ЕКСПЕРИМЕНТАЛЬНА ТА КЛІНІЧНА ФАРМАКОЛОГІЯ

Recommended by Doctor of Medicine, Professor S. Yu. Shtrygol'

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The study of acute toxicity of 1,3-oxazole-4-il-phosphonic acid derivative in intraperitoneal administration

The derivative of 1,3-oxazole-4-yl-phosphonic acid (OVP-1) has the vasodilatory activity and is studied as a potential antihypertensive agent. The toxicological assessment plays an important role in the preclinical study of new drugs.

Aim. To study the acute toxicity of OVP-1 in intraperitoneal administration and determine its average lethal dose.

Materials and methods. The intraperitoneal route of administration was chosen as it provides the systemic action of drugs. The experiment was conducted on female and male of white non-linear mice. Five experimental groups of animals corresponded to such dose levels of OVP-1 as 1000 mg/kg, 3000 mg/kg, 3500 mg/kg, 4000 mg/kg, and 4500 mg/kg. The solution of 1,3-oxazole-4-yl-phosphonic acid derivative and the blank solution (the mixture of Tween-80 and water for injection in the ratio of 1 : 10) was injected once in the abdominal cavity of mice. They were monitored for their mortality, behavior, and clinical characteristics over the following 14 days. LD_{50} of OVP-1 was determined by the number of dead animals before autopsy.

Results and discussion. As a result of the experiment the peculiarities of the toxic effect of 1,3-oxazole-4-yl-phosphonic acid derivative on the organism of the experimental mice have been studied. Based on clinical observations there were no changes in the behavior and appearance of mice for 14 days. However, taking into account the rare cases of tachypnea, piloerection and catalepsy, it can be assumed that OVP-1 in toxic doses can affect the central nervous system, the autonomic system, and there is a probability of occurrence of cardio-pulmonary insufficiency.

Conclusions. It has been determined that LD_{50} of OVP-1 derivative in intraperitoneal administration to mice of both sexes is 3350.67 ± 54.62 mg/kg and belongs to the toxicity class VI – relatively harmless compounds, which makes this compound promising for further preclinical pharmacological studies.

Key words: *derivative of 1,3-oxazole-4-yl-phosphonic acid; OVP-1; acute toxicity; intraperitoneal administration; mice; mid-lethal dose*

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Дослідження гострої токсичності похідного 1,3-оксазол-4-іл-фосфонової кислоти при внутрішньоочеревинному введенні

Похідне 1,3-оксазол-4-іл-фосфонової кислоти (ОВП-1) має вазодилатаційну активність та вивчається в якості потенційного антигіпертензивного засобу. Суттєве значення у доклінічному вивченні нових лікарських засобів має їх токсикологічна оцінка.

Мета роботи – дослідити гостру токсичність ОВП-1 при внутрішньоочеревинному введенні та визначити його середньолетальну дозу.

Матеріали та методи. Внутрішньоочеревинний шлях введення був обраний як той, що забезпечує системну дію лікарських засобів. Експеримент проводили на самках та самцях білих нелінійних мишей. 5 дослідних груп тварин відповідали рівням доз ОВП-1 – 1000 мг/кг, 3000 мг/кг, 3500 мг/кг, 4000 мг/кг, 4500 мг/кг. Введення розчину похідного 1,3-оксазол-4-іл-фосфонової кислоти та контрольного розчину (суміші твіну-80 та води для ін'єкцій у співвідношенні 1 : 10) здійснювали в черевну порожнину мишей одноразово. Протягом наступних 14 діб спостерігали за їхньою смертністю, поведінкою та клінічними характеристиками. Середньолетальну дозу ОВП-1 визначали за кількістю загиблих тварин до розтину.

Результати та їх обговорення. В результаті експерименту досліджені особливості токсичної дії похідного 1,3-оксазол-4-іл-фосфонової кислоти на організм експериментальних мишей. Ґрунтуючись на даних клінічних спостережень відзначена відсутність змін у поведінці та зовнішньому вигляді мишей впродовж 14 діб. Проте враховуючи поодинокі випадки тахіпное, пілоерекції та каталепсії можна зробити припущення, що ОВП-1 в токсичних дозах може впливати на ЦНС, вегетативну систему, та існує імовірність виникнення серцево-легеневої недостатності.

