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Preliminary assessment of the safety of genetically modified food products

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Abstract. Numerous studies on genetically modified food products indicate their safety and their potential to alleviate hunger worldwide. However, the issue of GM products and feeds remains relevant in the realm of food security. The purpose of this study is to present a comprehensive scheme of methods for determining the safety of GM food products and to develop a new method for assessing the safety of GM food products. This new method is based on the use of a biotest and the employment of *Paramecium caudatum infusoria.* In the study, GTS 40-3 and GTS 40-3-2 (Venus variety) soybeans, MON 00603 (NK 603) maize line, and non-GMO soybeans and maize were used as controls. Infusoria were prepared by culturing them in room temperature water (18-23°C) without direct sunlight, with the addition of non-GMO yeast powder as a nutrient solution and senna extract. Subsequently, the initial material containing approximately 100 infusoria was transferred to 100 ml flasks and incubated for 2-3 days. At the initial stage of the experiment, from day 1 to day 15, daily records and visual assessments were conducted. The assessment included the quantity of infusoria, motility (chemotaxis), size, shape, and the percentage of dead infusoria. Venus soybeans (GTS 40-3-2) exhibited a toxic effect on the protozoa, which died in repeated trials on the 14th-15th day of feeding. It was observed that infusoria fed with soybeans containing the GTS 40-3-2 (Venus) transformation event changed their shape after 3 days from the start of the experiment. Over the course of 10 days from the beginning of the study, a decrease in movement, motility, and the quantity of infusoria was noted. On the 14th-15th day, mortality was observed. Negative changes were also observed in infusoria that were fed with MON 00603 (NK 603) maize. The application of this method in the practice of assessing the safety of GM food products can provide a preliminary evaluation of long-term effects over a relatively short period

Keywords: feeds; biotest; infusoria; toxicity; long-term effects

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Introduction

Nowadays, food and feed safety issues receive growing attention. Whether transgenic products which were created to ensure food and feed security are safe is a particularly sensitive issue. The issue of simplifying safety controls for products derived from genetically modified animals is currently being raised (Fan *et al.*, 2022). In a 90-day feeding study of phytase-transgenic maize 11TPY050, authors found no unexpected adverse effects in SD rats. Y. Li *et al.* (2021) investigated the effects of genetically modified (GM) rice BPL9K-4 in rats and found no toxicity.

Accumulating evidence points to potential and actual biological risks associated with the commercial use of GM plants. The American organisation "Center for Food Safety" (CFS) (n.d.) has highlighted six key negative impacts of genetic modifications that could affect consumer health. These include concerns related to toxicity, allergic reactions, antibiotic resistance, immune system suppression, potential cancer risks, and inadequate nutrient absorption. While researchers continue to debate the risks associated with consuming GM foods, many ordinary consumers in certain countries unwittingly participate in a "global experiment" by including GM products in their diets over several years. The long-term consequences of this experiment may only become apparent in future generations, possibly 200-300 years from now. In contrast, in many Asian countries like Kazakhstan, consumers do not have the luxury of choice because food market sellers do not disclose whether products are genetically modified or not.

Currently, the examination of GM-containing foods is conducted in several areas: medical and genetic evaluation (study of the claimed gene embedded at the molecular and cellular level and its impact on the plant and other objects); technology assessment (study of sensory, consumer, and other properties); medical and biological assessment, the results of which are in clinical trials, issued as an opinion on the quality and safety of GM products (Ahmad *et al.*, 2021).

A stepwise safety and quality assessment of genetically modified sources is also implemented. At the core of this approach is the principle of comparative or real equivalence, which entails comparing GM foods with their conventional counterparts. This involves analysing the chemical composition of the product and comparing it to the conventional version, assessing basic nutrients, anti-nutritive and toxic substances, allergens specific to the food type, and examining the properties of the transferred genes. If the compositional equivalence evaluation reveals no differences between the GM food and the conventional counterpart, it is classified as first-class safety, indicating it is considered entirely safe for consumer health. In cases where differences are detected (second safety class) or there is a complete mismatch with conventional counterparts (third safety class), the safety assessment of genetically modified food should be further investigated. Stages of food safety research include the study of nutritional and toxicological characteristics of the product (Kopko *et al.*, 2022).

