THE TOXIC EFFECT OF ORGANIC COORDINATION COMPOUNDS *IN VIVO* STUDIES

ROŞCOV Elena, TODERAŞ Ion, GULEA Aurelian, GLIGA Olesea

Abstract. The influence of the coordination compounds TIA-84 and TIA-86 on the viability of *Paramecium caudatum* ciliates was studied. The LC_{50} toxic effect of the organic coordination compounds TIA-84 and TIA-86 in *in vivo* studies was found to be 3.8 ± 0.1 µM (TIA-84) and 1.2 ± 0.02 µM (TIA-86) time for 24 h and of 3.2 ± 0.5 µM (TIA-84) and 1.0 ± 0.03 µM (TIA-86) for 48 h. The studied compound TIA-84 has shown lower toxicity comparatively to analogue TIA-86 compound.

Keywords: biotesting, coordinating organic compounds, cyst formation, toxicity, viability, *Paramecium caudatum*.

Rezumat. Efectul toxic al compușilor organici coordinativi în studiile *in vivo.* A fost studiată influența compușilor coordinativi TIA-84 și TIA-86 asupra viabilității ciliatelor *Paramecium caudatum*. Efectul toxic $LC_{50,}$ al compușilor organici coordinativi TIA-84 și TIA-86 în studiile *in vivo*, s-au găsit valori de 3,8 ± 0,1 µM (TIA-84) și 1,2 ± 0,02 µM (TIA-86) timp de 24 de ore și de 3,2 ± 0,5 µM (TIA-84) și 1,0 ± 0,03 µM (TIA-86) timp de 48 de ore. Compusul studiat TIA-84 a arătat o toxicitate mai mică comparativ cu compusul analog TIA-86.

Cuvinte cheie: biotestare, compuși organici coordinativi, închistare, toxicitate, viabilitate, *Paramecium caudatum*.

INTRODUCTION

Coordination chemistry is one of the branches of modern chemistry, which can provide numerous organic coordination compounds with predetermined properties. The synthesis and study of organic coordination compounds with applications in the field of biology appear more and more in numerous publications in specialized literature. Research on this type of compounds is important especially due to their applicability in pharmacy, medicine, agronomy, and nutrition. A series of coordinated organic compounds synthesized, before being used in other fields, must be tested for their toxicity (GUDUMAC et al., 2011, 2012; PANTEA et. al., 2022; TAGADIUC et. al., 2010).

Toxicity represents the degree to which a chemical substance or a particular mixture of substances can damage an organism, and is a characteristic resulting from the biological manifestation of the respective organism (VENKATESWARA et al., 2007).

Since animal cruelty-free, including for those used in the study, is the fundamental principle of all toxicity research, it is recommended to perform such investigations *in vivo* on invertebrate organisms using the bioassay method (SHCHETKINA, 2007). Bioanalysis or biological standardization is a type of scientific experiment. A bioassay involves using an animal or living tissue to determine the biological activity of a tested substance, such as a medical drug. Bioassays are usually performed to measure the effects of a substance on the living organism and are essential for the development of new drugs and in the monitoring of environmental pollutants. A bioassay can also be used to determine the concentration of a particular constituent of a mixture (VAN NOORDWIJK, 1989).

Biological responses to chemical stressors occur first at the molecular, biochemical and cellular levels and subsequently at higher levels. Therefore, at the lowest levels, stress indicators often allow earlier detection of harmful effects. There are numerous investigations on changes in population dynamics (e.g. growth inhibition) of various unicellular tested organisms caused by toxic substances or pollutants (TAHEDL & HADER, 1999). Since most of these tests require hours or days, pollutant responses at biochemical, physiological, or behavioural levels should be used to generate a rapid biomonitoring system intended as an online biological alarm system.

To look into the toxicity of coordinating organic compounds, investigations were performed *in vivo*, using the freshwater ciliate *Paramecium caudatum* Ehrenberg, 1833. As this species can be easily cultured and manipulated, it is an ideal candidate for use in a bioassay. These methods are highly sensitive, rapid, reliable, versatile and low-cost. They are easy to perform, amenable to instrumentation and automation, and their results are easy to interpret (PUZYREVA et al., 2016).

