# Long-term exposure to food colorants E171 and E173 leads to the accumulation of titanium and aluminum in the brain

*Oysara* Zaripova[1], *Muslima* Abdullaeva[1], *Umar* To'raev[1], *Gulnoz* Rakhimova[1], *Laylo* Kadirova[1], Zebiniso Rajabova[1], *Dilnoza* Temirova[1], *Timur* Habibullaev[1], *Maftuna* Yusupova[1], *Gulnoza* Tursunova[1,*], Baxtiyor Sanoev[2], *Mirmuhsin* Istamov[3]

[1]Department of Pathological Physiology, Bukhara State Medical Institute, Bukhara, Uzbekistan,
[2]Department of Pathological Anatomy, Bukhara State Medical Institute, Bukhara, Uzbekistan,
[3]Department of Psychiatry, Narcology and Medical Psychology, Bukhara State Medical Institute, Bukhara, Uzbekistan.

**Abstract.** Today, human lifestyle, the increase in living conditions, social welfare, the improvement of working conditions, and the increase in annual income require the development of all sectors of society. In particular, in the manufacturing industry, the quality o,f the product and uniqueness, color, packaging, and long-term storage are highly valued by consumers, in addition to the shelf life. Food additives are actively used in industry on a large scale in order to fully meet the above requirements. In particular, artificially synthesized food dyes are attracting entrepreneurs with their low cost and stability. Artificial dyes, which are very convenient to use in industry and have many advantages, are completely replacing natural coloring agents. The fact that synthetic dyes available in the manufacturing industry seriously affect health indicators shows how urgent the issue is. At present, the number of diseases associated with poor nutrition is steadily increasing. This issue is one of the key global priorities under the attention of the World Health Organization. Therefore, in order to prevent such diseases, the development of programs aimed at promoting healthy eating and limiting the consumption of processed and semi-processed food products, as well as colorful items enriched with artificial additives, is gaining importance in the state policy designed to meet the needs of modern society. Among the artificial food additives used to alter the organoleptic properties of food products, food colorants occupy a significant place. These additives are organic compounds not found in nature, i.e., they are synthetically produced, and almost all of them have been used in the global food industry for decades. Unlike natural colorants, synthetic food dyes do not possess biological activity and do not contain flavoring substances or vitamins. Nevertheless, they have important technological advantages over natural dyes: they are less sensitive to processing and storage conditions and provide bright, easily reproducible colors. Typical representatives of such artificial, metal- containing food dyes are E171 and E173, which contain titanium dioxide and aluminum, respectively. This scientific research specifically studies the effects and accumulation of the nanoparticle-sized titanium dioxide (E171) and aluminum-based (E173) food dyes in the brain.

**Keywords:** Food colorants, E171, Titanium dioxide, E173, Aluminum, accumulation, Blood-brain barrier, Nanomaterials

*Corresponding author: gulnoza_tursunova@bsmi.uz

## 1 Introduction

Our research focuses on studying the passage and accumulation of titanium (found in the food colorant E171) and aluminum (found in the food colorant E173) through the blood-brain barrier. Additionally, information will be provided on how these food additives enter the body, are absorbed and exert their effects. The titanium dioxide compound occurs in three crystalline forms in industry: rutile, anatase, and brookite. However, the European Union permits only rutile and anatase to be used as the food colorant E171. Following the results of this model, the International Agency for Research on Cancer (IARC) classified titanium dioxide as belonging to the group of respiratory carcinogens.

The entry of titanium into the body occurs mainly through the enteral route via food products. In addition, this whitening pigment is used in the cosmetics industry: in sunscreens, whitening paints, personal hygiene products, and toothpaste production. In the construction materials industry, titanium dioxide of various particle sizes is used; during its application, factory dust is generated, which exerts pathogenic effects on the body through the inhalation route.