Висновки. Встановлено, що LD_{50} похідного 1,3-оксазол-4-іл-фосфонової кислоти (ОВП-1) при внутрішньоочеревинному введенні мишам обох статей становить 3350.67 ± 54.62 мг/кг та відноситься до VI класу токсичності «відносно нешкідливі сполуки», що робить дану сполуку перспективною для подальших доклінічних фармакологічних досліджень.

Ключові слова: *похідне 1,3-оксазол-4-іл-фосфонової кислоти; ОВП-1; гостра токсичність; внутрішньоочеревинне введення; миші; середньолетальна доза*

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Исследование острой токсичности производного 1,3-оксазол-4-ил-фосфоновой кислоты при внутрибрюшинном введении

Производное 1,3-оксазол-4-ил-фосфоновой кислоты (ОВП-1) имеет вазодилатирующую активность и изучается в качестве потенциального антигипертензивного средства. Важную роль в доклиническом изучении новых лекарственных средств имеет их токсикологическая оценка.

Цель работы – исследовать острую токсичность ОВП-1 при внутрибрюшинном введении и определить его среднелетальную дозу.

Материалы и методы. Внутрибрюшинный путь введения был выбран как тот, который обеспечивает системное действие лекарственных средств. Эксперимент проводили на самках и самцах белых нелинейных мышей. 5 подопытных групп животных соответствовали уровням доз ОВП-1 – 1000 мг/кг, 3000 мг/кг, 3500 мг/кг, 4000 мг/кг, 4500 мг/кг. Введение раствора производного 1,3-оксазол-4-ил-фосфоновой кислоты и контрольного раствора (смесь твина-80 и воды для инъекций в соотношении 1 : 10) проводили в брюшную полость мышей однократно. На протяжении следующих 14 суток наблюдали за их смертностью, поведением и клиническими характеристиками. Среднелетальную дозу ОВП-1 определяли по количеству погибших животных до вскрытия.

Результаты и их обсуждение. В результате эксперимента исследованы особенности токсического действия производного 1,3-оксазол-4-ил-фосфоновой кислоты на организм экспериментальных мышей. Основываясь на данных клинических наблюдений отмечено отсутствие изменений в поведении и внешнем виде мышей на протяжении 14 суток. Однако учитывая единичные случаи тахипноэ, пилоэрекции и каталепсии можно предположить, что ОВП-1 в токсических дозах может влиять на ЦНС, вегетативную систему, и существует вероятность возникновения сердечно-легочной недостаточности.

Выводы. Установлено, что LD_{50} производного 1,3-оксазол-4-ил-фосфоновой кислоты (ОВП-1) при внутрибрюшинном введении мышам обоего пола составляет 3350,67 ± 54,62 мг/кг и относится к VI классу токсичности «относительно безвредные соединения», что делает данное соединение перспективным для дальнейших доклинических фармакологических исследований.

Ключевые слова: *производное 1,3-оксазол-4-ил-фосфоновой кислоты; ОВП-1; острая токсичность; внутрибрюшинное введение; мыши; среднелетальная доза*

The study of the biological activity of new physiologically active substances is an up-to-date direction in modern experimental pharmacology [1]. The preclinical toxicological assessment plays the essential role in their study as potential drugs; it allows providing safety in subsequent clinical trials and medical use [2].

According to the preliminary experimental data diethyl ester of 5-piperidino-2-{N-[N-benzoyl-(4-methylbenzal) glycyl] aminomethyl}-1,3-oxazole-4-yl-phosphonic acid (in short – 1,3-oxazole-4-yl-phosphonic acid derivative or OVP-1) (Fig. 1) has the vasodilating activity and is promising for further study as an antihypertensive agent [3]. Earlier the study of acute toxicity of the above compound in mice of both sexes was started by oral administration, and it was found that the substance under research belonged to the toxicity class V – practically non-toxic substances [4]. Since the preclinical study of acute toxicity involves several routes of administration of pharmacologically active compounds, it has become logical to continue studying acute toxicity of this substance in intraperitoneal administration, this route for small rodents is equal to the intravenous one [2]. The

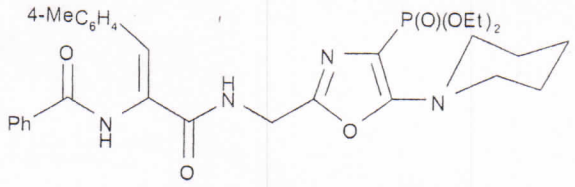

Fig. 1. The chemical formula of diethyl ester of 5-piperidine-2-{N-[N-benzoyl-(4-methylbenzal) glycyl] aminomethyl}- 1,3 - oxazole-4-yl-phosphonic acid

aim of the work was to study acute toxicity of one of phosphorylated derivatives of 1,3-oxazole [5] in intraperitoneal administration and determine its average lethal dose.

