomic approach allowed us to detect modifications in urine samples already detectable after 4 days in animals treated at the lowest dose. This metabonomic pattern testifies an **oxidative stress as well as renal and hepatic alterations.**

- **In vitro intestinal epithelium responses to titanium dioxide nanoparticles, Pedata P et al., *Food Research International*, 119 : 634-642, 2019 :**

  The well-established Caco-2 cell line differentiated for 21 days on permeable supports was used as a predictive model of the human intestinal mucosa to identify the biological response triggered by TiO2 particles. Exposure to 42 µg/mL TiO2 nanoparticles disrupted the tight junctions-permeability barrier with a prompt effect detectable after 4 h incubation time and wide effects on barrier integrity at 24 h. Transport and ultrastructural localization of TiO2 nanoparticles were determined by ICP-OES, TEM and ESI/EELS analysis, respectively. Nano-sized particles were efficiently internalized and preferentially entrapped by Caco-2 monolayers. Storage **of TiO2 nanoparticles inside the cells affected enterocytes viability and triggered the production of pro-inflammatory cytokines**, including TNF-α and IL-8. Taken together these data indicate that **nano-sized TiO2 particles exert detrimental effects on the intestinal epithelium layer.**

- **Exposure to Titanium Dioxide Nanoparticles During Pregnancy Changed Maternal Gut Microbiota and Increased Blood Glucose of Rat, Mao Z et al., *Nanoscale Research Letters*, 14:26, 2019 :**

  Our study pointed out that TiO2 NPs induced the alteration of gut microbiota during pregnancy and increased the fasting blood glucose of pregnant rats, which might **increase the potential risk of gestational diabetes of pregnant women.**

- **The food additive E171 and titanium dioxide nanoparticles indirectly alter the homeostasis of human intestinal epithelial cells in vitro, Dorier M et al., *Environ. Sci.: Nano*, Advance Article, 2019 :**

  Epithelial cells repeatedly exposed to TiO2 developed an inflammatory profile, together with increased mucus secretion. Epithelial integrity was unaltered, but the content of ATP-binding cassette (ABC) family xenobiotic efflux pumps was modified. Taken together, these data show that **TiO2** moderately but **significantly dysregulates several features that contribute to the protective function of the intestine**."

- **Impacts of Additive Food E171 (Titanium Dioxide) on the Gut Microbiota and Colorectal Carcinogenesis in ApcMIN/+ Murine Model, Brugiroux S et al., *Gastroenterology*, 156, (6), 1 : S-679, 2019 :**

  Additive E171 promotes colonic tumorigenesis and induces change in gut microbiota composition. Underlying carcinogenic mechanisms focusing on microbiota dysbiosis implication are in progress. This study supports the **carcinogenic properties of TiO2 in the context of colorectal cancer**.

- **Repeated administration of the food additive E171 to mice results in accumulation in intestine and liver and promotes an inflammatory status, Talamini L et al., *Nanotoxicology,* 2019 :**

  Repeated oral administration of E171 to mice at a dose level (5 mg/kg body weight for 3 days/week for 3 weeks) comparable to estimated human dietary exposure, resulted in TiO2 deposition in the liver and intestine; **titanium accumulation in liver** was associated with **necroinflammatory foci containing tissue monocytes/macrophages**; three days after the last dose, **increased superoxide production and inflammation** were observed **in the stomach and intestine**. Overall, the present study indicates that the risk for human health associated with dietary exposure to E171 needs to be carefully considered".

- **Gestational exposure to titanium dioxide nanoparticles impairs the placentation through dysregulation of vascularization, proliferation and apoptosis in mice, Zhang L et al., *Int J Nanomedicine*, 13: 777–789, 2018 :**

  Gestational exposure to TiO_2 NPs significantly impairs the growth and development of placenta in mice, with a mechanism that seems to be involved in the dysregulation of vascularization, proliferation and apoptosis.

