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Original Article

Initial assessment of the biological effects of hafnium tetrafluoride in rats following single intratracheal instillation

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ABSTRACT

Hafnium and its compounds assume ever greater importance in modern high-tech industries. There are very few and outdated data on their toxicity. This work examines the effect of hafnium tetrafluoride on airway reactions and hematological and some biochemical blood parameters in white rats under conditions of acute inhalation experiment. The substance was administered once intratracheally at the doses corresponding to concentrations of 2.0 mg/m^3 and 1.0 mg/m^3, and the test parameters were monitored on Day 2 and Day 7. Intratracheal administration of hafnium tetrafluoride did not cause visible deterioration in the general condition of the animals or animal death. Exposure to hafnium tetrafluoride caused a significant decrease in erythrocyte count in the absence of other hematological parameter shifts and an increase in AST activity 24 hours after animal intoxication. Peroxidation parameters of bronchoalveolar lavage fluid (BALF) did not change. BALF cytological composition did not differ significantly in the control and experimental groups, although hemosiderin granules in macrophages were observed, as well as degenerated cells of cylindrical epithelium on Day 2 after exposure. The obtained results showed a damaging effect of hafnium tetrafluoride at concentrations of 2.0 mg/m^3 and 1.0 mg/m^3 on lungs, which were self-cleaned under the influence of adaptive mechanisms within 7 days after exposure. There is a need for an in-depth study of the biological effects of hafnium compounds and search for safe levels of their exposure in industrial use.

KEYWORDS: hafnium tetrafluoride (IV), CAS RN13709-52-9, inhalation, airway reactions, systemic toxicity.

INTRODUCTION

Hafnium was only discovered in the 20s of the last century. It belongs to the group of the trace elements and does not form independent minerals, occurs in the form of impurities in zirconium compounds and is considered its homologue. Hafnon (Hf, Zr) SiO4 is the only known hafnium mineral and contains up to 70-75% HfO2. This element is an accessory mineral of zirconium in its ores and minerals with a constituent share of 8-13%. Naturally, Hf/Zr ratio is 2-3%. Accordingly, until the end of the XX century, it was perceived only as a by-product of the latter's extraction. Hafnium is found in all living organisms, including humans. The maximum content of hafnium was found in the trachea ($4 \cdot 10^{-3}$ mg/kg) and the minimum content in the large intestine ($3 \cdot 10^{-5}$ mg/kg). The biological significance of hafnium remains unclear [1].

Hafnium and its compounds are becoming increasingly important in the modern global economy; in the coming years an increase in demand up to 40% is expected for it. This is due to its widespread introduction in the important and knowledge-intensive industries. In particular, hafnium

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tetrafluoride is used in the manufacture of special optic devices including night vision devices, thermal imagers, and it is an intermediate product in obtaining metallic hafnium [2].

Given an increase in the hafnium compound production, a question of assessing their hazardous effect on people's health arises. At the same time, the literature records on toxicity and safe exposure to hafnium compounds are currently limited. The vast majority of experimental data on toxic effects hafnium compounds date back to the 60s and 70s of the last century, and information on hafnium tetrafluoride is virtually non-existent.

It should be noted that quite often the assessment of the hazards of hafnium compounds is based on data on zirconium compounds. Since the hafnium ionic radius is close to that of zirconium due to lanthanide electron contraction effect at the sixth energy level, it is suggested that the properties of these two elements are proximate [2]. However, these elements cannot be equated. The most striking contrast between zirconium and hafnium is found in nuclear chemistry – zirconium has a low cross-section neutron absorption, while hafnium easily absorbs neutrons. In addition, these elements have different melting and boiling points, as well as solubility in solvents [3].

Many publications point to the low toxicity of both zirconium and hafnium. It is noted that hafnium in terms of hazard level is moderately toxic along with beryllium, cadmium and chromium [4]. At the same time, in addition to the above negative effects of hafnium compounds on the body, there are publications showing concern about zirconium as a cause of pathological changes in the body of people in contact with it [5]. That is, currently, there is no consensus on the toxic potential of these metals.

The purpose of the current research is to study the effects of hafnium tetrafluoride on the cytological parameters of pulmonary lavage and blood homeostasis in white rats in an acute experiment with intratracheal instillation.

