



TOXICOLOGICAL AND HYGIENIC ASSESSMENT OF TITANIUM DIOXIDE NANOPARTICLES AS A COMPONENT OF E171 FOOD ADDITIVE (REVIEW OF THE LITERATURE AND METAANALYSIS)

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The review focuses on exposure values, biological availability, toxic effects, and risks caused by nanoparticles of TiO₂ under their oral introduction into a body as a food coloring agent or E171 food additive, or as a significant component in its structure. According to toxicological assessment performed by JECFA in 1969, TiO₂ is considered to be insignificantly hazardous. However, at present experts employed by several foreign and international organizations that deal with food safety believe that the assessment should be reviewed as there are new scientific data on adverse effects produced by nano-sized TiO₂ on a human body. Overall intake of TiO₂ by people with food products, cosmetics (tooth pastes) and medications can vary from 0.5 to 5 mg a day; children aged 3–9 and teenagers aged 10–17 are the most exposed population groups. Despite insignificant intestinal absorption of TiO₂ nano- and micro-sized particles, a lot of scientific works revealed their overall toxic effects produced on a body under oral and intragastric introduction. Detected effects produced by TiO₂ include organotoxic (mostly hepatotoxic) ones, genotoxicity, immune toxicity, reproductive toxicity, and neurotoxicity. Still, there haven't been any data on carcinogenic effects produced by TiO₂ when it is introduced into the gastrointestinal tract. Presumably, some effects produced by TiO₂ nanoparticles are mediated by their local impacts on the lymphoid tissue associated with an intestinal mucosa as well as on the structure and activity of intestinal microbiocenosis, and nanoparticles are not necessarily absorbed in the intestines in the process. We performed meta-analysis of 64 articles (published over 2007–2019) which complied with criteria related to scientific authenticity and completeness; the meta-analysis revealed that a probable NOAEL for nano-sized TiO₂ amounted to less than 10 mg/kg of body weight a day, and a daily reference safe dose of the substance is estimated as being equal to 0.1 mg/kg of body weight. Given all the above-mentioned, a risk caused by TiO₂ intake as E171 food additive depends on nanoparticles fracture in its composition and it can be unacceptably high if this fracture exceeds 10 % of the overall TiO₂ mass. Therefore, it is necessary to control and regulate TiO₂ nanoparticles contents in the structure of E171 food additive that is applied in food industry.

Key words: titanium dioxide, food additive, nanoparticles, exposure, biological availability, toxicity, intestinal microbiocenosis, risks.

Titanium dioxide (TiO₂) is widely used in various food products as a coloring agent E171 due to its intense white color. Production volumes of the substance exceeded 5 million tons in 2008 [1]. Both Russian and international legislation allows using E171 in food products manufacturing (The Customs Union Technical Regulations “Requirements to safety of food additives, flavoring agents and technological auxiliary substances” (TR CU 029/2012)¹,

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¹TR CU 029/2012. Requirements to safety of food additives, flavoring agents and technological auxiliary substances (last edited on September 18, 2014): The Customs Union Technical Regulations [Web-source] // KODEKS: an electronic fund of legal and reference documentation. – URL: <http://docs.cntd.ru/document/902359401> (date of visit March 23, 2019).

“Codex General Standard For Food Additives” (CODEXSTAN 192-1995)², Regulation (EU) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives³). Significant amounts of TiO₂ can obviously be found in covers of medications or in cosmetics (toothpastes). Besides, according to the Eurasian Union legislation, TiO₂ as a component of titanium enamels can be used in manufacturing package and materials that contact food (the Customs Union Technical Regulations “On safety of package”, TR CU 005/2011⁴). Therefore, population exposure to TiO₂ via the gastrointestinal tract can be rather considerable. And there is a question here related to TiO₂ safety for consumers and health risks that can be probably caused by it. It is especially true for nanoparticles (NPs) that can be contained in E171; that is, particles with their sizes being less than 100 nm [2]. In all cases that are practically significant these NPs are represented by two alternative crystal forms of TiO₂, namely anatase and rutile. Anatase NPs are, as a rule, spherical or oval and their sizes vary from 10 to 100 nm (most typically 20-30 nm); rutile NPs often have irregular shape or are rod-like with their cross section being less than 10 nm and their length being 40-50 nm or more. Both NPs tend to agglomerate or aggregate significantly in water suspensions and as components in food products; these processes depend on concentrations of a nanomaterial and disperse medium structure. Particle size analysis performed on widely spread E171 trademarks revealed that they often had anatase or rutile in their structure [3]. Due to their small size these NPs have potentially much greater penetrability than their micro-analogues (microparticles or MPs) and it allows them to penetrate through biological barriers including those lo-

cated in the gastrointestinal tract [4]. They also possess considerably greater chemical potential per a mass unit, greater catalytic activity and greater solubility as well. It means that we can't neglect toxic effects possibly produced on a human body by TiO₂, NPs contained in food products, cosmetics or medications.

Our research goal was to analyze data taken from scientific literature on exposure values, biological availability, toxic effects, and risks caused by TiO₂ nanoparticles when they were introduced into a body via the gastrointestinal tract as a food additive E171 or as a significant component in its structure.

Exposure volumes and scenarios

TiO₂ is used in food products manufacturing as a coloring agent that makes a product white due to a decrease in a volume of the “grey” component in a spectrum of light radiation that is back-scattered by this product [2]. In 1969 the Joint FAO/WHO Expert Committee on Food Additives (JECFA) developed the first and still the only one officially accepted and valid toxicological assessment of TiO₂ as a food additive [5]. An issue related to probable negative impacts exerted by NPs contained in titanium dioxide was not considered within that assessment procedure. There was a conclusion that it was not necessary to fix safe daily levels of consumption for this substance, mostly due to its extremely low solubility. According to European Food Safety Authority, pigment

TiO₂ (or so called “titanium white”) with its particles sized 01-1.0 μm is safe for a human body due to its poor solubility in water and biological media and total absence of its absorption in the gastrointestinal tract [6,7].

In the USA TiO₂ was allowed as a coloring agent in 1966 [8]. US FDA* allows using TiO₂ in a food product in a quantity not exceeding 1% of its total mass [9]. Besides, in

² CODEX STAN 192-1995. General Standard For Food Additives [web-source]. – URL: http://www.fao.org/gsfaonline/docs/CXS_192e.pdf (date of visit March 23, 2019).

³ Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives (Text with EEA relevance) [web-source]. – URL: <https://eur-lex.europa.eu/eli/reg/2008/1333/oj> (date of visit March 23, 2019).

⁴ TR CU 005/2011. “On safety of package” (last edited on October 18, 2016): The Customs Union Technical Regulations [web-source] // KODEKS: an electronic fund of legal and reference documentation. – URL: <http://docs.cntd.ru/document/902299529> (date of visit March 23, 2019).

* United States Food and Drug Administration.

