UDC: 615.9 + 661.162.6 + 547.823 + 593.17 DOI: 10.33273/2663-4570-2021-91-1-40-50

MORPHOFUNCTIONAL CHANGES IN CELLS OF TETRAHYMENA PYRIFORMIS W INFUSORIA UNDER THE INFLUENCE OF PLANT GROWTH REGULATORS – DERIVATIVES OF PYRIDINE-N-OXIDE

O. Vasetska
L.I. Medved's Research Centre of Preventive Toxicology, Food and Chemical Safety, Ministry of Health, Ukraine (State Enterprise), Kyiv, Ukraine

ABSTRACT. The aim of the Research. *To identify morphological changes in Tetrahymena pyriformis W infusoria under the acute exposure to plant growth regulators (PGR) – derivatives of pyridine-N-oxide and compare them to functional disorders of cells.*
Materials and Methods. In the research we used the 2-methylpyridine-N-oxide, 2,6-dimethylpyridine-N-oxide and their complexes with organic acids (succinic, maleic) or metal salts ($ZnCl_2$, ZnI_2, $CoCl_2$, $MnCl_2$) (a total of 15 substances), synthesized at the Institute of Bioorganic chemistry and Petrochemistry, NAS, Ukraine. Studies were performed on Tetrahymena pyriformis W infusoria in isotoxic doses – at the level of toxic concentrations – LC_{50}, LC_{16} and inactive concentrations (LC_0). Morphological changes in cells of infusoria were assessed visually with the use of a light microscope. Structural changes in infusoria were compared to functional changes in cells (motor activity and energy state) obtained under the same experiment.
Results and Conclusions. *It is demonstrated that 2-methylpyridine-N-oxide, 2,6-dimethylpyridine-N-oxide and their complexes with organic acids (succinic, maleic) or metal salts ($ZnCl_2$, ZnI_2, $CoCl_2$, $MnCl_2$) cause functional and morphostructural changes in infusoria, the extent of which depends on the current concentration. Morphostructural changes in infusoria under the influence of the studied PGRs are characterized by a change of shape, growth of the contractile vacuole, vesiculation, damage to the integrity of the cytoplasmic membrane, emission of cytoplasm and structural elements of cells into the nutrient medium.*
Complexes of methyl derivatives of pyridine-N-oxide with metal salts in the studied concentrations reduce speed and increase energy expenditure on movement, cause changes in behavioural reactions and structure of cells to a greater extent than 2-methylpyridine-N-oxide, 2,6-dimethylpyridine-N-oxide and their complexes with organic acids. Both functional and morphological changes in infusoria are more evident under the influence of studied PGR occurring at concentrations corresponding to LC_{50}. At lower concentrations the changes in the functional activity of infusoria were observed. Comparison of the obtained functional and morphostructural indicators of the state of infusoria shows that complexes of methyl derivatives of Pyridine-N-oxide with metal salts have more toxic effects on infusoria than complexes of methyl derivatives of pyridine-N-oxide with organic acids. Reduced motor activity and an increase in energy consumption per a unit of a path of motion, together with the morphological changes of cell structure, are the indicators of toxicity of xenobiotics for infusoria and criteria for assessing their viability.
Key Words: *methyl derivatives of Pyridine-N-oxide, Tetrahymena pyriformis W, morphofunctional changes.*

Introduction. Large-scale use of chemicals, including pesticides and agrochemicals, for various purposes in the national economy is a real threat because of their potential entry into the environment, as they can harm aquatic and terrestrial bioresources, as well as human health. Today in Ukraine a significant proportion of registered plant growth regulators (PGR) are the drugs based on methyl derivatives of pyridine-N-oxide (ivin, poteitin, kapanin, zeastymulin, agrostimulin, betastimulin, triman, etc.) [1], as they contribute to the increase in crops yield, reduction of the amount of pesticide applied without reducing efficiency, production of ecologically clean agricultural products [2-7].

Complexes of 2-methylpyridine-N-oxide, 2,6-dimethylpyridine-N-oxide with organic acids and metal salts synthesized at the Institute of Bioorganic Chemistry and Petrochemistry of the National Academy of Sciences of Ukraine demonstrated high auxin or cytokinin activity on various crops. According to the parameters of acute toxicity for laboratory animals, they are low- or moderately toxic substances. For 2,6-dimethylpyridine-N-oxide (Ivin) and complex of 2-methylpyridine-N-oxide with MnCl2 (Triman) membrane-associated trophic activity, intensification of protein synthesis processes in animals are proven. The ability, in particular of Ivin, to reduce acute toxicity and toxic effects in the body of rats due to exposure to pesticides of different chemical groups was detected [2, 8-10].

It is known that chemicals, which are characterized by high biological activity when released into the environment, have a pro-

nounced toxic effect on aquatic and terrestrial environments. The most dangerous for the environment are salts of heavy metals, surface active substances (SAS), and pesticides. Thus, heavy metals, accumulating in the soil, have a phytotoxic effect on plants, inhibit metabolic functions, limit growth, cause chlorosis, etc. [11]. Salts of heavy metals have a pronounced toxic effect on cyanobacteria Cynechocystis SP atthe level of 1,5 mkg/l^{-1}, zinc and copper oxides are toxic in low concentrations to marine bacteria Vibrio fischeri and freshwater protozoa Tetrahymena Thermophila [12, 13]. With decreasing aquatic temperatures, the toxicity of heavy metals increases significantly [12]. SAS in high concentrations cause lysis of infusoria, and in low – a toxic effect. This is due to the interaction of SAS with lipid bilayer of membranes, which in its turn, leads to changes of osmotic properties and disruption of cell lysis [14]. Oxidative stress caused by chemicals is also among the reasons of structural changes and impairment of infusoria [15].

Toxic effects of heavy metals, some pesticides and various chemical factors on infusoria and other water bodies are mainly reported in the literature as a concentration-effect relationship with the determination of lethal and effective concentrations [9, 16-21] without studies of structural cell disorders being conducted. For some substances, including pesticides, it is noted that along with physiological changes there are changes in the structure of infusoria (loss of their shape, growth of the contractile vacuole, damage to the integrity of the cytoplasmic membrane and organelles). Inhibition of growth and phagocytic activity of infusoria were observed. Important in the mechanism of toxic action of pesticides on infusoria is the imbalance of antioxidant enzymes [22-24].

