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Adverse effects linked to ingestion of titanium dioxide (TiO_2 / E171)

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(Non-exhaustive) bibliography of scientific articles published after those considered in EFSA re-evaluation of E171 published in 2021:

2025

- **Titanium dioxide nanoparticles disturb glucose homeostasis in association with impaired enteroendocrine cell differentiation, Zou K et al., *Food and Chemical Toxicology*, 202, 115504, 2025**

→ Gut hormones secreted by enteroendocrine cells play a critical role in maintaining glucose homeostasis. However, the adverse endocrine effects related to glucose homeostasis caused by food additives are not well understood. This work aims to investigate the effects of titanium dioxide nanoparticles (TiO2 NPs) in comparison to titanium dioxide microparticles (TiO2 MPs) on glucose homeostasis, with a specific focus on the enteroendocrine cells and gut hormones. Our research found that **exposure to 1 % (w/w) TiO2 NPs, unlike TiO2 MPs, resulted in elevated blood glucose levels and impaired glucose tolerance in mice**. Notably, 1 % (w/w) TiO2 NPs significantly influenced the differentiation of the intestinal epithelium while not causing any notable histological changes or affecting cell proliferation in the mouse ileum. Furthermore, **the levels of gut hormones**, including glucagon-like peptide-1 (GLP-1), peptide YY (PYY) and cholecystokinin (CCK), **released from mouse ileum tissues were also significantly reduced following exposure to 1 % (w/w) TiO2 NPs**. Using the intestinal organoid model, we also discovered that 20 μg/mL **TiO2 NPs impaired enteroendocrine cell differentiation, reduced basal GLP-1 secretion levels, and disrupted the GLP-1 secretion response to nutrient stimuli**. Our research highlights the **detrimental effects of TiO2 NPs as potential intestinal endocrine disruptor** and underscores the need to optimize their particle size for safe use in the food industry.

2024

- **Effect of chronic prenatal exposure to the food additive titanium dioxide E171 on respiratory activity in newborn mice, Colnot E, O'Reilly J and Morin D, *Frontiers in Pediatrics*, 12:1337865, 2024**

→ In this study, using whole body plethysmography from postnatal day (P) 0 to P7, we assessed the respiratory function of newborn mice born to mothers fed with E171 during pregnancy. We also evaluated the potential alterations to respiratory centers by using brainstem-spinal cord electrophysiological recordings from P0 to P6. Our study reveals that **E171-prenatally exposed animals displayed an abnormally elevated breathing rate** from P3 onwards. From P5 to P6, **the respiratory-related burst frequency generated by the isolated brainstem-spinal cord preparations was significantly higher in E171-exposed animals than in non-exposed animals**. These findings demonstrate **prenatal toxicity of E171 to the developing respiratory function** and may contribute to policy-making regarding the use of TiO_2 nanoparticles.

- **Multigenerational inheritance of breathing deficits following perinatal exposure to titanium dioxide nanoparticles in the offspring of mice, Boulain M et al., *Discover Nano*, 19, 16, 2024**

→ In this study, we utilized perinatal exposure of female mice to TIO2NPs through voluntary food intake and observed impaired metabolism in newborn male and female F1 offspring. The **exposed newborn mice exhibited reduced body weight gain and a slower breathing rate compared to non-exposed animals**. Additionally, a higher proportion of exposed F1 newborns experienced **apneas**. Similar observations were made when the exposure was limited to the postnatal period, highlighting lactation as a critical period for the adverse effects of TIO2NPs on postnatal metabolism. Importantly, **the breathing deficits induced by TIO2NPs were transmitted from F1 females to the subsequent F2 generation**. Moreover, re-exposure of adult F1 females to TIO2NPs exacerbated the breathing deficits in newborn F2 males. Our findings demonstrate that **perinatal exposure to TIO2NPs disrupts postnatal body weight gain and respiration in the offspring, and these deficits are transmissible to future generations**.

2023

- **Perinatal foodborne titanium dioxide exposure-mediated dysbiosis predisposes mice to develop colitis through life, Carlé C et al., *Particle and Fibre Toxicology*, (20), 45, 2023**

→ We showed that **perinatal exposure to TiO2 early in life alters the gut microbiota composition, increases the intestinal epithelial permeability and enhances the colonic cytokines and myosin light chain kinase expression**. Moreover, **perinatal exposure to TiO2 also modifies the abilities of intestinal stem cells to survive, grow and generate a functional epithelium. Maternal TiO2 exposure increases the susceptibility of offspring mice to develop severe DSS-induced colitis later in life**. Finally, **transfer of TiO2-induced microbiota dysbiosis to pregnant germ-free mice affects the homeostasis of the intestinal mucosal barrier early in life and confers an increased susceptibility to develop colitis in adult offspring**. Our findings indicate that foodborne TiO2 consumption during the perinatal period has **negative long-lasting consequences on the development of the intestinal mucosal barrier toward higher colitis susceptibility**.