Evaluation of the properties of food involves the study of nutritional value of the new product; consumption rates; methods of use in nutrition; bioavailability; the introduction of specific nutrients (especially if the expected nutrient intake exceeds 15% of the daily requirement); impact on the intestinal microflora (if the genetically modified source contains living organisms). Toxicological characterisation involves determining the following indicators: toxicokinetics; genotoxicity; the potential allergenicity; potential colonisation in the gastrointestinal tract (in the presence of genetically modified live microorganisms as a source); the results of subchronic (90-day) toxicological experiments on laboratory animals and studies on human volunteers.

Such a quality assessment system and safety evaluation of GM food sources, which is based on the principle of compositional equivalence, can be recommended for products devoid of proteins and DNA (Wang *et al*., 2022). The development of control methods and effective protocols, including the detection of new foreign proteins in raw materials and finished products that may trigger allergies, is a significant concern for improving product quality and, most importantly, ensuring human health safety (Kopko *et al.*, 2022). One solution to this issue involves the development of new methods for assessing food safety, including the study of long-term effects on dozens of generations of model organisms.

The application of biological test methods for assessing substance safety is grounded in the fact that living organisms require specific chemical compositions for their life, growth, reproduction, and functioning. Any alterations in this composition lead to immediate or delayed responses in test organisms, such as changes in behaviour (movement), morphological characteristics, reproductive function, or even death. B.S. Bandarra *et al.* (2023) employed biological test subjects to assess the ecotoxicity of industrial waste in their studies. A. Mehl & G.E. Morlock (2023) also proposed the use of bioassays to detect antibiotic residues in food, as this method can identify compounds that may remain undetected by other modern techniques like HPLC-MS.

The aforementioned studies support the feasibility of using protozoa as bioassays to assess the overall toxicity of food. In this regard, patents for inventions were developed (Balji & Adilbekov, 2022). The purpose of this study is to explore the potential of conducting preliminary safety assessments of GM products using model bioassays involving *Paramecium caudatum* infusoria.

Materials and Methods

The analyses were conducted at two different laboratories: the Food Safety Laboratory within the Veterinary Sanitation Department of Kazakh Agro-Technical University, named after S. Seifullin, and the Laboratory of Food Product Analysis at the Republican State Enterprise (RSE) under the Right of Economic Management (REM) called the "National Reference Center for Veterinary Medicine", affiliated with the Ministry of Agriculture of The Republic of Kazakhstan. The experimental studies utilised soybeans of the GTS 40-3 and GTS 40-3-2 (Venus variety) varieties, maize from the MON 00603 (NK 603) line, and non-genetically modified (GM) soy and maize as control samples. Real-time PCR was employed to analyse the test samples.

The analysis involved the determination of contaminants of anthropogenic and biogenic origin in both GM and non-modified samples. The concentration of toxic elements such as arsenic, cadmium, lead, and mercury was determined through Foodstuffs' Determination of Trace Elements using Inductively Coupled Plasma Mass Spectrometry (ICPMS) after pressure digestion, following the BS EN 15763:2009 (2009) standard. Sample preparation adhered to the All-Union standard 31671:2012 (2012), titled "Food Products: Determination of Trace Elements with Sample Preparation Involving Mineralization at Elevated Pressure". Detection was conducted using the Agilent Technologies 7700 ICPMS with inductively coupled plasma mass spectrometry.

