Біологічний вісник МДПУ імені Богдана Хмельницького 6 (2), стор. 174–180, 2016
Biological Bulletin of Bogdan Chmelnitskiy Melitopol State Pedagogical University, 6 (2), pp. 174–180, 2016

ARTICLE UDC 619:615

STUDY OF ACUTE AND CHRONIC TOXICITY OF 'INJECTABLE MEVESEL' INVESTIGATIONAL DRUG

B. Gutyj, M. Paska, N. Levkivska, R. Pelenyo, N. Nazaruk, Z. Guta

Lviv National University of Veterinary Medicine and Biotechnologies named after S.Z. Gzhytskyj, Pekarska Str., 50, 79010 Lviv, Ukraine, Email: bvh@ukr.net

The results of study of toxicity of the newly created «Injectable Mevesel» during acute and chronic experiments are presented. There were no lethal cases at intragastric and intramuscular injections, only short-time inhibition of laboratory animals receiving the drug at a dose of 10.0 ml was observed. There were no lethal cases of test animals during the experiment in the conditions of study of accumulation properties of «Injectable Mevesel». Total average dose of the drug administered made up 162500 mg/kg, and accumulation coefficient was respectively 5.3. In the study of morphological blood parameters of rats after intramuscular injection of «Injectable Mevesel» in increasing doses, probable increase in neutrophils count by 36.1%, and probable reduction in lymphocytes count by 15.2% were found. Administration of the drug in increasing doses significantly affects the functional state of internal organs of experimental animals (liver) and causes significant degradation of the membranes of hepatocytes, as evidenced by increased activity of intracellular ALT, AST enzymes and alkaline phosphatase. Therefore, new domestic drug «Injectable Mevesel» created by us belongs to class 4 toxicity criteria, i.e. low-toxic substances.

Key words: *pharmacology, toxicology, blood, rats, mice, 'Injectable Mevesel' drug.*

ДОСЛІДЖЕННЯ ГОСТРОЇ ТА ХРОНІЧНОЇ ТОКСИЧНОСТІ ЕКСПЕРИМЕНТАЛЬНОГО ПРЕПАРАТУ «МЕВЕСЕЛ-ІН'ЄКЦІЙНИЙ»

Б. Гутий, М. Паска, Н. Левківська, Р. Пеленьо, Н. Назарук, З. Гута

Львівський національний університет ветеринарної медицини та біотехнологій імені С. З. Ґжицького, вул. Пекарська, 50, Львів 79010, Україна, Email: bvh@ukr.net

Наведено результати дослідження токсичності новоствореного препарату «Мевесел-ін'єкційний» у гострому і хронічному досліді. За умов внутрішньошлункового і внутрішньо м´язового введення загибелі білих щурів не було, лише встановлено короткочасне пригнічення лабораторних тварин, яким задавали препарат у дозі 10,0 мл. За умов дослідження властивостей мевеселу-ін'єкційного в дозах щодо кумуляції загибелі дослідних тварин протягом досліду не виявлено. Сумарно введена середня доза препарату становила 162500 мг/кг, а коефіцієнт кумуляції був відповідно - 5,3. При дослідженні морфологічних показників крові щурів після внутрішньо м´язового введення препарату "Мевесел-ін'єкційний" у зростаючих дозах, встановлено вірогідне збільшення кількості нейтрофілів на 36,1 % та вірогідне зменшення кількості лімфоцитів на 15,2 %. Введення препарату у зростаючих дозах суттєво впливає на функціональний стан внутрішніх органів дослідних тварин (печінки) та викликає деструкцію мембран гепатоцитів, про що вказує підвищення активності внутрішньоклітинних ензимів АлАТ, АсАТ і лужної фосфатази. Отже, створений нами новий вітчизняний препарат "Мевесел-ін'єкційний" належить до 4-го класу токсичності, тобто до малотоксичних речовин.

Ключові слова: *фармакологія, токсикологія, кров, щурі, миші, препарат "Мевесел-ін'єкційний".*

Citation:
Gutyj, B., Paska, M., Levkivska, N., Pelenyo, R., Nazaruk, N., Guta, Z. (2016). Study of acute and chronic toxicity of 'Injectable Mevesel' investigational drug. *Biological Bulletin of Bogdan Chmelnitskiy Melitopol State Pedagogical University,* 6 (2), 174–180.