* Joint FAO/WHO Expert Committee on Food Additives.

the USA TiO_2 is allowed as “a material that contacts food” (as a component in food products packages). In Japan there are no limits imposed on TiO_2 application as a coloring agent [10]. In India a quantity of TiO_2 that can be added to a food product is limited to 1% in chewing gums and to 0.01% in dried mixtures used to make drinks [11]. As per data provided by X.-X. Chen [et al.], if any standard for TiO_2 contents in chewing gums is not fixed in a country, its quantity can reach up to 0.2% of a total mass of a product, and 93% out of this quantity can be a nanoform of the substance [12]. In the European Union [13] and in Russia, according to TR CU 029/2012¹, TiO_2 is allowed in manufacturing all types of food products in conformity with technical specifications, excluding products enlisted in Appendix 9. TiO_2 can also be applied to dye covers of medications (The RF Public Healthcare Ministry Order issued on March 19, 1998 No. 80⁵) and in cosmetics (toothpastes).

In 2013 EU Scientific Committee on Consumer Safety (EU-SCCS) published an expert opinion on a food additive E171 consisting of NPs [14]. This opinion being based on results of only two publications [15, 16], the Committee concluded that a minimal dose corresponding to LOAEL for nano- TiO_2 amounted to 5 mg/kg of a body weight a day. Experts also concluded that the assessment made by JECFA* in 1969 was no longer valid due to new scientific data being discovered and it was necessary to perform a new examination on TiO_2 safety when the substance was applied as a coloring agent in food products.

Therefore, it is crucial to determine which part of orally introduced TiO_2 is represented by NPs if we want to assess exposure and risks related to it. As per data provided by A. Weir [et al.], [17] an average diameter of particles contained in popular pigment TiO_2 , applied in food manufacturing can be equal to 110 nm; and according to electronic microscopy, at least 36% out of overall number of particles have their diameter varying from 30 to 100 nm.

However, as a mass of a particle grows proportionally to a cube of its diameter, an overall mass of a nano-sized component in food TiO_2 will be significantly lower. According to recommendations issued by the European Commission, to be called a nano-material, a substance should contain nanoparticles in a quantity not lower than 50% out of their total number [18]. If this criterion is applied, most E171 trademarks that are currently used can be considered a nanomaterial.

It was shown that when water extractions from such products as sweets, confectionary, chewing gums, and toothpastes were put through membrane filters, only about 5% Ti passed through pores with their diameter being 0.45 or 0.5 μm [17]. The result can have two interpretations; first of all, it can occur due to practical absence of nanoparticles in a sample, or it can be due to a massive aggregation or agglomeration of NPs contained in it or their adsorption on matrix components with their size being much greater than that within a nano-range.

According to research results provided by R.J. Peters [et al.] [19], most E171 samples obtained from food products contained NPs sized less than 100 nm in a quantity equal to 10-15% out of total particles number. TiO_2 was detected in 24 out of 27 types of examined food products and products for personal hygiene in significant quantities ranging from 0.02 to 9.0 mg/g, and 5-10% particles in these products were within a nano-range and it was qualitatively consistent with analysis performed on pure E171 samples.

Titan (Ti) as chemical element is known to be widely spread in the Earth crust; it is a natural component in animal tissues though it is found there in minimal quantities [20]. There are no data on any specific biological role played by Ti or the element being essential for animals. Nonetheless, data on background Ti levels in biological objects should be taken into account when assessing results of research that focused on attempts to determine NPs contents in food products and biological

⁵ On application of coloring agents in medications: The RF Public Healthcare Ministry Order issued on March 19, 1998 No. 80 [web-source]. – URL: <http://www.consultpharma.ru/index.php/ru/documents/drugs/374-80?showall=1> (date of visit March 23, 2019).

materials basing on data obtained via elemental analysis. Introduction of overall Ti being a natural food component into a human body can amount to approximately 300-400 μg a day, while introduction with drinking water is considered insignificant. A human body is exposed to significantly greater quantities of Ti due to various consumer products containing TiO_2 (E171) which was deliberately added to them both as microparticles (MPs, diameter is 100-2,500 nm) and as NPs.

As per early data provided by Dietary intake of food additives in the UK [21], in the UK average daily TiO_2 consumption amounted to 5.4 mg/kg of body weight. Later Weir [et al.] [17] performed more precise estimation and obtained another value varying from 0.2 to 2 mg/kg and it is rather close to the above-mentioned assessment for LOAEL without taking into account percentage of nanoparticles in TiO_2 contained in food products.

According to M.B. Herringa [et al.] [22], in Western European countries up to 57% of orally introduced TiO_2 came from toothpastes. There are some other products that can also be a source of TiO_2 NPs, for example, chewing gums (14%), coffee creamers (13%), dried milk for coffee (8%), glazed chocolates (3%), mayonnaise (7%), spicy sauces (5%), and instant cappuccino (3%). Younger children obviously face additional exposure with toothpaste as they tend to swallow it and it is quite significant for this age group. According to these data, TiO_2 was consumed as E171 coloring agent in the following quantities: 0.67 mg/kg b.w. by children aged 2-6; 0.17 mg/kg b.w. by people aged 7-69; and 0.06 mg/kg b.w. by people older than 70. Average estimated consumption of TiO_2 NPs amounted to 0.19 $\mu\text{g}/\text{kg}$ b.w. by people older than 70; 0.55 $\mu\text{g}/\text{kg}$ b.w. by people aged 7-69; and 2.16 $\mu\text{g}/\text{kg}$ b.w. by children aged 2-6. 95%-percentile of consumption amounted to 0.74, 1.61, and 4.16 $\mu\text{g}/\text{kg}$ b.w. in these age groups respectively. The authors didn't estimate quantities in which this coloring agents was consumed by children younger than 2. When performing their calculations, the authors assumed that average NPs

contents in commercial samples of E171 amounted to approximately 0.31% as per a sample mass [1].

A work published by EFSA* [6] focused on exposure to TiO_2 as a component in covers of medications and biologically active additives to food (BAA) sold as pills or capsules. In such cases TiO_2 contents can reach up to 3% out of the overall mass of a pill, and up to 12.5% out of this quantity can be rutile. Average daily consumption of medications usually amounts to approximately 20-200 mg; BAA, 10-1,000 mg. Daily exposure to TiO_2 can therefore be estimated as 15-37.5 mg respectively, or 0.625 mg/kg b.w. The same work contained another estimation of exposure to rutile in confectionary and it varied from 0.071 to 0.495 mg/kg b.w. a day.

M.-H. Ropers [3] stated that in the USA amount of TiO_2 consumed with food amounted to approximately 0.2-0.7 mg/kg of b.w. a day while in the UK, Holland, and Germany it could reach 1 mg/kg b.w. These data were obtained via taking into account all scenarios of introduction with food. For example, in Holland median estimation of E171 introduction varied from 1.1 to 1.4 mg/kg b.w., and the upper introduction limit for children amounted to 3.2 mg/kg b.w. Similar results were obtained in Germany. In spite of all the differences between analyzed exposure scenarios, data obtained in all the research works coincide about the most exposed populations groups and these are children aged 3-9 and teenagers aged 10-17. EFSA experts believe that a contribution made by chewing gums is insignificant in comparison with other confectionary should we take into account consumed breath fresheners in pills, spicy sauces, and salad seasonings [7]. A research work conducted in Germany contained a conclusion on food products that made maximum contribution (up to 75%) into consumed TiO_2 quantities; they were spicy sauces, salad seasonings, soft drinks, and cheese [23].