For the determination of aflatoxin B1 concentration in fodder, an enzyme-linked immunosorbent assay (ELISA) was employed, utilising the CELER AFLA B1 kit (Tecna R & D Diagnostics Biotechnology, Italy). Nitrates were detected using the potentiometric method as specified in the All-Union Standard 13496:19-93 (1997) titled "Fodder, Mixed Fodder, Animal Feed Raw Material: Methods for Determination of Nitrates and Nitrites". Total radioactivity (α, β, γ-rays) was assessed using the MKS-151 radiometer-dosimeter. Initial preparation of infusoria involved cultivating them in settled

water at room temperature (18-23°C), shielded from direct sunlight, with the addition of yeast powder (non-GM) as a nutrient solution and hay extract. After approximately 7-10 days, the concentration of paramecium reached approximately 10,000 u/ml. Following two weeks, starting material was extracted from the infusoria, consisting of around 100 infusoria in a 10 mcl volume, and transferred to flasks containing 100 ml of water for 2-3 days. Subsequently, daily records and visual assessments were carried out from the 1st to the 15th day, focusing on the following parameters:

1. The amount of test objects.
2. The motility (chemotaxis) of test objects.
3. The size of test objects.
4. The form of the test objects.
5. The percentage of test objects death.
6. Reproduction (reproductive function) of test objects.

Transitions were observed over a period of 6 months, twice a week. The foliar application was conducted with the GM soybean, maize and soy; as a control with soy and maize which do not contain genetic alterations 2 times per week.

The experimental setup was organized into groups, with flasks containing bioassay objects as follows:

Flask 1 – Control. Infusoria were fed with non-genetically modified soybeans.

Flask 2 – Control. Infusoria were fed with non-genetically modified maize.

Flask 3 – Infusoria were fed with GM soy from transformation event GTS 40-3.

Flask 4 – Infusoria were fed with GM soy from transformation event GTS 40-3-2 (Venus *grade).*

Flask 5 – Infusoria were fed with GM maize from the MON 00603 (NK 603) line.

The feed for infusoria was provided daily in the form of powder, with a quantity of 150 mg per flask.

Results are presented as the mean observations per flask. All analyses were performed in duplicate. Due to the limited number of samples, no statistical analyses were performed (Customs Union Commission..., 2010).

Results and Discussion

Initially, both GM soybean, maize, and control samples were examined for the presence or absence of contaminants of biogenic and technogenic origin, such as toxic elements, aflatoxin B1, pesticides, and nitrates, which could potentially affect the test organisms. Background radiation levels were also determined for all samples, and they did not exceed the natural background radiation, measuring no more than 0.14 microns per hour.

Bioassay objects, *Paramecium caudatum*, were exposed to the investigated GM product samples; for control in a parallel experiment, similar soy and maize samples without GM modifications were used as controls. The long-term effects on the infusoria, spanning 5-10 generations or more, were visually assessed over a relatively short period ranging from 15 days to 6 months. A notable factor favouring the selection of these bioassays is that the culture of *Paramecium caudatum* can be utilised for extended periods due to their periodically changing reproductive pathways (sexual reproduction and fission). Certain changes that occur with the test bioassays were established. Morphological alterations result in distinct shapes and sizes compared to the control groups.

Specifically, infusoria fed with soybeans containing the transformational event GTS 40-3-2 (Venus) exhibited changes in their shape after 3 days from the start of the experiment. These experimental infusoria became more rounded in contrast to the control group, as depicted in Figure 1. Within 10 days of the experiment in this group, a slow movement of infusoria was observed, along with reduced motor activity and population size. On the 14th to 15th day of the experiment, infusoria mortality was observed. Similar results were obtained in duplicate.