The effect of PGRs on the state of infusoria is still insufficiently studied. It is shown that methyl derivatives of pyridine-N-oxide under the acute exposure are moderate or low toxic substances for Tetrahymena Pyriformis W infusoria, in high concentrations they cause functional changes (increase or reduce the frequency and speed, total energy consumption for motion), under chronic exposure the curve of concentration-infusoria growth dependence has a mono -, bi - or polymodal form [9, 20, 21]. Along with functional disorders morphological changes in the structure of cells are an important characteristic of toxic effects on infusoria. So the determination of morphostructural changes in infusoria under the acute exposure to pyridine-N-oxide derivatives based PGRs, which are introduced into agricultural practice in Ukraine is an actual task.

The Aim of the Research. To identify morphological changes in Tetrahymena pyriformis W infusoria under the acute exposure to plant growth regulators (PGR) – derivatives of pyridine-N-oxide and compare them to functional disorders of cells.

Materials and Methods. In the study we used the 2-methylpyridine-N-oxide, 2,6-dimethylpyridine-N-oxide and their complexes with organic acids (succinic, maleic) or metal salts ($ZnCl_2$, ZnI_2, $CoCl_2$, $MnCl_2$) (a total of 15 substances), synthesized at the Institute of Bioorganic chemistry and Petrochemistry, NAS, Ukraine, that showed high growth regulating activity and are recommended for agricultural practice in Ukraine as PGR on different crops.

The study is conducted in Tetrahymena pyriformis W infusoria. Tetrahymena pyriformis W infusoria, as a test system in vitro, is widely used in toxicology as an alternative test object to study the toxicity of many pollutants of water bodies, pesticides, heavy metals, extracts of polymeric materials, preservatives and disinfectants, organic and inorganic compounds [23, 25, 26]. As a rule studies on infusoria are conducted under the criterion of "cell death" to determine the parameters of toxicity and predict the danger to humans and the environment. But in addition to the criterion of "cell death" functional and morphological changes are important characteristics of infusoria, and their determination will complement to existing understanding of toxic effects of the impact of chemicals on the cell body.

Studies of morphological changes in Tetrahymena pyriformis W infusoria were performed in isotoxic concentrations: at the level of toxic concentrations – LC_{50}, LC_{16} and inactive concentrations (LC_0), which were determined earlier [8] and shown in the table.

At high concentrations (at the level of LC_{50}) the microscopic examination of the effect of 2-methylpyridine-N-oxide, 2,6-dimethylpyridine-N-oxide and their complexes with organic acids revealed morphostructural changes in infusoria, which are presented in Fig. 1.

Table

Toxicity values of the researched N-pyridine oxide derivatives

Chemical name	LC_0 (mg/ml)	LC_{16} (mg/ml)	LC_{50} (mg/ml)
2-methylpyridine-N-oxide	9,45	18,90	26,00
2-methylpyridine-N-oxide with succinic acid	3,41	6,82	8,52
Di-2-methylpyridine-N-oxide with succinic acid	1,64	3,27	5,82
2,6-dimethylpyridine-N-oxide	8,89	17,79	34,27
2,6-dimethylpyridine-N-oxide with succinic acid	1,44	2,88	5,67
Di-2,6-dimethylpyridine -N-oxide with succinic acid	2,49	4,99	6,87
2,6-dimethylpyridine-N-oxide with maleic acid	2,05	4,09	5,78
Di-2-methylpyridine-N-oxide with $ZnCl_2$	1,49	2,98	3,83
Di-2-methylpyridine-N-oxide with ZnI_2	2,30	4,60	6,50
2-methylpyridine-N-oxide with $CoCl_2$	4,26	8,52	13,35
Di-2-methylpyridine-N-oxide with CoI_2	1,03	2,06	3,27
2-methylpyridine-N-oxide with $MnCl_2$	4,83	9,66	13,92
Di-2,6-dimethylpyridine-N-oxide with $ZnCl_2$	1,56	3,12	5,54
Di-2,6-dimethylpyridine-N-oxide with ZnI_2	0,87	1,74	2,76
Di-2,6-dimethylpyridine-N-oxide with $CoCl_2$	3,55	7,10	11,93

As can be seen in Fig. 1, intact (control) infusoria have a spindle-shaped or slightly elongated body shape with a clearly defined smooth cytoplasmic membrane and well-visible cell organelles. In the upper left part of the figure there are the cells which are in the process of division. The cells moved linearly, the trajectory changed by a sharp turn, spiral swimming was observed.

Under the influence of 2-methylpyridine-N-oxide in high concentrations (at the level of LC_{50}) the body of infusoria in most cases was elongated, some cells had a pear-shaped body. In cells that are located in the centre of the figure the vesiculation of the lateral surface of the body was detected, vesicles are arranged in chain. There was a slowdown in the speed of movement alongside with frequent changes of direction, reduction in the number of food vacuoles. Morphological transformation of body shape of infusoria, which was accompanied by frequent changes of direction, can be the evidence of the change of taxis [27].

Under the influence of the complex of 2-methylpyridine-N-oxide with succinic acid some cells become elongated in the forebody and curved towards the end or pulled out like "proboscis" (the "sickle shaped" form). These cells forebodies seemed to be fixed to glass slides and they quickly moved clockwise around its radial axis. The cytoplasmic membrane was not damaged.

Under the influence of the complex of di-2-methylpyridine-N-oxide with succinic acid, the nature of morphological changes was similar to the complex of 2-methylpyridine-N-oxide with succinic acid, but the field of view was dominated by cells with elongated and sickle shaped body form, which quickly rotated around its axis. Contractile vacuoles were empty, food vacuoles were not traced. The cytoplasmic membrane was not damaged. Atrophied cells were observed.

Under the influence of 2,6-dimethylpyridine-N-oxide in most cells the body shape of infusoria was spindle- or pear-like, some parts of the cell increased in volume, and membrane of some of these cells was deformed, vacuolisation of the cell wall in a small rounded form or in a form of single large spherical mass of ectoplasm, emissions of vesicles into the incubation medium were observed. Food vacuoles were seen clearly.