The most used indicator for assessing acute toxicity is the lethal concentration 50 (LC_{50}) (GARBUZ et al., 2022). Mortality is not the only endpoint to consider, but there is also a growing interest in developing behavioural parameters to assess the sub-lethal effects of the toxic compound. The latter are considered promising tools in toxicology (SHCHETKINA et al., 2007) and these studies are becoming prominent in toxicity assessments in unicellular organisms. In the toxicity test, as a rule, the death/survival of cells, the intensity of movement, the change in the external shape of the body, and the change in their reproduction intensity were recorded.

Therefore, the toxicity of the organic coordination compounds TIA-84 (Ligand-derived allyl-S-methyl-thiosemicarbazone of salicylic aldehyde with copper nitrate) and TIA-86 (Ligand-derived allyl-S-methyl-thiosemicarbazone of salicylic aldehyde with copper chloride) on *Paramecium caudatum* Ehrenberg 1833, was evaluated under *in vivo* conditions for 24 and 48 h.

MATERIAL AND METHODS

Paramecium caudatum Ehrenberg 1833 culture procedure.

In this study, the single cell organisms *Paramecium caudatum* were used as test subjects to detect the toxicity of organic coordination compounds. The infusoria culture was kept in 100 ml flasks, and periodically fed with dry yeasts (1g/1l). During the acclimatization, the paramecium culture was kept for 2-3 days in the thermostat at the constant temperature of $25^0 \pm 1^0$ C, during which their multiplication occurred. After metabolic debris were removed by filtration, = cells were counted and brought up to 2.0 - 3.0 x 10^3 cells/ml with dechlorinated tap water. For the bioassay analysis, the culture from the exponential development phase was used, having the highest sensitivity to organic coordination compounds action (KARPUKHINA et al., 2015).

In the present investigation, paramecia were pipetted into Eppendorf tubes (1 ml). Dilutions of organic coordination compounds ligand-derived allyl-S-methyl-thiosemicarbazone of salicylic aldehyde with copper nitrate (TIA-84) and ligand-derived allyl-S-methyl-thiosemicarbazone of salicylic aldehyde with copper chloride (TIA-86) of 100, 10 and 1 μM were prepared. Triplicates of each assay were performed and mean values were used for statistics.

The reaction of a tested object, when exposed to the action of toxic substances or other unfavourable environmental factors can be expressed by the death of the test objects (survival), a decrease in the intensity of reproduction, a decrease in mobility, or other behavioural characteristics typical for the test -the given object. The toxicity expression is reproduced by means of the LC_{50} indicator which represents the lethal concentration (from the English "Lethal Concentration" LC_{50}) for 24 and 48 hours (GARBUZ et al., 2022).

The use of the binocular microscope at maximum magnification allowed us, arbitrarily, to establish the changes occurring at the cellular level of the paramecia that were in the environment with various concentrations of the tested organic coordination compounds. Observations were made in the solution droplets, collected from each Eppendorf, on concave glass slides.

Finally, the direct toxicity evaluation of TIA-84 and TIA-86 was studied using the NR- colorimetric assay of quantification of membrane permeability and lysosomal activity of *Paramecium caudatum*, which is one of the most widely used test objects in laboratory research that aims at the direct determination of the toxicity of chemical compounds used in toxicological medicine (TODERAS et al., 2018), and for the cultivation and maintenance of paramecium culture in laboratory conditions, classic hydrobiological methods were used: SUKHANOVA (1968), KOKOVA (1982).

RESULTS AND DISCUSSIONS

The study was carried out in the Systematics and Molecular Phylogeny Laboratory of the Institute of Zoology. The complex coordination compounds ligand-derived allyl-S-methyl-thiosemicarbazone of salicylic aldehyde with copper nitrate (TIA-84) and ligand-derived allyl-S-methyl-thiosemicarbazone of salicylic aldehyde with copper chloride (TIA-86) were provided by Mr. Academician, PhD, university professor, Gulea Aurelian (Faculty of Chemistry and Chemical Technology, Director of the Laboratory of Advanced Biopharmaceutical and Technical Materials of the State University of Moldova).