Поступило в редакцию / Submitted: 17.06.2016
Принято к публикации / Accepted: 30.07.2016

crossref **http://dx.doi.org/10.15421/201649**

INTRODUCTION

In order to increase the body's antioxidant status of young cattle at the development of oxidative stress, a new drug "Injectable Mevesel", consisting of methionine, sodium selenite, unitiol, A, D_3 and E vitamins was developed. It should also be noted that Injectable Mevesel is a liposomal drug preparation (Gutyj et al., 2013). According to the literature data, it is known that liposomes reduce general toxic effect on animals' body of appropriate substances for pharmaceutical use and increase their therapeutic effect. With the help of liposomes we can reduce the dosage of drugs without reducing the effectiveness of their action, increase the ability of drug substances contained in liposomes to penetrate cells, reduce immune and allergic reactions; extend time of presence in the body of medicinal drugs introduced in the liposomes (Seredenko et al., 1993; Dudnichenko et al., 2001).

An important step in developing a new drug is toxicological study which begins with vivisection. This is the first phase, which aims at obtaining information on the hazards of the investigated material in terms of short-term studies and prospects for further tests. According to the accepted regulations, before recommending production and widespread use of a new drug, one should also conduct a chronic study of its safety in the conditions of biological experiment on laboratory animals. Defining the overall toxicity parameters of new functional drugs is an integral part of the complex research, determining their safety and efficacy (Kosenko et al., 1997).

The objective of this research was:

1. To determine the toxicity of «Injectable Mevesel» drug on laboratory animals after a single administration (acute toxicity).

2. To study accumulation properties of the drug in white rats.

3. To study the toxicity of «Injectable Mevesel» drug on laboratory animals after prolonged administration (chronic toxicity).

The study was conducted in accordance with the guidelines "Toxicological control of new means of protecting animals" and "Preclinical studies of veterinary drugs" (Malanin et al., 1988; Kosenko et al., 1997).

MATERIALS AND METHODS

Determination of acute toxicity of drug parameters.

Experiments to study acute toxicity of "Injectable Mevesel" drug were performed on 30 white rats, 2 – 3 months of age, weighing 160 – 180 g, and 30 white mice, 2 – 3 months of age, weighing 19 – 21 g. Drug was administered to test animals once intragastrically and intramuscularly.

We conducted the experiment on 16 groups of animals, 6 animals each. The drug was administered to white mice and rats in the following doses: 5000, 15000, 25000 and 50000 mg/kg.

After the drug administration we monitored laboratory animals for 14 days. On the first day of the experiment the animals were under constant supervision. We took into account the following indicators: general condition, appearance, behavior of animals, intensity and nature of motor performance, presence of seizures, coordination, response to external stimuli (tactile, sound, light), condition of hair, visible mucous membranes, relation to the food, rhythm, respiratory rate, time of occurrence and nature of intoxication, its severity, course, time of death or recovery of animals.

During the experiment we took into account loss of laboratory animals and, depending on the dose, calculated median lethal dose (LD_{50}) of the dosage form by G. Koerber (1931) (Litvinova et al., 2001).

Study of accumulation properties.

Properties of the drug accumulation were studied on 12 white rats weighing 150-160 g. Rats were divided into 2 groups: control and study ones. To determine the degree of accumulation of the drug we used K.S. Lima et al method (1961) (Malanin et al., 1988).

The drug was administered to test animals intramuscularly once a day. The experiment lasted for 20 days. We administered "Mevesel" to rats starting with the dose of 0.1 LD_{50} (5000 mg/kg), the dose being increased by 1.5 times every 4 days. At the end of the experiment "Injectable Mevesel" dose was 5,3 LD_{50}. During the experiment we took into account general condition and death of rats. Depending on the dose, we calculated accumulation coefficient by the formula proposed by Yu.S. Kagan and V.V. Stankevich (1964):

$C_{acc} = LD_{50\,n} / LD_{50\,1}$,

where C_{acc} is accumulation coefficient, $LD_{50\ n}$ and $LD_{50\ 1}$ are median lethal doses with multiple and single administration respectively.

To identify the effect of drug in the said doses upon body at the end of the experiment with regard to accumulation on the next day after the last administration of "Injectable Mevesel", animals from each group were weighed, decapitated with mild ether anesthesia, and blood samples for hematological and biochemical research were taken. Animal organs were taken after dissection, then we weighed and calculated ratios of weight compared to the control group (Sidorov, 1967; Shtabskiy and Kagan, 1974).