It is appropriate to discuss the negative impact of certain food additives on lifestyle and health, particularly on children and middle-aged individuals, who are the most exposed to these substances. The concern is not limited to acute poisoning or the absence of allergic symptoms, but also extends to the long-term consequences of such exposure, including carcinogenic, mutagenic, and other biological effects. The effects of food additives on public health service users are still insufficiently explained, especially regarding the changes that occur over time (such as mutagenicity, carcinogenicity, etc.). Only through long-term research can accurate and comprehensive information on these effects be obtained. According to recent findings, the titanium dioxide additive found in the food colorant E171 contributes to tumor formation and progression by inducing inflammation, activating immunological responses, altering cell cycle processes, and modifying cancer-related signaling pathways. These conclusions are significant in the ongoing discussions regarding the safety of E171 and may help identify the molecular mechanisms related to its potential genotoxicity and carcinogenicity. Additional toxicological studies are required to assess the safety of E171 and other metal- based nanomaterials used as food additives or in packaging materials [7].

Like other fine-particle food additives, titanium dioxide is mainly found in food products. It is found in large quantities in carbonated drinks, various colored sweets, packaged salty and fatty crackers, potato chips, paper candies, pastries, dairy and cheese products, and sausage products [1]. Titanium dioxide is actively used not only in the food industry, but also in the pharmaceutical industry, in the production of personal hygiene products, and cosmetics as a bleaching agent. Titanium dioxide is a water- insoluble substance with low toxicity [2]. In the body, titanium accumulates in the form of proteins. Enterally administered titanium dioxide interacts with the aggressive environment of the stomach significantly affecting the properties of proteins and enzymes changes. In a laboratory study, chronic (90 days) enteral administration of titanium dioxide to rats resulted in spleen damage, thrombocytopenia, lymphopenia, decreased hemoglobin levels, and decreased immunoglobulin levels [4]. In another laboratory study, administration of titanium dioxide at a dose of 10 mg/kg resulted in severe liver damage, nephron apoptosis, and impaired immunoregulation. When titanium dioxide was administered enterally at a dose of 100 mg/kg for 10 days, it was found that CD4 lymphocytes increased in all areas of the intestine, and the secretion of cytokines IL-4, IL-12, IL-23, and TNF increased mainly in the colon wall [5]. Many studies have been devoted to the effects of titanium dioxide on the body, especially on the lungs, and the changes that occur in it. In this, solutions were sought to the questions related to the entry of small- sized titanium dioxide through the respiratory tract in the workplace and the development of pathological processes. Experiments conducted on rats with high doses of titanium dioxide for two years showed that tumors developed in the lungs of the rats, which indicated the carcinogenic properties of titanium dioxide [6] Fine-particle titanium dioxide, which enters the body through the intratracheal route, damages the cellular structure of alveolar macrophages and leads to impaired function. In addition, it reduces the chemotoxic properties of alveolar macrophages. Small amounts of titanium dioxide increase the phagocytic properties of macrophages, while large amounts reduce this property. With an increase in the amount of fine-particle titanium dioxide, the production of NO and TNF increased, since more pro-inflammatory mediators were synthesized under the influence of fine-particle titanium dioxide than with conventional titanium dioxide [7] Small amounts of titanium dioxide increased the sensitivity of the upper respiratory tract by twofold, and the number of cells responsible for inflammation increased by up to threefold. Histological examination revealed edema, epithelial destruction, and inflammation [8]. Free titanium dioxide causes denaturation of cytoplasmic proteins [9]. E 173 Aluminum is one of the most common elements on Earth, ranking third after oxygen and silicon. Aluminum compounds occur in nature in a variety of forms. Aluminum compounds are part of more than 280 minerals and are actively used in various fields of human activity. Despite the widespread use of aluminum compounds, their negative consequences remain one of the most important and necessary problems of modern medicine. In particular, its complications related to the brain are the cause of much discussion. There is still much debate about whether Alzheimer's disease, autism, Parkinson's disease, multiple sclerosis, and similar profound changes in the brain are caused by aluminum and its compounds (11). Aluminum in drinking water, inhalation and food products despite entering in large quantities through mucous membranes in small quantities reabsorption occurs. luminum is not essential for the human body and metabolic processes (13). This element has a strong toxic effect on the body and the brain. The many ways in which aluminum enters the body and its widespread use further emphasize its toxic properties [11,12,13]. This toxic effect is especially pronounced in Alzheimer's disease, autism, progressive sclerosis, and other brain dysfunctions. The attention of international scientific journals has been focused on aluminum and the above-mentioned neurological diseases [12].

