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Grzegorz Oloś[1)], *Olga Zhuk*[1)], *Volodymyr G. Zinkovsky*[1)],
Inna I. Seifullina[2)], *Elena E. Martsinko*[2)], *A.G. Pesaroglo*[2)]

ACUTE TOXICITY OF NEW GERMANIUM ORGANIC DERIVATIVES

[1)] Department of Biotechnology and Molecular Biology, University of Opole, Opole, Poland
[2)] Department of Chemistry, Odessa National University, Odessa, Ukraine

The aim of the work was to examine toxicity of eight new germanium organic derivatives on two hydrobiont species: Daphnia magna and Danio rerio. Mean Live Time (MLT) and LC_{50} indexes were estimated. Toxic effect trends were demonstrated. Ranges of toxicant concentrations causing 100% and 0% lethal effect were set out. Hyperbolic relationship was found between the live time of tested organisms and concentrations of germanium organic compounds. Compounds showing highest and lowest toxicity to hydrobionts were indicated. Mathematical models and computer simulations have proved to be consistent with the observations and the results.

Key words: germanium organic derivatives, acute toxicity, hydrobionts, mean live time.
Hasła kluczowe: organiczne pochodne germane, toksyczność, hydrobionty, średni czas przeżywania.

INTRODUCTION

Germanium and its dioxide have been used mainly in Japan and later in other countries as an ingredient of many medicines, ointments and dietary supplements (1). In many cases a prolonged use of inorganic germanium and its derivatives led to the peripheral nephropathy, miophathy, anemia, weight loss, (2, 3, 4, 5), liver dysfunction (6), increase of acid phosphatase activity and loss of cytochrome *c* oxidase activity (7). On the other hand, some studies showed that germanium dioxide inhibited mutagenic activity of compounds such as cadmium chloride, 3-amino-1-metyl-5H-pyrido[4,3-b]indol, benzo[a]pyrene and phenylmercury acetate (8).

However, germanium organic derivatives show considerably more promising potential for therapeutical applications. These compounds display antiviral (9), antifungal (10), antiarteriosclerotic (11) hepatoprotective and antyarthretic activities, while their toxicity is lower toxicity than that of the inorganic derivatives (12, 13, 4, 14). Due to those biological properties of the germanium organic compounds we decided to design and find new germanium organic derivatives. The aim of this work was to determinate the acute toxicity and safety profile for hydrobionts exposed to selected new germanium organic derivatives as ingredients of future drugs and possible environmental pollutants.

MATERIALS AND METHODS

Tests of acute toxicity of eight germanium organic derivatives were carried out. These compounds were synthesized at the Odessa National University. Their structure was confirmed by X-ray structural analysis.

Compounds used in the experiment:

I) $(HPam)_2[Ge(HCitr)_2]\cdot 3H_2O$, Piracetamium bis(cytrato)germanate (IV)
II) $(HPam)_2[Ge_2(OH)_2(\mu\text{-}Tart)_2]$, Piracetamium bis(μ-tartrato)dihydroxydigermanate (IV)
III) Ge-H_4Togl-Pam , Piracetamium bis(threehydroxyglutarato)germanate (IV)
IV) $K_2[Ge(HCitr)_2]\cdot 2H_2O$, Potassium bis(cytrato)germanate (IV)
V) $\{K_2[Ge_2(\mu\text{-}Tart)_2(\mu\text{-}OH)_4]\cdot 4H_2O\}_n$, Potassium bis(μ-tartrato)tetra(μhydroxy)digermanate (IV)
VI) Ge-H_4Togl-K , Potassium bis(threehydroxyglutarato)germanate (IV)
VII) $(NicH)_2[Ge(OH)_2(Oedph)]\cdot H_2O$, Niacinium hydroxyethylidenedifosfonatodihydroxygermanate (IV)
VIII) $Mg[Ge(OH)_2(Oedph)]\cdot H_2O$, Oedph – anion oxyethylidenedifosfonic acid

The organisms used in the experiment

Toxic effect was studied on two hydrobiont species: *Daphnia magna* and *Danio rerio*.