As per data obtained via Monte Carlo method in the USA population consumed TiO_2 in all its forms in quantities being equal up to 1 mg/kg b.w. a day [17].

* European Food Safety Authority.

Table 1

Estimated daily oral exposure to titanium dioxide used as a component in food products, cosmetics, medications, and BAA

Reference	Exposure, mg/kg b.w.	Region, country	Population group	Note
MAFF, 1993	5.4	Great Britain	All groups	Median
Weir [et al.], 2012	1.0 2.0	The USA Western Europe	All groups – « –	Median Median
EFSA, 2004	1.1	EU countries	All groups	Median
Rompelberg [et al.], 2016	0.59	Holland	Children aged 2-6	Median
	1.29	– « –	– « –	95%-percentile
	0.08	– « –	Children aged 7–16 and adults	Median
	0.50	– « –	– « –	95%-percentile
Ropes [et al.], 2017	0.7	The USA	All groups	Median
	1.4	Western Europe	All groups	Median
	3.2	Western Europe	Children younger than 16	Median

Therefore, oral exposure of population to TiO₂ NPs was estimated by different authors and the results were not quite similar depending on a chosen scenario, selection of examined products, probable TiO₂ contents in them, and, above all, uncertainties related to a question which fraction of a pigment was represented by NPs. Completely nano-sized TiO₂ products are assumed to be scarcely used as a food additive E171 because maximum whitening (light-diffusing) effects are produced by the substance with its average particle size being equal to approximately 200 nm [1].

Table 1 contains basic results as regards estimating oral daily exposure of adults and children to all TiO₂, forms.

Biological availability, distribution and accumulation

There are few works with attempts to directly examine penetrability of intestinal mucous tunics for TiO₂ NPs and MPs. To do this, some researchers applied *in vitro* models of intestinal epithelium that applied monolayers with cells being functionally and morphologically identical to enterocytes, for example, Caco-2 line cells. Z.M. Song et al [24] showed in their work that when TiO₂ NPs influenced a cellular monolayer in a concentration equal to 10-100 µg/ml (which corresponded to an oral introduction into a human body equal to approximately 1-10 mg/kg b.w.), they penetrated through the epithelial layer in small quantities, both via transcytosis and paracellular transport through inter-cellular contacts due to damaged

γ-catenane structure. As per data provided by B.A. Koeneman [et al.] [25], NPs were able to accumulate in Caco-2 cells but they didn't penetrate through a monolayer in significant quantities. G. Janer [et al.] [26] applied models for mucous tunics of rats' intestines and Caco-2 cells monolayer to show that NPs penetrated epithelial cells in minimal quantities excluding specialized M-cells of Payer's plaques. G.E. Onishchenko et al. [27] applied transmission electronic microscopy (TEM) to examine effects produced by rutile NPs on a mucous tunic of a rat's intestine when they were introduced in a quantity equal to 50 mg into an isolated loop of the ileum with preserved blood supply and innervations. The authors detected massive NPs deposition on the apical surface of enterocytes and their penetration into apical cytoplasm in small quantities. So, there are data confirming TiO₂ NPs ability to penetrate through intestinal mucous tunics in rather limited quantities.

It is rather difficult to quantitatively estimate TiO₂ absorption in the gastrointestinal tract; the task can probably be solved by detecting NPs or MPs of the substance with analytical TEM or via elemental analysis of Ti contents in tissues. Historically, P.U. Jani [et al.] were the first to do that [28], as they applied histological and chemical procedures to detect TiO₂ MPs sized 500 nm in the liver, spleen, and lymphoid tissue of the intestines taken from female Sprague Dawley rats after a single oral introduction in a dose equal to

12.5 mg/kg b.w. MPs were not detected either in the heart or kidneys.

TiO₂ MPs (150 nm) and NPs (27 and 80 nm) were introduced into male and female mice in a dose equal to 5,000 mg/kg b.w.; after this single introduction Ti was detected in the liver, spleen, and kidneys [29]. Y.Cui [et al.] also highlighted that Ti quantities grew in the liver of mice that had been orally exposed to TiO₂ NPs during 60 days [30]. X. Sang [et al.] detected growing Ti contents in the spleen [31, 32] and thymus of mice [33] under longer exposure (90 days) to TiO₂ NPs in a dose equal to 10 mg/kg b.w. Anatase NPs accumulated in the spleen and ovaries of female Sprague-Dawley rats under 5-day introduction in a dose equal to 1-2 mg/kg b.w. As per data provided by G.E. Onishchenko [et al.] [27], when rutile was introduced during 28 days into Wistar rats in a dose equal to 100 mg/kg b.w., it resulted in its accumulation in the liver. Data obtained by R. Shrivastava [et al.] [35] indicate that TiO₂ NPs are likely to penetrate through the blood-brain barrier and enter cytoplasm and nucleuses of brain cells after a single intragastric introduction in a dose equal to 500 mg/kg b.w. Y. Ze [et al.] [36] also detected these NPs in mice's brains after 90-day introduction in a dose varying from 2.5 to 10 mg/kg of b.w. TiO₂ NPs accumulation in the stomach mucous tunic of mice was detected after their introduction in a dose equal to 500 mg/kg b.w. during 5 days [37]; the authors believed it could probably result in gastritis. Similar data were obtained regarding accumulation in mucous tunics of rats' small intestine [38]. There was a sub-acute 14-week experiment [39] when anatase NPs were introduced into mice's stomachs in doses up to 320 mg/kg b.w.; Ti was biologically distributed in the liver, spleen, small intestine, kidneys, and pancreas. F. Hong [et al.] [40] introduced TiO₂ NPs in a dose equal to 25-100 mg/kg b.w. into female mice during their pregnancy (17 days); after that Ti accumulation was detected in fetuses and placenta [40]. A recent work by J. Yang [41] contains data on profoundly described anatase NPs sized 21 nm and their accumulation in mice's livers after 14-day oral introduction.

Some other researchers failed to detect TiO₂ NPs absorption in the gastrointestinal tracts and their biological accumulation at detection limits of analytical procedures which they applied in their experiments. Thus, Cho [et al.] applied ICP-MS and didn't detect Ti accumulation in the liver, kidneys, spleen, brain, and urine excretion after a mixture of anatase and rutile NPs (80:20) had been orally introduced into rats in a dose equal to 1,000 mg/kg b.w. during 13 days [42]. Geraets [et al.] performed a 5-day experiment on male and female Wistar rats orally introducing TiO₂ NPs (with diameter 38-67 nm) or MPs (with diameter 132-267) into them. They revealed trace Ti quantities (not more than 0.001% of an introduced dose) only in the liver and spleen and it was rather close to a detection limit for this analytical procedure [43]. L. Martins [et al.] didn't reveal any Ti accumulation in the liver, kidneys, or blood after NPs had been introduced into Wistar rats during 45 days in a dose equal to 0.5 mg/kg b.w. [44]. E.M. Donner [etal.] [45] also stated they didn't reveal any biological accumulation in internal organs in their research during which rats were exposed to a single introduction of 6 different anatase or rutile NPs or MPs in a dose varying from 500 to 2,000 mg/kg b.w.; the same results were obtained by N. Gu[etal.] [46] after TiO₂ MPs had been introduced into mice in a dose equal to 64 mg/kg b.w.