Materials and methods

The object of the study was 1,3-oxazole-4-yl-phosphonic acid derivative synthesized in the Institute of Bioorganic Chemistry and Petrochemistry of the National Academy of Sciences of Ukraine [5]. The intraperitoneal route of administration was chosen as the one that provides the systemic action of drugs [2].

The animals were kept on a regular balanced diet and free access to water [6] in the conditions of the vivarium of the Bogomolets National Medical University. The experiment was performed on 42 females and 42 males of white non-linear mice weighing 20 ± 2 g at the age of 2.0-2.5 months divided into 7 groups (5 experimental, 1 control, 1 intact) with 6 animals of both sexes in each. The quarantine period was 5 days. The studies were carried out in accordance with the requirements of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" [7] and Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruel Treatment".

Five experimental groups of animals corresponded to such dose levels of OVP-1 as 1000 mg/kg, 3000 mg/kg, 3500 mg/kg, 4000 mg/kg, and 4500 mg/kg. The control group of mice was injected with the mixture of Tween-80 and water for injection in the ratio of 1 : 10 in the volume equivalent to that introduced to the animals of the main experimental groups. The solution of OVP-1 and the blank solution were injected once in the

abdominal cavity of mice. They were monitored for their mortality, behavior, and clinical characteristics over the following 14 days. The animals were weighed just before the injection of the test substance and on day 3, 7, and 14 of the experiment. On day 15 mice were withdrawn from the experiment by dislocation of cervical vertebrae, after that they were dissected; and the liver, spleen, kidneys, heart, lungs and brain were taken for weighing. The average lethal dose LD_{50} of OVP-1 was determined by the number of dead animals before autopsy.

Statistical calculations, as well as a graphical representation of the results were performed using BioStat 2009 v 5.8.4. (manufacturer – Analyst Soft), and probit analysis – according to Finney. Depending on the normality of the distribution of the compared series the experimental groups were compared to the control and intact groups, or using Student's t-test for independent samples and with the initial value using the paired Student's t-test, or Mann-Whitney U-criterion for groups with small samples. The significance level for all criteria was 0.05.

Results and discussion

Clinical observations. In most animals of the experimental and control groups the motor activity inhibition was observed immediately after administration and over the following hour. It can be considered as a response to the injection intervention. In OVP-1 1000 mg/kg group all animals showed no behavioral or appearance changes except for one male, who began to breathe heavily 15 min after the injection of OVP-1 solution over the following 20 min, after that the normal breathing was restored. In OVP-1 3000 mg/kg group in 30 min after introduction of the solution of the compound under research tachypnea was observed in 2 males (No. 2, No. 3) over the following 30 min, in 1 female of the same group (No. 4) the same symptoms were observed, but in 20 min after the OVP-1 administration. On day 6 after the start of the experiment the male No. 3 of this group died. In OVP-1 3500 mg/kg group tachypnea was observed in 30 min after introduction of OVP-1 solution (males No. 1, No. 5, No. 6, females No. 2, No. 3), piloerection (males No. 1, No. 5). Death in this group was noted on day 2 after the injection of OVP-1 (male No. 1), on day 3 (male No. 4), on day 6 (male No. 6, female No. 3) and on day 8 (male No. 2, female No. 2). In OVP-1 4000 mg/kg group tachypnea in 30 min after introduction of OVP-1 solution (males No. 3, No. 6, females No. 3, No. 5), piloerection (male No. 6), and catalepsy on day 2 (the animal studied remained in the same position) (males No. 3, No. 6, female No. 1) were observed. Death in this group was noted in 22 h after the injection of the test solution (male No. 1, female No. 2), on day 2 (males No. 3, No. 4, No. 6, females No. 1, No. 4), on day 5 (males No. 2, No. 5, female No. 3), on day 6 (female No. 5) and on day 8 (female No. 6). In OVP-1 4500 mg/kg group, in addition to tachypnea (male No. 3), abrupt movements and death were observed as a result of the respiratory arrest (in 20 min – males No. 1, No. 4; 30 min – females No. 2, No. 3, No. 6; in 40 min – females No. 1, No. 4; in 60 min – males No. 3, No. 5, No. 6, female No. 5; in 90 min – male No. 2). In the control group clinical changes were not observed within 14 days.