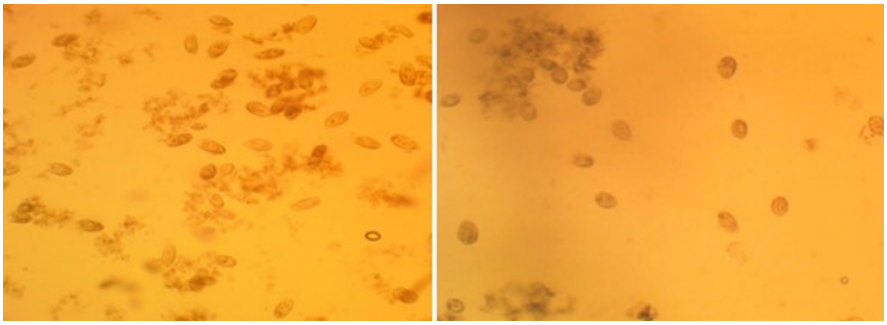

Figure 1. Changes in the shape of infusoria after feeding non-GM soy (left panel) and GM soy (right panel)

Source: author's development

Maize from the MON 00603 (NK 603) line induced the following changes in infusoria. After 3 days from the start of the experiment, infusoria slightly increased in size and took on an oval shape.

Subsequently, every 7 days, their population decreased. After 60 days of the experiment, slow movement of protozoa was observed, and after 4 months, mortality occurred in all bioassays (Fig. 2).

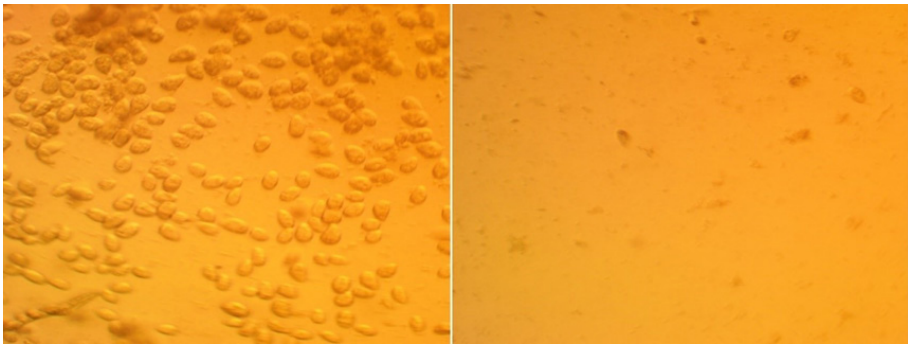

Figure 2. Changes in the shape of infusoria after feeding with GM maize (right panel) at the beginning of the experiment (left panel) and at the end of the experiment (right panel)

Source: author's development

In the control groups, no morphological changes were observed over the course of 6 months. In the control group, there was positive chemotaxis of infusoria, and even after 4 months of observation, the number of protozoa decreased to one-third. However, after 6 months from the start of feeding, no complete deaths of infusoria were observed in the control groups.

The study also examined the long-term impact on the reproductive function of the protozoa. However, it remained unclear from this study whether infusoria fed GM soybean and maize had altered the morphological characteristics and lifespan of the protozoa. It may be assumed that different genetically modified events in the studied cultures have varying effects on the lifespan of Paramecium caudatum protozoa. In the control group, where infusoria were fed non-GM soy and maize, the duration of reproductive function extended beyond 6

months, although the population decreased in both GM-fed and non-GM-fed groups. Nevertheless, complete infusoria death was not observed in the control groups. The experimental studies were concluded after 6 months, as the protozoa in the control groups ceased to exist after 15 days (GTS 40-3-2 Venus) or after 4 months of initial GM feeding.

Various biological organisms are employed to assess the safety of GM foods, with experiments commonly conducted on rats and mice. For instance, a 90-day subchronic feeding study assessed the food safety of stacked trait GM maize GH5112E-117C, which contains insect-resistance gene Cry1Ah and glyphosate-resistant gene G2-aroA, in comparison to non-GM Hi-II maize fed to Sprague-Dawley rats. The study used three different dietary concentrations (12.5%, 25%, and 50% w/w) of GM maize or its non-GM counterpart. The results indicated no biologically significant differences in clinical outcomes, body weights, food consumption, haematology, clinical chemistry, organ weights, and histopathology between the stacked trait GM maize groups and the non-GM maize groups. The results of the 90-day subchronic feeding study demonstrated that the stacked trait GM maize GH5112E-117C is as safe as the conventional non-GM maize Hi-II. Safety tests deemed the GM variety to be "substantially equivalent" to conventional soybeans. Feeding rats diets containing genetically modified or conventional (native) maize did not affect their final body weight or growth. These findings are confirmed by the results obtained by I. Kosieradzka *et al.* (2008).