- **Assessment of titanium dioxide nanoparticles toxicity via oral exposure in mice: effect of dose and particle size, Ali SA et al., *Biomarkers*, 24(5) : 492-498 , 2019 :**

  The effect of five days oral administration of TiO2NPs (21 and 80 nm) with different doses was assessed in mice via measurement of **oxidative stress markers; glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA) and nitric oxide (NO), liver function indices; aspartate and alanine aminotransferases (AST and ALT), chromosomal aberrations and liver histopathological pattern**. The results **revealed drastic alterations** in all the measured parameters and showed positive correlation with the gradual dose increment. In addition, **the smaller particle size of TiO2NPS (21 nm) had more adverse effect** in all the selected biochemical parameters, genetic aberrations and histological investigations.
  Toxicity of TiO2NPs increases in a dose-dependent manner and vice versa with particles size. The evaluated biomarkers are good indicators for TiO2NPs toxicity. **More detailed studies are required before the recommendation of TiO2NPS as food additives.**

- **Genotoxicity analysis of rutile titanium dioxide nanoparticles in mice after 28 days of repeated oral administration, Manivannan J et al., *The Nucleus*, 1-8, 2019**

  In this study Swiss albino male mice were gavaged TiO_2-NP at sub-acute concentration (0.2, 0.4 and 0.8 mg/kg body weight) over a period of 28 days. Results revealed that TiO_2-NP administered was of rutile form with mean average size of 25 nm by transmission electron microscopy. The values of PDI and Zeta potential from DLS of TiO_2-NP in suspension specified that the nanomaterial was stable without much agglomeration. Chromosomal aberration assay showed that **TiO_2-NP** was **genotoxic and cytotoxic**. DNA damage evaluation by comet assay confirmed that **long term exposure to TiO_2-NP at low concentrations can induce genotoxicity systemically in organs, such as liver, spleen, and thymus cells**. Structural chromosomal aberration test from bone marrow cells revealed the **clastogenicity of TiO_2-NP at sub chronic low concentrations**.

- **Titanium dioxide nanoparticles tested for genotoxicity with the comet and micronucleus assays *in vitro*, *ex vivo* and *in vivo*, Kazimirova A et al., *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 2019**

  The genotoxicity of TiO2 nanoparticles (NPs) was assessed with the cytokinesis-block micronucleus (CBMN) assay in TK6 lymphoblastoid cells, lymphocytes from human volunteers, and bone marrow erythrocytes from rats exposed in vivo; and with the comet assay (detecting both strand breaks and oxidised purines) in human and rat peripheral blood mononuclear cells (PBMCs). NPs were dispersed using three different methods giving different size distribution and stability.
  On average, TiO2 NPs caused no increase in micronuclei in TK6 cells, rat bone marrow erythrocytes or human lymphocytes (though lymphocytes from 3 out of 13 human subjects showed significant increases).
  PBMCs from rats treated in vivo with a single dose of NPs dispersed by a method with low agglomeration showed an increase in strand breaks after 1 day.
  TiO2 NPs dispersed in a stable, non-agglomerated state induced DNA strand breaks at 75 µg/cm2 after 4 h exposure of human PBMCs and at 15 µg/cm2 and 75 µg/cm2after 24 h exposure, but no increase in DNA oxidation was seen. Overall, NPs in an agglomerated state did not cause DNA damage. However, at the individual level, **significant increases in strand breaks were seen in PBMCs from most of the volunteers**. Cells from one volunteer showed positive effects in all conditions and both tests, while cells from another volunteer appeared to be completely resitant to TiO2 NPs. The implication is that **some individuals may be more sensitive than others to effects of this nanomaterial.**
  Differences seen in results obtained with the micronucleus and the comet assay may be due to the mechanisms underlying the genotoxic effects of TiO2 NPs and the different endpoints represented by the two assays.

- **Impact of the Food Additive Titanium Dioxide (E171) on Gut Microbiota-Host Interaction, Pinget G et al., *Front. Nutr.*, 2019**

  We investigated the impact of food grade TiO_2 on gut microbiota of mice when orally administered via drinking water. While TiO_2 had minimal impact on the composition of the microbiota in the small intestine and colon, we found that **TiO_2 treatment could alter the release of bacterial metabolites *in vivo* and affect the spatial distribution of commensal bacteria *in vitro* by promoting biofilm formation. We also found reduced expression of the colonic mucin 2 gene**, a key component of the intestinal mucus layer, **and increased expression of the beta defensin gene, indicating that TiO_2 significantly impacts gut homeostasis. These changes were associated with colonic inflammation**, as shown by decreased crypt length, **infiltration of $CD8^+$ T cells, increased macrophages as well as increased expression of inflammatory cytokines**. These findings collectively show that **TiO_2 is not inert, but rather impairs gut homeostasis which may in turn prime the host for disease development.**

