The effect of titanium dioxide nanoparticles on behavioral responses and blood values of the laboratory animals

T V Kazakova¹, O V Marshinskaia ¹

¹ Federal Research Centre of Biological Systems and Agrotechnologies RAS, 29, 9 Yanvarya str., Orenburg, Russia
E-mail: vaisvais13@mail.ru

Abstract. Nanoparticles of titanium dioxide (NP-TiO₂) are the most widely used nanomaterial in various industries. However, the safety of NP-TiO₂ has not been studied in full. The research was carried out with the «Wistar» rats (n=21). The nanoparticles of titanium dioxide obtained by the plasma chemical synthesis, were once administered intraperitoneally at doses of 15 mg/kg and 75 mg/kg (90 nm). On the 1st, 7th, and 14th days of the experiment, the emotional and motion activity was assessed using the Open Field test and the Infrared Actimeter system; the blood sampling was performed to evaluate the hematological and biochemical parameters. It was revealed that a single intraperitoneal injection of titanium dioxide nanoparticles at doses of 15 mg/kg and 75 mg/kg resulted the changes in the behavioral responses of animals, which were accompanied throughout the experiment by the increased motion activity and emotional stress. There were noted the changes in protein, fat and mineral metabolism, the enzymatic activity increased. Despite the fact that the administered doses were significantly different, the effects of exposure were similar, that indicates the absence of a dose-dependent effect. The greatest effect of exposure was recorded at the first day of the experiment.

1. Introduction

Scientific research in the field of nanotechnology and assessing their risk to the human health and the environment have become preferential throughout the world, which is associated with the intensive development of this industry. Nowadays, there are already more than two thousand sold consumer goods containing the nanoparticles [1].

Titanium dioxide nanoparticles (NP-TiO₂) are the most widely used nanomaterial due to its unique physicochemical properties in the nanoform [2]. According to H.C. Winkler and his co-authors, the annual consumption of NP-TiO₂ reaches four million tons [3]. Titanium dioxide is used extensively in the food industry and is known as the food colorant E 171 [4, 5]. According to the EFSA Panel on Food Additives and Nutrient Sources added to Food, the highest concentrations of titanium dioxide particles were revealed in chewing gum, nuts and ready-to-serve salads [6, 7]. The recent studies have shown that E 171 contains the titanium dioxide particles less than 100 nm in size. This reduction in size may change the characteristics of the material compared to the larger particles. In addition, NP-TiO₂ are used as a white pigment in paints and personal care products; as well as in biomedicine; in the textile and cosmetic industries [8, 9, 10].

In accordance with the European Union regulations, it is necessary to label the products containing the nanoparticles. This labeling should include the name of the chemicals used, size, physico-chemical information and toxicity [8, 11]. The Russian Federation has also developed the state standards in the field of nanotechnology safety [12]. Despite this fact, the particle distribution in size and morphology
are still not indicated on the packaging labels [3, 13, 14]. According to the European Chemicals Agency, 198 out of 205 companies do not meet the safety criteria. It is reported that only 3.4% of companies provided information on the nanomaterials that were used [15]. Nowadays, there is no legislation that fully regulates the use of nanomaterials.

Thus, the safety of titanium dioxide nanoparticles has not been studied in full, however, this nanomaterial continues to be used in various industries.

2. Purpose of research
To assess the effect of titanium dioxide nanoparticles on behavioral responses and blood values of the laboratory animals.

3. Materials and methods of research
The research was carried out on the basis of the Center for the Collective Use of Biological Systems and Agrotechnologies of the Russian Academy of Sciences on the 21 male Wistar rats, which were once intraperitoneally administered with the various solutions in a volume of 0.29 ml. Depending on the solution injected, the animals were divided into 3 groups: control (n=7) – NaCl solution 0.9%; I experimental (n=7) – a solution of NP-TiO\(_2\) at a dose of 15 mg/kg (1/10 LD\(_{50}\)); II experimental group (n=7) – a solution of NP-TiO\(_2\) at a dose of 75 mg/kg (1/2 LD\(_{50}\)). The NP-TiO\(_2\) were produced by the plasma chemical synthesis (“Platina” LLC, city of Moscow). Physico-chemical characteristics of the injected nanoparticles: composition – 99.8% TiO\(_2\) and Cl\(_2\) <0.2%, size – 90 nm, specific surface area – 16.5 m\(^2\)/g, Z potential – 96.5 ± 27.1 mV.