A 49-day feeding study was conducted to assess the impact of genetically modified (GM) maize strain C0030.3.5 on Japanese quails (Coturnix japonica), focusing on body performance and egg quality. Furthermore, the bodily fats of transgenic proteins in the Japanese quails were investigated. The findings indicated that male and female quails that consumed GM diets exhibited normal parameters in terms of body weight, haematology, serum chemistry, relative organ weight, and histopathological appearance, and no discernible differences in laying performance or the nutrient composition of eggs between the groups with varying diets (Zhang *et al.*, 2021).

Y.-Ch. Wang *et al*. (2023) delved into the impact of GM-Lac on juvenile Asian seabass. This study aimed to explore the potential of a combination of lactic acid bacteria (LAB), specifically Lactobacillus and Bifidobacterium, as a probiotic supplement in the field of aquaculture. In summary, GM-Lac, which includes four LAB strains (GMNL-93, -141, -277, and -550), was proposed as a natural antimicrobial agent suitable for prophylactic use and as a probiotic feed supplement in fish aquaculture practice for enhancing growth performance and intestinal health, reducing the host susceptibility to pathogenic infection, and lowing the food conversion ratio (FCR). Moreover, the rise in muscle amino acid levels was regarded as a promising advantage offered by GM-Lac in enhancing the taste of freshwater-cultured fish. These studies confirm the effective use of GM microorganisms in aquaculture. There is a widespread scientific consensus regarding the safety of approved foods and feed products derived from genetically modified (GM) plants, fish, livestock, poultry, and animals fed GM diets. This consensus is based on assessments by regulatory agencies, independent scientists, and scientific organisations from various countries (Vergolyas & Goncharuk, 2016; Blair & Regenstein, 2020).

Within the available literature, there is substantial data supporting the safe utilisation of GM products. There is a lot of information about the beneficial properties of genetically modified foods, for example, that GM crops are more productive than conventional crops, they reduce the use of toxic chemicals, pathogen infestation, the need for production costs, have a longer shelf life of perishable products, etc. (Udovic *et al*., 2013; Ahmad *et al*., 2021). In addition, GM crops can contribute to increased

biodiversity by incorporating new traits into field crops, expanding the range of germplasm used in cultivation (Schulman, 2020). In general, the literature presented shows the enormous economic, environmental, and health benefits of GM crops. However, there is evidence stating that most of the studies on the safety of GM products are methodologically incorrect and unreliable. Of the 37 identified adverse events associated with GM consumption, approximately 59.46% were categorised as serious. These serious adverse events encompassed risks such as mortality, tumour development or cancer, a significantly reduced number of pup deliveries, diminished learning and reaction capabilities, and structural abnormalities in organs, including the stomach, intestinal adenoma, mammary glands, pituitary, liver, and kidney. The interventions or exposures in the studies related to adverse events predominantly focused on GM soybeans, maize, and rice, specifically in the context of certain GM events.

In some developing countries, there is no legal framework regulating the control of the safety of GM products, their circulation and use. Study by S.S. Al Mazrooei & D.R. Alreshidi (2023) introduced and improved a PCR-screening system for detecting GMO regulatory elements p35S and Tnos. This system uses a modified in-house DNA extraction method and appropriate control samples. This screening is seen as a prerequisite for the regulation of GMOs in biotech-derived products. Their findings revealed the presence of GMOs in food and feed products available in the Kuwaiti market for both human and animal consumption. Animal studies are at the lowest hierarchy of evidence, and there are flaws in study design making the results not convincing. The evidence on the effect of GM consumption on humans is still insufficient. Research results indicated that in European countries, over 62.5% of the population holds a negative attitude toward the use of GM products, while in North America, Latin America, and Asia, this sentiment is below 50%. Further clinical trials and cohort studies focusing on the effects of GM food consumption in human populations are deemed necessary. It is essential to thoroughly investigate safety before approving GM products for food consumption. It also suggests the necessity of labelling GM food so that consumers can make their own choices (Shen *et al.*, 2022).