Experimental Procedures

Environmental conditions for both tested hydrobionts were the same: water hardness about 4–6 mg/l, dissolved oxygen concentration of 6.8–7.2 mg/l, pH 7.7–8.2 and temperature 19–2°C. Static system was used, in which tested solutions were not exchanged throughout the exposures. The following concentrations of study compounds were used in experiments on *Daphnia magna*: 100 mg/l, 200 mg/l, 400 mg/l, 800 mg/l, 1500 mg/l, 3000 mg /l. The experiment lasted for 72h and during that time each individual death was recorded.

In experiments with *Danio rerio,* 10 fish were placed in 2000-ml containers. In this study the acute toxicity of germanium compound No.2 was examined. The following concentrations of study compounds were used: 6.75 g/l, 3.37 g/l, 1.69 g/l, 0.90 g/l, 0.45 g/l, 0.22 g/l. The test lasted seven days.

Data analysis

The probability of the development of lethal effects in the hydrobionts was calculated as the ratio of the number of individuals that died within a specified period of time (n) to the total number of individuals in the group (N), P = n/N. The average time of death was calculated as the arithmetic mean.

The value of the median lethal concentration (LC_{50}), which is a measure of acute toxicity, represents the concentration causing death of 50% of test individuals during an uninterrupted, specific exposure time, which was 48h in our experiment. LC_{50} value and statistical error (m) was calculated from the formula (Kerber's method):

$$\ln LC50 = \frac{1}{2}\left[\left(\ln C_n + \ln C_{n+1}\right) - \sum P_i\left(\ln C_{i+1} - \ln C_{i-1}\right)\right]$$

$$m_{LC_{50}} = \sqrt{\sum \frac{P_i(1-P_i)\cdot(\ln C_{i+1} - \ln C_{i-1})}{4\cdot(n-1)}}$$

where:
lnC_n – logarithm of the first concentration causing death of 100% of individuals;
lnC_{n+1} – the next nearest logarithm concentration causing 100% death of individuals;
lnC_i – i logarithm of the concentration at which the lethal effect was observed;
P – probability of the development of lethal effect for lnCi concentration;
n – number of individuals in the experimental group.
$m_{LC_{50}}$ – standard error.

LC_{50} value was calculated for 48-hour exposure of *Daphnia magna*. Values of LC_{50} for tested compounds were compared by Student's *t*-test for unpaired data. The results were considered to be statistically significant when $P < 0.05$. MLT – Mean Live Time in groups of tested organisms was calculated as the arithmetic mean and determined during the total duration of the experiment.

RESULTS

The experiments on *Daphnia magna* using all germanium compounds showed concentration-dependent toxicity. Compound No.7 was found to be characterized by highest toxicity. For all studied compounds, lethal effects were recorded at high concentrations. The curves of occurrence and rate of lethal effect depending on the concentration of differently structured toxicants („MLT – C" dependence) (fig. 1A) indicate that the examined germanium compounds may be classified into three groups: 1) highly toxic germanium compound No.7, for which the trend is characterized by the highest average speed of change of survival time for organisms depending on the concentration, 2) compounds No.1 and No.2, causing rapid death of individuals only at their high concentrations (3 g/l), 3) germanium compounds No3, 4, 5, 6, 8, showing the lowest toxicity.

As you can see from fig. 1 in the last section of complex relationships valid for germanium organic compounds 3, 4, 5, 6 and 8, lnMLT decreases linearly relative to lnC, in contrast to the relationships for the more toxic compounds No. 7, 1 and 2.