Taking into account the above results of the experiment it can be noted that the most frequent pathological symptom observed only on the first day after intraperitoneal administration of OVP-1 is tachypnea that may indicate the probability of occurrence of cardio-pulmonary insufficiency [2] when using OVP-1 solution in highly toxic doses. The occurrence of rare cases of piloerection and catalepsy can be assessed as the effect of the compound under research on the central nervous system and the vegetative system [2].

The weight of animals. The results of the dynamics of changes in the body weight of animals during the experiment are shown in Tab. 1. In general, there is a positive dynamics of weight gain in all experimental groups of animals. It may indicate a rapid recovery of the experimental animals after intraperitoneal administration of OVP-1 solution.

Table 1

The dynamics of the body mass of mice after intraperitoneal administration of OVP-1, g (M ± m)

Groups of animals	Mass of animals, g			
	Output data	3-d day	7-th day	14-th day
1	2	3	4	5
Males				
Intact	20.10 ± 0.20[1]	20.40 ± 0.16[1]*	21.02 ± 0.21[1]*	21.40 ± 0.28[1]*
Control (water for injection + Tween-80)	19.77 ± 0.37[1]	20.15 ± 0.33[1]	20.67 ± 0.39[1]*	21.38 ± 0.39[1]*
No. 1 (OVP-1, 1000 mg/kg)	20.47 ± 0.41[1]	20.97 ± 0.41[1]	21.22 ± 0.37[1]*	21.57 ± 0.39[1]*
No. 2 (OVP-1, 3000 mg/kg)	18.68 ± 0.19[1]	19.50 ± 0.20[1]*	20.40 ± 0.24[2]*	21.04 ± 0.18[2]*
No. 3 (OVP-1, 3500 mg/kg)	20.53 ± 0.25[1]	21.03 ± 0.33[3]*	21.27 ± 0.24[4]*	21.35 ± 0.03[5]*
No. 4 (OVP-1, 4000 mg/kg)	20.28 ± 0.27[1]	20.95 ± 0.19[5]*	–	–
No. 5 (OVP-1, 4500 mg/kg)	19.30 ± 0.25[1]	–	–	–

Continuation of Table 1

1	2	3	4	5
Females				
Intact	20.03 ± 0.30[1]	20.58 ± 0.24[1]*	21.35 ± 0.22[1]*	21.83 ± 0.24[1]*
Control (water for injection + Tween-80)	20.50 ± 0.40[1]	20.90 ± 0.38[1]	21.45 ± 0.42[1]*	21.95 ± 0.36[1]*
No. 1 (OVP-1, 1000 mg/kg)	19.63 ± 0.43[1]	19.98 ± 0.42[1]	20.28 ± 0.45[1]*	20.68 ± 0.49[1]*
No. 2 (OVP-1, 3000 mg/kg)	19.27 ± 0.27[1]	19.72 ± 0.25[1]*	20.0 ± 0.29[1]*	20.37 ± 0.31[1]*
No. 3 (OVP-1, 3500 mg/kg)	19.11 ± 0.19[1]	19.88 ± 0.24[1]*	20.52 ± 0.26[2]*	20.83 ± 0.29[3]*
No. 4 (OVP-1, 4000 mg/kg)	19.90 ± 0.31[1]	20.24 ± 0.08[4]	21.1 ± 0.0[6]	–
No. 5 (OVP-1, 4500 mg/kg)	19.28 ± 0.31[1]	–	–	–

Note:* – the value is statistically significantly different from the initial one ($p \leq 0.05$); [1] – n = 6; [2] – n = 5; [3] – n = 4; [4] – n = 3; [5] – n = 2; [6] – n = 1.