The intraperitoneal method of administration of drugs is characterized by the ease of implementation and high absorption, so that the injected fluid quickly enters the bloodstream. The choice of doses is determined by the LD\(_{50}\) value for this method of TiO\(_2\) injection [2].

On the 1st, 7th, and 14th days of the experiment, the emotional and motion activity was assessed using the Open Field test and the Infrared Actimeter system; the blood sampling was also performed. Determination of the morphological values of the blood was carried out with the help of automatic hemanalyzer URIT-2900 Vet Plus (China). Determination of the blood biochemical values was performed using the CS-T240 biochemical analyzer (China).

The main data obtained in the research were processed using “Excel” and “Statistica 6.0”. The statistical significance of the effects was evaluated by the Mann-Whitney U-test.

4. Results of research
Changes in the behavioral activity of animals are clearly illustrated by the results of the Open Field test and the Infrared Actimeter system.

On the first day of the research, the animals of the experimental groups showed an increase in the total horizontal activity (THA) – by 9.8% (p<0.05) and by 20% (p<0.05) in groups I and II, respectively; an increase in total vertical motion activity (VMA) – by 50% (p<0.01) in both experimental groups. The level of emotional stress, assessed by the indicators of total grooming, was higher in animals of the experimental groups – by 200% and 400% (p<0.01).

The highest activity was observed in the I experimental group on the seventh day of the experiment: the level of THA was higher by 24% (p<0.01); VMA at 100% (p<0.01), total grooming at 400% (p<0.01). The research activity of animals in the experimental groups was lower than in the control one by 40% (p<0.01) and 60% (p<0.05), respectively.

A similar situation persisted by the 14th day of the experiment – the highest activity was observed in the group receiving titanium dioxide nanoparticles at a dose of 15 mg/kg: the level of THA was higher by 24% (p<0.01); VMA at 100% (p<0.01), total grooming at 400% (p<0.01). The research activity of animals in the experimental groups was lower than in the control one by 40% (p<0.01) and 60% (p<0.05), respectively.

The results of the Open Field test and the Infrared Actimeter system were confirmed. On the first day of the experiment, the activity of animals was also statistically significantly higher in the
experimental groups (by 56%); on the seventh day – higher by 257% (p<0.01) and 171% (p<0.01); on the 14th day – by 158% (p<0.01) in the I and II experimental groups.

The greatest changes in hematomal parameters were characteristic of the first experimental group. When assessing the morphological values of the blood on the first day, statistically significant differences were found between the experimental and control groups by all indicators (Table 1).

Table 1. Morphological blood values of the laboratory animals on the 1st, 7th and 14th day of the experiment, Me (q25-q75).

