# METHODS

# Evaluation of Growth Parameters in *Paramecium caudatum* Test Model for Standardization of Biological Screening

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We standardized *Paramecium caudatum* test model on the basis of identification of a quasichemical model representing the processes of population growth in the presence of nickel(II) sulfate, a toxic substance. The density of paramecium population depends on the time of exposure at different concentrations of nickel(II) sulfate. Ecotoxicological equation for population development was used to analyze the experimental data and to determine the standard kinetic parameters of population development in the presence of a toxic substance. An algorithm is developed for evaluation of quality of paramecium culture.

**Key Words:** *bioscreening; cell standard; cell cycle; test model; chemical toxicity*

*Paramecium caudatum* test model (Protozoa subkingdom) is widely used to study the biologic activity of various drugs [1]. *P. caudatum* has morphological signs of a cell and responds to environmental stimuli similarly to multicellular organisms. However, the kinetic parameters describing the dynamics of cell population of *P. caudatum* in the presence of drugs have not yet been determined. These data are necessary to standardize the test object, to predict the toxicometric parameters of chemical agents, and to screen them for biological activity.

Our aim was to standardize *P. caudatum* on the basis of a quasichemical model describing the growth of cell population in the presence of toxicants [3-5].

To standardize the model, we used nickel(II) sulfate ($NiSO_4$) as the toxicant.

For evaluation of the quality of natural, drinking, and waste waters, we used potassium dichromate containing Cr(VI) in the anionic form as the reference toxicant to control suitability of the biological object. This salt does not form coordinated compounds with natural ligands in the organism. Therefore, the mechanisms of action of Ni(II) forming complexes differs from that of Cr(VI). At the same time, it can be hypothesized that the mechanisms underlying biological activity of $Ni^{2+}$ and $Cu^{2+}$, $Zn^{2+}$, and $Mn^{2+}$ cations are analogous. These models were tested on different biologic objects [6].

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## MATERIALS AND METHODS

Paramecium culture was grown in Lozin—Lozinskii medium (pH 6.5-7.0) containing (in %): 0.01 NaCl, 0.001 KCl, 0.001 $CaCl_2$, 0.001 $MgCl_2$, 0.002 $NaHCO_3$ [5] and fed water extract of swollen oat seeds. Every day 10-15 drops of the extract were added to the test tube with paramecia. Every 10 days the culture was transferred into fresh medium and kept at 24-25°C. The glassware was sterilized by boiling [7].

$NiSO_4$ was analytical grade. Initial solution was prepared on Lozin—Lozinskii medium by serial dilution of $NiSO_4$ to final concentrations of $5\times10^{-6}$ and

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$2\times10^{-5}$ mol/liter. Equal volumes of the solutions (20 ml) were placed into sterile flasks.

Paramecia were washed in Lozin—Lozinskii medium and transferred to 50-ml flasks (5-8 cells per each) containing growth medium (control) or test solutions. Every days 3 aliquots (0.02 ml each) were taken and analyzed under an MBS-9 binocular microscope. Experiments were carried for 40 days under sterile conditions. The food was given in equal amounts.

To evaluate the growth parameters of *P. caudatum* culture, the test system was simulated in the two-stage quasichemical model [3-5], which describes the growth of paramecium population in the presence of $NiSO_4$ by the following equations:

$$\begin{gathered} C_1+M_1\rightarrow Cm\ (p) \\ C_m+M_2\rightarrow fC_1\ (b) \\ C_1\rightarrow C_d\ (g) \\ C1+Cm\rightarrow Ca+C_1 \\ C_1+X_1\rightarrow CX_{11}\ (d_{11}) \\ C_m+X_1\rightarrow CX_{21}\ (d_{21}), \end{gathered} \qquad (1)$$

where $C_1$ and $C_m$ are young cell immediately after division and mitotic cells, respectively; $M_1$ and $M_2$ are substrates in the oat seed extract for young and mitotic cell, respectively; $X_1$ is $NiSO_4$ (toxicant); $CX_{11}$ and $CX_{21}$ are poisoned young and mitotic cells, respectively; $C_d$ is a dead cell; $C_a$ is an autoinhibited cell. The kinetic coefficients are: $f$ (reproduction), $p$ (growth of the population chain), $b$ (birth or branching), $a$ (autoinhibition), $d_{11}$ and $d_{21}$ (toxic effect), $g$ (death).