The results of autopsy. Tab. 2 shows the mass ratios of internal organs. In the process of autopsy no significant macroscopic changes in the internal organs of the majority of animals were observed. However, there was a statistically significant increase in the mass of internal organs in individual groups of mice, in particular in OVP-1 1000 mg/kg group there was an increase in weight of the liver by 16.6 % and of the kidneys by 12.9 % in males in relation to the intact group; in OVP-1 3000 mg/kg group there was an increase in the heart weight by 16.3 % in males in relation to the control group, in OVP-1 3500 mg/kg group there was an increase in weight of the liver by 32.8 %, of the spleen by 19.7 %, of heart by 20.0 % in males in relation to the intact group; in OVP-1 3500 mg/kg group there was an increase in weight of the heart by 18.0 % and spleen by 11.8 % in females in relation to the intact group.

In males and females of the control group the weight of the liver was increased by 15.8 % and 14.8 %, respectively, compared to the intact group.

Statistical calculations of the average lethal dose LD_{50}. Data on the mortality of animals are given in Tab. 3. The curves for the "log10 dose-effect" relationship are shown in Fig. 2 and 3. The statistical analysis of data by Finney's probit analysis has shown that LD_{50} of OVP-1 in intraperitoneal injection is 3312.07 ± 175.51 mg/kg for males, and 3389.32 ± 409.11 mg/kg for females. There is no statistically significant difference between LD_{50} for females and males ($p > 0.05$). Therefore, the total LD_{50} value for females and males of

Table 2

Mass ratios of internal organs of mice after intraperitoneal administration of OVP-1

Group of animals	Mass ratio (M ± m)						
	liver	right kidney	left kidney	spleen	heart	lungs	brain
Males							
Intact	5.12 ± 0.11	0.62 ± 0.02	0.62 ± 0.02	0.76 ± 0.01	0.50 ± 0.01	0.77 ± 0.01	1.94 ± 0.01
Control (water for injection + Tween-80)	5.93 ± 0.32**	0.65 ± 0.02**	0.65 ± 0.02**	0.83 ± 0.03**	0.49 ± 0.02	0.82 ± 0.02**	1.97 ± 0.04
No. 1 (OVP-1, 1000 mg/kg)	5.97 ± 0.32**	0.70 ± 0.03*/**	0.70 ± 0.03*/**	0.83 ± 0.03**	0.55 ± 0.02*/**	0.83 ± 0.03*	2.11 ± 0.07*/**
No. 2 (OVP-1, 3000 mg/kg)	5.18 ± 0.16*	0.65 ± 0.01**	0.66 ± 0.01**	0.80 ± 0.01**	0.57 ± 0.02*/**	0.75 ± 0.00	2.01 ± 0.02*/**
No. 3 (OVP-1, 3500 mg/kg)	6.8 ± 0.05*/**	0.67 ± 0.01*/**	0.67 ± 0.00*/**	0.91 ± 0.01*/**	0.6 ± 0.01*/**	0.83 ± 0.01*/**	2.14 ± 0.08*/**
Females							
Intact	5.33 ± 0.17	0.63 ± 0.02	0.63 ± 0.02	0.76 ± 0.01	0.50 ± 0.01	0.76 ± 0.01	1.96 ± 0.01
Control (water for injection + Tween-80)	6.12 ± 0.29**	0. 69 ± 0.09	0.68 ± 0.03**	0.82 ± 0.03	0.54 ± 0.02**	0.82 ± 0.03**	1.95 ± 0.01
No. 1 (OVP-1, 1000 mg/kg)	5.6 ± 0.35*	0.63 ± 0.03	0.63 ± 0.03	0.79 ± 0.03**	0.51 ± 0.02*	0.79 ± 0.03	1.89 ± 0.03*/**
No. 2 (OVP-1, 3000 mg/kg)	5.1 ± 0.25*	0.63 ± 0.02	0.63 ± 0.03	0.76 ± 0.02*	0.57 ± 0.02	0.76 ± 0.02*	1.97 ± 0.03
No. 3 (OVP-1, 3500 mg/kg)	5.4 ± 0.28*	0.62 ± 0.01	0.62 ± 0.01	0.85 ± 0.02**	0.59 ± 0.02*/**	0.79 ± 0.01**	2.10 ± 0.04*/**

Note:* – a statistically significant change in relation to the control group ($p \leq 0.05$); ** – a statistically significant change in relation to the intact group ($p \leq 0.05$).

