Kanat Sarsenbayev et al /J. Pharm. Sci. & Res. Vol. 11(5), 2019, 1742-1748

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Study of mutagenicity, embryotoxic action, immunotoxicity and allergenicity of Xenon difluoride

Kanat Sarsenbayev[1*], Mariana Sarsembayeva[2], Kulzada Lakhanova[3], Gulnar Kylyshbaeva[4], Assiya Mamytova[2], Zharas Berdenov[1]

[1]*Faculty of Science, Eurasian National University after L.N. Gumilyov, Astana, Kazakhstan*
[2]*Department of biology, South Kazakhstan Pedagogical University, Shymkent, Kazakhstan*
[3]*Department of biology, International Kazakh-Turkish University, Turkestan, Kazakhstan*
[4]*Department of biology, International Humanitarian Technical University, Shymkent, Kazakhstan*

Abstract

Xenon difluoride is a strong oxidant. Referring to artificial antibacterial substances, it also has an extremely chemically active xenon radical. This compound is one of the promising for creating very rare aerosol anti-infective agents. Despite more than 50 years of experience in studying the chemical activity of this compound, its biological activity in standard preclinical studies has not been studied. The aim of the research was to study mutagenicity, embryotoxic action, immunotoxicity and allergenicity of Xenon difluoride as perspective aerosol anti-infection substance. The work was done on rats and mice according to the methods officially approved in Russia and Kazakhstan. The study in rats and mice showed no signs of immunotoxicity and allergy of xenon difluoride. Xenon difluoride has an embryotoxic effect with the intragastric administration to pregnant rats at a conventionally maximal therapeutic dose of 50 mg/kg at different gestation times. This is manifested by high embryonic death and disruption of the processes of skeleton formation. According to the micronucleus test, xenon difluoride in the form of a 0.2% solution, administered intranasally, does not have a mutagenic effect. The mutagenic effect of xenon difluoride is manifested with the intragastric administration of the maximum tolerated, sublethal dose (100 mg/kg). The high oxidizing ability of xenon difluoride is shown. The boundaries of mutagenicity and embryotoxicity are determined. The absence of allergenicity and immunotoxicity in the tested concentrations of xenon difluoride was shown.

Keywords: xenon difluoride; preclinical studies; mutagenicity; embryotoxic action; immunotoxicity; allergenicity

INTRODUCTION

Xenon difluoride is a strong oxidant. Referring to artificial halogen-containing substances, it also has an extremely chemically active xenon radical. Synthesis of this compound takes place under conditions of a glowing electric discharge, i.e. with energy absorption. When interacting with water or aqueous solutions, the molecule disintegrates. The mechanism of the reaction of hydrolysis of XeF_2

$XeF_2+H_2O \rightarrow 4HF+2Xe+FOH + 3.7$ kJ / mol

with a reaction rate constant $k_1 = (6.1\pm0.6)\cdot10^{-14}$ cm^3/s (Medy, 1990).

Highly reactive compounds are formed in this case, such as hypofluorides (a new class of compounds with xenon oxidation degree equal respectively +2, +4, +6 : $Xe(OF)_2$, $XeOF_2$ и $XeOF_4$, hydrogen peroxide and radicals: peroxide and hydroxide, having a high oxidative, disinfecting, degassing ability and detoxification ability. Once in the body, free radicals find themselves in a very favorable environment for the further development of chain reactions. Furthermore, in the presence of molecular oxygen, such reactions tend to branch, accompanied by damage to cell membranes, proteins, nucleic acids (Neyding et al., 1974).

When fluorinating water that contains organic macromolecular compounds (proteins, nucleic acids, lipids), their fluorinated derivatives are inevitably formed. Fluoroorganic compounds can spontaneously turn into very long-living and highly reactive free-radical forms. Although the stationary content of free radicals in water is small, they give rise to chain reactions during which new free radicals arise. Known enzymatic and non-enzymatic agents of antioxidant protection of the body are not suitable for inactivation of free radicals of this kind. It is very likely that in order to fight them, the body starts its most effective, but at the same time, a very dangerous defense system. Since the outer radical can be inactivated (turn into a more or less stable molecule) only through the recombination reaction with another free radical, the cells begin to actively generate active forms of oxygen – superoxide radical, nitric oxide and others, which easily enter recombination reactions with other radicals. Such protection, however, requires a significant expenditure of oxygen, which is accompanied by some degree of tissue hypoxia. In addition, the excessive production of reactive forms of oxygen can lead to the development of new chain processes that deplete the body's antioxidant reserves. Therefore, the treatment of tissues with xenon difluoride, which during the breakdown forms highly active short and long-lived radicals, can with a high degree of probability lead to the development of chronic diseases of varying severity (Nekrasov, 1973).