Study of "Injectable Mevesel" toxicity in chronic experiment.

In the study of chronic toxicity we were guided by the results obtained in the course of acute toxicity. The drug was administered daily by intramuscular injection. During the experiment we monitored clinical condition and behavior of animals.

Chronic toxicity was studied in 40 white rats weighing 90 – 110 g. We formed 4 groups equal in number and weight, 10 rats per each. The first (1st) group of animals was the control one. We injected them isotonic sodium chloride solution. Animals of other three groups were administered "Injectable Mevesel" in the following dosages: 2nd group – 250 mg/kg (1/20 LD_{50}), 3rd group – 100 mg/kg (1/50 LD_{50}) and 4th group – 50 mg/kg (1/100 LD_{50}). In the chronic experiment "Injectable Mevesel " was administered to rats for 30 days.

On the 31-st day from the start of drug administration we tested detoxifying liver function of 5 white rats from each group using hexenalum sample (Rozin, 1964). For this purpose laboratory animals were injected intraperitoneally with 1% solution of hexenalum in a dose of 45 mg/kg. Then we recorded average sleep time from the moment when the animal took lateral position.

Simultaneously, we conducted swimming test on 5 other rats by M.L.Rylova (Rylova, 1964). A glass aquarium was used for the experiment. Height of water in aquarium measured 50 cm. Water temperature was 12-13 °C. We attached clog to the test animals (metal batch) of 5% of body weight. Before the experiment, rats were weighed, and clogs were attached to their tails that corresponded to their weight. Then both test and control animals of about the same weight were allowed to swim. The animals were monitored to constantly swim. An indicator of efficiency was the time for which the animal could survive on water. Animals swam until complete sinking to the bottom.

On the next day laboratory animals were decapitated with the help of light ether anesthesia, then we performed hematological and biochemical research according to commonly recognized methods, made dissection and determined body mass coefficients as compared to the control group.

RESULTS

Study of acute toxicity.

Under the conditions of intragastric and intramuscular administration, there were no cases of death of white rats, only short-term inhibition of laboratory animals, to whom the drug was administered in a dose of 10.0 ml, due to the injection of large amounts of drug. On the following day no changes in the clinical condition of animals of study groups were observed.

Materials of research data obtained, and calculations of the drug LD_{50} are presented in Table 1.

The drug LD_{50} with intramuscular administration to white rats exceeds 50000 mg/kg. "Injectable Mevesel" belongs to low-toxic agents: class 4 according to GOST 12.1.007-76.

Under the conditions of intramuscular administration, there were no cases of death of white rats, only short-term inhibition of laboratory animals, to whom the drug was administered in a dose of 1.0 ml, due to the injection of large amounts of drug. On the following day no changes in the clinical condition of animals of study groups were observed.

Laboratory findings are shown in Table 1.

The drug LD_{50} with intramuscular administration to white mice exceeds 50000 mg/kg.

" Injectable Mevesel" belongs to low-toxic agents: class 4 according to GOST 12.1.007-76. LD_{50} with its intramuscular administration to laboratory animals (white mice and rats) exceeds 50000 mg/kg.

Results of "Injectable Mevesel" accumulation properties study.

During study of the properties of "Injectable Mevesel" in the doses with regard to accumulation, there

Table 1. Parameters of "Injectable Mevesel" drug toxicity on white rats and white mice

Number of animals in the group	Dose of drug, mg/kg	Number of lost animals		
		total	%	median lethal time
White rats				
6	5000	0	0	0
6	15000	0	0	0
6	25000	0	0	0
6	50000	0	0	0
White mice				
6	5000	0	0	0
6	15000	0	0	0
6	25000	0	0	0
6	50000	0	0	0

were no lethal cases of test animals. In total, the average dose of drug administered was 162500 mg/kg, and accumulation coefficient was respectively 5.3, indicating mild accumulation properties of the drug.

After a long-term intramuscular administration of drug to rats in increasing doses, liver mass coefficient increased by 30% ($P < 0.05$) (Table 2).

Table 2. Coefficients of internal organs mass of white rats during study of accumulation properties of Injectable Mevesel (M±m, n=12)

Internal organs	Control	Test
Liver	33.0±0.4	43.5±2.41*
Heart	3.4±0.16	4.2±0.31
Spleen	3.7±0.34	8.3±1.8
Lungs	8.4±0.89	11.2±1.89
Right kidney	3.3±0.20	4.2±0.30
Left kidney	3.7±0.21	4.0 ±0.21

* here and then $P<0.05$

Parameters of mass coefficients of lungs, heart, kidneys and spleen over the course of research were similar to those of rats of control group.