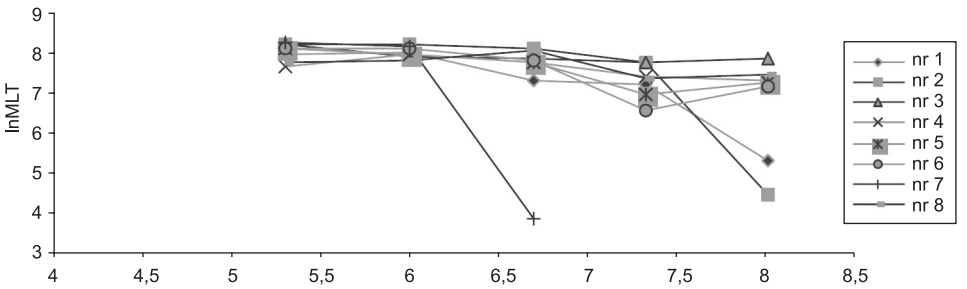

Fig. 1. Relationship between logarithms of concentrations of No.1 to No.8 germanium organic compounds and mean lethal time (MLT) for *Daphnia magna* (lnMLT, lnC).

Ryc. 1. Zależność logarytmów stężeń organicznych pochodnych germanu 1-8 i średniego czasu przeżywania badanych organizmów *Daphna magna* (lnMLT, lnC).

The most essential characteristics of the toxic effects in hydrobiont organisms is the value of median lethal dose (LC_{50}). Using the Kerber's method we were able to calculate the LC_{50} for the exposure of the animals within 48 hours, see table I.

Our analysis of the data showed statistically significant differences among all examined substances in Lethal Concentration effect ($P \leq 0.01$).

Table 1 shows that the analysis of the effects of the value of the LC_{50} are comparable with the effects of graphical analysis in fig.1. This indicates that the toxicity of compounds No.2 and 7 is higher than that of the remaining analyzed germanium organic compounds. Comparable estimates of the concentration ranges and temporal trends of the toxic (lethal) effect of germanium organic compounds in the study groups of *Daphnia magna* showed characteristic features of these substances and, therefore, they may serve as a basis for further toxicological testing.

Table I. Experimental LC_{50} values of germanium organic compounds on *Daphnia Magna.*

Tabela I. Obliczone eksperymentalnie wielkości stężeń półefektywnych efektu letalnego (LC_{50}) organicznych związków germanu w różnych stężeniach, oddziaływujące na osobniki *Daphnia magna*.

Substance	LC_{50}(mg/l)
1	373,2±1,20
2	177,3±1,34
3	154,4±1,36
4	181,3±1,32
5	273,9±1,35
6	486,1±1,26
7	200,7±1,26
8	367,2±1,30

Examinations o compound No. 2, (HPam) 2 [GE2 (OH) 2 (μ-Tart) 2], were carried out using *Danio rerio*. Concentration zones causing no toxic effects were defined (from 0 to maximum no-effect concentration). At concentrations from 0.2250 g/l to 0.4500 g/l no lethal effect was observed in the tested groups after 7 days (fig. 2).

Death of all fish occurred at C => 0.9 g/l. Threshold concentrations causing lethal effect in *Danio rerio* individuals range from 0.4500 to 0.9000 g/l. Curves representing the ratio of the probability of lethal effect (P) to logarithm of the exposure time for constant toxicant concentrations do not change their slopes in the range of tested

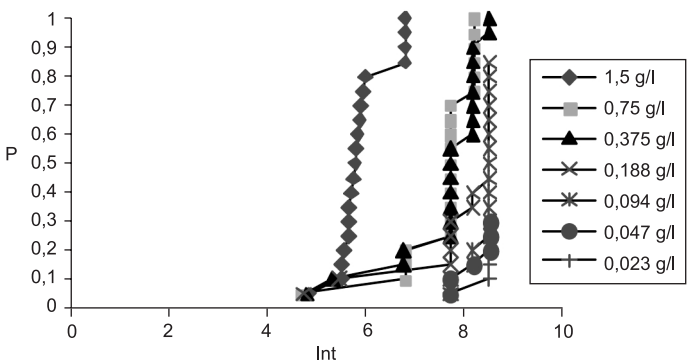

Fig. 2. Relationship between the probability of lethal effect (P) and logarithm of time (lnT) for different concentrations of germanium organic compound No.2 for *Daphnia magna*.