The following study is devoted to the study of the safety of xenon difluoride, which was developed by “Xenon” LLP, within the requirements of preclinical studies (Aleynikov, 2002).

The set of chemical properties and characteristics of xenon difluoride allows to assume the presence of antibacterial, fungicidal and antiviral properties (Fisenko, 2000) and, therefore, can be used as a basis for disinfection or sterilization in the field of sanitation and medical industrial hygiene.

The aim of the research was to study mutagenicity, embryotoxic action, immunotoxicity and allergenicity of Xenon difluoride as a perspective aerosol antiinfection substance.

MATERIALS AND METHODS

Methods for the study of immunotoxicity

The study was planned and conducted in accordance with the guidelines (Tussupbekova, 2005). The study was conducted on mongrel white mice of both sexes. The animals were on the usual ration of the vivarium.

Aqueous solutions of xenon difluoride were administered intragastrically at a dose of 10 mg/kg (1/10 of LD50) and an order of magnitude less than 1 mg/kg. The period of administration of the drug is 5 days.

At the end of the experiment, the animals were sacrificed by decapitation. In the course of the experiment, the following indicators were studied: the overall cellularity of immunocompetent organs (spleen, thymus, bone marrow), the number of antibody-forming cells, the delayed type hypersensitivity reaction, and the phagocytic activity of macrophages.

To assess the humoral immune response, the number of Antibody-forming cells (AFC) in the spleen of mice was determined by immunization with a T-dependent antigen. This method is based on obtaining local hemolysis - on anti-erythrocyte antibodies of secreted AFC immunized animals to lyse in the presence of complement erythrocytes of a ram. Xenon was administered according to the schedule for 5 days. On the 5th day, the erythrocytes of the ram were injected intraperitoneally. After immunization on day 5, the number of AFC was determined. The animals are sacrificed with the help of a cervical dislocation, the spleens are extracted and the cell suspension is prepared by means of a glass homogenizer. The suspension is carried out in a Hanks solution. Cell slurry of splenocytes together with erythrocytes of ram and complement of guinea pig are incubated at 37°C for 45 minutes. Then, the absolute and relative AFC content is microscopized and counted.

Influence on the cell link was carried out in the Delayed-type hypersensitivity reaction (DTHR). Xenon difluoride was administered according to the scheme for 5 days at a dose of 10 and 1 mg/kg. As a control, a group was taken which received distilled water as a solvent. On the last day of drug administration, the animals are immunized with erythrocytes of a ram. After 5 days, the permitting injection of the antigen is introduced into the animal's hind leg, a physiological solution is introduced into the contralateral. After 24 hours, the animals are sacrificed and the weight of the "experienced" and "control" paws is determined. The index of inflammation is determined by the formula:

$II = Me\text{-}M_c/M_c \times 100\%$ (1)

, where II - Index of inflammation, M_e and M_c are the mass of the "experienced" and "control" paws.

Determination of the overall cellularity of immunocompetent organs was performed on the 7th and 14th day of observation. Mice according to the scheme received xenon difluoride in doses of 10 and 1 mg/kg. As a control, the groups that received distilled water were taken. Mice were sacrificed by cervical dislocation, spleen, thymus and bone marrow were removed. Using a glass homogenizer, a cell suspension is prepared in Hanks solution. The suspensions are washed and centrifuged. The concentration of nucleated cells in 3% acetic acid is counted. Determine the absolute and relative values.

To assess the effect of the drug on the nonspecific part of the immune system, the phagocytic activity of macrophages in the NTT test was determined. In animals after the intraperitoneal administration of Hanks solution, exudate of peritoneal macrophages was collected. Glass slides with macrophage suspension, working solution of NTT, Hanks solution in a wet chamber were thermostated at 37 °C. Then, after fixation, stained with 1% neutral red. The macrophages were counted in a spontaneous and induced test.