The main part of aluminum enters the body through water, food dyes and used in packaging, preparation, and storage of food products enters through the details. Unprocessed food products the amount of aluminum in its composition is less than 5-7 mg/g. Aluminum entering the body with water is 0.3%, and with food - 0.1%. The daily intake of aluminum is 15 mg / day [14]. E171 and E173 are used as food dyes and give the product a white color. The fact that these dyes can change the analeptic properties of the product is very useful for entrepreneurs. But there is another side to the matter. All food additives, including food dyes that give color, can cause various pathological reactions in the body. processes are developing [6].

This article aims to demonstrate the effects and accumulation of the bleaching dyes E171 and E173 (when taken in large quantities) on the brain. The active substances of these dyes are: titanium dioxide (171) and aluminum (E173). The effects of titanium dioxide and aluminum substances on the nervous system, including the brain, have been proven in numerous experiments. For example, titanium dioxide powder was given to experimental rats for a long time and changes in the brain were observed. When the EEG of the rat brain was performed, it was observed that active epileptic foci appeared in the brain and the rats became very aggressive. [1], [2], [3]. Aluminum is believed to be one of the main causes of neurodegenerative diseases: Alzheimer's disease, Parkinson's disease, autism [4], [5].

## 2 Material and Methods

We will study the extent to which titanium and aluminum accumulate in the brain and how this accumulation is related to changes in the brain. We selected 40, white, inbred rats for the experiment and divided them into 4 groups.

1. Control group (10)
2. Group that received E171 (10)
3. Group that received E173 (10)
4. The group that received E171 and E173 (10)

The above group of rats was given enteral titanium dioxide and aluminum in the form of powder.
The standard for determining the mass amounts of macro- and microelements in the researched samples is carried out in relation to samples with known amounts of elements. The accumulation of active elements from food colorants in the brain was studied using the neutron activation analysis method. The instrumental neutron activation analysis technique is based on irradiating samples in the neutron flux of a BBP-CM type nuclear reactor, recording the gamma spectra of radionuclides formed in the sample with a semiconductor detector, and determining the mass concentrations of elements according to the intensity of the corresponding analytical peaks in the radionuclides gamma spectra. The determination of macro- and microelement mass concentrations in the studied samples is carried out relative to standards, i.e., reference samples with known element concentrations. Neutron activation analysis (NAA/INAA) does not have a single fixed "minimum and maximum" for all substances — the sensitivity and working range depend on the element, the sample matrix, irradiation/decay/counting conditions, neutron flux, geometry, and detector. However, there are typical ranges.
Typical ranges

- Detection limit (LOD) in INAA for "favorable" elements: from picograms–nanograms per gram ($10^{-12}$–$10^{-9}$ g/g; e.g., Au, Ir, Eu, Sm, Br).
- For "average" elements**:** ppb–ppm ($10^{-9}$–$10^{-6}$ g/g).
- For "unfavorable" elements: tens–hundreds of ppm ($10^{-5}$–$10^{-4}$ g/g or higher), unless radiochemical separation (RNAA) is applied to improve LOD.
- Upper quantitative range usually extends up to fractions of a percent and several percent by mass**,** but at higher concentrations, self-absorption, detector dead-time, or overload may occur, requiring dilution or adjusted measurement conditions.

For elements relevant to food colorants (typical estimated LODs in instrumental NAA; highly dependent on matrix, neutron flux, and counting time): Al (E173, aluminum): about 0.01–1 µg/g ($10^{-8}$–$10^{-6}$ g/g), usually determined quite reliably via $^{27}Al(n,\gamma)^{28}Al$, Ti (E171, titanium dioxide): with pure INAA, detection is worse, about 10–100 µg/g ($10^{-5}$–$10^{-4}$ g/g); with radiochemical NAA (RNAA), it can be improved to ~0.1–1 µg/g. Our research intends to analysis by instrumental neutron activation analysis the amounts of titanium and aluminum for 90 days, laboratory white rats were fed with food dyes E171 ($TiO_2$- titanium dioxide) and E173 (Al, aluminum) and the following results were obtained. During the study, 12 white rats were used. Rats that received food dyes as an experiment were divided into 4 groups

Group I E171 (titanium dioxide) was given 200 mkg/kg orally per day for 90 days as an experiment . Our instrumental neutron activation analysis revealed that the test sample contained an average of 3.63 mkg of titanium dioxide in the dry mass of the brain trace amount of titanium (Ti) was detected. In the brains of rats selected for control, this amount was 0.28 mkg.