In accordance with the quasichemical model (1), growth and inhibition of isolated *P. caudatum* population under the effect of substrates $M_1$ and $M_2$, as well as toxicant $NiSO_4$ at constant concentrations can be described by differential kinetic equation [3-5]:

$$dc_1/dt=p_xc_1(K_1-c_1)/(K_2+c_1), \qquad (2)$$

where:

$$p_x=p+d_{11}x_1;\ b_x=b+d_{21}x_1;\ b_x=b+d_{12}x_1.$$
$$K_1=c_1(fbp-p_xb_x)/ap_x;\ K_2=b_x/a$$
$$\text{or } K_2=C_f/[(1+K_1/K_2)^{1/2}-1].$$

Here $C_f$ is inflexion point, where the growth rate is maximal.

Equation (2) was used to find the kinetic coefficients of the growth of paramecium population.

Coefficient $p$ (growth of the population chain) was calculated from the initial logarithmic phase of the growth plot of paramecium population in the control. The experimental values of population chain growth in the medium with toxicant $p_1$, and $p_2$, were found in the same way.

Reproduction coefficient $f$ was assumed to be 2, because paramecia can proliferate by asexual way [2].

The birth (branching) kinetic coefficient $b$ was determined from the ratio $b=1/t_{div}$, where $t_{div}$ is the duration of cell division. Paramecium divide 1-2 times per day [2], so $b=0.083$ day$^{-1}$.

The processes of autoinhibition are manifested in deceleration of the population growth with increasing population density. Therefore, it is necessary to evaluate the autoinhibition coefficient $a$, which was determined in accordance with limiting value of population density in the control $K=b/a$ [3-5].

Under our experimental conditions at the initial phase of population growth $c_1<<K_1$, and $c_1<<K_2$. Thus, taking into consideration equation (1), one can assume

$$dc_1/dt=p_x(K_1/K_2)c_1,$$

which yields the population growth coefficients in the presence of inhibitor:

$$p_1'=p_{x1}(K_1/K_2);\ p_2'=p_{x2}(K_1'/K_2'). \qquad (3)$$

Equations (3) are equivalent to the following system:

$$\begin{aligned} p_1'&=(fbp-(p+d_{11}x_1)(b+d_{21}x_1))/(b+d_{21}x_1) \\ p_2'&=(fbp-(p+d_{12}x_2)(b+d_{22}x_2))/(b+d_{22}x_2). \end{aligned} \qquad (4)$$

In the presence of a toxicant, the population growth coefficients ($p_x$) and the birth coefficients ($b_x$) are calculated in accordance their definition (2):

$$p_x=p+d_{11}x_1;\ b_x=b+d_{21}x.$$

The obtained kinetic coefficients are used to plot the theoretical curve of paramecium population growth in the control and in the presence of a toxicant using ecotoxicologic equation [5]:

$$t(c_1)=\ln[(c_1/c_0)\{(K_1-c_0)/(K_1-c_1)\}(1+n)]/np_x, \qquad (5)$$

where $n=K_1/K_2$, $c_0$ is the initial number of cells.

In toxin-free medium, the growth of paramecium population can be described by function $c_1(t)$:

$$c_1(t)=K[1+H(t)-\{H(t)(2+H(t)\}]^{0.5}, \qquad (6)$$

where $H(t)=0.5(K-c_0)^2\exp(-pt)/Kc_0$.

The equations (5) and (6) are partial solutions to (1).

To analyze the experimental data we used GNUPLOT and EUREKA software. Standard deviation of experimental and theoretical values was 5-11 cell/cm$^3$.

## RESULTS

Addition of $NiSO_4$ to the growth medium inhibited the growth of *P. caudatum* (Fig. 1). At the initial growth phase, the corresponding regression curves can be described by the following equations: $\ln c_1=0.298t+2.08$ [at $x_1(NiSO_4)=0$]; $\ln c_1=0.25t+2.08$ [at $x_1(NiSO_4)=5\times10^{-6}$ mol/liter]; $\ln c_1=0.22t+1.25$ [at $x_1(NiSO_4)=2\times10^{-5}$

