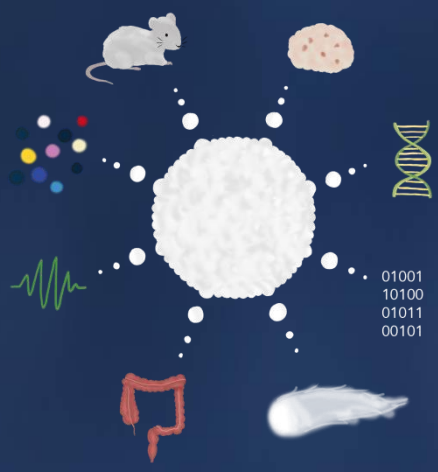
Beyond the white

Addendum I



The focus of this thesis is to study the facilitation of colorectal cancer (CRC) development after ingestion of food additive titanium dioxide (E171) and extend the current knowledge by investigating mechanisms that underlie these effects. The results showed that E171 induces oxidative stress in an acellular environment, DNA damage *in vitro*, gene expression changes *in vitro* and *in vivo* towards an impairment of the immune system, DNA repair, induction of oxidative stress, dysregulation of olfactory/GPCR receptor family, and facilitation of development of CRC. In addition, the relative contribution of the nanoparticle (NPs) (<100 nm) fraction (40% of E171) and the microparticle (MPs) (>100 nm) fraction (60% of E171) were investigated *in vitro*. Results showed that NPs also induce oxidative stress in an acellular environment whereas MPs induced oxidative stress *in vitro*. Both MPs and NPs provoked DNA damage and gene expression changes indicating effects in signalling, inflammation, immune system, transport, and cancer. This valorisation chapter reflects on how the scientific results described in this thesis can be applied to create societal value.

I- Societal and economic relevance

The societal relevance of the present thesis is regarding the consumers. E171 is present in a large number of products as shown in Chapter 1 and the exposure is occurring every day for all age groups. Recently, consumers have become more insistent over the need for greater transparency in their food and drinks. These changes have propelled the movement to simpler and natural ingredients affecting product formulation and consumer purchase interest. The consumers are looking for more information and can easily find them on the internet. However, the related information is not necessary scientific based conclusions and this has an impact on the consumers. It is important that they can find the scientific based conclusions about the possible risks engendered by the ingestion of these products as shown in this thesis.

Additionally, this thesis indicates a link between ingestion of E171 and enhancement of CRC. CRC is, worldwide, the second most common cancer in women and the third in men (1). With a morbidity of 614,000 cases for women and 740,000 for men, CRC represents 9.2% and 10% of all cancers respectively (1). CRC is also the fourth leading cause of cancer death in the world accounting for about 700,000 deaths in 2012. While taking into account the demographic projection, the global burden of CRC is estimated to increase by 60% to more than 2.2 million new cases and 1.1 million deaths by 2030 (1). The distribution of CRC burden varies around the world with almost 55% of all cases and about 60% of all death occurring in developed regions. Indeed, incidence rates are

estimated to be the highest in Australia/New Zealand (32.2 and 44.8 per 100,000 women and men respectively) and the lowest in Western Africa (3.8 and 4.5 per 100,000 women and men respectively). In view of these figures and studies on the effects of diet, CRC has been strongly associated with the adverse effects of lifestyle and diet (2).

The economic burden of CRC was recently estimated in Spain between €20,478 and 27,000€ per patient for 5 years after diagnosis (3). A rough estimation according to the number of cancer worldwide (1.4 million in 2012) would show a global economic burden between €5.5 and 7.3 billion per year for the year 2012 and between €9.0 and 11.9 billion per year for the year 2030. This estimation does not include extra costs due to the side effects of the chemotherapy which were estimated monthly in US per patient at $1,480 for hematologic, $1,253 for respiratory, and $1,213 for endocrine and metabolic adverse effects (4).

The results of this thesis contribute to both goals, more knowledge and transparency over food additive E171 and contributing in identifying underlying causes of the western diet leading to an increase of development of CRC.