Ryc. 2. Zależność między prawdopodobieństwem wystąpienia efektu letalnego (P) a logarytmem czasu (lnT) przy różnych stężeniach organicznej pochodnej germanu nr 2 dla *Daphnia magna.*

concentrations. This suggests a single acute toxic effect to hydrobiont organisms. A characteristic feature of the toxic effects of germanium compound in the concentration range 0.9000–6.7500 g/l is 100% lethality of animals in study groups (fig. 2).

A special method of kinetic analysis of the data in the coordinates (lnQ, T) indicates that the potency of toxic effect is increasing with time and the trend of death cases accelerate. The curves of fig. 3 are close to the vertical. This allows to estimate threshold completion time of effect (the curve tends to $-\infty$) and the beginning of the threshold effect (the curve tends to 0). As you can see in fig. 3, both parameters decrease with the rise of toxicant concentration.

Using the previously developed method (16), early lethal effect in study groups exposed to various concentrations of germanium organic compound No. 2 can be estimated. This method consists of presenting the data in the (1/lnQ, 1/T) coordinates, where Q is the fraction of organisms in the test group which in the presence of toxicant has not died during exposure time (T) (fig. 9). The trend of the lethal effect of germanium compound No.2 in the range of its various concentrations is charac-

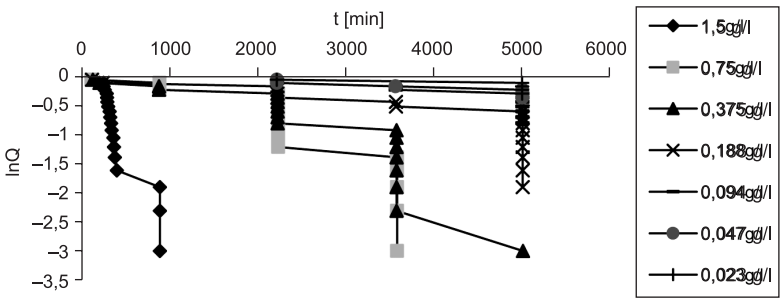

Fig. 3. Relationship between survival of *Daphnia magna* (lnQ) and time (t) under conditions of different concentrations of germanium organic compound No. 2.

Ryc. 3. Zależność przeżywalności osobników (lnQ) *Danio rerio* od czasu (T) w warunkach wpływu różnych stężeń organicznej pochodnej germanu nr 2.

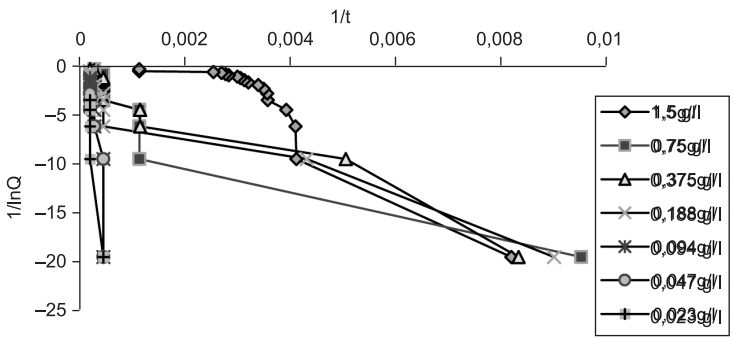

Fig. 4. Relationship between the reciprocal of the survival of *Daphnia magna* and time (1/lnQ), (1/t) for germanium organic compound No. 2.

Ryc. 4. Zależność odwrotności przeżywalności i czasu (1/lnQ), (1/T) dla osobników *Danio rerio* w obecności organicznej pochodnej germanu nr 2.

terized by a variably-decreasing starting time of lethal effect relative to increasing content of toxicant in the environment. Therefore, the delay of the development of toxic effects (lag-period) increases more than tenfold. Taking into account the proportional increase in the value of the beginning and the end of the lethal effect curves, it seems reasonable to conclude that the trend of this process is hyperbolic.