Study of allergenicity

Induction of delayed-type hypersensitivity (DTH) by administration of chemical allergens to mice in complete CFA was performed in non-linear white mice weighing 18-20 g. Sensitization was carried out once by intradermal injection into the base of the tail with 60 µl emulsion of the drug in the CFA. A 10 mM solution of xenon difluoride in CFA was taken in a 1: 1 ratio. The emulsion is prepared on a Hanks solution. To detect sensitization after 5 days, 40 µl of a 10 mM solution of the test preparation in Hanks solution was injected into the hind paw in mice. The indicators were considered after 6 and 24 hours. The intensity of the reaction (IR) was calculated from the formula:

$IR=Me\text{-}Mc/Mc \times 100\%$ (2)

where Me – the size of the experimental foot in cm, Mc – the size of the control foot in cm.

Indirect mast cell degranulation test

In the experiments, Sprague Dawley imbred rats weighing 250-300 g were used. Three groups of animals were taken: 1) the group receiving xenon difluoride at a dose of 1/10 of LD50 by the intragastric administration - 32.9 mg/kg, 2) the control group receiving solvent - distilled water, 3) intact animals. The animals were sacrificed by rapid bloodletting under ether anesthesia. Then 8 ml were injected intraperitoneally heated to 37 °C Tyrode solution without glucose. Then, after 1-1.5 minutes of gentle massage of the abdominal wall, the incision was made with scissors along the midline of the stomach, 1.5-2.0 cm long. The carcass was turned upside down and the exudate was collected, draining from the intestine loops into a test tube moistened with heparin (centrifugation 3000 rpm for 3-5 minutes with the addition of heparin). Preparations were prepared on skimmed subject glasses, stained with 0.3% alcohol solution of neutral red and dried at room temperature. To 0.03 ml. suspended mast cells were added with 0.03 ml. serum of the experimental animal and 0.03 ml of the test preparation previously diluted 100-fold (1% xenon difluoride solution). The preparations are covered with a coverslip, whose edges are lubricated with petroleum jelly, then incubated for 15 minutes in a thermostat at a temperature of 37 °C. The preparations are microscopically enlarged x20. The evaluation of the results was carried out by a differential method of counting, by calculating the degranulation index of mast cells by the formula:

$DIMC = (1a + 2b + 3c + 3d) / 100$ (3)

Where a, b, c, d - the average of three repetitions of the number of degranulated cells with different degrees of degranulation (weak, pronounced, sharp and completely degranulated cells).
The indices a, b, c, d are counted from a hundred cells. The reaction is considered positive if the DIMC exceeds 2.0.

Methods for investigating embryotoxicity
The study was planned and conducted in accordance with the guidelines (Salmi et al., 2008). Experiments were carried out on white outbred rats in the amount of 29 females weighing 180-220 g at the age of 2.5 months. The content of rats was carried out in accordance with the rules used for experimental and other scientific purposes.
For the administration of the preparation, animals were divided into 4 groups in each group of 5 animals: group 1 from day 1 to day 6, group 2 from group 6 to day 16, group 3 from day 16 to day 20 at the highest dose 50 mg/kg 2 ml, 4 group - from 6 to 16 days of pregnancy 0,5% therapeutic dose intranasal by 20 µl into each nostril. Control animals received an equivalent volume of solvent. Terms of investigation of the state of the offspring: fruits - on the 20th day of pregnancy.
Mating was recorded with vaginal smears (duration of two estrous cycles).
To study the state of offspring in the antenatal period of development, females were killed on the 20th day of pregnancy, then the animals were opened and counted the number of yellow bodies in the ovaries and implantation sites in the uterus, the number of live and dead fetuses (per female). Pre and postimplantation mortality rates were calculated from formulas:

$$\text{Preimplantation mortality} = \frac{\#\ of\ yellow\ bodies - \#\ of\ implantation\ sites}{\#\ of\ yellow\ bodies} \times 100\% \quad (4)$$

$$\text{Postimplantation mortality} = \frac{\#\ of\ implantation\ sites - \#\ \text{of live fetuses}}{\#\ of\ implantation\ sites} \times 100\% \quad (5)$$

Fruits were weighed, an external examination was performed and their cranio-caudal size was determined. After that, some of the fruits were placed in Bowen's solution for fixation and subsequent examination of the internal organs condition according to Wilson's method in the modification of A.P. Dyban. The remaining part of the fruit was fixed in 96% alcohol and stained using the Dawson method in the modification of A.P. Dyban to assess the processes of ossification.