| Indicators | Control group | I group | II group |
|------------|---------------|---------|----------|
| RBC, 10¹²/L |               |         |          |
| 1st day    |               |         |          |
| 4.38 (3.84-4.78) | 7.93 (7.52-8.23) | 5.13 (4.7-5.71) |
| HGB, g/L    |               |         |          |
| 128.2 (99.2-130.3) | 149.9 (144.5-154.7) | 134.0 (109.1-160.9) |
| HCT, %      |               |         |          |
| 31.5 (30.3-35.4) | 38.8 (38.4-41.1) | 32.6 (27.3-36.8) |
| PLT, 10⁹/L  |               |         |          |
| 130.7 (130.3-132.4) | 136.3 (123.5-145.6) | 226.0 (217.6-244.9) |
| WBC, 10⁹/L  |               |         |          |
| 4.38 (3.94-5.42) | 9.44 (9.23-9.48) | 7.42 (4.7-8.78) |
| GRAN, 10⁹/L |               |         |          |
| 2.4 (2.3-2.53) | 3.1 (2.67-3.31) | 3.58 (3.42-3.76) |
| LYM, 10⁹/L  |               |         |          |
| 1.38 (1.04-1.87) | 5.44 (5.01-5.54) | 4.23 (3.05-4.34) |
| MO, 10⁹/L   |               |         |          |
| 0.49 (0.31-0.6) | 1.56 (1.48-1.64) | 1.03 (0.85-1.08) |
| 7th day     |               |         |          |
| RBC, 10¹²/L |               |         |          |
| 5.78 (5.04-5.92) | 7.45 (7.16-7.5) | 8.8 (8.41-8.94) |
| HGB, g/L    |               |         |          |
| 118.1 (114.0-122.7) | 152.0 (146.7-154.6) | 199.0 (194.2-201.8) |
| HCT, %      |               |         |          |
| 46.8 (43.0-46.8) | 39.1 (39.1-39.3) | 44.2 (42.8-45.4) |
| PLT, 10⁹/L  |               |         |          |
| 128.0 (119.3-150.4) | 141.8 (136.3-142.1) | 208.9 (203.9-213.2) |
| WBC, 10⁹/L  |               |         |          |
| 5.32 (5.10-5.83) | 7.15 (7.09-7.25) | 9.92 (9.7-10.3) |
| GRAN, 10⁹/L |               |         |          |
| 3.12 (2.8-4.08) | 4.00 (3.79-4.44) | 3.19 (3.1-3.27) |
| LYM, 10⁹/L  |               |         |          |
| 2.17 (2.13-2.2) | 4.3 (3.81-4.4) | 4.4 (4.25-5.18) |
| MO, 10⁹/L   |               |         |          |
| 0.63 (0.6-0.73) | 1.45 (1.27-1.49) | 2.1 (1.98-2.46) |
| 14th day    |               |         |          |
| RBC, 10¹²/L |               |         |          |
| 5.95 (5.74-5.98) | 7.2 (6.81-7.37) | 5.7 (5.66-5.8) |
| HGB, g/L    |               |         |          |
| 114.2 (108.7-118.0) | 138.5 (135.8-142.0) | 121.4 (120.8-121.8) |
| HCT, %      |               |         |          |
| 31.0 (30.2-32.4) | 38.8 (37.3-40.0) | 30.7 (30.5-31.2) |
| PLT, 10⁹/L  |               |         |          |
| 146.3 (144.2-147.7) | 169.0 (165.6-169.4) | 235.7 (233.2-247.3) |
| WBC, 10⁹/L  |               |         |          |
| 6.8 (6.04-7.33) | 7.3 (7.08-8.15) | 8.8 (8.36-8.87) |
| GRAN, 10⁹/L |               |         |          |
| 1.58 (1.51-1.6) | 2.33 (1.85-2.65) | 2.5 (2.42-2.57) |
| LYM, 10⁹/L  |               |         |          |
| 2.6 (2.18-2.96) | 3.48 (3.28-3.5) | 3.72 (3.6-3.96) |
| MO, 10⁹/L   |               |         |          |
| 0.58 (0.37-0.65) | 1.87 (1.81-1.92) | 1.66 (1.6-1.72) |

a Reliability of differences in performance with the control group – p<0.05; b Reliability of differences in performance with the control group – p<0.01.

By the seventh day, statistically significant changes were indicative only for the first experimental group: the red blood cell level was by 30% higher (p<0.05); platelet level by 11% (p<0.01); leukocytes by 34% (p<0.01); granulocytes by 28% (p<0.05) and monocytes by 130% (p<0.05) relative to the control group.

On the 14th day of the experiment in the I experimental group, the level of most of the indicators was higher than in the control one: erythrocytes and hemoglobin by 21% (p<0.01); hematocrit by 25% (p<0.01); platelets by 15% (p<0.01); granulocytes by 47% (p<0.01) and monocytes by 222% (p<0.05). In the II experimental group, higher rates of granulocytes and leukocytes were noted by 58% (p<0.01) and 29% (p<0.05), respectively.

When analyzing the biochemical blood values on the first day, regarding the control group differences were found in the protein metabolism: in the I experimental group – an increase in the level of total protein by 47% (p<0.01); creatinine by 58% (p<0.01) and urea by 95% (p<0.01). In the II group – an increase at the level of total protein by 68% (p<0.01). In the experimental groups, there were a higher cholesterol values compared with the control group – by 119% (p<0.01) and 164% (p<0.01), respectively. High activity of aminotransferases was noted in the experimental groups: in the I group -
ALaT by 178% (p<0.01); in the II group – ASaT by 282% (p<0.05). In both experimental groups, high SOD activity was observed by 20% (p<0.05). The introduction of NP-TiO$_2$ led to an increase in the serum Mg, Ca and P, while statistically significant differences with the control were obtained in the I experimental group for P level (higher by 177%), and in group II for P level (higher by 219%) and Mg (higher by 48%).