MATERIALS AND METHODS

Chemicals

Hafnium(4+) tetrafluoride was provided by the State Enterprise "EASTERN MINING AND PROCESSING WORKS", purity grade: 99.9%.

In terms of physicochemical properties, it is a white powder, odourless, insoluble in water, with a dispersion of particles of 63 microns (74%). Relative molecular weight Mr: 254.48; mass fraction: Hf 70.138 %; F 29.861%.

Experimental animals and their management

An acute experiment was performed in 60 Wistar female rats, aged 3-4 months with a body weight of 150-220 g, provided by the vivarium of the Danylo Halytsky National Medical University in Lviv. To study the toxic effects, the animals were divided into groups of 10 individual animals using "blind ranking" method; each animal received group-based and individual labels characterizing its group affiliation and individual number in the experiment. Animals received standard pelleted feed with unrestricted access to drinking water. The animal studies were guided by the provisions of the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes [6].

Experimental design

The substance was administered once intratracheally [7] to white rats at doses corresponding to a concentration of 2.0 mg/m^3 (Group I) and 1.0 mg/m^3 (Group II). The choice of concentrations is based on the pre-set value of LC_{50}, which is 20.5 mg/m^3 [8]. Normal saline was used as a vehicle to suspend the hafnium tetrafluoride prior to instillation. Control animals were treated with the vehicle.

Rats were anesthetized by intramuscular administration of XylaVet at a dose of 5 mg/kg/body weight and Zoletil 50 at a dose of 0.5 mg/kg/body weight. Subsequently, the animals were fixed in a lateroprone position on a board at an angle of 60°, holding their upper incisor teeth. The tongue was gently stretched with coated tweezers. The animals were intubated through the mouth and trachea with glottis opened [9]. Intratracheal instillations were well tolerated, and no side effects were observed during the study period.

Cytological analysis of bronchoalveolar lavage fluid (BALF)

Deep airway reactions to hafnium tetrafluoride were evaluated along with the cytological characteristics of the fluid obtained by bronchoalveolar lavage on Days 2 and 7 after a single intratracheal instillation

of the suspension. BALF was prepared through lung infusion twice with 1 ml of saline per 25 g of body weight. Immediately after BALF collection, the samples were placed on ice and stored refrigerated. To calculate the total number of cells, the BALF sample was centrifuged for 10 minutes at 2000 rpm, the supernatant was discarded, and smears were made of the precipitation. Smears were stained according to Romanovsky-Giemsa. 200 cells were counted from each smear during microscope observation [10]. The ratio of neutrophil leukocytes (NL) and alveolar macrophages (AM) was calculated.

Clinical observations and hematological and blood biochemical analyses

Clinical signs were evaluated every day in all rats. Blood was collected by cardiac puncture into a serum collection tube. Part of the blood was used to count blood cells, and the rest of the blood with an anticoagulant was centrifuged at 3000 rpm for 10 minutes at 4 °C followed by plasma collection. Determinations were performed on Days 2 and 7 after administration.

Erythrocytes, leukocytes and their types were calculated. The levels of hemoglobin and biochemical blood parameters (alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase (ALT) activity, and creatinine level) were determined using an Assay Kit (manufactured by LLC NPP "Phylicit-Diagnostics", Ukraine) according to the instructions. Oxidative stress processes were evaluated by lipid peroxidation: catalase activity (nmol H_2O_2/mLxmin) was defined by the assay based on the rate of hydrogen peroxide/ammonium molybdate complex formation [11], diene conjugates (units/ml) – using the method based on rearrangement of double bonds in polyunsaturated fatty acids to form diene conjugates followed by their spectrophotometric determination at 233 nm [12], malonyldialdehyde (mmol/L) was tested by thiobarbituric acid (TBA) assay based on the release of a color complex due to TBA reaction with MDA [13].

In addition, De Ritis ratio [14] and antioxidant-prooxidant index (API) based on MDA concentration and catalase activity ratio were calculated.

Statistics

The study results were analysed and processed using Microsoft Excel 10.0 computer system. The normality of distribution was verified using Shapiro-Wilk test. Jonckheere trend test and Kruskal-Wallis test were used to compare non-parametric indicators. Data are described using median and interquartile range. The critical level of significance in testing statistical hypotheses was taken as $p \leq 0.05$ [15].