Cytogenetic methods
The protocol for carrying out the micronuclear test corresponds to the methodological recommendations (Preckel et al., 2006). The experiments were carried out on white laboratory rats, which were kept in conditions of free access to water and food with a 12-hour light regime.
The experiment was divided into several series corresponding to different experimental and control groups. In the first series, the drug was administered at a dose corresponding to the therapeutic dose (single intranasal administration of 0.2% aqueous XeF_2 solution) and sublethal dose (100 mg/kg XeF_2 intragastrically in 4% aqueous solution), only to males (3 rats) with cell fixation material one day after the administration. In the second series, the drug was administered at a dose corresponding to the therapeutic dose for 4 days to females and males (3 rats each), intranasal administration of 0.2% aqueous XeF_2 solution. As a negative control, animals were used intragastrically to receive an amount of a solvent corresponding to the amount of the solvent for the intragastric administration of the sublethal dose. As a positive control, cyclophosphamide was used, which was administered intraperitoneally at a dose of 20 mg/kg.
Bone marrow of the rat was obtained from the femur (Lachmann et al., 1990). Smears of the bone marrow were painted with the help of dyes of azur-eosin by Romanovsky and eosin-methylene blue by May-Grunwald. Analysis of bone marrow smears was performed at a 1000-fold magnification with an immersion objective. A binocular microscope MC300 from Micros (Austria) was used.
From each animal, 2000 polychromatic red blood cells were analyzed for micronuclei. Also counted 500 red blood cells with differentiation for polychrome and mature to determine the relationship between them.

Statistical methods
Statistical processing of the obtained results was carried out by parametric statistics methods with an assessment of the significance of differences in the Student. In a number of cases, nonparametric estimation methods were used: the Chi-square test, the Mann-Whitney U test, the Kolmogorov-Smirnov test (Zhang, 2000).
The calculation of toxic doses was carried out by the method of test analysis (European Medicines Agency, 1997).

$$y = \square(\alpha + \beta \cdot x) \quad (6)$$

where y is the proportion of dead animals, x is the dose of the drug, Φ is the distribution function of the standard normal variable, and α and β are regression coefficients.
An approximate 95% confidence interval for this toxic dose was calculated using the result of Feller's theorem, as a solution of the equation for λ ($\lambda = -\alpha/\beta$):

$$\lambda^2(b^2 - t^2 v(b)) + 2\lambda(ab - t^2 c(a, b)) + (a^2 - t^2 v(a)) = 0 \quad (7)$$

Calculation of the sample var(y(x)) for this dose was carried out according to the formula:

$$\text{var}(y(x)) = \{\varphi(\alpha + \beta \cdot x)\}^2 \{v(\alpha) + 2\, xc(\alpha, \beta) + x^2 v(\beta)\} \quad (8)$$

where $\varphi(\alpha + \beta \cdot x)$ is the density of the standard normal distribution at the point x.

Results and Discussion

Study of mutagenicity
The mutagenicity study was carried out using a micronuclear test, which is the generally accepted screening test for mutagenicity (Al Tmimi et al., 2015). A 0.2% xenon difluoride solution administered intranasally in an amount of 40 µl was used as the therapeutic dose (TD). As can be seen from Table 1, a significant increase in the number of micronuclei was observed in the case of a

positive control, i.e., under the action of cyclophosphamide, a substance known to have a significant mutagenic activity, and also under the action of a sublethal dose of xenon difluoride of 100 mg/kg. In the case of a sublethal dose of xenon difluoride, the average value of the number of micronuclei is about 2-2.5 times lower than in the case of cyclophosphamide. At the same time, there is no significant change in the number of micronuclei as in the case of a single action of the therapeutic dose of the drug, and with a similar effect within 4 days.

Table 1. Micronuclei test results

Group	X±c.o.	p (χ^2)	p (U)	p (K)
100 mg/kg XeF_2 ♂	11.67±1.07	<0,0001	0.049	<0.1
TD 1-fold ♂	4.17±0.70	1	1	>0.1
TD 4-fold ♂	4.75±0.84	0.68	0.500	>0.1
TD 4-fold ♀	4.25±0.72	0.16	0.184	>0.1
TD 4-fold ♂&♀	4.50±0.53	0.48	0.420	>0.1
cyclophosphamide 20 mg/kg ♂	29.08±4.44	<0,0001	0.049	<0.1
cyclophosphamide 20 mg/kg ♀	17.58±1.79	<0,0001	0.049	<0.1
cyclophosphamide 20 mg/kg ♂&♀	23,33±2,87	<0,0001	0,011	<0,025
Control -, water ♂	4,17±0,36	-	-	-
Control -, water ♀	5,90±0,91	-	-	-
Control -, water ♂&♀	4,95±0,51	-	-	-

Note: p (χ^2) — the probability corresponding to the chi-square test; p (U) is the probability corresponding to the U Mann-Whitney criterion; p (K) is the probability corresponding to the Kolmogorov-Smirnov criterion.