All the information obtained as a result of the research allows us to distinguish the key elements in the behaviuor and condition of the infusoria, by using toxic substances in the test.

Analysing the experimental results allowed us to observe the following changes:

1. The locomotor behaviour of the paramecium cyst formation.

The toxic effect of organic coordination compounds on the locomotor behaviour of paramecium was investigated for 10-30 minutes. The gradual increase in their mobility, initiated by the increase in speed in the form of spins was observed. The characteristic increase in speed compared to that of the control shows the negative response of the organism to the toxic compound. The gradual decrease in motility speed with increasing exposure time may be due to the effect of the tested chemicals on cellular metabolism. Eventually, the movement slows down completely, initiating the formation of cysts. Thus, cells treated with 100 μM of the complex coordination compounds TIA-84 and TIA-86 lead to cyst formation followed by their death (Table 1). After 10-30 min, the paramecia acquired a spherical shape and remained motionless from a few hours to a few days (Fig. 1^1).

Introducing the formed cysts into a fresh culture medium, free of toxic substances, they could not be activated, which allows us to conclude that, once the encystation occurs, organisms die.

2. Destruction and/or damage of biological membranes.

Cells treated with 10 μM of the TIA-86 preparation, rather quickly (from a few minutes to several tens of minutes) were subjected to penetration of the plasma membrane. The affected paramecia showed a change in their shape, by developing irregular damages of the cell membrane before complete cell lysis occurred. First of all, the destruction of the plasma membrane takes place, followed by the destruction of the micro- and macronucleus, the cytoplasm until the complete destruction of the ciliate cells (Fig. 1^2).

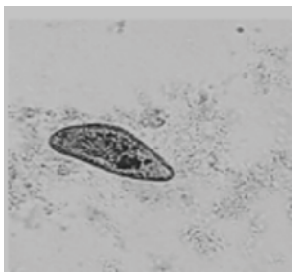
3. Dysregulation of the osmoregulation mechanism (swelling of cells).

Paramecia exposed to 100 μM of the complex coordination compounds TIA-84 and TIA-86 for 10 min were significantly affected leading to the disruption of the osmoregulation mechanism, expressed by the swelling of the

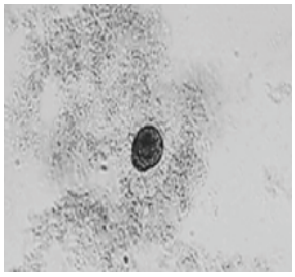
infusoria until obtaining a spherical shape. Digital microscope photographs of the disruption of the osmoregulation mechanism of a paramecium are shown in figure 1[3]. Paramecia in the control groups did not show any change in shape.

4. Increase in viscosity and differentiation of cytoplasm.

In the tested paramecia, approximately after 20 min, there is a pronounced bleaching of the ectoplasm with a strong separation of it from the endoplasm. In parallel, a difference is observed between the cytoplasmic structures and the nucleus. An increased volume of ectoplasm was observed, either at one location or at multiple locations, with an uneven accumulation of fluid leading to vesicle formation followed by membrane breakdown. However, the endoplasmic contents of the paramecium remained intact (nucleus and other cell organelles) until cell lysis. In case of irreversible disruption of the osmoregulation process of the ectoplasm, the cytoplasm, in the form of clots, comes to the surface of the cell, forming an air bubble (Fig. 1[4]).

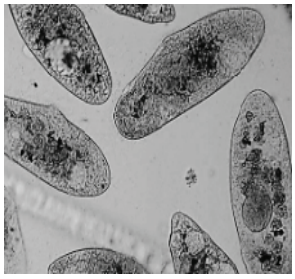


A

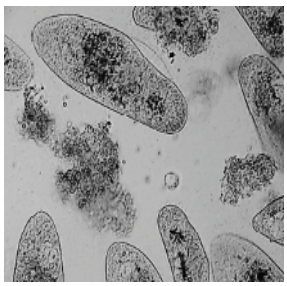


B

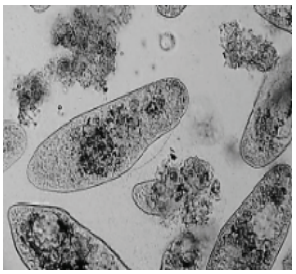
[1]The stages of the *P. caudatum* cell cyst formation under the action of TIA-84 and TIA-86 preparations at 100 μM concentration (A, B).