RESULTS

Clinical observations, hematology and blood biochemistry

Intratracheal instillation of hafnium fluoride at concentrations of 2.0 mg/m^3 and 1.0 mg/m^3 did not cause visible deterioration of the general condition of the animals or their death throughout the entire observation period.

Analysis of hematological peripheral blood parameters in rats 24 hours after intratracheal instillation of hafnium fluoride at a concentration of 2.0 mg/m^3 showed a significant decrease in the number of erythrocytes compared with the control group of animals and no significant differences in hemoglobin concentration between comparison groups. The second experimental group of animals showed a tendency towards decrease in the number of blood erythrocytes; however, it was insignificant compared to the group of control animals. On Day 7 of the experiment, this indicator recovered; it had no statistically significant differences in both the I and II groups vs. control (Fig. 1), but at the same time the hemoglobin concentration increased in the II experimental group (Fig. 2).

The total leukocyte blood count in groups I and II of experimental animals throughout the experiment had no significant differences as compared to the control group [16]. No statistically significant shifts were observed in the leukogram cells ratio of experimental animals compared with the control group (Table 1).

The effect of hafnium fluoride on serum AST and ALT activity on Day 2 after instillation is shown in Fig. 3 and 4. Hafnium fluoride concentrations of 2.0 and 1.0 mg/m^3 showed a significant increase in AST activity 24 hours after administration of the drug in the animals. On Day 7, the activity of transferases in the blood of experimental animals had no significant differences from that in the control group. For all tested concentrations, De Ritis ratio in the experimental groups exceeded 1 on both the

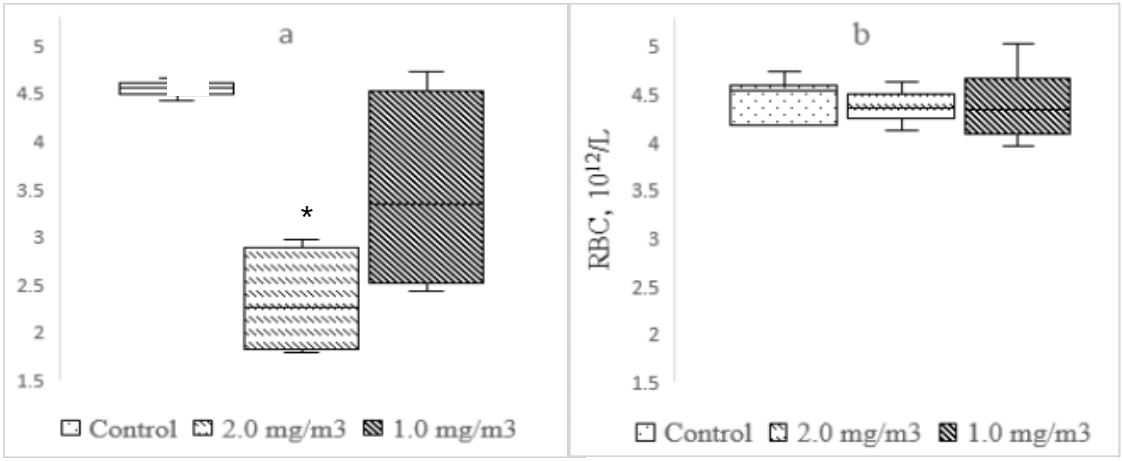

Fig. 1. Comparison of erythrocyte count in experimental animals following hafnium tetrafluoride intratracheal instillation at concentrations of 2.0 mg/m^3 and 1.0 mg/m^3 vs. control. a) Day 2 of the experiment, b) Day 7 of the experiment. Results are shown as box-and-whisker plots (the middle horizontal line within the box represents the median; boxes extend from the 25th to the 75th percentile; and the whiskers represent 95% confidence intervals).
*The median values differed significantly ($P < 0.05$ vs. control, Kruskal-Wallis test with Bonferroni adjustment).