On the 7th day, the largest changes were revealed in the mineral metabolism at the first experimental group – the level of magnesium and iron was lower by 17% (p<0.05) and 9% (p<0.05) relative to the control group; indicators of calcium and phosphorus are higher by 43% (p<0.01) and 52% (p<0.01), respectively. Changes in protein metabolism persisted – creatinine level is by 30% higher (p<0.01), urea is by 15% higher (p<0.01); high transferase activity was noted – ALaT – by 151% (p<0.01), ASaT – by 341% (p<0.01) above the control values. In the II experimental group there were no statistically significant changes.

On the 14th day in the II experimental group, the glucose level was higher by 11% (p<0.05); total protein by 46% (p<0.05); by 83% of the total bilirubin (p<0.05); iron level was lower by 26% (p<0.05) than in the control group. In both experimental groups, higher cholesterol levels were also noted – by 2% (p<0.01) in the first group and by 17% (p<0.01) in the second one; high activity of the ALaT and ASaT enzymes.

5. Discussion of results

The PubMed database contains more than a thousand articles devoted to the studying the effect of titanium dioxide nanoparticles on biological objects, however, it should be noted that these experimental studies are often incomparable and the results can even contradict each other, since different methods and dosages of nanoparticles injection, shapes and sizes are used [16]. In our study, the intraperitoneal method of injection was chosen in doses of the corresponding 1/10 LD$_{50}$ and 1/2 LD$_{50}$.

A single intraperitoneal injection of titanium dioxide nanoparticles in doses of 15 mg/kg and 75 mg/kg results to changes in the behavioral responses of animals, which were accompanied throughout the experiment by the increased motion activity and emotional stress. According to the analysis of literature data, NP-TiO$_2$ can penetrate the blood-brain barrier and accumulate in the brain [17, 18]. The main mechanisms of neurotoxicity when exposed to titanium dioxide nanoparticles are oxidative stress, inflammatory reactions, apoptosis, genotoxicity, and direct disruption of cellular components. In addition, there are minor mechanisms - violation of the distribution of microelements, signaling, regulation of neurotransmitters [19, 20]. Thus, titanium dioxide nanoparticles can influence the body's behavioral responses. It should be borne in mind that nanoparticles have a low rate of elimination and, therefore, can accumulate in the body [16]. Therefore, prolonged or chronic exposure to NP-TiO$_2$ can cause abnormalities in neurons and glial cells and may further cause the central nervous system dysfunctions, including neurodegenerative diseases and mental disorders [21, 22, 23].

The research revealed the significant differences between the control and experimental groups in many hematological parameters. Elevated levels of erythrocytes, hemoglobin, hematocrit, leukocytes and platelets against the background of high cholesterol indicate hyperviscose syndrome. According to M.A. Abdelhalim, an increase in blood viscosity correlates with the progression of coronary and peripheral vascular diseases [24, 25]. There is an activation of the immune system, which is accompanied by an increase in the level of white blood cells by increasing the number of granulocytes, lymphocytes and monocytes in response to the introduction of TiO$_2$ nanoparticles. The obtained data are consistent with the research of scientists at the Beijing University [26]. Titanium dioxide nanoparticles activate platelet synthesis, which can later affect the microcirculation.

The biochemical values of blood of the experimental groups showed significant changes compared with the control group. In both groups, there were significant increases in serum ALaT, ASaT, total protein, creatinine and urea, indicating that destructive changes in liver and kidney function are really possible [27]. Researchers have reported the possibility of accumulation of titanium dioxide nanoparticles in the liver, spleen, lungs and kidneys after the intravenous injection [28].

The ability of TiO$_2$ to stimulate the formation of reactive oxygen species is the most important mechanism underlying the toxicity of these nanoparticles [29]. One of the main enzymes of the antioxidant system is SOD. On the first day of the experiment, a significant increase in the activity of
this enzyme was observed in the experimental groups, which is a natural protective reaction against the highly toxic oxygen radicals. However, then a decrease in the activity of superoxide dismutase was observed, which is consistent with the studies of L. Wang and his co-authors [30]. Perhaps a decrease in SOD, leading to increased oxidative stress, may underlie the nanotoxic effect [31].