Therefore, long-term intramuscular daily administration of "Injectable Mevesel" drug during 20 days affected functional status of liver.

It was established during study of morphological blood parameters of rats after intramuscular "Injectable Mevesel" administration at increasing doses that basic blood parameters of the test group were similar to those in rats of control group (Table 3).

Table 3. Morphological blood parameters of white rats on the 21-st day of the experiment for accumulation properties of "Injectable Mevesel" (M±m, n=12)

Parameters	Group	
	control	test
Hemoglobin, g/l	76.6±5.74	76.7±5.27
RBC, T/l	4.8±0.40	4.4±0.12
Hematocrit, %	32.0±2.08	27.0±1.70
Cell-color ratio	0.72±0.05	0.78±0.05
Mean cell hemoglobin, pg	16.0±1.15	17.5±1.01
Mean cell volume. mcm^3	67.0±4.01	61.7±2.39
WBC, g/l	3.7±0.76	5.4±1.53
Eosinophiles, %	4.6±0.67	4.7±0.67

Neutrophiles, %	21.3±1.83	33.3±1.80*
Lymphocytes, %	72.3±2.90	61.3±1.33*
Monocytes, %	1.7±0.33	1.3±0.67

Probable changes were found only in counting leukocyte profile. Presumable increase in number of neutrophils by 36.1%, and presumable reduction in number of lymphocytes by 15.2% were established compared with the control group.

It was established that long-term administration of the drug at increasing doses significantly influenced some biochemical parameters of test animals (Table 4).

Table 4. Biochemical blood parameters of white rats on the 21-st day of the experiment for accumulation properties of "Injectable Mevesel"

Parameters	Animal group	
	control	24-th day after administration
Total proteins, g/l	8.1±0.35	7.6±0.41
ALT, U/l	70.5±6.43	63.5±14.60*
AST, U/l	202.4±10.54	256.8±23.11*
LF, U/l	157.5±31.8	340.2±39.48*
Total lipids, g/l	8.4±1.00	7.0±1.73
Urea, mmol/l	6.2±0.35	5.7±0.46
Creatinine, mmol/l	107.9±15.5	152.6±17.1
Glucose, mmol/l	6.6±0.22	3.2±0.72*

Thus, ALT activity apparently decreased compared with rats in the control group by 11.0%, AST activity apparently increased by 12.7%, and alkaline phosphatase increased by 2.2 times, due to the malfunction of liver tissue functioning and negative effect of increasing doses of the drug on animals.

Therefore, under the conditions of long-term (20 days) daily administration of increasing doses of "Injectable Mevesel", it causes significant degradation of the membranes of hepatocytes, as evidenced by increased activity of intracellular enzymes ALT, AST and alkaline phosphatase.

Results of "Injectable Mevesel" toxicity at chronic experiment.

During the experiment for study of chronic toxicity, loss of rats was not recorded.

Results of hexenalum sample and swimming test, conducted after the end of administering drug during chronic experiment, are shown in Table 5.

Table 5. Results of functional tests (M±m, n=20)

Animal group	Drug dose	Hexenalum sample	Swimming test
		Median sleep time, min	Median swimming time, min
1	control	28.8±1.66	12.84±1.51
2	1/20 LD_{50}	36.6±1.66*	9.01±1.32*
3	1/50 LD_{50}	31.2±0.65	11.22±1.82
4	1/100 LD_{50}	29.8±1.99	13.13±1.68

Statistically significant ($P<0.05$) increase in median sleep time with simultaneous reduction in median swimming time ($P<0.05$) were observed in animals of the 2nd group. These changes testify to the impairment of detoxifying liver function and general inhibiting effect upon organism, caused by long-term administration of the drug in a dose of 1/20 LD_{50}. The drug in doses of 1/50 LD_{50} and 1/100 LD_{50} did not affect the results of functional tests, which is connected with normal functioning of liver tissue and absence of negative effect upon animals of the 3rd and 4th groups.

On the 31-st day of the experiment, at administration of the drug in doses of 1/20 LD_{50}, 1/50 LD_{50} and 1/100 $LD_{50,}$ apparent changes in liver mass cooefficient in comparison with the control group have been recorded (Table 6).