Therefore, chemical analysis procedures, even highly sensitive ones (ICP-MS) didn't allow obtaining unambiguous results as regards probable TiO₂ NPs absorption in the gastrointestinal tract and their accumulation in organs and tissues. It can partly be due to artifacts associated with natural background Ti contents in biological objects and, owing to it, its excessive introduction into organs and tissues as a component in NPs can only be measured with great inaccuracy. On the other hand, part of negative results that have been described above can be explained by an incorrect choice on a dose of a nanomaterial (too low or, on the contrary, too high a dose resulting in total NPs aggregation), or by exposure duration being insufficient. This uncertainty could

be eliminated via applying NPs with a radionuclide marker; however, Ti is a chemical element without any long-living radionuclides. An attempt to mark TiO₂ NPs with iodine isotope [23] failed as this marker easily detached from NPs in a biological medium. Yu.P. Buzulukov [et al.] [47, 48] tried to mark rutile NPs with scandium radionuclide via bombarding with fast neutrons with an energy exceeding 1.9 MeV on a cyclotron as per the following reaction: $^{47}_{22}\text{Ti}(n,p)^{47}_{21}\text{Sc}$. Marked NPs were introduced into male Wistar rats; there was a single introduction in a dose equal to 400 mg/kg b.w. Registered radioactivity in internal organs didn't allow detecting markers in the spleen, pancreas, gonads, kidneys, lungs, heart, brain, or urine. The authors detected only trace quantities of markers (approximately 0.002% out of an introduced dose) in the liver and blood. More than 99.9% of radioactivity was excreted with feces, and about 0.06% of the introduced radioactivity was detected in bone-muscular-skin carcasses; the authors explained it by fur near anuses being probably contaminated with feces. Therefore, a single result obtained via a radioisotope procedure is consistent with research works where it is stated that TiO₂ NPs have very insignificant (very close to zero) biological availability when they are introduced into the gastrointestinal tract.

Hazard characteristic

1. General effects and target organs.

Obviously, TiO₂ NPs and MPs really have very insignificant biological availability but still there is a great number of works containing data on revealed overall toxic effects produced by them on a human body under oral and intragastric introduction. Chronologically, the first work was published by Wang [et al.] [29]; they introduced NPs (25 and 80 nm) or MPs (150 nm) into male and female mice; there was a single introduction in a dose equal to 5,000 mg/kg b.w. The authors didn't detect any signs of acute toxicity (lethality or drastic changes in animals' behavior) but they revealed perivascular degeneration and localized necrosis of hepatocytes, shifts in LDH activity and AST/ALT ratio in blood plasma, increased urea contents, and pathological changes in the

kidneys. A single intragastric introduction of TiO₂ NPs in a dose equal to 160-1,000 mg/kg b.w. into Wistar rats resulted in higher contents of taurine, citrate, hippurate, histidine, trimethylamine-N-oxide, citrulline, α -ketoglutarate, phenyl acetyl glycine and acetate in urine and in lower contents of lactate, betaine, methionine, threonine, pyruvate, 3-D-hydroxybutyrate, choline and leucine. Similar metabolome shifts were also detected in blood plasma [49].

When anatase NPs were orally introduced into female CD-1 (ICR) mice for 30 days in doses from 62.5 to 250 mg/kg b.w., it caused higher ALT, AST and alkaline phosphatase activity, higher concentrations of nitrogen oxide, bilirubin, and interleukin-s, shifts in blood leukogram and contents of CD3(+), CD4(+), CD8(+) cells, populations, NK-lymphocytes, and B-cells populations as well [15].

R.V. Raspopov [et al] [50] introduced anatase NPs, rutile NPs, and TiO₂ pigment MPs into growing male Wistar rats during 30 days; the substances were introduced intragastrically in doses equal to 1 and 100 mg/kg b.w. The lowest dose caused greater penetrability of intestinal walls for protein macromolecules, greater excretion of 8-oxo-G (a product of DNA oxidative destruction), lower contents of reduced thioles and CYP2B1 activity, greater overall activity of glutathione-S-transferases in the liver, and lower alkaline phosphatase activity in blood plasma. Effects revealed in the research were both those specific for NPs only and those produced by both particles types, MPs included. Other researchers applied proteomics to examine Wistar rats after exposure to anatase NPs in doses equal to 1-10 mg/kg b.w. and revealed abnormal isoform of glutathione-S-transferase expressed in the liver as well as occurrence of 53 protein spots and disappearance of 19 others that were not exactly identified [51].

Y.Cui [et al.] introduced TiO₂NPs into mice during 60 days and revealed enhanced hepatocytes apoptosis, oxidative stress development, lower expression of metallothionein, heat shock protein HSP70, P53, and transferrin [52]; transcriptome changes in expression

of TLR2 и 4, IKK1, IKK2, NF- κ B, NF- κ BP52, NF- κ BP65, TNF- α , NIK, I κ B and IL-2 genes [30].

In another research Wistar rats were exposed to TiO₂ NPs in a dose equal to 300 mg/kg and it resulted in greater lipoperoxides contents in the liver, greater ALT and AST activity in blood plasma, and lower antioxidant enzymes activity. Histological picture of damages done to the liver included apoptosis, centrilobular necrosis, and inflammation cells proliferation [53]. Changes in expression of p53, BAX, caspase-3 и -9 and Bcl-2 and signs of oxidative damage to DNA were observed in the liver of mice exposed to TiO₂ NPs in a dose equal to 100 mg/kg b.w. for 14 days [54].

Anatase NPs (21 nm) turned out to be hepatotoxic for mice in a dose equal to 150 mg/kg b.w.; it became obvious in a 14-day experiment through greater transaminase activity in blood plasma, liver edema, malonic dialdehyde accumulation in liver tissues, liver macrophages activation, greater TNF- α and IL-6 production, expression of nucleus erythroid-2-related factor 2 and NF- κ B together with inhibited expression of Bcl-2[55]. Yang [et al.] revealed disorders in metabolic functions of the liver caused by exposure to orally introduced anatase NPs sized 21 nm [41].

Y. Wang [et al.] [56] showed that introduction of TiO₂ NPs sized 75 nm into young male Sprague-Dawley rats caused shifts in mineral metabolism that became apparent via lower Mo, Co, Mn and P contents in the liver as well as lower Rb and Na contents in the kidneys.

