

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Infusoria *Tetrahymena Pyriformis* In Toxicological Studies.

Vasiliy Ivanovich Dorozhkin^{1*}, Svetlana Alekseevna Lavina¹, Lyubov Lvovna Zakharova¹,
Dmitry Nikolaevich Urazaev², and Mikhail Vladimirovich Arisov².

¹All-Russian Scientific Research Institute of Veterinary Sanitation, Hygiene and Ecology – branch of All-Russian Scientific Research Institute of Experimental Veterinary Science of the Russian Academy of Sciences, Zvenigorodskoe shosse 5, Moscow 123022, Russia.

²All-Russian Scientific Research Institute of Fundamental and Applied Parasitology of Animals and Plants - Branch of the Federal State Budget Scientific Institution «Federal Scientific Center - All-Russian Scientific Research Institute of Experimental Veterinari Medicina K.I. Skryabin and Y.R. Kovalenko the Russian Academy of Sciences, B. Cheremushkinskaya str., 28, Moscow 117218, Russia.

ABSTRACT

The article presents the results of many years of research using ciliates of *Tetrahymena pyriformis* to assess the quality and safety of veterinary and environmental monitoring facilities, which demonstrate the viability of using this microorganism as an indicative toxicological test.

Keywords: biotesting, ciliate *Tetrahymena pyriformis*, objects of veterinary and environmental control, integrated assessment, LD₅₀, ciliate enzymes.

**Corresponding author*

SHORT REVIEW

Biotest assessment (biotesting, biological evaluation) is one of the methods for studying environmental objects (soil, water, air, food, feed, etc.), which allows to study the effect of various adverse factors on a living organism and assess the cumulative adverse effects of such effects. Analytical research methods that are highly sensitive and at the same time sufficiently selective, selective and often costly, aim to identify the presence of specific toxic components without taking into account their interaction and the holistic effects on a living organism. Biological analysis allows to identify the influence of various components of the environment on the body in the relationship and interdependence, which makes it possible to obtain an integral response to the impact in the form of a response of a biological object.

Since the use of higher animals for the formulation of toxicological experiments for a variety of reasons (methodical, ethical, economic) is difficult and often impossible, the world tends to replace in experimental studies of higher animals with alternative test models - plants, invertebrates, microorganisms, cultures fabrics, etc. One of these organisms is the ciliate *Tetrahymena pyriformis*.

The ciliates of *Tetrahymena pyriformis* belong to the class of protozoa and, by genetic relationship, occupy an intermediate position between microbes and higher animals. In tetrahimenes and higher organisms, many aspects of metabolism, the phases of digestion, the need for amino acids and other food components are similar. Ciliates are sensitive to various adverse environmental factors, chemical substances and compounds of veterinary-sanitary and environmental importance - toxic elements, pesticides, bacteria, and mold toxins, pharmaceuticals, ionizing radiation, etc.

The advantage of using ciliates *Tetrahymena pyriformis* in comparison with a number of other similar test organisms is the greater similarity of their toxic-biological reaction with the similar reaction of higher animals, high metabolic rate, the rapid growth of ciliates, the ability to work with a sterile culture and a standard strain. In addition, they have a better distinguishable growth phase of the culture, which allows the use of tetrachimenes in toxicological assessment during the period of intensive growth, when they are most sensitive to toxic substances.

The following vital indicators of protozoa are mainly used as test functions: survival rate of ciliates, mobility and movement pattern, generative (growth) and chemotactic (behavioral) reactions, morphological and biochemical indicators. Together, they give a real idea of the degree of toxicity of the object under study (animal products, feed, soil, natural and waste waters, organic waste from livestock enterprises, polymeric and construction materials, etc.), and also provide the possibility of interspecific extrapolation of the results [1, 2].

In studies conducted over several decades, it has been proven that biotesting using the ciliate *Tetrahymena pyriformis* is an inexpensive, highly sensitive method that allows you to visualize the biological effect of various toxic factors on the body. The use of the developed instrument base allows for the scrying assessment of a large number of samples with a high degree of performance. Automation of the method and the use of the developed devices allow us to expand the methodological capabilities of biological analysis [3,4].

First of all, the ciliates of *Tetrahymena pyriformis* are a significant indicator of the toxicity of various environmental objects. The methods developed by us allow us to assess the quality and safety of meat and meat products, including those with altered sanitary and technological properties. For example, poultry meat with pseudomonosis and listeriosis has a biological value of about 10% lower than healthy poultry meat. The practical significance of such data indicates the receipt of less high-grade and low-quality products from such meat and the need to find ways and technological methods to increase its biological usefulness [1]

Exposure of various pharmaceutical preparations changes various functions of *Tetrahymena pyriformis* both in its native form and in the form of metabolites, the amount of which happens in the body slightly, hardly perceptible by analytical devices. We have found, for example, that furazolidone has an inhibitory effect on *Tetrahymena* at a concentration of 0.35 $\mu\text{g} / \text{ml}$ of medium. At the same time, the remnants of the drug in the liver of animals had the same effect on the ciliates at a concentration of 0.011 $\mu\text{g} / \text{ml}$. The 30-fold increase in toxicity is associated with the metabolism of the drug with the formation of an acetylated derivative, which has become possible to determine by means of bio-analysis [5].