- **Long-term exposure from perinatal life to food-grade TiO_2 alters intestinal homeostasis and predisposes to food allergy in young mice, Issa M et al., *Allergy*, 2023**

→ We aimed to assess the effect of chronic perinatal exposure to food-grade titanium dioxide (fg-TiO2). Dams were fed a control versus fg-TiO2-enriched diet from preconception to weaning, and their progeny received the same diet at weaning. A comprehensive analysis of baseline intestinal and systemic homeostasis was performed in offspring 1 week after weaning by assessing gut barrier maturation and microbiota composition, and local and systemic immune system and metabolome. The effect of fg-TiO2 on the susceptibility of progeny to develop oral tolerance versus FA to cow's milk proteins (CMP) was performed starting at the same baseline time-point, using established models. Sensitization to CMP was investigated by measuring β-lactoglobulin and casein-specific IgG1 and IgE antibodies, and elicitation of the allergic reaction by measuring mouse mast cell protease (mMCP1) in plasma collected after an oral food challenge. **Perinatal exposure to fg-TiO2 at realistic human doses led to an increased propensity to develop FA and an impaired induction of oral tolerance only in young males**, which could be related to global baseline alterations in intestinal barrier, gut microbiota composition, local and systemic immunity, and metabolism. **Long-term perinatal exposure to fg-TiO2 alters intestinal homeostasis establishment and predisposes to food allergy, with a clear gender effect.**

- **Titanium dioxide E171 consumption exacerbates *Listeria monocytogenes* infection in mice, Yue Teng et al, *Food Quality and Safety*, 2023**

→ The aim of this study was to investigate the impact of dietary exposure of titanium dioxide E171 on *Listeria monocytogenes* infection in mice. **Pre-exposure to E171 resulted in increased bacterial counts in the liver, spleen, ileum, colon, mesenteric lymph nodes, and feces of mice after *L. monocytogenes* infection**. Moreover, **E171 exposure increased the levels of pro-inflammatory cytokines while attenuating the levels of anti-inflammatory cytokines in mice infected with *L. monocytogenes***. Meanwhile, **mice in the E171+LM group exhibited considerably more severe colonic inflammation and worse intestinal barrier function than mice in the LM group**. The 16S rRNA gene sequencing revealed a shift in the composition of the gut microbiota of mice in the E171+LM group, characterized by a decrease in the relative abundance of Firmicutes and a decrease in the Firmicutes-to-Bacteroidetes ratio. The levels of acetate, butyrate, and isobutyrate were markedly decreased within the cecum of mice in the E171+LM group in comparison to mice in the LM group. In conclusion, **these results suggest that E171 exposure could exacerbate *L. monocytogenes* infection in mice**, which may provide useful information for future risk assessment of this commonly used food additive.

- **TiO2 nanoparticles combined with polystyrene nanoplastics aggravated reproductive toxicity in female mice via exacerbating intestinal barrier disruption, Zhang et al., *Journal of the Science of food and agriculture*, 2023**

→ TiO2 nanoparticles (NPs) have been shown to aggravate the progress of metabolic diseases. Nanoplastics (NPLs) are an emerging contaminant and have been shown to induce ovarian disorders in mammals. We investigated the potential effects and mechanisms of co-exposure to polystyrene (PS) NPLs and TiO2 NPs on the ovary in female mice. Our results revealed **that the co-exposure of TiO2 NPs and PS NPLs caused significant injury to ovarian structure and function, but individual exposure had no effect**. Moreover, compared to the TiO2 NPs group, **co-exposure aggravated the intestinal barrier damage in mice, increasing the bioaccumulation of TiO2 NPs in the ovary**. After being supplemented with the oxidative stress inhibitor N-acetyl-l-cysteine, the expression of ovarian antioxidant genes increased, and the ovarian structural and functional injury in co-exposure mice reverted to normal levels. The present study demonstrated that **co-exposure to PS NPLs and TiO2 NPs can cause more severe female reproductive dysfunction**.

- **The food additive titanium dioxide hinders intestinal production of TGF-β and IL-10 in mice, and long-term exposure in adults or from perinatal life blocks oral tolerance to ovalbumin, Lamas B et al., *Food and Chemical Toxicology*, 179, 2023**

  → We investigated whether fg-TiO2 may compromise the establishment of **oral tolerance (OT)** to food proteins using a model of OT induction to ovalbumin (OVA) in mice, and whether a perinatal exposure could trigger this effect. **Long-term exposure to TiO2 as food additive alters anti-inflammatory cytokine profile, and leads to oral tolerance failure** regardless of the timing of TiO2 exposure throughout life.