Group II was also given 200 mkg/kg of food dye E173 (Al – aluminum) to laboratory white rats for 90 days as an experiment. In our instrumental neutron activation analysis, the test sample contained an average of 4.21 mkg of aluminum in the dry mass of the brain. Element aluminum (Al) was detected in the amount of 0.53 mkg in the control group rats.

Group III E171 ( titanium dioxide) 200 mkg/kg and 200 mkg/kg of E173 (Al – aluminum) food dyes orally for 90 days as an experiment . Our instrumental neutron activation analysis showed that the study sample contained an average of 3.7 mkg of titanium (Ti) and 4.5 mkg of titanium (Ti) in the dry mass of the brain traces of aluminum (Al) elements were detected.

Control group. In the control group, the amount of titanium dioxide was 0.28 mkg, while aluminum amounted to 0.53 mkg (Table 1).

**Table 1. The increase of aluminum and titanium in the brain compared to the control**

| **Group** | **Substance** | **Average value (μg)** | **Approx. error range (±15%)** |
|---|---|---|---|
| E171 | $TiO_2$ | 3.63 mkg | 3.09 – 4.17 |
| E173 | Al | 4.21mkg | 3.58 – 4.84 |
| E171+E173 | $TiO_2$ | 3.70 mkg | 3.15 – 4.25 |
| E171+E173 | Al | 4.50mkg | 3.83 – 5.18 |
| Control | $TiO_2$ | 0, 28mkg | 0.24 – 0.32 |
| Control | Al | 0.53 mkg | 0.45 – 0.61 |

The results obtained in the above experimental groups, compared with the control group, revealed differences in titanium: an average of 3.63/0.28 mkg, 13 times more, and aluminum: an average of 4.21/0.53 mkg, 8 times more. In addition, in the group given aluminum and titanium at the same time, compared with the control group, it was found that: titanium increased by 3.7/0.28 mkg, 13.2 times, and aluminum increased by 4.5/0.53 mkg, 8.5 times. The stage following accumulation is the study of changes in the layers of the frontal region of the cerebral hemispheres under the influence of food colorants. The figure below shows a comparison of the size of the frontal cortex layers of the brain hemispheres between the control group and the group that received the food dye E171 at a dose of 200 μg/kg for three months. (Figure 1).