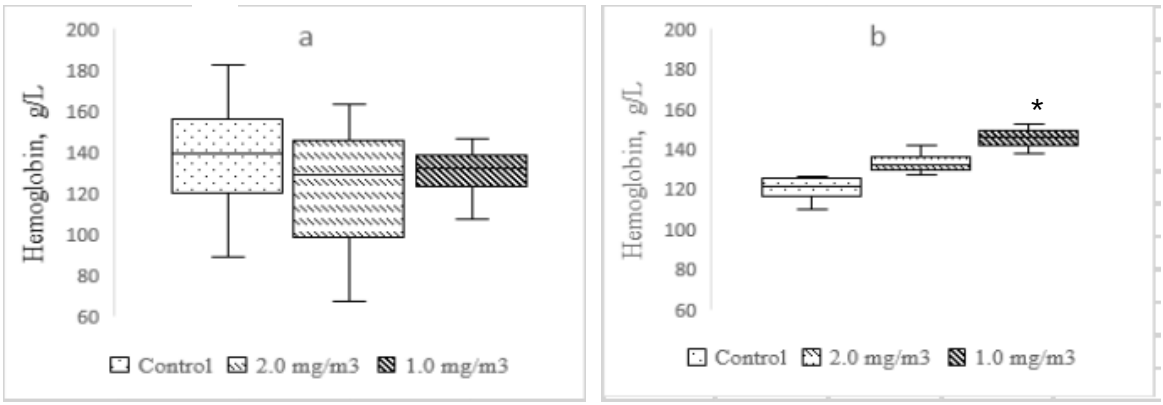

Fig. 2. Hemoglobin concentration in experimental animals following hafnium tetrafluoride intratracheal instillation at concentrations of 2.0 mg/m^3 and 1.0 mg/m^3 vs. control. a) Day 2 of the experiment, b) Day 7 of the experiment. Results are shown as box-and-whisker plots (the middle horizontal line within the box represents the median; boxes extend from the 25th to the 75th percentile; and the whiskers represent 95% confidence intervals).
*The median values differed significantly ($P < 0.05$ vs. control, Kruskal-Wallis test with Bonferroni adjustment).

Days 2 and 7 of the experiment. Throughout the experiment, this ratio was below 1 in the control group. Serum creatinine concentration and alkaline phosphatase activity in rats of experimental groups vs. control had no significant differences on Day 7 of the experiment.

Study results for lipid peroxidation product content and catalase activity in blood of experimental animals are shown in Table 2. These indicators did not change throughout the experiment. However, it was noted that the catalase activity in group I of experimental animals increased, although the increase was insignificant.

Cell profile of BALF

Table 3 shows the comparative evaluation results for BALF cell composition in response to hafnium tetrafluoride intratracheal instillation. In terms

Table 1. Peripheral blood leukogram in rats after hafnium tetrafluoride exposure.

Indicator	Experimental groups		
	Control	Group I (2.0 mg/m^3)	Group II (1.0 mg/m^3)
Day 2			
WBC, 10^9/L (white blood cells)	6.50 (4.03-10.98)	6.0 (5.0-15.40)	9.15 (7.95-10.15)
basophils,%	0 (0-0.25)	0 (0-0.25)	0 (0-0)
eosinophils,%	2.0 (2.0-2.25)	1.5 (1.0-2.0)	1.0 (1.0-1.25)
neutrophils,%	16.5 (14.5-18.75)	18.0 (15.75-20.25)	14.50 (13.5-16.0)
monocytes,%	5.0 (4.25-5.25)	5.0 (3.0-7.25)	5.0 (4.75-5.25)
lymphocytes, %	76.0 (74.8-77.5)	75.0 (71.8-78.3)	78.5 (76.5-80.8)
Day 7			
WBC, 10^9/L	8.4 (7.4-8.8)	9.5 (7.8-10.2)	8.9 (7.7-9.4)
basophils, %	0 (0-0)	0 (0-0)	0 (0-0.25)
eosinophils, %	2.0 (2.0-2.0)	2.0 (2.0-2.0)	2.0 (1.75-2.25)
neutrophils, %	20.0 (18.0-21.0)	18.0 (16.0-20.0)	21.0 (19.0-23.5)
monocytes, %	2.0 (2.0-2.0)	2.0 (2.0-2.0)	2.0 (2.0-2.25)
lymphocytes, %	76.0 (75.0-78.0)	78.0 (76.0-80.0)	75.5 (73.25-76.5)

Note: Data reported as medians and interquartile ranges (IQR).