- **Getting fat and stressed: Effects of dietary intake of titanium dioxide nanoparticles in the liver of turbot *Scophthalmus maximus*, Fonseca et al., Journal of Hazardous Materials, Volume 548, 2023**

  → Here, we study the effects of a sublethal concentration of citrate-coated TiO2 NPs of two different primary sizes over time in flatfish turbot, Scophthalmus maximus (Linnaeus, 1758). Bioaccumulation, histology and gene expression were assessed in the liver to address morphophysiological responses to citrate-coated TiO2 NPs. Our analyses demonstrated a **variable abundance of lipid droplets (LDs) in hepatocytes dependent on TiO2 NPs size, an increase in turbot exposed to smaller TiO2 NPs and a depletion with larger TiO2 NPs**. The expression patterns of genes related to oxidative and immune responses and lipid metabolism (nrf2, nfκb1, and cpt1a) were dependent on the presence of TiO2 NPs and time of exposure supporting the variance in hepatic LDs distribution over time with the different NPs. The citrate coating is proposed as the likely catalyst for such effects. Thus, our findings highlight the need to scrutinize the risks associated with exposure to NPs with distinct properties, such as primary size, coatings, and crystalline forms, in aquatic organisms.

- **Dysregulation along the gut microbiota-immune system axis after oral exposure to titanium dioxide nanoparticles: A possible environmental factor promoting obesity-related metabolic disorders, Lamas B et al., *Environmental Pollution*, 330, 2023**

  → Once absorbed, TiO_2 NPs may further interact with immune intestinal cells involved in gut microbiota regulation. Since obesity-related metabolic diseases such as diabetes are associated with alterations in the microbiota-immune system axis, this raises questions about the possible involvement of long-term exposure to food-grade TiO_2 in the development or worsening of these diseases. The current purpose is to review the dysregulations along the gut microbiota-immune system axis after oral TiO_2 exposure compared to those reported in obese or diabetic patients, and to highlight potential mechanisms by which foodborne **TiO_2 NPs may increase the susceptibility to develop obesity-related metabolic disorders.**

- **Estimation of genomic and mitochondrial DNA integrity in the renal tissue of mice administered with acrylamide and titanium dioxide nanoparticles, Mohammed et al. *Scientific reports*, 2023**

  → The Kidneys remove toxins from the blood and move waste products into the urine. However, the accumulation of toxins and fluids in the body leads to kidney failure. For example, the overuse of acrylamide and titanium dioxide nanoparticles (TiO2NPs) in many food and consumer products increases human exposure and risks; however, there are almost no studies available on the effect of TiO2NPs coadministration with acrylamide on the integrity of genomic and mitochondrial DNA. Accordingly, this study was conducted to estimate the integrity of genomic and mitochondrial DNA in the renal tissue of mice given acrylamide and TiO2NPs. To achieve this goal, mice were administrated orally TiO2NPs or/and acrylamide at the exposure dose levels (5 mg/kg b.w) and (3 mg/kg b.w), respectively, five times per week for two consecutive weeks. Concurrent oral administration of TiO2NPs with acrylamide caused remarkable elevations in the tail length, %DNA in tail and tail moment with higher fragmentation incidence of genomic DNA compared to those detected in the renal tissue of mice given TiO2NPs alone. Simultaneous coadministration of TiO2NPs with acrylamide also caused markedly high elevations in the reactive oxygen species (ROS) production and p53 expression level along with a loss of mitochondrial membrane potential and high decreases in the number of mitochondrial DNA copies and expression level of β catenin gene. Therefore, from these findings, we concluded that **concurrent coadministration of acrylamide with TiO2NPs augmented TiO2NPs induced genomic DNA damage and mitochondrial dysfunction through increasing intracellular ROS generation, decreasing mitochondrial DNA Copy, loss of mitochondrial membrane potential and altered p53 and β catenin genes expression.** Therefore, further studies are recommended to understand the biological and toxic effects resulting from TiO2NPs with acrylamide coadministration.

- **TiO2 nanoparticles abrogate the protective effect of the Crohn's disease-associated variation within the PTPN22 gene locus, Schwarzfischer M et al., *Gut*, 72(6):1101-1114,** 2023