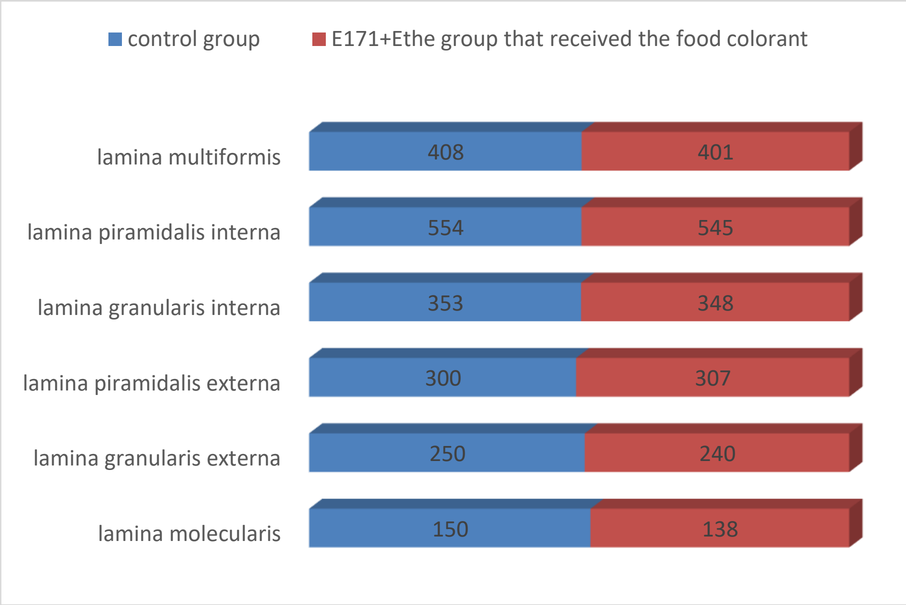


**Fig. 1. Comparison of the parameters of the frontal cortex of the cerebral hemispheres between the group that received the food colorant E171 and the control group (the indicators are given in micrometers (μm)).**

The second figure below shows a comparison of the size of the frontal cortex layers of the brain hemispheres between the control group and the group that received the food dye E173 at a dose of 200 μg/kg for three months (Figure 2).

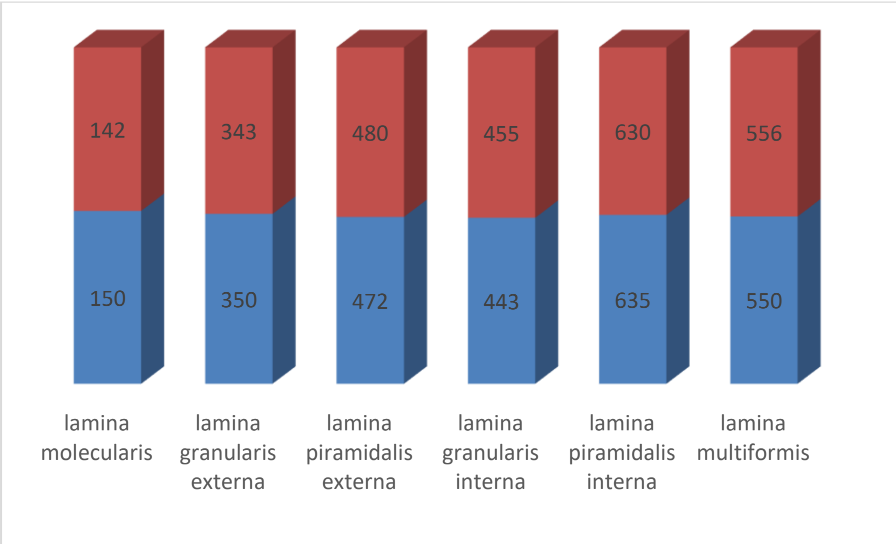


**Fig. 2. Comparison of the parameters of the frontal cortex of the cerebral hemispheres between the group that received the food colorant E173 and the control group (the indicators are given in micrometers (μm)).**

## 3 Discussion

E171, which consists of titanium dioxide nanoparticles, has been shown in several experimental studies to accumulate in various organs, including the liver, spleen, and brain. Long-term exposure may lead to oxidative stress, disruption of cellular homeostasis, and inflammatory responses. Animal studies have demonstrated that E171 can alter gut microbiota composition, increase intestinal permeability, and trigger immune cell activation, all of which contribute to chronic inflammation. Furthermore, the potential genotoxicity of titanium dioxide nanoparticles remains a subject of debate, suggesting that they could indirectly promote inflammatory and precancerous changes. E173 (aluminum) is less commonly used but poses similar toxicological concerns. Aluminum particles can interfere with cellular signaling, induce oxidative stress, and damage the blood-brain barrier. Continuous exposure may contribute to neuroinflammatory processes and has been linked in some studies to neurodegenerative disorders. Moreover, aluminum accumulation in tissues can disrupt the balance of essential minerals, leading to systemic inflammation and metabolic disturbances. In summary, although E171 and E173 are currently permitted as food additives in certain regions, accumulating evidence suggests they may pose potential health risks, particularly through mechanisms involving oxidative stress and inflammation. Further toxicological and epidemiological studies are necessary to fully understand their long-term effects and to establish safe consumption limits.