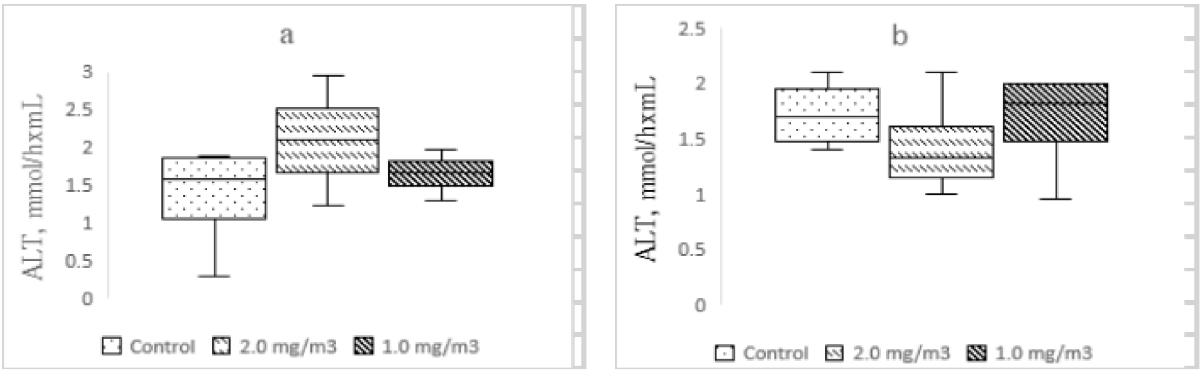

Fig. 3. Blood ALT activity in experimental animals following intratracheal hafnium tetrafluoride instillation at concentrations of 2.0 mg/m^3 and 1.0 mg/m^3 vs. control animals. a) Day 2 of the experiment, b) Day 7 of the experiment. Results are shown as box-and-whisker plots (the middle horizontal line within the box represents the median; boxes extend from the 25th to the 75th percentile; and the whiskers represent 95% confidence intervals). P <0.05 vs. control, Kruskal-Wallis test with Bonferroni adjustment.

of quantitative characteristics, the cytological composition of bronchoalveolar lavage had no differences in the control and experimental groups both 24 hours and 7 days after intoxication. However, it should be noted that 50% and 25% of animals treated with hafnium tetrafluoride at concentrations of 2.0 mg/m^3 and 1.0 mg/m^3, respectively, showed hemosiderin granules in macrophages and degenerated cylindrical epithelial cells on the first day after exposure.

DISCUSSION

The first phase of acute hafnium tetrafluoride exposure showed signs of damage to the lower