  → A genetic variation in the protein tyrosine phosphatase non-receptor type 22 (PTPN22) gene has been associated with autoimmune disorders while protecting from the Inflammatory bowel disease (IBD) subtype **Crohn's disease**. Mice expressing the murine orthologous PTPN22-R619W variant are protected from intestinal inflammation in the model of acute dextran sodium sulfate (DSS)-induced colitis. We previously identified food-grade titanium dioxide (TiO2, E171) as a neglected IBD risk factor. Here, we investigate the interplay of the PTPN22 variant and TiO2-mediated effects during IBD pathogenesis. Acute DSS colitis was induced in wild-type and PTPN22 variant mice (PTPN22-R619W) and animals were treated with TiO2 nanoparticles during colitis induction. Disease-triggering mechanisms were investigated using bulk and single-cell RNA sequencing. **In mice, administration of TiO2 nanoparticles abrogated the protective effect of the variant, rendering PTPN22-R619W mice susceptible to DSS colitis**. In early disease, cytotoxic CD8+ T-cells were found to be reduced in the lamina propria of PTPN22-R619W mice, an effect reversed by TiO2 administration. Normalisation of T-cell populations correlated with increased Ifng expression and, at a later stage of disease, the promoted prevalence of proinflammatory macrophages that triggered severe intestinal inflammation. Our findings indicate that **the consumption of TiO2 nanoparticles might have adverse effects on the gastrointestinal health of individuals carrying the PTPN22 variant.** This demonstrates that environmental factors interact with genetic risk variants and **can reverse a protective mechanism into a disease-promoting effect**.

- **Food-grade titanium dioxide translocates across the buccal mucosa in pigs and induces genotoxicity in an *in vitro* model of human oral epithelium, Vignard J et al., *Nanotoxicology*, 2023**

→ The data provide evidence that under realistic exposure conditions in terms of dose and duration of exposure, **food-grade TiO2 may translocate through the oral mucosa** in an in vivo pig model of buccal mucosa that is close to the human mouth. We also report the high permeability of human buccal epithelial cells to TiO2 particles in vitro. After these cells were exposed to the food additive for 2 h, **TiO2 particles generated oxidative and genotoxic stresses that were detrimental to proliferating cells mainly.** This raises the issue of **possible adverse consequences regarding the constant turnover of the buccal mucosa or during wound repair and regeneration.**

- **DNA Damage and Apoptosis as In-Vitro Effect Biomarkers of Titanium Dioxide Nanoparticles (TiO_2-NPs) and the Food Additive E171 Toxicity in Colon Cancer Cells: HCT-116 and Caco-2, Ferrante et al., *International Journal of Environmental Research and Public Health*, 2023**

→ This study investigated the DNA damage and apoptosis in colon cancer cells HCT-116 and Caco-2 induced by engineered titanium dioxide nanoparticles (TiO_2-NPs) (60 nm) and titanium dioxide food additive E171. MTT assays showed that both chemical forms significantly reduced cancer cell viability in a dose-dependent manner. In particular **the food additive E171 induced a pronounced inhibitory effect on the growth of HCT-116 and Caco-2 cell lines** (E171 IC50: 3.45 mg/L for HTC-116 and 1.88 mg/L Caco-2; TiO_2-NPs 60 nm IC50: 41.1 mg/L for HTC-116 and 14.3 mg/L for Caco-2). A low level of genotoxicity was observed in Caco-2 cells, especially when treated with TiO_2 60 nm. Western blot analysis showed that HCT116 and Caco-2 treated cells did not overexpress apoptotic markers such as cleaved Caspase 3 and cleaved Parp. Moreover, further analysis by quantitative real-time PCR (qRT-PCR) showed that TiO_2-NPs and E171 did not promote the expression of Bax or downregulation of Bcl-2, nor did they increase the Bax/Bcl-2 ratio. The assay data provide clear evidence that **TiO_2 can cause DNA damage** but does not induce apoptosis or decrease long-term cell proliferation. In addition, the results show that **E171 has a slightly higher level of cytotoxicity and genotoxicity**. This suggests that **exposure to E171 may be hazardous to health and that further research on biological effects is needed** to promote safer practices in the use of this compound.

2022

- **A review of research on the impact of E171/TiO2 NPs on the digestive tract, Baranowska-Wójcik E et al., *Biology*, 72: 126988, 2022**

→ A nano-sized (diameter < 100 nm) fraction of TiO2 is present, at a certain percentage, in the E171 (in the EU) pigment, whose presence raises particular concerns in terms of its potential negative health impact. The consumption of E171 is increasingly associated with **disorders of the intestinal barrier**, including **intestinal dysbiosis**. It may **disrupt the normal functions of the gastrointestinal tract (GIT)** including: enzymatic digestion of primary nutrients (lipids, proteins, or carbohydrates). The aim of this review is to provide a comprehensive and reliable overview of studies conducted in recent years in terms of the substance's potentially negative impact on human and animal alimentary systems.