Table 6. Coefficients of internal organs mass of white rats on the 31-st day during study of chronic toxicity of Injectable Mevesel (M±m, n=6)

Internal organs	Drug doses			
	Control	1/20 LD_{50}	1/50 LD_{50}	1/100 LD_{50}
Lungs	8.4±0.34	9.6±1.41	8.2±0.37	9.5±0.71
Liver	33.1±0.49	48.3±2.80*	31.3±0.73*	29.8±0.49*
Right kidney	3.3±0.20	3.7±0.21	3.3±0.11	3.1±0.20
Left kidney	3.7±0.21	3.9±0.20	3.4±0.19	3.3±0.11
Heart	3.4±0.16	3.8±0.48	3.8±0.41	3.6±0.42
Spleen	5.5±0.34	5.8±0.30	4.8±0.42	4.9±0.46

Therefore, administration of "Injectable Mevesel" in doses of 1/20 LD_{50}, 1/50 LD_{50} and 1/100 LD_{50} for 30 days significantly affects functional state of internal organs of experimental animals (liver).

In the study of morphological blood parameters in rats after intramuscular administration of "Injectable Mevesel" in different doses, a downward trend in hemoglobin values and color index, and apparent reduction in the number of white blood cells compared to control group, have been recorded in all experimental groups (Table 7).

Table 7. Morphological blood parameters of white rats on the 31-st day of the experiment for chronic toxicity of "Injectable Mevesel" (M±m, n=24)

Parameters	Group			
	C	1/20	1/50	1/100
Hemoglobin, g/l	76.6±5.74	70.6±4.27	93.3±13.03	93.3±2.23
RBC, T/l	4.8±0.40	5.5±0.42	5.4±0.60	5.5±0.37
Hematocrit, %	32.0±2.08	28.0±2.50	36.7±4.20	27.0±4.45
Color index	0.72±0.05	0.57±0.06	0.77±0.02	0.77±0.05
Mean cell hemoglobin, pg	16.0±1.15	12.8±0.26	17.1±0.45	17.1±1.09
MCHC, g/dl	23.8±0.38	24.9±0.93	25.3±0.67	34.6±0.67***
Mean cell volume, mcm^3	67.0±1.15	51.5±2.12*	67.8±1.57	69.7±3.27
WBC, 10^9/л	3.7±0.76	3.5±1.32	3.7±1.35	4.2±1.62**
Eosinophiles, %	4.7±0.67	6.0±1.15	6.0±1.16	4.3±0.68
Neutrophiles, %	21.3±2.90	22.7±1.87	31.0±3.02	11.75±2.39
Lymphocytes, %	72.3±2.90	70.0±3.06	61.3±1.70*	62.7±1.33*
Monocytes, %	1.7±0.33	1.3±0.70	1.7±0.70	2.0±0.31

* P<0.05; ** P<0.01; *** P<0.001

Thus, during administration of "Injectable Mevesel", lymphocytes count in doses of 1/50 LD_{50} and 1/100 LD_{50} significantly reduced by 8.5 % and 8.7 % respectively. In the 4th group increase in RBC and presumable increase in MCHC have been recorded during administration of "Injectable Mevesel" in a dose of 1/100 LD_{50}.

Apparent increase in WBC count and apparent reduction in lymphocytes in the 3rd and 4th groups animals as compared to the control group have been recorded in the study of leukocyte profile.

Sufficiently high level of alkaline phosphatase in blood serum of rats, 2.1 times higher respectively than in rats of the control group was recorded at administration of "Injectable Mevesel" in a dose of 1/20 LD_{50}. "Injectable Mevesel" in doses of 1/20, 1/50 and 1/100 LD_{50} reduced ALT level in blood serum in comparison with the rats of control group, and simultaneously increased AST level in animals of the 2nd group (Table 8).

In studying toxic effects of "Injectable Mevesel" in chronic experiment, it has been established according to the values of haematological and biochemical parameters that, in spite of low toxicity, the drug in a dose of 1/20 and 1/50 LD_{50} affected the hematopoietic function (increase in the number of white blood cells, reduction of lymphocytes in the 3rd and 4th animal groups) and caused abnormal liver function.