C

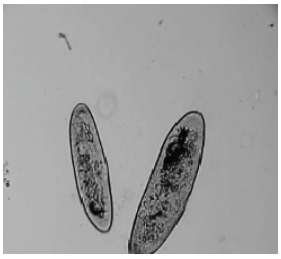


D

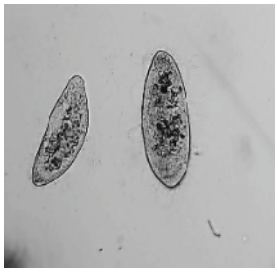


E

[2]The stages of damage of the plasma membrane in *P. caudatum* infusoria under the action of the preparation TIA-86 in a concentration of 10 μM (C, D, E).

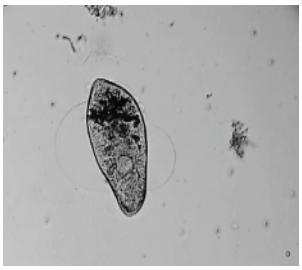


F

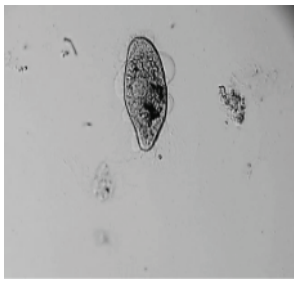


G

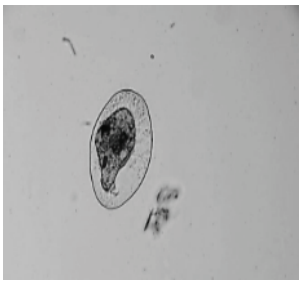
[3]Stages of swelling of the *P.c audatum* following osmoregulation failure under the action of TIA-84 and TIA-86 preparations at 100 μM concentration (F and G).



H



I



J

[4]The flow of the cytoplasm outside the ectoplasm (H, I and J).

Figure 1. The activity of the tested compounds TIA-84 and TIA-86 on the ciliate *Paramecium caudatum.* (personal photos).

As a continuation of these studies, the effect of lethal concentrations (100, 10 and 1 µM) of TIA-84 and TIA-86 upon the population growth rate of *P. caudatum* was assessed for 24 and 48 h. It is obvious that organic coordination compounds significantly affected the viability of paramecia in a concentration-dependent manner. The analysis of the obtained experimental data proved that paramecia exposed to 100 µM of the coordinating compound TIA-84 showed a significant inhibitory effect on viability. 100% of cells underwent cyst formation after 24 hours. The test concentration of 10 µM of the same mixture showed a significant decrease in cell viability, which is 23% after 24 hours and 38.5% after 48 hours.

The toxic concentration at 1 µM showed higher viability values, ranging between 84.5% after 24 hours and 65.4% after 48 hours, compared to the control group (Table 1).

Table 1. Evaluation of the activity of compounds TIA 84 and TIA 86 on the viability of *Paramecium caudatum* after exposure for 24 and 48 hours at the temperature of 25^0 C.

COD	C (µM /L)	Viability (%), 24 hours	SD (%)	LC_{50} (µM /L)	SD (µM /L)	Viability (%), 48 hours	SD (%)	LC_{50} (µM/L)	SD (µM /L)
TIA-84	100	0 (cysts)		**3,8**	0,1	0 (cysts)		**3,2**	0,5
	10	23,0	1,8			38,5	6,8		
	1	84,5	2,8			65,4	1,0		
TIA-86	100	0 (cysts)		**1,2**	0,02	0 (cysts)		**1,0**	0,03
	10	0 (cysts+damage)				0 (cysts+damage)			
	1	82,64	2,4			55,28	6,4		

The toxic effect LC_{50} upon paramecia viability was determined to be 3.8 ± 0.1 µM after 24 hours and 3.2 ± 0.5 µM after 48 hours. These values (viability and lethal toxic concentration, 50%) were decreasing from the first day of experimental sample processing, which demonstrated that TIA 84 is toxic to paramecium cells (Fig. 2).