- **Perinatal exposure to foodborne inorganic nanoparticles: A role in the susceptibility to food allergy?, Issa M et al., *Front. Allergy*, Sec. Food Allergy, 3, 2022**

→ Among the environmental factors, foodborne inorganic NP present in ultra-processed food exhibit a large spectrum of intrinsic (physico-chemical) properties able to **imbalance essential components of intestinal homeostasis, including microbiota composition and function, gut barrier integrity, and the local immune system (GALT)**, which may predispose the progeny to **chronic diseases related to immune dysregulation, such as food allergy (FA)**. Metal oxides and silicate NP such as TiO_2, SiO_2, and Ag may cross the placental barrier and be excreted in breast milk. Their anti-microbial effects could **impair microbiota set-up in early life and the concomitant maturation of immune and epithelial barrier functions starting at birth**, whose development continues throughout the neonatal period. The consequences of early exposure to NP during the "first 1,000 days" of life require further studies to decipher whether perinatal NP exposure could predispose to the development of FA among other immune-related disorders. **Additional studies are thus urgently needed to quantify and further characterize the human fetal and neonatal exposure to NP, and to determine the potential hazard for fetal/neonate development and their long-term health effects.**

- **Oral exposure to Ag or TiO2 nanoparticles perturbed gut transcriptome and microbiota in a mouse model of ulcerative colitis, Wang et al., *Food and Chemical Toxicology*, 169(11):113368, 2022**

→ The effects of ingested nAg or nTiO2 on inflamed colon were revealed in a mouse model of chemical-induced acute ulcerative colitis. Mice (eight/group) were exposed to nAg or nTiO2 by oral gavage for 10 consecutive days. We characterized disease phenotypes, histology, and alterations in colonic transcriptome (RNA sequencing) and gut microbiome (16S sequencing). Oral exposure to nAg caused only minor changes in phenotypic hallmarks of colitic mice but induced extensive responses in gene expression enriching processes of apoptotic cell death and RNA metabolism. Instead**, ingested nTiO2 yielded shorter colon, aggravated epithelial hyperplasia and deeper infiltration of inflammatory cells**. Both nanoparticles **significantly changed the gut microbiota composition, resulting in loss of diversity and increase of potential pathobionts. They also increased colonic mucus and abundance of Akkermansia muciniphila**. Overall, nAg and nTiO2 induce dissimilar **immunotoxicological changes at the molecular and microbiome level in the context of colon inflammation.**

- **Adverse Outcome Pathways Associated with the Ingestion of Titanium Dioxide Nanoparticles—A Systematic Review, Rolo D. et al., *Nanomaterials*, 12(19) : 327, 2022**

→ The aim of this review was to provide an integrative analysis of the published data on cellular and molecular mechanisms triggered after the ingestion of TiO_2-NPs, proposing plausible AOPs that may drive policy decisions. A systematic review according to Prisma Methodology was performed in three databases of peer-reviewed literature: Pubmed, Scopus, and Web of Science. A total of 787 records were identified, screened in title/abstract, being 185 used for data extraction. The main endpoints identified were oxidative stress, cytotoxicity/apoptosis/cell death, inflammation, cellular and systemic uptake, genotoxicity, and carcinogenicity. From the results, AOPs were proposed where **colorectal cancer, liver injury, reproductive toxicity, cardiac and kidney damage**, as well as **hematological effects** stand out as possible **adverse outcomes**. The recent **transgenerational studies** also point to concerns with regard to **population effects**. Overall, **the findings further support a limitation of the use of TiO_2-NPs in food, announced by the European Food Safety Authority (EFSA).**

- **Chronic maternal exposure to titanium dioxide nanoparticles alters breathing in newborn offspring, Colnot et al., *Particle and Fibre Toxicology*, 19, 57, 2022 :**

→ Due in particular to their extremely small size, TiO2 nanoparticles (NPs) are prone to cross biological barriers and potentially lead to adverse health effects. The presence of TiO2 NPs found in human placentae and in the infant meconium has indicated unequivocally the capacity for a materno-fetal transfer of this nanomaterial. Although chronic exposure to TiO2 NPs during pregnancy is known to induce **offspring cognitive deficits associated with neurotoxicity**, the impact of a gestational exposure on a vital motor function such as respiration, whose functional emergence occurs during fetal development, remains unknown. Using in vivo whole-body plethysmographic recordings from neonatal mice, we show that a **chronic exposure to TiO2 NPs during pregnancy alters the respiratory activity of offspring**, characterized by an abnormally elevated rate of breathing. Correspondingly, using ex vivo electrophysiological recordings performed on isolated brainstem-spinal cord preparations of newborn mice and medullary slice preparations containing specific nuclei controlling breathing frequency, we show **that the spontaneously generated respiratory-related rhythm is significantly and abnormally accelerated in animals prenatally exposed to TiO2 NPs**. Moreover, such a chronic prenatal exposure was found **to impair the capacity of respiratory neural circuitry to effectively adjust breathing rates in response to excitatory environmental stimuli such as an increase in ambient temperature**. Our findings thus demonstrate that **a maternal exposure to TiO2 NPs during pregnancy affects the normal development and operation of the respiratory centers in progeny.**

- **Food-Grade Titanium Dioxide Induces Toxicity in the Nematode Caenorhabditis elegans and Acute Hepatic and Pulmonary Responses in Mice, Sitia et al., *Nanomaterials* (Basel), 2022**