Aminazine used to relieve the stress of birds during transport, in the form of residual amounts (0.1-0.3 mg/kg) in the muscle tissue of birds, had an inhibitory effect on the ciliate *Tetrahymena pyriformis*, which numerically amounted to 15-20% compared to the meat of birds that did not receive the drug. The results of the research allowed us to estimate the timing of excretion of the drug from the body and establish the corresponding waiting periods for the slaughter of poultry [1, 2].

The method of assessing toxicity is applicable not only to meat but also to environmental objects. So *Tetrahymena pyriformis* proved to be a highly sensitive test in assessing the quality of wastewater and waste of livestock enterprises, which allowed to establish the class of waste and, accordingly, their safety for the environment [6].

Methods based on various *Tetrahymena pyriformis* reactions are used to assess the toxicity of such objects like water, air, feed, polymeric and building materials, etc. [7-10].

Comparative evaluation of the experimental effect of LD50 of various substances on the organism of white rats and ciliates revealed a high correlation between this indicator for the above-mentioned biological objects, which indicates the similarity of the main stages of metabolism, as well as the possibility of interspecific extrapolation of the indicator and a reduction in the cost of a preliminary assessment of toxicity toxic substances [1, 11].

Further in-depth study of the effect of toxic substances on the ciliates showed that *Tetrahymena pyriformis* enzymes are capable of decomposing various substrates, such as acetylcholine, butyrylcholine, phenylacetate, naphthyl acetate, and several others. This made it possible with the application of the methods developed by us to compare the effect of various substances - organophosphorus, organochlorine and pyrethroid compounds, heavy metal salts - on the conversion activity of these substrates in ciliates and higher animals. The study of the kinetics of enzymatic reactions has greatly expanded the understanding of the influence of toxicants on the biochemical processes occurring in the body under their influence. A high degree of correlation of changes in the activity of enzymes in ciliates and higher animals under the action of toxic substances was demonstrated, which, using appropriate methodological approaches, makes it possible to differentiate their effect on the object, and also allows to speed up and significantly reduce the cost of conducting such experiments [12, 13, 14].

In conclusion, it should be noted that ciliates of *Tetrahymena pyriformis* are a promising object of study in toxicology. Their use is not limited to narrow limits of toxicity assessment but allows to conduct rather complex experiments with a wide range of objects, extrapolate the results to higher organisms, obtain data with low costs and high performance by automating the process and increasing the number of studied parameters.

REFERENCES

- [1] Dolgov V.A. Methodological aspects and practical application of the accelerated biological assessment of feed, animal products and other objects of veterinary, sanitary and environmental control: Diss. ... Dr. wet Of science. M., 1992.
- [2] Ignatiev A.D., ShabliV.Ya. The use of the ciliate tetrahimene pyriformis as a test object for biological research in agriculture. M.: VASHNIL, 1978
- [3] Guidelines for the use of ciliates of *Tetrahymena pyriformis* as a test culture in the device "Biotester-2".M. : Veterinary Department of the Ministry of Agriculture of the Russian Federation, 2000. 13-7-2 / 2157.
- [4] Automated method for determining the toxicity of animal products, feed and environmental objects (guidelines). M., RAAS, 2006.
- [5] Dolgov V.A., Lavina S.A., Kozak S.S., Nikitchenko D.V. Biotesting food, feed and environmental objects. Bulletin of RUDN. Series Agronomy and Livestock. Number 3, 2014. pp. 69-78.
- [6] Tyurin V.G., Dolgov V.A., Lopata F.F., Kochish O.I. Role of environmental factors in obtaining safe product of animal husbandry. Food safety and sustainable development of the agro-industrial complex materials of the International scientific-practical conference. 2015. pp. 534-537.
- [7] Guidelines for the use of the rapid method of biological evaluation of products and feed. M.: VASHNIL, 1990.

- [8] Guidelines for the accelerated assessment of air toxicity at veterinary-sanitary and environmental monitoring facilities. - Moscow, Russian Academy of Agricultural Sciences, 2008.
- [9] Guidelines for determining the toxicity of polymeric and building materials for agricultural purposes. Moscow, Russian Academy of Agricultural Sciences, 2008.
- [10] Guidelines for the accelerated determination of the toxicity of animal products and feed. M.: Veterinary Department of the Ministry of Agriculture of the Russian Federation, 2000. 13-7- 2/2156.
- [11] Methodological manual on biotest assessment of the quality and safety of various objects of veterinary-sanitary and environmental monitoring. Moscow, Russian Academy of Agricultural Sciences, 2010.
- [12] Dolgov V.A., Lavina S.A. Parameters of toxicity of various substances for ciliates tetrahmene and white rats. In the collection: Veterinary and sanitary aspects of the quality and safety of agricultural products. Proceedings of the I-th International Conference on Veterinary-Sanitary Expertise. Voronezh State Agrarian University; 2015. pp. 235-238.
- [13] Lavina S.A., Dolgov V.A., Effect of heavy metal salts on the ciliate infuson systems Tetrahymena pyriformis. Russian journal Problems of Veterinary Sanitation, Hygiene and Ecology, 2001. 2. P. 48
- [14] Avalanche S.A. New features in the analysis of pyrethroid insecticides. Veterinary Medicine 2003. 2. P. 48.