According to the micronuclear test protocol, the ratio of the number of polychromatophilic erythrocytes to the number of normochromatophilic erythrocytes was determined for all smears. The calculation was carried out for 500 cells. This indicator reflects the intensity of hemopoiesis, a possible deficit of cells located at different stages of erythropoiesis. The obtained data are given in Table 2.

Table 2. The ratio of polychromatophilic erythrocytes to normochromatophilic

Group	X±c.o.
100 mg/g XeF_2, ♂	1.14±0.10
TD once, ♂	1.68±0.18
TD within 4 days, ♂&♀	1.56±0.25
Control +, cyclophosphamide 20 mg/kg, ♂&♀	1.35±0.21
Control -, water, ♂&♀	1.15±0.09

Thus, according to the micronuclear test, xenon difluoride in a 0.2% solution does not have a mutagenic effect. The mutagenic effect of xenon difluoride is manifested in a sublethal dose of 100 mg/kg for rats.

Study of the embryotoxic action

The study was conducted on white pedigree rats (29 females weighing 180-220 g at the age of 2.5 months). Mating was recorded with vaginal smears (duration of two estrous cycles).

Four groups of experimental animals (5 each) were formed: 1 group - the administration of the study drug continued from 1 to 6 days at a dose of 50 mg / kg intragastric, 2 group - the administration of the study drug continued from 6 to 16 days at a dose of 50 mg/kg intragastric , 3 group - administration of the study drug continued from 16 to 20 days at a dose of 50 mg/kg intragastric, 4 group - the administration of the study drug continued from 6 to 16 days of pregnancy in a dose of 0.5% intranasal to 20 µl in each nostril. Control animals (3 rats, total 9) received an equivalent volume of solvent at the same time in the same regime as the animals of the experimental groups. Terms of investigation of the state of the offspring: fruits - on the 20th day of pregnancy.

The dynamics of the body weight of pregnant rats is presented in Table 3.

Table 3. The dynamics of the body weight of pregnant rats

Animal groups	1 week	2 weeks	3 weeks
Control	190.0 ± 1.29	220.0 ± 1.48	235.5 ± 2.40
XeF_2	182.0 ± 1.13	205.5 ± 1.49	219.7 ± 2.06

As can be seen, there is no significant difference in the body weight between experimental and control animals, only a tendency to a slight decrease in body weight in rats that received xenon difluoride during pregnancy.

The study of the indices of embryonic death in females after the administration of xenon difluoride made it possible to establish that preimplantation mortality in animals exceeded the control values by 2.7 times (Table 4). An increase in the indices of embryonic death in females of experimental groups may indicate that xenon difluoride causes the appearance of dominant lethal mutations in oocytes at different stages of maturation. At the same time, high embryonic death, observed in female experimental groups 1.9 times, may be a consequence of a violation of the fertility relationship caused by the toxic effect of xenon difluoride on the mother's body.

Table 4. The indices of the embryotoxic effect of xenon difluoride

Studied indicators	Control	XeF_2
Number of embryos per rat	8.0 ± 0.67	4.6* ± 0.33
Number of implantation sites per rat	9.5 ± 0.49	9.6 ± 0.30
Number of yellow bodies per rat	10.33 ± 0.67	9.55 ± 0.17
Preimplantation death	18.52 ± 10.31	51.83* ± 5.71
Postimplantation death	2.22 ± 2.24	1.31 ± 1.55
Cranio-caudal fetal size	3.73 ± 0.12	3.62 ± 0.09
Fetal weight	4.27 ± 0.34	3.71 ± 0.11