→ We evaluated the impact of acute E171 administration on invertebrate and vertebrate animals. In the nematode, *Caenorhabditis elegans*, the administration of up to 1.0 mg/mL of **E171** did not affect the worm's viability and lifespan, but **significantly impaired its pharyngeal function, reproduction, and development**. We also investigated whether the intravenous administration of E171 in mice (at the dose of 6 mg/kg/body weight) could result in an acute over-absorption of filter organs. A **significant increase of hepatic titanium concentration** and the **formation of microgranulomas** were observed. **Interstitial inflammation and parenchymal modification were found in the lungs, coupled with titanium accumulation.** This was probably due to the propensity of TiO_2 NPs to agglomerate, as demonstrated by transmission electron microscopy experiments showing that the incubation of E171 with serum promoted the formation of compact clusters. **Overall, these data emphasize the actual risk for human and animal exposure to E171.**

- **Ingestion of titanium dioxide nanoparticles: a definite health risk for consumers and their progeny, Cornu R, *Arch Toxicol.*, 2022**

→ Daily oral intake by rats of amounts of E171 that are relevant to human intake has been associated with an **increased risk of chronic intestinal inflammation and carcinogenesis**. Furthermore, maternal-foetal transfer of TiO2 NPs during pregnancy, as well as exposure of the offspring by breastfeeding, have been recently described. To provide some answers to this public health problem and help global regulatory agencies finalize their decisions, we reviewed in vitro and in vivo studies that address the effects of TiO2 NPs through oral exposure, especially their **effects on the gastrointestinal tract, one of the most exposed tissues**. Our review also highlights the **effects of exposure on the offspring during pregnancy and by breastfeeding.**

- **The effects of the food additive Titanium dioxide (E171) on tumor formation and gene expression in the colon of a transgenic mouse model for colorectal cancer, Bischoff et al., *Nanomaterials* (Basel), 7;12(8), 2022**

→ We developed a transgenic mouse model to examine the effects of E171 on colorectal cancer (CRC), using the Cre-LoxP system to create an *Apc*-gene-knockout model which spontaneously develops colorectal tumors. A pilot study showed that **E171 exposed mice developed colorectal adenocarcinomas, which were accompanied by enhanced hyperplasia in epithelial cells, and increased tumor size**. In the main study, tumor formation was studied following the exposure to 5 mg/kg_{bw}/day of E171 for 9 weeks (Phase I). E171 exposure showed a statistically nonsignificant increase in the number of colorectal tumors in these transgenic mice, as well as a statistically nonsignificant increase in the average number of mice with tumors. Gene expression changes in the colon were analyzed after exposure to 1, 2, and 5 mg/kg_{bw}/day of E171 for 2, 7, 14, and 21 days (Phase II). Whole-genome mRNA analysis revealed the **modulation of genes in pathways involved in the regulation of gene expression, cell cycle, post-translational modification, nuclear receptor signaling, and circadian rhythm**. The processes associated with these genes might be involved in the **enhanced tumor formation** and suggest that **E171 may contribute to tumor formation and progression by modulation of events related to inflammation, activation of immune responses, cell cycle, and cancer signaling**.

- **Investigation of the genotoxicity of digested titanium dioxide nanomaterials in human intestinal cells, Vieira et al., *Food and Chemical Toxicology*, Volume 161, 2022**

→ We analyzed the genotoxicity and the intracellular reactive oxygen species induction by physiologically relevant concentrations of three different TiO2 NMs (NM-102, NM-103 and NM-105) in Caco-2 and HT29-MTX-E12 intestinal cells, while considering the potential influence of the digestion process in the NMs' physiochemical characteristics. The results evidenced **a DNA-damaging effect dependent on the NM, more relevant for the rutile/anatase NM-105**, possibly due to its lower hydrodynamic size in the cells medium. In addition, the results of the micronucleus assay suggest **effects on chromosomal integrity, an indicator of cancer risk**, in the HT29-MTX-E12 cells, **for all the tested TiO2 NMs**, especially after the *in vitro* digestion. **This work supports the evidence for concerns on the use of TiO2 NMs as a food additive, recently reported by EFSA, and for their use in applications in consumer products that may drive human exposure through ingestion.**

- **Oral administration of TiO2 nanoparticles during early life impacts cardiac and neurobehavioral performance and metabolite profile in an age- and sex-related manner, Mortensen et al., *Particle and Fibre Toxicology*, 19, 2022**