II- Target group

Outside of the academic community, the industries using TiO_2 in their products are interested by our research results. Due to the pressure of the consumers, certain industries are investigating the potential adverse effects of TiO_2 and opting for alternatives. Indeed, because of the incertitude regarding the potential adverse effects of TiO_2, some big companies have already claimed that they stopped using TiO_2 in all or some of their products like Lutti®, Verquin®, and Dunkin' Brands, Inc®. Furthermore, the possible changes in the regulation of E171 are well monitored by the industries and E171 is considered as an example of what could also happen with other food additives.

Additionally, the national organisation like Anses (Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail), NVWA (Nederlandse Voedsel- en Warenautoriteit), BfR (Bundesinstitut für Risikobewertung), and FDA (Food and Drug Administration) as well as regulators are also interested by the results of this thesis. Following the change of classification of TiO_2 by the IARC in 2010 from non-carcinogen to possible carcinogen (group 2B) concerns of the different routes of exposure like ingestion rose. Therefore, in 2016, the EFSA made a re-evaluation of TiO_2 as a food additive (5). Based on the available genotoxicity database, EFSA concluded that,

absorption, distribution, and excretion of micro- and nanosized TiO_2 particles after oral exposure are unlikely to represent a genotoxic hazard *in vivo*. In addition, in June 2017, the risk assessment committee of the European Chemical Agency (ECHA) classified TiO_2 as a suspected human carcinogen (category 2) via inhalation (6). Furthermore, national organisation could include the results of this thesis in a risk assessment which could lead to possible changes of the use of TiO_2 in food products.

The research presented in this thesis is of interest to the whole population for all age groups. Indeed, TiO_2 is present in various food products from dairy products (milk, cheese, and ice cream), milk replacements (powdered milk), sweets (M&M's®, Mentos®, chewing-gums, and cookies), drinks (soft drinks, sport drinks, and syrups), dressings and sauces (salad dressings), and food supplements (multivitamin pills) (7-11). The exposure varies depending on the age group. Children are the most exposed to TiO_2 due to their higher ingestion of sweets and toothpaste. Depending on the diet (US, UK, Dutch or a global European diet) the estimation ranged from 0.67 mg TiO_2/kg_{bw}/day in the Netherlands (children between 2-6 years old) to 5.5 mg/kg_{bw}/day as estimated by the European Food Safety Authority (EFSA) (children between 3 and 9 years old). For the adults, the estimated concentrations were lower from 0.17 mg TiO_2/kg_{bw}/day between 7-69 years in the Netherlands to 4.1 and 4.0 mg TiO_2/kg_{bw}/day for adolescents (10-17 years old) and adults (18-64 years old) respectively as estimated by the EFSA (5,10,11).

III- Translation of the results

The results from this thesis provide an opportunity for further research and a basis for a future risk assessment of TiO_2 as food additive. Following new evidence provided in the last 2 years, the European Commission asked the EFSA to include 4 new studies in their re-evaluation of titanium dioxide as food additive. In addition, due to the new evidence about the adverse effects of E171, the French government asked the Anses to perform more research about potential adverse effects of ingestion of E171.

IV- Innovation of the research

When the IARC decided, in 2010, to re-evaluate TiO_2 to assess its potential impact on development of cancer, enough evidence was found from the inhalation route where tumours in the lungs of rats were observed (12). Based on these results, the IARC decided to change the classification of TiO_2 from non-carcinogen to possible carcinogen to humans. During this evaluation, the IARC stated that there was not enough evidence regarding the other routes of exposure (dermal contact and ingestion) as well as the modes of action of TiO_2 underlying these adverse effects (kinetics, *in vivo* and *in vitro* genotoxicity, cytotoxicity, presence of inflammation, and penetration of TiO_2 through the skin).

Since the change of classification by the IARC, the ingestion route via the ingestion of food products containing E171 has been studied. Within these studies, a collaborative group has observed that after ingestion of 5 mg/kg_{bw}/day of E171 for 10 weeks in a chemically induced colorectal cancer mouse model with azoxymethane (AOM) and dextran sodium sulphate (DSS), the number of tumour was increased when E171 was also orally administrated (13). From the results of this study, the mechanisms behind this development of tumours were studied. **Chapter 2, 3, and 4** present the first whole-genome mRNA analysis of the colon of mice after oral exposure to E171 in 3 different mouse models: a normal BALB/c, a chemically induced CRC with AOM/DSS, and a transgenic model. These analyses provide potential underlying molecular mechanisms modulated after oral exposure to E171. The mechanisms of *in vitro* genotoxicity, cytotoxicity and oxidative stress on a human colon cell line (Caco-2) in **Chapter 5** confirmed previous findings of other cells lines. Additionally, the contribution of each fraction of E171 was identified and shows that the NPs and the MPs fraction contribute to these adverse effects. The mechanisms were also investigated *in vitro* in Caco-2 cells in **Chapter 6** to assess the contribution of the MPs and NPs at the mRNA level. To the best of our knowledge, this was the first time that the contribution of each fraction of E171 was assessed.