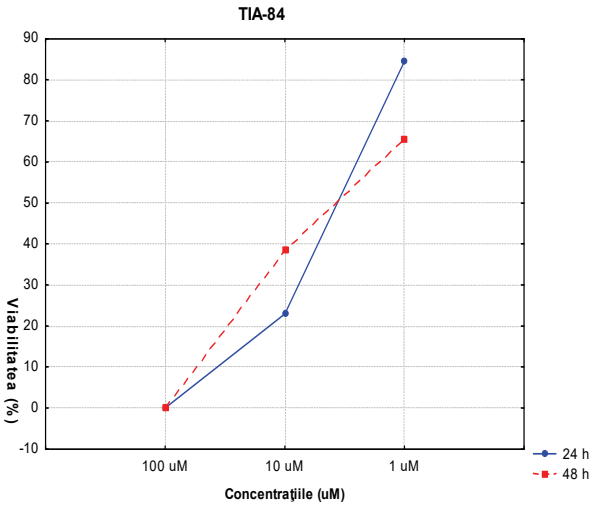


Figure 2. The graph expressing the dependence of the viability of paramecia exposed to the action of organic coordination compound TIA-84, of concentrations 100, 10 and 1 µM, after incubation for 24 and 48 hours.

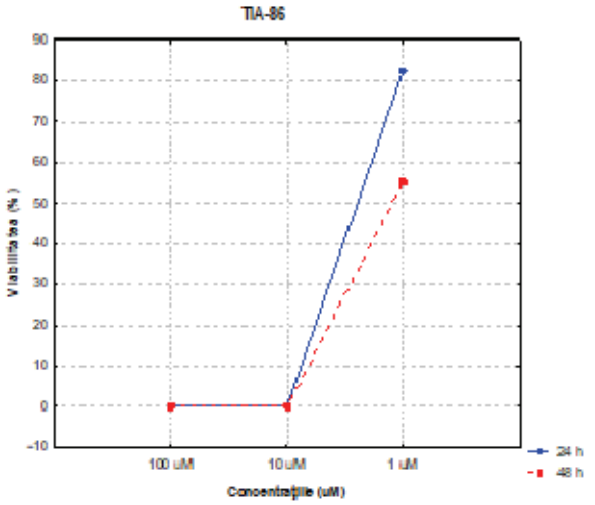


Figure 3. The graph expressing the dependence of the viability of paramecia exposed to the action of organic coordination compound TIA-86, of concentrations 100, 10 and 1 µM, after incubation for 24 and 48 hours.

With the action of the TIA-86 solution after 24 hours and after 48 hours, at the concentration of 100 µM, cyst formation was observed (Fig.1[1]). The activity of the 10 µM concentration attests to cell damage, initially the cytoplasmic membrane is destroyed, followed by the destruction of the entire cell (Fig. 1[2]), accompanied, in part, by the same cyst formation process.

Cell viability, at the concentration of 1 µM toxicant, after 24 hours is 82.64%, and after 48 hours it decreases to 55.28% compared to the control group (Table 1).

The LC_{50} value after 24 hours was determined to be 1.2 ± 0.02 µM, and after 48 hours to be 1.0 ± 0.03 µM. We conclude that the TIA 86 preparation is also toxic for infuser cells (Fig. 3).

Testing of the complex coordination compounds TIA-84 and TIA-86 demonstrated a high toxic activity on the test organism *Paramecium caudatum*. The TIA-86 compound showed more pronounced activity with lower values than the TIA-84 compound (Fig. 4).