→ To investigate the effects of early life exposure to orally ingested TiO2 NP, male and female Sprague–Dawley rat pups received four consecutive daily doses of 10 mg/kg body weight TiO2 NP (diameter: 21 ± 5 nm) or vehicle control (water) by gavage at three different pre-weaning ages: postnatal day (PND) 2–5, PND 7–10, or PND 17–20. Cardiac assessment and basic neurobehavioral tests (locomotor activity, rotarod, and acoustic startle) were conducted on PND 20. Pups were sacrificed at PND 21. Select tissues were collected, weighed, processed for neurotransmitter and metabolomics analyses. **Heart rate was found to be significantly decreased in female pups** when dosed between PND 7–10 and PND 17–20. Females dosed between PND 2–5 showed **decrease acoustic startle response** and when dosed between PND 7–10 showed **decreased performance in the rotarod test and increased locomotor activity**. Male pups dosed between PND 17–20 showed **decreased locomotor activity**. The **concentrations of neurotransmitters and related metabolites in brain tissue and the metabolomic profile of plasma were impacted by TiO2 NP administration for all dose groups**. Metabolomic pathways perturbed by TiO2 NP administration included pathways involved in amino acid and lipid metabolism. **Oral administration of**

TiO2 NP to rat pups impacted basic cardiac and neurobehavioral performance, neurotransmitters and related metabolites concentrations in brain tissue, and the biochemical profiles of plasma. The findings suggested that **female pups were more likely to experience adverse outcome following early life exposure to oral TiO2 NP than male pups**. Collectively the data from this exploratory study suggest **oral administration of TiO2 NP cause adverse biological effects in an age- and sex-related manner**, emphasizing the need to understand the short- and long-term effects of early life exposure to TiO2 NP.

2021

- **Impact of Food Additive Titanium Dioxide on Gut Microbiota Composition, Microbiota-Associated Functions, and Gut Barrier: A Systematic Review of In Vivo Animal Studies, Rinninella E et al., *International Journal of Environmental Research and Public Health,* 18(4):2008, 2021**

→ This systematic review aims to assess the potential associations between food TiO2 exposure and microbiota composition and functions. A total of 18 animal studies were included (n = 10 mice, n = 5 rats, n = 2 fruit flies, n = 1 silkworm). Studies varied significantly in protocols and outcomes assessment. **TiO2 exposure might cause variations in abundance in specific bacterial species and lead to gut dysfunctions such as a reduction in SCFAs levels, goblet cells and crypts, mucus production, and increased biomarkers of intestinal inflammation**.

- **Titanium dioxide particles from the diet: involvement in the genesis of inflammatory bowel diseases and colorectal cancer, Barreau et al., Particle and Fibre Toxicology, 18(26), 2021**

→ The gastrointestinal tract is a complex interface between the external environment and the immune system. Its ability to control uptake across the mucosa and to protect the body from damage of harmful substances from the lumen is defined as the **intestinal barrier function (IBF)**. The IBF involves four elements: the intestinal microbiota, the mucus layer, the epithelium and the immune system. Its dysfunction is linked with human diseases including inflammatory, metabolic, infectious, autoimmune and neurologic disorders. Data indicate that **TiO2 is able to alter the four compartments of IBF and to induce a lowgrade intestinal inflammation associated or not with preneoplastic lesions**. However, since multiple evidence has shown that the intestinal homeostasis in adults results directly from the perinatal establishment of the IBF, it is of major importance to investigate the impact of TiO2 for the early phases of the intestinal development, i.e., during the embryogenic and lactating periods. Moreover, since IBD is a complex disease involving genetic, psychologic and environmental factors, it is important to check the impact of this material in models exhibiting IBD susceptibility like models mutated in the genes encoding the NOD2, ATG16L1, IRGM or IL-23 receptors. Finally, to demonstrate the likely involvement of these particles in the IBD genesis, it is also crucial to investigate whether exposure to these particles in combination with episodes of stress, which are described to be involved in the IBD, may induce an intestinal inflammation and linked colorectal cancer similar to those observed in IBD, in mice with genetic susceptibility to IBD.

- **Possible Adverse Effects of Food Additive E171 (Titanium Dioxide) Related to Particle Specific Human Toxicity, Including the Immune System, Bischoff, NS et al., Int. J. Mol. Sci. 22, 207, 2021**

→ **The existing body of evidence raises concern for human health regarding the long-term ingestion of E171**. The wide-spread human exposure in combination with the reported **tumor-promoting and pro-inflammatory responses in animal experiments** indicates the necessity to fill in the identified knowledge gaps that are crucial in the hazard identification and risk assessment process. A particular concern was identified for children due to their proportionally higher TiO_2 intake, and **patients with IBD**, given their **potential risk of increased absorption, as a consequence of impaired intestinal health.**

Animal experiments have shown that chronic exposure to E171 can lead **to translocation and bioaccumulation of TiO_2 via the bloodstream, in various organs, including the liver, kidney, placenta, and brain**. Across different types of models, gene expression patterns have been reported that are associated with **inflammation and tumor development**. *In-vivo, ex-vivo,* and *in-vitro* experiments, mainly conducted with TiO_2 nanoparticles, show that **TiO_2 can result in the formation of ROS, which is associated with the induction of genetic damage, the initiation, and stimulation of inflammation, and the promotion of tumor formation**. The **endocrine and reprotoxic effects** found in rodent studies indicate the need for additional research to reduce uncertainties. These complex interplays of molecular mechanisms involving **local persistent inflammation, disturbance of the oxidative–antioxidative balance, immune suppression, apoptosis, changes in microbiotic health, and colon cancer-related pathways** need to be further investigated to better understand the molecular biological process, their interaction, and involvement following the chronic exposure to E171.