The titanium dioxide nanoparticles affect the mineral metabolism. In the study, there were violations in the calcium-phosphorus metabolism, indicators of magnesium and iron. It is reported that the effect of titanium dioxide nanoparticles reduces the number of microvilli of the intestinal epithelium, reducing the absorption of nutrients. According to scientists at Binghamton University, the iron absorption decreased when the NP-TiO$_2$ were exposed [32]. According to the works of researchers at Shenyang University of Pharmacy, NP-TiO$_2$ are able to induce apoptosis of mouse hippocampal neurons through the oxidative stress and indirect calcium imbalance [33].

6. Conclusion
The research demonstrated the negative effects of NP-TiO$_2$ on the body, which indicate a potential risk to the human health, as well as to the biota of the environment. Despite the fact that the doses administered were significantly different, the effects of exposure were similar, which indicates the absence of a dose-dependent effect. The greatest effect of exposure was recorded on the first day of the experiment with a further tendency to decrease, which is possibly due to the adaptation reactions and partial removal of nanoparticles from the body.

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References
[1] Brohi R D, Wang L, Talpur H S, Wu D, Khan F A, Bhattarai D, Rehman Z and Farmanullah F 2017 Toxicity of Nanoparticles on the Reproductive System in Animal Models: A Review Frontiers in Pharmacology 8 606
[2] Shi H, Magaye R, Castranova V and Zhao J 2013 Titanium dioxide nanoparticles: a review of current toxicological data Particle and Fibre Toxicology 10 15
[3] Winkler H C, Notter T, Meyer U and Naegeli H 2018 Critical review of the safety assessment of titanium dioxide additives in food Nanobiotechnology 16 51
[4] Peters R J, Bemmel G, Herrera-Rivera Z, Helsper H P, Marvin H J, Weigel S, Tromp P C, Oomen A G, Rietveld A G and Bouweemeester H 2014 Characterization of titanium dioxide nanoparticles in food products: analytical methods to define nanoparticles J. of Agricultural and Food Chemistry 62(27) 6285–93
[5] Pedata P, Ricci G, Malorni L, Venezia A et al 2019 In vitro intestinal epithelium responses to titanium dioxide nanoparticles Food Res. International 119 634–42
[6] EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) 2016 Re-evaluation of titanium dioxide (E 171) as a food additive EFSA J. 14(9)
[7] Dudefoi W, Terrisse H, Popa A F, Gautron E, Humbert B and Ropers M H 2018 Evaluation of the content of TiO$_2$ nanoparticles in the coatings of chewing gums Food additives & contaminants 35(2) 211–21
[8] Lua P J, Fanga S W, Cheniga W L, Huanga S C, Huangb M C and Chenga H F 2018 Characterization of titanium dioxide and zinc oxide nanoparticles in sunscreen powder by comparing different measurement methods J. of Food and Drug Analysis 26(3) 1192–200
[9] Kvan O, Gavrish I, Lebedev S, Korotkova A, Miroshnikova E, Bykov A, Serdaeva V, Davydova N. 2018 Effect of probiotics on the basis of Bacillus subtilis and Bifidobacteriumlongum on the biochemical parameters of the animal organism. Environmental Science and Pollution Research 25(3) 2175-2183
[10] Ghosal K, Agatemor C, Špitálsky Z, Thomas S and Kny E 2019 Electrosprining tissue engineering and wound dressing scaffolds from polymer-titanium dioxide nanocomposites Chemical Engineering J. 358 1262–78
[11] Mech A, Rasmussen K, Jantunen P, Aicher L, Alessandrelli M, Bernauer U et al 2018 Insights into possibilities for grouping and read-across for nanomaterials in EU chemicals legislation Nanotoxicology
[12] Hmelnickij I K, Larin A V and Luchinin V V 2015 Current state of regulatory and methodological support of nanotechnology safety in the Russian Federation Biotechnosphere 95–103
[13] Candás-Zapico S, Kutscher D J, Montes-Bayón M, Bettmer J 2018 Single particle analysis of TiO$_2$ in candy products using triple quadrupole ICP-MS Talanta (180) 309–15