In infusoria under the exposure to complexes of 2,6-dimethylpyridine-N-oxide with succinic acid and di-2,6-dimethylpyridine-N-oxide with succinic acid the morphostructural changes were similar. In both cases, there were

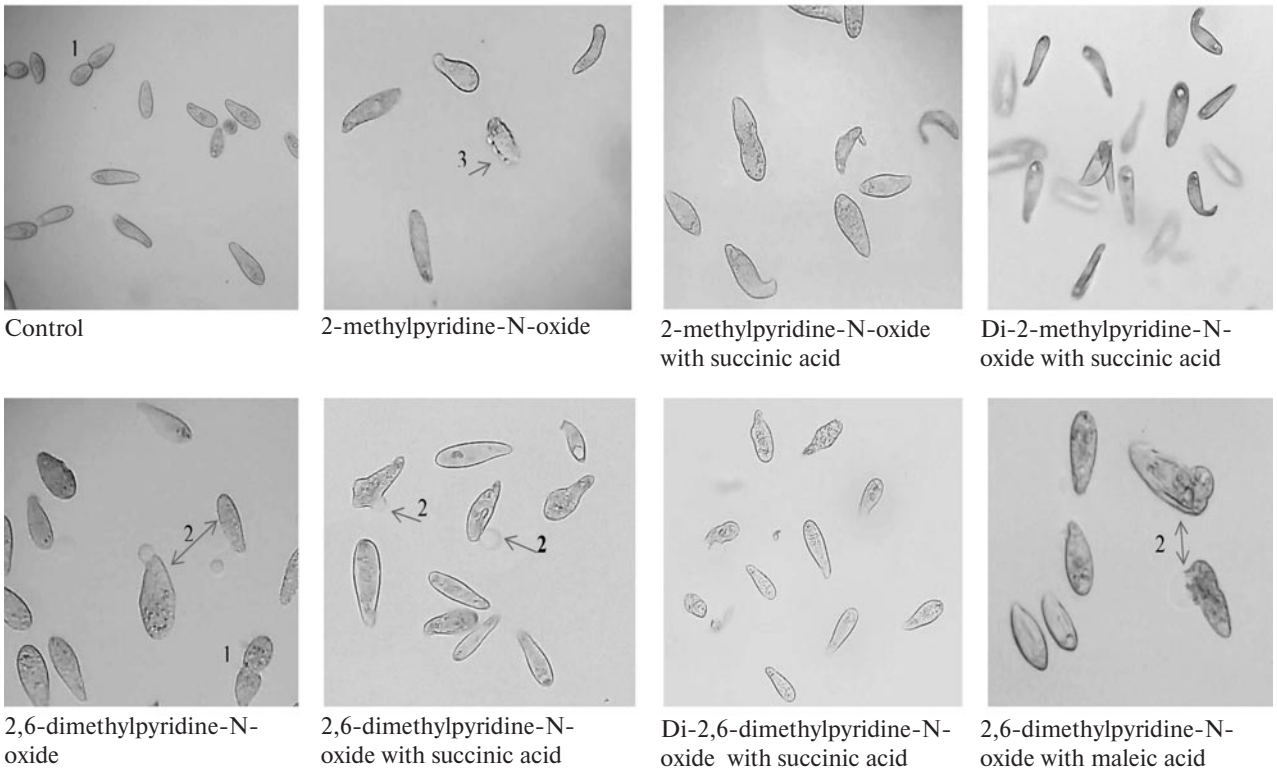
infusoria with distorted body shape (retort- or bottle-shaped), but in most of them the structural elements of the cells were well traced. In some cells the integrity damage of the cytoplasmic membrane and the release of cytoplasm into the nutrient medium were observed.

Significant morphological changes in infusoria were also observed due to the effect of complexes of 2,6-dimethylpyridine-N-oxide with maleic acid. Some infusoria and contractile vacuole increased in size. Cell vacuolisation, damaged cell membrane, the release of the cytoplasm and cell contents in the nutrient medium were observed.

As can be seen in Fig. 2, under the influence of 2-methylpyridine-N-oxide, 2,6-dimethylpyridine-N-oxide in the concentrations corresponding to LC_0 there was a probable increase in the velocity of infusoria; an insignificant increase in this indicator under the influence of complexes of 2-methylpyridine-N-oxide with succinic acid and 2,6-dimethylpyridine-N-oxide with maleic acid was observed. Probable changes in the total energy consumption for the movement of infusoria were not detected, but there was a slight increase in energy consumption per a unit of a path of motion with the greatest effect for 2-methylpyridine-N-oxide and its complex with succinic acid.

At concentrations corresponding to LC_{16}, a probable increase in the velocity of infusoria, the total energy consumption for infusoria movement, and an increase in energy consumption per a unit of a path of motion were observed only for 2-methylpyridine-N-oxide with succinic acid.

In average lethal concentrations under the influence of all studied PGRs a noticeable reduction in speed and increase in energy consumption per a unit of a path of motion were observed. Thus, reduction of speed of movement ranged from 22,4% to 51,4% and was the least expressed under the influence of di-2,6-dimethylpyridine-N-dioxide with succinic acid and most pronounced under the impact of 2,6-dimethylpyridine-N-oxide and its complex with succinic acid. Energy consumption per a unit of a path of motion increased from 47,84% to 127,3%, and the changes were the least expressed under the impact of 2,6-dimethylpyridine-N-oxide with succinic acid



Control | 2-methylpyridine-N-oxide | 2-methylpyridine-N-oxide with succinic acid | Di-2-methylpyridine-N-oxide with succinic acid

2,6-dimethylpyridine-N-oxide | 2,6-dimethylpyridine-N-oxide with succinic acid | Di-2,6-dimethylpyridine-N-oxide with succinic acid | 2,6-dimethylpyridine-N-oxide with maleic acid

Fig. 1. Morphostructural changes in Tetrahymena pyriformis W infusoria under the influence of methyl derivatives of pyridine-N-oxide and their complexes with organic acids at concentrations corresponding to LC_{50}. (1 – a cell at a stage of division, 2 – ectoplasm emission, 3 – vacuolisation)

and 2,6-dimethylpyridine-N-oxide with maleic acid and the most expressed under the impact of di-2,6-dimethylpyridine-N-oxide with succinic acid, 2-methylpyridine-N-oxide and its complex with succinic acid.