Using ciliates like Paramecium caudatum as test subjects for assessing the safety of GM foods offers several advantages over traditional subjects like rats, mice, quails, or earthworms:

1. Simplicity and availability: Ciliates are single-celled organisms with a straightforward structure, making them easy to culture and examine in a laboratory. Their short life cycle allows for quicker experiments and faster results.

2. Sensitivity to toxic substances: Ciliates are highly responsive to environmental changes, including the presence of toxic substances. This sensitivity makes them valuable as indicators or biomarkers for evaluating the potential toxicity of GM foods and other substances.

3. Cost efficiency: Ciliates are much smaller and require fewer resources (food, space, etc.) to keep compared to larger animals such as rats or quails. This cost-effectiveness makes them a practical choice for large-scale safety studies of GM products.

4. Ethical considerations: Using ciliates as test subjects avoids ethical concerns associated with more complex animals like rats or mice, which may require special care and protection.

However, it should be noted that the use of ciliates has some limitations. Their simple structure might restrict the study of complex biological processes or higher-level effects on organisms. In addition, research results from ciliates may not always directly apply to human health or the environment.

In general, the use of infusoria instead of rats, mice, quails, or earthworms can be useful for simple and cost-effective experiments to determine the safety of GM products, but requires additional research and evaluation in the

context of the specific goals and requirements of the study. The increased utilisation of bioassay methods through the use of simple organisms has long been a goal in the food industry for assessing the overall toxicity of food products and their impact on reproductive properties over multiple generations. The subjects of control and monitoring in food processing facilities are complex due to their variable nature, characterised by numerous multi-ingredients that can undergo changes in their properties under the influence of external factors. As chemical and biochemical reactions progress, the formation of new compounds with potentially harmful biological activity can occur, which may not be easily detectable using modern analytical equipment. Altering the genetic structure of a product can also lead to the emergence of new properties that may not be favourable for reproductive functions within the body.

One of the ways to solve this problem is to use biological methods, based on the fact that for the life, growth, reproduction, and functioning of living beings, there is a need for an appropriate environment with a strictly defined chemical composition. When altering the composition, such as by introducing an additional (specifically defined) compound or substance, the test organism typically provides a corresponding response over time, and sometimes, this response occurs almost immediately. Protozoa are single-celled organisms that possess all the essential functions of life, including metabolism, irritability, movement, reproduction, and more. As a result, they closely simulate the effects of chemical or complex biological components within ecosystems. The ultimate indicator of the effectiveness of an introduced substance on these organisms is their survival or death. Initially, researchers examined behavioural responses in infusoria, which represent one of the simplest types of reactions. Later, reproductive function in several generations was also studied, i.e., the ability to breed and maintain its population.

In the future, it is important to explore the impact of various GM products on protozoa, particularly assessing their reproductive capabilities over 7-10 generations. The results obtained do not show the dramatic negative impact of GM foods on the studied protozoa (except for GTS 40-3-2 Venus soy). When provided with a uniform diet, infusoria gradually decrease their active lifespan and reproductive function over time. Although vital functions of infusoria in experimental groups fed GM soy and maize ceased after 4 months, whereas in control groups, this occurred after more than 6 months, it may suggest a potential delayed impact of GM foods on protozoa. However, the author indicates that, when using a safety assessment of GM foods on the protozoa bioassay, it is needed to consider the results in the first 1-2 months. If no adverse effects are observed during this initial period, it may be inferred that the tested GM sample is safe at that time. The application of this developed rapid method for determining the safety of GM products through bioassays will empower food safety laboratories to expedite studies on GM products while providing a preliminary assessment of their long-term effects without the need for analytical equipment or laboratory animals.