- **The mechanism-based toxicity screening of particles with use in the food and nutrition sector via the ToxTracker reporter system, Brown DM et al., *Toxicol. In Vitro*, 4;61, 2019 :**

  The rapid expansion of the incorporation of nano-sized materials in consumer products overlaps with the necessity for high-throughput reliable screening tools for the identification of the potential hazardous properties of the nanomaterials. The ToxTracker assay (mechanism-based reporter assay based on embryonic stem cells that uses GFP-tagged biomarkers for detection of DNA damage, oxidative stress and general cellular stress) is one such tool, which could prove useful in the field of particle toxicology allowing for high throughput screening. Here, ToxTracker was utilised to **evaluate the potential hazardous properties** of two particulates currently used in the food industry (vegetable carbon (E153) and **food-grade TiO_2 (E171)**). Due to the fact that ToxTracker is based on a stem cell format, it is crucial that the data generated is assessed for its suitability and comparability to more conventionally used relevant source of cells - in this case **cells from the gastrointestinal tract and the liver**. Therefore, the cell reporter findings were compared to data from traditional assays (cytotoxicity, anti-oxidant depletion and DNA damage) and tissue relevant cell types**. The data showed E171 to be the most cytotoxic, decreased intracellular glutathione and the most significant with regards to genotoxic effects**. The ToxTracker data showed comparability to conventional toxicity and oxidative stress assays; however, some discrepancies were evident between the findings from ToxTracker and the comet assay.

- **Food-grade titanium dioxide (E171) by solid or liquid matrix administration induces inflammation, germ cells sloughing in seminiferous tubules and blood-testis barrier disruption in mice, Rodríguez-Escamilla JC et al., *Journal of applied toxicology*, 2019 :**

  We aimed to compare the effects of E171 consumption in a solid matrix (0.1%, 0.5% and 1% in pellets) and liquid suspension (5 mg/kg body weight) on testis structure, inflammation infiltrate and blood-testis barrier disruption of male BALB/c mice. Results showed that an **increase in germ cell sloughing and the infiltrate of inflammatory cells in seminiferous tubules, together with disruption of the blood-testis barrier** were similar in testis of both groups even if the dose received in mice in liquid matrix was 136 or 260 times lower than the dose reached by oral intake in solid E171 pellets in 0.5% E171 and 1% E171, respectively. This study highlights the attention on matrix food containing E171 and **possible adverse effects on testis when E171 is consumed in a liquid matrix**".

- **Telomere length and genotoxicity in the lung of rats following intragastric exposure to food-grade titanium dioxide and vegetable carbon particles, Jensen DM et al., *Mutagenesis*, 29;34(2):203-214, 2019 :**

  Vegetable carbon (E153) and titanium dioxide (E171) are widely used as black and white food colour additives. The aim of this study was to assess gastrointestinal tight junction and systemic genotoxic effects in rats following exposure to E153 and E171 for 10 weeks by oral gavage once a week. The expression of tight junction proteins was assessed in intestinal tissues. Levels of DNA strand breaks, oxidatively damaged DNA and telomere length were assessed in secondary organs. Hydrodynamic suspensions of E153 and E173 indicated mean particles sizes of 230 and 270 nm, respectively, and only E153 gave rise to intracellular production of reactive oxygen species in colon epithelial (Caco-2) cells. **Rats exposed to** E153 (6.4 mg/kg/week) or **E171 (500 mg/kg/week) had decreased gene expression of the tight junction protein TJP1** ($P < 0.05$). E153 (6.4 mg/kg/week) also decreased OCLN ($P < 0.05$) in the colon and occludin protein expression in the small intestine ($P < 0.05$). Furthermore, E153 or **E171 exposed rats had shorter telomeres in the lung** ($P < 0.05$). **Plasma from particle-exposed rats also produced telomere shortening in cultured lung epithelial cells.** There were unaltered levels of oxidatively damaged DNA in the liver and lung and no changes in the DNA repair activity of oxidatively damaged DNA in the lung. Altogether, these results indicate that **intragastric exposure to** E153 and **E171 is associated with reduced tight junction protein expression in the intestinal barrier and telomere length shortening in the lung in rats.**