Studies indicate that given methyl derivatives of pyridine-N-oxide and their complexes with organic acids in concentrations at the level of LC_0 and LC_{16} do not cause morphological changes in the structure of infusoria and cause a slight increase in speed of movement and energy consumption. At average lethal concentrations, they significantly reduce the speed of movement and increase the consumption of energy, cause changes in behavioural reactions and cell structure, which leads to a decrease in the viability of infusoria.

Under the influence of the complexes of 2-methylpyridine-N-oxide and 2,6-dimethylpyridine-N-oxide with metal salts, the behaviour of Tetrahymena pyriformis W infusoria in the nutrient medium was the same and depended on the active concentration of substances.

Under the action of low concentrations (at the level of LC_0 and LC_{16}) visible morphological changes of infusoria cells were not detected, the movement slowed down, the body shape of infusoria did not differ from the control sample.

As can be seen in Fig. 3, at high concentrations (at LC_{50}) for the complexes of 2,6-dimethylpyridine-N-oxide and 2-methylpyridine-N-oxide with metal salts morphological changes of infusoria cells are more pronounced than for the impact of complexes with organic acids.

Thus, when the impact of the mixture of di-2-methylpyridine-N-oxide with $ZnCl_2$ the body shape of the cells was pear-like, contractile vacuole increased and the some cells were deformed (with elongated and narrowed forebody and extended rear body with an enlarged contractile vacuole, near which other structural elements of the cell were concentrated). The integrity of the cytoplasmic membrane of cells was not damaged.

Under the impact of di-2-methylpyridine-N-oxide complex with ZnI_2 infusoria of different shapes were found: pear-shaped, increased in size with a large contractile vacuole, elongated and flattened laterally, at the bottom there were infusoria with defective cytoplasmic membrane.

The complex of 2-methylpyridine-N-oxide with $CoCl_2$ had a pronounced damaging effect. Most of the cells were deformed, the cytoplasmic membrane of the cells was damaged, and the remains of damaged cells and structural components were observed in the nutrient medium.

Due to the effect of the mixture of di-2-methylpyridine-N-oxide with CoI_2, the shape of most cells was deformed (oval, round, "sickle-shaped", "cone-shaped"). There were cells with signs of atrophy, in some cells there was a protrusion of the cytoplasm and the integrity of the cytoplasmic membrane was damaged.

Due to exposure to the complex of 2-methylpyridine-N-oxide with $MnCl_2$ infusoria body shape was mostly elongated, there were cells of "sickle-like" and rounded shapes. Cells with cytoplasmic release into the nutrient medium and with cytoplasmic membrane damage were observed.

The effect on infusoria of the complexes of di-2,6-dimethylpyridine-N-oxide with $ZnCl_2$, as well as of di-2-methylpyridine-N-oxide with CoI_2, was characterized by a significant change in the body shape of most cells. The body shape of the infusoria was deformed (flattened, elongated, cylindrical), the cytoplasmic membrane looked tortuous, and some cells had signs of dehydration. The damage to the integrity of the cytoplasmic membrane of cells, cytoplasmic emissions and cell contents emissions into the nutrient medium were recorded. The contractile vacuole was without any inclusions.

Due to the effect of the complex of di-2,6-dimethylpyridine-N-oxide with ZnI_2, infusoria also were of a different shape (pear-shaped, elongated, rounded, horseshoe-shaped). The contractile vacuole was without any inclusions. The vesiculation of cytoplasm, cytoplasm emission in nutrient medium and damage to the integrity of the cytoplasmic membrane of cells were detected.

Complex of di-2,6-dimethylpyridine-N-oxide with $CoCl_2$ in most cells caused form deformation, damage to the integrity of the cytoplasmic membrane, vesiculation of cytoplasm and its release into the nutrient medium; damaged infusoria and the remains of the structural components of cells were observed.

As can be seen in Fig. 4, there was likely reduction in the the speed of movement of infusoria under the impact of all complexes with methyl derivatives of pyridine-N-oxide

with metal salts in all concentrations studied, except for the complex of 2-methylpyridine-N-oxide with ZnI_2. The severity of the effect depended on the active concentration of the test substance.

At the lowest concentrations corresponding to LC_0, the least expressed changes in the speed of movement of infusoria were identified under the impact of complexes of di-2,6-dimethylpyridine-N-oxide with ZnI_2, 2-me-