V- Implementation of the valorisation

The knowledge acquired in this thesis offers a potential mechanistic explanation of the enhancement of tumours in the colon of mice and can be used for further translational and applied purposes, for instance for risk assessment in food safety of food additive E171. With this risk assessment, competent regulatory authorities could decide if policy changes are necessary. Risk assessments may set conditions for more healthy food. Consumers will be better informed regarding products that are added in the food.

References

1. Globocan (2012) CANCER FACT SHEETS: COLORECTAL CANCER.
2. Arnold, M., Sierra, M.S., Laversanne, M., Soerjomataram, I., Jemal, A., and Bray, F. (2017) Global patterns and trends in colorectal cancer incidence and mortality. *Gut*, **66**, 683-691.
3. Andrade, P., Sacristan, J.A., and Dilla, T. (2017) The Economic Burden of Cancer in Spain: A Literature Review. *Health Economics & Outcome Research: Open Access*, **3**.
4. Latremouille-Viau, D., Chang, J., Guerin, A., Shi, S., Wang, E., Yu, J., and Ngai, C. (2017) The economic burden of common adverse events associated with metastatic colorectal cancer treatment in the United States. *J Med Econ*, **20**, 54-62.
5. EFSA (2016) Re-evaluation of titanium dioxide (E 171) as a food additive. *EFSA Journal*, **14**, e04545-n/a.
6. Charles, S., Jomini, S., Fessard, V., Bigorgne-Vizade, E., Rousselle, C., and Michel, C. (2018) Assessment of the in vitro genotoxicity of TiO2 nanoparticles in a regulatory context. *Nanotoxicology*, 1-18.
7. EFSA (2016) Scientific Opinion on the re-evaluation of titanium dioxide (E 171) as a food additive. *EFSA Journal*, **14**, 4545-4638.
8. Lomer, M.C.E., Thompson, R.P.H., Commisso, J., Keen, C.L., and Powell, J.J. (2000) Determination of titanium dioxide in foods using inductively coupled plasma optical emission spectrometry. *Analyst*, **125**, 2339-2343.
9. Peters, R.J., van Bemmel, G., Herrera-Rivera, Z., Helsper, H.P., Marvin, H.J., Weigel, S., Tromp, P.C., Oomen, A.G., Rietveld, A.G., and Bouwmeester, H. (2014) Characterization of titanium dioxide nanoparticles in food products: analytical methods to define nanoparticles. *J Agric Food Chem*, **62**, 6285-6293.
10. Weir, A., Westerhoff, P., Fabricius, L., Hristovski, K., and von Goetz, N. (2012) Titanium dioxide nanoparticles in food and personal care products. *Environmental science & technology*, **46**, 2242-2250.
11. Rompelberg, C., Heringa, M.B., van Donkersgoed, G., Drijvers, J., Roos, A., Westenbrink, S., Peters, R., van Bemmel, G., Brand, W., and Oomen, A.G. (2016) Oral intake of added titanium dioxide and its nanofraction from food products, food supplements and toothpaste by the Dutch population. *Nanotoxicology*, **10**, 1404-1414.
12. IARC (2010) IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS: Carbon Black, Titanium dioxide and Talc. **Volume 93**.
13. Urrutia-Ortega, I.M., Garduno-Balderas, L.G., Delgado-Buenrostro, N.L., Freyre-Fonseca, V., Flores-Flores, J.O., Gonzalez-Robles, A., Pedraza-Chaverri, J., Hernandez-Pando, R., Rodriguez-Sosa, M., Leon-Cabrera, S., Terrazas, L.I., van Loveren, H., and Chirino, Y.I. (2016) Food-grade titanium dioxide exposure exacerbates tumor formation in colitis associated cancer model. *Food Chem Toxicol*, **93**, 20-31.