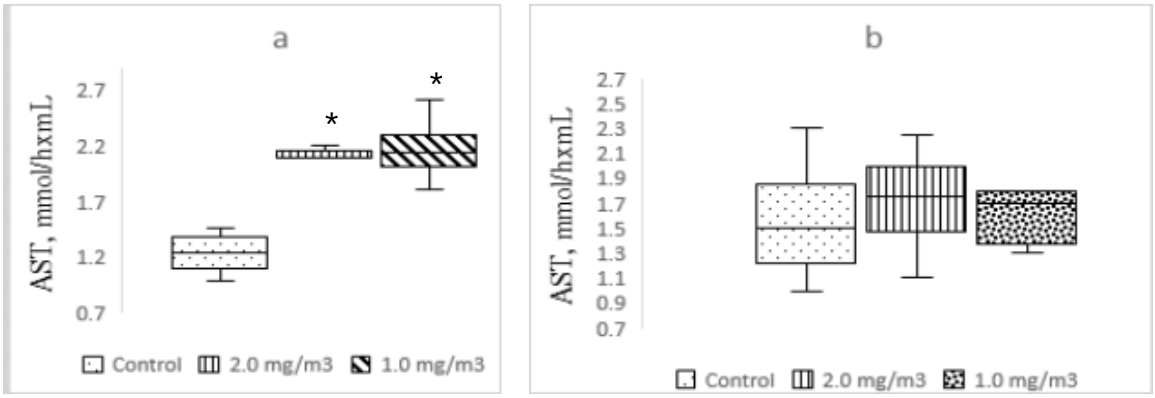

Fig. 4. Blood AST activity in experimental animals following intratracheal hafnium tetrafluoride instillation at concentrations of 2.0 mg/m^3 and 1.0 mg/m^3 vs. control animals. a) Day 2 of the experiment, b) Day 7 of the experiment. Results are shown as box-and-whisker plots (the middle horizontal line within the box represents the median; boxes extend from the 25th to the 75th percentile; and the whiskers represent 95% confidence intervals). *The median values differed significantly ($P < 0.05$ vs. control, Kruskal-Wallis test with Bonferroni adjustment).

Table 2. Level of malonyldialdehyde (MDA), diene conjugates (DC) and catalase activity in rats after single intratracheal instillation of hafnium tetrafluoride.

Indicator	Experimental groups		
	Control	Group I (2.0 mg/m^3)	Group II (1.0 mg/m^3)
Day 2			
DC, UA/mL	1.74 (0.51-6.49)	3.13 (2.84-9.17)	2.43 (1.69-5.67)
MDA, mmol/L	6.22 (5.16-7.14)	9.0 (8.07-11.90)	11.24 (5.42-13.40)
catalase, nmol H_2O_2/mL x hr	0.68 (0.47-0.88)	5.40 (2.84-13.77)	0.54 (0.27-0.68)
antioxidant-prooxidant index, API	0.12 (0.01-0.13)	0.6 (0.3-1.05)	0.07 (0.03-0.11)
Day 7			
DC, UA/mL	1.94 (1.92-1.96)	1.70 (1.65-1.77)	1.76 (1.64-1.88)
MDA, mmol/L	45.75 (45.50-46.30)	44.18 (42.99-45.49)	45.75 (43.78-47.08)
catalase, nmol H_2O_2/mLx hr	0.81 (0.27-1.89)	9.99 (1.55-21.13)	0.54 (0.27-2.90)
antioxidant-prooxidant index, API	0.02 (0.01-0.04)	0.21 (0.04-0.55)	0.01 (0.01-0.12)

Note: Data reported as medians and interquartile ranges (IQR).

lung in the form of hemosiderosis and bronchial epithelium exfoliation. This indicates the presence of cytotoxic activity of this compound. In addition to hemosiderin deposition in BALF macrophages, a significant decrease was observed in erythrocyte blood count, probably due to their hemolysis.

The results of the study on changes in biochemical parameters indicate liver tissue dysfunction due to intoxication. This is consistent with available data; that is an acute hepatocellular trauma causes immediate increase in serum AST levels, reaching levels higher than initial ALT, due to higher AST activity in hepatocytes and its release during liver damage [17]. However, it is not unlikely that this presentation is observed due to erythrocyte hemolysis. There is evidence that sometimes AST increase may have a non-hepatic origin due to enzyme release from the blood cells, which occurs during hemolysis [14].

Table 3. Bronchoalveolar lavage cytological composition in rats following intratracheal instillation of hafnium tetrafluoride.

Indicator	Experimental groups		
	Control	Group I (2.0 mg/m^3)	Group II (1.0 mg/m^3)
Day 2			
eosinophils,%	0.5 (0-2.0)	1.0 (0.5-2.5)	0.0 (0; 4.0)
neutrophils,%	11.0 (3.5-19.0)	29.0 (15.5-29.0)	16.5 (13.5-19.5)
macrophages,%	63.5 (45.0-67.0)	64.0 (41.0-69.0)	71.0 (45.5-77.0)
WBC,%	10.0 (6.0-16.5)	17.0 (13.0-22.5)	11.0 (5.5-22.0)
NL/MA	0.29 (0.24-0.32)	0.15 (0.13-0.17)	0.19 (0.15-0.23)
Day 7			
eosinophils,%	2.0 (1.0-2.0)	2.0 (1.5-2.5)	1.5 (1.0-2.0)
neutrophils,%	18.0 (17.0-19.0)	10.5 (9.5-11.5)	13.5 (11.5-15.0)
macrophages,%	67.0 (57.0-69.0)	71.5 (70.8-73.0)	71.0 (65.75-75.5)
WBC,%	16.0 (12.0-21.0)	16.0 (14.25-17.0)	15.0 (11.5-18.5)
NL/MA	0.18 (0.06-0.53)	0.76 (0.43-0.92)	0.44 (0.36-0.56)

Note: Data reported as medians and interquartile ranges (IQR).