Table 8. Biochemical blood parameters of white rats on the 31-st day of the experiment for chronic toxicity of "Injectable Mevesel" (M±m, n=24)

Parameters	Animal groups			
	Control	1/20 LD_{50}	1/50 LD_{50}	1/100 LD_{50}
Total proteins, g/l	8.4±0.25	8.76±0.78	9.0±0.29	9.1±0.47
LF, U/l	157.5±31.8	329.9±38.9*	187.8±51.1	176.3±16.05
ALT, U/l	70.5±6.43	52.0±7.13	54.4±6.73	53.1±7.97
AST, U/l	202.4±10.54	284.9±9.94**	202.9±24.34	182.3±44.26
Total lipids, g/l	8.4±1.00	8.0±2.87	7.3±0.62	8.2±0.98
Urea, mmol/l	6.2±0.35	4.8±0.38	4.9±0.30	7.4±0.53
Creatinine, mmol/l	107.9±15.5	107.0±8.47	114.8±12.6	128.5±10.90
Intermediate mass molecules	1.19±0.01	1.12±0.06	1.16±0.01	1.15±0.03

* P<0.05; ** P<0.01

CONCLUSIONS

New domestic drug "Injectable Mevesel" belongs to class 4 toxicity criteria, i.e. low-toxic substances.

"Injectable Mevesel" refers to drugs with subtle accumulation properties. Accumulation coefficient for white rats is 5.3. Administration of the drug in increasing doses significantly affects the functional state of internal organs of experimental animals (liver) and causes significant degradation of the membranes of hepatocytes, as evidenced by increased activity of intracellular ALT, AST enzymes and alkaline phosphatase.

At administration of "Injectable Mevesel" to white rats for 30 days affected leucopoiesis and caused hepatic impairment irrespective of the dosage.

REFERENCES

Gutyj, B.V., Hufriy, D.F., Khariv, I.I. (2013). *Technical conditiona of Ukraine: TU 21.2-00492990-003:2013 «Injectable Mevesel» drug; approved by the State Veterinary and Phytosanitary Service of Ukraine on 21.10.2013.* Lviv. (in Ukrainian).

Dudnichenko, A.S., Krasnopolskyy, Yu.M., Shvets, V.I. (2001). *Liposomal Medications in Experiment and Clinical Picture*. Kharkiv: Ra-Karavella. (in Russian).

Seredenko, M.M., Nazarenko, A.I., Kukoba, T.V. (1993). Vplyv liposom na stan tkanynnogo dyhannja u tvaryn pry gostrij gipoksychnij gipoksii'. *Physiological Magazine, 4*, 100-103. (in Ukrainian).

Kosenko, M.V., Malyk, O.H., Kotsyumbas, I.Ya., Paterega, I.P., Chura, D.O. (1997). *System of toxicological control of agents for protecting animals: Guidelines*. Kiev. (in Russian).

Malanin, L.P., Morozov, A.P., Selivanov, A.S. (1988). *Metodicheskie ukazanija po opredeleniju toksicheskih svojstv preparatov, primenjaemyh v veterinarii i zhivotnovodstve. Veterinary Drugs: Reference Book.* Moscow: Agropromizdat. (in Russian)

Litvinova, N.V., Filonenko-Patrusheva, M.A., Frantsuzova, S.B., Khrapak, V.V. (2001). *Preclinical study of drugs: Guidelines*. Kiev: Avicenna. (in Russian).

Rozin, D.G. (1964). Sovremennaja ocenka toksichnosti hlorproizvodnyh uglerodov zhirnogo rjada po geksenalovomu testu na belyh myshah. *Pharmacology and Toxicology, 5*, 613-614. (in Russian)

Rylova, M.L. (1964). *Metody issledovanija hronicheskogo dejstvija vrednyh faktorov sredy v jeksperimente.* Moscow. (in Russian)

Shtabskiy, B.M., Gzhegotskiy, M.I., Gzhegotskiy, M.R. (1980). K metodike opredelenija srednesmertel'nyh doz i koncentracij himicheskih veshhestv. *Hygiene and sanitary, 10*, 49-51. (in Russian)

Shtabskiy, B.M., Kagan, Yu.S. (1974). K ocenke kumuljativnyh svojstv himicheskih veshhestv po indeksu i standartizovannomu kojefficientu kumuljacii. *Hygiene and sanitary, 3*, 65-68. (in Russian)

Sidorov, K.K. (1967). *O nekotoryh metodah kolichestvennoj ocenki kumuljativnogo jeffekta. Toksikologija novyh promyshlennyh himicheskih veshhestv*. Moscow: Medicine. (in Russian)