Note:* - $p < 0.05$

It should be noted that the consequence of cytogenetic disorders in germ cells can be not only the formation of genetically inferior gametes leading to the death of zygotes and embryos but also the appearance of offspring characterized by a violation of the development of external and internal organs, inhibition of ossification processes. In this regard, for more information on the mutagenic properties of chemical compounds in toxicological studies, a detailed study of the state of offspring is practiced. We studied the state of offspring obtained from female rats,

who suffered the introduction of the maximum tolerated a higher dose of xenon difluoride during pregnancy. When the macroscopic examination of the offspring of control groups (72 fetuses), gross external anomalies of development were not found, there was no teratogenic effect.
At macroscopic survey of all examined offspring of experimental groups (92 fetuses). The average weight and cranio-caudal size of the fruit were reduced in comparison with the control. Analysis of the internal organs of the fetus (Wilson method) showed that the progeny showed a significant increase in the number of fetuses with hemorrhages in various organs and tissues, edema of the subcutaneous tissue, cerebral microcephaly, ectopic kidneys (Table 5).

Table 5. Anomalies of fetal development (on the 20th day of the prenatal period)

Studied indicators	Control	XeF_2
Hemorrhage into the abdominal cavity	2	10
Ectopia of the kidneys	4	9
Hemorrhage in the pericardium	4	11
Microcephaly of the brain	-	5

The inhibition of ossification of the fetuses was also revealed (Table 6). To a large extent, this concerned the bones of the sternum and skull. The centers of ossification of the thoracic spine have been found asymmetrically and irregularly. In the experimental group, incomplete development of the cranial bones and too wide cerebral sutures are also noted.

Table 6. The parameters of the fetuses skeleton development (on the 20th day of the prenatal period)

The absence of centers of ossification:	control	XeF_2
Sternum (%)	3.7	3.3
Front limb (в%)	-	-
2nd metacarpal bone	3.7	15.9
3d metacarpal bone	2.9	8.2
4th metacarpal bone	1.7	6.05
Hindquarters (%)	-	-
2nd metatarsal bone	4.06	13.7
3d metatarsal bone	8.25	9.9
4th metatarsal bone	6.05	6.6
Lone Bone (%)	2.6	8.2

Thus, with a six-fold administration of xenon difluoride at a dose of 50 mg/kg (dose corresponding to half the maximum tolerated dose) to pregnant rats, an increase in preimplantation mortality of embryos, an increase in embryonic death rates, a decrease in body weight and craniocaudal size. We can state the presence of the embryotoxic effect of xenon difluoride.

Study of immunotoxicity and allergenicity
Evaluation of the effect on the humoral unit of the immune system of xenon difluoride was carried out by determining the amount of antibody-forming cells in the spleen.
According to the obtained data on the amount of AFC in the spleen, the animals receiving the two dose levels of 10 mg/kg and 1 mg/kg did not show the toxic effect of the drug on the antibody formation process. The results are shown in Table 7.

Table 7. The number of antibody-forming cells in the spleen upon administration of xenon difluoride

Groups	AFCC × 10^6/organ	%	Absolute
10 mg/kg	199.80±27.03	23.22±1.80	41.58±4.79
1 mg/kg	205.40±27.03	23.62±1.52	47.78±4.51
Control	212.20±17.59	25.04±1.63	52.73±4.01
Intact	213.20±12.66	25.40±2.36	53.57±2.37

In a parallel study of the standard values of peripheral blood, the same animals failed to detect significant changes in the indices compared to the animals in the control group. Although it can be noted in the group receiving 10 mg/kg of xenon difluoride, a statistically unreliable increase in the number of neutrophils and an increase in the number of basophils in this group compared with the control (Table 8).
The effect on the cellular link of the immune system was studied in a delayed type hypersensitivity reaction. The results were taken into account by the index of inflammation in percentage terms. According to the data obtained in Table 4, xenon difluoride in doses of 10 mg/kg and 1 mg/kg has no toxic effect on cellular immunity. The indices in both experimental groups are comparable with the results of the control group. According to the data obtained on the seventh day of the experiment, significant changes in the parameters of the experimental and control groups were not detected, although there was a slight decrease in viable cells in the spleen and bone marrow. The results are shown in Table 9.