TiO₂ NPs introduced in a dose equal to 2-50 mg/kg b.w. turned out to be cardiotoxic for Sprague-Dawley rats in 30-day or 90-day experiments; the effects were a smaller interval between systolic and diastolic blood pressure, leukocytosis, greater activity of lactate dehydrogenase, a-hydroxybutyrate dehydrogenase, and higher TNF- α and IL-6 contents in blood plasma [57]. Functional disorders in the cardiovascular system were observed not only under exposure to NPs, but also to pigment TiO₂ that contained NPs in a small fraction only and was introduced into mice orally in a dose up to 500 mg/kg b.w. Not only cholinergic

vasorelaxation intensified in coronary artery also, but also did serotonergic vasoconstriction that competed with it [58].

Some researchers assessed influence exerted by TiO₂ nanoforms on carbohydrate-energy metabolism in animals. 14-week anatase NPs introduction (64-320 mg/kg b.w.) into mice caused hyperglycemia, insulin resistance, increased ARS1 phosphorylation and decreased Akt under effects produced by JNK1 and p38 MAPK respectively. The process was accompanied with oxidative stress and greater anti-inflammatory cytokines concentrations [59].

TiO₂ NPs introduction into female mice in a dose up to 50 mg/kg b.w. during 14 days caused oxidative stress, hyperglycemia, and shifts in levels of thyroid hormones, estradiol, and prolactin [60]. A probable mechanism of influence exerted by TiO₂ NPs on carbohydrate metabolism was examined in research works [59, 61]; the authors applied transcriptomics to determine metabolic pathways (KEGGs) that were a target for such influence. Thus, multiple oral introductions of these NPs into mice in a dose equal to 50-200 mg/kg b.w. influenced a xenobiotics transformation system and caused a stress in endoplasmic liver reticulum.

An extent to which TiO₂ NPs influence animals can depend on their sex and age. Specifically, increased glycemia level and higher glutathione peroxidase activity were detected in young male Sprague-Dawley rats (initial age being 3 weeks) that were orally exposed to TiO₂ NPs in a dose equal to 30 mg/kg b.w. during 30 days; no such effects were detected in older animals (6 weeks) [62]. Changes in reduced glutathione, lipid peroxidation products, IL-1 α , IL-4, and TNF α caused by exposure to TiO₂ NPs in a dose up to 50 mg/kg b.w. in a 90-day experiment were more apparent in female mice than in male ones, animals' age in both sex groups being comparable [63].

We should pay special attention to some research works where authors didn't reveal any toxicity of various TiO₂ forms under oral introduction. Gu [et al.] [46] exposed mice to pigment titanium white (MPS size being greater than 100 nm) multiple times but didn't

reveal any signs of hyperglycemia or intensified lipid peroxidation. Warheit [et al.] [64] declared their research work to be an arbitration one and performed it in full conformity with OECD test guidelines TG 407, 408, 425. Growing male rats were exposed to a single introduction of NPs (73 nm) or MPs (145; 173 nm) (an acute toxicity test) in a dose equal to 24,000 mg/kg b.w. or to multiple introductions (28 or 29 days) in doses equal to 1,000 mg/kg b.w. or more. The authors tested integral parameters and internal organs morphology and didn't reveal any signs of toxicity. Despite the research having strict methodological substantiation, later on it was criticized [22] mostly due to a limited set of examined parameters as well as inadequate choice on a dose of a nanomaterial; chosen doses were extremely high and it could presumably lead to its massive aggregation and formation of extended three-dimensional structures (gelation) in the gastrointestinal tract lumen.

2. Genotoxicity

TiO₂ NPs (33 nm) and MPs (160 nm) were introduced intragastrically into CBAB6F1 mice in doses varying from 40 to 1,000 mg/kg b.w. for 7 days; researchers detected occurrence of micronucleuses in the bone marrow and liver cells. Both MPs and NPs caused an increase in mitotic index in glandular mucous tunic of the stomach and large intestine epithelium. NPs introduction also caused apoptosis of cells in the stomach mucous tunic and multi-nucleus spermatids occurrence in the testicles [65]. A 5-day TiO₂ NPs introduction into mice in a dose equal to 5-500 mg/kg b.w. led to apoptosis, DNA fragmentation, and mutations in gene p53 exons together with biochemical signs of oxidative stress in the stomach mucous tunic [37]. Anatase NPs introduction into Wistar rats (100-200 mg/kg b.w., 60 days) caused various disorders in erythrocytes system in blood, including occurrence of cells with a micronucleus, together with damage to chromosomes in bone marrow cells and DNA fragmentation revealed via a comet assay [66]. A micronucleus test performed on lymphocytes culture revealed genotoxicity of anatase NPs in their concentration being equal

to 1.6 µg/ml, while MPs became genotoxic only in concentrations equal to 40 µg/ml and higher [67].

There were some data on TiO₂ NPs having no genotoxicity; they were obtained via an experiment on male Sprague-Dawley rats exposed to anatase NPs (10-200 mg/kg b.w., 30 days). No chromosome aberrations or mitosis disorders in bone marrow cells were revealed under those experimental conditions [68]. Rats were exposed to a single introduction of 6 various NPs and MPs types in doses equal to 500-2,000 mg/kg b.w. and there was no increase in number of cells with a micronucleus in their bone marrow and blood reticulocytes [69]. And finally, according to Martins [et al.] [44], there were no genotoxicity signs revealed in male Wistar rats after a 45-day exposure to NPs in a low dose (0.5 mg/kg b.w.).

Therefore, data on TiO₂ NPs genotoxicity are rather controversial at present. Effects do not directly correlate with particles size and introduction duration, or a type of experimental animals, and it indicates that further research on the subject is required.

3. Immunotoxicity

When considering specific or remote effects produced by oral TiO₂ NPs introduction, one should linger on their interaction with the immune system. Tassinari [et al.][34] reported there were disorders in white pulp structure in the spleen in female (but not male) Sprague-Dawley rats after a 5-day intragastrical introduction of anatase NPs (1-2 mg/kg b.w.). These disorders were also accompanied with changes in morphology of the thyroid gland, adrenal cortex, and ovaries; in the authors' opinion, it indicated that there was a systemic endocrine disorder developing in experimental animals.

Male Wistar rats were immunized with chicken ovalbumin during a 28-day experiment and then exposed to rutile NPs in a dose equal to 100 mg/kg b.w. It resulted in lower number of immature cells and B-lymphocytes and growing phagocytic activity of neutrophils in peripheral blood. IgG antibodies level increased in animals exposed to NPs and the authors thought it to be accelerating B-lymphocytes maturing into plasmatic cells caused by this exposure [70].