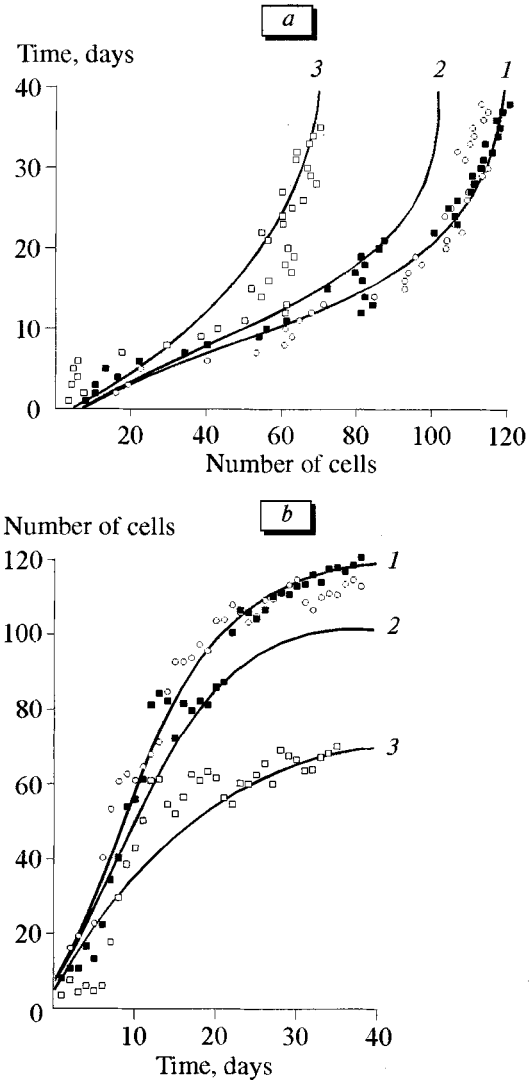


**Fig. 1.** Experimental points and theoretical plots describing growth of *Paramecium caudatum* at various concentrations of $NiSO_4$: *1*) 0; *2*) $5\times10^{-6}$ mol/liter; *3*) $2\times10^{-5}$ mol/liter. The curves were drawn according to equation (5) and (6) in *a*) and *b*), respectively.

mol/liter]. The angular coefficients are $p$=0.298; $p_{11}$= 0.25; $p_{21}$=0.22, respectively (Fig. 2). At the limiting value of the intact culture density $K$=114.7, the autoinhibition coefficient $a$ is 0.0007 ml/h. Solution of system (4) yields the toxicity (inhibition) coefficients: $d_{11}$=1734 and $d_{21}$=40.

Then the coefficients of population birth and growth in the presence of a toxicant can be found: $b_{x1}$=0.0832 $day^{-1}$; $b_{x2}$=0.0837 $day^{-1}$; $p_{x1}$=0.311; and $p_{x2}$=0.334. The coefficients defined in this way are standard for paramecium population under these conditions. At various concentrations of the toxicant, the theoretical and experimental points of functions (5) and (6) coincided within the limits of experimental errors (Fig. 1). The following procedure may be proposed on the basis of the described method of calculation of the

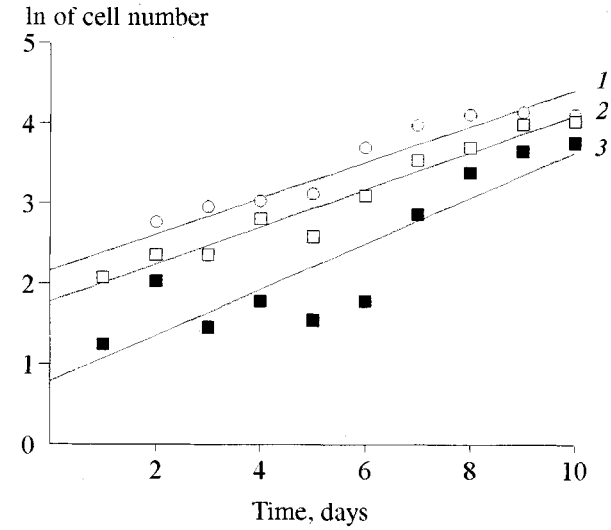


**Fig. 2.** Growth kinetics of *Paramecium caudatum* in the half-logarithmic coordinates at various concentrations of $NiSO_4$: *1*) 0; *2*) $5\times10^{-6}$ mol/liter; *3*) $2\times10^{-5}$ mol/liter.

standard parameters of cell culture growth (*P. caudatum*) in the medium with a toxicant:

1. One cell strain grown in a standard medium under constant temperature and illumination is used for standardization.
2. Experimental curves of the population growth in the presence and absence of a toxicant are obtained (the curve is plotted by experimental points using the method of least squares).
3. The experimental value of population growth coefficients $p$, $p_1$', and $p_2$' are determined in the half-logarithmic scale.
4. The coefficients of autoinhibition ($a$), reproduction ($f$), and birth ($b$) are calculated.
5. The toxicity coefficients $d_{11}$ and $d_{21}$ are found from equation (4), then population growth ($p_x$) and birth ($b_x$) coefficients for the culture in a medium with toxicant are calculated.
6. The theoretical curves of population growth are plotted according to the ecotoxicological equation (5) and experimentally determined kinetic coefficients.

The experimental data were analyzed on the basis of ecotoxicological equation. The standard kinetic parameters of paramecium growth in the presence of $NiSO_4$ were found. The algorithm is elaborated, which can evaluate the quality of paramecium culture in the toxicometrical experiments.

The ecotoxicological equation can determine the kinetic parameters not only for the unicellular organisms, but also for plants and multicellular animals.

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