Table 8. Hematologic indices in mice on the 5th day after the introduction of xenon difluoride, in the (antibody-forming cells) AFC definition groups

Mice	L, m/mm^3	Neutroph	Basoph	Eosinoph	Monoph	Lymph	Eryth., M/mm^3	Hb, g/l
10 mg/kg	3.82±0.85	39.83±6.3	0.35±0.2	0.7±0.5	17.5±0.6	41.6±6.6	4.7±0.2	128.2±6.8
1 mg/kg	5.63±1.3	22.84±3.2	0.28±0.08	0.5±0.3	17.0±0.6	59.3±2.8	9.1±0.2	127.6±6.8
Control	4.66±1.21	29.75±2.9	0.18±0.02	0.38±0.3	17.3±1.4	52.4 ±3.7	10.8±1.3	148.2±20.2

Table 9. General cellularity of immunocompetent organs on the 7th day after the administration of xenon difluoride

Groups	IS,%
10 mg/kg	25.62±6.6
1 mg/kg	27.36±10.4
Control	24.8±1.3

As follows from the data presented in Table 10 the administration of xenon difluoride in doses of 10 and 1 mg/kg does not affect the overall cellularity of immunocompetent organs and the number of viable cells.

Evaluation of the cellularity of immunocompetent organs was carried out on the 7th and 14th day of observation after a 5-day administration of xenon difluoride in doses of 10 and 1 mg/kg.

The phagocytic activity of macrophages in the NTT-test under the influence of xenon difluoride in two test doses does not undergo significant changes in comparison with the control data. The indices of spontaneous and pyrogen-induced phagocytosis of macrophages on the 7th and 14th days after the administration of xenon did not show the effect of xenon difluoride on the nonspecific part of the immune system. On the 7-14th day at a dose of 10 mg/kg, the spontaneous phagocytosis figures were reduced 2-fold compared to the control, but after stimulation, the figures obtained did not show a decrease in the reserve capacities of the macrophage cell, which indicates the absence of toxic effect of xenon difluoride on the phagocytic activity of cells (Table 11).

It can be stated that the results of the study of the effect of xenon difluoride on immunotoxicity under the conditions of our experiment do not give grounds to state the presence of any violations in the immune response.

In the study of the allergic effect of xenon difluoride in the delayed type hypersensitivity (DTH) induction test, as can be seen from Table 28, the obtained results did not reveal the ability of Xenon difluoride to cause DTH when administered intradermally to the PAF. Despite a slight increase in the intensity of the reaction after 6 hours in the experimental group, after 24 hours this indicator decreases and was comparable with the indices in rats of the intact group.

Table 10. General cellularity of immunocompetent organs after administration of xenon difluoride

Groups	General cellularity, $\times 10^6$/organ	Viability, %	Viability, $\times 10^6$/organ
Spleen			
1 mg/kg	263.20±19.74	72.60±3.21	190.35±13.90
10 mg/kg	261.40±21.45	73.00±2.14	189.89±13.63
control	264.00±21.03	74.20±3.86	194.77±15.74
intact	267.60±15.23	73.20±1.71	195.58±9.79
Thymus			
1 mg/kg	67.60±3.64	69.40±2.78	47.70±1.98
10 mg/kg	67.40±11.15	68.80±3.21	46.34±2.82
control	68.80±2.36	69.60±3.86	47.70±1.98
intact	69.80±2.36	70.00±1.71	48.73±1.37
Bone marrow			
1 mg/kg	213.80±13.3	71.80±2.57	153.67±8.21
10 mg/kg	212.20±14.37	72.00±2.36	153.43±12.27
control	215.40±15.02	72.60±4.07	155.92±13.09
intact	216.20±7.08	72.20±3.7	156.18±8.81

Table 11. Phagocytic activity of macrophages in the NTT-test (nitrosinium tetrazolium test) under the influence of xenon difluoride

Timing	7 days		14 days	
Groups	**Spontaneous**	**Stimulated**	**Spontaneous**	**Stimulated**
10 mg/kg	2.8±0.42 ($p_к$<0,01)	4.6±0.64	3.2±0.42	6.4±1.07
1 mg/kg	4.6±0.64	6.8±1.07	4.4±0.21	6.6±0.85
Control	4.4±0.21	6.8±0.42	4.6±0.64	6.8±0.85
Intact	4.2±0.42	6.4±0.64	4.4±0.42	6.8±0.85

Note: $p_к$ — validity with the control group

The indirect mast cell degranulation test with the serum of animals receiving xenon difluoride also gave a negative result. The serum of the experimental animal in the presence of xenon difluoride, and in its absence, did not cause mast cell degranulation (Table 12).