Anatase NPs were introduced into mice during 90 days in a dose equal to 2.5-10 mg/kg; it intensified lymphocytes apoptosis, caused greater production of macrophage inflammation factors MIP-1 α , MIP-2, Eotaxin, MCP-1, as well as IFN- γ , VCAM-1, IL-13, IFN-inducible protein-10, greater expression of CD69, tyrosine protein kinase and phosphatase, basic growth factor for fibroblasts, FasI and GzmB with simultaneous decrease in expression of NKG2D, NKp46, and 2B4 [33]. The same model was applied to demonstrate necrosis and oxidative stress developing in the spleen, expression of cyclooxygenase-2 with simultaneous growth in production of prostaglandin PGE2, higher levels of mRNA for ERK, AP-1, CRE, Akt, JNK2, MAPKs, PI3-K, and c-Jun и c-Fos [32]. The authors also reported there was an increase in TNF- α , macrophage migration inhibitory factor, IL-2, IL-4, IL-6, IL-8, IL-10, IL-18, IL-1 β , cross-reaction protein, TGF- β , expression of Bax and CYP1A1 together with suppression of Bcl-2 и HSP-70 in blood of the experimental animals [31].

30-day experiments that were similar in their procedure allowed revealing spleen edema in experimental mice accompanied with reinforced lipid peroxidation and expression of heme oxygenase via p38-Nrf-2 signal pathway [71].

4. Reproductive toxicity

There are experimental data, although rather few, on impacts exerted by orally introduced TiO₂ NPs on mammals' reproductive system. Male Kunming mice were introduced these NPs which were smaller than 50 nm in size during 52 days in a dose up to 250 mg/kg b.w. The exposure caused greater number of anomalies in sperm in the testicles and a decrease in number of sperm cells and bubbles in testicular tubes. There was a simultaneous decrease in circulating testosterone level and expression of 17 β -hydroxysteroid dehydrogenase in the testicles [72]. Wistar rats were orally exposed to NPs in a dose equal to 50 mg/kg b.w. for 2 or 3 weeks and it resulted in greater expression of γ -glutamyl transferase and a decrease in c-kit and steroidogenic acute regulatory protein (StAR). Overall number of developing sperm cells reduced but a number of

ones with defects only grew. There were signs of oxidative stress in the prostate and testicles, decrease in reduced glutathione contents, increase in TNF- α concentration, greater expression of Fas, Bax and caspase-3 together with falling Bcl-2 contents. Hormonal disorders included higher levels of gonadotropin and estradiol and lower testosterone concentration [73].

Pregnant female mice were exposed to TiO₂ NPs during 17 days (during pregnancy) in a dose equal to 25-100 mg/kg b.w.; it led to skeletal anomalies in fetuses with signs of cartilage being underdeveloped and lower ossification. There was a growth in number of fetuses with dysplasia; there were fetuses with exencephaly, spina bifida, twisted tails, scoliosis, underdeveloped ribs and breast bone [40].

But as opposed to the above-mentioned work, D.B. Warheit [et al.] introduced 6 types of TiO₂ NPs and MPs into pregnant female Wistar rats in a dose up to 1,000 mg/kg b.w. and didn't detect any anomalies in fetuses [74]. A hypothesis on reproductive toxicity TiO₂ NPs might require further research.

5. Neurotoxicity

There has been no reliable evidence on probable translocation of orally introduced TiO₂ NPs into the brain so far; still, some research shows that their impacts lead to functional disorders in the CNS. Thus, when ICR mice were introduced NPs during 60 days in doses 5-50 mg/kg b.w., researchers detected changes in their behavioral reactions and spatial recognition memory. NPs inhibited activity of metal-dependent ATPase (ion pumps for Na⁺/K⁺ и Ca²⁺/Mg²⁺), acetyl cholinesterase and nitrogen oxide synthase in the brain; There were disorders in metabolism of dopamine, norepinephrine, and their metabolites. According to the authors, detected changes could be related to shifts that occurred under exposure to NPs in mineral substances status, including contents of Ca, Mg, Na, K, Fe and Zn in the brain [16]. Probable effects produced by TiO₂ NPs on metabolism of dopamine and norepinephrine in the brain cortex were also mentioned by Shrivastava [et al.] [35] who performed their experiments on mice. There was a single introduction of NPs in a dose

500 mg/kg b.w. A significantly lower dose, 2.5-10 mg/kg b.w., introduced into mice for 90 days, caused grave morphological changes in the brain, loss of spatial orientation, weaker long-term memory together with a decrease in expression of NR2A sub-units, B-receptor of N-methyl-D-aspartate (NMDA), inhibited expression of CaMKIV, CREB-1, CREB-2 and FosB/DFosB in the hippocampus [36]. Very small anatase NPs (5-10 nm) introduced into Wistar rats in doses 50-200 mg/kg b.w. during 60 days inhibited acetyl cholinesterase activity in the CNS and caused greater IL-6 production by glia tissue. There was a growth in accumulation of glial fibrillar acidic protein GFAP in the brain cortex [75].

6. Carcinogenicity

Inhaled TiO₂ NPs are likely to be carcinogenic as IARC* assigns them into 2B group (substances that are presumably carcinogenic for humans) [76]. However, probable carcinogenic effects produced by these NPs when they are introduced into the gastrointestinal tract have not been examined in great details. There was a single research work [77] when TiO₂ coloring agent which was a mixture of rutile and anatase NPs and MPs was introduced as a part of ration (2.5–5% of total mass) into Fischer 344 rats and B6C3F142 mice during 103-104 weeks. There was a slight growth in frequency of tumors (adenoma and carcinoma) with various localization in female rats exposed to high NPs doses with a probability of the zero hypothesis being accepted $p=0.043$; it wasn't sufficient to substantiate carcinogenic effects according to Bonferroni criteria. Therefore, the authors didn't obtain any compelling data that TiO₂ could have carcinogenic effects when introduced into the gastrointestinal tract.

7. Effects produced by titanium dioxide in the intestine lumen

When we discuss probable reasons and mechanisms related to obvious oral toxicity of TiO₂ NPs, we should dwell on its effects in the gastrointestinal tract lumen; to become apparent, these effects do not necessarily require system translocation. We should linger on the

following aspects: impacts exerted by NPs on intestinal uptake, interaction between NPs and mucous tunics in the gastrointestinal tract and gut-associated lymphoid tissue (GALT) in particular, and their influence on intestinal microbiome as well.

TiO₂ NPs didn't exert any impacts on fat acids absorption by intestinal cells in an acute experiment. However, in a chronic experiment researchers detected a considerable decrease in their absorption in the gastrointestinal tract. NPs are known to be able to influence mineral substances absorption in the gastrointestinal tract. First of all, NPs penetrate a food product matrix and form complexes with proteins, fats, and carbohydrates thus creating a "crown" on its surface. These changes in a product surface can influence nutrients biological availability, their solubility, and recognition by structural elements in a body that are responsible for intestinal uptake of nutrients; as a result, all these changes influence NPs toxicity [78].

Koeneman [et al.] applied *in vitro* model for Caco-2 cells monolayer and showed that NPs introduced in concentration equal to 10-100 µg/ml didn't cause any cells death, morphological disorders in micro-villi, or adhesion contacts failure [24]. Rutile NPs introduced in a concentration equal to 100 µg/ml didn't produce any effects on Caco-2 cells monolayer as it was shown by M. Fisichella [et al.] [79]. Similar results were obtained by M.R. Jo [et al.] [80] who performed comparative analysis of impacts exerted by much larger MPs (117 and 153 nm) on Caco-2 cells layers and rats' small intestine *in vivo*. G.E. Onishchenko [et al] [27] didn't detect any ultrastructural changes in erythrocytes of rats' ileum under intraintestinal introduction of rutile NPs.