To estimate the hyperbolic relationship between the rate of lethal effect occurrence and toxicant concentration, the calculated values of MLT (fig. 3) were used. According to the Haber's rule, these dependencies are n-power hyperboles. It is not precise because the curves of the trend of the toxic effect must include an independent factor, which is characterized by a zone of concentration which does not cause the lethal effect. Table II shows that the zone of concentrations of germanium organic compound No. 2 without causing 100% lethal effect is within the range up to 0.9 g/L.

Whether or not the lethal effect curve takes the form of a n-power hyperbole can be checked in two ways:

– by presentation of research data in double logarithmic coordinates (lnMLT, lnC);

– by presenting the research data in the (P, ln ($t \cdot C^n$)) coordinates, where –*, n – sizes of n-powers of the specified variables are chosen experimentally by computer simulation.

Fig. 5 shows that the research data given in double log coordinates are arranged linearly. This, using the regression method, it is possible to estimate the size of the power of the toxic effect ("Habber's hyperbole"). The line has the regression coefficient of –2.04. No deviation from the hyperbolic dependence of research data was noticed.

Analysis of the results shows that the power $n = 2$ focuses most of research curves (Fig. 6). Thus, the trend of the effect of the Germanium organic compound No. 2 to *Danio rerio* organisms takes hyperbole shape at the power of 2 (fig. 6).

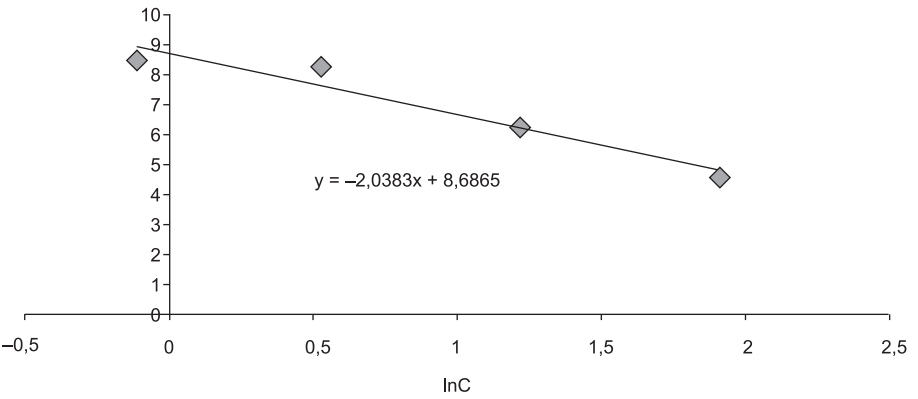

Fig. 5. Relationship between logarithm of MLT (lnMLT) and logarithms of concentrations of germanium organic compound No. 2 on *Danio rerio*.

Ryc. 5. Zależność pomiędzy logarytmem średniego czasu przeżywania (lnMLT) od logarytmów stężenia organicznej pochodnej germanu nr 2 dla *Danio rerio*.

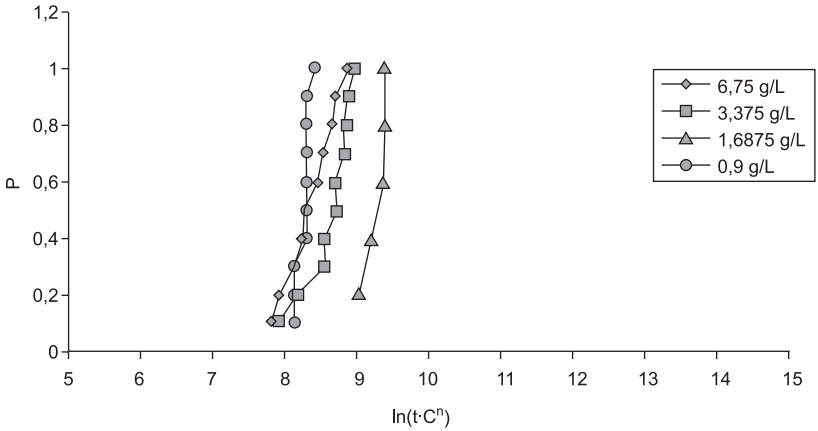

Fig. 6. Concentration of the curves representing the probability of the lethal effect (P) against the logarithm of product of time and squared concentration of germanium organic compound No. 2; graph coordinates [P,ln(t*C^2)] characterized by the best convergence of the experimental data.