Chronic carcinogenicity studies in laboratory animals might have limitations in identifying influences on the incidence of rare tumors, such as colon cancer in rats. For this purpose, specific disease models may provide complementary information. While oral exposure of TiO_2 via drinking water (oral gavage) and via the diet is both relevant, the effect of the food matrix on bioavailability and adverse health effects is still poorly understood and potentially has an influence on particle properties and toxicokinetics, hence should be considered in the hazard identification of E171. Finally, human dietary intervention studies are needed to demonstrate or discard adverse responses to E171, under relevant exposure conditions, and to better understand the potential cellular and molecular mechanism of action in humans.

- **TiO2 – do we have to worry about it? One of the important aetiological factors in inflammatory bowel disease, Jarmakiewicz-Czaja S et al., Prz Gastroenterol, 4;16(2):106–110, 2021**

→ TiO2 is absorbed through food ingestion and the respiratory tract. There are also reports that demonstrate the ability to accumulate in some internal organs, like spleen, liver, and kidneys, depending on its size and structure. It may have **pro-inflammatory, cytotoxic, and genotoxic effects**. A **change in the composition of the host's intestinal microflora** is also observed after exposure to high doses of TiO2.

- **Toxic effects of TiO2 NPs in the blood-milk barrier of the maternal dams and growth of offspring, Yao et al, Ecotoxicology and Environmental Safety, 2021 :**

→ This study presented the **deleterious pathological effects of oral exposure to TiO2 NPs in the mammary gland tissues and blood-milk barrier** via the **production of reactive oxygen species (ROS)** in dams and **developmental concerns in offspring**.

2020

- **Food-grade titanium dioxide (E171) induces anxiety, adenomas in colon and goblet cells hyperplasia in a regular diet model and microvesicular steatosis in a high fat diet model, Medina-Reyes E et al., *Food and Chemical Toxicology*, 146, 111786, 2020**

  → Food-grade titanium dioxide (E171) is a white additive widely used in solid and liquid food products. There is still debate about E171 toxic effects after oral consumption since this additive is deposited in colon, liver, spleen, testis and brain. The consumption of E171 commonly occurs with Western diets that are characterized by a high fat content. Thus, E171 could worsen adverse effects associated with a high fat diet (HFD) such as anxiety, colon diseases and testicular damage. We aimed to evaluate the effects of E171 on anxiety-like behavior, colon, liver and testis and to analyze if the administration of a HFD could exacerbate adverse effects. E171 was administered at ~5 mg/kgbw by drinking water for 16 weeks and mice were fed with a Regular Diet or a HFD. **E171 promoted anxiety, induced adenomas in colon, goblet cells hypertrophy and hyperplasia and mucins overexpression**, but had no toxic effects on testicular tissue or spermatozoa in regular diet fed-mice. **Additionally, E171 promoted microvesicular steatosis in liver in HFD fed-mice and the only HFD administration decreased the spermatozoa concentration and motility**. In conclusion, **E171 administration increases the number of adenomas in colon, induces hypertrophy and hyperplasia in goblet cells and microvesicular steatosis.**

- **Possible effects of titanium dioxide particles on human liver, intestinal tissue, spleen and kidney after oral exposure, Brand W et al., *Nanotoxicology, 14(7):985-1007,* 2020**

  → **TiO_2 can trigger a number of key events in liver and intestine: Reactive Oxygen Species (ROS) generation, induction of oxidative stress and inflammation**. TiO_2 seems to be able to exert these early effects in animal studies at Ti liver concentrations that are only a factor of 30 and 6 times higher than the median and highest liver concentration found in humans, respectively. This **confirms earlier conclusions** that **adverse effects on the liver in humans as a result of (oral) TiO_2 exposure** cannot be excluded.

- **TiO2 genotoxicity: An update of the results published over the last six years, Carrière et al., *Mutation Research Genetic Toxicology and Environmental Mutagenesis*, 854-855, 2020**

  → This review summarizes data retrieved from their genotoxicity testing during the past 6 years, encompassing both *in vitro* and *in vivo* studies, mostly performed on lung and intestinal models. It shows that TiO_2 particles, **both nano- and micro-sized, produce genotoxic damage to a variety of cell types, even at low, realistic doses.**