Conclusions

Nearly all of the available results that negatively characterise GM foods have been disproven in scientific literature, often because researchers had flawed experimental designs. An additional alternative method for determining the safety of GM foods without the use of animals, including the impact on several generations of protozoa in a relatively short period of time was proposed. A new method based on the application of bioassay and Paramecium caudatum infusoria was developed in this study. Soybeans GTS 40-3 and GTS 40-3-2 (Venus variety) and maize line MON 00603 (NK 603) were tested as a feed for infusoria and showed some adverse effects. Those effects included changes in the shape,

reduced movement, reduced motor activity, and total number of infusoria. Feeding the infusoria with GM diets led to the death of the organisms within 15 days in the case of soybeans GTS 40-3-2 Venus and after 4 months with maize line MON 00603. These outcomes underscore the critical significance of safety testing for GM foods and feeds.

This method can be applied as a preliminary assessment of GM foods safety. Further, more comprehensive studies can be conducted based on the results from the method based on the application of bioassay and Paramecium caudatum infusoria. An advantage of this proposed method is its ability to conduct preliminary assessments of GM food safety across numerous generations of biological test subjects within a relatively short time frame and without incurring high costs. Future research should extend to higher animals and humans, notably human observational cohort studies, to mitigate any potential health risks for the broader population. Furthermore, to address public concerns, information should be available in easily accessible and understandable formats.

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Conflict of Interest

None.

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Попередня оцінка безпеки генетично модифікованих харчових продуктів

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Анотація. Численні дослідження генетично модифікованих продуктів харчування показують, що вони безпечні та можуть захистити людей у всьому світі від голоду. Проте проблема ГМ-продуктів і кормів продовжує залишатися актуальною у сфері харчової безпеки. Метою даного дослідження було представлення узагальненої схеми методів визначення безпечності ГМ харчових продуктів та розробка нового методу визначення безпечності ГМ харчових продуктів. Цей новий метод заснований на застосуванні біотесту та використанні інфузорії *Paramecium caudatum*. У дослідженні використовували соєві боби GTS 40-3 і GTS 40-3-2 (сорт Венус), лінію кукурудзи MON 00603 (NK 603) і без ГМО сою та кукурудзу як контроль. Інфузорії готували культивуванням у воді кімнатної температури (18-23 °C) без прямих сонячних променів з додаванням дріжджового порошку (не ГМ) як поживного розчину та екстракту сени. Потім вихідний матеріал, що містив близько 100 інфузорій, переносили у колби з водою об'ємом 100 мл і витримували 2-3 доби. Далі на початковому етапі експерименту з 1-ї по 15-ту добу проводили щоденні записи та візуальні оцінки. Оцінка включала кількість інфузорій, рухливість (хемотаксис), розмір, форму та відсоток мертвих інфузорій. Соя сорту Венера (ГТС 40-3-2) виявила токсичну дію на найпростіші, які гинули в повторних дослідах на 14-15 добу після початку згодовування. Було помічено, що інфузорії, згодовані соєвими бобами, що містять трансформаційну подію GTS 40-3-2 (Venus), змінили форму через 3 дні від початку експерименту. Протягом 10 діб від початку досліду спостерігалося зниження руху, рухової активності та кількості інфузорій. На 14-15 добу досліду спостерігали загибель. В інфузоріях, яким підживлювали кукурудзу лінії MON 00603 (NK 603), також спостерігалися негативні зміни. Застосування цього методу в практиці оцінки безпеки ГМ харчових продуктів може дати попередню оцінку віддалених ефектів за відносно короткий час

Ключові слова: корми; біопроба; інфузорії; токсичність; віддалені ефекти