On Day 7, the experimental animals showed in response to the hafnium fluoride effects the active adaptive processes. A significant increase was identified in the hemoglobin level, but there was no noticeable change in erythrocyte count compared with the control animals ($p > 0.05$), which indicates hyperchromia. Given the close relationship of all hemostasis components, we can assume that the blood cell reaction serves as a kind of compensator aimed to reduce the factors influencing changes in their quantitative indicators. The above weekly time period reflects the existing time delay of the body's response to exposure.

The mechanism of hafnium tetrafluoride toxicity may be based on a provision that toxic metals can bond to the same enzymes as the metals involved in maintaining homeostasis [18]. As for hafnium, the available literature reports that it binds with transferrin, the main transport protein of iron [19]. Accordingly, this promotes a pool of catalytically active iron ions.

Adaptive or damaging effect of xenobiotics takes place in a body through the cell membrane system. The membrane condition determines to a greater extent the course of physiological and biochemical processes and thus constitutes an original component in a complex chain of adaptive modifications at all levels. The metabolic disorders, we have identified, underlie the decrease in detoxicative hepatic function, since the main detoxifying systems are localised in hepatocytes.

In general, the data we have obtained are consistent with the reports on the toxic effects of hafnium compounds. In particular, hafnium hydride when administered intratracheally showed a pronounced fibrogenic and general toxic effect [20]. Exposure to hafnium carbide and hafnium nitride dust showed in the liver – hepatic circulatory disorders, and granular dystrophy; in kidneys – granular dystrophy of the convoluted tubule epithelium; in myocardium – eosinophilic and granular dystrophy of muscle fibers; in spleen - plethora and hyperplasia of reticuloendothelium; in lungs – thickening of the interalveolar septa with moderate emphysematosis and vascular disorders in the form of erythrostasis, chronic interstitial pneumonia with severe fibrosis, generalized purulent bronchitis, and bronchiectasis [21].

Defense industry workers were diagnosed with pulmonary interstitial fibrosis with scattered loose granulomas and focal macrophages. Subsequent spectroscopy revealed the presence of hafnium in macrophages. Industrial contact with hafnium was indicated in the patients´ medical history [22]. In addition, it has been suggested that hafnium compounds may cause benign pneumoconiosis [23].

The mechanism of toxicity of slightly soluble metals, in particular hafnium compounds, can be explained by the formation in the body of organometallic compounds. The hafnium cations are almost never present in the solutions in the form of monoatomic ions and do not form typical ionic bonds. It was shown that hafnium tetrafluoride in an aqueous medium forms hydroxide polynuclear particles [24]. It is generally believed that toxicants forming colloidal particles, in particular trivalent and tetravalent cations (lanthanum, cesium, and hafnium), are captured by the reticuloendothelial system of the specialised cells of the organs and tissues. Larger particles are usually characterized by their subsequent retention in the liver [25]. In addition, hafnium has been shown to be a good complexing agent. Its ability to form coordination bonds with oxygen-containing ligands leads to the formation of new organic compounds [26], which can disrupt the body's homeostasis and cause the development of a pathology.

CONCLUSION

The obtained results showed significant damaging activity of hafnium tetrafluoride in concentrations of 2.0 mg/m^3 and 1.0 mg/m^3 on lungs and blood erythrocytes. It was established that the toxic damage of hafnium tetrafluoride on lungs reduces due to pulmonary mechanisms of self-clearance. There is an urgent need for the in-depth study of the biological effects of hafnium compounds and search for safe levels of their exposure in industrial use.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