Table 3

Death of mice after a single intraperitoneal administration of OVP-1

Group of animals	Total number of animals in the group	Number of dead animals	Number of dead animals, %
Males, n = 42			
No. 1 (OVP-1, 1000 mg/kg)	6	0	0
No. 2 (OVP-1, 3000 mg/kg)	6	1	16,7
No. 3 (OVP-1, 3500 mg/kg)	6	4	66,7
No. 4 (OVP-1, 4000 mg/kg)	6	6	100
No. 5 (OVP-1, 4500 mg/kg)	6	6	100
Blank (water for injection+Tween-80)	6	0	0
Intact	6	0	0
Females, n = 42			
No. 1 (OVP-1, 1000 mg/kg)	6	0	0
No. 2 (OVP-1, 3000 mg/kg)	6	0	0
No. 3 (OVP-1, 3500 mg/kg)	6	2	33,3
No. 4 (OVP-1, 4000 mg/kg)	6	6	100
No. 5 (OVP-1, 4500 mg/kg)	6	6	100
Blank (water for injection+Tween-80)	6	0	0
Intact	6	0	0

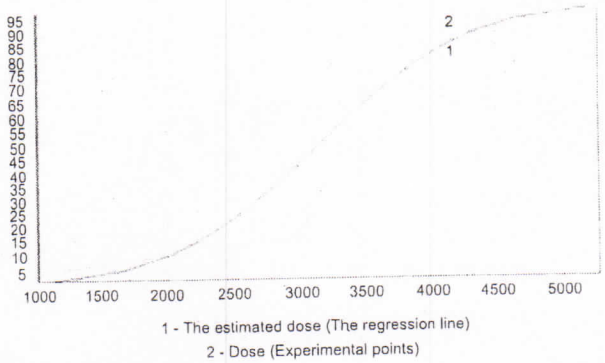

Fig. 2. The curve of dependence of the effect (ordinate axis) on log10 dose (abscissa axis), %, in intraperitoneal administration of OVP-1 in male mice

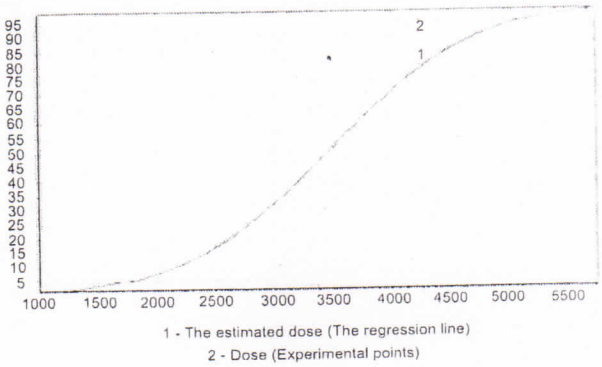

Fig. 3. The curve of dependence of the effect (ordinate axis) on log10 dose (abscissa axis), %, in intraperitoneal administration of OVP-1 in female mice

white non-linear mice is **3350.67 ± 54.62 (CI 95 %: 3338.08 ÷ 3363.32) mg/kg.**

The results obtained have shown that this substance can be referred to the toxicity class VI – relatively harmless compounds according to the classification given in the recommendations for preclinical studies [2].

CONCLUSIONS

1. LD_{50} of 1,3-oxazole-4-yl-phosphonic acid (OVP-1) derivative in intraperitoneal administration to mice of both sexes is 3350.67 ± 54.62 mg/kg.

2. The results of the studies indicate that in intraperitoneal administration to mice of both sexes OVP-1 belongs to the toxicity class VI – relatively harmless compounds, which makes this compound promising for further preclinical pharmacological studies.

3. The peculiarities of the toxic effect of 1,3-oxazole-4-yl-phosphonic acid derivative on the organism of the experimental mice have been studied. Based on clinical observations there were no changes in the behavior and appearance of mice for 14 days. However, taking into account the rare cases of tachypnea, piloerection and catalepsy, it can be assumed that OVP-1 in toxic doses can affect the central nervous system, the autonomic system, and there is a probability of occurrence of cardio-pulmonary insufficiency. The results obtained regarding clinical changes and the increase in the mass ratios of individual internal organs of animals require further thorough study.

Conflict of Interests: authors have no conflict of interests to declare.

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