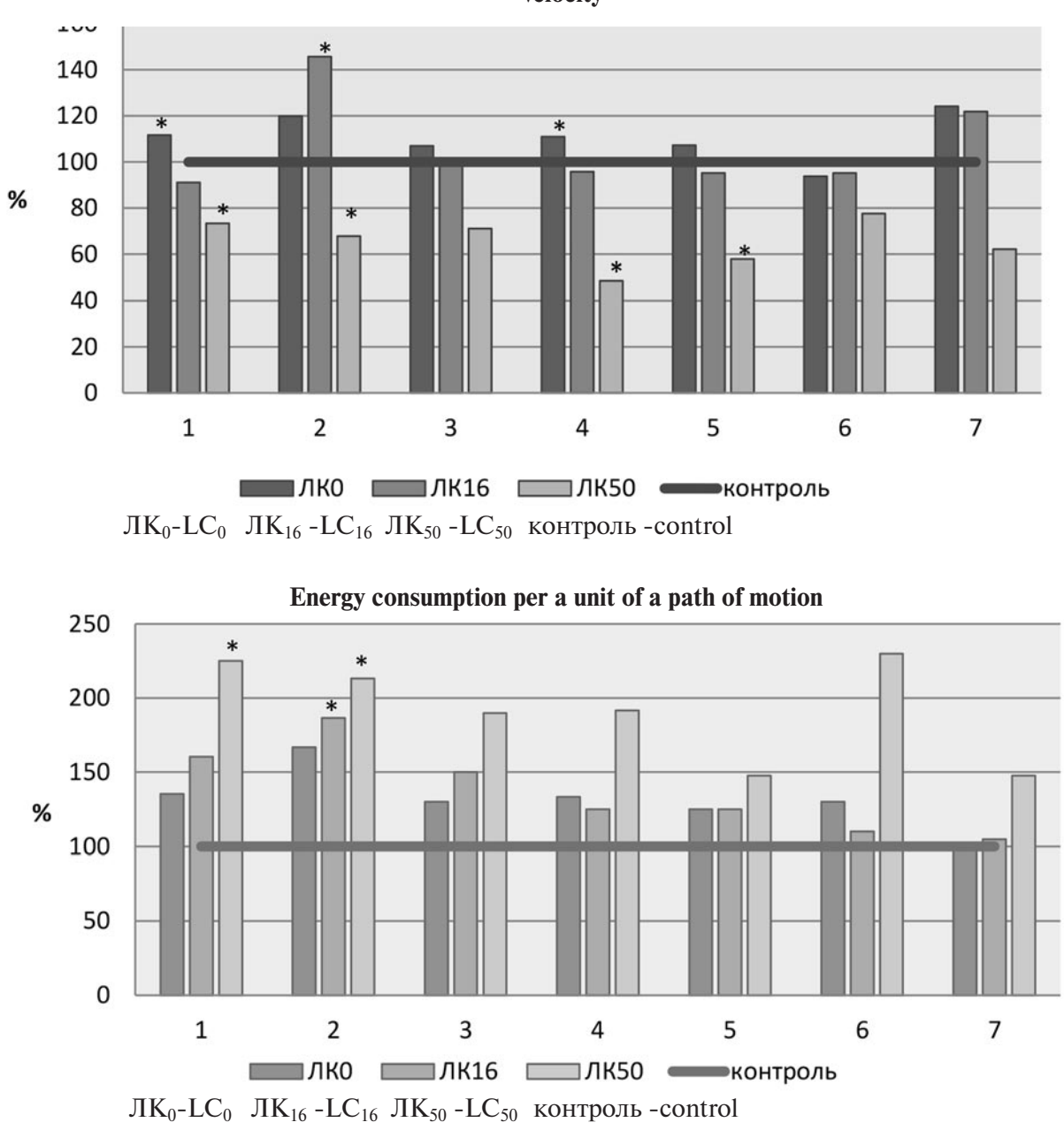
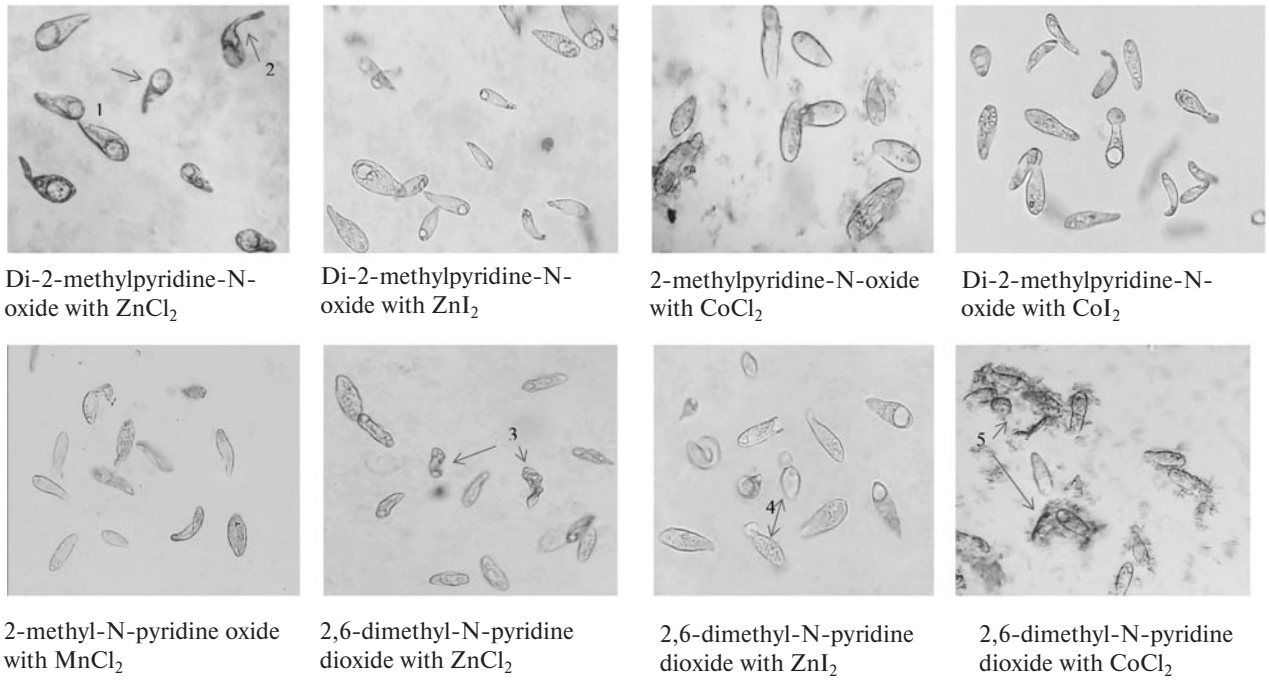


Fig. 2. Velocity and energy consumption per a unit of a path of motion of Tetrahymena pyriformis W infusoria under the influence of methyl derivatives of pyridine-N-oxide and their complexes with organic acids (* – $P \leq 0.005$):
1 – 2-methylpyridine-N-oxide,
2 – 2-methylpyridine-N-oxide with succinic acid,
3 – di-2-methylpyridine-N-oxide with succinic acid,
4 – 2.6-dimethylpyridine-N-oxide,
5 – 2.6-dimethylpyridine-N-oxide with succinic acid,
6 –di-2.6-dimethylpyridine-N-oxide with succinic acid,
7 – 2.6-dimethylpyridine-N-oxide with maleic acid.