Table 12. DTH in Freund's complete adjuvant during the trial of xenon difluoride

Group	Experim. (cm)	Control (cm)	IR, %
After 6 hours			
PAF	0.46±0.02	0.33±0.02	44.00±11.38
XeF_2+PAF	0.46±0.02	0.32±0.03	49.57±15.17
Intact	0.43±0.03	0.33±0.03	32.20±7.51
After 24 hour			
PAF	0.48±0.03	0.39±0.02	23.86±4.70
XeF_2+PAF	0.47±0.02	0.39±0.03	22.86±3.49
Intact	0.41±0.02	0.32±0.02	27.20±8.15

CONCLUSIONS

As a result of the conducted studies, it was established that xenon difluoride has moderate toxicity and can be attributed to the third hazard class of chemical substances (GHS-system) (Booker et al., 2013). The study in rats and mice showed no signs of immunotoxicity and allergy of xenon difluoride.

Xenon difluoride has an embryotoxic effect with the intragastric administration to pregnant rats at a conventionally maximal therapeutic dose of 50 mg/kg at different gestation times. This is manifested by high embryonic death and disruption of the processes of skeleton formation.

According to the micronucleus test, xenon difluoride in the form of a 0.2% solution, administered intranasally, does not have a mutagenic effect. The mutagenic effect of xenon difluoride is manifested with the intragastric administration of the maximum tolerated, sublethal dose (100 mg/kg).

In conclusion, freshly prepared aqueous solutions of xenon difluoride in concentrations of 0.2% and 0.5% can be considered safe. All doses below 40 mg/kg, administered intragastric, are conditionally therapeutic, not having signs of toxicity under the conditions of this experiment.

REFERENCES

[1] Aleynikov, A. N. *Method for obtaining xenon difluoride, a method for its purification from explosive impurities and its use*. 2002, Patent №2232711. Russia (Ru)

[2] Al Tmimi, L., Van de Velde, M., Herijgers, P., Meyns, B., Meyfroidt, G., Milisen, K., Fieuws, S., Coburn, M., Poesen, K., Rex, S. *Xenon for the prevention of postoperative delirium in cardiac surgery: study protocol for a randomized controlled clinical trial*. 2015, Trials, 16, 449.

[3] Booker, R.D., Sum, A.K. *Biophysical changes induced by xenon on phospholipid bilayers*. 2013, Biochimica et Biophysica Acta, 1828(5), 1347-1356.

[4] Fisenko, V.P. *Manual on experimental (preclinical) study of new pharmacological substances*, 2000, Remedium. Moscow. (Ru)

[5] *ICH Harmonized Tripartite Guideline Genotoxicity: a Standard Battery for Genotoxicity Testing of Pharmaceuticals S2B* (16 July 1997). European Medicines Agency. London, UK.

[6] Lachmann, B., Armbruster, S., Schairer, W., Landstra, M., Trouwborst, A., Van Daal, G. J., Kusuma, A., Erdmann, W. *Safety and efficacy of xenon in routine use*. 1990, Lancet, 335 (8703), 1413-1415.

[7] Medy, D. *Chemical Encyclopedia. 1990,* Vol. 2. Soviet encyclopedia. Moscow. (Ru)

[8] Nekrasov, V. B. *Fundamentals of general chemistry*. 1973, Chemistry. Moscow. (Ru)

[9] Neyding, A. B., Sokolov, V.B. *Connections of noble gases*. 1974, Advances in Chemistry, 43(12), 2146-2194. (Ru)

[10] Preckel, B., Weber, N.C., Sanders, R.D., Maze, M., Schlack, W. *Molecular mechanism transducing the anesthetic, analgesic, and organ-protective actions of xenon, Anesthesiology*. 2006, 105(1), 187-197.

[11] Salmi, E., Laitio, R. M., Aalto, S., Maksimow, A. T., Långsjö, J. W., Kaisti, K. K., Aantaa, R., Oikonen, V., Metsähonkala, L., Någren, K., Korpi, E. R., Scheinin, H. *Xenon does not affect gamma-aminobutyric acid type A receptor*. 2008, Anesthesia & Analgesia, 106(1), 129-34.

[12] Tussupbekova, M. M. *Bases of histological technique and methods of histological investigation of autopsy, operational-biopsy and experimental material*. 2005 Methodical recommendations. Karaganda State Medical Academy. Karaganda. (Ru)

[13] Zhang, Z. *Reliability and error analysis on xenon*. 2000, The Keio Journal of Medicine, 49 Suppl 1, A29-32.