REFERENCES

1. Baranovskaya, N. V., Rikhvanov, L. P., Ignatova, T. N., Narkovich, D. V. and Denisova, O. A. 2015, The human geochemistry essays. TPU Publishing House, 378.
2. Jones, J. V. III, Piatak, N. M. and Bedinger, G. M. 2017, Critical mineral resources of the United States—Economic and environmental geology and prospects for future supply, K. J. Schulz, J. H. DeYoung, R. R. Jr Seal II, D. C. Bradley (Editors), Reston, VA: U.S. Geological Survey, 26.
3. Xu, L., Xiao, Y., Sandwijk A., Xu, Q. and Yang, Y. 2015, Journal of Nuclear Materials, 466, 21-28.
4. Bhargava, P., Gupta, N., Vats, S. and Goel, R. 2017, Austin J Environ Toxicol, 3(1), 1018.
5. Voevodin, V. N., Lavrinenko, S. D., Oshkaderov, S. P. and Strebkova, O. A. 2014, Metallophysics and advanced technologies, 36(7), 935-946.
6. Directive 2010/63/EU on the protection of animals used for scientific purposes. 2010, EU Official Journal. L276.
7. Oka, Y., Mitsui, M., Kitahashi, T., Sakamoto, A., Kusuoka, O., Tsunoda, T., Mori, T. and Tsutsumi M. 2006, J. Toxicol. Pathol., 19, 107-109.
8. Turkina, V. A. and Lukasevych, N. F. 2020, Actual problems of preventive medicine, 19, 215-219.
9. Ribeiro, R. S. A., Ferreira, I. M. E. S. R., Figueiredo Jr. I. and Verícimo, M. A. 2015, Jornal Brasileiro de Patologia e Medicina Laboratorial, 51(3), 183-188.
10. Matichin, A. A., Katel'nikova, A. E. and Kryshen', K. L. 2019, Laboratory Animals for Science, 4, 37-43.
11. Korolyuk, M. A., Ivanova, L. I., Maiorova, I. G. and Tokarev, V. E. 1988, Lab Delo, 1, 16-19.
12. Recknagel, R. O. and Glende, E. A. 1984, Methods Enzymol, 105, 331-337.
13. Gönenç, A., Erten, D., Aslan, S., Akıncı, M., Şimşek, B. and Torun, M. 2006, Cell Biology International, 30(4), 376-380.
14. Botros, M. and Sikaris, K. A. 2013, Clin. Biochem. Rev., 34(3), 117-130.
15. Glantz, S. A., Bryan, K. S. 1990, Primer of Applied Regression and Analysis of Variance, McGraw-Hill, Health Professions Division, New York.
16. Sorokina, A. V., Alekseeva, S. V., Eremina, N. V. and Durnev, A. D. 2019, Bulletin of the Scientific Centre for Expert Evaluation of Medicinal Products, 9(3), 197-206.
17. Kim, W. R., Flamm, S. L., Di Bisceglie, A. M. and Bodenheimer, H. C. 2008, Hepatology, 47, 1363-1370.
18. Guo, W., Zhang, J., Li, W., Xu, M. and Liu, S. 2015, J. Environ. Sci., 34, 155-164.
19. Taylor, D. M., Lehmann, M., Planas-Bohne, F. and Seidel, A. 1983, Radiat Res., 95(2), 339-358.

20. Shkurko, G. A. 1973, Gigiena truda, Y. I. Kundiev (Ed.), Kiev, «Zdorov'ya», 74-76 [in Rus.].
21. Zhislin, L. E. and Oveckaya, N. M. 1969, Gigiena truda, Y. I. Kundiev (Ed.), Kiev, «Zdorov'ya», 36-40 [in Rus.].
22. Sanyal, S., Crawford, J. A., Abraham, J. L. and Gimino, V. 2018, Am. J. Respir. Crit. Care Med., 197, A6086.
23. Wright, P. H. 1993, Postgrad. Med. J., 69, 129-135.
24. Pchelkin, V. A., Goryunov, E. F., Lavrikov, A. S., Galaev, A. V. and Kosenko, K. A. 1988, Zhurnal Neorganicheskoj Khimii, 33(8), 1919-1923.
25. Lewis, R. J. and Sax's, N. 2012, Dangerous Properties of Industrial Materials, 12th Edition, New York, 5772 p.
26. Kostrikin, A. V., Kosenkova, O. V., Spiridonov, F. M., Komissarova, L. N., Lin'ko, I. V. and Zaitsev, B. E. 2010, Russian journal of inorganic chemistry, 55(6), 866-875.