Di-2-methylpyridine-N-oxide with $ZnCl_2$ | Di-2-methylpyridine-N-oxide with ZnI_2 | 2-methylpyridine-N-oxide with $CoCl_2$ | Di-2-methylpyridine-N-oxide with CoI_2

2-methyl-N-pyridine oxide with $MnCl_2$ | 2,6-dimethyl-N-pyridine dioxide with $ZnCl_2$ | 2,6-dimethyl-N-pyridine dioxide with ZnI_2 | 2,6-dimethyl-N-pyridine dioxide with $CoCl_2$

Fig. 3. Morphostructural changes in Tetrahymena pyriformis W infusoria under the influence of methyl derivatives of pyridine-N-oxide and their complexes with salts of metals at concentrations corresponding to LC_{50}.(1 – increased vacuole, 2 – elongated body in the shape f proboscis, 3 – damaged membrane (dehydrated cell), 4 – cytoplasm and vacuole emission, 5 – destroyed cells)

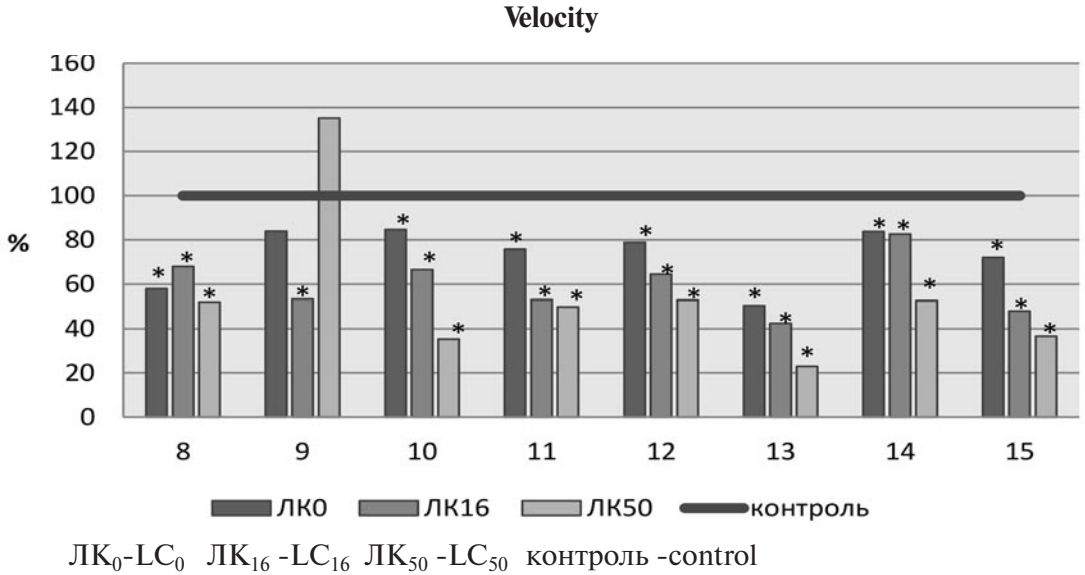
thylpyridine-N-oxide with $MnCl_2$, 2-methylpyridine-N-oxide of $CoCl_2$, di-2-methylpyridine-N-oxide with ZnI_2 and CoI_2, and the most expressed under the influence of complexes of di-2,6-dimethylpyridine-N-oxide with $ZnCl_2$ and of di-2-methylpyridine-N-oxide with $ZnCl_2$.

At concentrations corresponding to LC_{16}, the least pronounced decrease in the velocity of infusoria was due to the effect of complexes of di-2,6-dimethylpyridine-N-oxide with ZnI_2, di-2-methylpyridine-N-oxide with $ZnCl_2$ and 2-methylpyridine-N-oxide with $CoCl_2$, and the most pronounced decrease in the speed of movement was under the influence of complexes of di-2,6-dimethylpyridine-N-oxide with $ZnCl_2$, di-2,6-dimethylpyridine-N-oxide with $CoCl_2$.

At these same concentrations increase in movement energy consumption was not expressed, major changes were observed under the impact of complexes of 2-methylpyridine-N-oxide with $MnCl_2$, di-2,6-dimethylpyridine-N-oxide with ZnI_2. Under the influence of di-2-methylpyridine-N-oxide complex with ZnI_2, unlike other complexes, decrease in movement energy consumption was observed.

Under the influence of average lethal concentrations of the studied complexes of methyl derivatives of pyridine-N-oxide with metal salts, the decrease in the speed of infusoria ranged from 47,1% to 77,0% and was most pronounced in di-2,6-dimethylpyridine-N-oxide with $ZnCl_2$, 2-methylpyridine-N-oxide with $CoCl_2$ and di-2,6-dimethylpyridine-N-oxide with $CoCl_2$. The increase in energy consumption per a unit of a path of motion ranged from 40,0% to 90,2% and was most pronounced under the influence of the complexes of di-2-methylpyridine-N-oxide with $ZnCl_2$, di-2,6-dimethylpyridine-N-oxide with $ZnCl_2$, di-2,6-dimethylpyridine-N-oxide with ZnI_2, 2-methylpyridine-N-oxide with $CoCl_2$ and 2-methylpyridine-N-oxide with $MnCl_2$, di-2,6-dimethylpyridine-N-oxide with $CoCl_2$. For the complexes of di-2-methylpyridine-N-oxide with ZnI_2 and with CoI_2 a decrease in energy consumption per a unit of a path of motion was observed.

As it was discovered in previous studies [20] and is shown in Fig. 2 and Fig. 4, 2-methylpyridine-N-oxide, 2,6-dimethylpyridine-N-oxide and their complexes with organic acids and metal salts are characterized by an increase or decrease in the frequency and speed of movement of infusoria, which reflects the response of cells to a chemical factor. The frequency and speed of infusoria under the