## 4 Conclusion

Around the world, it is known that the brain has a natural blood-brain barrier, and the peculiarity of this barrier is its selective permeability. Accordingly, various toxic substances, poisonous products and many types of drugs circulating in the blood cannot pass through this barrier. However, there are exceptions, and there are 3 types of entry mechanisms for substances that have the property of crossing the barrier : 1) slow diffusion 2) active transport mechanism 3) endocytosis. The ability of the two food dyes titanium dioxide and aluminum shown in the experiment to cross the brain barrier has been proven in many experiments. If aluminum crosses the barrier by binding to proteins through the active transport pathway, then the physical properties of titanium dioxide help it in this way. Small particles (nanoparticles) of size 5- 100 nm can easily pass through the brain barrier and show the property of accumulation. However, the question of how titanium enters the brain is a matter of much debate among scientists. During the 90 days of possible entry, it is shown that the active substances in the enterally administered dyes (E 171 and E 173): aluminum and titanium, accumulate in the brain to a high degree, and changes in the brain may be associated with the degree of this accumulation.

## References

**1.** Koynova , A. N. (2019). Industry pishchevyx dobavok: sostoyanie and perspective development. *Pishchevaya industry* , (3 (41)), 36-39.

**2.** Raoul, P., Cintoni , M., Palombaro , M., Basso, L., Rinninella , E., Gasbarrini , A., & Mele, MC (2022). Food additives, a key environmental factor in the development of IBD through gut dysbiosis. *Microorganisms* , *10* (1), 167.

**3.** Abdullaeva MA , Zaripova OO .( 2023) E 171 and E 173 food of paints brain to the activity and behavior influence on character.10 (60)

4. Cornu R., Béduneau A., Martin H. Ingestion of titanium dioxide nanoparticles: a definite health risk for consumers and their progeny //Archives of toxicology. - 2022. - T. 96. - no. 10. – S. 2655-2686.

5. Khasanova D., Azimova Z. Methody opredeleniya morfologicheskix osobonnostey legkix pod vliyaniem krasiteley, primenyaemyx v pishchevoy promyshlennosti, i ix phytokorrektsiya //Profilakticheskaya meditsina i zdorove. - 2023. - T. 2. – no. 6. - S. 19-29.

6. Vliyanie pishchevykh krasiteley E 171 i E 173 na mozgovuyu activitiy i povedencheskiy charakter patientov Abdullaeva M.A. https://orcid.org/0000-0001-8090-8681 Zaripova O.O. https://orcid.org/0009-0007-1694-4064 Bukhara State Medical Institute named Abu Ali ibn Siny, Uzbekistan, g. Bukhara, ul. A. Navoi.

7. Juhee P. et al. An update on the sample preparation and analytical methods for synthetic food colorants in food products //Food Chemistry. - 2024. - S. 140333.

8. Exley C., House E. R. Aluminium in the human brain //Monatshefte für Chemie-Chemical Monthly. – 2011. – T. 142. – №. 4. – C. 357-363.

9. Kumar, Vijay, and Kiran Dip Gill. "Aluminium neurotoxicity: neurobehavioural and oxidative aspects." *Archives of toxicology* 83 (2009): 965-978.

10. Crisponi G. et al. The meaning of aluminium exposure on human health and aluminium-related diseases //Biomolecular concepts. – 2013. – T. 4. – №. 1. – C. 77-87.

11. Muslima, A., & Dilrabo, X. (2024). Results of the study of the influence of viral liver damage in white rats under experimental conditions on liver tissue (trichrome Masson and immunohistochemistry marker cd68). *BIO Web of Conferences*, *121*, 03013. https://doi.org/10.1051/bioconf/202412103013

12. Abdullaeva, M., Turaeva, N., Yadgarova, S., Khalimova, D., Eshonkulova, E., Yormatova, M., & Nazarov, Q. (2024). Multimedia data processing in an intelligent medical system featuring embedded elliptic curve cryptography. *Deleted Journal*, *3*. https://doi.org/10.56294/hl2024.199

13. DYA, Z., MA, A., NA, S., FK, A., ZS, N., EU, U., & GJ, SH. (2024). Optimising diagnostic measures for early menopause and premature menopause. Reproductive Health Eastern Europe, 14(5), 617-628. https://doi.org/10.34883/pi.2024.14.5.006