Ryc. 6. Skupienie krzywych prawdopodobieństwa efektu letalnego (P) od logarytmu czasu i stężenia w potędze (n = 2), wykres we współrzędnych (P,ln(t*C^)) charakteryzujący się najwi.ększym skupieniem danych badawczych.

Research shows that germanium organic compound No. 2 present at concentrations above 0.9 g/l in the aqueous environment resulted in the development of toxic (lethal) effects in *Danio rerio* organisms. The time of lethal effect trend of toxicant takes hyperbole shape at the power of $n = 2$.

CONCLUSIONS

The purpose of this study was to examine the toxicity of eight germanium organic derivatives with particular emphasis on the germanium organic complex No. 2 – tartaricgermanium acid complex with piracetam. Two hydrobiont species, *Daphnia magna* and *Danio rerio* were used in the experiment. The concentrations of 100 mg/l to 3000 mg/l and experiment time of 1 hour to 96 hours were used, which were characteristic values for toxic trend (acute effect, lethal effect) of tested substances. Hyperbolic relationship was found to occur between the survival time of tested organisms and concentrations of germanium organic compounds No. 3, 4, 5, 6, and 8. Compounds No. 2, 3 and 7 have the highest toxicity confirmed through estimation of the volume of effective concentrations by Kerber's method.

In the case of *Daphnia magna* it was noted that under the specified conditions of the experiment there was a single mechanism of action of the toxicant causing death of *Daphnia magna* organisms. It was noted that this hydrobiont showed high sensitivity to the germanium organic compound No. 2. The presence of only one acute lethal effect of tartaricgermanium acid complex with piracetam to these organisms, as well as simple form of toxic trend (linear hyperbola) make this species suitable for biomonitoring studies in ecotoxicology of germanium compounds. Germanium

organic derivate No. 2 proved to be slightly toxic to vertebrate animals. Concentration of toxicant without causing the lethal effect for *Danio rerio* organisms was below 0.9 g/l of tested substance. The mean live time (MLT) of *Danio rerio* organisms decreased hyperbolically with increasing concentrations of the tested substance. Using mathematical regression method and computer simulations it has been proved that the lethal effect trend for *Danio rerio* may be represented by hyperbole of the power of n = 2. Models and mathematical operations used in our study have confirmed their effectiveness, making them useful for application in similar studies. Low toxicity to the fish points to the necessity of performing further studies of toxicity of germanium compounds using rodents as the test species. Studies on mammals are necessary to ensure safe use of those substances in future medical practice.

G. Oloś, O. Zhuk, V. G. Zinkovsky, I. I. Seifullina, E. E. Martsinko, A. G. Pesaroglo

TOKSYCZNOŚĆ OSTRA NOWYCH, ORGANICZNYCH POCHODNYCH GERMANU

Streszczenie

W pracy dokonano oceny toksyczności ośmiu organicznych pochodnych germanu z użyciem dwóch gatunków hydrobiontów: *Daphnia magna* i *Danio rerio*. Oznaczono wartości średniego czasu przeżywania (MLT) oraz stężenia śmiertelnego medialnego (LC_{50}). Zostały zdemonstrowane dynamiki efektu toksycznego. Zakresy toksyczne stężeń powodujące 100% i 0% skutek śmiertelny zostały określone. Oszacowano hiperboliczne zależności między szybkością wystąpienia efektu letalnego względem stężeń toksykantów. Oznaczono związki o najniższej i najwyższej toksyczności dla badanych organizmów. Modele matematyczne i symulacje komputerowe okazały się zgodne z obserwacjami i wynikami.

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Adres: 45-035 Opole, ul. Kominka 4.