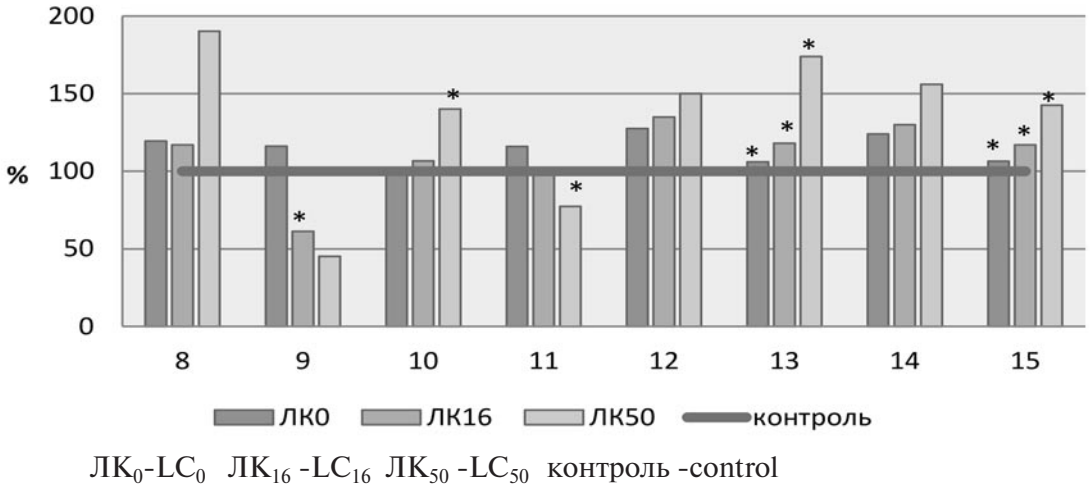


Fig. 2. Velocity and energy consumption per a unit of a path of motion of Tetrahymena pyriformis W infusoria under the influence of methyl derivatives of pyridine-N-oxide and their complexes with metal salts (* – $P \leq 0.005$):
8 – di-2-methylpyridine-N-oxide with $ZnCl_2$,
9 – di-2-methylpyridine-N-oxide with ZnI_2,
10 – 2-methylpyridine-N-oxide with $CoCl_2$,
11 – di-2-methylpyridine-N-oxide with CoI_2,
12 – 2-methylpyridine-N-oxide with $MnCl_2$,
13 – di-2.6-dimethylpyridine-N-oxide with $ZnCl_2$,
14 – di-2.6-dimethylpyridine-N-oxide with ZnI_2,
15 – di-2.6-dimethylpyridine-N-oxide with $CoCl_2$

impact of the researched methyl derivatives of pyridine-N-oxide and their complexes with organic acids reduced only at concentrations corresponding to LC_{50}, under the influence of complexes of methyl derivatives of pyridine-N-oxide with metal salts – in all studied concentrations with a maximum effect at average lethal concentrations. Differences in energy consumption per a unit of a path of motion are also discovered. Under the influence of 2-

methylpyridine-N-oxide, 2,6-dimethylpyridine-N-oxide and their complexes with organic acids an increase in energy consumption for infusoria movement with the greatest effect in average lethal concentrations. Complexes of methyl derivatives of pyridine-N-oxide with metal salts did not increase this indicator as significantly as complexes with organic acids, and under the exposure to complexes of di-2-methylpyridine-N-oxide with ZnI_2 (at 2 higher concentrations) and with CoI_2 (at the average lethal concentration) there was a decrease in movement energy consumption.

Compared to the complexes of methyl derivatives of pyridine-N-oxide with organic acids, complexes with metal salts demonstrate a significant reduction in the speed of movement of infusoria and energy consumption for movement which indicates a more toxic effect of the latter.

Impact on morphostructures of infusoria of methyl derivatives of pyridine-N-oxide and their complexes with organic acids and salts of metals is characterized by a change in shape and impaired osmoregulation of cells, increase of contractile vacuole, vesiculation, damage to the integrity of the cytoplasmic membrane, cytoplasm and structural elements of cells emissions into a nutrient medium. However, under the influence of complexes of methyl derivatives of pyridine-N-oxide with salts of metals a change of body shape of infusoria and destructive changes occur more often than in complexes of methyl derivatives of pyridine-N-oxide with organic acids. It should be mentioned that "sickle-like" body shape of infusoria and rapid clockwise movement of these cells was unique to 2-methylpyridine-N-oxide and its complexes with organic acids and salts of metals. For most PGRs studied at average lethal concentrations an increase in the degree of functional changes infusoria was consistent with the expressed structural changes.

Conclusions. 1. Morphostructural changes in infusoria under the influence of the researched PGRs are characterized by the change in shape, increase of the contractile vacuole, vesiculation, and damage to the integrity of the cytoplasmic membrane, cytoplasm and structural elements of cells emissions into a nutrient medium.

2. Complexes of methyl derivatives of pyridine-N-oxide with salts of metals in the studied concentrations reduce the speed of movement and increase movement energy expenditure, cause changes in behavioural responses and cell structure to a greater extent than complexes of 2-methylpyridine-N-oxide, 2,6-dimethylpyridine-N-oxide with organic acids.

3. The most pronounced functional and morphological changes in infusoria under the influence of the studied PGRs occurred at concentrations corresponding to LC_{50}. Changes in the functional activity of infusoria were observed at lower concentrations.

4. Comparison of the obtained functional and morphostructural indicators of infusoria shows that complexes of methyl derivatives of pyridine-N-oxide with metal salts have greater toxic effects on infusoria than the complexes of methyl derivatives of pyridine-N-oxide with organic acids.

5. Decrease in motor activity and increase in energy consumption per a unit of a path of motion together with morphological changes of cell structure are the criteria of xenobiotic toxicity for infusoria and assessment of their viability.

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МОРФО-ФУНКЦІОНАЛЬНІ ЗМІНИ КЛІТИН ІНФУЗОРІЙ TETRAHYMENA PYRIFORMIS W ЗА ВПЛИВУ РЕГУЛЯТОРІВ РОСТУ РОСЛИН – ПОХІДНИХ N-ОКСИД ПІРИДИНУ

О.П. Васецька
ДП «Науковий центр превентивної токсикології, харчової та хімічної безпеки імені академіка Л.І. Медведя МОЗ України», м. Київ, Україна