[14] Lebedev S, Gavrish I A, Gubaydullina I Z 2019 Different chrome sources influence on morphobiochemical indicators and activity of digestive enzymes in wistar rats Sel'skokhoyazvstvennaya biologiya [Agricultural Biology] 54 (2) 304-315
[15] *Titanium dioxide* Retrieved from: https://pubchem.ncbi.nlm.nih.gov/compound/26042#section=Safety-and-Hazards
[16] Song B, Liu J, Feng X, Wei L and Shao L 2015 A review on potential neurotoxicity of titanium dioxide nanoparticles Nanoscale Res. Letters 10 342
[17] Shao L Q and Song B 2017 Toxicity of Titanium Dioxide Nanoparticles on Brain Neurotoxicity of Nanomaterials and Nanomedicine 5 99–125
[18] Czajka M, Sawicki K, Sikorska K, Popek S, Kruszewski M a)nd Kapka-Skryczczak L 2015 Toxicity of titanium dioxide nanoparticles in central nervous system Toxicology in Vitro 29(5) 1042–52
[19] Wu T and Tang M 2018 The inflammatory response to silver and titanium dioxide nanoparticles in the central nervous system Nanomedicine (Lond) 13(2) 233–49
[20] Song B, Zhang Y, Liu J, Feng X, Zhou T and Shao L 2016 Unraveling the neurotoxicity of titanium dioxide nanoparticles: focusing on molecular mechanisms Beilstein J. of Nanotechnology 7 645–54
[21] Valentini X, Deneufbourg P, Paci P, Rugira P, Laurent S, Fraa A, Stanicki D, Ris L and Nonclercq D 2018 Morphological alterations induced by the exposure to TiO2 nanoparticles in primary cortical neuron cultures and in the brain of rats Elsevier Toxicology Reports 5 878–89
[22] Ze Y, Sheng L, Zhao X, Ze X, Wang X, Zhou Q et al 2014 Neurotoxic characteristics of spatial recognition damage of the hippocampus in mice following subchronic peroral exposure to TiO$_2$ nanoparticles J. of Hazardous Materials 264(15) 219–29
[23] Sheida E, Sipailova O, Miroshnikov S, Sizova E, Lebedev S, Rusakova E and Notova S 2017 The effect of iron nanoparticles on performance of cognitive tasks in rats Environmental Sci. and Pollution Res. 24(9) 8700–10
[24] Abdelhalim M A 2011 The effects of size and period of administration of gold nanoparticles on rheological parameters of blood plasma of rats over a wide range of shear rates Lipids in Health and Disease 191
[25] Sheyda E., Sipaylova O., Kvan O., Notova S., Nesterov D., Rusakova E., Kosyan D., Duskaev G. 2014 Functional properties of antimicrobial peptides extracted from hens’ platelets. *Life Science Journal* 11(9).25 180-184
[26] Chen Z, Wang Y, Zhuo L, Chen S, Zhao L, Luan X, Wang H and Jia G 2015 Effect of titanium dioxide nanoparticles on the cardiovascular system after oral administration Toxicology Letters 239(2) 123–30
[27] Sarhan M and Hussein M 2014 Effects of intraperitoneally injected silver nanoparticles on histological structures and blood parameters in the albino rat Int. J. of Nanomedicine 9 1505–17
[28] Sizova E.A., Yausheva E.V., Miroshnikov S.A., Lebedev S.V., Duskaev G.K. 2015 Element status in rats at intramuscular injection of iron nanoparticles. Biosciences Biotechnology Research Asia 12 119-127.
[29] Li M, Yin J-J, Wamer W G and Lo Y M 2014 Mechanistic characterization of titanium dioxide nanoparticle-induced toxicity using electron spin resonance *J. of Food and Drug Analysis* **22**(1) 76–85

[30] Hou J, Wang L, Wang C, Zhang S, Liu H, Li S and Wang X 2019 Toxicity and mechanisms of action of titanium dioxide nanoparticles in living organisms *J. of Environmental Sci.* **75** 40–53

[31] Niska K, Pyszka K, Tukaj C, Radomski M W and Inkielewicz-Stepniak I 2015 Titanium dioxide nanoparticles enhance production of superoxide anion and alter the antioxidant system in human osteoblast cells *Int. J. of Nanomedicine* **10** 1095–107

[32] Guo Z, Martucci N J, Moreno-Olivas F, Tako E and Mahler G J 2017 Titanium Dioxide Nanoparticle Ingestion Alters Nutrient Absorption in an In Vitro Model of the Small Intestine *NanoImpact* 70–80

[33] He Q, Zhou X, Liu Y, W Gou, Cui J, Li Z, Wu Y and Zuo D 2018 Titanium dioxide nanoparticles induce mouse hippocampal neuron apoptosis via oxidative stress- and calcium imbalance-mediated endoplasmic reticulum stress *Environmental Toxicology and Pharmacology* **63** 6–15