Still, there are some research works where TiO₂ NPs ability to produce certain effects on the intestinal epithelium is described. For example, there was a research work [38] that described an effect produced by NPs, namely, an increase in villi length in small intestine mucous tunics of rats orally exposed to them. There was also an experiment on Caco-2 cells

* International Agency for Research on Cancer.

when researchers observed morphological disorders in the brush border under exposure to pigment TiO₂ (a mixture of 25% NPs and 75% MPs) in a low concentration (lower than 0.35 µg/ml) [81].

All the results obtained in the above-mentioned works are related to effects produced by NPs on “absorptive” epithelium of intestinal mucous tunics. However, when NPs interact with GALT of Peyer’s plaques, a totally different effect can occur. Thus, when male C57Bl/6J mice were exposed to MPs (260 nm) and NPs (66 nm) in a dose equal to 100 mg/kg b.w. for 10 days, researchers detected an increase in CD4⁺ number in lymphoid tissue of the duodenum, empty intestine, and the ileum. There was an increase in production of IL-4, IL-12, IL-23, TNF-α, IFN-γ and TGF-β by Peyer’s plaque cells against the control group [82]. The above-mentioned anti-inflammatory cytokines are known to act systemically; taking this into account, we can state that this single result is of great interest when toxic effects produced by TiO₂ NPs are explained as they have extremely low biological availability and absorbability in the gastrointestinal tract. However, to confirm these data, further research is required.

As per data provided by J.J. Faust [et al.] [83], impacts exerted by TiO₂ NPs in a chronic experiment caused a significant damage to barrier functions performed by intestines. The process was accompanied with active oxygen forms occurrence, intensified inflammation, and alkaline phosphatase being more active. There was also a decrease in transport of iron, zinc, and fat acids through intestinal mucous tunics due to weaker absorbing capacity of epithelial cells in the brush border after exposure to TiO₂ nanoparticles.

A possibility in principle that TiO₂ NPs can have mediated effects on a human or an animal body through influencing a structure and functions performed by components of the intestinal microbiome becomes obvious due to multiple data on biocide effects produced by these NPs on various groups of microorganisms *in vitro* (an overview of basic results is given in the work [84]). Besides, it was deter-

mined that NPs could increase pathogenic properties of opportunistic micro-pathogens which are parts of the intestinal microbiota [85]. And it is important that growth of various microorganisms or their groups is not inhibited in the same way and it can result in a nanomaterial interfering significantly with a subtle adjustment of symbiotic and competitive relations between multiple components in the microbiome. However, there are few data on direct ability of TiO₂ NPs to influence intestinal microbiocenosis under natural conditions.

Preparations containing anatase and rutile NPs as well as pigment TiO₂ were introduced into Wistar rats during 30 days in doses equal to 1 and 100 mg/kg b.w. [86]. Under those conditions, exposure to both low and high doses led to an increase in quantity of hemolytic and common streptococci and staphylococci and a decrease in quantity of bifid bacteria. Changes in the immune background in animals became apparent via an increase in IL-10 production. These detected changes were not related to a crystal form or sizes of particles and were detected after exposure both to NPs and MPs. W. Dufouir [et al.] [87] applied both MPs and NPs in their research and revealed that there were minimal changes in the microbiome state *in vitro* in gasification test and production of fat acids C16:0, C18:0, cisC15:1w5 and cisC18:1w9c under exposure to NPs in concentration being equal to 0.1-0.25 mg/ml; but there was a change in microbe populations structure as quantity of bacteroids dropped but that of *Clostridium sp* increased. Mice were orally exposed to anatase and rutile NPs during 28 days and it resulted in gradually developing changes in the intestinal microbiome including such phyla as *Proteobacteria*, *Prevotella*, *Rhodococcus* and *Bacteroides*. Intensity of an effect was different for two crystal forms of NPs and was not accompanied with apparent morphological changes in intestinal walls [88]. We can conclude that searching for changes in a structure and functional activity of the intestinal microbiome caused by exposure to TiO₂ NPs is a promising research trend that can allow establishing mechanisms of biological ef-

fects produced by this poorly absorbed nanomaterial; however, research works in the field are rather scarce.

8. Meta-analysis of data on toxicity

Therefore, we analyzed data from 64 sources (published in 2007-2019); all the analyzed data are scientifically authentic and complete according to MG 1.2.2522-09 (publications in reviewed scientific journals that contain detailed description of a research object and applied biological model; are accomplished with quantitative techniques; contain statistically authentic results; and are not questioned in later publications). Distribution of all the analyzed research works as per years of their publication is given in Figure 1a. Applied biological models included a single oral introduction (15 works), multiple sub-acute introduction (42), and chronic introduction (1) into female rats and mice from different lines as well as experiments *in vitro* on monolayers of intestinal epithelium cells and cultures of the gastrointestinal microbiota. 50 articles focused on TiO₂ NPs; 3 articles, on MPs; and 9 works, MPs and NPs being compared. 35 works out of the overall number contained data on adverse (toxic) effects produced on a body by TiO₂ doses varying from 0.1 to 1,000 mg/kg b.w.; 6 works contained no data on toxicity; data obtained in 27 works didn't allow unambiguous assessment of LOAEL, or a focus was on bioaccumulation only. Basic target organs were the liver (17 works); the gastrointestinal tract (9 works); the spleen and immune system organs (8 works); as well as the heart, kidneys, and brain. Special attention should be paid to data on effects produced by

TiO₂ (both in nano- and micro-form) on a structure and biological properties of microorganisms in the intestinal microbiota; such effects were revealed in 4 research works. Distribution of publications as per assessed LOAEL is given in Figure 1b.

As we can see from the data presented above, LOAEL was 10 mg/kg b.w. and even lower in 10 out of 41 revised publications; it roughly corresponds to a 25-percentile of the overall number of the analyzed sources. Re-

markably, all works that reported absence of any toxic properties possessed by TiO₂ NPs were predominantly performed with introducing extremely high doses of the nanomaterial (1,000 mg/kg b.w. and even more) into experimental animals. It can possibly indicate that authors obtained false negative results due to NPs aggregation [22]. We should also note that toxic effects were less frequently observed after an acute (single) introduction of the nanomaterial into animals than after sub-acute one (30-90 days). Therefore, we can be almost certain to conclude that TiO₂ NPs, both anatase and rutile ones, when introduced in a dose not higher than 10 mg/kg b.w. can produce adverse effects on a body. Obviously, there are organs and tissues in a body that are primary targets for them, first of all, the spleen and immune system as well as mucous tunics in the stomach and small intestine, the liver, and the brain. Thus, NOAEL for TiO₂ in its nano-form is less than 10 mg/kg b.w. that is qualitatively consistent with previously performed assessments that were based on much smaller number of sources [14].