РЕЗЮМЕ. Мета. *Визначити морфологічні зміни інфузорій Tetrahymena pyriformis W за гострого впливу деяких регуляторів росту рослин (РРР) – похідних N-оксид піридину та зіставити їх з функціональними порушеннями клітин.*
Матеріали та методи. *У роботі використані N-оксид-2-метилпіридин, N-оксид-2,6-диметилпіридин та їх комплекси з органічними кислотами (бурштиновою, малеїновою) або солями металів ($ZnCl_2$, ZnI_2, $CoCl_2$, $MnCl_2$) (всього 15 речовин), синтезованих в Інституті біоорганічної хімії та нафтохімії НАН України. Дослідження проведені на інфузоріях Tetrahymena pyriformis W в ізотоксичних дозах – на рівні токсичних концентрацій – $ЛК_{50}$, $ЛК_{16}$ і недіючих концентрацій ($ЛК_0$). Морфологічні зміни клітин інфузорій оцінювали візуально за допомогою світлового мікроскопу. Порушення структури інфузорій порівнювали з функціональними змінами клітин (руховою активністю та енергетичним станом), отриманих у тому ж експерименті.*
Результати та висновки. *Показано, що N-оксид-2-метилпіридин, N-оксид-2,6-диметилпіридин та їх комплекси з органічними кислотами (бурштиновою, малеїновою) або солями металів ($ZnCl_2$, ZnI_2, $CoCl_2$, $MnCl_2$) викликають функціональні та морфоструктурні зміни інфузорій, ступінь яких залежить від діючої концентрації. Порушення морфоструктури інфузорій за впливу досліджених РРР характеризуються зміною форми, збільшенням скорочувальної вакуолі, везикуляцією, пошкодженням цілісності цитоплазматичної оболонки, викидами цитоплазми і структурних елементів клітин у живильне середовище.*
Комплекси метильних похідних N-оксид піридину з солями металів у досліджених концентраціях знижують швидкість руху і підвищують витрати енергії на рух, викликають зміни поведінкових реакцій і структури клітин більш, ніж N-оксид-2-метилпіридин, N-оксид-2,6-диметилпіридин та їх комплекси з органічними кислотами. Найвираженіші як функціональні, так і морфологічні зміни інфузорій за впливу досліджених РРР відбуваються в концентраціях відповідних $ЛК_{50}$. У менших концентраціях спостерігались зміни функціональної активності інфузорій. Співставлення отриманих функціональних і морфоструктурних показників стану інфузорій свідчить, що комплекси метильних похідних N-оксид піридину з солями металів чинять токсичну дію на інфузорії більше, ніж комплекси метильних похідних N-оксид піридину з органічними кислотами. Зниження рухової активності та збільшення енерговитрат на одиницю шляху руху, разом з морфологічними змінами структури клітин, є одним із критеріїв токсичності ксенобіотиків для інфузорій та оцінки їхньої життєздатності.
Ключові слова: *метильні похідні N-оксид піридину, Tetrahymena pyriformis W, морфо-функціональні зміни.*

МОРФО-ФУНКЦИОНАЛЬНЫЕ ИЗМЕНЕНИЯ КЛЕТОК ИНФУЗОРИЙ TETRAHYMENA PYRIFORMIS W ПРИ ВЛИЯНИИ РЕГУЛЯТОРОВ РОСТА РАСТЕНИЙ – ПРОИЗВОДНЫХ N-ОКСИД ПИРИДИНА

О.П. Васецкая
ГП «Научный центр превентивной токсикологии, пищевой и химической безопасности имени академика Л.И. Медведя МЗ Украины», г. Киев, Украина

РЕЗЮМЕ. Цель исследований. *Определить морфологические изменения инфузорий Tetrahymena pyriformis W при остром влиянии некоторых регуляторов роста растений – производных N-оксид пиридина и сопоставить их с функциональными нарушениями клеток.*
Материалы и методы. *В работе использованы N-оксид-2-метилпиридин, N-оксид-2,6-диметилпиридин и их комплексы с органическими кислотами (янтарной, малеиновой) или солями металлов ($ZnCl_2$, ZnI_2, $CoCl_2$, $MnCl_2$) (всего 15 веществ), синтезированных в Институте биоорганической химии и нефтехимии НАН Украины. Исследования проведены на инфузориях Tetrahymena pyriformis W в изотоксических дозах – на уровне токсичных ($ЛК_{50}$, $ЛК_{16}$) и недействующих концентраций ($ЛК_0$). Морфологические изменения клеток инфузорий оценивали визуально с помощью светового микроскопа. Нарушение структуры инфузорий сравнивали с функциональными изменениями клеток (двигательной активностью и энергетическим состоянием), полученных в том же эксперименте.*
Результаты и выводы. *Показано, что N-оксид-2-метилпиридин, N-оксид-2,6-диметилпиридин и их комплексы с органическими кислотами (янтарной, малеиновой) или солями металлов ($ZnCl_2$, ZnI_2, $CoCl_2$, $MnCl_2$) вызывают функциональные и морфоструктурные изменения инфузорий, степень которых зависит от действующей концентрации. Нарушение морфологической структуры инфузорий при влиянии исследованных РРР характеризуется изменением формы, увеличением сократительной вакуоли, везикуляцией, повреждением целостности цитоплазматической оболочки, выбросами цитоплазмы и структурных элементов клеток в питательную среду.*
Комплексы метильных производных N-оксид пиридина с солями металлов в исследованных концентрациях снижают скорость движения и повышают расход энергии на движение, вызывают изменения поведенческих реакций и структуры клеток в большей степени, чем N-оксид-2-метилпиридин, N-оксид-2,6-диметилпиридин и их комплексы с органическими кислотами. Наиболее выраженные как функциональные, так и морфологические изменения инфузорий при влиянии исследованных РРР происходят в концентрациях соответствующих $ЛК_{50}$. В меньших концентрациях наблюдались изменения функциональной активности инфузорий. Сопоставление полученных функциональных и морфоструктурных показателей состояния инфузорий свидетельствует, что комплексы метильных производных N-оксид пиридина с солями металлов оказывают токсическое действие на инфузории в большей степени, чем комплексы метильных производных N-оксид пиридина с органическими кислотами. Снижение двигательной активности и увеличение энергозатрат на единицу пути движения вместе с морфологическими изменениями структуры клеток является одним из критериев токсичности ксенобиотиков для инфузорий и оценки их жизнеспособности.
Ключевые слова: *метильные производные N-оксид пиридина, Tetrahymena pyriformis W, морфо-функциональные изменения.*

Received 10/26/2020