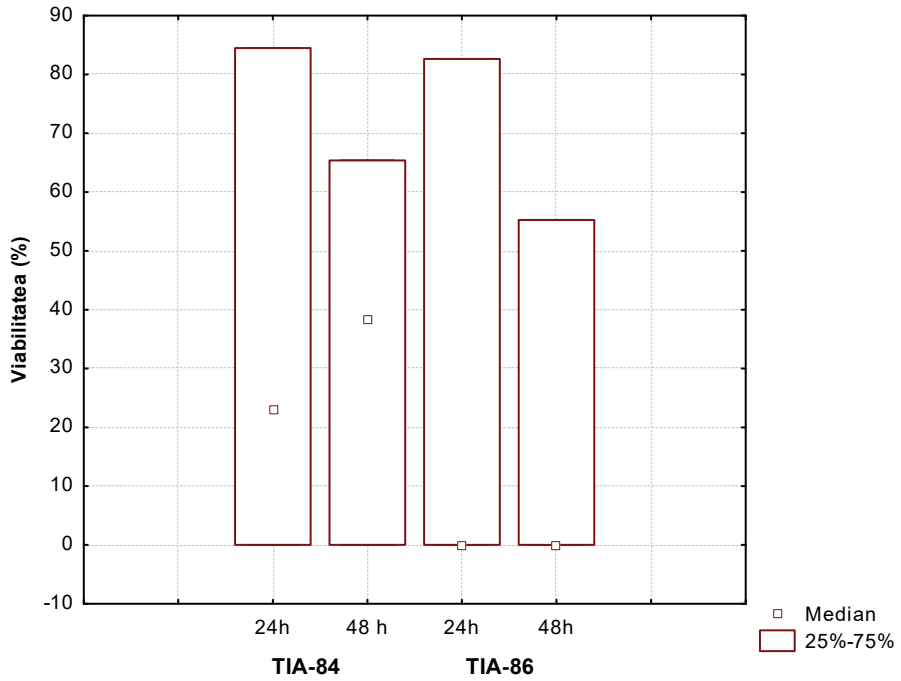


Figure 4. The graph expressing the dependence of the viability of paramecia exposed to the action of organic coordination compounds TIA-84 and TIA-86, on the incubation time of 24 and 48 hours.

The effect of organic coordination compounds with several biological properties can be studied on paramecia, which is a single-cell organism, while such extensive tests cannot be performed on human cell lines. Preliminary toxicological evaluations of any compound, behavioural changes, morphological, and short-term survival of paramecia would be more appropriate than when using multicellular organisms. These ciliates are preferred for their simplicity in experimental manipulation and also for cost-effectiveness.

We can conclude that the paramecium toxicity test could be considered a rapid and new method to evaluate organic coordination compounds effects based on LC_{50} values (24 and 48 hours).

CONCLUSIONS

1. The test objects with complex TIA-84 and TIA-86 (100 µM) showed the reduced activity, cellular volume initially decreased and the zygote nucleus was formed by fusion of the migratory and stationary nucleus as well as cyst formation.
2. The average concentration of 10 µM (for TIA-86) leads to the destruction of the cytoplasmic membrane and cytoplasm, causing complete cell lysis.
3. The low concentration of 1 µM shows higher viability indices after 24 h from 84.5% for TIA-84 and 82.64% for TIA-86, with a decrease after 48 h to 65.4% for TIA-84 and 55.28% for TIA-86.
4. Direct toxic evaluation of compounds, performing *Paramecium caudatum* colorimetric bioassay demonstrated that the LC_{50} after 24 h treatment for TIA-84 is 3.8 ± 0.1 µM and for TIA-86 is 1.2 ± 0.02 µM. The LC_{50} after 48 h treatment for TIA-84 is 3.2 ± 0.5 µM and for TIA-86 it is 1.0 ± 0.03 µM.
5. The toxicity analysis revealed that organic coordination compound ligand-derived allyl-S-methyl-thiosemicarbazone of salicylic aldehyde with copper nitrate (TIA-84) is the least toxic compared to organic coordination compound ligand-derived allyl-S-methyl-thiosemicarbazone of salicylic aldehyde with copper chloride (TIA-86).

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