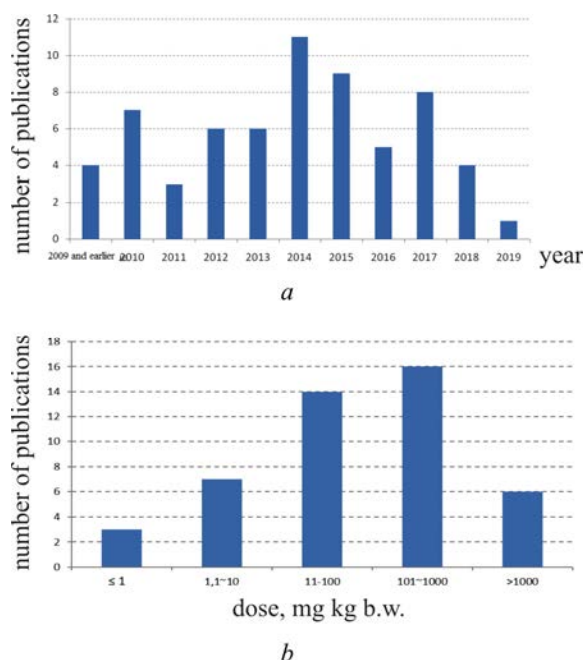


Figure 1. Distribution of publications (quantity) on oral toxicity of titanium dioxide nanoparticles a) as per a year when they were published, b) as per assessed LOAEL

9. Risk assessment

Non-carcinogenic risk caused by impacts exerted by a nanomaterial on a human body is estimated according to MG 1.2.0038-11 "Assessment of risks caused by impacts exerted by nanomaterials and nanoparticles on a human body"⁶ via calculating a hazard quotient as per the following formula:

$$HQ = E_d/RfD$$

where RfD is a safe reference dose that is given in mg/kg b.w. * day; and E_d is assessed exposure in the same units. As we can see from Table 1, a dose equal to 1 mg/kg a day is a realistic estimation for TiO₂ consumption as a food additive E171 by most people. And also, as it was shown in the Section 8, NOAEL for TiO₂ in its nanoform was not higher than 10 mg/kg b.w. a day. If we want to determine RfD basing on this value, we should introduced two 10-fold assurance factors; the first one allows for uncertainty (discrepancies in experimental data obtained from various publications), and the second one is a similarity coefficient that is applied when data obtained via experiments on small animals (laboratory rats and mice) are transferred onto a human body. Therefore, RfD for TiO₂ in its nanoform should be equal to not more than 0.1 mg/kg b.w. a day

Table 2 contains results of assessing risks caused by oral TiO₂ NPs introduction taking into account 4 possible scenarios of NPs contents in E171. The first scenario is optimistic and means that NPs account for not more than 0.3% of the overall mass of a sample as it was shown in [2, 22]; the last one is extremely pessimistic and means that the overall TiO₂ sample was a nanomaterial.

These data prove that application of TiO₂ that contains NPs in an amount higher than 1% of an overall mass can result in such effects produced on a human body that cause unacceptable health risks.

10. Conclusion and recommendations

So, all the available literature data allow us to conclude that TiO₂ NPs in their two most

Table 2

Assessment of risks caused by titanium dioxide as a component of E171 under oral exposure

No.	Scenario (NPs contents, % in overall mass)	Hazard quotient (HQ)	Risk (assessment)
1	0.3	0.03	Acceptable
2	1.0	0.1	Acceptable
3	10	1.0	Unacceptable
4	100	10.0	Extremely unacceptable

widely spread forms (anatase and rutile) exert various adverse effects on a body both after a single introduction into the gastrointestinal tract (an acute experiment) and after multiple ones. The most frequent effects are produced on the liver as it becomes obvious via damage to liver tissues, oxidative stress signs, changes in biochemical parameters of blood plasma, as well as via shifts occurring in proteome and transcriptome of the organ. The immune system is another target, the bone marrow and spleen in particular, where researchers detected changes in genetic apparatus of cell nucleus, shifts in production of anti-inflammatory cytokines and growth factors. There were also signs that NPs had neurotoxicity and reproductive toxicity; however, LOAEL in those cases, as a rule, was substantially higher than 10 mg/kg b.w. Any toxic effects produced by TiO₂ NPs were not revealed in relatively small number of works. A reason for this can be extremely high doses of nanomaterials chosen by their authors; massive NPs aggregation that occurs in such a situation can be a factor that imposes certain limitations on biological effects occurrence. There is a well-known contradiction between various toxic effects produced by TiO₂ in its nanoform and data on the substance being obviously poorly absorbable and therefore poorly biologically available in the gastrointestinal tract. A key insight into this paradox can obviously be provided by data contained in relatively few works on effects produced by

⁶ MG 1.2.0038-11. Assessment of risks caused by impacts exerted by nanomaterials and nanoparticles on a human body [web-source] // KODEKS: an electronic fund of legal and reference documentation. – URL: <http://docs.cntd.ru/document/1200097700> (date of visit March 23, 2019).

NPs on the intestinal microbiome. Impacts exerted by NPs can intensify pathogenic properties of opportunistic micro-pathogens that are part of the intestinal microbiome. Disorders in quantitative and qualitative structure of intestinal microflora can, in its turn, lead to functional disorders in intestinal lymphoid tissue (GALT), acid balance in the intestinal lumen, inflammatory cells proliferation, and putrefaction. Such impacts can cause significant shifts in production of pro- and into-inflammatory cytokines, and absorption of nutrients and biologically active metabolites in microflora; all these impacts combined can explain a lot of observed systemic effects produced by nano-sized TiO₂. However, getting better insight into these significant peculiarities related to effects produced by nano-sized TiO₂, will require further research; by analogy, it can be applied to other nanomaterials that are poorly absorbed or not absorbed at all under oral introduction (silicon oxide NPs, silica-alumina clays, and carbon nanotubes).

We assessed risks caused by TiO₂ NPs that could penetrate a body with food, cosmetics, or medications, basing on literature data on population exposure to all TiO₂ forms as a food additive E171 and experimental determi-

nation of LOAEL/NOAEL. The assessment revealed that a risk depended on a scenario for NPs contents in a product. In particular, when NPs contents amounted to 10% and more out of a total mass, a risk could become unacceptable. Given that, we can conclude that it is necessary to regulate and control nano-sized material contents in E171 that is applied in food industry. To do that, the following measures seem advisable.

1. A specification that describes E171 food additive should be revised; it should impose a limitation on mass contents of a nano-sized component in it that is not to exceed 1%.

2. When assessing conformity of a product that contains E 171, an applicant should provide obligatory data on its particles size distribution.

3. There should be new sanitary-epidemiologic surveillance techniques developed and implemented into every day practice; these techniques should allow estimating TiO₂ particles size, both in pure E17 samples and in food products containing it.

4. Application of E171 should be strictly prohibited by law in food products for preschool children, pregnant women and feeding mothers, as well as in specific food products aimed for preventive and therapeutic diets.

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Received: 27.03.2019

Accepted: 23.05.2019

Published: 30.06.2019